XXIVth North American Testis Workshop

"From Testis Differentiation to Sperm Production"

April 19 – 22, 2017

The Hilton Miami Downtown | Miami, Florida

Program Chair: Leslie L. Heckert, PhD
Program Vice-Chair: Vassilios Papadopoulos, DPharm, PhD

Welcome to the XXIVth North American Testis Workshop

Welcome to the 2017 XXIVth North American Testis Workshop. The theme for 2017 is *"From Testis Differentiation to Sperm Production,"* a title that, we feel, captures the field's many sub-specialties. This year, we gather in the exciting city of Miami, Florida, to continue the Workshop's longstanding tradition of bringing together trainees and new and established scientists from around the world to discuss various aspects of testicular physiology, biochemistry, molecular and cell biology. The XXIVth Workshop will commence with a keynote lecture by Dr. Dirk de Rooij of Utrecht University, The Netherlands. Dr. de Rooij's address *"Comparison Between Human and Rodent Spermatogonial Renewal and Differentiation"* will provide insights into what defines these progenitors of spermatogenesis and set the stage for presentations and discussions throughout the meeting. The program features three benchmark lectures; one on the various roles of piRNAs in spermatogenesis by Dr. Haifan Lin, a second on male infertility by Dr. John Aitken and a third on paternal environment and its effects on future generations by Dr. Oliver Rando. Other program highlights include six highly interactive sessions, which emphasize different aspects of testis biology and include short talks selected from submitted abstracts, and two poster sessions where participants meet in a social setting to discuss data, encourage young scientists and explore new collaborations.

We would like to thank members of the program committee (listed below), who provided invaluable assistance and insight in developing the scientific program for the XXIVth Testis Workshop. We also thank members of the Abstract Review Committee for their help in identifying presentations for the short talks and poster awards. We are sincerely 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 National Institute of Environmental Health Sciences, the Burroughs Wellcome Fund, and Dr. Paul Cheney and the Kansas Idea Network of Biomedical Research Excellence (NIGMS). To Donna Rostamian and her team at WJ Weiser & Associates, thank you for the amazing "behind the scenes" job managing the overwhelming number of venue and program arrangements. And, to the Testis Workshop Executive Committee, thank you for your unwavering dedication and support. The committee, represented by past and future program chairs, oversees management and long-term planning of the workshop.

The Testis Workshop "works" because of the many excellent scientists who attend and contribute to each meeting. Thank you all for participating and we hope you enjoy the meeting and Miami venue, with its sandy beaches and seascapes.

Leslie Heckert, PhD
Chair of the Program Committee

Vassilios Papadopoulos, DPharm, PhD
Vice-Chair of the Program Committee

Testis Workshop Committees

Program Committee

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Mitch Eddy, PhD
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Faculty

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April 19 - 22, 2017

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East Carolina University

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Washington State University

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University of Texas at San Antonio

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Sarah Kimmins, PhD
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Kate Loveland, PhD
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Humphrey Yao, PhD
NIEHS/NIH

Program Schedule

The XXIV North American Testis Workshop
"From Testis Differentiation to Sperm Production"
April 19 – 22, 2017
The Hilton Miami Downtown

Program Chair Leslie L. Heckert, PhD and Program Vice-Chair Vassilios Papadopoulos, DPharm, PhD
*All sessions will be held in **Symphony Ballroom III/IV** unless otherwise noted.*
Speakers and times are subject to change.

WEDNESDAY, APRIL 19, 2017

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

7:00 p.m. - 7:15 p.m. **Welcome and Opening Remarks**
Program Chair: Leslie L. Heckert, PhD
University of Kansas Medical Center

7:15 p.m. - 8:15 p.m. **KEYNOTE ADDRESS:**
Comparison Between Human and Rodent Spermatogonial Renewal and Differentiation
Dirk De Rooij, PhD
Universiteit Utrech, the Netherlands

8:15 p.m. - 9:30 p.m. **Testis Workshop Welcome Reception**
Location: Upper Pool Terrace

THURSDAY, APRIL 20, 2017

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

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

8:00 a.m. - 8:55 a.m. **Benchmark Lecture I**

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

8:05 a.m. - 8:55 a.m. **Uniting the Genome: Multifaceted Roles of piRNAs during Spermatogenesis**
Haifan Lin, PhD
Yale University Stem Cell Center

SESSION I: GERMLINE ESTABLISHMENT & HOMEOSTASIS

8:55 a.m. - 9:00 a.m. **Chair and Introduction to Session I**
Jon M. Oatley, PhD

9:00 a.m. - 9:40 a.m. **Dynamics of Stem Cell Replacement in the Drosophila Testis Niche**
Erika Matunis, PhD
Johns Hopkins University

9:40 a.m. - 10:20 a.m. **Defining Spermatogonial Stem Cell Transcriptomes at the Single-Cell Level**
Brian P. Hermann, PhD
University of Texas at San Antonio

10:20 a.m. - 10:40 a.m. **Break**
Location: Symphony Ballroom Foyer

10:40 a.m. - 11:20 a.m. **Differential Requirements for the 'Mechanistic Target of Rapamycin' (mTOR) and mTORC1 Component Raptor in Spermatogonial Development in the Mouse**
Christopher Geyer, PhD
East Carolina University

11:20 a.m. - 11:35 a.m. **Short Talk #1**
A High - Throughput Screen to Identify Novel Transcription Factors That Regulate Mouse Speratogonial Stem Cell Maintenance
Presented by: Tessa Lord, BBiotech, PhD
Washington State University

11:35 a.m. - 11:50 a.m. **Short Talk #2**
Conservation of a Gene Expression Barcode that Defines Spermatogonial Stem Cells in Mice and Humans
Presented by: Anukriti Singh, BTech
University of Texas

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

SESSION II: GERM CELL DIFFERENTIATION AND MAINTENANCE - THE ROLE OF RNA &RBP'S

1:10 p.m. - 1:15 p.m. **Chair and Introduction to Session II**
Wei Yan, PhD
University of Nevada

1:15 p.m. - 1:55 p.m. **Distinct Functions of Nanos2 and Nanos3 During Spermatogenesis**
Yumiko Saga, DSc
National Institute of Genetics Japan

1:55 p.m. - 2:35 p.m. **GASZ Interacts with Mitofusins to Regulate Spermatogenesis**
Yuan Wang, PhD
East China Normal University

2:35 p.m. - 2:55 p.m. **Break**
Location: Symphony Ballroom Foyer

2:55 p.m. - 3:35 p.m. **Regulation of Testis Transcriptome by the MOV10 RNA Helicase**
Jeremy Wang, MD, PhD
University of Pennsylvania

3:35 p.m. - 3:50 p.m. **Short Talk #3**
Clonal Development of Spermatogonia in Rhesus Testes
Presented by: Adetunji Fayomi, DVM
University of Pittsburgh

3:50 p.m. - 4:05 p.m. **Short Talk #4**
The RHOX10 Homeobox Transcription Factor Promotes Prospermatogonia Migration
Presented by: Wei-Ting Hung, PhD
UC San Diego

4:05 p.m. - 6:05 p.m. **Poster Session I**
Location: Symphony Ballroom I/II

Program Schedule

FRIDAY, APRIL 21, 2017

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

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

8:00 a.m. - 8:55 a.m. **Benchmark Lecture II**

8:00 a.m. - 8:05 a.m. **Chair and Introduction**
Dolores J. Lamb, PhD, HCLD

8:05 a.m. - 8:55 a.m. **New Insights into the Causes and Consequences of Male Infertility**
John R. Aitken, PhD, ScD, FRSE, FSRB
University of Newcastle Australia

SESSION III: TESTIS DEVELOPMENT & DIFFERENTIATION

8:55 a.m. - 9:00 a.m. **Chair and Introduction to Session III**
Jannette Dufour, PhD
Texas Tech University Health Sciences Center

9:00 a.m. - 9:40 a.m. **Control of Post-Natal Testis Development by the Sertoli Cells**
Peter O'Shaughnessy, PhD
University of Glasgow Veterinary School, Ireland

9:40 a.m. - 10:20 a.m. **New Insights into Fate Deamination and Maintenance of the Testis**
Humphrey H. Yao, PhD
NIEHS/NIH

10:20 a.m. - 10:40 a.m. **Break**
Location: Symphony Ballroom Foyer

10:40 a.m. - 11:20 a.m. **Evidence that Nucleocytoplasmic Transport Proteins Mediate Environmental Cues Required for Male Fertility**
Kate Loveland, PhD
Monash University and Hudson Institute of Medical Research

11:20 a.m. - 11:35 a.m. **Short Talk #5**
3 Dimensional Human Testis Organoid System Created from Immature Testicular Cells
Presented by: Nima Pourhabibi Zarandi, MD
Wake Forest School of Medicine

11:35 a.m. - 11:50 a.m. **Short Talk #6**
Regulation of CYP26B1 Expression in the Testis
Presented by: Parag Parekh, PhD
University of Texas

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

SESSION IV: TRANSCRIPTIONAL & ENDOCRINE REGULATION IN THE TESTIS

1:10 p.m. - 1:15 p.m. **Chair and Introduction to Session IV**
Ralph Meyer, PhD
Utah State University

1:15 p.m. - 1:55 p.m. **Spermatogenesis Requires Classical and Nonclassical Testosterone Signaling**
William H. Walker, PhD
University of Pittsburgh

1:55 p.m. - 2:35 p.m. **The CAMKI-MEF2-NUR77-AMPK Cascade in the Regulation of Leydig Cell Steroidogenesis**
Jacques J. Tremblay, PhD
Laval University, Canada

2:35 p.m. - 2:50 p.m. **Short Talk #7**
ADCY2 is a Candidate Gene for the Development of Congenital Genitourinary Anomalies Through Partial Disruption of Steroidogenesis
Presdented by: Marisol O'Neill, MS
Baylor College of Medicine

2:50 p.m. - 3:10 p.m. **Break**
Location: Symphony Ballroom Foyer

SESSION V: SPERM DEVELOPMENT & MATURATION

3:10 p.m. - 3:15 p.m. **Chair and Introduction to Session V**
Jacquetta Trasler, MD, PhD
McGill University Health Centre

3:15 p.m. - 3:55 p.m. **Spermatogenesis as a Model System to Define Katanin Function**
Moira K. O'Bryan, BSc, PhD
Monash University, Australia

3:55 p.m. - 4:35 p.m. **Intercellular Networks and Luminal Acidification in the Epididymis**
Sylvie Breton, PhD
Massachusetts General Hospital

4:35 p.m. - 4:50 p.m. **Short Talk #8**
Mutation of a Single Amino Acid of Meiosis-Expressed Gene 1 by CRISPR/CAS9 System Results in Impaired Spermiogenesis and Male Infertility in Mice
Presented by: Shiyang Zhang
Virginia Commonwealth University

4:50 p.m. - 6:50 p.m. **Poster Session II**
Location: Symphony Ballroom I/II

SATURDAY, APRIL 22, 2017

7:00 a.m. - 2:00 p.m. **Registration/Information Desk Open**
Location: Symphony Ballroom Registration

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

8:00 a.m. - 9:00 a.m. **Benchmark Lecture III**

8:00 a.m. - 8:05 a.m. **Chair and Introduction**
Prabhakara Reddi, PhD
University of Illinois Urbana-Champaign

8:05 a.m. - 8:55 a.m. **Daddy Issues: Effects of the Paternal Environment on Future Generations**
Oliver J. Rando, MD, PhD
University of Massachusetts

SESSION VI: GENETICS & EPIGENETICS OF MALE REPRODUCTION

8:55 a.m. - 9:00 a.m. **Chair and Introduction to Session VI**
Vassilios Papadopoulos, DPharm, PhD
University of Southern California

9:00 a.m. - 9:40 a.m. **Humanized Infertility Alleles in Mice Reveal Novel Gene Activities**
John Schimenti, PhD
Cornell University

9:40 a.m. - 10:00 a.m. **Break**
Location: Symphony Ballroom Foyer

10:00 a.m. - 10:40 a.m. **Environmental Programming of the Sperm Epigenome**
Sarah Kimmins, PhD
McGill University, Canada

10:40 a.m. - 11:20 a.m. **Niacin: A Dietary Factor Influencing Sperm Quality and Epigenetic Information**
Mirella Meyer-Ficca, PhD
Utah State University

11:20 a.m. - 11:35 a.m. **Introduction to the NICHD Contraceptive Research Program**
Daniel S. Johnston, PhD
NIH, NICHD

11:35 a.m. - 11:50 a.m. **Concluding Remarks & Acknowledgments**
Vassilios Papadopoulos, DPharm, PhD

11:50 a.m. - 12:00 p.m. **Announcement of the 25th North American Testis Workshop**
Vassilios Papadopoulos, DPharm, PhD

WEDNESDAY, APRIL 19, 2017
7:15 p.m. - 8:00 p.m.

KEYNOTE ADDRESS:
COMPARISON BETWEEN HUMAN AND RODENT SPERMATOGONIAL RENEWAL AND DIFFERENTIATION
Dirk De Rooij, PhD
Fondazione Pasteur Cenci Bolognetti, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences - Section of Histology and Medical Embryology, Sapienza University of Rome, Rome, 00161, Italy

Undifferentiated spermatogonia in primates are generally subdivided into A-pale (Ap) and (Ad) A-dark spermatogonia according to the staining of the nuclei with hematoxylin. However, this distinction is difficult to make as cells with an intermediate morphology are frequent. A more meaningful subdivision of spermatogonia can be made based on the expression of GFRA1, UTF1, Ki67 and KIT (Di Persio et al, submitted). GFRA1+/UTF1- A spermatogonia are at the beginning of the spermatogenic lineage and include stem cells. Then UTF1 becomes expressed and cells become quiescent. Subsequently, GFRA1 expression stops and the cells loose UTF1, start to express KIT and become B spermatogonia. Human A spermatogonia consist mostly of single cells and some pairs. There are 3 generations of B spermatogonia. Rodent undifferentiated spermatogonia, consist of singles, pairs and chains. Spermatogonial stem cells are single ID4+/GFRA1+ cells and when these cells loose ID4 expression their chance of self-renewal decreases and they form pairs and chains of up to 16 cells that will differentiate into KIT+ A1 spermatogonia. In rodents, there are 6 generations of differentiating spermatogonia.
There are large differences between human and rodent spermatogenesis. First, in human there are 11 times more undifferentiated spermatogonia (Ap-d) per Sertoli cell than in mice. Second, there are no chains of Ap-d spermatogonia in the human while they are numerous in mice. Third, in human only few of the Ap-d spermatogonia divide during an epithelial cycle, in mice all undifferentiated spermatogonia divide 2 to 3 times per epithelial cycle. Fourth, in human there are 3 generations of differentiating type spermatogonia versus 6 in mice. Interestingly, only about half of the differentiating type spermatogonia are positive for the proliferation marker KI67 versus 100% in mice, indicating that proliferation of differentiating spermatogonia is down-regulated in the human. Fifth, in human the maximal clonal size of spermatocytes is 8 or 16 versus about 1000 cells in mice. Still, thanks to the very large numbers of Ap-d spermatogonia present, human sperm production per Sertoli cell is comparable to that in mice.

THURSDAY, APRIL 20, 2017
8:00 a.m. - 8:55 a.m.

Benchmark Lecture I:
UNITING THE GENOME: MULTIFACETED ROLES OF piRNAs DURING SPERMATOGENESIS
Haifan Lin, PhD
Toshiaki Watanabe, Ee-chun Cheng, and Haifan Lin
Yale Stem Cell Center and Department of Cell Biology,
Yale University School of Medicine, New Haven, CT

The eukaryotic genome has vast intergenic regions containing transposons, pseudogenes, repetitive sequences, and noncoding genes that produce numerous long non-coding RNAs (lncRNAs) and PIWI-interacting RNAs (piRNAs). Yet the functions of the intergenic regions remain largely unknown. In mammals, a unique set of piRNAs, pachytene piRNAs, is abundantly expressed in the germline in late spermatocytes and early spermatids. Recently, we showed that piRNAs derived from transposons and pseudogenes mediate the degradation of a large number of mRNAs and lncRNAs in mouse late spermatocytes. In particular, they have a large impact on the lncRNA transcriptome, as a quarter of lncRNAs expressed in late spermatocytes are upregulated in mice deficient in piRNA pathway. Furthermore, our genomic and in vivo functional analyses revealed that retrotransposon sequences are frequently found in the 3' UTR of mRNAs that are targeted by piRNAs for degradation. Similarly, the degradation of spermatogenic cell-specific lncRNAs by piRNAs is mediated by retrotransposon sequences. Moreover, we have shown that pseudogenes regulate mRNA stability via the piRNA pathway. The degradation of mRNAs and lncRNAs by piRNAs requires MIWI and, at least in part, depends on its slicer activity. Together, these findings reveal a highly complex and global RNA regulatory network through which transposons and pseudogenes regulate target mRNA and lncRNA stability via the piRNA pathway to promote meiosis-spermiogenesis transition. This represents a novel paradigm of gene regulation in any system.

THURSDAY, APRIL 20, 2017
9:00 a.m. - 9:40 a.m.

SESSION I: Germline Establishment & Homeostasis
DYNAMICS OF STEM CELL REPLACEMENT IN THE DROSOPHILA TESTIS NICHE
Erika Matunis, PhD
Salman Hasan and Erika Matunis
The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Hunterian G1, Baltimore, MD 21205

Stem cells are generally thought to have mechanisms for resisting damage, but few such mechanisms are well understood. We use the Drosophila tests niche as a model for studying the cellular and molecular mechanisms underlying stem cell biology. In this well-defined tissue, spermatogonial stem cells, also called germline stem cells (GSCs) tissue attach to a cluster of quiescent somatic cells called the hub. The hub generates a niche via secreting signals that locally activate signaling effectors, including transcription factor and Signal Transducer and Activator of Transcription homologue STAT92E, in adjacent stem cells. We had previously found that as in mammals, spermatogonia in Drosophila are more susceptible to death under normal and stress conditions, than are stem cells. In Drosophila, we showed previously that GSCs are stress resistant, due to enriched levels of the Drosophila Inhibitor of Apoptosis (DIAP) protein, which is up regulated by STAT92E. Here, we establish a paradigm to induce DNA-double strand breaks at a level that depletes half of the GSCs. This allows us to follow the fate of stem cells that leave the niche, and those that repopulate it. We find that DNA damage sensing in this tissue requires the highly conserved Chk2 Kinase and its downstream target p53. Unexpectedly, upon DNA damage, p53 activation appears to promote the detachment of GSCs from the niche, rather than the activation of cell death. P53 typically promote activation of highly conserved DNA repair pathways. This includes homologous recombination (HR), which is accurate, and the mutagenic pathway of non-homologous end joining (NHEJ). Interestingly we find differential requirements for these repair pathways in GSCs in vivo, and are testing whether stem cells unable to perform individual pathways are preferentially retained in the niche compared to neighboring wild-type cells, and imagine this process in real time.

THURSDAY, APRIL 20, 2017
9:40 a.m. - 10:20 a.m.

SESSION I: Germline Establishment & Homeostasis
DEFINING SPERMATOGONIAL STEM CELL TRANSCRIPTOMES AT THE SINGLE-CELL LEVEL
Brian P. Hermann, PhD, Department of Biology, University of Texas at San Antonio

Spermatogonial stem cells (SSCs) are undifferentiated spermatogonia that sustain mammalian spermatogenesis by producing progeny that will either retain stemness (self-renew) or become progenitors that are committed to differentiation. The mechanisms that drive these alternate fates remain poorly understood partly because 1) SSCs are rare, 2) undifferentiated spermatogonia (including SSCs) are heterogeneous, and 3) SSCs cannot be prospectively distinguished from progenitors. One possible strategy to identify a unique gene expression signature characteristic of SSCs would be to examine single-cell transcriptomes of spermatogonial populations highly enriched for SSCs in comparison to those which comprise progenitors. For this purpose, we have initiated a series of single-cell RNA-seq studies comparing populations of mouse undifferentiated spermatogonia that differ in their content of functional SSCs based on transplantation. These experiments have revealed cohorts of differentially-expressed genes between mouse SSCs and progenitor spermatogonia that inform upon the underlying mechanisms driving their developmental fate. We have also begun exploring whether such differentially-expressed genes constitute a putative mouse SSC barcode that may be useful for prospective functional categorization of undifferentiated spermatogonia. This talk will discuss key finding from our single-cell spermatogonial transcriptome profiling in mice and explore the possibility of conservation among spermatogonia from higher mammalian species, including humans.
Data will be presented from studies supported by NIH grants HD062687 (BPH), HD061665 (JMO and GM092334 (JRM), NSF grant 1337513 (BPH), the Max and Minnie Tomerlin Voelcker Fund, the Helen Freeborn Kerr Foundation, the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation, and The University of Texas at San Antonio.

THURSDAY, APRIL 20, 2017
10:40 a.m. - 11:20 a.m.

SESSION I: Germline Establishment & Homeostasis
DIFFERENTIAL REQUIREMENTS FOR THE 'MECHANISTIC TARGET OF RAPAMYCIN' (MTOR) AND MTORC1 COMPONENT RAPTOR IN SPERMATOGONIAL DEVELOPMENT IN THE MOUSE
Christopher Geyer, PhD

Nicholas D. Serra1, Ellen K. Velte1, Bryan A. Niedenberger1, Randall H. Renegar1, and Christopher B. Geyer1,2
1Department of Anatomy and Cell Biology, Brody School of Medicine, 2East Carolina Diabetes and Obesity Institute at East Carolina University, Greenville, NC.
Objectives: Spermatogonial self-renewal, proliferation, and differentiation must be finely coordinated during spermatogenesis, as dysregulation of these processes can lead to loss of the germline or formation of testicular tumors. While considerable progress has been made in understanding mechanisms regulating self-renewal, those controlling spermatogonial differentiation are largely undefined. To address this gap in knowledge, our laboratory has focused on identifying the pathways that direct spermatogonial differentiation. In previous work, we found that retinoic acid (RA) activates the PI3K/AKT/mTOR kinase pathway and stimulates the translation of repressed mRNAs encoding proteins required for differentiation (e.g. KIT, SOHLH1, SOHLH2). Methods: Here, we investigate the germ cell-autonomous requirement for 'mTOR complex 1' (mTORC1) in spermatogonial differentiation by analyzing the reproductive phenotypes of conditional germ cell knockout mice for Mtor or Raptor (essential component of mTORC1). Results: Testes from both lines of adult KO mice were much smaller than littermate controls, and there were no sperm in the cauda epididymides. Histological and immunostaining analyses revealed a small population of spermatogonia was still present in Mtor KO testes, but the differentiation marker KIT was reduced or undetectable in these cells. This phenotype was evident as early as postnatal (P) day 8, with KO testes containing only apparently undifferentiated spermatogonia similar to rapamycin treatment. Raptor KO germ cells also fail to complete spermatogenesis, but in contrast to Mtor KO, a small number of spermatogonia express detectable KIT and enter but fail to complete meiosis. The remaining pool of spermatogonia in Raptor KO is gradually lost, leaving adult Raptor KO with Sertoli-only testes. Conclusions: Our findings reveal that mTOR is dispensable for the maintenance of undifferentiated spermatogonia, but is cell-autonomously required for their proliferation and differentiation. RAPTOR is dispensable for differentiation and meiotic entry in the first wave of spermatogenesis, but is required for the survival of undifferentiated spermatogonia and complete spermatogenesis.
Funding provided by a grant from the NIH/NICHD (HD072552) to C.B.G.

THURSDAY, APRIL 20, 2017
1:15 p.m. - 1:55 p.m.

SESSION II: Germ Cell Differentiation and Maintenance - The Role of RNA & RBP's
DISTINCT FUNCTIONS OF NANOS2 AND NANOS3 DURING SPERMATOGENESIS
Yumiko Saga, D.Sc, National Institute of Genetics , Mishima, Japan

Spermatogenesis is a complex but an orderly regulated differentiation system for sperm production, which depends on a self-renewing ability of spermatogonial stem cells (SSCs). We have been interested in RNA-mediated mechanisms centered by an RNA-binding protein Nanos2. Nanos2 is a male specific RNA binding protein involved in the posttranscriptional RNA repression. The expression starts from embryonic stage and all male gonocytes strongly express Nanos2 but the expression becomes restricted in most primitive type of undifferentiated spermatogonia including SSCs in adult testes. Recently we have revealed that Nanos2 works with other mRNP components to ensure the primitive status of SSCs through a dual mechanism that involves 1) post-transcriptional repression of differentiation genes, and 2) repression of mTORC1 signaling. In addition, we found a mechanism to destabilize Nanos2 upon differentiation of SSCs, which is achieved by the action of an E3-ubiquitin ligase. Based on these studies, we propose Nanos2 as an intrinsic factor essential for the SSC maintenance. On the other hand, the function of Nanos3 during spermatogenesis has not been addressed before. In this workshop, I also like to report our recent progress on Nanos3 function during spermatogenesis and would like to discuss possible molecular differences between Nanos2 and Nanos3.

THURSDAY, APRIL 20, 2017
1:55 p.m. - 2:35 p.m.

SESSION II: Germ Cell Differentiation and Maintenance
GASZ INTERACTS WITH MITOFUSINS TO REGULATE SPERMATOGENESIS
Yuan Wang, PhD
Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences & School of Life Sciences, East China Normal University, Shanghai, China

Objective: Nuage is an electron-dense cytoplasmic structure in germ cells that contains ribonucleoproteins and participates in piRNA biosynthesis. Despite the observation that clustered mitochondria are associated with a specific type of nuage called intermitochondrial cement (pi-body), the importance of mitochondrial functions in nuage formation and spermatogenesis is yet to be determined.
Methods: With bioinformatic prediction and immunofluorescence, we explored the subcellular localization of germ cell specific genes including GASZ. We established mouse models with targeted deletion of mitochondrial localization signal of GASZ or with conditional knockout of Mitofusins (MFNs) in germ cells.
Results: We show that a germ cell specific protein GASZ contains a functional mitochondrial targeting signal, and is largely localized at mitochondria both endogenously in germ cells and in somatic cells when ectopically expressed. In addition, GASZ interacts with itself at the outer membrane of mitochondria and promotes mitofusion in a Mitofusin/MFN-dependent manner. In mice, deletion of the mitochondrial targeting signal reveals that mitochondrial localization of GASZ is essential for nuage formation, mitochondrial clustering, transposon repression, and spermatogenesis. MFN1 deficiency also leads to defects in mitochondrial activity and male infertility.
Conclusion: Our data thus reveal a crucial requirement for GASZ and MFN-mediated mitofusion during spermatogenesis.

THURSDAY, APRIL 20, 2017
2:55 p.m. - 3:35 p.m.

SESSION II: Germ Cell Differentiation and Maintenance - The Role of RNA & RBP's
REGULATION OF TESTIS TRANSCRIPTOME BY THE MOV10 RNA HELICASE
Yongjuan Guan, Anastassios Vourekas, Panagiotis Alexiou, Manolis Maragkakis, Zissimos Mourelatos, and P. Jeremy Wang
University of Pennsylvania

Objectives: Our goal is to study the function of MOV10 – an RNA helicase in testicular development. We previously studied the germ cell-specific homologue of MOV10 – MOV10L1 (MOV10-like 1). We find that MOV10L1 functions in the piRNA pathway. CLIP-seq analysis reveals that MOV10L1 binds to piRNA precursors to initiate piRNA biogenesis. Therefore, MOV10L1 is a master regulator of piRNA biogenesis and its disruption leads to male sterility and transposon de-repression. In contrast with MOV10L1, MOV10 is broadly expressed and its function in testis remains unknown.
Methods: We examined the expression and subcellular localization of MOV10 in testis. IP/mass spectrometry was performed to identify MOV10-associated proteins in testes. Using the CLIP-seq (crosslinking coupled with immunoprecipitation), we comprehensively identified MOV10-bound RNAs in vivo. We have generated a conditional knockout allele to probe the requirement of MOV10 in testicular development.
Results: MOV10 is present in multiple adult tissues including testis and ovary but not in brain. In testis, MOV10 is expressed in both germ cells and Sertoli cells. MOV10 is cytoplasmic. MOV10 appears to be expressed in spermatocytes but not in post-meiotic germ cells. IP/mass spectrometry identified a number of MOV10-associated proteins such as UPF1, MEIOC, YTHDC2, etc. Notably, UPF1 is a key factor in the NMD (nonsense mediated decay) pathway. We succeeded in MOV10 CLIP-seq using 3-week-old testes. Four CLIP-seq libraries were constructed and sequenced. We find that MOV10 preferentially binds to 3'UTRs. Strikingly, many MOV10-targeted transcripts have long 3'UTRs. Such transcripts are highly expressed in brain but at a very low level in testis. So far, interbreeding of Mov10+/- mice failed to produce homozygous knockout offspring, suggesting that Mov10 is required for embryo development. Germ cell-specific and Sertoli cell-specific deletions of Mov10 are ongoing and phenotype results are expected to be available by the meeting time.
Conclusions: We find that MOV10 binds to 3'UTRs in vivo (testis). A contrasting expression pattern emerges for MOV10-bound transcripts. The abundance of MOV10-bound transcripts with longer 3'UTRs is high in brain but low in testis, suggesting that MOV10 may function together with UTF1 to degrade transcripts with long 3'UTRs through the NMD pathway. By doing so, MOV10 regulates the testis transcriptome globally.
Supported by NICHD R01HD069592.

FRIDAY, APRIL 21, 2017
8:00 a.m. - 8:55 a.m.

Benchmark Lecture II
NEW INSIGHTS INTO THE CAUSES AND CONSEQUENCES OF MALE INFERTILITY
Robert John Aitken, ScD
Priority Research Centre for Reproductive Science, Faculty of Science, University of Newcastle, NSW

Defective sperm function is a dominant cause of male subfertility and yet we have very few insights into the etiology of this condition or its consequences for the offspring. Historically, a great deal of work has been done on the functional impairments in human spermatozoa, highlighting errors in sperm motility, sperm-egg recognition and sperm-oocyte fusion that impede fertilization. In the past decade, new technologies, driven by improvements in the design and sensitivity of mass spectrometers, have started to generate important insights into the biochemical lesions present in human spermatozoa that underpin their functional deficiencies. Such studies have, for example, identified certain patients with a specific defect in the heat shock protein, HSPA2, that results in the disruption of sperm-egg recognition. The importance of this particular molecular chaperone is that it appears to be essential for the surface expression of a zona recognition molecule, arylsulfatase A, late in sperm capacitation. The loss of HSPA2 has, in turn, been linked to the induction of oxidative stress, which generates lipid aldehydes such as 4-hydroxynonenal (4HNE) that bind to this molecule, inducing its dissociation from the regulatory co-chaperone, BAG6. This event then precipitates a ubiquitination response that targets HSPA2 for destruction in the proteasome. Another group of patients with errors in sperm motility and sperm head-tail association are deficient in outer dense fibre 1 (ODF1), a thiol rich protein which is also vulnerable to oxidative stress. The origin of oxidative stress in defective spermatozoa appears to be largely mitochondrial and to be precipitated by the binding of electrophilic metabolites such as 4HNE or thiolactone (the activated congener of homocysteine) to proteins in the mitochondrial electron transport chain, particularly succinic acid dehydrogenase. The free radicals generated by the mitochondria, and possibly other sites, are also capable of attacking sperm nuclear DNA, inducing the formation of highly mutagenic lesions that may have a profound impact on the mutational load carried by the offspring. Together, these studies shed new light on the pathophysiology of male infertility.
This work was supported by the National Health and Medical Research Council of Australia. The author has no competing interests to declare.

FRIDAY, APRIL 21, 2017
9:00 a.m. - 9:40 a.m.

SESSION III: Testis Development & Differentiation
CONTROL OF POST-NATAL TESTIS DEVELOPMENT BY THE SERTOLI CELLS
Peter O'Shaughnessy, PhD
Peter O'Shaughnessy, PhD[1], Diane Rebourcet, PhD[1,2] and Lee Smith, PhD[2]
[1]College of Medical, Veterinary and Life Sciences, University of Glasgow and [2]MRC Centre for Reproductive Health, University of Edinburgh. UK

It has been recognised for a number of years that the Sertoli cells are essential regulators of testicular fate in the differentiating gonad although the subsequent role that they play in fetal and prepubertal testis development has been less clear. Similarly, in the adult, the Sertoli cells are known to be essential for spermatogenesis but what function they play in regulating other somatic cells has been less certain. To study Sertoli cell function during development and in the adult we have developed mouse models which allow specific ablation of the Sertoli cell population at different stages of development or in the adult using diphtheria toxin (DTX). When doses of DTX were used that cause complete ablation of the Sertoli cells during development results showed that the Sertoli cells are required to maintain the peritubular myoid cells (PTMC) in prepubertal life and for the development of normal adult Leydig cell (ALC) numbers. When the Sertoli cell population was completely ablated in the adult there was a rapid loss of all germ cells and a 75% decline in ALC numbers although PTMC numbers were not clearly affected. In subsequent studies with lower doses of DTX we have shown that we can induce partial loss of the Sertoli cell population during development or in the adult. Results from these studies show that the size of the Sertoli cell population that forms during development determines the number of germ cells and Leydig cells that will be present in the adult testis. Similarly, the number of germ cells and Leydig cells that can be maintained in the adult is directly dependent on the size of the adult Sertoli cell population. Overall, results show that the Sertoli cells remain essential regulators of testis development long after initial testis differentiation and that the number of Sertoli cells that form during testis development determines the overall cellular composition of the adult testis.

FRIDAY, APRIL 21, 2017
9:40 a.m. - 10:20 a.m.

SESSION III: Testis Development & Differentiation
NEW INSIGHTS INTO FATE DEAMINATION AND MAINTENANCE OF THE TESTIS
Humphrey Hung-Chang Yao, PhD
Reproductive Developmental Biology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Transformation of the gonadal primordium into a testis or an ovary defines the first morphogenetic event in mammalian sex determination. The Y---chromosome---derived SRY gene and its downstream regulator SOX9 initiate the testicular program and gear bipotential somatic cells toward a Sertoli cell fate, rather than an ovarian granulosa cell destiny. Once established and differentiated, Sertoli cells require the transcription factor DMRT1 to maintain their identities, and without DMRT1, Sertoli cells transdifferentiate into FOXL2---positive granulosa cells. These evidence support the model that establishment and maintenance of the Sertoli cell lineage occurs cell autonomously, via a coordinate action of transcription factors. However, in the case of freemartinism, where a female twin shares a placenta with a male twin, the ovary of the female twin develops testis cell types and structures. This phenomenon led us to hypothesize that somatic cell fate in the fetal testis is sensitive to secreted factors with hormonal properties. Candidates for such factors are anti---Müllerian hormone (AMH) and activin B, both hormones produced by Sertoli cells. Fetal mouse testes lacking either one of these two genes developed normally; however, Amh/activin B double knockout testes exhibited progressive sex reversal with the disappearance of SOX9---positive Sertoli cells, appearance of FOXL2---positive granulosa cells, and disintegration of testis cord structures. When Foxl2, the fate maintenance factor for granulosa cells, was inactivated in the Amh/activin B double knockout testes, the sex reversal phenotypes were prevented. These genetic evidence reveal another level of somatic cell fate regulation in the fetal testis: Sertoli cell---derived AMH and activin B act in an autocrine/paracrine manner to maintain Sertoli cell fate by suppressing Foxl2 expression and the consequent emergence of the granulosa cell program.
This study was supported by NIH Intramural Research Program.

FRIDAY, APRIL 21, 2017
10:40 a.m. - 11:20 a.m.

SESSION III: Testis Development & Differentiation
EVIDENCE THAT NUCLEOCYTOPLASMIC TRANSPORT PROTEINS MEDIATE ENVIRONMENTAL CUES REQUIRED FOR MALE FERTILITY
Kate Loveland, PhD
Kate L. Loveland[1, 2, 3], Yoichi Miyamoto[4,6], Penny A.F. Whiley[1], Julia Young[1, 3], David Jans[5], Yoshihiro Yoneda[3,6]
[1]Hudson Institute of Medical Research, Clayton, Victoria, Australia; [2]School of Clinical Sciences, Monash University, Clayton, Victoria, Australia; [3]Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia; [4]Nuclear Transport Dynamics Project, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan; [5]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia; [6]National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan

Objectives: The importin (IMP) proteins mediate regulated nucleocytoplasmic transport, and we proposed many years ago that co-ordinated expression of a cell-specific transcription factor and its cognate IMP could drive a developmental switch by enabling proteins to enter the nucleus to influence gene activity. Our previous work demonstrated that expression of individual IMPs is tightly regulated during spermatogenesis, and IMP binding partners differ between spermatogenic subtypes. We also reported that certain IMPs are nuclear-localized in meiotic and haploid male germ cells and also localized to distinct regions in mature mouse sperm. These observations led us to propose that IMPs serve roles additional to protein trafficking into the nucleus.
Methods and Results: Because cellular stressors such as hydrogen peroxide cause nuclear sequestration of IMPs, we examined whether nuclear-localization of IMP proteins is involved in determining the responses of germ cells to oxidative stress. Examining two unique mouse models revealed that levels of one particular importin, in spermatids IMP□lpha4, determines their survival under oxidative stress conditions. Exploring the function of nuclear-localized importins led us to identify genes that are transcriptionally regulated by IMP□s, one of which is highly expressed in the testis. In experiments using the GC-1 spermatogenic cell line, we found that transcription at this gene locus is regulated by oxidative stress. Establishment of a knockout mouse line demonstrated that this locus is essential for male germ cell formation and fertility.
We also examined the function of an IMP□□□□□□□□□member that we had previously shown exhibits tight regulation during spermatogenesis in both fetal and postnatal testes by creating a mouse with a floxed allele. Complete absence of this allele is fetal lethal, while conditional knockouts demonstrate it is essential for male germ cell survival.
Conclusions: These investigations of IMPs in spermatogenesis has uncovered new genes required for male fertility and identified potential new mechanisms by which IMP proteins contribute to cell viability and differentiation.
Funding provided by: Australian Research Council and National Health and Medical Research Council

FRIDAY, APRIL 21, 2017
1:15 p.m. - 1:55 p.m.

SESSION IV: Transcriptional & Endocrine Regulation in the Testis
SPERMATOGENESIS REQUIRES CLASSICAL AND NONCLASSICAL TESTOSTERONE SIGNALING
William H. Walker, PhD
University of Pittsburgh Department of Obstetrics, Gynecology and Reproduction Sciences and Magee Women's Research Institute

Testosterone acts via the androgen receptor (AR) to activate two intracellular signaling pathways in Sertoli cells, the classical and the non-classical. The classical pathway regulates gene expression. The non-classical pathway causes the activation of kinases and downstream target genes.
Objectives: To determine whether each testosterone pathway was required to maintain spermatogenesis and to identify essential processes required for spermatogenesis that are supported by each pathway.
Methods: Exp. 1: Rats were treated with a GnRH antagonist to decrease testosterone production after which testosterone was administered followed by immunofluorescence assays of testis tissue sections to determine whether kinase activation occurred via non-classical signaling in vivo. Exp. 2: Sertoli cells within rat testis explants lacking endogenous AR activity were transduced with adenovirus constructs expressing mutant ARs that selectively activate only one signaling pathway. RT-qPCR assays were performed to determine whether expression of germ cell genes was regulated by either pathway. Exp. 3: Mouse testes were injected with adenovirus constructs expressing inhibitors of the classical or non-classical testosterone signaling pathway. Four days after injection, the integrity of the blood testis barrier (BTB) and effects on spermatogenesis was assessed.
Results: Non-classical testosterone signaling activity was observed in vivo as MAP kinase activation was induced rapidly in Sertoli cells after restoration of testosterone levels in rat testes. In testis explants lacking AR activity, spermatogonia-specific genes (Zbtb16 and c-Kit) were induced after expression of classical or non-classical pathway selective AR mutants in Sertoli cells. Selective inhibitors of either pathway disrupted the BTB. Inhibition of the classical pathway caused premature release of meiotic and post-meiotic germ cells. Inhibition of the non-classical pathway blocked meiosis and caused the loss of spermatocytes.
Conclusions: Non-classical testosterone signaling occurs in Sertoli cells in vivo. Both the classical and non-classical testosterone signaling pathways in Sertoli cells are required to maintain spermatogenesis.
Funding provided by: NIH-HD43143 and PA Department of Health Research Formula Funds.

FRIDAY, APRIL 21, 2017
1:55 p.m. - 2:35 p.m.

SESSION IV: Transcriptional & Endocrine Regulation in the Testis
THE CAMKI-MEF2-NUR77-AMPK CASCADE IN THE REGULATION OF LEYDIG CELL STEROIDOGENESIS
Jacques J. Tremblay, PhD,
Centre for Research in Reproduction, Development and Intergenerational Health, Department of Obstetrics, Gynecology, and Reproduction, Faculty of Medicine, Université Laval, Québec City, Québec, Canada G1V 0A6

Steroid hormones regulate essential physiological processes; inadequate levels are associated with various pathologies. Fine regulation of steroidogenesis is therefore essential. Testosterone is synthesized in Leydig cells from cholesterol through the sequential action of transporters/enzymes, including STAR. Like steroidogenesis, Star expression is controlled primarily by pituitary LH. LH acts by binding to its G protein-coupled receptor leading to increased cAMP and Ca2+ concentrations. This activates different kinases which phosphorylate target proteins such as transcription factors leading to increased expression of genes involved in steroid hormone synthesis, including Star. CAMKI is activated downstream of cAMP/Ca2+ in Leydig cells and is essential for hormone-induced steroidogenesis and Star expression. Hormone-induced Star expression in Leydig cells involves the nuclear receptor NUR77. NUR77 expression and phosphorylation status are altered in response to LH/cAMP in Leydig cells. The cAMP responsiveness of the Nur77 promoter requires the CAMK pathway, which acts in part through the MEF2 transcription factor. MEF2 is an important regulator of gene expression during organogenesis, cell differentiation, as well as in response to stimulation in various tissues, but it has only recently been reported in the testis. Once appropriate levels have been reached, steroid hormone production must be reduced. In the negative feedback loop, LH production is reduced and steroidogenesis passively decreases as cAMP is degraded into AMP by phosphodiesterases. We recently reported that the increase in intracellular AMP leads to activation of AMPK, which actively shuts down steroid hormone synthesis in Leydig cells. Interestingly, NUR77 is also targeted by the AMPK pathway leading to its repression. The fact that both the activating (CAMKI/MEF2) and repressing (AMPK) pathways converge on NUR77 further highlights its central role in steroidogenesis. The CAMKI-MEF2-NUR77-AMPK cascade therefore defines a two-step mechanism required for the fine regulation of steroid hormone production in Leydig cells. Supported by CIHR and NSERC.

FRIDAY, APRIL 21, 2017
3:15 p.m. - 3:55 p.m.

SESSION V: Sperm Development & Maturation
SPERMATOGENESIS AS A MODEL SYSTEM TO DEFINE KATANIN FUNCTION
Moira K. O'Bryan, BSc, PhD
The Development and Stem Cells Program of Monash Biomedicine Discovery Institute, Monash University, Clayton, Australia.

The katanin microtubule-severing proteins are essential regulators of microtubule dynamics in a diverse range of species and tissues. Within mammals each of the katanin subunits is notably enriched within the seminiferous epithelium and we have recently shown that both the katanin regulatory protein, KATNB1 (1), and a novel katanin severing protein, KATNAL1 (2), serve essential and distinct roles in germ cell development. More recently, we have defined critical roles for the poorly characterised katanin protein KATNAL2 in multiple aspects of spermatogenesis: the initiation of sperm tail growth from the basal body, sperm head shaping via the manchette, acrosome attachment and ultimately sperm release. We show that depending on context, KATNAL2 can partner with the regulatory protein KATNB1 or act autonomously. Moreover, our data indicate KATNAL2 may regulate tubulin subunits other than the classical □-□-tubulin microtubule polymers, suggesting the katanin family has a greater diversity of function than previously realised. Collectively our data supports the concept that in higher order species the presence of multiple katanins has allowed for subspecialisation of function within complex cellular settings such as the seminiferous epithelium. This study also highlights the utility of using spermatogenesis, and male fertility, as an efficient and valuable model system within which to define the function of pathways of importance to multiple aspects of human health

FRIDAY, APRIL 21, 2017
3:55 p.m. - 4:35 p.m.

SESSION V: Sperm Development & Maturation
INTERCELLULAR NETWORKS AND LUMINAL ACIDIFICATION IN THE EPIDIDYMIS
Sylvie Breton, PhD, Massachusetts General Hospital/Harvard Medical

Spermatozoa acquire their ability to fertilize an oocyte as they transit through the epididymis. The luminal fluid of the epididymal duct is acidic and has a low bicarbonate (HCO3-) concentration: both factors are important for keeping sperm quiescent during their maturation and storage. Several populations of epithelial cells that line the epididymal lumen work in a concerted manner to regulate luminal pH. We will discuss the relative contribution of clear cells (CCs) versus principal cells (PCs) in the maintenance and regulation of the epididymal acidic luminal environment. We showed that CCs secrete H+ via the vacuolar proton pump V-ATPase located in their apical membrane. Interestingly, PCs have the dual role of being either H+ or HCO3- secretors, depending on physiological cues. We showed the contribution of CFTR in HCO3- secretion and the Na+/H+ exchanger NHE3 in H+ secretion by PCs. In addition to being directly involved in the regulation of luminal pH, PCs also indirectly control luminal acidification via crosstalk with CCs. During sexual arousal, stimulated PCs secrete HCO3- into the lumen, which contributes to luminal alkalinization, a process that primes sperm before ejaculation. Luminal HCO3- then activates the bicarbonate-sensitive soluble adenylyl cyclase sAC in CCs, which stimulates V-ATPase-dependent H+ secretion, a process that restores the luminal pH to its resting acidic value. We showed that, in addition to HCO3-, PCs also secrete adenosine triphosphate (ATP) into the lumen. Our recent data show that PCs and CCs engage in crosstalk that relies not only on HCO3-, but also on ATP and its hydrolysis product adenosine, which then act as extracellular mediators that activate H+ secretion by CCs. In the second part of this talk, we will dissect the regulation of CCs via activation of purinergic receptors located on their apical surface. By studying how epithelial cells lining the epididymal duct maintain and regulate luminal pH, we wish to provide new frameworks for the evaluation and treatment of male infertility, as well as for the control of male fertility. Our goal is to improve the quality of life of infertile men, as well as to provide more options to those who wish to participate in family planning.

SATURDAY, APRIL 22, 2017
8:00 a.m. - 9:00 a.m.

Benchmark Lecture III
DADDY ISSUES: EFFECTS OF THE PATERNAL ENVIRONMENT ON FUTURE GENERATIONS
Oliver J. Rando, MD, PhD, University of Massachusetts
Xinyang Bing, Ana Boskovic, Colin Conine, Upasna Sharma, Markus Vallaster, Andrew Tapper, and Oliver J. Rando
University of Massachusetts Medical School, Worcester, MA USA

It is increasingly appreciated that ancestral environmental conditions can affect phenotypes in future generations. We have previously shown that paternal diet can affect offspring metabolism in mice, and that paternal dietary information is carried in the gametes. I will discuss our latest efforts to understand the mechanistic basis for paternal effects in mammals, focusing largely on surprising aspects of the biogenesis and function of small RNAs in sperm. I will also discuss our efforts to determine the "bandwidth" of the sperm epigenome, focusing on pharmacological studies designed to interrogate the specificity of the offspring response to paternal drug exposures.

SATURDAY, APRIL 22, 2017
9:00 a.m. - 9:40 a.m.

SESSION VI: Genetics & Epigenetics of Male Reproduction
HUMAN INFERTILITY ALLELES ELUCIDATE MOLECULAR BIOLOGY OF SPERMATOGENESIS
John Schimenti, PhD, Cornell University
Priti Singh, Robert Fragosa, Tina Tran, Jisnu Das, Haiyuan Yu & John Schimenti
Cornell University, Ithaca, NY

About half of infertility cases in people have a genetic basis. Despite extensive knowledge gained from gene knockouts in mice, the genetic causes for the vast majority of idiopathic human infertilities are unknown. Traditional methods for studying inheritance, such as GWAS or linkage analysis, have been confounded by heterogeneity of infertility phenotypes and the great number of genes involved in gametogenesis. Consequently, there is little known about the relative contributions to infertility of: environment vs genetics; Mendelian vs multigenic inheritance; spontaneous vs inherited mutations; or dominant vs recessive alleles. To begin addressing these questions, we have been using CRISPR/Cas9-mediated genome editing in mice to model nonsynonymous human SNPs in essential fertility genes. In addition to overviewing this project designed to address the genetics of human infertility, we will present two alleles, in the Cdk2 and Spo11 genes, that cause novel phenotypes that provide unique insight into the processes of spermatogonial stem cell maintenance and meiotic recombination, respectively.

SATURDAY, APRIL 22, 2017
10:00 a.m. - 10:40 a.m.

SESSION VI: Genetics & Epigenetics of Male Reproduction
ENVIRONMENTAL PROGRAMMING OF THE SPERM EPIGENOME
Sarah Kimmins, PhD
Departments of Animal Science1 and Pharmacology and Therapeutics2, McGill University, Montreal, Quebec, Canada.

Effects of the paternal environment including stress, diet and toxicants have been linked to negative outcomes for offspring including birth defects and increased risks for complex diseases. These paternal effects may occur via non-genetic inheritance, through epigenetic mechanisms including DNA methylation, post-translational modifications of histones and noncoding RNAs. The genome in spermatozoa is packaged in a highly unique manner where the majority is packaged by sperm-specific nucleoproteins, the protamines. Intriguingly there are regulatory regions of many genes that retain histones and these carry post-translational modifications. If histone methylation is altered in sperm development there are dire consequence for embryo development that is transgenerational. In mice, environmental exposures such as diet and toxicants alter the sperm epigenome, but whether there are similar responses in men is unknown. Our overall objectives are to: 1) determine when histone methylation patterns that are transmitted to the embryo are established in spermatogenesis, and 2) identify a robust epigenetic signature that is implicated in epigenetic transmission that can be used to assess environmental exposures in mice and men. Using epigenomic approaches such as chromatin immunoprecipitation followed by sequencing (ChIP-seq), we have been characterizing the effects of environmental exposures on spermatogonia and sperm from mice and men. Our long-term goals are to identify windows in spermatogenesis that are sensitive to reprogramming and to determine the potential for interventions to correct environment induced epimutations.
Funded by the Canadian Institutes of Health Research

SATURDAY, APRIL 22, 2017
10:40 a.m. - 11:20 a.m

SESSION VI: Genetics & Epigenetics of Male Reproduction
NIACIN: A DIETARY FACTOR INFLUENCING SPERM QUALITY AND EPIGENETIC INFORMATION
Mirella L. Meyer-Ficca, PhD

Laura Kessler[1,2], Kirsten Z. Jensen[1], James B. Kirkland[3], Ralph G. Meyer[1] and Mirella L. Meyer-Ficca[1]
[1]Utah State University, School of Veterinary Medicine, Department of Animal, Dairy and Veterinary Sciences, Logan, UT; [2]University of Konstanz, Konstanz, Germany; [3]University of Guelph, Guelph, ON, Canada

Niacin (Vitamin B3), the dietary precursor of NAD+, is an essential vitamin in humans, where lack of dietary niacin causes NAD+ depletion and pellagra, a disease characterized by diarrhea, dermatitis and mental impairment. Beyond the central role of NAD+ in metabolic redox-reactions, it is an essential substrate permitting the activity of various NAD-dependent epigenetic regulator proteins, such as poly(ADP-ribose) polymerases (PARPs) and histone deacetylases (sirtuins). In contrast to humans, normal mice do not become NAD+-deficient in the absence of dietary niacin. This is thought to be due to species-specific differences in the efficiency of tryptophan conversion to NAD+. We generated a novel mouse model with human-like limitations of NAD+ synthesis and dietary niacin requirements. Similar to humans, mice with Acquired Niacin DependencY (ANDY) responded to dietary niacin-deficiency with significantly decreased blood and tissue NAD+ content, which reversed with niacin supplementation. Decreased NAD+ levels accompanied a significant loss of body mass and further pellagra-like phenotypical changes. A niacin-rich but otherwise identical control diet caused excessive gain of body fat mass despite unchanged daily calorie intake.
Such diet-induced restricted availability of NAD+, the substrate of PARP enzymes, caused reduced poly(ADP-ribose) (PAR) formation in mouse testis. Testicular PARP activity is necessary for proper chromatin remodeling in spermatids, as impaired PARP activity reduces chromatin compaction and increases histone content in sperm from genetic and pharmacologic mouse models of altered PAR metabolism. In ANDY mice, short term dietary niacin restriction caused increased sperm histone retention, teratozoospermia and decreased motility as measured by computer assisted sperm analysis. Continuous long-term niacin deficiency led to progressive testicular degeneration and cessation of spermatogenesisIn conclusion, ANDY mice may be a model for human niacin deficiency and NAD+ depletion, allowing the study of dietary niacin restriction and supplementation, and providing a tool to dissect how nutritional factors, now including vitamin B3, exert epigenetic control through regulation of NAD+ dependent epigenetic modifiers

Podium/Short Talk #1

A HIGH-THROUGHPUT SCREEN TO IDENTIFY NOVEL TRANSCRIPTION FACTORS THAT REGULATE MOUSE SPERMATOGONIAL STEM CELL MAINTENANCE

Tessa Lord, Melissa J. Oatley and Jon M. Oatley
Washington State University
(Presented By: Tessa Lord)

Introduction: Precise regulation over spermatogonial stem cell (SSC) function is integral for continuation of spermatogenesis. SSCs must balance self-renewal with the production of progenitors that are poised for differentiation, lest the self-renewing reservoir becomes exhausted, and azoospermic infertility ensues. Despite this, few regulating factors have been identified; primarily due to limitations in distinguishing SSCs from their closely related progenitor counterparts. To address this, our lab has created a mouse line containing an Id4-Gfp transgene, in which Gfp+ cells (specifically Gfp 'bright') encompass the SSC population, while Gfp- cells are progenitors. Using this mouse line, the objective of the current study was utilize a large scale, high-throughput approach to identify novel transcription factors that regulate SSC function.
Methods: Primary cultures of undifferentiated spermatogonia were established from the testes of Id4-Gfp mice. In these cultures, SSCs are marked as Gfp+ and progenitors are Gfp-, thus, changes in the dynamics of the Gfp+/- populations can be used as a readout for alterations of SSC maintenance. Using a large scale siRNA library, we knocked down expression of 1440 transcription factors in these cultures, over three biological replicates. Experiments were conducted in a 96 well plate format, using a flow cytometer with an automated plate reader to assess the effects of transcription factor knockdown on Gfp content at a rate of 80 wells per hour. Transcription factors were ranked by Z score; calculated on the basis of fluctuations in Gfp content as compared to a non-targeted siRNA control.
Results: Using a Z score cut-off of ±1.5, 23 novel candidates were identified that appear to be involved in the SSC-to-progenitor transition; i.e. their knockdown caused an accumulation of Id4-Gfp bright spermatogonia. Further, 10 novel candidates were identified that are likely to be involved in SSC maintenance, with their knockdown resulting in loss of the Gfp-bright population. From these candidates, two have been selected for further investigation using CRISPR directed gene inactivation, on the basis of testis-specific expression profiles.
Conclusion: Our high throughput methodology has yielded over 30 novel transcription factor candidates that will provide investigative inroads for assessing control over SSC maintenance and progenitor production, and potentially provide insight into underlying causes of azoospermic infertility.

Podium/Short Talk #2

CONSERVATION OF A GENE EXPRESSION BARCODE THAT DEFINES SPERMATOGONIAL STEM CELLS IN MICE AND HUMANS.

Anukriti Singh, BS[1], Kazadi Mutoji, PhD[1], Thu Nguyen, MS[1], Heidi Gildersleeve, BS[1], Birgit Westernströer, PhD[1], Jon Oatley, PhD[2], Sherman Silber, PhD[3], John McCarrey, PhD[1] and Brian Hermann, PhD[1]
[1]Department of Biology, University of Texas at San Antonio; [2]Center for Reproductive Biology, Washington State University; [3]The Infertility Center of St. Louis
(Presented By: Anukriti Singh, B.Tech)

Spermatogonial stem cells (SSCs) are undifferentiated spermatogonia that sustain mammalian spermatogenesis by producing progeny that will either retain stemness (self-renew) or become progenitors that are committed to differentiation. The mechanisms that drive these alternate fates remain poorly understood partly because 1) SSCs are rare, 2) undifferentiated spermatogonia (including SSCs) are heterogeneous, and 3) SSCs cannot be prospectively distinguished from progenitors. We reasoned that single−cell transcriptomes of cells highly enriched for SSCs could help identify a gene expression "barcode" characteristic of SSCs. To this end, we performed single-cell RNA-Seq on ID4-EGFP+ spermatogonia postnatal day 6 (P6) and adult mice and subdivided these cells based on intensity of EGFP epifluorescence into EGFP-bright (SSCs) and EGFP-dim (progenitors), which matches their functional distinctions based on transplantation (Helsel et al., 2017). Thousands of genes were differentially-expressed between the EGFP-bright and dim subpopulations at both stages, including a subset of genes which were conserved across postnatal development. While EGFP-bright and dim subpopulations were heterogeneous in their gene expression profiles, they were phenotypically separable by 206 differentially-expressed genes [≥2-fold change (FC)] that constitute a putative mouse SSC barcode. Among genes that were upregulated in EGFP-bright (SSCs) were components of the cellular response to GDNF (Gfra1, Ret, Tcl1, Etv5, Fos) and FGFs (e.g., Dusp1, Dusp6). In EGFP-dim (progenitors), genes involved in the regulation of translation (e.g., Eif4ebp1), retinoic acid response (Rbp1) and pyrimidine metabolism (Upp1) were enhanced. In addition, we compared the mouse SSC barcode to single−cell transcriptomes of adult human undifferentiated spermatogonia isolated from 9 individuals, which were stratified based on ID4 mRNA levels. Spermatogonia with the highest ID4 levels in neonatal mice, adult mice, and adult humans exhibited significant conservation of this gene expression barcode (27 genes, ≥2-FC; 327 genes, ≥1.5-FC). Expression of exemplary candidate genes was subsequently validated by immunostaining. Collectively, these findings point to the first putative gene expression signature (barcode) distinguishing SSCs across postnatal testis development, and which may ultimately reveal the identity and phenotype of human SSCs.

Podium/Short Talk #3

CLONAL DEVELOPMENT OF SPERMATOGONIA IN RHESUS TESTES

Adetunji Fayomi, Karen Peters BS[1] and Kyle Orwig PhD[2]
[1]Magee Womens Research Institute; [2]Magee Womens Research Institute, Univeristy of Pittsburgh School of Medicine
(Presented By: Adetunji Fayomi, DVM)

Introduction and Objectives: Undifferentiated spermatogonia in rodent testes are described by clone size (Asingle, Apaired, Aaligned) and molecular markers that they express. Spermatogonia in nonhuman primate (NHP) testes are described by nuclear morphology and intensity of staining with hematoxylin (Adark, Apale). There is limited information about how the dark and pale descriptions of nuclear morphology correlate with clone size or molecular markers in primates, which makes it difficult to compare rodent and primate data. The aim of this study is to learn molecular characteristics of Adark and Apale spermatogonia and use them as molecular markers to characterize stage-specific clonal development of undifferentiated and differentiating spermatogonia in the rhesus seminiferous epithelium.
Methods: We used colorimetric immunohistochemistry (IHC) to characterize UTF1, ENO2 and cKIT expression in Adark, Apale and B spermatogonia of the Rhesus testis. We performed IHC co-staining in section and whole mount to determine the extent of overlap between these markers and correlate their expression with clone size. 5-ethynyl-2'-deoxyuridine (EDU)-labeling was used to mark cells at S-phase and establish a tool for staging NHP seminiferous tubules in whole mount.
Results Obtained: We found that UTF1 and ENO2 are markers of Adark and Apale undifferentiated spermatogonia in the Rhesus tests. We also demonstrated that irrespective of the stage of seminiferous epithelium, most of the undifferentiated spermatogonia (UTF1+/cKIT- cells) exist as clones of 1, 2 or 4 cells. Clones of 4 cells were more prevalent in stage V, which coincide with the stage with the highest frequency of UTF1+/cKIT+ transitioning spermatogonia. UTF1+ spermatogonia rarely express cKIT during stages I-IV of the seminiferous epithelium. cKIT expression occurs mostly in Larger UTF1+ clones (2-4 cells). Highest frequency of EDU+/UTF1+ clones were observed in stages X-XI.
Conclusions: Similar to rodents, rhesus spermatogonia develop in interconnected clones of cells and increased clone size is associated with increased spermatogonial differentiation (cKIT+). Undifferentiated (UTF1+/cKIT-) spermatogonia were observed in clones of 1-4 cells and rarely in clones of 8, suggesting that Rhesus has fewer transit amplifying divisions in the pool of undifferentiated spermatogonia than rodents.
Supported by NIH grant R01 HD076412 and P01 HD075795

Podium/Short Talk #4

THE RHOX10 HOMEOBOX TRANSCRIPTION FACTOR PROMOTES PROSPERMATOGONIA MIGRATION

Wei-Ting Hung PhD, Hye-Won Song PhD and Miles F. Wilkinson PhD
UC San Diego
(Presented By: Wei-Ting Hung, PhD)

Introduction & Objective: Spermatogonia stem cells (SSCs) are generated from prospermatogonium (ProSG) at approximately the same time when these SSC precursor cells migrate from the center of seminiferous tubules to the periphery – the "stem cell niche". We recently reported that the RHOX10 transcription factor promotes this migration event, as well as the differentiation of ProSG into SSCs (Song et al. Cell Reports 2016). Here, we report our investigation into the underlying mechanism of RHOX10 action in ProSG.
Methods: Using single cell-RNA sequencing (scRNAseq) analysis, we identified RHOX10-regulated genes in the ProSG subset of Id4-eGFP+ cells from early postnatal testes. Ingenuity Pathway Analysis (IPA) was performed on these RHOX10-regulated genes to identify statistically enriched functional categories.
Results: Four hundred and eight genes were downregulated in Rhox10-KO secondary transitional (T2) ProSG relative to control T2-ProSG, as defined by scRNAseq analysis. Molecular and cellular functions significantly enriched among these RHOX10-regulated genes are "cellular movement," "cell death and survival," and "cellular growth and proliferation," as defined by IPA. Enrichment for "cellular movement" genes is consistent with the function of RHOX10 in ProSG migration. Because RHOX10 promotes cell migration, we next performed IPA on only the genes involving in cellular movement, which revealed enrichment for the PTEN, PI3K/AKT, NF-kappa-b, and PKC signaling pathways. To determine the roles of these signaling pathways in germ cell migration, experiments are ongoing to establish an in vitro 3D culture system to reflect the seminiferous environment. In this system, Sertoli cells are cultured in a microwell to provide the seminiferous epithelium framework. GFP-tagged germ cells with selected target genes genetically modified are introduced into the microwells. Mimic and rescue experiments are then conducted to identify RHOX10-downstream targets critical for germ cell migration. The long-term goal is to identify RHOX10-based molecular circuits that drive ProSG migration and differentiation.
Conclusions: RHOX10-regulated genes in a specific ProSG subset were identified using scRNAseq analysis. IPA analysis revealed several significantly enriched functions that will guide us in ongoing in vitro experiments to define the molecular mechanism by which RHOX10 promotes ProSG migration and differentiation.

Podium/Short Talk #5

3 DIMENSIONAL HUMAN TESTIS ORGANOID SYSTEM CREATED FROM IMMATURE TESTICULAR CELLS

Nima Pourhabibi Zarandi MD[1], Guillermo Galdon MD[1], Hooman Sadri-Ardekani MD, PhD[2] and Anthony Atala MD[2]
[1]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine; [2]Wake Forest Institute for Regenerative Medicine, and Department of Urology, Wake Forest School of Medicine
(Presented By: Nima Pourhabibi Zarandi, MD)

Introduction: Creating miniature 3 dimensional (3D) organ-like structures from human cells mimicking the function of native organs and eventually develop a "body on a chip" is eagerly desired. We have recently developed an in vitro 3D human testis organoid system from mature human testicular cells with the potential for in vitro differentiation of spermatogonial stem cells (SSC) and androgen production. The main objective of this study is to show the feasibility of establishing the same 3D organoid system, using immature testicular cells. This has a potential application of fertility preservation in prepubertal male cancer survivors and genetically impaired boys who are at risk of infertility.
Material and Methods: Isolated cells from immature (prepubertal) testicular tissue were cultured in 2 Dimensional (2D) condition for 50 days and 5 passages. Specific genes expression assay was used to prove the presence of all 4 cell types including SSCs, Sertoli, Leydig and peritubular cells, as well as confirming undifferentiated condition of spermatogonial cells. Flow cytometry analysis showed the quantity of each cell type. We integrated 2D cultured cells into 3D spherical culture via hanging drop method, using 10,000 cells per organoid. Over 5 weeks of 3D culture the functionality of organoids was evaluated using live/dead cell staining, ATP production assay, post-meiotic genes expression and androgen production.
Results: Specific markers for spermatogonia including ZBTB16 (PLZF) , PGP9.5 (UCHL1), THY1 (CD90), CD9, FGFR3 and SSEA4; GATA4, SOX9, Clusterin and CD49f for Sertoli cells; STAR, TSPO and Cyp11A1 for Leydig cells; and CD34 for peritubular cells; all together approved the presences of different cell types in the cells that isolated, cultured and integrated into 3D organoid. The 3D testis organoids system maintained their structure, viability, metabolic activity and produced androgen over 5 weeks of culture. PRM1 expression showed that this 3D system was able to differentiate SSCs to post meiotic germ cells.
Conclusion: Human 3D testicular organoid system was generated successfully by using isolated human SSC, Sertoli, Leydig and peritubular cells from immature testis and maintained long term in 3D culture. The system was able to produce androgen and push SSCs toward early differentiation. Future directions will include optimizing the system and testing implanted organoids in vivo, as well as evaluating their use as a novel testicular toxicity model.

Podium/Short Talk #6

REGULATION OF CYP26B1 EXPRESSION IN THE TESTIS

Parag Parekh PHD[1], Thomas Garcia PHD[1,2], Reham Waheeb DVM, PHD[3], Vivek Jain MS[1,2], Pooja Gandhi MS[1], Gunapal Shetty PHD[1], Marvin Meistrich PHD[1] and Marie-Claude Hofmann PHD[1]
[1]University of Texas MD Anderson Cancer Center, Houston, TX; [2]University of Houston Clear Lake, Houston, TX; [3]University of Alexandria, Damanhur, Egypt
(Presented By: Parag Parekh, PhD)

Cytochrome P45026B1 (CYP26B1) regulates the concentration of all-trans-retinoic acid (RA) and plays a key role in germ cell differentiation by controlling local distribution of RA. Interestingly, little is known about the mechanisms of Cyp26b1 gene regulation. In Sertoli cells, it is maintained by SF1 and SOX9 during gonad development and throughout life but inhibitors that would balance its expression, possibly accounting for the pulses of RA in the adult seminiferous epithelium, are not known. Our previous data from Sertoli-cell specific NOTCH gain- and loss-of-function mouse models indicated that expression of Cyp26b1 is inversely correlated to NOTCH pathway activity. We hypothesized that 1) Spatiotemporal Cyp26b1 downregulation is directly dependent on canonical NOTCH signaling; and 2) A subset of premeiotic germ cells is responsible for Cyp26b1 downregulation through the NOTCH ligand JAG1. Germ cell-Sertoli cell co-cultures experiments demonstrated that JAG1, mainly expressed by Aundiff spermatogonia, activated NOTCH signaling in primary Sertoli cells and induced the transcriptional repressors and canonical NOTCH target genes Hes/Hey. Upregulation of Hes/Hey gene expression by JAG1 was associated with significant decreases in Cyp26b1 expression, while simultaneous downregulation of Hes/Hey by RNAi led to significant increases. Further, Luciferase and ChIP-PCR assays demonstrated that HES/HEY directly bind to the Cyp26b1 promoter to downregulate its expression. Investigation of stage-specific NOTCH activity using transgenic mice, together with qPCR analysis of Hes/Hey and Cyp26b1 expression, indicated lowest expression of Cyp26b1 at stages VI-VIII of the seminiferous epithelium, when NOTCH activity and RA production are highest. To elucidate which germ cells activate NOTCH signaling in Sertoli cells in vivo, we performed germ cell depletion experiments using moderate doses of busulfan. We found that elimination of undifferentiated spermatogonia will downregulate NOTCH signaling and upregulate Cyp26b1 expression in Sertoli cells. In conclusion, we believe that NOTCH signaling, induced by JAG1-expressing Aundiff in Sertoli cells, is a mediator of germ cell differentiation by controlling Cyp26b1 expression and possibly RA pulses.
Supported by NIH R01HD081244

Podium/Short Talk #7

ADCY2 IS A CANDIDATE GENE FOR THE DEVELOPMENT OF CONGENITAL GENITOURINARY ANOMALIES THROUGH PARTIAL DISRUPTION OF STEROIDOGENESIS

Marisol O'Neill MS and Dolores J. Lamb PhD
Baylor College of Medicine
(Presented By: Marisol Ann O'Neill, M.S.)

Introduction and Objectives: Genitourinary (GU) anomalies are among the most common types of birth defects yet their genetic causes are poorly understood. Using array comparative genome hybridization (aCGH), we identified Adenylyl Cylcase 2 (ADCY2) copy number variants (CNVs) in a patient with hypospadias and a patient with ambiguous genitalia. ADCY2 converts ATP into cAMP which is a necessary step in androgen production. We hypothesize that ADCY2 is a dosage-sensitive gene which regulates steroid synthesis during GU development.

Methods: The incidence of ADCY2 CNVs in a GU abnormal population was determined by aCGH and Taqman ADCY2 copy number assays on DNA from patients with GU anomalies. ADCY2 expression was localized in embryonic murine GU tracts by immunohistochemistry (IHC). The effects of Adcy2 copy number changes in Leydig cells were evaluated by overexpressing Adcy2 in murine Leydig cell lines TM3, MLTC-1, and MA-10. Changes in genes involved in steroidogenesis were quantified by qPCR. Steroidogenic acute regulatory protein (StAR) phosphorylation was quantified by immunoprecipitation and western blot. Localization and expression of the luteinizing hormone receptor was visualized by immunofluorescence.

Results: DNA from 262 patients with congenital GU anomalies was analyzed by Taqman CNV assay; five patients (1.9%) were identified with ADCY2 duplications; this is significantly higher ($p<0.001$) than the incidence of ADCY2 CNVs in the general population (0.16%). aCGH verified the CNV region. ADCY2 is highly expressed in embryonic murine Leydig cells, urethra, and bladder. The expression of ADCY2 in Leydig cells suggests a role in steroidogenesis. Overexpression of Adcy2 in murine Leydig cell lines resulted in a dysregulation of steroidogenesis. We observed a 40% decrease in StAR production ($p<0.05$) and phosphorylation, as well as qualitative downregulation of LHR. Mevalonate kinase (MVK), an LHR mRNA binding protein, was up-regulated by three fold in ADCY2 overexpressing cells.

Conclusion: We identified and enrichment of ADCY2 CNVs in patients with congenital GU anomalies. ADCY2 is highly expressed in Leydig cells during development and results suggest ADCY2 may contribute to the development of GU anomalies through down-regulation of the LHR in a cAMP dependent manner.

Funding: NIH grants T32DK007763 and R01DK078121 to DJL

Podium/Short Talk #8

MUTATION OF A SINGLE AMINO ACID OF MEIOSIS-EXPRESSED GENE 1 BY CRISPR/CAS9 SYSTEM RESULTS IN IMPAIRED SPERMIOGENESIS AND MALE INFERTILITY IN MICE

Shiyang Zhang, Wei Li MD[1], Hong Liu Master student[2], Ling Zhang MD, PhD[3], Yuhong Li MD, PhD[2], Rex Hess PhD[4] and Zhibing Zhang MD, PhD[1]
[1]Virginia Commonwealth University; [2]Virginia Commonwealth University/Wuhan University of Science and Technology; [3]Wuhan University of Science and Technology; [4]University of Illinois
(Presented By: Shiyang Zhang, Master)

Mouse meiosis-expressed gene 1 (mMEIG1) is a key player in the regulation of mouse spermiogenesis and sperm flagella formation. In male germ cells, it is expressed in the whole cell body of spermatocytes and round spermatids, but is recruited to the manchette of elongating spermatids by another spermiogenesis regulator, PACRG. The MEIG1/PACRG complex is essential to transport cargo, including sperm associated antigen 16 (SPAG16) to build sperm flagella. Nuclear magnetic resonance (NMR) studies revealed that mMEIG1 adopts a unique fold that provides a large surface for interacting with other proteins. Among the 12 exposed and conserved amino acids, four of them, W50, K57, F66, particularly Y68 mediate binding to PACRG. To study the role of Y68 in vivo, we mutated this amino acid using the CRISPR/cas9 system. DNA sequencing of the RT-PCR product revealed that only the amino acid was mutated in the mutant mice. Western blot analysis demonstrated that MEIG1 protein was expressed, however, the level was reduced in the testis compared to the controls. All homozygous mutant mice examined were completely infertile, and sperm count was dramatically reduced. The developed sperm displayed multiple abnormalities, including short and bend tails, round heads. All mutant sperm examined were immotile. Histologic studies showed impaired spermiogenesis in the mutant mice. Immunofluorescent staining revealed that the mutant MEIG1 is still present in the whole cell body of spermatocytes, but accumulated in the acrosome region of round spermatids. No MEIG1 signal was discovered in the manchette of the elongating spermatids. Similarly, SPAG16L is expressed in the cytoplasm of spermatocytes and round spermatids, and is present in the manchette of the elongating spermatids of the control mice. In the mutant mice, SPAG16 is still expressed in the cytoplasm of spermatocytes and round spermatocytes; it is no longer present in the manchette of elongating spermatids. These findings suggest that Y68 is a key amino acid that controls MEIG1 migration to the manchette to transport cargo proteins for sperm flagella formation.

Poster Sessions

**All posters will be displayed throughout the meeting, but presenters are only required to be present during their designated session, as indicated below.*

***Poster Session I**
Thursday, April 20, 2017
4:05 p.m. – 6:05 p.m.
Location: Symphony Ballroom I/II

Short Talk #1 Poster
(Poster Reference #38)
A HIGH-THROUGHPUT SCREEN TO IDENTIFY NOVEL TRANSCRIPTION FACTORS THAT REGULATE MOUSE SPERMATOGONIAL STEM CELL MAINTENANCE
Tessa Lord , Melissa J. Oatley and Jon M. Oatley
Washington State University
(Presented By: Tessa Lord)

Short Talk #2 Poster
(Poster Reference #28)
CONSERVATION OF A GENE EXPRESSION BARCODE THAT DEFINES SPERMATOGONIAL STEM CELLS IN MICE AND HUMANS.
Anukriti Singh BS[1], Kazadi Mutoji PHD[1], Thu Nguyen MS[1], Heidi Gildersleeve BS[1], Birgit Westernströer PHD[1], Jon Oatley PHD[2], Sherman Silber PHD[3], John McCarrey PHD[1] and Brian Hermann PHD[1]
[1]Department of Biology, University of Texas at San Antonio; [2]Center for Reproductive Biology, Washington State University; [3]The Infertility Center of St. Louis
(Presented By: Anukriti Singh, B.Tech)

Short Talk #3 Poster
(Poster Reference #60)
CLONAL DEVELOPMENT OF SPERMATOGONIA IN RHESUS TESTES
Adetunji Fayomi, Karen Peters BS[1] and Kyle Orwig PhD[2]
[1]Magee Womens Research Institute; [2]Magee Womens Research Institute, Univeristy of Pittsburgh School of Medicine
(Presented By: Adetunji Fayomi, DVM)

Short Talk #4 Poster
(Poster Reference #13)
THE RHOX10 HOMEOBOX TRANSCRIPTION FACTOR PROMOTES PROSPERMATOGONIA MIGRATION
Wei-Ting Hung PhD, Hye-Won Song PhD and Miles F. Wilkinson PhD
UC San Diego
(Presented By: Wei-Ting Hung, PhD)

Poster #2
ANDROGEN INSENSITIVITY SYNDROME: LOSS OF GERM CELLS IN EARLY CHILDHOOD
Paula Aliberti BS[1], Roxana Marino Biochemist[1], Natalia Perez Garrido Biochemist[1], Pablo Ramirez Biochemist[1], Maria Sol Touzon Biochemist[1], Mariana Costanzo MD[1], Gabriela Guercio MD PhD[1], Diana M. Warman MD[1], Roberto Ponzio MD, Prof[2], Roberta Siurano PhD[2], Alberto J. Solari MD PhD, Prof[2], Marco A. Rivarola MD PhD[1], Alicia Belgorosky MD PhD[1] and Esperanza Berensztein PhD[1]
[1]Garrahan Pediatric Hospital, Buenos Aires, Argentina; [2]School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
(Presented By: Esperanza Beatriz Berensztein, PhD)

Poster #4
HUMANIN TRANSGENIC MICE ARE PROTECTED FROM CYCLOPHOSPHAMIDE-INDUCED MALE GERM CELL APOPTOSIS
YanHe Lue MD[1], Hemal Mehta MS[2], James Hoang BS[1], Kelvin Yen PhD[2], Junxiang Wan PhD[2], Ronald Swerdloff MD[1], Pinchas Cohen MD[2] and Christina Wang MD[1]
[1]Division of Endocrinology, LABioMed at Harbor-UCLA; [2]USC Leonard Davis School of Gerontology, University of Southern California
(Presented By: YanHe Lue, MD)

Poster #6

IMPACT OF SOCIAL HABITS AND LIFESTYLE INTERVENTION ON SPERM DNA INTEGRITY: CLINICAL IMPLICATIONS

Surabhi Gautam, MSc, Shilpa Bisht, MSc, Madhuri Tolahunase, MSc, Manoj Kumar, MSc, PhD, Bhavna Chawla, MS and Rima Dada, MD, PhD
All India Institute of Medical Sciences, New Delhi, India
(Presented By: Surabhi Gautam, PhD Scholar)

Poster #8

PRENATAL EXPOSURE TO 1,2-CYCLOHEXANE DICARBOXYLIC ACID DIISONONYL ESTER (DINCH) ON OFFSPRING LEYDIG CELLS AND TESTOSTERONE PRODUCTION

Enrico Campioli PharmD, PhD[1], Matthew Lau[2], Sunghoon Lee MSc[3], Lucas Marques[3] and Vassilios Papadopoulos DPharm, PhD4
[1]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University; [2]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University4Pharmacology & Therapeutics, McGill University; [3]Research Institute of the McGill University Health Centre and Department of Biochemistry, McGill University; 4Research Institute of the McGill University Health Centre and Department of Medicine, McGill University and Department of Pharmacology & Pharmaceutical Sciences, School of Pharmacy, University of Southern California
(Presented By: Enrico Campioli, PharmD, PhD)

Poster #10

THE ROLE OF SUBCLINICAL GENITOURINARY INFECTIONS IN MALE INFERTILITY

Juliana R Pariz MSc, PhD student[1],[2],[3],4, Rosa Alice C Monteiro BSc[1],4 and Jorge Hallak MD, PhD[1],[2],[3],4
[1]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil; [2]Dept. of Urology, USP, Brazil; [3]Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil; 4Oswaldo Cruz German Hospital, Brazil
(Presented By: Juliana Risso Pariz, BSc, MSc)

Poster #14

S-NITROSOGLUTATHIONE REDUCTASE (GSNOR) KNOCKOUT MICE: A NOVEL MODEL OF MALE INFERTILITY

HIMANSHU ARORA PhD, Shathiyah Kulandavelu PhD, Marilia Zuttion Masters, Bruno Nahar MD, Oleksandr Kryvenko MD, Emad Ibrahim MD, Nancy Brackett MD, Joshua Hare MD and Ranjith Ramasamy MD
University of Miami
(Presented By: Himanshu Arora, PostDoc)

Poster #16

A MULTIDISCIPLINARY MODEL OF EARLY FERTILITY PRESERVATION IN KLINEFELTER PATIENTS: DESCRIPTION AND UPDATE OF A PROGRAM

Stanley Kogan MD[1], Guillermo Galdon MD[2], Nima Pourhabibi Zarandi MD[2], David Crudo MD[3], Mark Pettinati PhD4, Shadi Quasem MD5, Yimin Shu MD, PhD6, David Childs MD7, Daniel Rukstalis MD8, Stuart Howards MD8, Hooman Sadri-Ardekani MD, PhD? and Anthony Atala MD?
[1]Wake forest Institute for Regenerative Medicine, Department of Urology; [2]Wake Institute for Regenerative Medicine; [3]Section of Pediatric Endocrinology; 4Section of Medical Genetics; 5Department of Pathology; 6Center for Reproductive Medicine; 7Department of Radiology; 8Department of Urology; ?Wake Institute for Regenerative Medicine, Department of Urology
(Presented By: Stanley Jay Kogan, MD)

Poster #18

GEMINI STUDY: DISSECTING GENETICS OF MALE INFERTILITY BY EXOME SEQUENCING OF SINGLETON PATIENTS

Liina Nagirnaja[1], Nicholas R. Y. Ho[2], Amy B. Wilfert[1], Kenan R. Omurtag[3], Emily S. Jungheim[3], Kenneth I. Aston4 and Donald F. Conrad[1]
[1]Department of Genetics, Washington University in St. Louis; [2]The Institute of Molecular and Cell Biology , Singapore; [3]Department of Obstetrics and Gynecology, Washington University School of Medicine; 4Department of Surgery, University of Utah
(Presented By: Liina Nagirnaja)

Poster #20

LEYDIG STEM CELL ISOLATION AND DIFFERENTIATION FROM HUMAN TESTIS BIOPSIES: POTENTIAL MODALITY TO INCREASE SERUM TESTOSTERONE

HIMANSHU ARORA PhD, Marilia Sanches Santos Rizzo Zutti PhD, Bruno Nahar MD, Joshua M. Hare MD and Ranjith Ramasamy MD
University of Miami
(Presented By: Himanshu Arora, PostDoc)

Poster #22
SPERM RNA AS A REGULATOR OF SUCCESSFUL EMBRYO IMPLANTATION
VIDHU DHAWAN MD[1], MANOJ KUMAR MD[2], DIPIKA DEKA MD[2], NEENA MALHOTRA MD[2], NEETA SINGH MD[2], VATSLA DADHWAL MD[2] and RIMA DADA MD, PhD[2]
[1]AIIMS; [2]AIIMS, NEW DELHI, INDIA
(Presented By: Vidhu Dhawan, MBBS, MD)

Poster #24
MRNIP IS A UBIQUITOUSLY-EXPRESSED GENE REQUIRED FOR MALE FERTILITY
Renata Prunskaite-Hyyrylainen PhD[1], Julio Castañeda PhD[2], Denise Archambeault PhD[3], Zhifeng Yu PhD[3], Ramiro Ramirez-Solis PhD[4] and Martin Matzuk MD, PhD[3]
[1]Baylor College of Medicine and University of Oulu; [2]Baylor College of Medicine and Osaka University; [3]Baylor College of Medicine; [4]Wellcome Trust Sanger Institute
(Presented By: Renata Prunskaite-Hyyrylainen, PhD)

Poster #26
TESTICULAR VOLUME AND TESTOSTERONE LEVELS ARE SIGNIFICANTLY POSITIVELY ASSOCIATED WITH BETTER QUALITY OF SEXUAL LIFE, FUNCTIONAL CAPACITY, COGNITION AND GENERAL MEN'S HEALTH BY SF-36, WHOQOL AND IIEF-15 QUESTIONNAIRES
Jorge Hallak MD, PhD[1,2,3,4], Juliana R Pariz MSc, PhD student[1,2,3,4] and Elaine MF Costa MD, PhD[1,2,3,4]
[1]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil; [2]Dept. of Urology, USP, Brazil; [3]Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil; [4]Oswaldo Cruz German Hospital, Brazil
(Presented By: Jorge Hallak, MD, PhD)

Poster #30
TESTICULAR CANCER IN CHILE: SEMINAL QUALITY IN PATIENTS BENEFICIARIES OF THE EXPLICIT HEALTH GUARANTEES LAW PROGRAM (AUGE), WHICH CONSULT THE MATERNAL AND CHILD RESEARCH INSTITUTE (IDIMI) FOR TEN YEARS (2006-2016)
Marina Fatima Diaz Fontdevlia, Biochemistry Doctor, Pamela Beatriz Inostroza Ballesteros, Biochemistry and Johanna Carrasco Rojas, Veterinary
Facultad De Medicina Universidad De Chile
(Presented By: Marina Fatima Diaz Fontdevila, Adjunt Professor)

Poster #32
STALLION SPERMATOZOA CAN USE CYSTEINE FROM THE MEDIA TO MAINTAIN FUNCTIONALITY
FERNANDO PEÑA PhD, CRISTINA ORTEGA PhD, PATRICIA MARTIN MUÑOZ DVM, JOSE MANUAL ORTIZ RODRÍGEZ DVM and CRUZ GIL ANAYA PhD
UNIVERSITY OF EXTREMADURA
(Presented By: Fernando Juan Pena Vega, PhD)

Poster #40
EPIGENETIC MODIFICATIONS IN THE MOUSE GERMLINE FOLLOWING IN VITRO MATURATION OF FRESH OR FROZEN/THAWED PREPUBERTAL TESTICULAR TISSUES
Antoine Oblette MSc[1], Julie Rondeaux MSc[1], Ludovic Dumont PhD[1], Véronique Sétif BSc[2], Amandine Bironneau BSc[2], Nathalie Rives MD-PhD[2] and Christine Rondanino PhD[1]
[1]Rouen University; [2]Rouen University Hospital
(Presented By: Christine Rondanino, PhD)

Poster #42
IDENTIFYING POTENTIAL MECHANISM STIMULATING RECOVERY OF SSCS AND PS AFTER TEMPORARY INHIBITION OF GDNF SIGNALING
Nicole Parker BS and William Wright PhD
Johns Hopkins Bloomberg School of Public Health
(Presented By: Nicole Parker, BS)

Poster #44
WTAP IS ESSENTIAL FOR MEIOSIS
Zhen Lin
(Presented By: Zhen Lin)

Poster #46

THE CHROMATIN MODIFIER BHC80 IS A DOWNREGULATOR OF NOTCH SIGNALING IN THE MAMMALIAN TESTIS

Pooja Gandhi MS[1], Parag Parekh PhD[1], Jaspreet Farmaha PhD[1,2], Brian Danysh PhD[1] and Marie-Claude Hofmann PhD[1]
[1]University of Texas MD Anderson Cancer Center, Houston, TX; [2]Purdue University, West Lafayette, IN
(Presented By: Parag Parekh, PhD)

Poster #48

ESSENTIAL ROLE OF HIGH AFFINITY COPPER TRANSPORTER 1 GENE IN FUNCTIONAL SPERMATOGENSIS AND CISPLATIN-INDUCED TESTICULAR TOXICITY

Rashin Ghaffari BS, Kristin R. Di Bona PhD and John H. Richburg PhD
The University of Texas at Austin, Austin, Texas
(Presented By: Rashin Ghaffari, BS)

Poster #50

SUMOYLATION MAY REGULATE TRANSCRIPTION AND PHOSPHORYLATION EVENTS IN MOUSE SPERMATOCYTES, AND IS REQUIRED FOR G2/M MEIOTIC TRANSITION IN VITRO.

Margarita Vigodner PhD[1], Benjamin Lucas PhD[2] and Elana Molcho BSc[2]
[1]Yeshiva Univeraity and AECOM; [2]Yeshiva University
(Presented By: Margarita Vigodner, PhD)

Poster #52

HORMONE INDUCED ACUTE STEROIDOGENESIS MEDIATES DYNAMIC RE-ORGANIZATION OF SUBCELLULAR MEMBRANE LIPIDS IN MA-10 MOUSE TUMOR LEYDIG CELLS.

Sathvika Venugopal PhD[1], Rachel Chan BSc[2], Esha Sanyal BSc[2], Lorne Taylor MSc[1], Kaur Pushwinder MSc[1], Edward Daly BSc[1] and Vassilios Papadopoulos DPharm, PhD, DSc [3]
[1]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University; [2]McGill University; [3]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University and Department of Pharmacology & Pharmaceutical Sciences, School of Pharmacy, University of Southern California
(Presented By: Sathvika Venugopal PhD)

Poster #54

THE ANTIOXIDANT AND ANTI INFLAMMATORY EFFICACY OF POMEGRANATE AND CINNAMOMUM ZEYLANICUMON EXTRACT ON 1,7 DIMETHYLBENZANTHRACENE (DMBA) INDUCED APOPTOSIS AND SPERMATOGENESIS IN RATS' TESTES

Arash Khaki DVM-PhD[1] and Nava Ainehchi PhD[2]
[1]Department of Pathobiology,Tabriz Branch,Islamic Azad University,Tabriz,Iran; [2]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
(Presented By: Arash Khaki, DVM, PhD)

Poster #56

TRANSCRIPTION FACTOR USF1 IN THE MAINTENANCE OF SPERMATOGONIAL STEM CELLS

Imrul Faisal Master of Science[1], Geert Hamer PhD[2], Pirkka-Pekka Laurila PhD[3], Matti Jauhiainen PhD[3] and Liisa Kauppi PhD[1]
[1]University of Helsinki; [2]University of Amsterdam; [3]University of Helsinki and National Institute for Health and Welfare
(Presented By: Imrul Faisal Master of Science)

Poster #58

EFFECT OF EARLY TYPE 2 DIABETES ON MALE FERTILITY

Jannette Dufour PhD[1], Robin Hannah Greer[1], Gurvinder Kaur PhD[1], Kandis Wright BS[1], Michael D. Tomison BS[1], Latha Ramalingam PhD[2], Eunhee Chung PhD[3], Naima Moustaid-Moussa PhD[2] and Chwan-Li Shen PhD[1]
[1]Texas Tech University Health Sciences Center; [2]Texas Tech University; [3]University of Texas San Antonio
(Presented By: Jannette Dufour, PhD)

#62

KNOCKDOWN OF IFT140 MAY DISRUPT SPERMATOGENESIS BY DYSREGULATING THE NFKB SIGNALING PATHWAY

Amin Herati MD[1], Peter Bulter BA[1] and Dolores Lamb PhD[2]
[1]Baylor College of Medicine, Scott Department of Urology, Center for Reproductive Medicine; [2]Baylor College of Medicine, Scott Department of Urology, Center for Reproductive Medicine, Department of Molecular and Cellular Biology
(Presented By: Amin S. Herati, MD)

Poster #64

THE EFFECT OF GROWTH FACTORS ON IN VITRO CULTURE OF PREPUBERTAL TESTICULAR CELLS IN DOMESTIC CATS – PRELIMINARY RESULTS

Erika C S Oliveira PhD[1],[2], Valdemiro A Silva Junior PhD[2], Gleide F Avelar PhD[3], Karla P S Oliveira Esquerre PhD[4], Barry T Hinton PhD[5], Buddhan Pukazhenthi PhD[1] and Nucharin Songsasen PhD[1]

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(Presented By: Erika Oliveira, PhD)

Poster #66

HISTONE H4 HYPERACETYLATION IS AN UNEXPECTEDLY EARLY EVENT IN EQUINE SPERMIOGENESIS

Chelsea C. Ketchum BS, Casey Larsen BS, Alexis McNeil BS, Mirella L. Meyer-Ficca PhD and Ralph G. Meyer PhD

Dept. of Animal, Dairy and Veterinary Sciences, School of Veterinary Medicine , Utah Agricultural Experiment Station, Utah State University

(Presented By: Ralph G. Meyer PhD)

Poster #68

GONADOTROPIN INDEPENDENT ANDROGEN SYNTHESIS IN THE HUMAN PREPUBERTAL TESTIS: BREAKING THE DOGMA

Paula Aliberti, Maria Sonia Baquedano PhD, Nora Isabel Saraco PhD, Roxana Marino, Marco Aurelio Rivarola MD, PhD, Esperanza Beatriz Berensztein PhD and Alicia Belgorosky MD, PhD

Endocrinology Service, Hospital de Pediatría Garrahan, Buenos Aires, Argentina

(Presented By: Esperanza Berensztein, PhD)

Poster #70

MUTATION OF THE OUABAIN BINDING SITE OF NA,K-ATPase a4 DOES NOT AFFECT SPERM FERTILITY

Victor Gustavo Blanco, Gladis Sánchez and Jeff P. McDermott

Department of Molecular and Integrative Physiology. University of Kansas Medical Center. Kansas City, KS 66160

(Presented By: Victor Gustavo Blanco, MD, PhD)

Poster #72

THE FUNCTION OF EPIDIDYMAL CYSTEINE-RICH SECRETORY PROTEINS (CRISPS)

Jinghua Hu MSc[1], Duanporn Jamsai PhD[2], Jo Merriner BSc[2], Anne O'Connor BSc[2], Mark Hedger PhD[3] and Moira O'Bryan PhD[2]

[1]Monash University; [2]Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia; [3]Hudson Institute of Medical Research, Wright Street, Clayton, VIC, Australia

(Presented By: Jinghua Hu)

Poster #74

THE INTACT PIRNA PATHWAY PREVENTS L1 MOBILIZATION IN MALE MEIOSIS

Simon Newkirk[1], Fiorella Grandi[2], Suman Lee[1], Valeriya Gaysinskaya[3], James Rosser[2], Nicole Vanden Berg[1], Cathryn Hogarth[2], Carol Marchetto[4], Alysson Muotri[4], Michael Griswold[2], Alex Bortvin[3], Ping Ye[5], Fred Gage[4], Jef Boeke[6] and Wenfeng An[1]

[1]South Dakota State University; [2]Washington State University; [3]Carnegie Institution of Washington; [4]The Salk Institute for Biological Studies; [5]Avera Cancer Institute; [6]New York University

(Presented By: Wenfeng An, PhD)

Poster #76

MULTIPLE GENOMIC MUTATIONS CONTRIBUTE TO MALE INFERTILITY IN MENDELIAN AND NON-MENDELIAN FASHION.

Alexander Yatsenko PhD[1], Nijole Pollock[2], Jiang Huaiyang[2], Yatsenko Svetlana[2], Rajkovic Alek[2], Jaffe Tomas[2], Olszewska Marta[3] and Kurpisz Maciej[3]

[1]Magee-Womens Research Institute; [2]Magee-Womens Research Institute, University of Pittsburgh; [3]Institute of Human Genetics

(Presented By: Alexander Yatsenko, MD, PhD)

***Poster Session II**
Friday, April 21, 2017
4:50 p.m. – 6:50 p.m.
Location: Symphony Ballroom I/II

**Not CME Accredited*

Short Talk #5 Poster
(Poster Reference #47)
3 DIMENSIONAL HUMAN TESTIS ORGANOID SYSTEM CREATED FROM IMMATURE TESTICULAR CELLS
Nima Pourhabibi Zarandi MD[1], Guillermo Galdon MD[1], Hooman Sadri-Ardekani MD, PhD[2] and Anthony Atala MD[2]
[1]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine; [2]Wake Forest Institute for Regenerative Medicine, and Department of Urology, Wake Forest School of Medicine
(Presented By: Nima Pourhabibi Zarandi, M.D.)

Short Talk #6 Poster
(Poster Reference #12)
REGULATION OF CYP26B1 EXPRESSION IN THE TESTIS
Parag Parekh PHD[1], Thomas Garcia PHD[1,2], Reham Waheeb DVM, PHD[3], Vivek Jain MS[1,2], Pooja Gandhi MS[1], Gunapal Shetty PHD[1], Marvin Meistrich PHD[1] and Marie-Claude Hofmann PHD[1]
[1]University of Texas MD Anderson Cancer Center, Houston, TX; [2]University of Houston Clear Lake, Houston, TX; [3]University of Alexandria, Damanhur, Egypt
(Presented By: Parag Parekh, PhD)

Short Talk #7 Poster
(Poster Reference #59)
ADCY2 IS A CANDIDATE GENE FOR THE DEVELOPMENT OF CONGENITAL GENITOURINARY ANOMALIES THROUGH PARTIAL DISRUPTION OF STEROIDOGENESIS
Marisol O'Neill MS and Dolores J. Lamb PhD
Baylor College of Medicine
(Presented By: Marisol Ann O'Neill, M.S.)

Short Talk #8 Poster
(Poster Reference #34)
MUTATION OF A SINGLE AMINO ACID OF MEIOSIS-EXPRESSED GENE 1 BY CRISPR/CAS9 SYSTEM RESULTS IN IMPAIRED SPERMIOGENESIS AND MALE INFERTILITY IN MICE
Shiyang Zhang, Wei Li MD[1], Hong Liu Master student[2], Ling Zhang MD, PhD[3], Yuhong Li MD, PhD[2], Rex Hess PhD[4] and Zhibing Zhang MD, PhD[1]
[1]Virginia Commonwealth University; [2]Virginia Commonwealth University/Wuhan University of Science and Technology; [3]Wuhan University of Science and Technology; [4]University of Illinois
(Presented By: Shiyang Zhang, Master)

Poster #1
LUTEOLIN INCREASES CAMP-DEPENDENT STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR) GENE EXPRESSION AND STEROIDOGENESIS WITHIN MA-10 LEYDIG CELLS
Michelle Cormier[1], Firas Ghouili BSc[1], Pauline Roumaud MSc[1], Mohamed Touaibia PhD[2] and Luc J. Martin PhD[1]
[1]Biology Department, Université de Moncton; [2]Chemistry and Biochemistry Department, Université de Moncton
(Presented By: Luc J. Martin, PhD)

Poster #3
EXPOSURE OF PERIPUBERTAL RATS TO MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) LEADS TO A PRO-INFLAMMATORY ENVIRONMENT IN THE TESTIS WITH THE INFILTRATION OF BOTH MACROPHAGES AND NEUTROPHILS
Jorine Voss PhD, Angela Stermer PhD, Rashin Ghaffari BSc and John Richburg PhD
University of Texas at Austin
(Presented By: Jorine Voss, BSc, MSc, PhD)

Poster #5
PRESENCE OF PERITUBULAR MACROPHAGES IN RAT TESTIS AND THEIR CHANGES AFTER IRRADIATION AND CHEMICAL TREATMENTS
Gunapala Shetty PhD[1], Sarah Potter PhD[2], Zhuang Wu MD[1], Truong Lam BS[1], Tony DeFalco PhD[2] and Marvin Meistrich PhD[1]
[1]University of Texas M.D. Anderson Cancer center; [2]Cincinnati Children's Hospital Medical Center
(Presented By: Gunapala Shetty, PhD)

Poster #7
FETAL EXPOSURE TO GENISTEIN (GEN) AND DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) AT ENVIRONMENTAL DOSES INDUCES INFLAMMATORY RESPONSES IN RAT TESTIS
Shahrzad Ghazisaeidi PhD student[1], Berenice Collet MSc[1], Annie Boisvert ResearchAssistant[1] and Martine Culty PhD[2]
[1]McGill University; [2]University of Southern California
(Presented By: Martine Culty, PhD)

Poster #9
EFFECTS OF PRENATAL EXPOSURE TO DI-N-BUTYL PHTHALATE ON THE DEVELOPMENT OF ADULT LEYDIG CELLS IN RAT DURING PUBERTY
Linxi Li PhD[1], Guoxin Hu PhD[2], Xiaomin Chen PhD[1], Huitao Li Msc[1] and Ren-Shan Ge MD[1]
[1]The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University; [2]School of Pharmaceutical Sciences of Wenzhou Medical University
(Presented By: Linxi Li, PhD)

Poster #11
LEYDIG STEM CELL AUTOGRAFT IN MICE: A NOVEL APPROACH TO INCREASE SERUM TESTOSTERONE WHILE PRESERVING FERTILITY
HIMANSHU ARORA PhD, Marilia Sanches Santos Rizzo Zutti Masters, Bruno Nahar MD, Joshua M. Hare MD and Ranjith Ramasamy MD
University of Miami
(Presented By: Himanshu Arora, PostDoc)

Poster #15
IN VITRO CULTURE OF KLINEFELTER MOUSE SPERMATOGONIAL STEM CELLS
Guillermo Galdon MD[1], Nima Pourhabibi Zarandi MD[1], YanHe Lue MD, PhD[2], Ronald Swerdloff MD[2], Stanley Kogan MD, FACS[1],[3],4, Hooman Sadr-Ardekani MD, PhD[1],[3],4 and Anthony Atala MD[1],[3],4
[1]Wake Forest Institute for Regenerative Medicine ; [2]Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute; [3]Department of Urology; 4Wake Forest School of Medicine
(Presented By: Guillermo Galdon MD)

Poster #17
SIMPLE AND HIGHLY EFFICIENT POLYETHYLENIMINE TRANSFECTION PROTOCOL FOR TRANSIENT TRANSFECTION IN MOUSE SPERMATOGONIAL STEM CELLS
Chatchanan Doungkamchan MD[1], Yi Sheng MD[2], Meena Sukhwani PhD[2],[3] and Kyle E. Orwig PhD[1],[2],[3]
[1]Molecular Genetics and Developmental Biology Graduate Program, Magee-Womens Research Institute, University of Pittsburgh School of Medicine; [2]Magee-Womens Research Institute, Pittsburgh; [3]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213
(Presented By: Chatchanan Doungkamchan, MD)

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SPERM DNA METHYLATION AND RECURRENT MISCARRIAGE
Tim Jenkins PhD, Kenneth Aston PhD, Erica Johnstone MD and Douglas Carrell PhD
University of Utah
(Presented By: Tim Jenkins, PhD)

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INVESTIGATING THE ANTIOXIDANT EFFECT OF ALLIUM CEPA AFTER EXPOSURE TO ESCHERICHIA COLI ON BIOCHEMICAL FACTORS, THE BLOOD ANTIOXIDANTS, AND TESTIS TISSUE IN RATS
Nava Ainehchi PhD[1], Arash Khaki DVMPhD[1] and solmaz Shahverdi MSc[2]
[1]Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; [2]Department of Pathobiology,Ahar,Branch,Islamic Azad University,Ahar,Iran
(Presented By: Nava Ainehchi, PhD)

Poster #23

TESTICULAR PATHOLOGY IS NOT ALTERED IN OBESE INFERTILE MEN WHO PRESENTSEMEN ANALYSES, SPERM FUNCTIONAL TESTS, ELECTRON MICROSCOPY AND TESTIS HISTOLOGY IN OBESE INFERTILE PATIENTS

Caroline Ranéa BSc, MSc student[1,2,3], Juliana R Pariz MSc, PhD student[1,4,5,6], Rosa Alice C Monteiro BSc[1,4,5,6], Inari Ciccone BSc, MSc student[1,4,5], Elaine MF Costa MD; PhD[1,3,7,8], Hector E Chemes MD, PhD[1] and Jorge Hallak MD, PhD[1,4,5,6]

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(Presented By: Caroline Ranea)

Poster #25

ERYTHROPOIETIN AND A FEEDER CELL-FREE HYDROGEL-LAMININ SCAFFOLD PROMOTE THE EXPANSION AND MAINTENANCE OF HUMAN SPERMATOGONIAL STEM CELLS IN CULTURE

Sarayu Ratnam[1], Robert Brannigan[2] and Christopher Payne[2]

[1]Ann & Robert H. Lurie Children's Hospital of Chicago; [2]Northwestern University Feinberg School of Medicine

(Presented By: Christopher Payne, PhD)

Poster #27

FERTILIN-?, CALMEGIN, IZUMO-1, P34H, ACE AND FIBRONECTIN PROTEINS ON THE SURFACE OF RAM SPERMATOZOA: DETERMINED NOT ONLY WITH THE QUANTITY BUT ALSO WITH THEIR DISTRIBUTION

Abit Aktas associate professor and Gul Ipek Gundogan Phd

Istanbul Yeni Yüzyil University, Faculty of Medicine, Department of Histology and Embryology Istanbul, Turkey

(Presented By: Abit Aktas, Sr.)

Poster #29

HISTAMINE H4 RECEPTOR AS A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF LEYDIG CELL TUMORS IN PREPUBERTAL BOYS

Adriana M. B. Abiuso[1], María Luisa Varela[2], Luis Haro Durand[3], Marcos Besio Moreno[1], Alejandra Marcos[1], Marco A. Rivarola4, Alicia Belgorosky[4], Omar P. Pignataro[1], Esperanza Berensztein[4] and Carolina Mondillo[1]

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(Presented By: Adriana M. B. Abiuso)

Poster #31

EFFECTS OF VITAMIN C ON REPRODUCTIVE PERFORMANCE OF TEDDY GOAT BUCKS

Muhammad Zubair, PhD

University of Poonch rwawalakot Azad Kashmir

(Presented By: Muhammad Zubair, Lecturer)

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POSITIVE EFFECT OF MELATONIN AND CAFFEINE SUPPLEMENTATION IN STRUCTURAL AND FUNCTIONAL CHARACTERISTICS IN PRE-FREEZE AND POST-THAW SEMEN SAMPLES

Juliana R Pariz MSc, PhD student[1,2,3,4], Priscilla R Costa MSc, PhD[5], Dayane G Reis BSc student[1,2,3,4], Victória S Coutinho BSc student[1,2,3], Donald P Evenson MD, PhD[6] and Jorge Hallak MD, PhD[1,7,8,9]

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

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A NOVEL METHOD FOR THE ISOLATION OF GERM CELLS AT DIFFERENT STAGES OF SPERMATOGENESIS
Nina Mayorek PhD, Yousef Mansour graduate student, Michael Klutstein PhD and Eli Pikarsky MD, PhD
The Hebrew University
(Presented By: Nina Mayorek, PhD)

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LACK OF TRIM28 IN EARLY GERM CELLS AFFECTS SPERMATOGENESIS AND RESULTS IN MALE INFERTILITY
Joel Tan BSc , Shu Ly Lim PhD and Daniel Messerschmidt PhD
Developmental Epigenetics and Disease Group, Institute of Molecular and Cell Biology, A*STAR
(Presented By: Joel Tan BSc)

Poster #37
DISTINCT MEIOTIC ARREST MECHANISMS ACT DURING HUMAN SPERMATOGENESIS
Sabrina Jan, MSc, Aldo Jongejan, PhD, Cindy Korver, Saskia van Daalen, Ans van Pelt, PhD, Sjoerd Repping, PhD and Geert Hamer, PhD
AMC Amsterdam
(Presented By: Ans van Pelt, PhD)

Poster #39
CLASSICAL RETINOIC ACID SIGNALING IS NECESSARY IN STEROIDOGENIC CELLS FOR NORMAL SPERMATOGENESIS AND EPIDIDYMAL FUNCTION.
Estela Jauregui, My-Thanh Beedle, Debra Mitchell, Traci Topping, Cathryn Hogarth and Michael Griswold
Washington State University
(Presented By: Estela J. Jauregui)

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SPERMATOGENOMICS: CORRELATING GENE EXPRESSION TO HUMAN MALE INFERTILITY
Arka Baksi MSc[1], Ruchi Jain PhD[2], Satish Bharadwaj PhD[3], Vasan S,S MD[4], Kondaiah Paturu PhD[2] and Rajan Dighe PhD
[1]MRDG,IISc,Bangalore; [2]MRDG, IISc, Bangalore; [3]Manipal Ankur fertility clinic; [4]Manipal Ankur Fertility clinic
(Presented By: Rajan Dighe, PhD)

Poster #43
RNMT IS REQUIRED FOR MOUSE SPERMATOGONIAL STEM CELL MAINTAINANCE
Yao Chen
(Presented By: Yao Chen)

Poster #45
STEM LEYDIG CELL DIFFERENTIATION IN VITRO: EFFECTS OF AGING AND NICHE CELLS*
Xiaoheng Li[1], Fenfen Chen[1], June Liu[2], Renshan Ge MD[1], Barry Zirkin PHD[2] and Haolin Chen PHD[2]
[1]The Second Affiliated Hospital and Yuying Hospital, Wenzhou Medical University, China; [2]Johns Hopkins Bloomberg School of Public Health
(Presented By: Xiao-Heng Li, MS)

Poster #49
CFAP69 IS REQUIRED FOR SPERM HEAD AND FLAGELLUM DEVELOPMENT IN MICE
Frederick Dong BA and Haiqing Zhao PhD
Johns Hopkins University
(Presented By: Frederick Dong)

Poster #51
A STANDARDIZED METHOD FOR MULTISPECIES PURIFICATION OF TESTICULAR GERM CELLS
Ana Cristina Lima PhD[1], Min Jung[1], Jannette Rusch PhD[1], Abul Usmani PhD[1], Alexandra M. Lopes PhD[2] and Don Conrad PhD[3]
[1]Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA; [2]Instituto de Investigação e Inovação em Saúde, Universidade do Porto – I3S, Porto, Portugal [4]Instituto de Patologia e Imunologia Molecular da Universidade do Porto, – IPATIMUP, Porto, Portugal; [3]Department of Genetics, [5]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA
(Presented By: Ana Cristina Lima, PhD)

Poster #53

INTRODUCTION OF TSPO GENE MUTATIONS IN MA-10 MOUSE LEYDIG TUMOR CELLS RESULT IN REDUCED STEROID HORMONE FORMATION

Jinjiang Fan PhD[1] and Vassilios Papadopoulos DPharm, PhD[2]
[1]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University; [2]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University and Department of Pharmacology & Pharmaceutical Sciences, School of Pharmacy, University of Southern California
(Presented By: Vassilios Papadopoulos, DPharm, PhD)

Poster #55

SINGLE-CELL GENE EXPRESSION ANALYSIS REVEALS DIVERSITY AMONG HUMAN SPERMATOGONIA

Nina Neuhaus Dr, Juyong Yoon Dr[1], Nicole Terwort[2], Sabine Kliesch Prof[3], Jochen Seggewiss Dr[4], Andreas Huge Dr[5], Voss Reinhard Dr[6], Stefan Schlatt Prof[2], Rashel Grindberg PhD[7] and Hans Schöler Prof[1]
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(Presented By: Stefan Schlatt)

Poster #57

CHARACTERIZING THE ROLE OF RETINOIC ACID IN THE NON-HUMAN PRIMATE TESTIS

Angel Thalhofer, Traci Topping, Cathryn Hogarth and Michael Griswold
Washington State University
(Presented By: Angel Brooke Thalhofer, Graduate Student)

Poster #61

IDENTIFICATION OF GENE TARGETS OF A TRANSCRIPTION FACTOR THAT PROMOTES SPERMATOGONIAL STEM CELL ESTABLISHMENT

Kun Tan PhD, Hye-Won Song PhD, Abhishek Sohni PhD and Miles Wilkinson PhD
Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA 92037, USA
(Presented By: Kun Tan, PhD)

Poster #63

TESTIS HISTOLOGY IN MEN SUBMITTED TO MICROSURGICAL CORRECTION OF SUBCLINICAL VARICOCELE WITH LONG REFLUX AS A VARIABLE TO UNDERSTAND IMPROVEMENT IN SPERM QUALITY POST-TREATMENT

Jorge Hallak MD, PhD[1],[2],[3],[4], Robertson T Dutra MSc[1],[2],[3] and Juliana R Pariz MSc, PhD student[1],[2],[3],[4]
[1]Androscience – High Complexity Clinical and Research Andrology Laboratory, Brazil; [2]Dept. of Urology, USP, Brazil; [3]Reproductive Toxicology Unit, Dept. of Pathology, USP, Brazil; [4]Oswaldo Cruz German Hospital, Brazil
(Presented By: Jorge Hallak, MD, PhD)

Poster #65

IN VITRO CULTURE OF SEMINIFEROUS CORDS OF DOMESTIC CATS – PRELIMINARY RESULTS

Erika C S Oliveira PhD[1],[2], Valdemiro A Silva Junior PhD[2], Gleide F Avelar PhD[3], Karla P S Oliveira Esquerre PhD4, Budhan Pukazhenti PhD[1] and Nucharin Songsasen PhD[1]
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(Presented By: Erika Oliveira, PhD)

Poster #67

THE IMPACT OF GRAND PATERNAL AGING ON SPERM DNA METHYLATION PATTERNS

Tim Jenkins PhD, Kenneth Aston PhD, James Hotaling MD and Doug Carrell PhD
University of Utah
(Presented By: Tim Jenkins, PhD)

Poster #69

FEWER AND DYSFUNCTIONAL FETAL LEYDIG CELLS PRODUCE LESS TESTOSTERONE AND CAUSE DELAYED TESTIS DESCENT AND ABNORMAL EXTERNAL GENITALIA IN GLI3XTJ MUTANT MICE

Jessica L. Muszynski[1], Samantha R. Lewis[1], Anna E. Baines[1], Stephanie L. Winske[1], Chad M. Vezina[1], Elena M. Kaftanovskaya [2], Alexander Agoulnik[2], Martin J. Cohn[3], and Joan S. Jorgensen[1]

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(Presented By: Joan S. Jorgensen, DVM/PhD)

Poster #71

SEASONAL CHANGES IN THE TESTICULAR ACTIVITY OF THE BRAZILIAN RATTLESNAKE CROTALUS DURISSUS (LINNEAUS, 1758)

Flávia Resende PhD student[1],[2], Anna Menezes Biology student[1],[2], Tailine Reis Biology Bachelor[1] and Gleide Avelar PhD[1]

[1]UFMG; [2]FUNED

(Presented By: Gleide Fernandes Avelar, PhD)

Poster #73

HIGH-RESOLUTION PHENOTYPING OF SPERMATOGENIC DEFECTS USING SINGLE-CELL SEQUENCING

Min Jung, Jannette Rusch PhD, Abul Usmani PhD and Don Conrad PhD

Department of Genetics, Washington University in St. Louis

(Presented By: Min Jung)

Poster #75

FX-MIR: A TESTES-EXPRESSED MICRORNA CLUSTER TARGETING FMR1 AND KEY SPERMATOGENESIS GENES

Hye-Won Song PhD, Madhuvanthi Ramaiah PhD, Terra-Dawn Plank PhD and Miles Wilkinson PhD

University of California, San Diego

(Presented By: Hye-Won Song, PhD)

Poster #77

EXPRESSION OF TWO NANOS SEQUENCES IN THE TESTIS OF A LITTLE SHARK, SCYLIORHINUS CANICULA

Laura Gribouval, PhD Student[1], Cécile Guidardiere[2], Pierrick Auvray Dr[2], Pascal Sourdaine Pr[1], and Aude Gautier Dr[1]

[1]UMR BOREA; [2]KELIA

(Presented By: Laura Gribouval, PhD Student)

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Poster #1

LUTEOLIN INCREASES CAMP-DEPENDENT STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR) GENE EXPRESSION AND STEROIDOGENESIS WITHIN MA-10 LEYDIG CELLS

Michelle Cormier[1], Firas Ghouili BSc[1], Pauline Roumaud MSc[1], Mohamed Touaibia PhD[2] and Luc J. Martin PhD[1]
[1]Biology Department, Université de Moncton; [2]Chemistry and Biochemistry Department, Université de Moncton
(Presented By: Luc J. Martin, PhD)

Introduction: Testicular Leydig cells are major contributors of androgen synthesis and secretion, which play an important role in testis development, normal masculinization, maintenance of spermatogenesis, and general male fertility. The rate-limiting step in testosterone biosynthesis involves the transfer of cholesterol to the mitochondrial inner membrane by the steroidogenic acute regulatory (Star) protein, a critical factor in steroid hormone biosynthesis. Once inside the mitochondria, cholesterol is metabolized by the steroidogenic enzyme Cyp11a1 to pregnenolone, which is further converted to testosterone by the action of other steroidogenic enzymes. Interestingly, Star protein level declines during Leydig cell aging, resulting in defective mitochondrial cholesterol transfer and lower testosterone production. It is possible to delay the age-related decline in testosterone production by increasing Star and/or Cyp11a1 gene expressions using supplementation with flavonoids, a group of the polyphenolic compounds widely distributed in fruits and vegetables.
Objective: Determine whether the distribution of hydroxyl groups among flavones could influence their potency to stimulate steroidogenesis within Leydig cells.
Methods: MA-10 Leydig cells were transfected with steroidogenic promoters luciferase reporter plasmids, followed by stimulations with increasing concentrations (0, 10, 50, 250 µM) of selected flavones for 6 h of incubation with or without 10 µM forskolin. Effects of flavones on steroidogenic genes expressions were also investigated at the mRNA (qPCR) and protein levels (Western blot). Changes in progesterone accumulation in response to flavones treatments were evaluated by ELISA assays.
Results: Low levels of apigenin, luteolin, chrysin and baicalein (10 µM) stimulated cAMP-dependent Star, Cyp11a1 and Fdx1 promoters' activations and may increase steroidogenesis within Leydig cells. Indeed, luteolin effectively improved cAMP-dependent accumulation of progesterone from MA-10 Leydig cells through increased of Star transcription and translation.
Conclusion: Luteolin at 10 µM increased cAMP-dependent Star gene expression and steroidogenesis within MA-10 Leydig cells. Thus, dietary luteolin could be potentially effective to maintain testosterone production within aging males.

Poster #2

ANDROGEN INSENSITIVITY SYNDROME: LOSS OF GERM CELLS IN EARLY CHILDHOOD

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(Presented By: Esperanza Beatriz Berensztein, PhD)

Androgen insensitivity syndrome (AIS) is a hereditary disease in which androgen receptor (AR) mutations in 46, XY patients present with partial (PAIS) or complete (CAIS) defects in virilization.
Possible complications in adult patients with AIS include infertility, psychological or social issues and testicular cancer. Nevertheless, scarce information is available about testis features in prepubertal (PP) patients with AIS.
Our aim was to analyze the consequences of lack of AR in germ cell (GC) health and survival along postnatal development.
We studied 14 patients with AIS (11 CAIS, 3 PAIS) corresponding to 12 families (median age 8.8, range 0.4-23y). The 9 PP gonads were inguinal (median age 2.3, 0.4-10.3y) while the 5 pubertal (PUB) were abdominal (median age 19, 16.2-23y). Clinical diagnosis of AIS was confirmed by hormonal and molecular studies. Control testes were collected at necropsy or biopsy from 16 subjects without endocrine disorders (median age 1.2, range 0.003-13.8y, 12 PP and 4 PUB).
Tissue samples of all the gonads were observed by our specialists. Electron microscopy (EM) of gonadal tissue from one CAIS patient (1.8y) was done. Expression of MAGE-A4 to identify GC and of OCT4 to identify pluripotential GC was assessed by IHC.
Many signs of testicular dysgenesis were found, as abundant gonocytes, huge GC, microlithiasis, thickened basal membrane and/or fibrous interstitium in PP AIS testes and Leydig cell hyperplasia, vacuolated SC, scarce/none GC, absence of meiosis, and a sex cord tumor in PUB testes. The presence of gonocytes was confirmed by EM. Normal testicular parenchyma according to age was found in all controls.
In AIS testes there was a significant loss in the number of GC with age ($R2= 0.4061$, $p= 0.0025$), clearly evident after the first 2y of age. As the staining decreases, foci of positive cords were found. No difference between PAIS and CAIS was found. In contrast, controls showed an increase of MAGE-A4 expression as a function of age ($R2= 0.4582$, $p= 0.0271$) with a homogeneous staining pattern. OCT4 expression was found in 3 CAIS samples and in none Controls.
We report the presence of signs of testicular dysgenesis, premalignant marker and early loss of GC in an androgen deprived milieu. We propose that the lack of AR expression and action in AIS might impair the survival and normal development of spermatogonia prior to puberty. However, the role of an abnormal testicular location on the survival of GC must not be ruled out.

Poster #3

EXPOSURE OF PERIPUBERTAL RATS TO MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP) LEADS TO A PRO-INFLAMMATORY ENVIRONMENT IN THE TESTIS WITH THE INFILTRATION OF BOTH MACROPHAGES AND NEUTROPHILS

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University of Texas at Austin
(Presented By: Jorine Voss, BSc, MSc, PhD)

Introduction and objectives: The testis is an immune-privileged organ that maintains an immune suppressive environment that results in low numbers of leukocytes in the testicular interstitium. We have previously shown that exposure of peripubertal (postnatal day (PND) 28) Fischer rats to an acute dose of MEHP (700 mg/kg, p.o.), a well-described Sertoli cell toxicant, leads to an accumulation of CD11b+ cells in the interstitial space of the testis that closely correlates with a robust incidence of germ cell (GC) apoptosis. CD11b is expressed on the outer membrane of many leukocytes of the innate immune system, including monocytes, macrophages, and granulocytes. Here we further characterized the phenotype of these immune cells.

Methods and results: PND 28 Fischer rats received an oral dose of 700 mg/kg MEHP, and after 12, 24 or 48 hours the interstitial cells were analyzed by flow cytometry and immunofluorescence. It was found that after 12 hours of MEHP exposure, there were two different CD11b+ populations. One was also positive for CD68, a monocyte and macrophage marker, and the other population were identified to be neutrophils by morphology and the expression of myeloperoxidase, which is abundantly expressed in neutrophil granules. By 24 hours, both populations of cells were still present, but had reduced to approximately half and by 48 hours less than a quarter of the accumulated cells remained. Although the majority of macrophages in the untreated peripubertal testis expressed both CD68 and CD163, a marker of resident or alternatively activated macrophages, the majority of the monocytes/macrophages that accumulate following MEHP exposure express CD68, but not CD163. Gene expression analysis of these infiltrating monocytes/macrophages showed upregulated expression of Tnfa and Il6 compared to macrophages from the interstitium of control-treated testis. The increase in inflammatory cells coincided with an increased expression of Il1a, Il6 and Ccl2 in the seminiferous tubules.

Conclusion: Together these results suggest that exposure to MEHP leads to an increase in pro-inflammatory signaling and an accumulation of various innate immune cells in the interstitium of the testis. Current experiments are targeted to evaluate the functional significance of the innate immune cell populations in the testis after MEHP exposure and whether they serve a protective role or further exacerbate phthalate-induced injury to the testis.

Poster #4

HUMANIN TRANSGENIC MICE ARE PROTECTED FROM CYCLOPHOSPHAMIDE-INDUCED MALE GERM CELL APOPTOSIS

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(Presented By: YanHe Lue, MD)

Humanin (HN) is a cytoprotective peptide encoded by a mitochondrial gene. We have previously demonstrated that the pharmacological administration of HN or its analogue HNG protects male germ cells against cyclophosphamide (CP)-induced apoptosis in mice. To examine the role of endogenous HN in the cytoprotection of male germ cells from chemotherapy, we generated HN transgenic (HNt) mice expressing a CMV-promoter driven humanin transgene. After genotyping by PCR, 1) groups of 7 adult (5-10 month-old) HNt and age-matched wildtype (WT) mice were used for the characterization of male reproductive phenotype, and 2) groups of 6 adult HNt and age-matched WT mice were treated with a single-dose of CP injection (i.p. 200mg/kg) to examine male germ cell apoptosis (quantified as apoptotic index (AI): the number of apoptotic germ cells/100 Sertoli cells). The plasma testosterone (T) was measured by RIA. HNt mice were viable, fertile and smaller in size (BW:28.5±2.2g) with an average of 18% decrease in body weight (BW) as compared to WT (BW:34.9±10.3g) mice. The testis weight (TW:88±10.1mg, $p=0.007$) in HNt mice was significantly lower than WT (TW:105.2±9.3mg) mice. There was no difference in cauda epididymal sperm count between HNt (1.3±0.07 million/mg cauda) and WT (1.3±0.03 million/mg cauda) mice. Testis histological examination in HNt mice showed normal histology with the baseline germ cell apoptosis rates reminiscent of WT levels. HNt mice have similar plasma T levels (0.6±0.4ng/ml) as WT (0.7±0.5ng/ml) controls. Two days after CP treatment, there were no marked changes in body and testis weight, and plasma T levels. The germ cell apoptosis rate in WT mice was significantly ($p<0.001$) increased at spermatogenic stages I-III (AI:46.1±4.6), VII-VIII (AI:20.6±0.9) and XI-XII (AI:56.9±4.8) as compared to non-treated WT mice (stages I-III AI: 9.5±2.1; VII-VIII:2.5±0.6; XI-XII:17.5±1.8). In HNt mice, CP treatment significantly increased germ cell apoptosis at stages XI-XII (AI: 23.7±2.9; $p=0.03$), but not at stages I-III (AI:14.9±2.3) and VII-VIII (AI:4.8±1.1) as compared to baseline levels of HNt mice (stages I-III AI:8.3±1.8; VII-VIII:3.8±0.8; XI-XII:13.3±3.2), suggesting that male germ cells in HNt mice were partially resistant to CP-induced apoptosis. Thus, we conclude that HN 1) is cytoprotective hormone; and 2) mimics the effects of caloric−restriction on metabolism and chemotherapy−protection.

Poster #5

PRESENCE OF PERITUBULAR MACROPHAGES IN RAT TESTIS AND THEIR CHANGES AFTER IRRADIATION AND CHEMICAL TREATMENTS

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(Presented By: Gunapala Shetty, PhD)

A recent study has identified a new and distinct population of macrophages at the peritubular region of the seminiferous tubules of adult mouse testes (DeFalco et al, Cell Reports, 12:1107, 2015). These macrophages contribute to the spermatogonial niche in the adult testis of mice and appear to be required for spermatogonial differentiation to proceed.
Based on our observations of a block in spermatogonial differentiation in irradiated rat testes, we initiated studies to identify changes in the numbers and phenotype of peritubular macrophages in this model to investigate their role in modulation of spermatogonial differentiation. We mainly used an antibody to ionized calcium-binding adapter molecule 1(IBA1) to localize the peritubular macrophages in testes of unirradiated and irradiated rats given various treatments after irradiation.
In the peritubular region of normal rat testes, we identified macrophages that had long processes and a ramified appearance characteristic of dendritic cells. After testicular irradiation, despite a progressive block in spermatogonial differentiation, the number of these peritubular macrophages appeared to increase with time. However, treatments of irradiated rats with a GnRH-antagonist plus flutamide, which induced differentiation, resulted in a further increase in macrophage numbers. Nevertheless, there was no correlation between the time at which the differentiation was maximal and the total number of peritubular macrophages, and no change in macrophage numbers when flutamide was replaced with testosterone, a combination that inhibits the differentiation. Interestingly, the number and ramification of peritubular macrophages further dramatically increased after treatment of irradiated rats with ethane dimethane sulfonate (EDS), which also transiently stimulated spermatogonial differentiation for 2 weeks. However this change persisted at 4 weeks after EDS treatment, at which time differentiation became blocked again. Thus the number of IBA1-positive peritubular macrophages only weakly correlates with the progression of spermatogonial differentiation in the irradiated rat model.
Further studies are in progress to closely examine the distribution of these macrophages with respect to the differentiating clones of spermatogonia in these treated irradiated rat testes and to determine whether there are changes in functional factors, that are produced in the peritubular macrophages that could affect spermatogonial differentiation.

Poster #6

IMPACT OF SOCIAL HABITS AND LIFESTYLE INTERVENTION ON SPERM DNA INTEGRITY: CLINICAL IMPLICATIONS

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(Presented By: Surabhi Gautam, PhD Scholar)

Introduction & Objective: A sedentary lifestyle, psychological stress, increased intake of fast & non-veg food, increased smoking, excessive alcohol intake and other such habits leads to supra physiological free radical levels. Various studies have emphasized that lifestyle intervention such as yoga/meditation might be effective in management of oxidative stress (OS). Thus study planned with aim to analyse effect of yoga based lifestyle intervention (YBLI) on oxidative stress and cellular ageing.
Methods: This study included 150 fathers of children with Retinoblastoma and grouped according to their lifestyle habits. Semen and blood samples were collected at (0,10,21,90days) and analyzed for Reactive Oxygen Species (ROS), DNA Fragmentation Index (DFI), 8-hydroxy-2'-deoxyguanosine (8-OHdG), Telomerase activity and Telomere length.
Results: From day 0 to day 90, there was an increase in the activity of telomerase ($p < 0.001$) and telomere length ($p > 0.05$) whereas the markers of oxidative stress such as ROS ($p < 0.0001$), DFI ($p < 0.05$) and 8-OHdG ($p < 0.01$) showed a sustained reduction.
Conclusion: YBLI is a simple tool of self-transformation which not only minimizes OS, improves DNA integrity but also causes reversal of markers of aging and improves quality of life. Thus Yoga should be adopted as an integral part of our lifestyle which may reduce incidence of childhood cancer.

Poster #7

FETAL EXPOSURE TO GENISTEIN (GEN) AND DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) AT ENVIRONMENTAL DOSES INDUCES INFLAMMATORY RESPONSES IN RAT TESTIS

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(Presented By: Martine Culty, PhD)

Introduction and objectives: Perinatal exposure to endocrine disruptors (EDs) may predispose adult males to reproductive abnormalities. Although humans are exposed to chemical mixtures, few studies have assessed the toxic effects of ED mixtures on male reproduction at environmentally relevant doses.
Our aim was to investigate whether fetal exposure to the mixture of two common chemicals, the plasticizer di-(2-ethylhexyl) phthalate (DEHP) and the phytoestrogen genistein (GEN) at doses relevant to humans, would induce inflammatory responses in neonatal and adult rat testes.
Methods: Pregnant SD rats were gavaged with corn oil, 0.1 or 10 mg/kg/day of DEHP, GEN or their mixture, from gestation day 14 to birth. Male offspring were sacrificed at Postnatal day (PND)3 and 120, and their testes either snap frozen or fixed. Expression levels of testicular somatic cell markers were assessed by quantitative real-time PCR (qPCR) and immunohistochemistry (IHC).
Results: qPCR analysis revealed significant increases in mast cell and macrophage mRNA markers in adult rats treated with GEN+DEHP at both doses, while at PND3, the two doses triggered opposite effects. In addition, expression of the Leydig and Sertoli cell marker Anxa1 was reduced in rats exposed to the lower dose at both ages, but increased by the higher dose. Moreover, collagen 1 and 4 expression were upregulated at PND120 by the higher dose mixture. IHC analysis showed morphological changes in the testes of adult rats exposed to GEN + DEHP at both doses.
Conclusion: These data suggest that fetal exposure to DEHP+GEN mixtures induce short and long lasting inflammatory responses in testis, which may contribute to testicular dysfunction. They also highlight differential age-related effects, and that GEN and DEHP do not follow classical dose-response effects.
Financial support: This work was supported by a grant from the Canadian Institutes of Health Research (CIHR) to MC. The Research Institute of McGill University Health Centre is supported in part by a center grant from Fonds de la Recherche en santé Quebec.

Poster #8

PRENATAL EXPOSURE TO 1,2-CYCLOHEXANE DICARBOXYLIC ACID DIISONONYL ESTER (DINCH) ON OFFSPRING LEYDIG CELLS AND TESTOSTERONE PRODUCTION

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(Presented By: Enrico Campioli, PharmD, PhD)

1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) is a plasticizer introduced in 2002 in the European market for use in plastic materials and articles that come into contact with food. Although DINCH received final approval from the European Food Safety Authority in 2006, there is limited knowledge about its potential endocrine-disrupting properties. Bisphenol A, a chemical used as an intermediate in polycarbonate plastic and epoxy resin synthesis, and phthalate plasticizers, have been shown to be associated with the development of endocrine and reproductive diseases and different types of cancer. Preliminary studies in our laboratory showed altered gene profile in the testis of PND 3 and 6 pups that had been exposed in utero to DINCH. Moreover, DINCH exposure resulted in a non-monotonic reduction of serum testosterone levels and seminal vesicle weight in the PND 60 progeny. The purpose of the present work was to assess whether in utero exposure to 1, 10 and 100 mg DINCH/kg/day from gestational day 14 until birth would affect the progeny testis function.
In utero exposure to DINCH did not affect body weight and anogenital distance of the male offspring at PND 3 and 200, but it affected the anogenital distance at PND 60. Gene markers of somatic and germ cell function in the testis, including steroid production and androgenic activity, were analyzed. PND 3 pups exhibited a modification in Nes and Cyp11a1, which are highly expressed in Leydig cells. Additional genes were modified in PND 60 animals: Star, Tspo, Cyp11a1, Ar, and Plzf. At PND 200 only Cyp11a1 and Pdgfra were significantly modified. Testosterone production was reduced significantly at both PND 60 and PND 200. Culture of PND 3 testes with DINCH did not affect testosterone production and thus had no effect on fetal Leydig cells. Seminal vesicle weights at PND 60 and 200 were negatively correlated to in utero DINCH dose. Interestingly we observed the random appearance in PND 200 animals of small and liquid testis containing degenerating tubules.
Taken together, these results suggest that DINCH might have a direct effect on Leydig cell function, causing a premature aging of the testis. Those effects are likely attenuated with the physiological aging of the animal.
(Supported by CIHR grant FRN-148688 and a CRC).

Poster #9

EFFECTS OF PRENATAL EXPOSURE TO DI-N-BUTYL PHTHALATE ON THE DEVELOPMENT OF ADULT LEYDIG CELLS IN RAT DURING PUBERTY

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(Presented By: Linxi Li, PhD)

Introduction: Fetal exposure to di-n-butyl phthalate (DBP) causes the adult disease such as lower testosterone production and infertility. However, the mechanism is still unknown. The objective of the present study is to determine how DBP affects the involution of fetal Leydig cells during neonatal period and how this event causes the delayed development of the adult Leydig cells during puberty.
Methods: The pregnant Sprague-Dawley dams were randomly divided into 3 groups and were gavaged with 0 (corn oil, the vehicle control), 100 or 500 mg/kg DBP from gestational day 12 to 21. The blood and testes were collected from male pups at postnatal day 4, 7, 14, 21, 28, and 56. Serum testosterone concentrations were assessed and the mRNA levels of Leydig cell- or gonadotroph cell-specific genes were measured.
Results: Prenatal exposure to DBP caused the aggregation of fetal Leydig cells, which slowly disappeared when compared to the control. This effect was associated with the reduction of testicular testosterone secretion and down-regulation of the mRNA levels of Leydig cell biomarkers including Scarb1, Star, Cyp11a1, Hsd3b1, Hsd11b, and Hsd17b3.
Conclusion: We demonstrated that the increasing aggregation of fetal Leydig cells with the increasing doses of DBP delayed their involution, thus leading to the delayed development of the adult Leydig cells.
Funding: This work is supported by NSFC (81373032 and 81601264), Zhejiang Provincial NSF (LQ16H040005 & 2016KYB199) and Health & Family Planning Commission of Zhejiang Province (2016KYB202). Corresponding author: Ren-Shan Ge.

Poster #10

THE ROLE OF SUBCLINICAL GENITOURINARY INFECTIONS IN MALE INFERTILITY

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

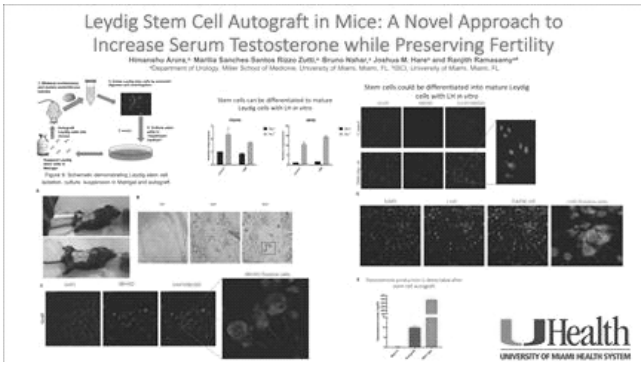
Introduction: Genitourinary tract infections are the most common disease affecting male reproductive health, frequently do not have clear symptoms, therefore are not properly investigated neither diagnosed nor treated. Bacteria, protozoa and yeasts may interact directly with spermatozoa, resulting in sperm agglutination, motility and morphological alterations to sperm.
Objective: To determine genitourinary infections frequency in asymptomatic patients in as part of a routine andrological evaluation.
Methods: 981 tests were performed in patients evaluated between 2012 and 2016 who presented with any alteration on anamnesis and/or physical examination: pain in the external genitalia, symptoms of urethritis, burning sensation in the perineum, urethral discharge, pain, etc. After initial evaluation, a prostatic massage followed by microbiological analysis on urethral secretion (collected by swab), urine (medium−jet urine) and semen (collected by masturbation). Were used Student's T test for statistical analysis and adopted $p<0.05$.
Results: Twenty−one percent (239/981 samples) had some microorganism both semen, secretion, urine. Of these, 6.28% (15/239) reported testicular pain and 43.52% (104/239) had a clinical sign on physical exam that could be associated with any kind of infection. When diagnosed during clinical evaluation, epididymitis was suspected in 24.26% patients (10.46% epididymitis only, prostatitis in combination with epididymitis 11.29%, and 2.51% orchiepididymitis). In addition, 10.46% had urethritis, 5.02% prostatitis and 3.76% orchitis. Enterococcus ssp, E. coli, Staphylococcus ssp and Klebsiella ssp. were the most frequent microorganisms. Antibiograms revealed that only 58% of the available antibiotics in the market were effective against these infections.
Conclusion: Male genitourinary tract infections should be a concern by the andrologists when seeking for a diagnosis and correct treatment for male infertility. Often difficult to diagnosis due to the lack of a readily available and well equipped andrology laboratory. Much higher incidence of epididymitis, support the hypothesis that the epididymis is a physiological barrier against testicular infections. Appropriate antibiotic treatment should be given before investigating every other possible cause of infertility, since the presence of infections impact negatively on seminal quality.
Financial support: Androscience
Ethics Committee Approval: FMUSP n°859215/2014

Poster #11

LEYDIG STEM CELL AUTOGRAFT IN MICE: A NOVEL APPROACH TO INCREASE SERUM TESTOSTERONE WHILE PRESERVING FERTILITY

HIMANSHU ARORA PhD, Marilia Sanches Santos Rizzo Zutti Masters, Bruno Nahar MD, Joshua M. Hare MD and Ranjith Ramasamy MD
University of Miami
(Presented By: Himanshu Arora, PostDoc)

Background: Leydig cell loss or dysfunction is associated with impaired testosterone production. Exogenous testosterone supplementation can be used to treat low testosterone, however it has several adverse effects including infertility due to negative feedback on the hypothalamic-pituitary-gonadal axis. We studied testosterone production in mouse models following autograft in skin with Leydig stem cells isolated from testes.
Methods: A total of 10 wild-type adult C57/BL6 mice were included in the study. Orchiectomy was conducted in seven mice (4 experimental and 3 negative controls) and the remaining three were used as positive controls. Leydig stem cells were harvested from testis by collagenase/trypsin digestion. Cells from each mouse were allowed to grow separately in the media containing DMEM, FBS (10%), P/S, ITS, Dexamethasone, EGF, PDGF-AA. After 10 days following orchiectomy, 1 X 106 cells from four animals were autografted in the subcutaneous tissue. After four weeks, grafts and blood were harvested. We evaluated testosterone production, graft morphology, and expression of Leydig cell markers.
Results: We successfully isolated and cultured up to 1 X 106 million Leydig stem cells / testis from all 7 animals. These cells were differentiated and converted into functional adult Leydig cells in vitro. Stem cell property of cultured cells was confirmed by IF and qPCR in which the expression of PDGFR-α was high in regular media vs differentiation induction media and expression of 3BHSD was low in regular media vs differentiation induction media. The autografts were able to survive in animals for at least one month. H&E and 3BHSD, LHR staining showed the presence of Adult Leydig cells subcutaneously. Testosterone levels were almost doubled in autograft mice as compared to negative controls.
Conclusions: Our results indicate that Leydig stem cells can be isolated and cultured from wild-type mice. Leydig stem cell autograft can a novel therapeutic approach to increasing serum testosterone while simultaneously preserving fertility.



Poster #12/Short Talk #6

REGULATION OF CYP26B1 EXPRESSION IN THE TESTIS

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(Presented By: Parag Parekh, PhD)

Cytochrome P45026B1 (CYP26B1) regulates the concentration of all-trans-retinoic acid (RA) and plays a key role in germ cell differentiation by controlling local distribution of RA. Interestingly, little is known about the mechanisms of Cyp26b1 gene regulation. In Sertoli cells, it is maintained by SF1 and SOX9 during gonad development and throughout life but inhibitors that would balance its expression, possibly accounting for the pulses of RA in the adult seminiferous epithelium, are not known. Our previous data from Sertoli-cell specific NOTCH gain- and loss-of-function mouse models indicated that expression of Cyp26b1 is inversely correlated to NOTCH pathway activity. We hypothesized that 1) Spatiotemporal Cyp26b1 downregulation is directly dependent on canonical NOTCH signaling; and 2) A subset of premeiotic germ cells is responsible for Cyp26b1 downregulation through the NOTCH ligand JAG1. Germ cell-Sertoli cell co-cultures experiments demonstrated that JAG1, mainly expressed by Aundiff spermatogonia, activated NOTCH signaling in primary Sertoli cells and induced the transcriptional repressors and canonical NOTCH target genes Hes/Hey. Upregulation of Hes/Hey gene expression by JAG1 was associated with significant decreases in Cyp26b1 expression, while simultaneous downregulation of Hes/Hey by RNAi led to significant increases. Further, Luciferase and ChIP-PCR assays

demonstrated that HES/HEY directly bind to the Cyp26b1 promoter to downregulate its expression. Investigation of stage-specific NOTCH activity using transgenic mice, together with qPCR analysis of Hes/Hey and Cyp26b1 expression, indicated lowest expression of Cyp26b1 at stages VI-VIII of the seminiferous epithelium, when NOTCH activity and RA production are highest. To elucidate which germ cells activate NOTCH signaling in Sertoli cells in vivo, we performed germ cell depletion experiments using moderate doses of busulfan. We found that elimination of undifferentiated spermatogonia will downregulate NOTCH signaling and upregulate Cyp26b1 expression in Sertoli cells. In conclusion, we believe that NOTCH signaling, induced by JAG1-expressing Aundiff in Sertoli cells, is a mediator of germ cell differentiation by controlling Cyp26b1 expression and possibly RA pulses.
Supported by NIH R01HD081244

Poster #13/Short Talk #4
THE RHOX10 HOMEOBOX TRANSCRIPTION FACTOR PROMOTES PROSPERMATOGONIA MIGRATION
Wei-Ting Hung PhD, Hye-Won Song PhD and Miles F. Wilkinson PhD
UC San Diego
(Presented By: Wei-Ting Hung, PhD)

Introduction & Objective: Spermatogonia stem cells (SSCs) are generated from prospermatogonium (ProSG) at approximately the same time when these SSC precursor cells migrate from the center of seminiferous tubules to the periphery – the "stem cell niche". We recently reported that the RHOX10 transcription factor promotes this migration event, as well as the differentiation of ProSG into SSCs (Song et al. Cell Reports 2016). Here, we report our investigation into the underlying mechanism of RHOX10 action in ProSG.
Methods: Using single cell-RNA sequencing (scRNAseq) analysis, we identified RHOX10-regulated genes in the ProSG subset of Id4-eGFP+ cells from early postnatal testes. Ingenuity Pathway Analysis (IPA) was performed on these RHOX10-regulated genes to identify statistically enriched functional categories.
Results: Four hundred and eight genes were downregulated in Rhox10-KO secondary transitional (T2) ProSG relative to control T2-ProSG, as defined by scRNAseq analysis. Molecular and cellular functions significantly enriched among these RHOX10-regulated genes are "cellular movement," "cell death and survival," and "cellular growth and proliferation," as defined by IPA. Enrichment for "cellular movement" genes is consistent with the function of RHOX10 in ProSG migration. Because RHOX10 promotes cell migration, we next performed IPA on only the genes involving in cellular movement, which revealed enrichment for the PTEN, PI3K/AKT, NF-kappa-b, and PKC signaling pathways. To determine the roles of these signaling pathways in germ cell migration, experiments are ongoing to establish an in vitro 3D culture system to reflect the seminiferous environment. In this system, Sertoli cells are cultured in a microwell to provide the seminiferous epithelium framework. GFP-tagged germ cells with selected target genes genetically modified are introduced into the microwells. Mimic and rescue experiments are then conducted to identify RHOX10-downstream targets critical for germ cell migration. The long-term goal is to identify RHOX10-based molecular circuits that drive ProSG migration and differentiation.
Conclusions: RHOX10-regulated genes in a specific ProSG subset were identified using scRNAseq analysis. IPA analysis revealed several significantly enriched functions that will guide us in ongoing in vitro experiments to define the molecular mechanism by which RHOX10 promotes ProSG migration and differentiation.

Poster #14
S-NITROSOGLUTATHIONE REDUCTASE (GSNOR) KNOCKOUT MICE: A NOVEL MODEL OF MALE INFERTILITY
HIMANSHU ARORA PhD, Shathiyah Kulandavelu PhD, Marilia Zuttion Masters, Bruno Nahar MD, Oleksandr Kryvenko MD, Emad Ibrahim MD, Nancy Brackett MD, Joshua Hare MD and Ranjith Ramasamy MD
University of Miami
(Presented By: Himanshu Arora, PostDoc)

Introduction: Nitrosative stress is regulated by S-nitrosylation of cysteine thiols. Mice lacking S-nitrosoglutathione reductase (GSNOR KO mice), a denitrosylase that regulates S-nitrosylation, show increased levels of S-nitroslyated proteins and exhibit nitrosative stress. Nitrosative stress, similar to oxidative stress, can affect spermatogenesis. We hypothesized that GSNOR KO male mice will exhibit impaired fertility and spermatogenesis.
Methods: Male wild-type (WT) and GSNOR KO mice (N=6 each) were studied after postnatal day 42, at a stage where they have completed the first wave of spermatogenesis. Testes were either fixed and/or frozen for further analysis. Histology of testes was quantified using Johnsen score, epididymal sperm counts was determined using an automated counter, serum testosterone levels was determined using ELISA and GSNOR protein within the testis was evaluated using immunofluorescence and Western blot analysis.
Results: GSNOR KO males exhibited significantly smaller testes as compared to WT (0.1± 0.0 grams vs. 0.07± 0.0 grams, $p<0.05$). Furthermore, serum testosterone levels was significantly lower in the GSNOR KO as compared to WT mice (370.18 ± 0.0ng/mL vs. 42.55 ± 21.7 ng/mL, $p<0.05$). Histological analyses using Johnsen score of GSNOR KO testes showed evidence of degeneration of seminiferous tubules, overall reduction in post-meiotic cells and disrupted spermatogenesis (9.5 vs. 6.5, $p<0.05$). We observed a ~2-fold reduction in epididymal sperm count in GSNOR KO males compared to WT males, indicating that spermatogenesis was

impaired, but not globally arrested (2054 ± 35.35 sperms vs. 1236 ± 86.26 sperms, $p<0.05$). Wild type testis showed extremely high levels of GSNOR protein expressed in the germ cells as well as Leydig cells.
Conclusion: This is the first study demonstrating the association between GSNOR and male fertility. GSNOR KO males exhibit small testes with impaired spermatogenesis and reduced fertility. Attempts to decrease nitrosative stress can reverse impaired spermatogenesis.

Poster #15
IN VITRO CULTURE OF KLINEFELTER MOUSE SPERMATOGONIAL STEM CELLS
Guillermo Galdon MD[1], Nima Pourhabibi Zarandi MD[1], YanHe Lue MD, PhD[2], Ronald Swerdloff MD[2], Stanley Kogan MD, FACS[1],[3],4, Hooman Sadr-Ardekani MD, PhD[1],[3],4 and Anthony Atala MD[1],[3],4
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(Presented By: Guillermo Galdon MD)

Introduction: Klinefelter Syndrome (KS) is characterized by masculine phenotype, supernumerary X chromosomes and a dramatic loss of spermatogonial stem cells (SSC) starting at the onset of puberty. In order to study this process and explore possible therapies, our current method of SSC isolation and propagation have been adapted to KS (41,XXY) mouse model aiming to expand these cells in vitro and overcome the in vivo loss of SSC.
Material and Methods: Putative SSCs were isolated and cultured from testes of normal (40, XY) mice aged 1-day old and 3-day old. The propagation of the cells was optimized comparing different culture medias, culture surfaces and seeding concentrations. Propagated cells were characterized using SSC specific markers assessed by Q-PCR, Digital-PCR and Flow Cytometry analyses. Histological images were used to examine the evolution of cells morphology in culture. The optimized SSC isolation, culture and evaluation system established from normal mouse was then applied to 3-day old KS mouse testicular cells.
Results: The presence of SSC population was demonstrated in normal and KS cultured testicular cells by qPCR, and FACS. Quantification of undifferentiated spermatogonia by using Digital-PCR showed >15% ZBTB16 (PLZF) positive cells in culture. Preliminary data culturing KS mouse testicular cells showed a viable culture of slowly growing cells up to 60 days. Ongoing work is focusing on optimization of culture system and full characterization of cultured KS testicular cells as well as testing their transplantation efficacy to restore fertility.
Conclusions: This work overcomes the initial quiescent stage of neonatal germ cells loss in KS mouse testis to successfully expand them in vitro. Extension of this novel method may lead to new therapeutic options for KS patients.

Poster #16
A MULTIDISCIPLINARY MODEL OF EARLY FERTILITY PRESERVATION IN KLINEFELTER PATIENTS: DESCRIPTION AND UPDATE OF A PROGRAM
Stanley Kogan MD[1], Guillermo Galdon MD[2], Nima Pourhabibi Zarandi MD[2], David Crudo MD[3], Mark Pettinati PhD[4], Shadi Quasem MD[5], Yimin Shu MD, PhD[6], David Childs MD[7], Daniel Rukstalis MD[8], Stuart Howards MD[8], Hooman Sadri-Ardekani MD, PhD[9] and Anthony Atala MD[9]
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(Presented By: Stanley Jay Kogan, MD)

Introduction: Klinefelter Syndrome affects 1/500-1/1000 males and is the most common genetic disorder compromising male fertility. Previous studies of its physiopathology have shown a dramatic loss of germ cells including spermatogonial stem cells (SSC) following the onset of puberty.
Material and Methods: To establish a multidisciplinary referral program to offer clinical and experimental fertility preservation options to Klinefelter patients of all ages. Klinefelter patients diagnosed at any age including prenatal, infancy, prepubertal, adolescence and adult are referred by either pediatric endocrinologists or medical genetics consultants to a male reproductive medicine and surgery clinic. After initial consultation, each patient is enrolled in a long term follow up program to monitor his endocrine profile (Testosterone, FSH, LH, E2, Inhibin B and AMH), pubertal development (Tanner stage) and testicular structure to detect early fibrosis with Elastography and Ultrasound. At Tanner stage III or higher, a one step fertility intervention is offered, including semen collection (by penile vibration stimulation or electroejaculation), microsurgical testicular sperm extraction (micro TESE) and SSC cryopreservation. The extracted sperm is stored in a clinical setting for future IVF/ICSI and his testicular tissue containing SSCs is stored in our experimental autologous testicular tissue bank for possible future in vitro or in vivo spermatogenesis trials.
Result: From December 2014 to January 2016, 15 patients have been enrolled in this program. Two patients (11 & 13 years old; XXYY and XXY respectively) met our criteria for intervention and went through electroejaculation and semen was collected successfully, however no sperm found in their semen. Micro TESE was performed immediately in both testes of each patient and no testicular sperm were found in either specimen by an embryologist presented in the operating room to evaluate the ejaculate and testicular biopsy samples. A biopsy from each testis was stored to preserve SSCs. Diagnostic pathology examination performed by a

dedicated testicular pathologist confirmed the absence of testicular sperms at all specimens and presence of spermatogonia in fewer than 10% of tubules in both patients.
Conclusion: We have established an effective, comprehensive and safe multidisciplinary team program for potential early fertility preservation in Klinefelter boys.

Poster #17
SIMPLE AND HIGHLY EFFICIENT POLYETHYLENIMINE TRANSFECTION PROTOCOL FOR TRANSIENT TRANSFECTION IN MOUSE SPERMATOGONIAL STEM CELLS
Chatchanan Doungkamchan MD[1], Yi Sheng MD[2], Meena Sukhwani PhD[2,3] and Kyle E. Orwig PhD[1,2,3]
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(Presented By: Chatchanan Doungkamchan, MD)

Introduction: In this study, we aimed to develop a simple transient transfection protocol for mouse spermatogonial stem cells (mSSCs) to facilitate downstream gene editing studies. Polyethylenimine (PEI) is a cationic transfection reagent that has been widely used to transiently transfect mammalian cells, but has not been tested in mSSCs. In this study, we developed a modified PEI protocol that allows simple, efficient, low toxicity transient transfection in mSSCs.
Methods: To assess transfection efficiency using PEI compared to Lipid-based reagent, established mSSC cultures from EF1a-EGFP mice were passaged; replated into 24-well plates; expanded until 80% confluent; and transfected with a chicken β-actin (CAG)-mCherry reporter plasmid. The transfection efficiency and cell viability were evaluated 48 hours after transfection by flow cytometry. Lipid-based reagent transfection was done using Superfect (Qiagen) according to manufacturer's protocol. PEI transfection protocol was done by separately mixing 1 µg plasmid DNA with 10 µL of 50 mM sodium chloride (NaCl); and 10 µL of PEI with 5 µL NaCl. The mixtures were allowed to equilibrate for three minutes before the PEI/NaCl mixture was added into DNA/NaCl mixture and incubated for 30 minutes. The mixture was then mixed with 350 µL Iscove's Modified Dulbecco's Medium (IMDM) media and added to the mSSCs culture for six hours before replacing transfection media with 1 mL of supplemented IMDM media. To improve transfection efficiency, we modified PEI protocol (mPEI) by replacing NaCl with plain IMDM media.
Results: Transfection efficiency with PEI (46.90%±2.54) was significantly higher than Superfect (1.92%±0.15, $p<0.0001$). The viability after PEI transfection (55.50%±5.97) was significantly higher than Superfect (37.86%±1.72, $p=0.0116$). The transfection efficiency was improved further using the modified PEI protocol (65.40%±0.90, $p=0.0023$) without decreasing viability (58.23%±3.06, $p=0.7048$). To test the long-term survival and proliferation in vitro, the mCherry-positive cells from modified PEI protocol were sorted and cultured for at least 3 passages. Transplant experiments are underway to test the stem cell function of PEI transfected, FACS-sorted mSSCs.
Conclusion: We developed a transient transfection protocol for mSSCs using PEI (mPEI) that is simple, cost-effective, highly efficient and feasible in most labs. This work was supported by discretionary funds to KEO.

Poster #18
GEMINI STUDY: DISSECTING GENETICS OF MALE INFERTILITY BY EXOME SEQUENCING OF SINGLETON PATIENTS
Liina Nagirnaja[1], Nicholas R. Y. Ho[2], Amy B. Wilfert[1], Kenan R. Omurtag[3], Emily S. Jungheim[3], Kenneth I. Aston4 and Donald F. Conrad[1]
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(Presented By: Liina Nagirnaja)

Introduction: Male infertility due to spermatogenic failure is a common disorder found in 1 % of men. Although the contribution of genetic predisposition is considered to be substantial, the genetic causes of severe male infertility have largely remained elusive. It is feasible that rare patient-specific mutations across a multitude of genes essential for sperm development may lead to the manifestation of the disease. A multi-center study Genetics of Male INfertility Initiative (GEMINI) has been established to map the genetic profile of severe male infertility in a large cohort of patients (currently n=1600) across continents.
Objectives: As a proof of principle, we aimed to perform a case-by-case mutation discovery among various infertility patients by applying a pipeline designed to identify rare variants of large effect in singleton cases.
Methods: Patient cohort (n=34) included 12 men with non-obstructive azoospermia, 15 with oligozoospermia, 3 with unexplained infertility and 4 women with premature ovarian failure (POF). Patient exome libraries were sequenced on Illumina HiSeq2500/3000. A case-by-case analysis included mutation calling (GATK tools), annotation and prioritization (PSAP method) and filtering using in-house pipeline. All mutations were confirmed by Sanger sequencing. A newly developed in vivo shRNA knock-down assay of selected novel male infertility genes was performed in mice to demonstrate their relevance in spermatogenesis.
Results obtained: Putative rare (MAF<0.01) disease-causing genetic variants were identified in 15/34 (44 %) patients. Mutations in five genes previously implicated in impaired fertility were observed both among male patients (e.g. PDE11A, INHBB) and two female

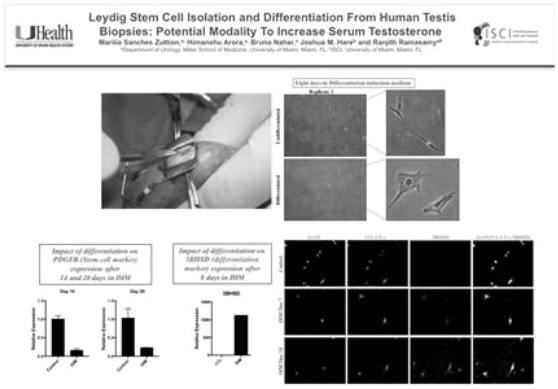
siblings with POF (MSH5). Additionally, mutations in 12 novel genes with unknown function in fertility were highlighted among 11 men. Out of all mutations, 25 % had not been reported previously in the ExAC database. An in vivo shRNA knock-down assay of 6 selected novel genes indicated impaired germ cell development in mice. Further validation is needed to determine the functional effect of the identified variants among humans.
Conclusions: The findings demonstrate a large network of patient-specific disease mutations potentially leading to severe infertility phenotypes. An inter-disciplinary collaborative effort, such as GEMINI, is valuable for uncovering the full profile of these rare genetic variants which will enable to improve the management of infertility.

Poster #19
SPERM DNA METHYLATION AND RECURRENT MISCARRIAGE
Tim Jenkins PhD, Kenneth Aston PhD, Erica Johnstone MD and Douglas Carrell PhD
University of Utah
(Presented By: Tim Jenkins, PhD)

Objective: To understand the role of sperm DNA methylation in the process of recurrent miscarriage where no clear female factors are present.
Design: Prospective study.
Materials and methods: A total of 23 couples were recruited based on the absence of female factor diagnoses and the occurrence of at least two early pregnancy losses. Sperm DNA methylation array data from a total of 16 known fertile sperm donors and 98 patients who have undergone IVF were also screened. All samples were assessed for DNA methylation levels via Illumina's 450k methylation array. Initially, sperm DNA methylation patterns in the 6 most well phenotyped recurrent pregnancy loss patients were compared to known fertile donors to assess methylation variability between the two groups. Further analysis utilized previously screened samples to further describe the alterations identified in the initial study.
Results: Our results indicate that sperm DNA methylation is quite similar between the recurrent pregnancy loss group and donors. There are however 6 total regions that were statistically different between the two groups based on our commonly used cutoff values (corrected p-value < 0.001 and log2 ratio > 0.2). Five of the six regions, though significant, displayed extremely subtle changes between the two groups. One region in the PFKP gene displayed a robust difference in fraction methylation (donor average = 0.529, patient average = 0.877) that required further investigation. We expanded our analysis to assess previously run array datasets, which included a total of 23 recurrent pregnancy loss patients, 16 known fertile donors, and 98 IVF patients. Interestingly in all populations the methylation signature at this location exists in either a fully methylated or partially methylated state. Nearly 70% of all recurrent pregnancy loss patients displayed a hypermethylated profile at this region while approximately 42 % of IVF patients and 31 % of donors displayed similar methylation profiles. These distributions were significantly different based on Fisher's exact analysis at a 95% confidence interval.

Poster #20
LEYDIG STEM CELL ISOLATION AND DIFFERENTIATION FROM HUMAN TESTIS BIOPSIES: POTENTIAL MODALITY TO INCREASE SERUM TESTOSTERONE
HIMANSHU ARORA PhD, Marilia Sanches Santos Rizzo Zutti PhD, Bruno Nahar MD, Joshua M. Hare MD and Ranjith Ramasamy MD
University of Miami
(Presented By: Himanshu Arora, PostDoc)

Background: Impaired testosterone production as a result of Leydig cell loss or dysfunction can occur in men with testicular failure. Testis failure is typically seen in men with Klinefelter syndrome and in men undergoing high dose chemotherapy or hematopoietic stem cell transplant. Currently, these patients are offered long-term testosterone supplementation that can cause infertility. We evaluated an approach for isolation and differentiation of Leydig stem cells from men with infertility that underwent testis biopsies.
Methods: A total of 6 men with testicular failure underwent testis biopsies for sperm retrieval. Using an IRB approved protocol, about 10mg of testicular tissue from each of these men were processed for Leydig stem cell isolation and culture. Leydig stem cells and Sertoli cells were analyzed by immunofluorescence (IF) and quantitative real time PCR (qPCR) for PDGFR-α and Sox-9 respectively. After stimulation by Luteinizing hormone (LH), we compared the levels of 3βHSD mRNAs (involved in testosterone production) using qPCR, and testosterone production in the media using radioimmunoassay from the adult Leydig cells.
Results: We successfully isolated and cultured Leydig stem cells from all 6 men with testicular failure who underwent testis biopsies. Leydig stem cells were maintained in the media without LH for up to 30 days. We conducted a minimum of five independent isolations within 30 days. We were able to culture up to 3 X 106 million cells / biopsy in 14 days. Of the cells cultured, up to 70% of the cells were Leydig stem cells and 10% of them were Sertoli-cell in origin on day 14. IF and qPCR data showed as the majority of cell population was undifferentiated, the expression of PDGFR-α was high. Upon stimulation by LH, the expression of 3βHSD was induced and that of PDGFR-α was reduced at both RNA as well as at protein levels.
Conclusions: Our results indicate that Leydig stem cells can be isolated and cultured from men with testicular failure. Leydig stem cells can be differentiated with LH and the adult Leydig cells can be functional. These results suggest that Leydig stem cell therapy can be used to increase serum testosterone without affecting fertility outcomes.



Poster #21

INVESTIGATING THE ANTIOXIDANT EFFECT OF ALLIUM CEPA AFTER EXPOSURE TO ESCHERICHIA COLI ON BIOCHEMICAL FACTORS, THE BLOOD ANTIOXIDANTS, AND TESTIS TISSUE IN RATS

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(Presented By: Nava Ainehchi, PhD)

Objective: Infectious infertility is considered by the World Health Organization (WHO) as a main problem in sexual life and public health. The aim of the present study was to investigate the antioxidant properties and the effect of Allium cepa (onion) juice on the tissue of testis and seminiferous tubules affected by Escherichia coli.
Materials and Methods: Thirty-Two adult Wistar male rats aging 2.5 to 3 months divided to four groups of 8 rats. Enterotoxigenic E. coli (serotype 0114) used to infect the rats. Onions prepared from the district Ilkhichi, Iran which were used for two groups. Following the infection, pathologic samples were prepared from the tissue of the sperms which were investigated through hematoxylin & eosin (H & E) staining. In addition, the motility, vitality, the number of sperms, total antioxidant capacity (TAC), luteinizing hormone (LH), and testosterone were evaluated as well.
Results: Results indicated that in the control group all the seminiferous tubules are sticking together and all the lines of sexual germ cells observed;while, in E. coli group were disunited and the line of sexual cells were destroyed. In the groups infected by E. coli and treated by A. cepa juice, the effects of bacteria reduced considerably. The number of sperms, sperms vitality and motility decreased significantly in E. coli infected group, while in the A. cepa juice + E.coli the effects of infectious was reduced. The results of the study showed that A. cepa juice significantly increases TAC and testosterone.
Conclusion: The results indicated A. cepa juice has protective effects against E .coli bacteria and fertility, testis tissue and antioxidants improvement and the effects of the bacteria decreased significantly.
Keywords: Antioxidant, Allium cepa, E. coli bacteria, Onion juice, Infertility, Testis tissue, Sperm parameters

Poster #22

SPERM RNA AS A REGULATOR OF SUCCESSFUL EMBRYO IMPLANTATION

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(Presented By: Vidhu Dhawan, MBBS, MD)

Introduction: Implantation, a remarkably dynamic event is a key rate limiting step in an ART laboratory set-up. The molecular and cellular mechanisms governing implantation and the factors contributing to its failure need to be elucidated. The role of paternal factors in embryonic development is being brought to surface. The delivery of transcripts has been seen to contribute to the transcriptome of embryo prior to activation of embryonic genome.
Objectives: The present study was designed to assess the expression pattern of spermatozoal FOXG1, SOX-3, as well as, PARP1 and OGG1 in male partners of couples experiencing recurrent implantation failure (RIF). Seminal oxidative stress and DNA Fragmentation Index (DFI) was also assessed.
Methods: Ejaculates were obtained from 30 male partners of couples experiencing RIF and 30 healthy volunteers with proven fertility. Semen analysis was assessed by WHO (2010) criteria. Reactive oxygen species (ROS) levels (RLU/sec/million sperm) were assessed by luminol-dependant chemiluminescence. The Sperm chromatin structure assay (SCSA) was performed by flow cytometry

to determine (DFI). RNA was isolated from semen samples, reverse transcribed and investigated by q-PCR analysis. The relative quantification of target genes was calculated with 2□□Ct method after normalization to β-actin.
Results: No significant difference was observed in age, seminal volume, liquefaction time, pH and sperm concentration between the male partner of RPL cases and the controls. The average DCt of FOXG1, SOX-3, OGG1 and PARP1 was found to be 5.41, 6.98, 4.5 and 6.1 with respect to 3.94, 4.4, 3.9 and 4.5 in controls. The mean ROS level was seen to be higher (142.78 ± 75.65) in 75% of RIF patients with respect to controls (26.7 ± 9.8). However, the DFI in all the patients of RIF (41.3 ± 5.1) was seen to be higher (>28) against that of fertile controls (27.4 ± 6.4) ($P < .0001$). The odds of occurrence of implantation failures was 4.2 times greater, whose ROS>25 RLU/sec/million sperm (OR 4.2, 95% CI: (1.14-15.3) (p=0.03). While no association was found with DFI>28% (p=0.989).
Conclusion: Dysregulation of genes responsible for early embryogenesis as well as those of base excision repair (BER) pathway may pose as an important causal factor for implantation failure. Normalization of the transcripts by adoption of various lifestyle modifications and correction of oxidative DNA damage may help in morphogenetic patterning of the early developing embryo.

Poster #23
TESTICULAR PATHOLOGY IS NOT ALTERED IN OBESE INFERTILE MEN WHO PRESENTSEMEN ANALYSES, SPERM FUNCTIONAL TESTS, ELECTRON MICROSCOPY AND TESTIS HISTOLOGY IN OBESE INFERTILE PATIENTS
Caroline Ranéa BSc, MSc student[1,2,3], Juliana R Pariz MSc, PhD student[1,4,5,6], Rosa Alice C Monteiro BSc[1,4,5,6], Inari Ciccone BSc, MSc student[1,4,5], Elaine MF Costa MD; PhD[1,3,7,8], Hector E Chemes MD, PhD[1] and Jorge Hallak MD, PhD[1,4,5,6]
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(Presented By: Caroline Ranea)

Introduction: It is estimated that one-third of adult men around the world are obese and one-third are overweight, routinely diagnosed by body mass index (BMI). There is a strong relationship between obesity and male infertility; however, its physiological mechanisms are not well elucidated.
Aims: To evaluate the effect of obesity (BMI and body fat percentage evaluation) in seminal and functional parameters, testicular histology and hormonal profile.
Methods: We included data from 83 medical records of infertile patients aged 21 to 45 y.o., classified according to body fat percentage (BFP) according to bioimpedance values [eutrophic≤19% (n=27), high>19%(n=56)] and BMI [eutrophic (n=34; 18.5<BMI≤24.9 kg/m2), overweight (n =31; 25.0<BMI≤29.9 kg/m2) and obesity (n =18; BMI≥30 kg/m2). Patients were submitted to semen analysis and complementary sperm function tests: anti-sperm antibodies (ASA), creatine kinase activity (CK), reactive oxygen species (ROS) and ultrastructural morphology by electron microscopy (EM). Testicular histology according to Johnsen and Copenhagen criteria and sexual hormones were included. Were used Student's T test and analysis of variance for statistical analysis.
Results: Grade A motility decreased in overweight and obesity groups when compared to the eutrophic group. Grade C motility increased in overweight and obesity groups, compared to the control group. We observed an increase in percentage of anti-sperm antibodies in the overweight group ($p<0.05$). Patients with BFP>19% had a reduction in progressive motility and reduced sperm maturation by CK activity ($p<0.05$). We did not observe significant alterations in the hormonal profile, testis histology and maturation of sperm chromatin in patients with excess of fat tissue.
Conclusion: Excessive body fat has a negative effect on the final steps of spermatogenesis, demonstrated by reduced total progressive motility, increased forms of immature sperm and high anti-sperm antibodies.
Financial support: Androscience/CNPq – PIBIC
Keywords: Male infertility, body mass index, body fat, semen, sperm.
Ethics Committee Approval: FMUSP Ethics Committee (n°859215/2014)

Poster #24
MRNIP IS A UBIQUITOUSLY-EXPRESSED GENE REQUIRED FOR MALE FERTILITY
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(Presented By: Renata Prunskaite-Hyyrylainen, PhD)

Infertility is a polygenic multifactorial disease with heterogeneous phenotypes that affects males and females. Globally, ~14% of couples experience primary or secondary infertility. It affects about 7-10% of all reproductive age men. Genetic factors can be identified in only ~15% of cases given this there is an increasing need to identify and characterize novel infertility associated genes. We also greatly lack an understanding of how sperm-specific genes function whereas numerous yet uncharacterized proteins could be potential contraceptive targets

We used bioinformatic methods to reveal novel, mouse testis-specific genes that have homologs in humans. For some of the genes we identified, the knockout mice were readily available through the knockout mouse project (KOMP) resources. We have analyzed 14 of these mouse lines and found that 9 of those had an unaltered fertility, indicating that these 9 genes alone are not necessary for fertility preservation (Miyata et al., PNAS, 2016). We found that disruption of two other genes caused subfertility and two mouse lines were infertile.
One of these infertile mouse lines is called 3010026O09Riktm1a(EUCOMM)Wtsi (Mrnip) carries a mutation in the mouse gene MRN complex interacting protein (Mrnip), which is the orthologue of humans Chromosome 5 open reading frame 45 (C5orf45) gene. The RT-PCR analysis has demonstrated that Mrnip is ubiquitously expressed in multiple tissues with the strongest expression in testis, kidney, and brain.
Mrnip knockout male did not sire any pups whereas female fertility was not altered. The testes weights and sperm counts were reduced in Mrnip KO mice as compared to heterozygote control mice.
Histological sections of testis and downregulated expression levels of Mvh indicated reduced amount of germ cells. Expression of genes critical for meiosis, Rec8, Mlh1, Sycp3 and Hspa2, was downregulated in Mrnip null adult males as studied by qRT-PCR. Analysis of juvenile Mrnip KO mice at P15 revealed no changes in testes gross morphology, whereas qRT-PCR data has pointed to an emerging trend of reduced expression of meiosis specific genes. Our current data indicates that infertility in Mrnip mice could be attributed to defects in meiosis progression.
This work was supported by Eunice Kennedy Shriver National Institute of Child Health and Human Development grant R01 HD088412, J.C. Baylor College of Medicine training grant 5T32HD007165-35, the Academy of Finland and the Sigrid Juselius Foundation.

Poster #25

ERYTHROPOIETIN AND A FEEDER CELL-FREE HYDROGEL-LAMININ SCAFFOLD PROMOTE THE EXPANSION AND MAINTENANCE OF HUMAN SPERMATOGONIAL STEM CELLS IN CULTURE

Sarayu Ratnam[1], Robert Brannigan[2] and Christopher Payne[2]
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(Presented By: Christopher Payne, PhD)

Conditions that permit the long-term culture of mouse spermatogonial stem cells (SSCs) were established more than a decade ago. However, human (h)SSC cultures are not yet optimized. Most studies reporting hSSC propagation cite a maximum limit of 2-6 weeks. Maintenance of hSSCs declines over this period of time. Changes to current culture conditions are warranted in order to promote hSSC propagation in culture beyond 6 weeks. To address this compelling need, we focused on revising the culture medium and substrate used for hSSCs. A serum-free medium based on the Iscove Modified Dulbecco formulation (IMDM) with supplementation promotes mouse SSC expansion and maintenance favorably in our hands, but does not support the culture of hSSCs. Likewise, a substrate of pure laminin maintains mouse SSCs under feeder cell-free conditions, but does not support hSSC maintenance. Our two objectives of this study were to identify a novel scaffold-based substrate on which hSSCs could be supported without the need for feeder cells, and to determine whether supplementing IMDM culture medium with additional growth factor(s) would promote hSSC expansion and maintenance beyond 2-6 weeks. Donor hSSCs were obtained from adult human testis tissue. When hSSCs were grown on pure laminin in IMDM, very few survived at 2 months (<1 x 103 cells on day 60 versus 4.7 x 104 cells on day 0). In contrast, hSSCs grown on a hydrogel-laminin scaffold (HyStem-C) exhibited a modest expansion in number (9.2 x 104 cells on day 60 versus 4.5 x 104 cells on day 0). Previous studies revealed that erythropoietin (EPO) induced an expansion of undifferentiated spermatogonia in mammalian testes, and that the EPO receptor was expressed in germline-derived cells in vitro. We therefore supplemented IMDM culture medium with various concentrations of recombinant EPO. After 2 months in culture, hSSCs exposed to 1 ng/ml EPO exhibited significantly greater cell numbers (3.6 ± 0.7 x 105 cells on day 60 versus 5.5 ± 1.8 x 104 cells on day 0) than control hSSCs (8.1 ± 0.6 x 104 cells on day 60 versus 4.9 ± 1.1 x 104 cells on day 0). Immunocytochemistry and western blot analysis confirmed that the EPO receptor is present in cultured hSSCs. Quantitative RT-PCR analysis revealed that hSSC self-renewal gene expression was maintained during culture. We conclude that EPO and the HyStem-C-laminin scaffold together promote the expansion and maintenance of human spermatogonial stem cells in culture for at least 2 months.

Poster #26

TESTICULAR VOLUME AND TESTOSTERONE LEVELS ARE SIGNIFICANTLY POSITIVELY ASSOCIATED WITH BETTER QUALITY OF SEXUAL LIFE, FUNCTIONAL CAPACITY, COGNITION AND GENERAL MEN'S HEALTH BY SF-36, WHOQOL AND IIEF-15 QUESTIONNAIRES

Jorge Hallak MD, PhD[1],[2],[3],4, Juliana R Pariz MSc, PhD student[1],[2],[3],4 and Elaine MF Costa MD, PhD[1],[2],[3],4
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(Presented By: Jorge Hallak, MD, PhD)

Introduction: The challenge for the 21st Century will be to provide better quality of life to an already extended lifespan. Prevention of diseases is much better than healing, as it avoids the need of being sick. Finding tools to evaluate general sexual and global health that are easily available and easy to stablish follow-up patterns must be an objective in today's modern Andrology.
Objective: To evaluate if good testicular function could have a positive effect in quality of life by applying questionnaires that are validated worldwide: Short-Form Health Survey (SF-36), The World Health Organization quality of life assessment (WHOQOL) and International Index of Erectile Function (IIEF-15).
Methods: A trained nurse applied all questionnaires as part of the initial evaluation of 212 men attending a private andrology clinic in São Paulo, Brazil.
Results: Testicular volume by physical examination as well as by ultrasound, was positively correlated with WHOQOL (p=0.05), SF-36 (p=0.045) and IIEF-15 (p=0.05). SF-36 demonstrated an improvement in General health (p=0.031), functional and cognitive capacity (p=0.020) with increase in testicular volume and testosterone levels.
Conclusion: Testicular volume measured by an orchidometer or pachymeter can be a useful and easily applicable tool in the daily practice of andrologists, family doctors and general practitioners to evaluate, diagnose medical conditions and counsel on medical therapies to improve testicular function and consequently general quality of life.

Poster #27

FERTILIN-?, CALMEGIN, IZUMO-1, P34H, ACE AND FIBRONECTIN PROTEINS ON THE SURFACE OF RAM SPERMATOZOA: DETERMINED NOT ONLY WITH THE QUANTITY BUT ALSO WITH THEIR DISTRIBUTION

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(Presented By: Abit Aktas, Sr.)

Spermatozoas at developing stages obtained from testis and 3 different regions of epididymis. Determination of existence and localisation of Fertilin-β, Calmegin, Izumo-1, P34H, ACE and Fibronectin were analyzed quantatively via their protein expression profiles by western blotting technique and indirect immunofluorescence technique. Localisation changes of ram spermatozoa during development and maturation have been determined and also ejaculate and structural features of freezed-thawed ram spermatozoas with and without in vitro capacitation/acrosome reaction also been evaluated.
Fertilin-β, Calmegin, P34H proteins in caput, corpus, cauda and mature spermatozoas showed marking in different density and distrubition with. Freezed-thawed samples had lower density and marking than both ejaculate and cauda samples.
Marking was not obtained except Izumo-1 protein from the samples undergo in vitro capatitation/acrosome reaction. Marking of Izumo-1 protein was seen as increasing band formation through equatorial region on acrosome, after in vitro capacitation, however after acrosome reaction, the band formation was only equatorial region. In contrast to expected marking on spermatozoa head, non specific marking was obtained on different localization changing with the region in fibronectin antibody and samples. ACE antibody did not mark the samples. Region specific differences of proteins at kDa level were obtained with western blotting and possible isoforms specific to ram spermatozoa or proteins with similar epitops were marked.
The present work was supported by the Research Fund of Istanbul University.
Project No. 47033

Poster #28/Short Talk #2

CONSERVATION OF A GENE EXPRESSION BARCODE THAT DEFINES SPERMATOGONIAL STEM CELLS IN MICE AND HUMANS.

Anukriti Singh BS[1], Kazadi Mutoji PHD[1], Thu Nguyen MS[1], Heidi Gildersleeve BS[1], Birgit Westernströer PHD[1], Jon Oatley PHD[2], Sherman Silber PHD[3], John McCarrey PHD[1] and Brian Hermann PHD[1]
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(Presented By: Anukriti Singh, B.Tech)

Spermatogonial stem cells (SSCs) are undifferentiated spermatogonia that sustain mammalian spermatogenesis by producing progeny that will either retain stemness (self-renew) or become progenitors that are committed to differentiation. The mechanisms that drive these alternate fates remain poorly understood partly because 1) SSCs are rare, 2) undifferentiated spermatogonia (including SSCs) are

heterogeneous, and 3) SSCs cannot be prospectively distinguished from progenitors. We reasoned that single−cell transcriptomes of cells highly enriched for SSCs could help identify a gene expression "barcode" characteristic of SSCs. To this end, we performed single-cell RNA-Seq on ID4-EGFP+ spermatogonia postnatal day 6 (P6) and adult mice and subdivided these cells based on intensity of EGFP epifluorescence into EGFP-bright (SSCs) and EGFP-dim (progenitors), which matches their functional distinctions based on transplantation (Helsel et al., 2017). Thousands of genes were differentially-expressed between the EGFP-bright and dim subpopulations at both stages, including a subset of genes which were conserved across postnatal development. While EGFP-bright and dim subpopulations were heterogeneous in their gene expression profiles, they were phenotypically separable by 206 differentially-expressed genes [≥2-fold change (FC)] that constitute a putative mouse SSC barcode. Among genes that were upregulated in EGFP-bright (SSCs) were components of the cellular response to GDNF (Gfra1, Ret, Tcl1, Etv5, Fos) and FGFs (e.g., Dusp1, Dusp6). In EGFP-dim (progenitors), genes involved in the regulation of translation (e.g., Eif4ebp1), retinoic acid response (Rbp1) and pyrimidine metabolism (Upp1) were enhanced. In addition, we compared the mouse SSC barcode to single−cell transcriptomes of adult human undifferentiated spermatogonia isolated from 9 individuals, which were stratified based on ID4 mRNA levels. Spermatogonia with the highest ID4 levels in neonatal mice, adult mice, and adult humans exhibited significant conservation of this gene expression barcode (27 genes, ≥2-FC; 327 genes, ≥1.5-FC). Expression of exemplary candidate genes was subsequently validated by immunostaining. Collectively, these findings point to the first putative gene expression signature (barcode) distinguishing SSCs across postnatal testis development, and which may ultimately reveal the identity and phenotype of human SSCs.

Poster #29

HISTAMINE H4 RECEPTOR AS A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF LEYDIG CELL TUMORS IN PREPUBERTAL BOYS

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(Presented By: Adriana María Belén Abiuso, Biologist)

Introduction: Leydig cell tumors (LCTs) are rare steroid-secreting tumors of the testicular stroma, with apparent increased incidence. Symptoms include feminization or virilization in prepubertal boys, and loss of libido, erectile dysfunction, infertility and/or gynecomastia in adults. Although the etiology of LCTs is unknown, multiple studies indicate that overexpression of aromatase (CYP19), as well as excessive estrogen (E2) and IGF-1 production, play a role in Leydig cell tumorigenesis. LCTs are usually benign; however, the malignant phenotype responds poorly to chemo/radiotherapy, highlighting the need to identify novel therapeutic targets for treatment. HRH4, the newest member of the HA receptor family, is considered a promising drug target for allergy, inflammation, autoimmune disorders, and cancer.

Objetive: To investigate the potential role of HRH4 as a therapeutic target for LCTs.

Methods: Most of the experiments described herein were perfomed in R2C rat Leydig tumor cells, a well-documented in vitro model for Leydigioma. The expression of HRH4, StAR and CYP19 was evaluated by qPCR and Western Blot. P4 and E2 levels were determined by radioimmunoassay, and cell proliferation was assessed as a function of 3H-Thymidine incorporation. The angiogenic capacity of R2C cells and the effect of HRH4 agonist treatment on this capacity were evaluated in vitro and in vivo, employing human umbilical vein endothelial cells and by means of the quail chorioallantoic membrane assay, respectively. Also, HRH4 immunoexpression was evaluated in 2 human LCTs (3,92 and 6,0 years old) versus 9 normal human testis samples (NHTS) belonging to four different age groups: neonatal, n=2; infantile, n=1; juvenile, n=3 and pubertal, n=3.

Results: E2 and IGF-1 negatively regulated HRH4 mRNA and protein levels in R2C cells. In agreement, HRH4 expression was weak in LCTs, whereas we observed moderate to strong HRH4 staining, confined to the interstitium, in all the NHTS analyzed. No HRH4 was detected in Sertoli cells nor in germ cells. Treament of R2C cells with two specific HRH4 agonists inhibited StAR expression, P4 and E2 synthesis, CYP19 expression, and cell proliferation. Finally, selective HRH4 activation negatively affected the angiogenic capacity of R2C cells.

Conclusion: Our results point to HRH4 as a potential therapeutic target for LCTs in prepubertal boys. Further studies are needed to determine if this conclusion can be extrapolated to adult patients.

Poster #30

TESTICULAR CANCER IN CHILE: SEMINAL QUALITY IN PATIENTS BENEFICIARIES OF THE EXPLICIT HEALTH GUARANTEES LAW PROGRAM (AUGE), WHICH CONSULT THE MATERNAL AND CHILD RESEARCH INSTITUTE (IDIMI) FOR TEN YEARS (2006-2016)

MARINA FATIMA DIAZ FONTDEVILA BIOCHEMISTRY DOCTOR, PAMELA BEATRIZ INOSTROZA BALLESTEROS BIOCHEMISTRY and JOHANNA CARRASCO ROJAS VETERINARY
FACULTAD DE MEDICINA UNIVERSIDAD DE CHILE
(Presented By: Marina Fatima Diaz Fontdevila, Adjunt Professor)

Introduction: the magnitude of cancer incidence in Chile has required the development of public policies, that promote earlier screening and effective treatments of various cancers. Chilean public health system cared for over 2000 patients with testicular cancer between 1988-2007 (15-40 years old). This pathology has increase in the world with high incidence in Caucasian, North European, Oceania and South American populations. Because, new anti oncogenic therapy, the mortality index in the word, has decrease, increasing the survivors, with secondary effects and better life quality. One of the most important secondary effect, is the loss of fertility. To preserve the fertility, of this patients, cryopreservation, of seminal or testicular samples may be offered. Since 2004, in Chile, a program of explicit health guarantees law (AUGE), for different pathologies, including testicular cancer started up. The cryopreservation of seminal or testicular samples, as sperm bank, is included, prior to their therapy, to preserving mature spermatozoa, for future use in assisted reproduction procedures. Since 2006, our center: the Institute of maternal and child research (IDIMI), attended patients for this public system.
Objectives: this research will analyze, seminal parameters of this patients.
Methods: to do this, a retrospective study of the parameters was performed and analyzed using SSPS statistical program.
Results: based on 172 patients, shows a increase, from 3 patients/year in 2006, to 18 /year in 2016. The semen has the following characteristics: azoospermia (8%), oligozoosepermia (53%), astenozoospermia (47%) and only 31%, normozoospermia. Five percentage, has died. The majority of patients were taking 2 or 3 samples to criopreserve (80 and 40 %) but 1 patient could not obtained any sample. The cancer affected the Right testis of 75 patients, and the Left of 66, 12 patients suffer the pathology in both (synchronous or non-synchronous). From these, 38 presented Seminoma, 17 no seminoma, 16 mixed, 19 patients another types of testicular and 82 were unregistered.
Conclusions: for the first time in Chile, this study shows, seminal parameters of testicular cancer patients from the program of explicit health guarantees in a public institute of reproductive medicine. It can't be inferred that the increase of testicular cancer from 2006 to 2016, was cause for increase of incidence, or for a increase in the clinical derivation.

Poster #31

EFFECTS OF VITAMIN C ON REPRODUCTIVE PERFORMANCE OF TEDDY GOAT BUCKS

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(Presented By: Muhammad Zubair, Lecturer)

The present study was conducted to investigate the effects of vitamin C on reproductive functions of Teddy bucks. For this purpose, 8 adult Teddy bucks were randomly divided into two treatment groups viz; A (control) and B (vitamin C with dose of 200 mg/kg BW/day). These treatments continued for 12 weeks. Semen quality parameters (volume, motility, sperm morphology and sperm DNA integrity) of experimental bucks of each group was evaluated on weekly basis, while testicular measurements (length, scrotal circumference and weights) were also recorded after every two weeks of experiment. At the end of study, testes were also removed and histomorphomatrical changes of testes including the diameter of semeniferous tubules, thickness of germinal epithelium and number of leydig cells were also measured. Serum concentrations of male sex hormones (testosterone, LH, FSH) and cortisol were recorded fortnightly. The data were subjected to two-way analysis of variance, followed by Duncan test for multiple mean comparisons. Supplementation of vitamin C improved significantly ($P<0.05$) the semen quality parameter, testicular measurements and serum levels of sex hormones. Likewise, the morphometrical changes were also improved with this vitamin. It was concluded from the present study that dietary supplementation of vitamin C has beneficial effects on the semen and hormones in male reproductive system.
Key Words: semen, teddy bucks, hormones and testicular measurements

Effects of vitamin C on Reproductive Performance of Teddy Goat Bucks

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ABSTRACT

The present study was conducted to investigate the effects of vitamin C on reproductive functions of Teddy bucks. For this purpose, 8 adult Teddy bucks were randomly divided into two treatment groups viz; A (control) and B (vitamin C with dose of 200 mg/kg BW/day). These treatments continued for 12 weeks. Semen quality parameters (volume, motility, sperm morphology and sperm DNA integrity) of experimental bucks of each group was evaluated on weekly basis, while testicular measurements (length, scrotal circumference and weights) were also recorded after every two weeks of experiment. At the end of study, testes were also removed and histomorphometrical changes of testes including the diameter of seminiferous tubules, thickness of germinal epithelium and number of leydig cells were also measured. Serum concentrations of male sex hormones (testosterone, LH, FSH) and cortisol were recorded fortnightly. The data were subjected to two-way analysis of variance, followed by Duncan test for multiple mean comparisons. Supplementation of vitamin C improved significantly ($P<0.05$) the semen quality parameter, testicular measurements and serum levels of sex hormones. Likewise, the morphometrical changes were also improved with this vitamin. It was concluded from the present study that dietary supplementation of vitamin C has beneficial effects on the semen and hormones in male reproductive system.

Key Words; semen, teddy bucks, hormones and testicular measurements

Poster #32

STALLION SPERMATOZOA CAN USE CYSTEINE FROM THE MEDIA TO MAINTAIN FUNCTIONALITY

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UNIVERSITY OF EXTREMADURA
(Presented By: Fernando Juan Pena Vega, PhD)

Although the redox regulation and oxidative stress are important concepts in spermatology, the molecular mechanisms behind these processes are poorly understood. Recent findings in stallion sperm function reveal that redox homeostasis is extremely important in horses due to a high mitochondrial activity in this species. We hypothesized that glutathione (GSH) is especially involved in the regulation of sperm functionality. To test this hypothesis initially we investigated relationship between sperm function and GSH content showing highly significant correlations between GSH, sperm viability, motility and velocities ($p<0.001$). Furthermore we depleted GSH with menadione and we were able to reverse GSH depletion with cysteine, but no with other antioxidants. Also pre – incubation with cysteine prevented menadione induced damage in sperm membranes, after 1 (live sperm in controls 80%, menadione treated 56% $p<0.001$, and preincubation with cysteine and treatment with menadione 80%) and three hours of incubation (controls 78%, menadione 30% $p<0.001$ and cysteine and menadione 83%). Similar results were also observed in motility and sperm velocities. Cysteine was able to reduce increases in 4-hydroxynonenal induced by menadione ($p<0.001$). If exogenous cysteine increase GSH one possibility is that stallion spermatozoa may synthetize this tri-peptide. To test this hypothesis we investigated the presence of Glutathione Synthetase and glutamate-cysteine ligase, we detected both enzymes in stallion spermatozoa using western blotting and inmunocitochemistry. Furthermore, the inhibition glutamate cysteine ligase reduced the recovery of GSH by addition of cysteine after depletion with menadione, suggesting that stallion spermatozoa may use exogenous cysteine to regulate GSH. This novel finding open new clues for the treatment of male infertility and for the development of better conservation technologies of stallion spermatozoa

Poster #33

POSITIVE EFFECT OF MELATONIN AND CAFFEINE SUPPLEMENTATION IN STRUCTURAL AND FUNCTIONAL CHARACTERISTICS IN PRE-FREEZE AND POST-THAW SEMEN SAMPLES

Juliana R Pariz MSc, PhD student[1],[2],[3],4, Priscilla R Costa MSc, PhD5, Dayane G Reis BSc student[1],[2],[3],4, Victória S Coutinho BSc student[1],[2],[3], Donald P Evenson MD, PhD6 and Jorge Hallak MD, PhD[1],7,8,?
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(Presented By: Juliana Risso Pariz, BSc, MSc)

Introduction: Cryopreservation process can damage spermatozoa and impair structural and functional characteristics. Plasma, nuclear membranes and cellular organelles can suffer from freeze and thaw process.
Objective: To evaluate the effect of melatonin (MEL) and caffeine (CAF) supplementation in structural and functional characteristics in pre−freeze and post−thaw seminal samples.
Methods: Twenty−six semen samples from men between 22 and 54 years−old. All samples were normozoospermic according to WHO criteria. Samples were cryopreserved using Human Tubal Fluid modified without any supplement or with MEL 2mM. After thawing, samples were analyzed as they were cryopreserved or supplemented also with CAF 2mM. Samples were incubated for 15 minutes before final analysis. At the end of the experiments, we obtained five groups: pre−freeze samples (Group I), post−thaw samples without any supplementation (Group II), post−thaw samples supplemented with MEL (Group III), CAF (Group IV) and MEL+CAF (Group V). Sperm count, motility, hyperactivity, reactive oxygen species (ROS), mitochondrial activity and DNA fragmentation (SCSA) were evaluated by StudentÂ´s T test and one−way analysis of variance ($p<0.05$).

Results: Pre−freeze and post−thaw results in non−supplemented samples: progressive motility (51.92vs7.27%; p<0.001). High mitochondrial activity sperm (25.30vs8.30%; p<0.001), sperm vitality (78.33vs41.67%; p<0.001), sperm hyperactivation (8.43vs0.69%; p=0.002). No statistical differences in ROS, SCSA were observed. Supplementation with CAF+MEL (Group V), improved progressive motility (16.47vs7.27%; p=0.017), motility grade b (15.38vs7.27%; p=0.025) and high mitochondrial activity sperm (16.86vs8.30%; p=0.05); reduction of lower mitochondrial activity sperm (10.24vs18.15%; p=0.018) when compared with samples without supplementation. In groups III and IV, were only one supplement was added, either CAF or MEL, no differences were noticed.
Conclusion: Cryopreservation has negative effects in sperm quality in normozoospermic samples. ROS and sperm DNA damage in pre−freeze and post−thaw samples did not show differences. Samples supplemented with CAF+MEL improved significantly post−thaw progressive motility and mitochondrial activity and could be a new resource in andrology.
Financial support: Androscience/Capes/SCSA Diagnostics
Keywords: Cryopreservation, sperm, caffeine, melatonin, ROS, SCSA.
Ethics Committee Approval: FMUSP 031/13

Poster #34/Short Talk #8
MUTATION OF A SINGLE AMINO ACID OF MEIOSIS-EXPRESSED GENE 1 BY CRISPR/CAS9 SYSTEM RESULTS IN IMPAIRED SPERMIOGENESIS AND MALE INFERTILITY IN MICE
Shiyang Zhang, Wei Li MD[1], Hong Liu Master student[2], Ling Zhang MD, PhD[3], Yuhong Li MD, PhD[2], Rex Hess PhD4 and Zhibing Zhang MD, PhD[1]
[1]Virginia Commonwealth University; [2]Virginia Commonwealth University/Wuhan University of Science and Technology; [3]Wuhan University of Science and Technology; 4University of Illinois
(Presented By: Shiyang Zhang, Master)

Mouse meiosis-expressed gene 1 (mMEIG1) is a key player in the regulation of mouse spermiogenesis and sperm flagella formation. In male germ cells, it is expressed in the whole cell body of spermatocytes and round spermatids, but is recruited to the manchette of elongating spermatids by another spermiogenesis regulator, PACRG. The MEIG1/PACRG complex is essential to transport cargo, including sperm associated antigen 16 (SPAG16) to build sperm flagella. Nuclear magnetic resonance (NMR) studies revealed that mMEIG1 adopts a unique fold that provides a large surface for interacting with other proteins. Among the 12 exposed and conserved amino acids, four of them, W50, K57, F66, particularly Y68 mediate binding to PACRG. To study the role of Y68 in vivo, we mutated this amino acid using the CRISPR/cas9 system. DNA sequencing of the RT-PCR product revealed that only the amino acid was mutated in the mutant mice. Western blot analysis demonstrated that MEIG1 protein was expressed, however, the level was reduced in the testis compared to the controls. All homozygous mutant mice examined were completely infertile, and sperm count was dramatically reduced. The developed sperm displayed multiple abnormalities, including short and bend tails, round heads. All mutant sperm examined were immotile. Histologic studies showed impaired spermiogenesis in the mutant mice. Immunofluorescent staining revealed that the mutant MEIG1 is still present in the whole cell body of spermatocytes, but accumulated in the acrosome region of round spermatids. No MEIG1 signal was discovered in the manchette of the elongating spermatids. Similarly, SPAG16L is expressed in the cytoplasm of spermatocytes and round spermatids, and is present in the manchette of the elongating spermatids of the control mice. In the mutant mice, SPAG16 is still expressed in the cytoplasm of spermatocytes and round spermatocytes; it is no longer present in the manchette of elongating spermatids. These findings suggest that Y68 is a key amino acid that controls MEIG1 migration to the manchette to transport cargo proteins for sperm flagella formation.

Poster #35
A NOVEL METHOD FOR THE ISOLATION OF GERM CELLS AT DIFFERENT STAGES OF SPERMATOGENESIS
Nina Mayorek PhD, Yousef Mansour graduate student, Michael Klutstein PhD and Eli Pikarsky MD, PhD
The Hebrew University
(Presented By: Nina Mayorek, PhD)

A novel method for the isolation of germ cells at different stages of spermatogenesis was developed using transgenic mice expressing tomato fluorescent protein exclusively in germ cells.
In this system the level of tomato expression decreases with the progression of spermatogenesis, thus allowing to separate different cell populations from pre puberty and sexually mature mice using FACS sorting. Combination with fluorescent-ckit antibody labeling allows to separate between undifferentiated and differentiating spematogonia.
The main advantage of this method is the absence of contamination by tomato negative somatic cells and no need in using DNA dyes. Our 4 hours protocol allows us to harvest at least 100,000 cells of each of the following populations: undifferentiated spermatogonia, differentiating spermatogonia, late spermatogonia, leptotene/zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes and round spermatids. Using this method we are going to study changes that occur along the process of spermatogenesis using high throughput methodologies.

Poster #36

LACK OF TRIM28 IN EARLY GERM CELLS AFFECTS SPERMATOGENESIS AND RESULTS IN MALE INFERTILITY

Joel Tan BSc , Shu Ly Lim PhD and Daniel Messerschmidt PhD
Developmental Epigenetics and Disease Group, Institute of Molecular and Cell Biology, A*STAR
(Presented By: Joel Tan BSc)

As the role of epigenetics in spermatogenesis becomes more apparent, scientists are beginning to find correlations between epigenetic defects and male infertility. Tripartite motif-containing 28 (TRIM28) is a prominent epigenetic transcriptional co-regulator that has been shown to regulate numerous biological processes such as cellular differentiation. However, little is understood about the role of TRIM28 in spermatogenesis except that ablating it leads to testicular degeneration in mice. We observed that Trim28-heterozygous (Trim28Het) male mice become infertile prematurely, pointing to a likely haploinsufficiency phenotype of Trim28. Mating experiments confirmed this observation. As these mice grew older, their testes progressively became smaller compared to the wild type. Histological analysis uncovered an increase in sertoli cell-only tubules, suggesting that the size reduction potentially resulted from the loss of germ cells. Using diverse genetic models we have shown the haploinsufficiency defects to be germ cell-autonomous. From our results, we believe that lack of TRIM28 causes loss of undifferentiated spermatogonia.

Poster #37

DISTINCT MEIOTIC ARREST MECHANISMS ACT DURING HUMAN SPERMATOGENESIS

Sabrina Jan MSc, Aldo Jongejan PhD, Cindy Korver, Saskia van Daalen, Ans van Pelt PhD, Sjoerd Repping PhD and Geert Hamer PhD
AMC Amsterdam
(Presented By: Ans van Pelt, PhD)

To prevent chromosomal aberrations to be transmitted to the offspring, strict male meiotic checkpoints exist to remove spermatocytes that fail certain quality checks. Nevertheless, although extensively studied in mice, the mechanisms that cause human male meiotic arrest have not been unraveled. Using patient samples from our clinic, we here distinguish three different types of human male meiotic arrest. Five patients, hereafter referred to as type I, display meiotic prophase arrest characterized by severe asynapsis of the homologous chromosomes and disturbed XY-body formation. Four patients, although also undergoing meiotic prophase arrest, display complete chromosome synapsis, normal XY-body morphology and meiotic crossover formation, hereafter referred to as type II. One patient, type III, progresses through the first meiotic prophase without visible problems and displays meiotic arrest at the metaphase stage. Using a novel protocol, which combines RNA sequencing with laser capture microdissection of individual cells from fixed human testicular specimens, we analyzed the transcriptome of pachytene spermatocytes from these groups of patients in comparison to early and late pachytene spermatocytes from fertile controls. Whereas pachytene spermatocytes of the metaphase arrest patient cluster closely to fertile controls, the two types of meiotic prophase arrest have clearly distinct transcriptome profiles that indicate two different molecular mechanisms of human meiotic prophase arrest.

Poster #38/Short Talk #1

A HIGH-THROUGHPUT SCREEN TO IDENTIFY NOVEL TRANSCRIPTION FACTORS THAT REGULATE MOUSE SPERMATOGONIAL STEM CELL MAINTENANCE

Tessa Lord , Melissa J. Oatley and Jon M. Oatley
Washington State University
(Presented By: Tessa Lord, B. Biotech, PhD)

Introduction: Precise regulation over spermatogonial stem cell (SSC) function is integral for continuation of spermatogenesis. SSCs must balance self-renewal with the production of progenitors that are poised for differentiation, lest the self-renewing reservoir becomes exhausted, and azoospermic infertility ensues. Despite this, few regulating factors have been identified; primarily due to limitations in distinguishing SSCs from their closely related progenitor counterparts. To address this, our lab has created a mouse line containing an Id4-Gfp transgene, in which Gfp+ cells (specifically Gfp 'bright') encompass the SSC population, while Gfp- cells are progenitors. Using this mouse line, the objective of the current study was utilize a large scale, high-throughput approach to identify novel transcription factors that regulate SSC function.
Methods: Primary cultures of undifferentiated spermatogonia were established from the testes of Id4-Gfp mice. In these cultures, SSCs are marked as Gfp+ and progenitors are Gfp-, thus, changes in the dynamics of the Gfp+/- populations can be used as a readout for alterations of SSC maintenance. Using a large scale siRNA library, we knocked down expression of 1440 transcription factors in these cultures, over three biological replicates. Experiments were conducted in a 96 well plate format, using a flow cytometer with an automated plate reader to assess the effects of transcription factor knockdown on Gfp content at a rate of 80 wells per hour. Transcription factors were ranked by Z score; calculated on the basis of fluctuations in Gfp content as compared to a non-targeted siRNA control.

Results: Using a Z score cut-off of ±1.5, 23 novel candidates were identified that appear to be involved in the SSC-to-progenitor transition; i.e. their knockdown caused an accumulation of Id4-Gfp bright spermatogonia. Further, 10 novel candidates were identified that are likely to be involved in SSC maintenance, with their knockdown resulting in loss of the Gfp-bright population. From these candidates, two have been selected for further investigation using CRISPR directed gene inactivation, on the basis of testis-specific expression profiles.
Conclusion: Our high throughput methodology has yielded over 30 novel transcription factor candidates that will provide investigative inroads for assessing control over SSC maintenance and progenitor production, and potentially provide insight into underlying causes of azoospermic infertility.

Poster #39 (Poster Award)
CLASSICAL RETINOIC ACID SIGNALING IS NECESSARY IN STEROIDOGENIC CELLS FOR NORMAL SPERMATOGENESIS AND EPIDIDYMAL FUNCTION.
Estela Jauregui, My-Thanh Beedle, Debra Mitchell, Traci Topping, Cathryn Hogarth and Michael Griswold
Washington State University
(Presented By: Estela J. Jauregui)

Spermatogenesis in mammals is a very complex, highly organized process, regulated in part by androgens and retinoic acid (RA). There is a significant amount known about how the RA and testosterone signaling pathways independently regulate this process, but there is almost no information regarding whether these two signaling pathways directly interact and whether RA is critical for Leydig cell function. Our objective was to determine whether Leydig cells require the classical RA signaling mechanism. To test this, we utilized a transgenic mouse line that expresses a dominant negative form of RA receptor alpha (RAR−DN) and the steroidogenic cell−specific Cre mouse line, Cyp17iCre, to generate male mice with steroidogenic cells unable to perform RA signaling. Morphological analysis of 30, 60, 90, and 180 dpp RAR−DN−Flox/Cyp17iCre−positive mice revealed that the testes of these animals display pachytene spermatocyte apoptosis and small seminal vesicles, similar to mice either lacking or containing only low levels of testosterone. Vacuoles were also present within the seminiferous epithelium at 30 and 60 dpp and elongated spermatids were missing in the 90 and 180 dpp mutant testes. Biotin permeability assay showed increase permeability of blood-testis barrier in 90 dpp mutant testes. In addition, qPCR measurements showed decreased levels of transcripts for steroidogenic enzyme. Mutant mice were infertile starting at 60 dpp. Surprisingly, the epididymides of 90 dpp RAR−DN−Flox/Cyp17iCre−positive mice also displayed an abnormal phenotype. Morphological analysis revealed that the epithelium lining the ducts of the cauda epididymis had undergone squamous metaplasia. Using a Cre-Lox responsive reporter strain we were able to detect Cre expression in the principal cells of the epididymis. As a result, our mutant mice also lacked classical RA signaling within the principal cells of the epididymis. Interestingly, preliminary data indicate that testosterone implants partially rescued the abnormal testis and epididymis phenotypes in our mutant animals. These data imply that the classical RA signaling mechanism is required in both the Leydig cells and principal cells for their normal function and, thus, for male fertility.

Poster #40
EPIGENETIC MODIFICATIONS IN THE MOUSE GERMLINE FOLLOWING IN VITRO MATURATION OF FRESH OR FROZEN/THAWED PREPUBERTAL TESTICULAR TISSUES
Antoine Oblette MSc[1], Julie Rondeaux MSc[1], Ludovic Dumont PhD[1], Véronique Sétif BSc[2], Amandine Bironneau BSc[2], Nathalie Rives MD-PhD[2] and Christine Rondanino PhD[1]
[1]Rouen University; [2]Rouen University Hospital
(Presented By: Christine Rondanino, PhD)

In prepubertal boys with cancer, fertility preservation relies on the freezing of testicular tissues. Organotypic culture is one of the approaches allowing the in vitro maturation of thawed tissues. Epigenetic modifications (DNA methylation, histones H3/4 posttranslational modifications) play an important role during spermatogenesis and during embryonic development. We previously found that the expression of DNA methyltransferases and DNA methylation are maintained in the germline after in vitro maturation of mouse prepubertal testicular tissues.
In this study, we investigated (i) the epigenetic marks H3K4me3, H3K9ac and H4K8ac, the expression of genes encoding the enzymes involved in these modifications and the progression of spermatogenesis in organotypic cultures, (ii) DNA methylation at imprinted genes in in vitro produced spermatozoa.
Fresh or thawed (after controlled slow freezing or vitrification) testicular tissues from 6.5 days postpartum (dpp) mice were cultured for 30 days. Prdm9, Jarid1b, Src1, Cdyl, Sirt1 and Hdac1 transcripts were quantified by RT-qPCR. The distribution of modified histones in germ cells and the advancement of spermatogenesis were analyzed after immunohistochemistry. Methylation at differentially methylated regions of the paternally (H19) and maternally (Igf2r) imprinted genes is being studied by bisulfite pyrosequencing. Testes and spermatozoa from 36.5 dpp mice were used as in vivo controls.
The in vitro maturation of fresh or thawed tissues allows the differentiation of spermatogonia into elongated spermatids. The modified histones H3K4me3, H3K9ac and H4K8ac are detected in spermatogonia, leptotene/zygotene spermatocytes, round and elongated spermatids in vivo and after in vitro maturation. If the level of the transcripts studied varies slightly following freezing/thawing and

organotypic culture, the proportion of germ cells containing H3K4me3, H3K9ac and H4K8ac is modified in cultures of fresh, slow frozen and vitrified tissues compared to in vivo controls. Pyrosequencing of PCR-amplified bisulfite-treated DNA extracted from the spermatozoa generated in vitro and in vivo is currently in progress.
In conclusion, despite differences with the in vivo model, DNA methylation and histones methylation/acetylation occur in in vitro matured germ cells. Future studies will be needed to analyze the nuclear quality of the gametes produced in organotypic cultures and embryonic development after oocyte microinjection.

Poster #41
SPERMATOGENOMICS: CORRELATING GENE EXPRESSION TO HUMAN MALE INFERTILITY
Arka Baksi MSc[1], Ruchi Jain PhD[2], Satish Bharadwaj PhD[3], Vasan S,S MD4, Kondaiah Paturu PhD[2] and Rajan Dighe PhD
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(Presented By: Rajan Dighe, PhD)

The differential gene expression during spermatogenesis and its correlation to infertility is not well understood due to lack of human testicular tissues and suitable culture conditions. The present study is an attempt to correlate gene expression in the testicular germ cells to infertility. The testicular germ cell patterns of 44 azoospermic patients were classified into two major groups of obstructuve azoospermia (OA) and non obstructuve azoospermia (NOA). When analyzed using Flow cytometry, the patients with OA (Group I) exhibited presence of diploid, tetraploid and haploid cells indicating complete spermatogenesis. The patients with NOA showed incomplete spermatogenesis with arrest at the meiotic stage showing presence of diploid and tetraploid cells, but not haploid cells (Group II), or at the pre-meiotic stage with only diploid cells (Group III). RT-PCR analysis of Group I revealed expression of markers specifc for the Leydig cells (LHCGR, HSD3B2 and HSD17B3), the Sertoli cells (FSHR, KITL), the spermatogonia (KIT), the tetraploid cells (CCNA1, LDHC) and the haploid cells (PRM1). Group II patients showed expression of CCNA1 and LDHC, but not of PRM1. Group III patients did not express any of the haploid or tetraploid specific markers. Having confirmed the cellular patterns in different patients, microarray analysis was carried out with samples from each group leading to identifcation of diploid/tetraploid/haploid specific genes, their network and probable pathways. The diplod and tetraploid specific genes mainly belonged to pathways related to cell cycle and division and stress response while the haploid specific genes belonged to pathways related to sperm assembly and architecture. Genes such as CDKN1A and GADD45A involved in cell cycle arrest and MCL1, an anti-apoptotic gene, were highly up-regulated in the diploid arrested patients while EGR2 and inflammatory cytokines were up-regulated in the tetraploid arrested patients. RFX2, a master transcriptional regulator for spermiogenesis, was down regulated in the tetraploid cells. Perturbations in expression of these genes could be contributing to the arrest of spermatogenesis. Thus, this study provides an understanding of the possible pathways involved in regulation of human spermatogenesis and their relation to infertility. (supported by Grants from DBT and DST, GOI, New Delhi

Poster #42 (Poster Award)
IDENTIFYING POTENTIAL MECHANISM STIMULATING RECOVERY OF SSCS AND PS AFTER TEMPORARY INHIBITION OF GDNF SIGNALING
Nicole Parker BS and William Wright PhD
Johns Hopkins Bloomberg School of Public Health
(Presented By: Nicole Parker, BS)

The testicular histology of some infertile men suggests they have lost significant numbers of spermatogonial stem cells (SSCs) and their immediate progeny, progenitor spermatogonia (PS). Developing therapies for these men, requires that we understand how numbers of cells are maintained in the normal testis, and how they are restored after some stem cells are lost. Using a chemical-genetic approach, we have shown that numbers of SSCs and PS decrease when glial cell line-derived neurotrophic (GDNF) signaling is temporarily inhibited. Restoration of GDNF signaling may reveal mechanisms responsible for restoring the cells. We hypothesized that this restoration of SSCs and PS is correlated with increased expression of GDNF. To test this hypothesis, we inhibited GDNF signaling for 9 days and testes were collected on days 10 through 28 of the experiment. One day prior to collection, mice were injected with the thymidine analogue EDU to label replicating cells. We then determined the numbers of spermatogonia expressing GFRα1, a marker of SSCs and PS, the fraction replicating, and GDNF levels. On day 10 numbers of cells were 12-fold lower than controls, and by day 28, the numbers of cells increased 6-fold. This recovery was preceded on day 14 by a 2-fold increase in GFRα1+ cell replication, in which there was a 4-fold increase in A single (As) spermatogonia, that includes the SSCs. However, this recovery was not associated with an increase in testis content of GDNF mRNA or protein. We also did not detect an increase in the expression of other transcripts encoding known paracrine regulators of SSCs. To begin to identify the molecular basis for recovery of SSCs and PS, we compared the transcriptomes of testes of control mice, and testes of day 14 mice. This analysis identified ~100 differentially expressed transcripts. One encoded the kinesin, Kif26a that was previously determined to have a role in modulating GDNF signaling in the enteric neurons. Preliminary results indicate Kif26a expression is predominately found in the spermatogonia. RNA sequencing demonstrated that the testis content of Kif26a mRNA at day 14 was 30% lower than controls. However, when Kif26a expression is normalized to GFRα1 expression, results suggest that Kif26a expression per spermatogonia is increasing. Further investigation of

Kif26a could provide insight on the mechanisms GDNF signaling uses to drive SSC and PS maintenance. Supported by (R01HD074542− 01).

Poster #43
RNMT IS REQUIRED FOR MOUSE SPERMATOGONIAL STEM CELL MAINTAINANCE
Yao Chen
(Presented By: Yao Chen)

Spermatogenesis is a highly organized and complex process that allows for the continuous production of millions of haploid spermatozoon throughout adult male life. Despite recent progress in our understanding of spermatogenesis, the regulatory mechanisms especially at the post-transcriptional level that govern spermatogenesis remain poorly understood. The m7G (7-methylguanosine cap) RNA modification has been reported to mediate several key RNA processing events, such as RNA maturation, translation initiation and alternative splicing. Here, we show that RNMT (RNA guanine-7methyltransferase), the only previously known m7G methyltransferase, is highly expressed in male germ cells. To determine the role of RNMT in spermatogenesis, we generated germ cell-specific RNMT knockout mice. We found that germ cell-specific RNMT deficient male mice quickly lost their germ cells, which become apparent at 3 days of age. In two-week-old mutant testes, germ cells were completely exhausted and the tubules contained only Sertoli cells. Our results indicate that RNMT is essential for the homeostasis in murine spermatogonial stem cells (SSCs).

Poster #44
WTAP IS ESSENTIAL FOR MEIOSIS
Zhen Lin
(Presented By: Zhen Lin)

Meiosis is essential for sexual reproduction and evolution. A single round of DNA replication followed by two divisions leads to generation of haploid gametes. Despite recent progress in our understanding of meiotic progression, current knowledge remains largely incomplete. Wilms' tumor 1-associating protein (WTAP), which was previously identified as a nuclear protein, has been suggested to function in alternative splicing, stabilization，m6A formation of mRNA, and cell cycle progression. We show that WTAP is highly expressed in germ cells especially in spermatocytes, suggesting that WTAP could be critical for meiosis. To explore the role of WTAP in meiosis, we deleted WTAP in differentiated spermatogonia by crossing a WTAP floxed line with the Stra8-GFPCre knockin mice. We report here that disruption of mouse WTAP in differentiated spermatogonia results in impaired spermatogenesis from defective meiosis. WTAP-deficient spermatocytes suffer apoptotic death during meiotic prophase I. We further find that WTAP-deficient spermatocytes display homologous synapsis defects. Our findings demonstrate that WTAP is required for meiotic progression.

Poster #45
STEM LEYDIG CELL DIFFERENTIATION IN VITRO: EFFECTS OF AGING AND NICHE CELLS*
Xiaoheng Li[1], Fenfen Chen[1], June Liu[2], Renshan Ge MD[1], Barry Zirkin PHD[2] and Haolin Chen PHD[2]
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(Presented By: Xiao-Heng Li, MS)

We reported that in response to LH, stem Leydig cells associated with the surface of seminiferous tubules differentiate into testosterone (T)-producing Leydig cells in vitro. In contrast, when stem cells were isolated from the tubules, they failed to differentiate, suggesting that the seminiferous tubules produce factor(s) required for their differentiation. In the present study, we examined the effects of the germ cell content of the seminiferous tubules and of aging on tubule-associated stem cells.
First, the abilities of stem cells associated with rat seminiferous tubules of stages VI-VIII and IX-XI to develop T-producing Leydig cells were compared. Then, tubules isolated from young (3 mo) rats, old (18-24 mo) rats with normal spermatogenesis (old-normal), and old rats with extensive loss of germ cells (old-regressed) were cultured in serum-free medium for 4 weeks in the presence of LH (1 ng/ml). Stem cells and Leydig cells were identified by CD90 and 3βHSD staining, respectively, and T production and steroidogenic proteins were assayed. After culture, T production by cells associated with stage VI-VIII tubules was 25% that of stage IX-XI cells. CYP11A1 expression was consistent with T production. The numbers of stem cells (CD90+) and Leydig cells (3βHSD+) were equal, but the intensity of 3βHSD staining and the expression of CYP11A1 were reduced at stages VI-VIII. Tubules of old-regressed testes produced significantly more T than old-normal or young tubules, and old-normal tubules produced more T than young tubules. 3βHSD staining indicated that the numbers and maturation of Leydig cells were increased on the surfaces of tubules from old-normal and old-regressed testes.
CONCLUSION: 1) After culture, the numbers of CD90+ stem cells and of 3βHSD+ Leydig cells did not differ between stages VI-VIII and IX-XI, suggesting that the spermatogenic cycle did not affect stem cell distribution or their commitment to the Leydig cell lineage. Differences in T production, however, suggest that the spermatogenic cycle can affect the development/maturation of Leydig cells. 2) Tubules from old-regressed testes produced more T than tubules from old-normal or young, and tubules from old-normal

produced more T than young, suggesting that both aging and loss of germ cells can affect stem cell numbers and differentiation. *Supported by NIH grant AG25037 (BZ) and by National Natural Science Foundation of China grants NSFC81471411 (HC) and NSFC81601266 (XL).

Poster #46

THE CHROMATIN MODIFIER BHC80 IS A DOWNREGULATOR OF NOTCH SIGNALING IN THE MAMMALIAN TESTIS

Pooja Gandhi MS[1], Parag Parekh PhD[1], Jaspreet Farmaha PhD[1,2], Brian Danysh PhD[1] and Marie-Claude Hofmann PhD[1]
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(Presented By: Parag Parekh, PhD)

NOTCH signaling is a highly conserved cell signaling system present in most multicellular organisms. The NOTCH receptor is a transmembrane protein that is cleaved upon ligand binding to release the NOTCH intracellular domain (NICD). NICD then migrates to the nucleus and forms a complex with the transcription factor RBPJ, which is then activated to promote transcription of target genes such as Hes1 and Hey1. We previously demonstrated that in the testis, NOTCH signaling is repressed in germ cells but active in Sertoli cells, where it plays the role of a negative regulator of Gdnf and Cyp26b1 expression. In order to better understand the specific regulation of RBPJ and its downstream targets in germ cells and Sertoli cells, we used the yeast-2-hybrid assay with RBPJ as bait and discovered that RBPJ might be functionally associating with a protein called BHC80. BHC80 is encoded by the gene Phf21a and is a component of a BRAF35/HDAC complex (BHC) that mediates repression of neuron-specific genes in non-neuronal cells. It has also been linked to LSD1-mediated gene repression in HeLa cells. Co-IP analysis confirmed the association between BHC80 and RBPJ in the testis. BHC80 is mainly expressed in the brain, but we found it expressed in the neonatal and adult testis through immunohistochemistry, in situ hybridization and qPCR. In vivo, BHC80 is well expressed in postnatal premeiotic germ cells and Leydig cells, where the NOTCH pathway is inactive, but expression levels are low in Sertoli cells. In order to understand the role of BHC80 in the context of NOTCH signaling, we used primary Sertoli cells and the Sertoli cell line SF7, which express moderate amounts of BHC80. Inhibition of BHC80 expression by shRNA indicated an increase of NOTCH activity, as seen by increased expression of the NICD/RBPJ targets Hes1 and Hey1, and downregulation of Gdnf and Cyp26b1 expression. Therefore, the functional role of BHC80 in the testis could be to mediate spatiotemporal NOTCH signaling inhibition in specific cellular subsets.
Supported by NIH R01HD081244

Poster #47/Short Talk #5

3 DIMENSIONAL HUMAN TESTIS ORGANOID SYSTEM CREATED FROM IMMATURE TESTICULAR CELLS

Nima Pourhabibi Zarandi MD[1], Guillermo Galdon MD[1], Hooman Sadri-Ardekani MD, PhD[2] and Anthony Atala MD[2]
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(Presented By: Nima Pourhabibi Zarandi, MD)

Introduction: Creating miniature 3 dimensional (3D) organ-like structures from human cells mimicking the function of native organs and eventually develop a "body on a chip" is eagerly desired. We have recently developed an in vitro 3D human testis organoid system from mature human testicular cells with the potential for in vitro differentiation of spermatogonial stem cells (SSC) and androgen production. The main objective of this study is to show the feasibility of establishing the same 3D organoid system, using immature testicular cells. This has a potential application of fertility preservation in prepubertal male cancer survivors and genetically impaired boys who are at risk of infertility.
Material and Methods: Isolated cells from immature (prepubertal) testicular tissue were cultured in 2 Dimensional (2D) condition for 50 days and 5 passages. Specific genes expression assay was used to prove the presence of all 4 cell types including SSCs, Sertoli, Leydig and peritubular cells, as well as confirming undifferentiated condition of spermatogonial cells. Flow cytometry analysis showed the quantity of each cell type. We integrated 2D cultured cells into 3D spherical culture via hanging drop method, using 10,000 cells per organoid. Over 5 weeks of 3D culture the functionality of organoids was evaluated using live/dead cell staining, ATP production assay, post-meiotic genes expression and androgen production.
Results: Specific markers for spermatogonia including ZBTB16 (PLZF) , PGP9.5 (UCHL1), THY1 (CD90), CD9, FGFR3 and SSEA4; GATA4, SOX9, Clusterin and CD49f for Sertoli cells; STAR, TSPO and Cyp11A1 for Leydig cells; and CD34 for peritubular cells; all together approved the presences of different cell types in the cells that isolated, cultured and integrated into 3D organoid. The 3D testis organoids system maintained their structure, viability, metabolic activity and produced androgen over 5 weeks of culture. PRM1 expression showed that this 3D system was able to differentiate SSCs to post meiotic germ cells.
Conclusions: Human 3D testicular organoid system was generated successfully by using isolated human SSC, Sertoli, Leydig and peritubular cells from immature testis and maintained long term in 3D culture. The system was able to produce androgen and push SSCs toward early differentiation. Future directions will include optimizing the system and testing implanted organoids in vivo, as well as evaluating their use as a novel testicular toxicity model.

Poster #48

ESSENTIAL ROLE OF HIGH AFFINITY COPPER TRANSPORTER 1 GENE IN FUNCTIONAL SPERMATOGENSIS AND CISPLATIN-INDUCED TESTICULAR TOXICITY

Rashin Ghaffari BS, Kristin R. Di Bona PhD and John H. Richburg PhD
The University of Texas at Austin, Austin, Texas
(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. Although it is known that cDDP disrupts spermatogenesis, its main cellular target and mechanism responsible for its lasting effects on fertility remain unknown. The high affinity membrane copper transporter 1 (CTR1; SCL31A1) has been shown to be involved in cDDP uptake in both in vivo and in vitro studies. Our preliminary evaluation on mice testes indicates that CTR1 is primarily expressed in primary spermatocytes and SCs. To examine the role CTR1 in the testis as well as to discern the relative contribution between CTR1 in SC and GC to the lasting cDDP-induced disruption in spermatogenesis, 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, Ctr1lox/Δ). Interestingly, GC-KOs exhibit a severe reduction in testis weight (~83% by PND 41) with almost complete depletion of GCs. On the other hand, SC-KO mice had indistinguishable testis weight and histology from their wild-type (WT; Ctr1fl/fl) littermates, with all stages of spermatogenesis present. 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 SC-KO mice had significantly (35%) reduced GC death compared to its WT littermates, with same testis to body weight ratio between all treated and untreated genotypes. Taken together, these observations reveal for the first time 1) the required role of CTR1 in GCs, but not in SCs for functional spermatogenesis and, 2) the significant participation of SC with its expression of CTR1 for mediating cDDP's ability to disrupt spermatogenesis. Future investigations will utilize SC-KO as a mouse model to study the molecular mechanism of acute SC induced GC death, and GC-KOs to explore the importance of CTR1 and/or copper on spermatogenesis.

Poster #49

CFAP69 IS REQUIRED FOR SPERM HEAD AND FLAGELLUM DEVELOPMENT IN MICE

Frederick Dong BA and Haiqing Zhao PhD
Johns Hopkins University
(Presented By: Frederick Dong)

Introduction: Cilia- and flagella-associated protein 69 (CFAP69) is a poorly characterized protein conserved in ciliated eukaryotes ranging from unicellular green algae to humans. Data from the International Mouse Phenotyping Consortium and our own studies demonstrate that in mice, Cfap69 is expressed in several tissues including the testes, and that male Cfap69 homozygous mutant mice are sterile. To understand the function of Cfap69 in male reproduction, we study the defects arising from its loss.
Methods: Knockout and reporter mice in all experiments carry the Cfap69-tm1b allele in which a lacZ trapping cassette is inserted after exon 4, and exon 5 is excised. This allele was generated by crossing Cfap69-tm1a and EIIa-cre mouse lines. The Cfap69-tm1a allele contains the lacZ trapping cassette, as well as a floxed exon 5. The EIIa promoter drives widespread, early embryonic cre expression including in the germline. Expression of Cfap69 was detected by X-gal staining in testis cryosections of Cfap69-tm1b/+ mice. Histology of the seminiferous epithelium was examined by toluidine blue staining of 1µM thick Embed812 sections. FITC-conjugated peanut agglutinin (PNA) was used to stain acrosomes and stage seminiferous epithelia. Scanning electron microscopy was used to assess sperm morphology.
Results: Male Cfap69-tm1b/+ and Cfap69-tm1b/tm1b mice show no obvious abnormalities in mating behavior under laboratory housing conditions, but Cfap69-tm1b/tm1b mice fail to sire offspring when mated with wildtype females, demonstrating they are sterile. Epididymal sperm from these mice have severe and diverse morphological defects of both the head and flagellum, with flagella frequently being tangled or coiled. In Cfap69-tm1b/+ mice, X-gal staining is observed in spermatids and spermatozoa in seminiferous epithelia, indicating Cfap69 expression begins in spermatids. The organization of mutant seminiferous epithelia appears normal and contains all cell types. Additionally, PNA staining of testis sections shows that all epithelium stages are present, indicating that the overall progression of spermatogenesis, spermiogenesis, and subprocesses such as acrosome development is preserved to some extent. However, developing spermatids and resultant spermatozoa are defective, with head and flagella abnormalities becoming conspicuous around stages IX-X.
Conclusions: CFAP69 functions during spermiogenesis in sperm head and flagella morphogenesis and is essential for male fertility.

Poster #50

SUMOYLATION MAY REGULATE TRANSCRIPTION AND PHOSPHORYLATION EVENTS IN MOUSE SPERMATOCYTES, AND IS REQUIRED FOR G2/M MEIOTIC TRANSITION IN VITRO.

Margarita Vigodner PhD[1], Benjamin Lucas PhD[2] and Elana Molcho BSc[2]
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(Presented By: Margarita Vigodner, PhD)

Introduction: Identification of SUMO targets in purified mouse spermatocytes by our laboratory has provided initial insights into the functional importance of sumoylation during male meiosis in mice. The identified proteins are involved in the regulation of transcription, stress response, microRNA biogenesis, cell cycle control, DNA breaks, and other processes. Notably, the largest identified group was of proteins regulating transcription, including those important for spermatogenesis. Interestingly, some kinases have also been identified by our screen as SUMO targets. This finding is consistent with growing evidence that phosphorylation and sumoylation interact at multiple levels.

Methods and Results: Our preliminary data in purified mouse spermatocytes demonstrate that the inhibition of sumoylation arrests the G2/M1 transition in mouse spermatocytes. In the control culture , treatment with Okadaic acid (OA, an inhibitor of the serine/threonine protein phosphatases PP1 and PP2A) induced condensation of chromosomes accompanied by massive H3Ser10 phosphorylation and full disassembly of the SC . In contrast, the addition of GA prevented chromosome condensation and disassembling of the SC. Similar to the results in somatic cells, inhibition of sumoylation significantly affected the global pattern of tyrosine phosphorylation. The timing of the meiotic arrest was similar to the one observed upon inhibition of tyrosine phosphorylation. It must be noted that, similar to sumoylation, the role of tyrosine phosphorylation in meiotic prophase and G2/M transition is not well understood. Global changes in serine/threonine (Ser/Thr) phosphorylation were less prominent, probably due to the poor quality of the antibodies. Therefore, we used specific antibodies against either activating (PLK1, AUR, ERK, AKT) or inhibitory (CDC) phosphorylation on several Ser/Thr kinases implicated in the regulation of meiosis as well as kinase assays for some of them. Our results revealed that the activity of PLK1 and Aurora kinases were negatively regulated by inhibition of sumoylation during OA−induced G2/M1 meiotic transition, while the activity of ERKs and AKT were not affected or increased.

Conclusion: Both AURB and PLK1 are being "at the right time and at the right place" to at least, in part, explain the meiotic arrest obtained in the spermatocyte culture. AURB is responsible for phosphorylation of H3 on Ser10 and PLK1 is responsible for the disassembling of the SC.

Poster #51

A STANDARDIZED METHOD FOR MULTISPECIES PURIFICATION OF TESTICULAR GERM CELLS

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(Presented By: Ana Cristina Lima, PhD)

Introduction & Objectives: The great cellular heterogeneity of the testis hinders the establishment of an in vitro system to study spermatogenesis, and therefore, robust techniques to isolate specific cell types from testicular tissue are required for studies of male germ cell biology. Fluorescence-activated cell sorting (FACS) has been the method of choice, currently allowing the isolation of up to 9 germ cell populations in the mouse. This is possible due to the unique changes in chromatin structure and amount throughout spermatogenesis, which can be captured by flow cytometry of testicular cells stained with DNA dyes. By combining and adapting previously published techniques, the aim of this work was to develop a standardized protocol to isolate enriched germ cell populations from testicular tissue of different mammalian species.

Methods: We collected fresh tissue of 5 representative mammalian species: mouse, rat, guinea-pig, dog and miniature pig. To overcome the need of species-specific adjustments during tissue dissociation, we used the Medimachine™ system to obtain single cell suspensions from testicular tissues by mechanical dissociation. These cell suspensions were stained with a combination of DNA dyes, Hoechst-33342 (Hoechst) and propidium iodide (PI), and processed by flow cytometry with a serial gating strategy that includes cell viability (PI negative) and DNA content (Hoechst intensity). Purity of the isolated cell populations was morphologically evaluated by microscopy.

Results: We show that testicular cell suspensions obtained by mechanical dissociation contain viable (measured by FACS) and intact single cells in various developmental stages (microscopy), indicating that this is a reliable method to dissociate testicular tissue of different mammalian species. Using our optimized FACS gating strategy we were able to isolate enriched populations of up to 6 germ cell types: spermatogonia (66%), primary (71%) and secondary (85%) spermatocytes, and spermatids (90%) - round (93%) and elongating (87%).

Conclusions: Execution of the entire workflow takes less than 2h, is straightforward and allows to simultaneously isolate 4 germ cell types. As the reduced processing time helps to preserve cell physiology and standardization eliminates methodological sources of variables, this method is ideal for downstream high-throughput comparative studies of male germ cell biology.

Poster #52

HORMONE INDUCED ACUTE STEROIDOGENESIS MEDIATES DYNAMIC RE-ORGANIZATION OF SUBCELLULAR MEMBRANE LIPIDS IN MA-10 MOUSE TUMOR LEYDIG CELLS.

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(Presented By: Sathvika Venugopal PhD)

Cholesterol, a unique lipid is an important component of all mammalian cell membranes. Cholesterol is also the precursor to all vertebrate steroid hormones. Acute steroidogenesis in MA-10 mouse tumor Leydig cells entails significant amounts of cholesterol trafficked from the plasma membrane to the mitochondria.

To analyze the compensatory mechanism used to balance the loss of the essential cholesterol in the plasma membrane and to understand the mechanism by which cholesterol is trafficked to the mitochondria from the plasma membrane, we isolated mitochondria, endoplasmic reticulum (ER), cytoplasm, plasma membrane (PM), PM-associated membranes (PAMs) and mitochondrial associated membranes (MAMs) from MA-10 cells in basal, hormone stimulated (treated for 2 hours with dibutyryl cAMP (dbcAMP), and steroidogenesis inhibited (treated with dbcAMP and cycloheximide) states. Lipids were isolated from each of these samples and subjected to lipidomic analyses by direct infusion (shotgun lipidomics) using electrospray ionization tandem mass spectrometry of major membrane lipid categories, which identified 2105 individual/isobaric species, including glycerophospholipids, lyso-glycerophospholipids, sphingolipids, cholesterol and its esters and ceramides. Each of the subcellular organelle membranes isolated had a unique lipid composition that significantly changed during induction of steroidogenesis by dbcAMP. As expected PM had the highest concentration of cholesterol content, followed by PAMs and MAMs. An increase in the amount of cholesterol was noted in the PAMs and ER after dbcAMP stimulation, indicating a route for cholesterol trafficking to the mitochondria. In addition, a drastic decrease in cholesterol ester levels was noted in the dbcAMP-stimulated cytoplasm, ER and whole cell lipid extracts when compared to basal rates, suggesting that a significant amount of cholesterol esters could be de-esterified and utilized for a replacement for the loss of cholesterol in PM. Increase in ceramide concentrations in the dbcAMP-stimulated PM and PAMs suggest its role in the signal transduction for induction of acute steroidogenesis.

The observed cAMP-induced dynamic lipid changes in MA-10 cell subcellular membrane lipidome suggest that hormone-induced acute steroidogenesis is a process that involves extensive organelle remodeling. This study is one of the first to analyze lipid reorganization during steroidogenesis. (Supported by CIHR grant FRN-148659 and a CRC).

Poster #53

INTRODUCTION OF TSPO GENE MUTATIONS IN MA-10 MOUSE LEYDIG TUMOR CELLS RESULT IN REDUCED STEROID HORMONE FORMATION

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(Presented By: Vassilios Papadopoulos, DPharm, PhD)

Translocator protein (18 kDa), TSPO, is an outer mitochondrial membrane protein shown to be involved in multiple biological functions, including mitochondrial cholesterol transport and steroid hormone biosynthesis. Although there is general agreement in the structure and pharmacology of TSPO and the impact of TSPO drug ligands on steroid production in gonads, adrenal and brain, recent studies of genetic deletion of Tspo in mice have provided conflicting data on the role of TSPO in steroidogenesis. Moreover, conflicting data have been generated in MA-10 mouse Leydig cells; knocking down TSPO expression using antisense oligonucleotides was shown to reduce the ability of the cells to form steroids, whereas CRISPR/Cas9-guided Tspo deletion was reported to have no effect on steroid synthesis.

We re-assessed the role of TSPO in steroidogenesis by introducing Tspo-specific mutations in MA-10 cells using CRISPR/Cas9. Experiments were performed using wild-type (WT) vs. Tspo-mutated cells (nG1) and a MA-10 sub-cell line Mito-H cells (generated by introducing reduction-oxidation sensitive green fluorescent protein roGFP) vs. cells with TSPO deficiency (G2G). Cells carrying a Tspo deletion (nG1 and G2G) were obtained via FACS of cells transfected with two guide RNAs designed specifically to target the exon2 of Tspo gene. The loss of TSPO was confirmed by RT-PCR, immunoblot analysis, confocal live cell imaging and immuofluorescence staining. TSPO mutant cells produced significantly lower progesterone than the corresponding WT cells; one failed to produce steroids in response to dbcAMP treatment and the other showed a 50% reduced response to dbcAMP. These changes correlated with disturbed neutral lipid homeostasis. The mitochondrial membrane potential ($\delta\psi m$) of the Tspo mutant cells was significantly reduced. Steroidogenic acute regulatory (STAR) protein levels were induced in Tspo mutant cells prior to and independently of dbcAMP simulation, suggesting an effort by the cells to compensate for the lack of TSPO or abnormal STAR mitochondrial import. Considering that $\delta\psi m$ is required for cAMP-stimulated steroidogenesis, these results (i) provide additional,

strong evidence for a role of TSPO in mitochondrial steroid biosynthesis, and (ii) suggest that TSPO or a TSPO-associated partner involved in δψm regulation is necessary for STAR action and import in steroidogenesis. (Supported by CIHR grants MOP-125983 and FRN-148659, and a CRC).

Poster #54

THE ANTIOXIDANT AND ANTI INFLAMMATORY EFFICACY OF POMEGRANATE AND CINNAMOMUM ZEYLANICUMON EXTRACT ON 1,7 DIMETHYLBENZANTHRACENE (DMBA) INDUCED APOPTOSIS AND SPERMATOGENESIS IN RATS' TESTES

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(Presented By: Arash Khaki, DVM, PhD)

Introduction: Pomegranate and cinnamon multifactorial properties such as antioxidant, anti-inflammatory and antidiabetic. The present study aims at examining the influence of combined pomegranate and cinnamon on spermatogenesis in DMBA-induced apoptosis in male Wistar rats

Materials and Methods: For this study, 70 male Wistar rats were randomly allocated into 7 groups of 10 animals each. Group 1 served as control and was given the basal diet and tap water without DMBA. Rats from Groups 3 to 7 assigned to receive 0.1nmol DMBA applicated on testis for 12 weeks. When the apoptogenesis process started, the rats of groups 3 and 4, received Pomegranate extract at a dose of 200 and 300 mg/kg/b.w, in group 5 Cinnamomum zeylanicum 75 mg/Kg/b.w and group6 and 7 Pomegranate 200 and 300 mg/kg/b.w and C. zeylanicum 75 mg/Kg/b.w, orally, respectively, 3 times per week. At the end of experiment, the rats were euthanized and the testis was removed. Histological evaluations for apoptosis were performed for testis.

Results: Sperm numbers, percentages of sperm viability and motility, and total serum testosterone increased in treated rats compared with DMBA group. Serum LH, FSH, anti-oxidants (TAC, SOD, GPX and catalase) all were increased at the end of treatment were higher compared to control group and also serum anti-oxidants (TAC, SOD, GPX and catalase) all were increased in compare to DMBA groups. Combined pomegranate and cinnamon showed more intense improvement in all parameters compare to pomegranate and cinnamon alone ($p<0.05$). Also apoptotic Sertoli cells was decreased significantly in all treated groups in comparison to DMBA group.

Conclusions: The present study thus demonstrated the anti-apoptotic potential of pomegranate and cinnamomum in DMBA induced testis apoptosis in rats

Keywords: Apoptosis, Testis, 1,7dimethylbenzanthracene, Pomegranate extract, Cinnamomum zeylanicumon, Rat.

Poster #55

SINGLE-CELL GENE EXPRESSION ANALYSIS REVEALS DIVERSITY AMONG HUMAN SPERMATOGONIA

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(Presented By: Stefan Schlatt)

Introduction: Spermatogonial stem cells (SSCs) can self-renew, as well as give rise to progenitor cells thereby maintaining spermatogenesis. Detailed analysis of individual spermatogonia in rodents has revealed an unexpected degree of heterogeneity among these cells.

Objectives: The aim of this study was to compare the expression profiles of individual human spermatogonia and to assess the potential of single-cell RNA-seq analysis for identifying novel SSC markers.

Methods: Human testicular biopsies with spermatogonial arrest (n=1) and qualitatively normal spermatogenesis (n=7) were selected to establish short-term cultures of human spermatogonia. On days 3-4, qPCR analyses of cell populations as well as single-cell gene expression analysis were performed following micromanipulation of spermatogonial clusters. Undifferentiated (OCT4, UTF1, MAGE A4), differentiating (DDX4, BOLL) as well as somatic marker genes (ACTA2, VIM) were analyzed. Additionally, two individual cells were selected for global transcriptional capture using shallow RNA-seq and subsequent immunohistochemical stainings were performed for candidate markers.

Results: Population analyses confirmed a significantly higher expression of the spermatogonial marker genes UTF1, FGFR3 and MAGE A4 in the germ cell enriched supernatant fraction. Single-cell expression data from the arrest patient (20 cells) showed distinct expression profiles of somatic cells and spermatogonia. Unexpectedly, spermatogonia had heterogeneous expression profiles. Also, from patients with normal spermatogenesis, heterogeneous expression patterns of undifferentiated (OCT4, UTF1 and MAGE A4) and differentiated marker genes (BOLL and PRM2) were obtained within each spermatogonia cluster (13 clusters with 85 cells). Shallow

RNA-seq analysis of individual human spermatogonia was validated, and a spermatogonia-specific heterogeneous protein expression of selected candidate markers (DDX5, TSPY1, EEF1A1 and NGN3) was demonstrated.
Conclusion: We conclude that single-cell expression analysis of human spermatogonia facilitates the identification of distinct spermatogonial subpopulations that were masked by traditional cell population analyses. While single-cell RNA-sequencing presents a powerful tool for the identification of novel spermatogonial markers, our data suggest that transcriptional heterogeneity among individual spermatogonia may be a hallmark of this cell population.

Poster #56
TRANSCRIPTION FACTOR USF1 IN THE MAINTENANCE OF SPERMATOGONIAL STEM CELLS
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(Presented By: Imrul Faisal Master of Science)

Introduction & Objectives: In mammalian testis, spermatogonial stem cells (SSCs) self-renew and differentiate into sperm under the tight control of autocrine and paracrine factors in cooperation with Sertoli and Leydig cells. While excess self-renewal of SSC is responsible for germ cell tumors, increased differentiation can lead to infertility and sterility. Thus, a balance of SSCs' self-renewal versus differentiation is essential for normal spermatogenesis.
Upstream stimulatory factor 1 (USF1) is a ubiquitously expressed transcription factor, and is reported to control transcription of multiple genes important in Sertoli cell differentiation, such as Fshr, Gata4, Shbg, Nr5a1 etc. However, no role for USF1 in the self-renewal or differentiation of SSCs, or paracrine regulation of spermatogenesis has been reported. According to our preliminary observations, Usf1-/- males are viable, sub fertile, have smaller testes. Thus, our data suggest that USF1 plays a significant role in spermatogenesis.
As USF1 is ubiquitously expressed and acts as a transcription factor for multitude of genes, it likely controls several factors important for the self-renewal and/or the differentiation of SSCs. Thus, this study characterizes intriguing role(s) of USF1 in spermatogenesis and fertility in vivo in Usf1-/- mice.
Methods: Spermatogenesis is characterized by morphology. Hormones involved in spermatogenesis are quantified by ELISA. Testicular metabolism are assessed by metabolomics profiling and pathway analysis. Transcriptional regulation in Usf1-/- testes are assessed by RNA-sequencing (RNA-Seq). High-throughput transcriptomics data are validated by RT-qPCR and RNA in situ hybridization. Protein levels are assessed by western blot, and/or immunofluorescence.
Results: According to my preliminary observations, Usf1-/- males are viable but sub fertile. Morphological analysis of Usf1-/- testes showed presence of several atrophic seminiferous tubules. Immunofluorescent staining for PLZF showed that SSCs are depleted with age in Usf1-/- testes. Serum FSH, LH, and testosterone are differentially regulated in Usf1-/- mice. Metabolites profiling in knockout mice testes suggests that multiple metabolic pathways are affected. In addition, transcriptome profiling by RNA-Seq reveals that Usf1-/- mice experience a global transcriptional misregulation in testis.
Conclusion: Our data strongly suggest that USF1 plays a critical role in mammalian spermatogenesis, especially self-renewal of SSCs.

Poster #57
CHARACTERIZING THE ROLE OF RETINOIC ACID IN THE NON-HUMAN PRIMATE TESTIS
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Washington State University
(Presented By: Angel Brooke Thalhofer, Graduate Student)

Retinoic acid (RA) is essential for germ cell development in the murine testis. In the absence of RA, germ cell development is halted at spermatogonial differentiation, commonly referred to as the A to A1 transition. This transition is marked by the co-expression of STRA8 and KIT, both RA responsive markers, and the expression of retinoic acid receptor gamma (RARG) in spermatogonia directly prior to their differentiation. However, whether or not an equivalent of the A to A1 transition exists in the primate testis and/or whether RA is involved is unknown. Attempts to analyze primate testis sections with our full-length murine STRA8 antibody yielded no positively stained cells, therefore we generated an antibody to the full-length human STRA8 recombinant protein (pSTRA8). Immunohistochemistry analysis using pSTRA8 on non-human primate testis cross sections revealed signal present in a sub population of Apale spermatogonia and preleptotene/leptotene spermatocytes. Our analysis also revealed that pSTRA8 is expressed in these cells types in specific stages along the length of the seminiferous tubules. These preliminary results imply that a subpopulation of primate spermatogonia may be RA responsive and that RA may play a role in primate spermatogonial differentiation. Further investigations will include co-localization of pSTRA8 with KIT, the RA receptors and other published markers of the Adark and Apale primate spermatogonial populations to assist with the accurate identification of whether an equivalent of the murine A to A1 transition occurs in the primate testis.

Poster #58

EFFECT OF EARLY TYPE 2 DIABETES ON MALE FERTILITY

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(Presented By: Jannette Dufour, PhD)

Diabetes is a major epidemic with the number of affected individuals increasing at unprecedented rates. In the United States, it is the 7th leading cause of death affecting 9.3% of the population. Type 2 diabetes (T2D), which traditionally appeared in individuals over 40, is now emerging in young adults. For instance, in Ohio and Arkansas, African-American children with T2D represent up to 70-75% of new pediatric diabetes cases. In males, diabetes is associated with infertility, low testosterone levels and altered testicular morphology. Since T2D is now affecting more people who are still within their reproductive years, it is important to learn if T2D in its early stages reduces an individual's ability to reproduce. Thus, we hypothesized that high blood glucose levels would affect cellular morphology and testosterone levels in the testis in an early type 2 diabetes mouse model. Male C57BL/6J mice were categorized into low fat diet (LFD) or high fat diet (HFD) groups. Mice in LFD or HFD were fed a diet containing 10 or 65% energy from fat, respectively, for 14 weeks. To determine impaired glucose tolerance and insulin resistance, intra peritoneal glucose tolerance and intra peritoneal insulin tolerance tests were performed, respectively. At the end of the study, body weight was measured and blood was collected. Testes and pancreas were collected for protein extraction and immunostaining. ELISA was performed to measure testicular and serum testosterone levels. Body weight was significantly higher in HFD mice compared to LFD. Additionally, HFD mice had impaired glucose tolerance and insulin resistance. In the Pancreas, total cellular insulin and islet cell morphology were not affected by the HFD, indicating an early diabetes model. Analysis of the testis tissue revealed that tubule diameter and Sertoli cells and Leydig cells were not different between LFD and HFD groups. No significant difference in the number of interstitial macrophages was detected. On the same note, testicular or serum testosterone levels were not different between the groups. Since no measurable differences in somatic cells, immune cells and testosterone levels were detected between HFD and LFD groups, it can be concluded that fertility is not affected during the early stages of T2D. This is promising for young adults who have early T2D or pre-diabetes. If they receive treatment early that prevents progression to chronic diabetes, their reproductive capabilities may not be affected.

Poster #59/Short Talk #7

ADCY2 IS A CANDIDATE GENE FOR THE DEVELOPMENT OF CONGENITAL GENITOURINARY ANOMALIES THROUGH PARTIAL DISRUPTION OF STEROIDOGENESIS

Marisol O'Neill MS and Dolores J. Lamb PhD
Baylor College of Medicine
(Presented By: Marisol Ann O'Neill, M.S.)

Introduction & Objectives: Genitourinary (GU) anomalies are among the most common types of birth defects yet their genetic causes are poorly understood. Using array comparative genome hybridization (aCGH), we identified Adenylyl Cylcase 2 (ADCY2) copy number variants (CNVs) in a patient with hypospadias and a patient with ambiguous genitalia. ADCY2 converts ATP into cAMP which is a necessary step in androgen production. We hypothesize that ADCY2 is a dosage-sensitive gene which regulates steroid synthesis during GU development.
Methods: The incidence of ADCY2 CNVs in a GU abnormal population was determined by aCGH and Taqman ADCY2 copy number assays on DNA from patients with GU anomalies. ADCY2 expression was localized in embryonic murine GU tracts by immunohistochemistry (IHC). The effects of Adcy2 copy number changes in Leydig cells were evaluated by overexpressing Adcy2 in murine Leydig cell lines TM3, MLTC-1, and MA-10. Changes in genes involved in steroidogenesis were quantified by qPCR. Steroidogenic acute regulatory protein (StAR) phosphorylation was quantified by immunoprecipitation and western blot. Localization and expression of the luteinizing hormone receptor was visualized by immunofluorescence.
Results: DNA from 262 patients with congenital GU anomalies was analyzed by Taqman CNV assay; five patients (1.9%) were identified with ADCY2 duplications; this is significantly higher ($p<0.001$) than the incidence of ADCY2 CNVs in the general population (0.16%). aCGH verified the CNV region. ADCY2 is highly expressed in embryonic murine Leydig cells, urethra, and bladder. The expression of ADCY2 in Leydig cells suggests a role in steroidogenesis. Overexpression of Adcy2 in murine Leydig cell lines resulted in a dysregulation of steroidogenesis. We observed a 40% decrease in StAR production ($p<0.05$) and phosphorylation, as well as qualitative downregulation of LHR. Mevalonate kinase (MVK), an LHR mRNA binding protein, was up-regulated by three fold in ADCY2 overexpressing cells.
Conclusion: We identified and enrichment of ADCY2 CNVs in patients with congenital GU anomalies. ADCY2 is highly expressed in Leydig cells during development and results suggest ADCY2 may contribute to the development of GU anomalies through down-regulation of the LHR in a cAMP dependent manner.
Funding: NIH grants T32DK007763 and R01DK078121 to DJL

Poster #60/Short Talk #3

CLONAL DEVELOPMENT OF SPERMATOGONIA IN RHESUS TESTES

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(Presented By: Adetunji Fayomi, DVM)

Introduction and Objectives: Undifferentiated spermatogonia in rodent testes are described by clone size (Asingle, Apaired, Aaligned) and molecular markers that they express. Spermatogonia in nonhuman primate (NHP) testes are described by nuclear morphology and intensity of staining with hematoxylin (Adark, Apale). There is limited information about how the dark and pale descriptions of nuclear morphology correlate with clone size or molecular markers in primates, which makes it difficult to compare rodent and primate data. The aim of this study is to learn molecular characteristics of Adark and Apale spermatogonia and use them as molecular markers to characterize stage-specific clonal development of undifferentiated and differentiating spermatogonia in the rhesus seminiferous epithelium.
Methods: We used colorimetric immunohistochemistry (IHC) to characterize UTF1, ENO2 and cKIT expression in Adark, Apale and B spermatogonia of the Rhesus testis. We performed IHC co-staining in section and whole mount to determine the extent of overlap between these markers and correlate their expression with clone size. 5-ethynyl-2'-deoxyuridine (EDU)-labeling was used to mark cells at S-phase and establish a tool for staging NHP seminiferous tubules in whole mount.
Results Obtained: We found that UTF1 and ENO2 are markers of Adark and Apale undifferentiated spermatogonia in the Rhesus tests. We also demonstrated that irrespective of the stage of seminiferous epithelium, most of the undifferentiated spermatogonia (UTF1+/cKIT- cells) exist as clones of 1, 2 or 4 cells. Clones of 4 cells were more prevalent in stage V, which coincide with the stage with the highest frequency of UTF1+/cKIT+ transitioning spermatogonia. UTF1+ spermatogonia rarely express cKIT during stages I-IV of the seminiferous epithelium. cKIT expression occurs mostly in Larger UTF1+ clones (2-4 cells). Highest frequency of EDU+/UTF1+ clones were observed in stages X-XI.
Conclusion: Similar to rodents, rhesus spermatogonia develop in interconnected clones of cells and increased clone size is associated with increased spermatogonial differentiation (cKIT+). Undifferentiated (UTF1+/cKIT-) spermatogonia were observed in clones of 1-4 cells and rarely in clones of 8, suggesting that Rhesus has fewer transit amplifying divisions in the pool of undifferentiated spermatogonia than rodents.
Supported by NIH grant R01 HD076412 and P01 HD075795

Poster #61

IDENTIFICATION OF GENE TARGETS OF A TRANSCRIPTION FACTOR THAT PROMOTES SPERMATOGONIAL STEM CELL ESTABLISHMENT

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(Presented By: Kun Tan, PhD)

Introduction & Objectives: The molecular mechanisms by which spermatogonial stem cells (SSCs) are initially established is poorly understood. We are in an optimal position to fill this gap, as we recently demonstrated that Rhox10—a member of the X-linked reproductive homeobox (Rhox) gene cluster—is critical for driving the differentiation of Pro-spermatogonia (ProSG) into SSCs. To understand the mechanisms underlying SSC establishment, we elected to identify RHOX10-regulated genes.
Methods: We identified genes regulated and bound by RHOX10 using a battery of approaches, including single-cell RNA sequencing (scRNAseq), luciferase reporter assay, mutagenesis, conventional ChIP, and ChIP-seq analyses.
Results: We performed scRNAseq analysis on Id4-eGfp+ cells isolated from testes at postnatal day 3, the time point when most of Id4-eGfp+ cells are SSCs and secondary transitional (T2) ProSG. To identify RHOX10-regulated genes, we compared genes differentially expressed in the SSC-enriched and T2-ProSG-enriched cell subsets (defined by cluster analysis) from Rhox10-KO and control mice. To identify candidate direct RHOX10-target genes that potentially promote SSCs establishment, we generated luciferase reporter constructs under the control of selected putative RHOX10-target gene promoters. This revealed that RHOX10 regulates the Oct4, Gfra1, Brachyury (T), and Tet1 promoters, which together have the potential to drive the expression of proteins critical for ProSG functions, including DNA demethylation, cell fate determination, and proliferation. To identify RHOX10 cis elements and determine whether RHOX10 binds to the promoters of these genes, we performed follow-up mutagenesis and ChIP analyses. To define RHOX10-binding sites genome-wide by ChIP-seq, we are currently in the process of generating knock-in mice that encode FLAG and HA tags at the C-terminus of the endogenous Rhox10 gene using the CRISPR/Cas9 approach.
Conclusions: We have identified RHOX10-regulated genes in specific undifferentiated spermatogonial cell subsets using single-cell genome-wide analysis and in vitro methods, including reporter analysis. The finding that RHOX10-regulated genes encode proteins critical for events that occur in ProSG—such as DNA methylation, cell migration, and differentiation—raises the possibility that studying RHOX10 will reveal insights into key steps in early gametogenesis.

Poster #62

KNOCKDOWN OF IFT140 MAY DISRUPT SPERMATOGENESIS BY DYSREGULATING THE NFKB SIGNALING PATHWAY

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(Presented By: Amin S. Herati, MD)

Introduction & Objective: A homozygous, six nucleotide deletion in exon 22 of the intraflagellar transport 140 (IFT140) gene in a consanguineous family of non-obstructive azoospermic brothers was identified using whole-exome sequencing. Significant changes in the expression of key genes in the NFkB pathway (decreased Fas and increased Bcl2) were present upon Ift140 knockdown in mouse male germ cells, suggesting the presence of an anti-apoptotic milieu. The objective of this study was to define the functional consequences of Ift140 knockdown on the NFkB pathway signaling genes.
Methods: Ift140 function was silenced in C18-4, a mouse spermatogonial cell line. Ift140 mRNA levels were evaluated using quantitative real-time PCR. Gene expression studies were performed in technical triplicates on an oligonucleotide array of 84 key genes related to NFkB-mediated signal transduction using Ift140 siRNA and a scrambled control. Changes in gene expression that were more than 1.5x up or down relative to control were considered dysregulated. Results were validated and analyzed for statistical significance using REST software (Qiagen). Knockdown and control cultures were treated with Sunitinib (2μM, 8 hours) to induce apoptosis, and a cell survival assay was performed. Two-way ANOVA was used to compare cell survival rates.
Results: Ift140 knockdown was 90.3% efficient in C18-4 cells at 72 hours. Twenty-four NFkB genes were down-regulated and eight genes were up-regulated. Dysregulated genes encoded 11 ligands and receptors upstream of the NFkB pathway, a member of the intermediate signaling complexes; a tyrosine kinase; and 6 pathway responsive genes that modulate the immune response and apoptosis. After Sunitinib treatment, 63.4% of Ift140-silenced cells survived 8 hours compared to 25.7% of scramble-control cells ($p<0.05$) underscoring the significance of increased expression of apoptosis-related NFkB signaling genes.
Conclusion: Statistically significant changes in the expression of key genes in the NFkB pathway occurred upon Ift140 knockdown. Dysregulated genes encoded inflammatory ligands and receptors that activate the NFkB pathway, an intermediate signaling complex member, tyrosine kinase, and pathway responsive genes that modulate the immune response and apoptosis. These changes suggest increased NFkB activity following Ift140 knockdown with negative feedback on activating ligand genes and increased expression of apoptosis related genes.

Poster #63

TESTIS HISTOLOGY IN MEN SUBMITTED TO MICROSURGICAL CORRECTION OF SUBCLINICAL VARICOCELE WITH LONG REFLUX AS A VARIABLE TO UNDERSTAND IMPROVEMENT IN SPERM QUALITY POST-TREATMENT

Jorge Hallak MD, PhD[1],[2],[3],[4], Robertson T Dutra MSc[1],[2],[3] and Juliana R Pariz MSc, PhD student[1],[2],[3],[4]
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(Presented By: Jorge Hallak, MD, PhD)

Introduction: Subclinical varicocele with long reflux can damage spermatogenesis and cause sperm abnormalities. The challenge in subclinical varicocele approach is the identification of subjects who will benefit from surgical treatment, since many patients do not show improvement in semen analysis.
Objective: To identify testicular histological pattern as prognostic value of improved reproductive capacity in patients with subclinical varicocele submitted to microsurgical correction.
Methods: We retrospectively analyzed testicular biopsies of 20 men diagnosed with subclinical varicocele. The diagnosis of subclinical varicocele was carried out through bilateral testicular palpation, auscultation of long and continuous venous reflux by Doppler stethoscope with and without Valsalva maneuver and confirmation by color Doppler sonography. The determination of the testicular histological pattern was performed by the creation of cut-off values that associate Johnsen scores, Copenhagen index and testicular volume with improvement in semen parameters. To determine cut-off points of predict fertility improvement, Receiver Operating Characteristic (ROC) curves were created combining histological scores, hormones and semen parameters.
Results: Johnsen score must be >8.2 (left testicle) and right testicular volume >12.8 mL to improve sperm concentration. Johnsen score must be >8.2 (bilateral testis) and Copenhagen index (digit II) must be <2.5 in both testis to total sperm motility improvement. To increase progressive motility, Johnsen score must be >9.1 bilaterally and right testicular volume >13.6 mL.
Conclusion: We could determine parameters to evaluate which patients can benefit from surgical treatment of subclinical varicoceles with very long reflux.
Financial support: Androscience/Capes-DS
Ethics Committee Approval: FMUSP n°047/2012

Poster #64

THE EFFECT OF GROWTH FACTORS ON IN VITRO CULTURE OF PREPUBERTAL TESTICULAR CELLS IN DOMESTIC CATS – PRELIMINARY RESULTS

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Presented By: Erika Oliveira, PhD

The ability to spur growth of early stage germ cells could be a valuable approach for preserving the male germ plasm of wild felids. Furthermore, several studies have demonstrated the value of domestic cats as a model for understanding human genetic and reproductive disorders. The addition of specific growth factors (GF) is required for the successful establishment of in vitro culture and expansion of feline spermatogenesis. Therefore, the present study was designed to test the influence of GDNF and RA on Sertoli and germ cell survival in in vitro cultured prepubertal cat testes. Seminiferous cords (SeC) from testes of seven immature kittens (2-4 months old) were mechanically dissociated from each other and from the interstitial tissue. SeC fragments were cultured in Dulbecco´s modified Eagle medium high glucose (DMEM), supplemented with gonadotropins, testosterone and different concentrations of GDNF (10 or 50ng/mL) and/or RA (1 or 5µM) in 24-well plate at 34°C under 5% CO2. All experiments were performed in triplicate. Control media did not contain GF. After 4wks of culture, SeC were fixed in 4% (w/v) paraformaldehyde, and processed for histological evaluation. Morphometric analyses also were performed to evaluate the viability of the SeC. Percentages of tunica propria (TP), Sertoli cells (SC), germ cells (GC), vacuoles/lume (V) and necrotic/degenerative SeC (D) were defined from the ~2500 points counted per animal. Statistical analysis was performed by ANOVA. The higher percentage of TP was observed at DMEM+1RA (8.8±3.7%) than the control (5.4±2.8%, p=0.005). In vitro culture in the presence of DMEM+1RA+50GDNF resulted in the higher proportion of SeC with SC (65.2±9.7%) and lower degeneration (17±15.2%) compared to the control (32.3±46.4, p=0.001 and 59.3±31.4, p=0.001; respectively). Higher percentages of GC were observed in SeC cultured in DMEM+1RA+10GDNF (2.6±1.6%) and DMEM+1RA+50GDNF (1.6±1.1%), than the control (0.8±0.8%, p=0.016 and p=0.260, respectively). Our results suggest that the combination of low RA with low/high GDNF may be suitable for maintenance and expansion of germ cells of prepubertal cats.

Key words: GDNF, Retinoic acid, Spermatogenesis, in vitro culture, Felide.

Financial support: CAPES

Poster #65

IN VITRO CULTURE OF SEMINIFEROUS CORDS OF DOMESTIC CATS – PRELIMINARY RESULTS

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Presented By: Erika Oliveira, PhD

The ability to spur growth of early stage gametic cells recovered from pre-pubertal animals could lead to significant advances in rescuing the genomes of rare genotypes or endangered species. The aim of this study was to determine the ability of two different culture media, Dulbecco modified Eagle's medium (DMEM) or Minimal essential medium (aMEM), with stimulation by gonadotropins, and two different in vitro conditions, agar block (AB) or a three dimensional agar culture system (SACS), in preserving seminiferous cords (SeC) from testes of 2-4 months old kittens cultured for 7 or 14d, at 34°C under 5% CO2 and performed in triplicates. SeC were fixed in 4% (w/v) paraformaldehyde solution, and processed for histological evaluation. Histological evaluation and a score count based on Johnsen's spermatogenesis criteria (1970) were used, and analyzed by a one-way ANOVA. Evaluation of fresh tissue exhibited 74±9% of normal SeC. Cultures performed for 7d in AB in both DMEM and aMEM were better ($P<0.05$) preserved SeC structure (48±23% and 40±21%, respectively) when compared to SACS cultures (15±20% and 28±4%, respectively).The evaluation of the remaining SeC revealed immature Sertoli cells and Sertoli cell vacuolization, thickeness of tunica propria and germ cells in process of necrosis characterized by acidophilic cytoplasm and pycnotic nuclei. Cultures performed for 14d in AB/DMEM system still preserved the structure of SeC (when compared to fresh control and 7d) and approximately 31±33% of the cords showed a normal morphology. Interestingly, the SeC kept in the SACS/DMEM system for 14d presented 2-fold the percentage of normal cords when compared to the same condition for 7d. A decrease of the percentage of normal SeC was observed in AB/aMEM and SACS/aMEM cultures (18± 22% and 14±15%, respectively). Hence, our preliminary results show the composition of the media and not the in vitro culture conditions had a direct influence on the preservation of seminiferous cords of kittens during 14d of in vitro culture. Thus, for practical purposes, we suggest the use of AB and DMEM for in vitro culture of prepubertal testis of domestic cats.

Key words: Feline, Testis, DMEM, SACS, Spermatogenesis

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior (Capes)

Poster #66

HISTONE H4 HYPERACETYLATION IS AN UNEXPECTEDLY EARLY EVENT IN EQUINE SPERMIOGENESIS

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(Presented By: Ralph G. Meyer PhD)

Sperm chromatin quality is important for successful fertilization and embryonic development.
Postmeiotic chromatin remodeling with its characteristic exchange of nucleosomes for transition proteins and protamines is key to proper sperm chromatin maturation and genetic integrity of healthy sperm. The presence of DNA strand breaks and incomplete sperm chromatin remodeling with excessive histone retention and a lack of protamine deposition are signs of poor sperm quality that have been associated with embryonic failure. We hypothesize that suboptimal sperm quality in stallions may be used as a model for certain aspects of human male fertility research. Because chromatin remodeling in spermatogenic cells has not yet been characterized in depth in stallions, we studied relevant key events in equine spermiogenesis using immunofluorescence imaging of histological testis sections. To this end, the expression and fates of testis-specific histone TH2B, transition protein 1 (TP1) and H2AZ were characterized along with the phosphorylation of H2AX and acetylation of histone H4. Acetylation of histone H4 in lysine residues K5, K8, and K12 (hyperacetylation) at the onset of spermatid elongation is a presumed hallmark of nucleosome removal at the onset of nuclear condensation in elongating spermatids in humans and mice. H4 hyperacetylation is recognized by the bromodomain protein Brdt which is involved in the removal of H4 from spermatid chromatin. Unexpectedly, H4 is already acetylated in all of these positions almost immediately after meiotic division in round spermatids in the stallion, in absence of TP1 deposition. We detected additional H4K16 acetylation during a brief phase of spermatid elongation in both, mice and stallions, concomitant with H2AX phosphorylation, which is an indicator of DNA strand break- mediated DNA relaxation. These results suggest that in horses H4 (hyper-) acetylation in positions K5, K8 and K12 does not immediately result in nucleosome replacement for transition proteins as would be suggested by findings in other species. The DNA repair-dependent H4K16 acetylation, which occurs during spermatid head elongation in most species, may therefore be more important for nucleosome replacement in mammalian spermiogenesis than previously thought. In summary, this study provides an initial characterization of equine nucleoprotein exchange in spermiogenesis and shows unexpected differences in the timing of H4 hyperacetylation compared to other species.

Poster #67

THE IMPACT OF GRAND PATERNAL AGING ON SPERM DNA METHYLATION PATTERNS

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(Presented By: Tim Jenkins, PhD)

Introduction: Aging has been shown in multiple tissues to affect epigenetic signatures. Interestingly in sperm, DNA methylation profiles are significantly altered as an individual ages and these alterations appear to located at sites associated with diseases with increased incidence in the offspring of older fathers. In some cases the increased incidence of disease can even occur in the grand offspring of older fathers. This study is designed to assess the impact and transmission of epigenetic marks over multiple generations in human sperm as this represents a potential mechanism to explain the increased incidence of disease in offspring and grand offspring of older fathers.
Materials and methods: Sperm DNA from 32 individuals were assessed in this study. All samples were selected based on grand paternal age difference, where both the individual's age and paternal age were held constant. Two groups were assessed, young grandpaternal age (YGPA; <25 years old) or old grandpaternal age (OGPA; >40 years of age). Sperm DNA methylation was assessed via EPIC methylation array. We performed differential methylation analysis at the global, regional, single CpG level, and at all regions previously shown to undergo age-assocaited methylation changes.
Results – Our results indicate that sperm DNA methylation is similar between groups. In fact, no differential methylation analysis yielded any significant results between these two groups. However, one finding was quite striking. When considering only regions where age-associated decreases in sperm DNA methylation occurs, we found similar patterns in the OGPA group. Specifically, just over 80% of all sites known to have age associated decreases in methylation were also hypomethylated in individuals with older paternal grandfathers.
Conclusions: Our data represent the first assessment of the impact of male age on grand offspring methylation signatures. In this study we found no dramatic shift in sperm methylation signatures associated with advanced grandpaternal age. However, we did note a trend toward lower methylation at regions known to experience age associated demethylation in the grand offspring of individuals whose paternal grandfather sired offspring at an older age. While this finding was not significant, it does appear to be suggestive and does not rule out the possibility that some methylation signatures may be propagated across generations either directly or through some unknown mechanism.

Poster #68

GONADOTROPIN INDEPENDENT ANDROGEN SYNTHESIS IN THE HUMAN PREPUBERTAL TESTIS: BREAKING THE DOGMA

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(Presented By: Esperanza Berensztein, PhD)

In contrast with the well documented gonadotropin stimulated testicular androgen synthesis, in humans, prepubertal (Pp) intratesticular testosterone has been reported (Rivarola 1989) but not elucidated. It has been proposed that the insulin-like growth factor family could provide essential signals for testis development (Griffeth 2014).
The aim of this study was to evaluate the expression of steroidogenic enzymes and androgen receptor (AR) in human Pp testes, and analyze a possible relation with the IGF family.
Eighteen Pp testes, confirmed by histology analysis, were collected at necropsy from 18 subjects without endocrine or metabolic diseases, and divided in 2 groups: Infancy n=8 (median age 1.3 mo, range 2 days-7 mo), and Childhood n=10 (median age 6 years, range 1-9 y). Protein expression of AR was analyzed by IHC, and of HSD3B2 and CYP11A1 by WB. The mRNA expression of the front and backdoor steroidogenic pathways (CYP17A1, HSD3B2, SRD5A1, SRD5A2, AKR1C1, AKR1C2, AKR1C3, AKR1C4) and the IGF family (IGF1, IGF1R, INSR) was assessed by RTqPCR.
Every sample expressed AR in peritubular and interstitial cells, including the Leydig cells present in neonatal and minipubertal samples. Sertoli cells were negative for AR in Infancy, by its expression increased gradually throughout Childhood starting at 3 years of age.
HSD3B2 and CYP11A1 protein expression was observed in all samples. SRD5A2 and AKR1C4 mRNAs were not detected in any sample while all other analyzed genes were expressed in every tissue.
CYP17A1 mRNA expression was significantly higher in Infancy than in Childhood ($p<0.01$), while IGF1R, SRD5A1 and AKR1C3 were significantly higher in Childhood ($p<0.05$).
Multiple correlation studies revealed in Infancy a strong negative correlation between IGF1 and CYP17A1 expression and a positive correlation between the enzyme AKR1C3 and both AKR1C1 and AKR1C2. In Childhood, a positive correlation with age was found for CYP17A1 as well as for AKR1C3. Both enzymes SRD5A1 and AKR1C1 correlated positively with AKR1C3 and AKR1C2. A negative correlation was obtained between both receptors. IGF1R correlated positively with CYP17A1 while INSR negatively with CYP17A1 and AKR1C3.
To our knowledge, we are the first to report the Pp testicular expression of genes involved in androgen synthesis and its gonadotropin independent increase throughout childhood. Also, our results hint to a possible role of the IGFs in the testis steroidogenic maturation previous to central puberty onset.

Poster #69

FEWER AND DYSFUNCTIONAL FETAL LEYDIG CELLS PRODUCE LESS TESTOSTERONE AND CAUSE DELAYED TESTIS DESCENT AND ABNORMAL EXTERNAL GENITALIA IN GLI3XTJ MUTANT MICE

Jessica L. Muszynski[1], Samantha R. Lewis[1], Anna E. Baines[1], Stephanie L. Winske[1], Chad M. Vezina[1], Elena M. Kaftanovskaya [2], Alexander Agoulnik[2], Martin J. Cohn[3], and Joan S. Jorgensen[1]
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(Presented By: Joan S. Jorgensen, DVM/PhD)

In humans, Greig cephalopolysyndactyly syndrome (GCPS) is an autosomal dominant disorder linked to craniofacial and limb development, but some male patients are also born exhibiting defects in sex differentiation including cryptorchidism and/or hypospadias. GCPS is caused by a mutation in a Hedgehog pathway mediator, GLI3 that results in a non-functional protein and a phenotype with complete penetrance, but with variable severity across tissues. Whereas it is established that Desert Hedgehog (DHH) signaling via Smo is essential for the onset of androgen synthesis and normal male sex differentiation, elimination of individual downstream mediators, GLI1 or GLI2, had no effect. GLI3 has not been tested; therefore, we hypothesized that GLI3 is required for DHH-mediated androgen synthesis and male sex differentiation. To test this hypothesis, we used the Gli3XtJ mouse model, which faithfully models GCPS. Reminiscent of the variability in GPCS phenotypes, our results showed unpredictable timing for testis descent in all Gli3XtJ mutants due to failed disintegration of the cranial suspensory ligament, and abnormal external genitalia phenotypes with varying severity. While transcript and immunohistochemical analyses indicated that germ, Sertoli, and peritubular myoid cells of Gli3XtJ testes were not different from wild type, fetal Leydig cell numbers were significantly decreased. Further, after correction for cell number, transcript levels specific to fetal Leydig cell function, including DHH mediators, Sf1, and steroidogenic enzymes were significantly lower in Gli3XtJ testes. Diminished fetal Leydig cell number and function culminated in variable, but significantly less testosterone production. Thus, we conclude that variances in the severity of male sex differentiation in Gli3XtJ embryos are caused by disparities in fewer fetal Leydig cells to produce androgens. These findings are important because they

represent a genetic component to fluctuant fetal Leydig cell numbers and function that suggests potential thresholds in androgen production requirements for normal differentiation of each male characteristic. Advanced understanding of the concentrations of androgen required for male sex differentiation would impact our ability to discover potential genetic and/or environmental interactions that are associated with the troubling increase in incidence in male birth defects within industrialized nations.
Supported by NIH R01-HD075079 and the University of Wisconsin-Madison

Poster #70
MUTATION OF THE OUABAIN BINDING SITE OF NA,K-ATPase a4 DOES NOT AFFECT SPERM FERTILITY
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(Presented By: Victor Gustavo Blanco, MD, PhD)

Two isoforms of the Na+ and K+ transporter Na,K-ATPase (NKA) are expressed in sperm, the widely distributed NKAα1 and the testis specific NKAα4. NKAα4 is critical for sperm motility and fertility and is characterized by having an affinity for the endogenous NKA regulator ouabain, which is approximately one thousand fold higher than that of NKAα1. The functional relevance of this intriguing property of NKAα4 and its importance for sperm function is unknown. To determine this, we engineering a mouse in which the ouabain binding site of NKAα4 was mutated to make it ouabain resistant (NKAα4-OR). This was achieved by exchanging amino acids H and N in the first extracellular loop of NKAα4 with the corresponding residues (D and R) in NKAα1, which gives NKAα1 its low ouabain sensitivity. NKAα4-OR mice were overall normal and completely fertile with litter intervals, number and size indistinguishable from those of wild type mice. Sperm from the NKAα4-OR mice expressed levels and activity of the mutant NKAα4 that were similar to those of wild type mouse sperm. Moreover, sperm from NKAα4-OR exhibited total motility, as well as different parameters of sperm motility that were all normal. Different from wild type sperm, sperm from NKAα4-OR mice showed that NKAα4 activity was insensitive to 1 μM ouabain, an amount of ouabain that completely inhibits the activity of wild type NKAα4. Also, different from wild type sperm, flagellar beat of NKAα4-OR sperm was insensitive to 1 μM ouabain. Not only total, but all parameters of sperm motility, including sperm hyperactivation, were unaffected by 1 μM ouabain in sperm from NKAα4-OR mice. In addition, sperm from NKAα4-OR mice exhibited practically no high affinity binding of the fluorescent ouabain derivative, bodipy ouabain, which is typical of NKAα4. These results show that modifying the high ouabain affinity of NKAα4 does not impair sperm function and that the high ouabain affinity site of NKAα4 is not an absolute requirement for sperm fertility.
[Supported by NIH grant U01HD080423].

Poster #71
SEASONAL CHANGES IN THE TESTICULAR ACTIVITY OF THE BRAZILIAN RATTLESNAKE CROTALUS DURISSUS (LINNEAUS, 1758)
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[1]UFMG; [2]FUNED
(Presented By: Gleide Fernandes Avelar, PhD)

The Reptilia class consists of almost 10.000 of species and despite this large number, the reproductive biology of most of them is unknown. The reproductive patterns of snake species from the neotropical region are poorly studied and morphological approaches are scarce in comparison to the temperate region. Crotalus durissus is a neotropical snake from the Viperidae family, which is widely distributed in the Americas. This study was based on the dissection of 60 males of C. durissus from Minas Gerais state, Brazil, collected along the four seasons of the year. The specimens were pre-anesthetized with dry ice, weighed and euthanized with an overdose of thiopental (100 mg/kg) via intra-cardiac. Testes were removed, weighed and the gonanadosomatic index (GSI) was obtained. Fragments were fixed by immersion in 5% buffered glutaraldehyde and dehydrated in a sequence of alcohol and embedded in glycol methacrilate for histological evaluation. The preliminary results indicate that the testes of the rattlesnake are composed for tubular and intertubular compartment. Seminiferous tubules were composed by germ cells in different steps of development and supporting Sertoli cells (SC) arranged in a consistent pattern between the basal lamina and the lumen. Similar to freshwater turtle, birds, humans and other primate species, several stages were observed per seminiferous tubule cross-section. The highest values of the GSI were obtained for those specimens collected during the spring and the summer. In the fall there was a decrease in GSI, and the lowest values for this parameter were found in the winter, seasons correlated to testicular regression. SC, spermatogonia, and occasionally spermatocytes and remaining spermatids were observed in the regressed testes. The spermatogenesis recovering takes place in the spring. SC, spermatogonia, primary and secondary spermatocytes, two generations of spermatids (round and elongated), including spermatozoa inside the lumen were recognized. During the summer, the spermatogenesis reached the peak of gamete production. Leydig cells, connective tissue cells, blood and lymph vessels were observed in the intertubular compartment. Taken together, our preliminary results indicate a striking seasonal influence over spermatogenesis process of C. durissus. However, further studies are being developed in order to better characterize the spermatogenic cycle, to determine the spermatogenic cycle length and spermatogenic efficiency as well.

Poster #72

THE FUNCTION OF EPIDIDYMAL CYSTEINE-RICH SECRETORY PROTEINS (CRISPS)

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(Presented By: Jinghua Hu)

It has been demonstrated that epididymal CRISP1 and CRISP4 are individually required for full sperm function, but mice null for either gene remain fertile, suggesting that there may be redundancy between the two genes. In contrast to mice, humans possess a single epididymal CRISP. In order to define the function of CRISPs in epididymis as a family, and thus their likely relevance to human fertility, we produced Crisp1/Crisp4 double knockout mouse model to study the influence of epididymal CRISPs absence on male fertility. Crisp1/Crisp4 homozygous null males are fertile, however, computer assisted sperm analysis (CASA) revealed that epididymal CRISPs are necessary for sperm to acquire the capacity for rapidly progressive motility, and a normal ability to undergo the progesterone-induced acrosome reaction in vitro and thus, likely normal acrosome reaction ability in vivo. Further, we have shown that with increased age, 23 weeks, double knockout mice epididymides contained significantly reduced numbers of sperm compared to their wild type counterparts. This, and an association between CRISP-related proteins and immune tolerance in lower order species, suggests that epididymal CRISPs may function to protect sperm against immune-mediated sperm elimination in epididymis. Ongoing studies are dissecting the relationship between epididymal CRISPs and immune regulation.

Poster #73

HIGH-RESOLUTION PHENOTYPING OF SPERMATOGENIC DEFECTS USING SINGLE-CELL SEQUENCING

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(Presented By: Min Jung)

Introduction: RNA sequencing of testis tissue provides great promise for improving the description of molecular defects in men with gonadal dysfunction. With thoughtful interpretation, gene expression data could be useful for classifying patients into a diagnostic hierarchy, defining i) disrupted cell types, ii) disrupted pathways, and, iii) in conjunction with genome sequencing data, disrupted gene(s) and causal genetic variants. However, the cellular diversity of testis severely limits the utility of expression measurements made on bulk tissue. Thus, the application of single-cell RNA-sequencing on male germ cells represents an amazing new set of scientific opportunities for research in male reproductive biology and translational medicine
Objectives: We aim to develop a single-cell framework that utilizes large-scale single-cell expression profiles from normal and disease models to elucidate the fundamentals of spermatogenesis and to phenotype spermatogenic defects.
Methods: Using Drop-seq, we generated single-cell expression measurements on over 30,000 cells from wild-type mice and over 20,000 cells from mouse mutants with infertility of mechanisms known (e.g. MLH3 deficiency) and unknown (spontaneous infertility after a transgene insertion). We constructed a pipeline for interpreting the single-cell data using publicly available single-cell computational tools such as RaceID2, Waterfall, and Monocle2, and an in-house cell-type assigner algorithm.
Results: We find that the increased resolution of single cell expression profiling reveals novel cell-type specific markers, 5 of which we have confirmed with immunofluorescence staining. As part of the framework, we developed a cell-type identifier that provides automated assignments of cells to 4 stages of germ cells and 3 types of somatic cells. Our cell-type assigner algorithm has 96% accuracy when benchmarked with data from flow-sorted germ cells. Finally, unsupervised ordering of male germs on a developmental timescale depicts the genetic landscape of both normal and infertile mice, inferring the cell-types and pathways that are dysregulated in the germ cell development of disease models.
Conclusions: Our single-cell framework has great potential for expanding our ability to dissect pathophysiology in tissues with extensive cellular heterogeneity and decrypt the spermatogenic failure in more patients.

Poster #74

THE INTACT PIRNA PATHWAY PREVENTS L1 MOBILIZATION IN MALE MEIOSIS

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(Presented By: Wenfeng An, PhD)

The PIWI-interacting RNA (piRNA) pathway is essential for retrotransposon silencing. In piRNA-deficient mice, L1 overexpressing male germ cells exhibit excessive DNA damage and meiotic defects. It remains unknown whether L1 expression simply highlights piRNA deficiency or actually drives the germ cell demise. Specifically, the sheer abundance of genomic L1 copies prevents reliable quantification of new insertions. Here, we developed a codon-optimized L1 transgene that is controlled by the endogenous mouse L1 promoter. Importantly, DNA methylation dynamics of a single-copy transgene were indistinguishable from those of endogenous L1s.

Analysis of Mov10l1-/- testes established that de novo methylation of the L1 transgene required the intact piRNA pathway. Consistent with loss of DNA methylation and programmed reduction of H3K9me2 at meiotic onset, the transgene showed >1200-fold increase in RNA expression and consequently >300-fold increase in retrotransposition in postnatal day 14 Mov10l1-/- germ cells compared to the wild type. Analysis of adult Mov10l1-/- germ cell fractions indicated a stage-specific increase of retrotransposition in the early meiotic prophase. However, extrapolation of the transgene data to endogenous L1s suggests that it is unlikely insertional mutagenesis alone accounts for the Mov10l1-/- phenotype. Indeed, pharmacological inhibition of reverse transcription did not rescue the meiotic defect. Cumulatively, these results establish the occurrence of productive L1 mobilization in the absence of an intact piRNA pathway but leave open the possibility of processes preceding L1 integration in triggering meiotic checkpoints and germ cell death. Additionally, our data suggest that many heritable L1 insertions originate from individuals with partially compromised piRNA defense.

Poster #75

FX-MIR: A TESTES-EXPRESSED MICRORNA CLUSTER TARGETING FMR1 AND KEY SPERMATOGENESIS GENES

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(Presented By: Hye-Won Song, PhD)

Introduction & Objective: Triplet-repeat expansions in the promoter of the Fragile-X gene, FMR1, cause Fragile-X Syndrome (FXS), the most common form of inherited human intellectual disability. FXS patients also exhibit macroorchidism and spermatogenic defects, including Sertoli cell (SC) expansion. In our investigation of regulatory mechanisms that act on FMR1 in SCs, we uncovered a testes-expressed microRNA (miRNA) cluster directly adjacent to FMR1 that has a strong predilection for targeting FMR1.
Methods: miRNA targets were identified using prediction programs and confirmed by mutagenesis of predicted target sites and luciferase reporter analysis. miRNA levels were quantified by Taqman.
Results: In a screen for miRNAs targeting mouse Fmr1, we discovered that it is regulated by many members of a miRNA cluster directly adjacent to the Fmr1. This was unexpected given that miRNAs act in trans, not in cis. By virtue of its location in the Fragile X region, we named this the "Fx-miR" cluster. Analysis of its expression revealed that the miRNAs in this cluster are primarily expressed in SCs in a temporally regulated manner. Comparison with all genes preferentially expressed in SCs (defined by our in vivo RiboTag analysis) revealed that the members of the Fx-miR cluster target Fmr1 more than any other SC-expressed gene. This predilection for targeting FMR1 is conserved, based on our analysis of human FX-MIR cluster miRNAs target human FMR1. Intriguingly, many members of the Fx-miR cluster also downregulate the mRNAs encoding eIF4E and CYFIP1, which together with the protein product of Fmr1, FMRP, form a translation regulatory complex. The Fx-miR cluster also downregulates other SC-expressed genes critical for spermatogenesis, including key post-transcriptional regulators (Ago2 and Upf2, which function in miRNA biogenesis and NMD, respectively), as well as Vegfa and the androgen receptor (Ar).
Conclusions: We have defined a large X-linked miRNA cluster expressed in SCs—Fx-miR—that has a predilection for regulating the neighboring gene – Fmr1. This has important clinical implications given that the protein encoded by Fmr1—FMRP—is a translational repressor critical for SC proliferation and normal spermatogenesis. The Fx-miR cluster also targets other key post-transcriptional regulators that function in SCs, as well as factors with other critical roles in SCs, including AR.

Poster #76

MULTIPLE GENOMIC MUTATIONS CONTRIBUTE TO MALE INFERTILITY IN MENDELIAN AND NON-MENDELIAN FASHION.

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(Presented By: Alexander Yatsenko, MD, PhD)

Infertility is a common disorder that affects nearly 15% of American couples, yet male factor contribution is mostly unknown; vast majority of men receive a descriptive diagnosis of idiopathic infertility (unknown etiology). Current clinical genetic testing is informative for small fraction of infertile males only. To improve this situation we examine comprehensively the contribution of multiple genomic aberrations and mutations in all known coding genes. To carry out the study we apply a combination of 2 powerful genomic techniques available to date, high-resolution genome-wide comparative genomic hybridization (CGH) microarray and massive sequencing that capture RefSeq coding genes, whole exome sequencing (WES). For the study we assemble 110 sporadic cases with male infertility; 60 males are diagnosed with non-obstructive azoospermia and 50 males have severe oligozoospermia. To rule out known causes of infertility we test patients for chromosome aberrations and AZFs. To identify genomic aberrations we utilize 400K whole-genome CGH array (Agilent). To detect coding mutations we perform WES with SureSelectV6 capture library (Agilent). We analyze genomic data using classical Mendelian and complex models. We identified novel heterozygous and hemizygous aberrations and point mutations in 9 patients and 12 infertile males, respectively. The findings constitute nearly 20% of the study patients. These genes include CIRBP, KISS1R, PRSS55, PTCHD3, SYCE1, SERPINE2, TAF7L, and TEX11. Most mutations we found in a set of male infertility genes. We build the set using testis-specific meiotic and post-meiotic expression, transgenic animal,

functional, and human studies data. Some of our findings are consistent with autosomal recessive and X-linked models. Interestingly, we found several instances of non-Mendelian inheritance of male infertility; we noticed combinations of multiple gene mutations in same infertile male and detected heterozygous aberrations in second gene in addition to found point mutation(s) in first gene. To confirm genetic cause of the top mutations we initiated functional knock-out and knock-in studies in Drosophila melanogaster (fruit fly). This approach is highly efficient and cost-effective in uncovering function for dozens of novel gene candidates and examine potential mutation effect for dozen of found genomic variants. To verify initial findings we perform functional and replication studies in independent infertile male population studies.

Poster #77

EXRESSION OF TWO NANOS SEQUENCES IN THE TESTIS OF A LITTLE SHARK, SCYLIORHINUS CANICULA

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(Presented By: Laura Gribouval, PhD Student)

Scyliorhinus canicula, a chondrichthyan fish, presents a huge interest in testis studies because of its phylogenic position at the base of Vertebrates, and its polarized testis, which lets an easy access to the different germinal stages of spermatogenesis. Previous studies have highlighted different spermatogonial cell types, including potential SSCs (Spermatogonial Stem Cells) in the testicular germinal niche (Loppion et al., 2008; Bosseboeuf et al., 2014). Morphological and preliminary molecular analyses have revealed the presence of large round cells isolated in a dense somatic tissue, that express Gfrα1, assuming a stem potential. However, these results must be confirmed by the detection of additional SSC markers.

Nanos proteins are well known for their implication in the germline cell development and the maintenance of adult mouse spermatogonial stem cells self−renewal. Nanos are highly conserved ARN−binding proteins and key regulatory proteins involved in the translational control. In several mammals and teleostean fishes, three Nanos paralogues have been identified whereas only two were found in birds. Nanos1 has been shown to prevent apoptosis in primordial germ cells in Xenopus. In mice, Nanos2 suppresses meiosis and promotes male germ cell differentiation, and Nanos3 maintains the germ cell lineage.

In S. canicula, in silico analysis revealed the presence of two nanos transcripts. Phylogenic approaches showed a structural conservation of the two zinc finger motifs specific to Nanos family and a segregation of both forms with the Nanos1 protein. Nanos1a was closer to tetrapod and chondrichtyan sequences whereas Nanos1b was closer to teleostean fishes sequences. Nanos cellular localization was determined by immunohistochemistry on testis paraffin sections and revealed a specific germinal cell labelling. These results were also confirm by in situ hybridization. RT−PCR analyses revealed a strong expression of Nanos1b in the testicular niche, this expression progressively decreased in the area with cysts containing spermatogonia and at more advanced stages of spermatogenesis.

In our laboratory, a culture protocol has been designed to maintain shark potential SSCs. First immunocytochemistry results confirmed the expression of Nanos in our colonies. It is therefore envisaged to transplant these cells in early embryos in order to confirm their stemness by determining their capacity to colonize a host gonad and to differentiate.

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