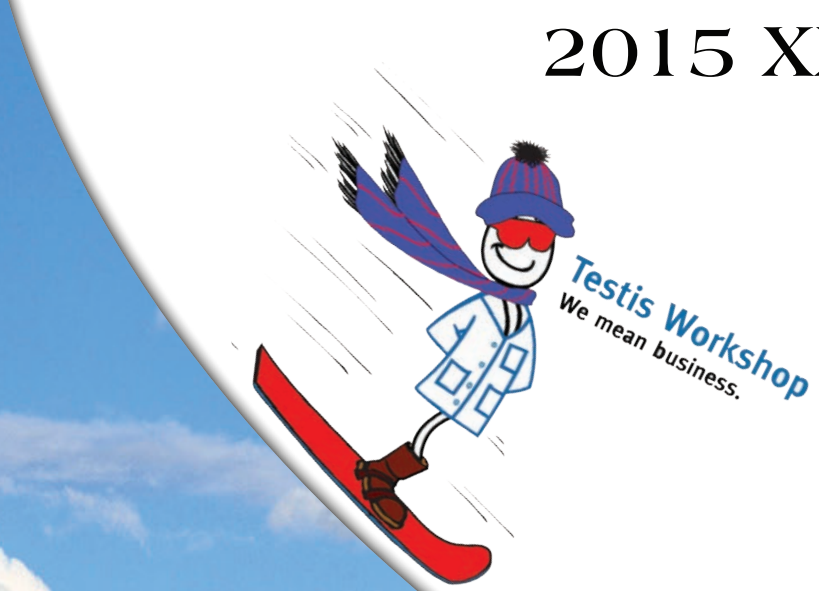
2015 XXIII[rd] North American Testis Workshop



"Healthy Sperm – Healthy Children"

April 15 – 18, 2015

The Little America Hotel
Salt Lake City, Utah

Chair: Jacquetta Trasler, MD, PhD
Vice-Chair: Leslie L. Heckert, PhD

2015
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Welcome to the 2015 XXIIIrd North American Testis Workshop

The theme for this year's Testis Workshop is "Healthy Sperm – Healthy Children." The program brings together trainee, new and established scientists from around the world to discuss the latest research on testicular physiology, biochemistry, molecular biology and cell biology, and this year includes a genetic/epigenetic and intergenerational emphasis. The program begins with the keynote lecture from Dr. Amander Clark introducing us to the latest findings on primordial germ cells, as they enter the complex process leading to the formation of spermatozoa, and along the way as they are influenced by many factors, including genes, the environment, hormones and somatic cells. The program has many highlights, including three benchmark lectures by international speakers: one on spermatogonial stem cells from Dr. Stefan Schlatt, a second on maternal-paternal interactions that influence offspring outcome from Professor Sarah Robertson and a third on androgen signaling from Professor Lee Smith. There are six highly interactive sessions with lectures on different aspects of testis biology and function, short talks chosen from the submitted abstracts and two poster sessions where all participants can meet in a social setting to discuss data, encourage young scientists and explore new collaborations.

A multi-disciplinary committee developed the program for the XXIIIrd Testis Workshop. We thank the members of the Program Committee, Vassilios Papadopoulos (Vice-Chair elect, McGill University), Amander Clark (UC Los Angeles), Barry Hinton (University of Virginia), Sarah Kimmins (McGill University), Kate Loveland (Monash Institute of Medical Research), Stuart Moss (NICHD), David Page (MIT), Gail Prins (University of Chicago), Takashi Shinohara (Kyoto University), Katja Teerds (U Wageningen), Christina Wang (UC Los Angeles), and Wei Yan (University of Nevada) for their engagement, enthusiasm and ideas. We thank the Abstract Review Committee (Chair Leslie Heckert, Mitch Eddy, Brian Hermann, Michael Griswold, Kate Loveland, Ralph Meyer, Vassilios Papadopoulos, Katja Teerds, Wei Yan) for their help identifying presentations for the short talks and travel awards. Sincere thanks also to the following organizations that have 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 National Institute of Aging, the Burroughs Wellcome Fund, Utah State University, University of Utah and Nova Source/Tessenderlo Kerley. We are grateful to Donna Rostamian and her team at WJ Weiser & Associates for a masterful job behind the scenes, managing the many details of overall venue and program arrangements. The Testis Workshop is governed by an Executive Committee, which is made up of past and future Testis Workshop Chairs, and is responsible for the management and long-term planning of the workshop. The members include Barry Zirkin, Mitch Eddy, Erwin Goldberg, Michael Griswold, Mary Ann Handel, Leslie Heckert, John McCarrey, Vassilios Papadopoulos, Bernard Robaire, Jacquetta Trasler and William Wright. In particular, we are grateful to Barry Zirkin (Chair, Executive Committee) and Vassilios Papadopoulos for their very successful fund raising campaign for this year's meeting.

Thank you all for joining us and contributing to the poster and oral sessions and the discussions. Enjoy the meeting and our venue in Salt Lake City with its Rocky Mountain views.

Jacquetta Trasler
Chair of the Program Committee

Leslie Heckert
Vice-Chair of the Program Committee

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Thank You

The organizers of the XXIII[rd] North American Testis Workshop gratefully acknowledge the financial support provided by the following sources, without which we would not have been able to sustain this conference.

- **Burroughs Wellcome Fund**
- **The Eunice Kennedy Shriver National Institute of Child Health and Human Development**
- **The National Institute of Environmental Health Sciences**
- **The National Institute of Aging**
- **NovaSource/Tessenderlo Kerley**
- **Utah State University – CAAS, ADVS and the SVM**
- **University of Utah**

2015 Testis Workshop Logo
happily modified by Pamela Wright, PB Wright Scientific Editing, LLC

Faculty

XXIII[rd] North American Testis Workshop
"Health Sperm – Healthy Children"
April 15 – 18, 2015

Alexei Aravin, PhD
Caltech

Tracy Bale, PhD
University of Pennsylvania

Douglas Carrell, PhD, HCLD
University of Utah

Diego Castrillon, MD, PhD
University of Texas Southwestern

Amander Clark, PhD
UCLA

Paula Cohen, PhD
Cornell University

Daniel Cyr, PhD
INRS

Jannette Dufour, PhD
Texas Tech University

Mitch Eddy, PhD
NIEHS

Eric Greer, PhD
Children's Hospital Boston and
Harvard Medical School

Leslie Heckert, PhD
University of Kansas Medical Center

Marie-Claude Hofmann, PhD
University of Texas – MD
Anderson Cancer Center

Csilla Krausz, MD, PhD
University of Florence

Kate Loveland, PhD
Monash University

Clinton MacDonald, PhD
Texas Tech University

Amanda MacFarlane, PhD
Health Canada

Ralph Meyer, PhD
Utah State University

Kyle Orwig, PhD
University of Pittsburgh

Vassilios Papadopoulos, PhD
McGill University,
Research Institute

Christopher Payne, PhD
Northwestern University

Sarah Robertson, PhD
University of Adelaide

Stefan Schlatt, PhD
WWU Münster, Germany

Lee B. Smith, PhD
MRC Centre for Reproductive
Health – Edinburgh

Katja Teerds, PhD
Wageningen University

Jorma Toppari, MD, PhD
University of Turku

Jacquetta Trasler, MD, PhD
McGill University

Mark Van Doren, PhD
Johns Hopkins University

William Wright, PhD
Johns Hopkins University

Wei Yan, MD, PhD
University of Nevada
School of Medicine

Carole Yauk, PhD
Health Canada

Barry Zirkin, PhD
Johns Hopkins University

Program Schedule

XXIII[rd] North American Testis Workshop
"Healthy Sperm – Healthy Children"

April 15 – 18, 2015
Chair: Jacquetta M. Trasler, MD, PhD
Vice-Chair: Leslie L. Heckert, PhD

*All sessions located in **Grand Ballroom A-B** unless otherwise noted*
Speakers and times are subject to change

WEDNESDAY, APRIL 15, 2015

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

7:00 p.m. – 7:15 p.m. **Welcome**
Jacquetta Trasler, MD, PhD
McGill University

7:15 p.m. – 8:15 p.m. ***Keynote Address***
Human Primordial Germ Cells: Intergenerational Epigenetic Gatekeepers
Amander Clark, PhD
UCLA

8:15 p.m. – 9:30 p.m. **Testis Workshop Welcome Reception**
Location: Grand Ballroom Reception A-B

THURSDAY, APRIL 16, 2015

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

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

8:00 a.m. – 8:45 a.m. ***Benchmark Lecture I***
Chair: William Wright, PhD
Johns Hopkins University

Therapeutic Promise of Primate Spermatogonial Stem Cells
Stefan Schlatt, PhD
WWU Münster, Germany

SESSION I: MALE FERTILITY ACROSS GENERATIONS-SETTING THE STAGE

8:45 a.m. – 8:50 a.m. **Chair and Introduction to Session I**
Ralph Meyer, PhD
Utah State University

8:50 a.m. – 9:25 a.m. **Identification of a Novel DNA Methylation in C. Elegans and Its Role in Transgenerational Inheritance**
Eric Greer, PhD
Children's Hospital Boston and Harvard Medical School

THURSDAY continued

9:25 a.m. – 10:00 a.m. **Paternal Stress Alteration of Sperm miRNAs Reprograms Stored Maternal mRNAs and Offspring Neurodevelopment**
Tracy Bale, PhD
University of Pennsylvania

10:00 a.m. – 10:25 a.m. **Break**

10:25 a.m. – 11:00 a.m. **Father's In Utero and Postnatal Folate Exposures Affect Offspring Health**
Amanda MacFarlane, PhD
Health Canada

11:00 a.m. – 11:15 a.m. **Short Talk #1**
Implications of Lifetime Folate Deficiency and Supplementation on Intergenerational Health
Lundi Ly, BSc
McGill University

11:15 a.m. – 11:30 a.m. **Short Talk #2**
The RHOX10 Homeobox Transcription Factor Drives the Initial Establishment of Spermatogonial Stem Cells
Hye-Won Song, PhD
University of California

11:30 a.m. – 1:00 p.m. **Lunch (on your own)**

SESSION II: HEALTHY STEM CELLS, HEALTHY AGING

1:00 p.m. – 1:05 p.m. **Chair and Introduction to Session II**
Kyle Orwig, PhD
University of Pittsburgh

1:05 p.m. – 1:40 p.m. **Sex Determination in the Somatic Gonad and Germline**
Mark Van Doren, PhD
Johns Hopkins University

1:40 p.m. – 2:15 p.m. **Biological Functions of Pax7+ Spermatogonia**
Diego Castrillon, MD, PhD
University of Texas Southwestern

2:15 p.m. – 2:40 p.m. **Break**

2:40 p.m. – 3:15 p.m. **Aging Effects on the Spermatogenic Cell Epigenome**
Christopher Payne, PhD
Northwestern University

3:15 p.m. – 3:50 p.m. **Aging and Leydig Cells**
Barry Zirkin, PhD
Johns Hopkins University

3:50 p.m. – 6:00 p.m. **Poster Session I**
Location: Grand Ballroom C

6:00 p.m. – 8:00p.m. **Post-Poster Social Event**
Location: Idaho
(not included in registration: ticket required)

FRIDAY, APRIL 17, 2015

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

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

8:00 a.m. – 8:45 a.m. ***Benchmark Lecture II***
Chair: Kate Loveland, PhD,
Monash University

Male-Female Signaling By Seminal Fluid: Effects on Metabolic Phenotype of Offspring
Sarah Robertson, PhD
University of Adelaide

SESSION III: REGULATION OF GENE EXPRESSION FROM PROGENITOR CELLS THROUGH MEIOSIS

8:45 a.m. – 8:50 a.m. **Chair and Introduction to Session III**
Wei Yan, MD, PhD
University of Nevada – School of Medicine

8:50 a.m. – 9:25 a.m. **Small RNAs Target Active Transposable Elements to Establish the Repressive Chromatin Mark in Germ Cells**
Alexei Aravin, PhD
Caltech

9:25 a.m. – 10:00 a.m. **Investigating the Role of Argonaute Proteins in Mammalian Meiosis**
Paula Cohen, PhD
Cornell University

10:00 a.m. – 10:25 a.m. **Break**

10:25 a.m. – 11:00 a.m. **How Polyadenylation Controls Gene Expression in Testis and Brain**
Clinton MacDonald, PhD
Texas Tech University

11:00 a.m. – 11:15 a.m. **Short Talk #3**
Evaluating L1 Transgenes Regulated by the Endogenous Mouse L1 Promoter
Wenfeng An
South Dakota State University

11:15 a.m. – 11:30 a.m. **Short Talk # 4**
Overexpression of ID4 Alters Cell Cycle Progression and Transition from the Stem Cell to Progenitor State in Mouse Spermatogonia
Jon M. Oatley, PhD
Washington State University

11:30 a.m. – 1:00 p.m. **Lunch (on your own)**

SESSION IV: DETERMINING AND PERTURBING TESTICULAR FUNCTION

1:00 p.m. – 1:05 p.m. **Chair and Introduction to Session IV**
Leslie Heckert, PhD
University of Kansas Medical Center

1:05 p.m. – 1:40 p.m. **Protecting Maturing Sperm from Environmental Toxicants: The Role of the Epididymis**
Daniel Cyr, PhD
INRS

FRIDAY continued

1:40 p.m. – 2:15 p.m. **Safeguarding Sperm: Identification of Mutagenic Hazards to Future Generations**
Carole Yauk, PhD
Health Canada

2:15 p.m. – 2:30 p.m. **Short Talk #5**
Direct Germline Editing in Spermatogonia Eliminates CRISPR/CAS9 Catalyzed Animal Mosaicism
F. Kent Hamra, PhD
University of Texas Southwestern Medical Center

2:30 p.m. – 2:45 p.m. **Short Talk #6**
The Role of Peritubular Myoid (PM) Cells in the Regulation of Spermatogonial Stem Cell (SSC) Self-Renewal, Proliferation and Differentiation in the Testis Niche
Liang-Yu Chen
NIEHS

2:45 p.m. – 3:10 p.m. **Break**

SESSION V: SOMATIC CELLS

3:10 p.m. – 3:15 p.m. **Chair and Introduction to Session V**
Katja Teerds, PhD
Wageningen University

3:15 p.m. – 3:50 p.m. **Sertoli Cells: Immune Privilege & Novel Roles in Cell Based Gene Therapy**
Jannette Dufour, PhD
Texas Tech University

3:50 p.m. – 4:25 p.m. **NOTCH Signaling in Sertoli Cells**
Marie-Claude Hofmann, PhD
University of Texas – MD Anderson Cancer Center

4:25 p.m. – 4:40 p.m. **Short Talk #7**
Cholesterol Trafficking for Steroid Biosynthesis in MA-10 Mouse Tumor Leydig Cells
Sathvika Jagannathan, MSc
McGill University

4:40 p.m. – 4:55 p.m. **Short Talk #8**
Requirement for Adenosine Deaminase Containing Proteins in Male Germ Cell Development
Elizabeth Snyder, PhD
The Jackson Laboratory

5:00 p.m. – 7:00 p.m. **Poster Session II**
Location: Grand Ballroom C

SATURDAY, APRIL 18, 2015

7:00 a.m. – 2:00 p.m. **Registration/Information Desk Open**
Location: Grand Ballroom A Foyer

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

8:00 a.m. – 8:45 a.m. ***Benchmark Lecture III***
Chair: Mitch Eddy, PhD

Androgen-Signaling in Lifelong Health and Well Being
Lee B. Smith, PhD
MRC Centre for Reproductive Health – Edinburgh

SESSION VI: HUMAN FERTILITY, INFERTILITY AND THE NEXT GENERATION

8:45 a.m. – 8:50 a.m. **Chair and Introduction to Session VI**
Vassilios Papadopoulos, DPharm, PhD
McGill University, Research Institute

8:50 a.m. – 9:25 a.m. **Endocrine Disruptors, Early Exposures and Sperm Function**
Jorma Toppari, MD, PhD
University of Turku

9:25 a.m. – 10:00 a.m. **Infertility, Aging, ART and Sperm Epigenetics**
Douglas Carrell, PhD, HCLD
University of Utah

10:00 a.m. – 10:25 a.m. **Break**

10:25 a.m. – 11:00 a.m. **Genetic Causes of Human Infertility:**
From X Chromosome High Resolution Array-CGH to Whole Exome Studies
Csilla Krausz, MD, PhD
University of Florence

11:00 a.m. – 11:15 a.m. **Short Talk #9**
The Testicular Transcriptome of the Rhesus Monkey (Macaca Mulatta) Associated with the Decision by Undifferentiated Type A Spermatogonia to Commit to a Pathway of Differentiation
Suresh Ramaswamy, PhD
University of Pittsburgh School of Medicine
Magee-Womens Research Institute

11:15 a.m. – 11:30 a.m. **Short Talk #10**
Testes Specific Protease 50 (TSP50) Modulates Male Fertility
Carolina Jorgez, PhD
Baylor College of Medicine

11:30 a.m. – 11:45 a.m. **Concluding Remarks & Acknowledgements**

11:45 a.m. – 11:55 a.m. **Announcement of the 24th North American Testis Workshop**

12:00 p.m. **MEETING ADJOURNED**

Speaker Abstracts

WEDNESDAY, APRIL 15, 2015
7:15 p.m. – 8:15 p.m.

Keynote Address
HUMAN PRIMORDIAL GERM CELLS: INTERGENERATIONAL EPIGENETIC GATEKEEPERS
Amander T. Clark, PhD
University of California, Los Angeles Department of Molecular Cell and Developmental Biology

DNA methylation reprogramming is required to reset the epigenome during pre-implantation embryo development, and during formation of the mammalian germline. These waves of reprogramming are thought to be critical for gamete quality, fertility and child health in the next generation. Therefore the germline acts as the Intergenerational epigenetic gatekeeper. My laboratory studies epigenetic reprogramming during germline development in the embryo with a focus on the initial wave of demethylation to create the germline epigenetic ground state at midgestation. The second major stage we study involves uncovering novel mechanisms that promote *de novo* methylation of the male germline prior to birth. In the last five years we have established at single base resolution using whole genome bisulfite sequencing the methylome of the human germline from 57 to 113 days of human life post fertilization. We discovered that the human germline represents the most demethylated cell type in humans described to date, with bulk methylation levels reducing to below 20% in CpG sequence contexts by 113 days of life. Within the landscape of global genome demethylation, we discovered that the human germline has sites of persistent methylation at CG islands (CGIs) located in intragenic regions including splice sites, exons, 3'UTRs and promoters as well as persistent methylation and some subclasses of transposons, notably the young and mobile LINE 1 human specific (L1HS) transposons. We also found that the human germline undergoes small amounts of *de novo* methylation between 67 and 113 days, particularly at CG Islands in exons, splice sites and promoters. This local re-methylation is also observed in the mouse germline. Demonstrating that the mouse model is suitable for studying the logic of germline methylation reprogramming as it relates to humans. To understand methylation reprogramming in mice and humans we use transgenic mice and embryonic stem cell models.

THURSDAY, APRIL 16, 2015
8:00 a.m. – 8:45 a.m.

Benchmark Lecture I
THERAPEUTIC PROMISE OF PRIMATE SPERMATOGONIAL STEM CELLS
Stefan Schlatt, PhD
U. Münster, Centre for Reproductive Medicine and Andrology, Germany

Due to the improved success rates of treating childhood cancers an increasing number of young adults are now long-term survivors of childhood malignancies. A known late effect of chemotherapy agents and radiation exposure in males is damage to the spermatogonial stem cells in the testis. Male infertility could be a consequence of defects in spermatogonial stem cells or the stem cell niches. Loss of germ cells may be an aftermath of diminished germ cell quality after gonadotoxic exposures if effective checkpoints exist to recognize and eliminate damaged germ cells. Several scenarios for potential checkpoints in the male germ lineage will be presented and models for germ cell selection are discussed. Irrespective of the physiological mechanisms the treatments used to cure the oncological disease often render the patients temporarily or permanently infertile. Advances in research methods, clinical treatments and patient management strategies have recently been made which open options for preservation of sperm and testicular tissue in prepubertal boys and adolescents prior to being able to ejaculate sperm. This benchmark talk will first provide an update on the physiology of spermatogonial stem cells in rodent and primate testes. Significant species-specific differences in spermatogonial physiology between rodents and primates must be considered when experimental data are interpreted and new technologies are developed for applications in clinical settings. We will then explore potential curative strategies like germ cell transplantation which has become an established research tool but its clinical application is still in infancy. Albeit its successful application has been shown in principle also for primates this strategy must still be regarded as experimental in a clinical context. Xeno- and auto-grafting of immature tissue fragments revealed a high regenerative potential of testicular tissue. Grafting was applied in rodents and primates and resulted in the generation of sperm. In vitro cell or organ culture to generate sperm in a culture dish has now been tested for a century and recently showed promising results at least in mouse models. Novel strategies to generate sperm from pluripotent or iPS cells reveal an almost futuristic scenario of generating gametes from somatic tissues. The advantages and challenges of potential stem cell based techniques will be visited and their potential future application will be discussed. Finally the newly established EU sponsored Marie Curie training program "GROWSPERM" will be presented which tries to implement a number of novel strategies to generate sperm from stem cells.

Speaker Abstracts

THURSDAY, APRIL 16, 2015
8:50 a.m. – 9:25 a.m.

IDENTIFICATION OF A NOVEL DNA METHYLATION IN C. ELEGANS AND ITS ROLE IN TRANSGENERATIONAL INHERITANCE

Eric Greer, PhD
Children's Hospital Boston & Harvard Medical School

Eric Lieberman Greer[1,2], Mario Andres Blanco[1,2], Erdem Sendinc[1,2], David Aristizábal-Corrales[1,2], Jianzhao Liu[3], Lei Gu[1,2], L. Aravind[4], Chuan He[3], and Yang Shi[1,2]
[1] Division of Newborn Medicine, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115; [2] Harvard Medical School, Boston, MA 02115; [3] Department of Chemistry and Institute for Biophysical Dynamics, Howard Hughes Medical Institute, The University of Chicago, Chicago, IL 60637; [4] National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 208943

Non-Mendelian inheritance influences a wide variety of phenotypes. How non-Mendelian information is transmitted from generation to generation remains largely unknown. Heritable chromatin modifications have been proposed mediators for some of these phenotypes. In mammalian cells, DNA methylation on the 5th position of the pyrimidine ring of cytosine (5mC) plays an important role in the inheritance of epigenetic information. However, DNA methylation was considered to be absent in *C. elegans* because of the lack of detectable 5mC as well as homologs of the cytosine DNA methyltransferases (DNMTs). Here I will present our recent work identifying a novel eukaryotic DNA methylation which can be detected on *C. elegans* DNA. This modification increases transgenerationally in a paradigm of epigenetic inheritance. We have identified a DNA demethylase, NMAD-1, as well as a potential DNA methyltransferase DAMT-1, which regulate DNA methylation levels in the nematode and control the epigenetic inheritance of the histone H3K4me2 demethylase *spr-5* mutant worms. Together these data suggest that this novel eukaryotic DNA methylation mark may be a potential carrier of epigenetic information across generations.

THURSDAY, APRIL 16, 2015
9:25 a.m. – 10:00 a.m.

PATERNAL STRESS ALTERATION OF SPERM miRNAS REPROGRAMS STORED MATERNAL mRNAS AND OFFSPRING NEURODEVELOPMENT

Tracy Bale, PhD
University of Pennsylvania

Ali Rodgers, Jennifer Chan, Christopher Morgan, and Tracy L. Bale
University of Pennsylvania, Department of Animal Biology, Philadelphia, PA

Neurodevelopmental disorders including autism and schizophrenia have been highly associated with parental factors, including lifetime stress experience. We have developed a mouse model of paternal stress in which adult male mice exposed to chronic stress prior to breeding produce offspring with hypothalamic-pituitary-adrenal (HPA) stress axis dysregulation. Paternal sperm was examined for changes in miRNA content where 9 specific miRNAs were identified as significantly increased in stressed sperm. To test the relevance and potential mRNA targets of these miRNAs, we synthesized and injected the 9 miRNAs into single cell zygotes and found that the resulting offspring recapitulated the stress phenotype found from paternal stress sires. In addition, we have now completed single cell amplification from injected zygotes using Fluidigm technology and ascertained the stored maternal mRNAs that are targets of these sperm miRNAs and thus affecting post-fertilization development that results in a reprogrammed brain that is stress hypo-responsive. Gene set enrichment analysis of the offspring paraventricular nucleus of the hypothalamus revealed global changes in transcription, including increased representation of glucocorticoid receptor-responsive gene sets and functional annotation clustering for enrichment of extracellular matrix genes, with an exceptional representation of collagens. Overall, these results demonstrate that paternal experience across the lifespan can induce germ cell epigenetic reprogramming and impact offspring HPA stress axis regulation, and may therefore offer novel insight into factors influencing neuropsychiatric disease risk. Identification of the specific miRNAs in germ cells that are altered long-term following stress experience may point to unique biomarkers that could identify at-risk populations.

Speaker Abstracts

THURSDAY, APRIL 16, 2015
10:25 a.m. – 11:00 a.m.

FATHER'S IN UTERO & POSTNATAL FOLATE EXPOSURES AFFECT OFFSPRING HEALTH
Amanda Mac Farlane, PhD
Health Canada

Amanda J. MacFarlane[1], (Ian) J. W. R. Zinck[1], Nathalie A. Behan[1], Marie Caudill[3], and Carole L. Yauk[2]
[1]Nutrition Research Division, Health Canada, Ottawa, ON; [2] Division of Nutritional Sciences, Cornell University, Ithaca, NY; [3] Mechanistic Studies Division, Health Canada, Ottawa, ON

Folic acid (FA) is required to maintain nucleotide synthesis and cellular methylation capacity, which can affect gene expression, potentially through heritable changes in DNA methylation. We hypothesized that father's diet may alter the health of his offspring, possibly by changes to gene expression through epigenetic modification of the sperm methylome.
We used a multigenerational mouse model to examine the effect of paternal FA deficiency and supplementation on his progeny's embryo development (F1), and gene expression and one carbon metabolism in their unexposed descendants (F3). Male Balb/c mice (F0) were exposed to a FA deficient (0mg/kg), sufficient (2mg/kg) or supplemented (6mg/kg) diet in early development (*in utero* and during lactation) or from weaning. They were bred to female mice fed a sufficient diet. F1 embryo analysis was performed at GD16.5. Tissue folate and liver choline metabolites were measured in F3 male descendants. Hepatic gene expression was assessed by microarray analysis and qPCR validation.
Fifty percent of F1 litters from FA deficient fathers had embryos with congenital anomalies (omphalocele and gastroschisis) or were small for gestational age (SGA), compared to 10% of sufficient fathers. Developmental anomalies or SGA were observed in 25% litters of litters from supplemented fathers (not significant). In comparison to the sufficient line, F3 male descendants of F0 males exposed to FA deficiency in early development or from weaning demonstrated ~15% higher hepatic and RBC folate, ~50% higher phosphocholine, ~30% higher betaine and tended to have higher choline (~30%). Hepatic expression of *Chka* was ~75% higher and *Mthfr* tended to be higher (~60%). F0 supplementation had similar but mostly non-significant effects on choline metabolites and gene expression as observed for F0 deficiency.
The data indicate that FA deficiency in fathers' may cause congenital anomalies and SGA in their offspring, and influences heritable metabolic programming of one carbon metabolism in their unexposed descendants. Ongoing analyses will examine the effect of paternal FA intake on the sperm methylome.

THURSDAY, APRIL 16, 2015
1:05 p.m. – 1:40 p.m.

SEX DETERMINATION IN THE SOMATIC GONAD AND GERMLINE
Mark Van Doren, PhD
Johns Hopkins University

My lab focuses on understanding the molecular mechanisms controlling somatic and germline sex determination, and how this information is used to control development of the male and female stem cell systems in the gonads. The testis and ovary form from a common set of somatic and germline cells. Thus, one of the most important aspects of gonad formation is how these cells determine their sex and how their developmental programs are regulated differently in males vs. females. Over the past several years, homologs of the Drosophila gene *doublesex* (*doublesex, mab-3* related transcription factors, DMRTs) have been shown to control sex-specific somatic gonad development in most or all animals where it has been studied. Though this provides a unifying molecular mechanism for controlling sexual dimorphism in the somatic gonad, much remains to be learned about how the DMRTs regulate specific aspects of testis development. One particularly interesting place to study *dsx*/DMRT function is in the formation of the sex-specific somatic stem cell niches that control the germline stem cells.
Also of importance is how the germline determine's its own sexual identity and how this influences germ cell development and their ability to produce sperm and eggs. Much less is known about sex determination in the germline compared to the soma, but one common theme is that germ cells utilize signals from the soma to determine their sex. In many animals, both male and female germ cells produce germline stem cells, but these stem cells behave differently in the two sexes. In other animals, such as mammals, a stem cell population exists only in males. It is currently unknown how germline sex determination controls sex-specific development of the germline stem cells. I will present our lab's recent work on the molecular mechanisms of somatic and germline sex determination, and on sex-specific development of the male and female gonadal stem cells.

Speaker Abstracts

THURSDAY, APRIL 16, 2015
1:40 p.m. – 2:15 p.m.

BIOLOGICAL FUNCTIONS OF PAX7+ SPERMATOGONIA
Diego Castrillon, MD, PhD
University of Texas Southwestern

Gina M. Aloisio, Yuji Nakada, Hatice D. Saatcioglu, Christopher G. Peña, Michael D. Baker, Edward D. Tarnawa, Jishnu Mukherjee, Hema Manjunath, Abhijit Bugde, Anita L. Sengupta, James F. Amatruda, Ileana Cuevas, F. Kent Hamra and Diego H. Castrillon
UT Southwestern, Department of Pathology, Dallas TX

The uncertainty as to the precise identity of the stem cells of the adult testis has been widely recognized as a major question in male infertility research. We conducted an RNA-based screen to find genes highly enriched in spermatogonial stem cells and identified the paired box transcriptional regulator Pax7 as a factor expressed in a rare subset of A_{single} spermatogonia (1). This finding was interesting because A_{single} spermatogonia were generally believed to be a homogenous population. However, our studies, along with others—including the recent identification of Id4 (2) and Erbb3 (3) as additional markers of restricted subsets of A_{single} spermatogonia—have shown that the A_{single} pool is much more heterogeneous than had generally been considered. Another surprising aspect of our discovery is that Pax7 was well-known as the defining marker of satellite cells (the stem cells of adult skeletal muscle, which become reactivated following injury) but Pax7 was not known to be expressed in any cells within the testis.
$Pax7^+$ spermatogonia divide rapidly and continuously. This combined with their rarity suggested that they might serve as robust testis stem cells in adult males. This hypothesis was explored through lineage tracing analyses in live mice, which confirmed that $Pax7^+$ spermatogonia are robust stem cells giving rise to mature spermatozoa.
Outstanding questions that will be discussed include the role and function of Pax7, the unique biological properties of $Pax7^+$ spermatogonia both in normal steady state spermatogenesis and under conditions of germline stress, and the implications of functional studies together with the apparent conservation of $Pax7^+$ spermatogonia across mammalian species.

THURSDAY, APRIL 16, 2015
2:40 p.m. – 3:15 p.m.

AGING EFFECTS ON THE SPERMATOGENIC CELL EPIGENOME
Christopher Payne, PhD
Northwestern University

Amber E. Kofman, Jessica M. Huszar and Christopher J. Payne
Department of Pediatrics and Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL

Advanced age has a negative impact on male germ cell maintenance and spermatogenesis. Spermatogonial stem cells from aging mice exhibit a decreased capacity for self-renewal. Aged sperm are more susceptible to gene expression changes than their younger counterparts. Increased paternal age is also significantly associated with decreased sperm motility. One potential mechanism contributing to these outcomes is an aging-induced perturbation of epigenetic modifications within male germ cells. Members of the histone deacetylase (HDAC) family of epigenetic erasers, including HDAC2 and Sirtuin 6, are implicated in the aging process. In many tissues, HDAC2 protects against cellular senescence and premature aging in response to oxidative stress. Sirtuin 6 (SIRT6) regulates DNA damage repair and telomere maintenance. SIRT6 also influences spermatid elongation through a mechanism that remains unclear. We previously discovered a reduction in gene expression of several HDACs in undifferentiated spermatogonia of 1-year-old mice (1). More recent observations suggest that insufficient levels of HDAC2 promote the depletion of undifferentiated spermatogonia as mice age. To address the question of how HDAC2 and SIRT6 modulate male germ cell maintenance and spermatogenesis, we have utilized gene-targeted mouse models and chromatin immunoprecipitation-DNA sequencing studies. Here we discuss the germ cell-specific ablation of HDAC2 and its impact on aging in undifferentiated spermatogonia. We also assess the genomic targets of SIRT6 in elongating spermatids and how they change between young and old animals. Collectively, this evidence supports the role of epigenetics in the aging process of spermatogenic cells.
1. Kofman, A.E., Huszar, J.M., Payne, C.J. (2013) *Stem Cell Rev.* 9:59-64.

Speaker Abstracts

THURSDAY, APRIL 16, 2015
3:15 p.m. – 3:50 p.m.

AGING AND LEYDIG CELLS
Barry Zirkin, PhD
Johns Hopkins Univrsity

Haolin Chen[1], Vassilios Papadopoulos[2] and Barry Zirkin[1]
[1]Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]The Research Institute of the McGill University Health Centre, and Departments of Medicine, Biochemistry and Pharmacology & Therapeutics, McGill University, Montreal, Quebec, Canada

Leydig cell testosterone (T) production is reduced with age, resulting in reduced serum T levels (hypogonadism). A number of cellular changes have been identified in the steroidogenic pathway of aged Leydig cells that are associated with reduced T formation, including reductions in luteinizing hormone (LH)-stimulated cAMP production, the cholesterol transport proteins steroidogenic acute regulatory (STAR) protein and translocator protein (TSPO), and downstream steroidogenic enzymes of the mitochondria and smooth endoplasmic reticulum. Many of the changes in steroid formation that characterize aged Leydig cells can be elicited by the experimental alteration of the redox environment of young cells, suggesting that changes in the intracellular redox balance may cause reduced T production. Hypogonadism is estimated to affect about 5 million American men, including both aged and young. This condition has been linked to mood changes, worsening cognition, fatigue, depression, decreased lean body mass, reduced bone mineral density, increased visceral fat, metabolic syndrome, decreased libido, and sexual dysfunction. Exogenous T administration is now used widely to elevate serum T levels in hypogonadal men and thus to treat symptoms of hypogonadism. However, recent evidence suggests that men who take exogenous T may face increased risk of stroke, heart attack, and prostate tumorigenesis. Moreover, it is well established that administered T can have suppressive effects on LH, resulting in lower Leydig cell T production, reduced intratesticular T concentration, and reduced spermatogenesis, making exogenous T administration inappropriate for men who wish to father children. There are promising new approaches to increase serum T by directly stimulating Leydig cell T production rather than by exogenous T therapy, thus potentially avoiding some of its negative consequences.

FRIDAY, APRIL 17, 2015
8:00 a.m. – 8:45 a.m.

Benchmarck Lecture II
MALE-FEMALE SIGNALING BY SEMINAL FLUID: EFFECTS ON METABOLIC PHENOTYPE OF OFFSPRING
Sarah A. Robertson, PhD
Univeristy of Adelaide

Sarah A. Robertson, John J. Bromfield and John E. Schjenken
The Robinson Research Institute and School of Paediatrics and Reproductive Health, University of Adelaide, SA 5005 Australia

In mammalian reproduction, the male seminal fluid is generally thought to participate in one straightforward way – by providing sperm to fertilise the oocyte. But the male contribution may not be so simple. In mouse and human systems we have explored how signalling factors present in sperm and seminal plasma interact with cells of the female reproductive tract after coitus, to alter gene expression and modify tissue function, and the consequences for fertility and pregnancy outcome. Cytokines elicited by seminal fluid activate the female immune response, in turn influencing embryo development and implantation, and offspring health (1).
We evaluated in mice the consequences for offspring of ablating the plasma fraction of seminal fluid by surgical excision of the seminal vesicle gland (2). Conception was substantially impaired and when pregnancy did occur, placental hypertrophy was evident in late gestation. After birth, the growth trajectory and metabolic parameters of progeny were altered, most profoundly in males which exhibited obesity, distorted metabolic hormones, reduced glucose tolerance and hypertension. Altered offspring phenotype was partly attributable to sperm damage and partly to an adverse effect of seminal fluid deficiency on the female tract. Moreover, embryos developed in female tracts not exposed to seminal plasma were abnormal from the early cleavage stages, but culture in vitro partly alleviated this. Absence of seminal plasma was accompanied by down-regulation of the embryotrophic factors *Lif, Csf2, Il6,* and *Egf* and up-regulation of the apoptosis-inducing factor *Trail* in the oviduct. These findings show that paternal seminal fluid composition affects the growth and health of male offspring, and reveal that its impact on the peri-conceptional environment involves not only

sperm protection, but also indirect effects on pre-implantation embryos via oviduct expression of cytokines, to influence epigenetic mechanisms of developmental programming in offspring

(1) Robertson (2005) *Cell Tissue Res* 322:43
(2) Bromfield et al. (2014) *Proc Natl Acad Sci USA* 111:2200

FRIDAY, APRIL 17, 2015
8:50 a.m. – 9:25 a.m.

SMALL RNAS TARGET ACTIVE TRANSPOSABLE ELEMENTS TO ESTABLISH THE REPRESSIVE CHROMATIN MARK IN GERM CELLS

Alexei Aravin, PhD
Division of Biology, 147-75, California Institute of Technology, 1200 E California Blvd. Pasadena, CA 91125

Transposable elements (TEs) occupy a large fraction of metazoan genomes and pose a constant threat to genomic integrity. This threat is particularly critical in germ cells, as changes in the genome that are induced by TEs will be transmitted to the next generation. Small noncoding piwi-interacting RNAs (piRNAs) recognize and silence a diverse set of TEs in germ cells. In mice, piRNA-guided transposon repression correlates with establishment of CpG DNA methylation on their sequences, yet the mechanism and the spectrum of genomic targets of piRNA silencing are unknown. We show that in addition to DNA methylation, the piRNA pathway is required to maintain a high level of the repressive H3K9me3 histone modification on long interspersed nuclear elements (LINEs) in germ cells. piRNA-dependent chromatin repression targets exclusively full-length elements of actively transposing LINE families, demonstrating the remarkable ability of the piRNA pathway to recognize active elements among the large number of genomic transposon fragments.

FRIDAY, APRIL 17, 2015
9:25 a.m. – 10:00 a.m.

INVESTIGATING THE ROLE OF ARGONAUTE PROTEINS IN MAMMALIAN MEIOSIS

Paula Cohen, PhD
Cornell University

Elizabeth Crate[1], Andrew Modzelewski[1], Stephanie Hilz[2], Rebecca Holmes[1], Andrew Grimson[2], and Paula Cohen[1]
[1]Departments of Biomedical Sciences and [2]Molecular Biology & Genetics, Cornell University, Ithaca, NY 14853

Argonautes are highly conserved proteins found in almost all eukaryotes and some bacteria and archaea. In mammals, there are eight argonaute proteins evenly distributed across two clades, the Ago clade (AGO1-4) and the Piwi clade (MILI, MIWI, MIWI2, and PIWI). All four members of the Ago clade are thought to share redundant functions in the microRNA pathway, yet only AGO2 possesses the catalytic “slicer” function required for RNA interference. Our labs have been investigating the functions of AGO3 and AGO4 in mammalian gametogenesis, on the basis of the high level of expression of their genes in mammalian testis. Interestingly, both of these proteins localize within the nucleus during meiotic prophase I, and are enriched at sites where chromosomes fail to undergo synapsis. This is most evident in the sex body, the heterochromatin-rich sub-domain of the nucleus that houses the X and Y chromosomes which, due to a lack of homology along most of their length, fail to synapse. Studies in our lab have demonstrated the AGO4 is required for the silencing of the sex chromosomes, through a process known as meiotic sex chromosome inactivation (MSCI). Loss of *Ago4* in homozygous null mice leads to disrupted MSCI and sex chromatin, associated with loss of meiotic cells at prophase I, leading to decreased epididymal sperm numbers, and low fertility of the males. This is associated with a dramatic loss of microRNAs, >20% of which arise from the X chromosome. Thus, AGO4 regulates MSCI in mammalian germ cells, implicating small RNA pathways in these processes. However, conditional deletion of Dicer or Dgcr8, both of which lie upstream of Argonautes in the small RNA biosynthetic pathway, indicate that the *Ago4* phenotype does not fully capture the spectrum of AGO-associated functions in prophase I, suggesting additional roles for other AGO members, including AGO3. Ongoing studies are focused on the shared functions of AGO3 and AGO4, together with elucidating the molecular mechanisms of AGO4 action in MSCI and other meiotic silencing events.

Speaker Abstracts

FRIDAY, APRIL 17, 2015
10:25 a.m. – 11:00 a.m.

HOW POLYADENYLATION CONTROLS GENE EXPRESSION IN TESTIS AND BRAIN
Clinton C. MacDonald
Texas Tech University Health Sciences Center, Cell Biology & Biochemistry, Lubbock, TX

Messenger RNA polyadenylation is important for all aspects of gene expression during reproduction and development. The cleavage stimulation factor CstF-64 (gene name: *Cstf2*) is an important regulatory component that is involved in polyadenylation. We discovered τCstF-64 (gene name: *Cstf2t*), the CstF-64 paralog that is expressed during male meiosis when X-linked genes like *Cstf2* are inactivated. Mice lacking *Cstf2t* are infertile, which led us to ask what were the molecular bases for this infertility. Using high-throughput sequencing technologies, we found that τCstF-64 was important for the correct polyadenylation of specific genes that control spermatogenesis, including *Atp1b3, Crem, Jund, Lipe, Map27k,* and *Skp1a.* Binding of τCstF-64 at locations downstream of the polyadenylation sites is associated with correct polyadenylation site choice in those genes. In male *Cstf2t*$^{-/-}$ mice, these errors in polyadenylation cumulatively result in misregulation of these and other gene products, resulting in infertility. We also found that τCstF-64 was necessary for the alternative splicing of exon 4 in *Crem.* While we have not yet eliminated the involvement of other splicing factors, we observed many τCstF-64-binding sites in the *Crem* pre-mRNA in and around exon 4, leading us to hypothesize a direct involvement for τCstF-64 in alternative splicing.
In addition to testis, τCstF-64 is also expressed in brain. Somewhat surprisingly, we found that female (but not male) *Cstf2t*$^{-/-}$ mice show significant improvements in spatial learning and memory in the absence of τCstF-64, i.e., female mice lacking *Cstf2t* score significantly better in tests of spatial learning and memory. This suggests that τCstF-64 has specific functions in hippocampal or other regions in a sex-dependent manner, and that τCstF-64 suppresses spatial learning and memory through regulation of mRNA processing. We speculate that advantages to females of loss of *Cstf2t* function might keep the gene in a population despite the obvious disadvantages of male infertility.

FRIDAY, APRIL 17, 2015
1:05 p.m. – 1:40 p.m.

PROTECTING MATURING SPERM FROM ENVIRONMENTAL TOXICANTS: THE ROLE OF THE EPIDIDYMIS
Daniel G. Cyr, PhD
INRS-Institut Armand-Frappier, University of Quebec, Laval, QC, Canada

Sperm maturation occurs in the epididymis, where sperm acquire the ability to swim and fertilize. Since sperm are transcriptionally inert, they must rely on the epididymis for protection from chemical insult. This is accomplished in part by an extensive blood-tissue barrier that limits the passage of molecules between cells and into the epididymal lumen and which contains a variety of transporters that modulate the composition of the lumen. We have shown that endocrine disrupting chemicals in fish exposed to municipal wastewater effluent and fed to female rats, can be transferred via lactation to impact the reproductive tract of developing rat pups. Among the endocrine disrupting chemicals released in wastewater effluent, alkylphenol ethoxylates such as nonylphenol can impact intercellular communication between Sertoli cells of the testis. We have shown that, at environmentally relevant doses, octylphenol, another alkylphenol, does not accumulate in either the testis or epididymis, and is rapidly eliminated, suggesting an active transport mechanism. Using rat epididymal principal cell lines we have shown the presence of a functional ABC-B1 transporter in the epididymis which is induced by alkylphenol ethoxylates. Surprisingly, we also observed that during epididymal transit, sperm acquire a functional ABC-B1 transporter critical to the detoxification process protecting sperm from chemical insult. The limited detoxification mechanism of sperm highlights the importance of the epididymis to detoxify environmental toxicants and protect maturing sperm. Studies by our lab and others have shown that epididymal basal cells play a significant role in protecting sperm from blood-borne reactive oxygen species induced by environmental contaminants. The roles of these basal cells in the epididymis, and their contribution to detoxification of environmental toxicants remain poorly understood. Given the limited detoxification capabilities of sperm, epididymal detoxification mechanisms are critical for the protection of sperm from chemical insult. Our understanding of these functions and how cells within the epididymis interact to provide a detoxification barrier is essential for our understanding of male reproductive toxicology. Supported by NSERC and CIHR.

Speaker Abstracts

FRIDAY, APRIL 17, 2015
1:40 p.m. – 2:15 p.m.

SAFEGUARDING SPERM: IDENTIFICATION OF MUTAGENIC HAZARDS TO FUTURE GENERATIONS
Carole Yauk, PhD
Health Canada

Yauk, C.L., O'Brien JM, Beal M, Meier, M., Gagné, R., Marchetti F.
Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada

Polycyclic aromatic hydrocarbons (PAHs) are by-products of combustion and are widespread in our environment. Humans are exposed throughout their lifetimes to PAHs through their diets, polluted air, and exposure to mainstream and sidestream tobacco smoke. A number of PAHs are well-established mutagens. We used benzo(a)pyrene (BaP), an established mutagenic and carcinogenic PAH, as a model agent in a series of experiments to explore: 1) whether germ cell mutations are induced by PAHs; and 2) the mechanisms leading to BAP induced mutations. The long-term objective is to develop a stronger regulatory paradigm to identify and assess germ cell mutagens. Our studies applied the Muta™Mouse model, which contains a *lacZ* transgenic mutation reporter gene that enables analysis of mutation frequency in any tissue. In the first experiment, mice were sub-chronically exposed to increasing doses of BaP and mutations arising in different phases of spermatogenesis were identified through analysis of *lacZ* mutant frequency in mature sperm. The data revealed that dividing spermatogonia are the most affected by BaP exposure, but that mutations do arise in spermatogonial stem cells. The mutation spectrum (the types and locations of mutations), measured through next-generation sequencing analysis of thousands of *lacZ* clones, revealed slight differences between somatic and germ cells, suggesting subtle differences in the creation or processing of BaP lesions in germ cell DNA. In a second experiment, exposure of male mice to BaP *in utero* caused significant dose-related decreases in testes weight, sperm concentration and motility in the F1 males. Furthermore, *lacZ* mutant frequency in cauda sperm increased at the middle and highest dose, but very few sperm were present at the highest dose due to overt toxicity. Overall, these studies demonstrate that BaP is both toxic and mutagenic in male germ cells. BaP exposure is expected to significantly impact mutational landscape in future generations. We are currently characterizing copy number variation and point mutations in the offspring of exposed males using genomics technologies. Overall, the studies suggest that increased heritable mutation induction from BaP exposure has the potential to increase the risk of tumorigenesis and other diseases in adult offspring.

FRIDAY, APRIL 17, 2015
3:15 p.m. – 3:50 p.m.

SERTOLI CELLS – IMMUNE PRIVILEGE AND NOVEL ROLES IN CELL BASED GENE THERAPY
Jannette Dufour, PhD
Texas Tech University

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

The immune privilege status of the testis has been recognized for over two centuries. This immune privileged environment is important for protecting the developing auto-antigenic germ cells from the host's immune system. Moreover, foreign tissue grafts transplanted into the testis enjoy prolonged survival. Sertoli cells (SC) are considered key players in creating this immune-privileged environment, which has led to their use in transplantation. For instance, SC survive and protect co-grafted cells, such as pancreatic islets, when transplanted as allografts or xenografts. Interestingly, we have found that successful co-transplantation of SC with islets appears to be associated with the formation of tubule-like structure. These tubules contain SC and are surrounded by smooth muscle alpha actin-positive peritubular myoid cells. Insulin-expressing islets were not located in the center of these tubules and yet enjoyed prolonged graft survival. This is similar to the testis where foreign tissue grafts and germ cells located outside of the blood-testis-barrier are still immune protected and suggests that, contrary to popular belief, testis immune privilege involves more than just sequestering auto-antigens behind the blood-testis barrier. In support of this, we have found that SC express several immunoregulatory proteins and survive transplantation by inducing regulatory cells (Tregs and macrophages). More recently, we have explored the potential use of SC in cell based gene therapy i.e. the ability of SC to deliver therapeutic proteins. When SC were engineered to transiently express insulin, using an adenoviral vector, they significantly decreased blood glucose levels after transplantation into diabetic SCID mice. To test their ability to stably produce insulin, SC were engineered to deliver insulin using a lentiviral vector. These cells survived and expressed insulin for over 50 days when transplanted as

allografts in diabetic mice. Overall, our data demonstrate that SC immunoprotect co-grafted cells and stably produce therapeutic proteins thereby strengthening the use of SC in cell based therapy. Supported by NICHD HD067400 and NIAID AI109398

FRIDAY, APRIL 17, 2015
3:50 p.m. – 4:25 p.m.

NOTCH SIGNALING IN SERTOLI CELLS
Marie-Claude Hofmann, PhD
University of Texas – MD Anderson Cancer Center

Thomas Garcia, Sean Kow, Pooja Gandhi, Brian Danysh, Marie-Claude Hofmann
Department of Endocrine Neoplasia and Hormonal Disorders, University of Texas MD Anderson Cancer Center, Houston, TX

Mammalian spermatogenesis is a highly specialized process controlled by the integration of paracrine and endocrine information. Because Sertoli cells and germ cells are in contact to each other, juxtracrine signaling is essential as well. The Notch signaling pathway is a conserved signaling system used by adjacent cells to communicate and regulate their developmental fate. In the postnatal testis, NOTCH pathway components are expressed in all testicular cells, but true activation of the system has been found only in Sertoli cells. Sertoli cells are the main component of the spermatogonial stem cell niche and provide factors indispensable for stem cell maintenance, self-renewal and differentiation. Using conditional gain- and loss-of-function mouse models, we demonstrated that NOTCH signaling in Sertoli cells is critical for the delivery of proper amounts of these factors[1,2]. Further, our studies show that canonical effectors of NOTCH signaling such as Hes1/Hey1 and HeyL down-regulate GDNF expression, counteracting the effects of FSH, and therefore regulating germ cell homeostasis. Additionally, our studies show that germ cells are the ligand-presenting cells, and are part of a negative feedback mechanism that controls their own numbers.

1. Garcia, T.X., et al. *Dev Biol* 377, 188-201 (2013).
2. Garcia, T.X., et al. *Development* 141, 4468-4478 (2014).

SATURDAY, APRIL 18, 2015
8:00 a.m. – 8:45 a.m.

Benchmark Lecture III
ANDROGEN SIGNALING IN LIFELONG HEALTH AND WELLBEING
Lee B. Smith, PhD
MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

The ageing of western societies, and the associated increase in obesity, brings with it an increase in prevalence of disorders such as cardiovascular disease, diabetes, loss of bone density, muscle strength, libido and erectile dysfunction, which are associated with reduced androgen levels in men. As Leydig cells are the source of androgens in men, establishing the mechanisms which control Leydig cell development, function and maintenance is crucial to our understanding of ageing and male health.
Adult Leydig cells develop from a stem cell population to a mitotically quiescent mature population in adulthood. We have recently used lineage-tracing to identify the adult Leydig cell stem cell population resident within the fetal testis, and demonstrated that attainment and maintenance of a full complement of Leydig cells in adulthood is dependent on the active support of Sertoli cells throughout postnatal and adult life, a completely new paradigm in testis biology [1, 2].
Whilst Sertoli cells control Leydig cell number, Leydig cell development, maturation and adult function is impacted by androgens. We show that androgenic fetal programming of Leydig cell stem cells leads to reduced Leydig cell function in adulthood [3], and that autocrine androgen action within the developing adult Leydig cell population is essential for correct maturation and retention of the Leydig cell population in adulthood [4].
Together, these new data reveal a complex system of intra-testicular signaling underpinning the correct development, maturation and lifelong support of Leydig cell function, with implication for many chronic androgen-related conditions.

1. Rebourcet et al. (2014) *Development* 141:2139-2149;
2. Rebourcet et al. (2014) *PLoS ONE* 9(8): e105687.
3. Kilcoyne et al. (2014) *PNAS* E1924–E1932
4. O'Hara et al. (2014) *FASEBJ* doi:10.1096/fj.14-255729

Speaker Abstracts

SATURDAY, APRIL 18, 2015
8:50 a.m. – 9:25 a.m.

ENDOCRINE DISRUPTORS, EARLY EXPOSURES AND SPERM FUNCTION
Jorma Toppari, MD, PhD
University of Turku, Departments of Physiology and Pediatrics, 20520 Turku, Finland

Endocrine disruptors cause both organizational effects that typically occur in early development and functional effects that occur at any time. Early exposures to antiandrogens lead to birth defects in organs that develop under androgen regulation, i.e. genitalia. Hypospadias and cryptorchidism are the most common human genital birth defects. Defects in androgen production or action can cause both conditions. In experimental animals, any antiandrogenic effect can lead to these disorders, and the antiandrogenic agents act in an additive manner. They do not need to have the same mechanism of action as long as the net effect is similar, i.e. inhibitors of androgen biosynthesis and androgen receptor antagonists have a cumulative effect when present together. While the list of antiandrogenic chemicals is increasing, we still lack knowledge how much the human fetus is exposed to these compounds. Furthermore, other endocrine disruptors may also contribute without affecting androgen signaling, e.g., estrogenic and dioxin-like compounds. The genital birth defects are the best characterized risk factors for testicular cancer and impaired semen quality, suggesting similar underlying mechanisms in pathogenesis. In addition to developmental effects, endocrine disruptors can have direct effects on sperm function by impairing the ion channel signaling. These effects come visible e.g. in disturbed movements of the sperm. Reproductive risk assessment should therefore consider both developmental and later functional effects of chemicals. Although much has been done during the last 25 years, we have only come to realize how little do we know about the reproductive effects of the chemicals that we use today.

SATURDAY, APRIL 18, 2015
9:25 a.m. – 10:00 a.m.

INFERTILITY, AGING, ART AND SPERM EPIGENETICS
Douglas T. Carrell, PhD, HCLD
University of Utah School of Medicine, Departments of Surgery (Urology), Ob-Gyn, and Human Genetics, Salt Lake City, UT

The fertilizing sperm provides both coding DNA and epigenetic marks and factors to the embryo upon fertilization. DNA methylation, histone modifications, and small non-coding RNAs are critical for spermatogenesis and are present in mature sperm. Recent evidence suggests that a subset of these epigenetic modifications have roles beyond sperm function, providing transcriptional cues for early embryonic development. Numerous associations have been observed between paternal age, lifestyle, or chemical exposures and offspring health, with alterations seen in sperm epigenetic marks. These studies implicate epigenetic mechanisms in paternally inherited offspring phenotype. This talk will outline the major epigenetic modifications established in sperm and explore possible modes of epigenetic inheritance from the sperm to the fertilized embryo. In addition, we provide evidence of environmental effects on the epigenetic state of paternal germline, and transmission of epigenetic alterations to offspring, including aging, smoking, and alcohol. Lastly, we will explore the possibility of using sperm epigenetic patterns to predict male infertility and/or poor embryogenesis in patients undergoing ART.

Speaker Abstracts

SATURDAY, APRIL 18, 2015
10:25 a.m. – 11:00 a.m.
GENETIC CAUSES OF HUMAN INFERTILITY: FROM X CHROMOSOME HIGH RESOLUTION ARRAY-CGH TO WHOLE EXOME STUDIES

Csilla Krausz
Department of Clinical, Experimental and Biomedical Sciences, University of Florence, Florence, Italy

Although numerous studies aimed at discovering new genetic factors of male infertility, studies based on mutational analysis of single candidate genes and on SNP arrays failed to provide major advances. The lack of progress is likely to be due to: i) inappropriate study design i.e. inclusion of subjects with heterogeneous semen phenotypes (from oligozoospermia to azoospermia with different testis histology); ii) non-rigorous selection for idiopathic cases; iii) inappropriate working hypothesis (analysis of common variants), iv) extremely low number of entirely sequenced genes.
The enrichment on the X chromosome of genes predominantly/ exclusively expressed in the testis makes this chromosome an especially promising target of investigation. We performed the first high resolution customized X chromosome array-CGH providing evidence for a significantly higher deletion load in men affected by severe spermatogenic failure in respect to normozoospermic men (1). This data is of relevance if we consider that epidemiologic studies revealed an association between spermatogenic failure and increased risk for reduced life expectancy and increased morbidity. Our follow-up studies led to the identification of recurrent deletions and duplications with potential clinical significance (2,3)
Azoospermia is a heterogeneous condition from a histological point of view. We performed the first systematic mutational *screening* of candidate genes involved in the development/differentiation of PGCs/spermatogonia, and in meiosis, in highly selected patients. We performed Whole Exome Sequencing in i) 2 brothers with SGA (consanguineous parents; ii) 2 unrelated SCOS patients (consanguineous parents); iii) 2 sporadic SCOS cases. Our pilot study provided us with promising results, identifying homozygous mutations in genes with potential role in the early phases of spermatogenesis or in meiosis in the patients from consanguineous families. In the 2 sporadic SCOS cases we observed predicted as “deleterious” variants which would fit with the digenic/oligogenic model observed in central hypogonadism.

Krausz et al, PlosOne 2012
Lo Giacco et al, J Med Genet. 2014
Chianese et al, Plos One 2014

Poster Session I

Poster/Short Talk #1
IMPLICATIONS OF LIFETIME FOLATE DEFICIENCY AND SUPPLEMENTATION ON INTERGENERATIONAL HEALTH
Lundi Ly, BSc[1], Donovan Chan[2], Mylene Landry[2], Nathalie Behan[3], Amanda MacFarlane[3] and Jacquetta Trasler[4]
[1]Department of Human Genetics, McGill University, Montreal, QC, Canada; [2]Research Institute of the McGill University Health Centre at the Montreal Children's Hospital; [3]Health Canada, Ottawa ON, Canada; [4]Departments of Human Genetics, Pediatrics, and Pharmacology and Therapeutics, McGill University
(Presented by: Lundi Ly, BSc)

Poster/Short Talk #2
THE RHOX10 HOMEOBOX TRANSCRIPTION FACTOR DRIVES THE INITIAL ESTABLISHMENT OF SPERMATOGONIAL STEM CELLS
Hye−Won Song, PhD[1], Kyle Orwig, PhD[2] and Miles Wilkinson, PhD[1]
[1]UCSD; [2]University Pittsburgh
(Presented by: Hye−Won Song, PhD)

Poster/Short Talk #3
EVALUATING L1 TRANSGENES REGULATED BY THE ENDOGENOUS MOUSE L1 PROMOTER
Simon Newkirk[1], James Rosser[1] and Wenfeng An[2]
[1]Washington State University; [2]South Dakota State University
(Presented by: Wenfeng An)

Poster/Short Talk #4
OVEREXPRESSION OF ID4 ALTERS CELL CYCLE PROGRESSION AND TRANSITION FROM THE STEM CELL TO PROGENITOR STATE IN MOUSE SPERMATOGONIA
Qi-En Yang[1], Melissa Oatley[1], Fred Sablitzky[2] and Jon Oatley[1]
[1]Washington State University; [2]University of Nottingham
(Presented by: Jon Oatley)

Poster/Short Talk #5
DIRECT GERMLINE EDITING IN SPERMATOGONIA ELIMINATES CRISPR/CAS9 CATALYZED ANIMAL MOSAICISM
Karen Chapman, BS[1], Gerardo Medrano, BS[1], Priscilla Jaichander, PhD[1], Jaideep Chaudhary, BS[1], Marcelo Nobrega, PhD[2], James Hotaling, MD[3], Carole Ober, PhD[2] and F. Kent Hamra, PhD[1]
[1]UT Southwestern Medical Center; [2]University of Chicago; [3]University of Utah
(Presented by: F. Kent Hamra, PhD)

Poster/Short Talk #6
THE ROLE OF PERITUBULAR MYOID (PM) CELLS IN THE REGULATION OF SPERMATOGONIAL STEM CELL (SSC) SELF-RENEWAL, PROLIFERATION AND DIFFERENTIATION IN THE TESTIS NICHE
Liang-Yu Chen, William Willis and Mitch Eddy
Gamete Biology Group/ RDBL/NIEHS / NIH
(Presented by: Liang-Yu Chen)

Poster/Short Talk #7
CHOLESTEROL TRAFFICKING FOR STEROID BIOSYNTHESIS IN MA−10 MOUSE TUMOR LEYDIG CELLS.
Sathvika Jagannathan, MSc[1], Seimia Chebbi, BSc[1], Francoise Hullin−Matsuda, PhD[2], Toshihide Kobayashi, PhD[2] and Vassilios Papadopoulos, PhD[1]
[1]Research Institute of the McGill University Health Centre and Department of Medicine, McGill University, Montreal, Quebec, H3G 1A4, Canada; [2]Lipid Biology Laboratory, RIKEN Advanced Science Institute, Wako, Saitama, Japan
(Presented by: Sathvika Jagannathan, MSc)

Poster/Short Talk #8
REQUIREMENT FOR ADENOSINE DEAMINASE CONTAINING PROTEINS IN MALE GERM CELL DEVELOPMENT
Elizabeth Snyder, PhD, Anuj Srivastava, PhD and Robert Braun, PhD
The Jackson Laboratory
(Presented by: Elizabeth Snyder, PhD)

Poster Session I

Poster/Short Talk #9
THE TESTICULAR TRANSCRIPTOME OF THE RHESUS MONKEY (MACACA MULATTA) ASSOCIATED WITH THE DECISION BY UNDIFFERENTIATED TYPE A SPERMATOGONIA TO COMMIT TO A PATHWAY OF DIFFERENTIATION
Suresh Ramaswamy, PhD[1], Gary Marshall, PhD[2], Seyedmehdi Nourashrafeddin, PhD[3], Rahil Sethi, PhD[4], Uma Chandran, PhD[4], William Walker, PhD[5] and Tony Plant, PhD[5]
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(Presented by: Suresh Ramaswamy, PhD)

Poster/Short Talk #10
TESTES SPECIFIC PROTEASE 50 (TSP50) MODULATES MALE FERTILITY
Carolina Jorgez, PhD, Nathan Wilken, BS, Alexander Pastuszak, MD, PhD and Dolores Lamb, PhD
Baylor College of Medicine
(Presented by: Carolina Jorgez, PhD)

Poster #11
GENERATION OF FERTILE OFFSPRING FROM KITW/KITWV MICE THROUGH DIFFERENTIATION OF GENE CORRECTED NUCLEAR TRANSFER EMBRYONIC STEM CELLS
Yan Yuan, PhD
(Presented by: Yan Yuan, PhD)

Poster #12
HORMONE INDUCED DIFFERENTIAL TRANSCRIPTOME ANALYSIS OF RAT SERTOLI CELLS DURING POSTNATAL TESTICULAR DEVELOPMENT
Indrashis Bhattacharya, PhD[1], Mukkesh Gautam, PhD[2], Bholashankar Pradhan, PhD[3] and Subeer Majumdar, PhD[3]
[1]HNB Garhwal University, Srinagar ,India; [2]The Ken & Ruth Davee Department of Neurology, Northwestern University, Chicago, USA.; [3]Division of Cellular Endocrinology, National Institute of Immunology, New Delhi, India.
(Presented by: Indrashis Bhattacharya, PhD)

Poster #13
CYSTEINE−RICH SECRETORY PROTEIN 2 (CRISP2) IS REQUIRED FOR FULL MALE FERTILITY
Shu Ly Lim, PhD, Duangporn Jamsai, PhD, Anne E. O'Connor, Bsc, Donna Merriner, Bsc and Moira O'Bryan, PhD
Department of Anatomy and Developmental Biology, Monash University, Vic, Australia
(Presented by: Shu Ly Lim, PhD)

Poster #14
DYNAMIC CHANGES IN THE EXPRESSION OF APOPTOSIS−RELATED GENES DURING RETINOIC ACID−INDUCED NEONATAL RAT GONOCYTE DIFFERENTIATION AND IN HUMAN TESTICULAR SEMINOMAS
Gurpreet Manku, PhD and Martine Culty, PhD
The Research Institute of the McGill University Health Centre, and Departments of Pharmacology & Therapeutics and Medicine, McGill University, Montreal, Quebec, Canada
(Presented by: Gurpreet Manku, PhD)

Poster #15
IDENTIFICATION AND CHARACTERIZATION OF A BOVINE SPERM ACROSOMAL STRUCTURAL PROTEIN AND ITS MECHANISM OF INTERACTION WITH ACROSOMAL HYDROLASES
Linda Smith, BS, Allen Mcnamara, BS, Luisa Hernandez−Encarnacion, Ilza Medina−Ortiz and Subir Nagdas, PhD
Fayetteville State University
(Presented by: Linda Smith, BS)

Poster #16
HISTONE H4K20 DEMETHYLASE REGULATES SPERMATOGENESIS
Charlie Degui Chen
(Presented by: Charlie Degui Chen)

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Poster #17
NOVEL ACTION OF FOLLICLE STIMULATING HORMONE (FSH) ON MOUSE TESTICULAR STEM CELLS
Hiren Patel, MSc[1] and Deepa Bhartiya, PhD[2]
[1]PhD Student at Stem Cell Biology Department, NIRRH, Parel, Mumbai; [2]Scientist 'E', Head of Stem Cell Biology Department, NIRRH, Parel, Mumbai
(Presented by: Hiren Patel, MSc[1])

Poster #18
IMPORTANCE OF SOMATIC NICHE IN REGULATING TESTICULAR STEM CELLS DIFFERENTIATION INTO SPERM
Sandhya Anand, MSc, Kalpana Sriraman, PhD, Hiren Patel, MSc, Smita Bhutda, MSc and Deepa Bhartiya, PhD
National Institute for Research in Reproductive Health
(Presented by: Sandhya Anand, MSc)

Poster #19
DMRT1 IN SERTOLI CELLS REGULATES ADULT LEYDIG CELL MATURATION
Leslie Heckert, PhD, Valentine Agbor, PhD and Tatiana Karpova, PhD
University of Kansas Medical Center
(Presented by: Leslie Heckert, PhD)

Poster #20
RETINOIC ACID TRIGGERS C−KIT GENE EXPRESSION IN SPERMATOGONIAL STEM CELLS ENTAILING ACTIVE PARTICIPATION OF AN ENHANCEOSOME CONSTITUTED BETWEEN TRANSCRIPTION FACTOR BINDING SITES RARE, PU.1 AND ETS
Swanand Koli, MSc[1], Ayan Mukherjee, PhD[2] and Kudumula Venkata Rami Reddy, PhD[3]
[1]Division of Molecular Immunology & Microbiology, National Institute For Research In Reproductive Health(NIRRH,ICMR); [2]Department of Biological Science, Kent State University; [3]Division of Molecular Immunology & Microbiology National Institute For Research In Reproductive Health(NIRRH,ICMR)
(Presented by: Swanand Koli, MSc)

Poster #21
DIFFERENTIAL EFFECTS OF COMBINED GENISTEIN AND DEHP ON TESTICULAR CELL LIPID HOMEOSTASIS AND STEROID PRODUCTION
Steven Jones, MSc[1], Annie Boisvert, MSc[1], Gurpreet Manku, PhD[1], Francoise Hullin−Matsuda, PhD[2], Peter Greimel, PhD[2], Toshihide Kobayashi, PhD[2] and Martine Culty, PhD[1]
[1]McGill University; [2]RIKEN
(Presented by: Steven Jones, MSc)

Poster #22
AN EMERGING ROLE FOR ACTIVIN B IN TESTICULAR FUNCTION AND PATHOLOGY
Mark Hedger, PhD[1], Wendy Winnall, PhD[2], Julie Muir[1] and Susan Hayward[1]
[1]MIMR−PHI Institute of Medical Research, Monash University; [2]Department of Microbiology and Immunology, University of Melbourne
(Presented by: Mark Hedger, PhD)

Poster #23
XENOSCREENING OF IRRADIATION EFFECTS IN A PRIMATE TESTIS: MINIMIZING ANIMAL INPUT AND MAXIMIZING READOUT
Ina Tröndle, Joachim Wistuba, PhD, Anu David, PhD, Westernströer Birgit, PhD, Sandhowe Reinhild, Nicole Terwort, Nina Neuhaus, PhD, Stefan Schlatt, PhD
Univ Münster
(Presented by: Ina Tröndle)

Poster #24
HUMAN INFERTILITY AND GENE EXPRESSION
Arka Baksi, MSc[1], Dhananjay Sathe, MS[2], S.S Vasan, MS[2] and Rajan Dighe, PhD[3]
[1]IISc; [2]Ankur Hospital; [3]Indian Institute of Science
(Presented by: Rajan Dighe, PhD)

Poster Session I

Poster #25
CONTRAST ENHANCED ULTRASOUND CONFIRMS TESTICULAR CIRCULATION TO BE HAMPERED BY DISTURBED VASCULARIZATION IN 41, XXY* MICE, A MODEL FOR KLINEFELTER SYNDROME
Oliver Damm, Dr[1], Ann−Sophie Warmeling, MSc[1], Reinhild Sandhowe-Klaverkamp[1], Steffi Werler, Dr[1], Katharina Körner, MSc[1], Stefan Schlatt, Prof, Dr[1], Jörg Stypmann, PD, Dr[2], Michael Kuhlmann, Dr[3], Richard Holtmeier[3], Michael Zitzmann, Prof, Dr[4], Frank Tüttelmann, PD, Dr[5], Jörg Gromoll, Prof, Dr[1] and Joachim Wistuba, Dr[1]
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(Presented by: Stefan Schlatt, Prof, Dr)

Poster #26
DNA METHYLATION AND CONSERVED GERM CELL EXPRESSION OF DAZ FAMILY GENES
Chenwang Zhang, BS, Peng Xue, MD, Gao Liuze, BS and Eugene Yujun Xu, PhD
Nanjing Medical University
(Presented by: Chenwang Zhang, BS)

Poster #27
EFFECT OF A TEMPORARY INHIBITION OF GDNF SIGNALING ON THE NUMBERS AND REPLICATION OF GFRA1+ AS, APR AND AAL SPERMATOGONIA
Nicole Parker, BS and William Wright, PhD
Johns Hopkins Bloomberg School of Public Health
(Presented by: Nicole Parker, BS)

Poster #28
MULTICELLULAR HUMAN TESTICULAR ORGANOID: A NOVEL IN VITRO GERM CELL AND TESTICULAR TOXICITY MODEL
Samuel Pendergraft, MS[1], Hooman Sadri−Ardekani, MD, PhD[2], Tanya Reid, BS[3], Anthony Atala, MD[2] and Colin Bishop, PhD[1]
[1](1) Molecular Medicine and Translational Science Graduate Program, Wake Forest University Health Sciences, Winston−Salem, NC (2) Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston−Salem, NC; [2](1) Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston−Salem, NC (2) Department of Urology, Wake Forest School of Medicine, Winston−Salem, NC; [3](1) Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston−Salem, NC
(Presented by: Samuel Pendergraft, MS)

Poster #29
THE UBIQUITIN LIGASE HUWE1 IS REQUIRED FOR ESTABLISHMENT AND MAINTENANCE OF SPERMATOGONIA
Ellis Fok, PhD[1], Rohini Bose, MSc[1], Wenming Xu, MD, PhD[2], Martine Culty, PhD[3], Makoto Nagano, DVM, PhD[3], Hsiao Chang Chan, PhD[4], Antonio Iavarone, MD[5], Anna Lasorella, MD[5] and Simon Wing, MD[1]
[1]Department of Medicine, McGill University; [2]Laboratory of Reproductive Medicine, Sichuan University; [3]Department of Obstetrics & Gynecology, McGill University; [4]School of Biomedical Sciences, The Chinese University of Hong Kong; Institute for Cancer Genetics, Columbia University Medical Center
(Presented by: Ellis Fok, PhD)

Poster #30 – WITHDRAWN

Poster #31
INACTIVATION OF UBIQUITIN LIGASE HUWE1 PERTURBS SPERMATOGONIAL DIFFERENTIATION AND TRANSITION TO MEIOSIS
Rohini Bose, MSc[1], Ellis Fok, PhD[1], Kai Sheng, BSc[1], Wenming Xu, PhD[2], Hsiao Chang Chan, PhD[3], Antonio Iavarone, MD[4], Anna Lasorella, MD[4] and Simon S. Wing, MD[1]
[1]McGill University; [2]Sichuan University; [3]The Chinese University of Hong Kong; [4]Columbia University
(Presented by: Rohini Bose, MSc)

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Poster #32 – WITHDRAWN

Poster #33
SUPPLEMENTATION WITH HIGH DOSE FOLATE AFFECTS THE SPERM EPIGENOME IN MEN PRESENTING WITH INFERTILITY

Mahmoud Aarabi, MD, PhD[1], Maria C. San Gabriel, PhD[2,3], Donovan Chan, PhD[4], Armand Zini, MD[2,3] and Jacquetta Trasler, MD, PhD[5]
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(Presented by: Mahmoud Aarabi, MD, PhD)

Poster Session II

Poster #34
KINETICS OF STEM CELL DIVISIONS IN PRIMARY CULTURES OF UNDIFFERENTIATED SPERMATOGONIA
Aileen Helsel and Jon Oatley
Washington State University
(Presented by: Aileen Helsel)

Poster #35
MAINTENANCE OF HUMAN SPERMATOGONIAL STEM CELLS ON LAMININ
Hanna Valli, PhD and Kyle Orwig, PhD
University of Pittsburgh School of Medicine
(Presented by: Hanna Valli, PhD)

Poster #36 – WITHDRAWN

Poster #37
STUDY OF RNA BIOMARKERS OF NORMAL SPERMIOGENESIS IN NORMAL SEMEN AND SPERM VIA TRANSCRIPTOME ANALYSIS
Alexander Yatsenko, MD, PhD[1], Archana Kishore, PhD[1], Andrew Georgiadis, BS[1], Randy Beadling, BS[1], Etta Volk[2], Joseph Sanfilippo, MD[3], Thomas Jaffe, MD[4], James Lyons−Weiler, PhD[5] and Tamanna Sultana, PhD[5]
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(Presented by: Alexander Yatsenko, MD, PhD)

Poster #38
REQUIREMENT FOR MOV10L1 RNA HELICASE ACTIVITY IN THE PROCESSING OF PIRNA PRECURSORS
Qi Fu[1], Anastassios Vourekas, PhD[1], Ke Zheng, PhD[2], Erica Goode and P. Jeremy Wang, PhD[1]
[1]The University of Pennsylvania; [2]Nanjing Medical University School
(Presented by: Qi Fu)

Poster #39
INHIBITION OF RALDH ENZYMES ALTERS TESTICULAR RA AVAILABILITY AND AFFECTS MULTIPLE STEPS OF SPERM PRODUCTION.
Travis Kent[1], Sam Arnold[2], Rachael Fasnacht[1], Ross Rowsey[1], Debra Mitchell[1], Cathryn Hogarth[1], Pat Hunt[1], Nina Isoherranen[2] and Michael Griswold[1]
[1]Washington State University; [2]University of Washington
(Presented by: Travis Kent)

Poster #40
NORMAL SPERM HEAD MORPHOGENESIS REQUIRES ADP−RIBOSYLTRANSFERASE 11 (PARP11) IN MICE
Mirella Meyer-Ficca, PhD[1], Motomasa Ihara, MD, PhD[2], Jessica Bader, BS[1], N. Adrian Leu, BS[3], Sascha Beneke, PhD[4] and Ralph G. Meyer, PhD[1]
[1]Utah State University; [2]Tohoku University Graduate School of Medicine, Sendai, Japan; [3]University of Pennsylvania; [4]University of Zurich, Switzerland
(Presented by: Mirella Meyer-Ficca, PhD)

Poster #41
THE FIRST WAVE OF MURINE SPERMATOGENESIS RELIES ON AN INTACT RAR SIGNALING MECHANISM
Cathryn Hogarth, PhD[1], Debra Mitchell, BSc[1], Jenny Onken, BSc[1], Minghan Tong, PhD[2] and Michael Griswold, PhD[1]
[1]Washington State University; [2]SIBCB
(Presented by: Cathryn Hogarth, PhD)

Poster #42
CHARACTERIZING THE SPERMATOGONIAL RESPONSE TO RETINOIC ACID DURING THE INITIATION OF SPERMATOGENESIS IN THE NEONATAL MURINE TESTIS.
Kellie O'Rourke, Cathryn Hogarth, Debra Mitchell, Jon Oatley and Michael Griswold
(Presented by: Kellie O'Rourke)

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Poster #43
CLASSICAL RA SIGNALING IS NECESSARY IN LEYDIG CELLS FOR NORMAL SPERMATOGENESIS.
Estela Arciniega, Debra Mitchell, Jennifer Onken, Cathryn Hogarth and Michael Griswold
Washington State University
(Presented by: Estela Arciniega)

Poster #44
HOW DO HISTONES CONVEY INFORMATION BETWEEN GENERATIONS?
Mirella L. Meyer−Ficca, PhD[1], Motomasa Ihara, MD, PhD[2], Fan Li, PhD[3], Brian D. Gregory, PhD[3], Richard M. Schultz, PhD[3] and Ralph Meyer, PhD[4]
[1]Utah State University, Logan, UT; [2]Tohoku University Graduate School of Medicine, Sendai, Japan; [3]University of Pennsylvania, Philadelphia, PA; [4]Utah State University
(Presented by: Ralph Meyer, PhD)

Poster #45 – Switched to Short Talk/Poster #2

Poster #46
CYCLOPHOSPHAMIDE (CPA) TREATMENT ALTERS THE EXPRESSION OF MEMBERS OF THE ZIP FAMILY ZINC TRANSPORTERS IN PACHYTENE SPERMATOCYTES.
Anne Marie Downey[1], Barbara Hales, PhD[2] and Bernard Robaire, PhD[3]
[1]Department of Pharmacology and Therapeutics, McGill University; [2]Department of Pharmcology and Therapeutics, McGill University; [3]Department of Pharmacology and Therapeutics, Department of Obstetrics and Gynecology, McGill University
(Presented by: Anne Marie Downey)

Poster #47
PATERNAL USE OF TOBACCO, ALCOHOL AND EXPOSURE TO PESTICIDES MAY DAMAGE SPERM CHROMATIN STRUCTURE: ROLE IN CHILDHOOD CANCER − RETINOBLASTOMA (NON-FAMILIAL SPORADIC)
Shiv Basant Kumar, MSc[1], Bhavna Chawla, MD[2] and Rima Dada, MD, PhD[1]
[1]Lab. for Molecular Reproduction and Genetics, Deptartment of Anatomy, All India Institute of Medical Sciences, New Delhi; [2]Ocular Oncology & Pediatric Ophthalmology Service, Rajendra Prasad Centre for Ophthalmic Sciences
(Presented by: Shiv Basant Kumar, MSc)

Poster #48
HUMANIN ANALOGUE (HNG) PREVENTS MALE GERM CELL APOPTOSIS AND ATTENUATES WHITE CELL SUPPRESSION INDUCED BY TEMOZOLOMIDE IN SEVERE COMBINED IMMUNO−DEFICIENCY (SCID) MICE BEARING MEDULLOBLASTOMA
Yue Jia, MD, PhD, Yanhe Lue, MD, Ronald S. Swerdloff, MD, Joseph L. Lasky, MD, PhD, Eduard H. Panosyan, MD, PhD, Jenny Dai−Ju, MD, PhD, Christina Wang, MD
LA BioMed Research Institute & Harbor−UCLA Medical Center
(Presented by: Yue Jia, MD, PhD)

Poster #49
COMPARISON OF GDNF MRNA EXPRESSION IN TESTES OF FERTILE AND INFERTILE MEN AND FERTILE MICE.
Dolly Singh, BS[1], Kyle Orwig, PhD[2], Darius A. Paduch, MD, PhD[3], Peter N. Schlegel, MD, FACS[3] and William Wright, PhD[4]
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(Presented by: Dolly Singh, BS)

Poster #50
OXIDATIVE STRESS: A DIAGNOSTIC APPROACH TOWARDS VARICOCELE
Arozia Moazzam, MD, PhD[1], Rakesh Sharma, PhD[2] and Ashok Agarwal, PhD[2]
[1]University of Lahore, Pakistan; [2]Cleveland Clinic, Ohio, USA
(Presented by: Arozia Moazzam, MD, PhD)

Poster Session II

Poster #51
IN VITRO SEMINIFEROUS TUBULE FORMATION FROM HUMAN ISOLATED TESTIS CELLS
Raquel Alvarenga, MSc[1], Gleide Avelar, PhD[1], Samyra Lacerda, PhD[1], Anne-Pascale Satie, PhD[2], Dominique Mahe-Poiron, PhD[2], Giulia Matusali, PhD[2], Nathalie Dejucq-Rainsford, PhD[2] and Luiz Renato França, PhD[1]
[1]Federal University of Minas Gerais; [2]Université de Rennes 1
(Presented by: Gleide Avelar, PhD)

Poster #52
SUMOYLATION REGULATES G2/MI TRANSITION IN MEIOTIC SPERMATOCYTES
Yuxuan Xiao, PhD[1], Benjamin Lucas, PhD[1], Tania Schiff, BSc[1] and Margarita Vigodner, PhD[2]
[1]Stern College, Yeshiva Universirty; [2]Stern College and Albert Einstein College of Medicine, Yeshiva University
(Presented by: Margarita Vigodner, PhD)

Poster #53 – WITHDRAWN

Poster #54
SUBPOPULATIONS OF NEONATAL MOUSE UNDIFFERENTIATED SPERMATOGONIA DEFINED BY BI−MODALLY EXPRESSED GENES
Thu Nguyen[1], Kazadi N. Mutoji, PhD[1], Christopher B. Geyer, PhD[2], Jon M. Oatley, PhD[3], John R. McCarrey, PhD[1] and Brian P. Hermann, PhD[1]
[1]Department of Biology, University of Texas at San Antonio; [2]Department of Anatomy and Cell Biology, East Carolina University; [3]Center for Reproductive Biology, Washington State University
(Presented by: Brian P. Hermann, PhD)

Poster #55
REGULATION OF LEVELS OF OCT4 PROTEIN IN MOUSE SPERMATOGENESIS
Yu Zheng, Jennifer Balke and Christina Dann, PhD
Indiana University
(Presented by: Christina Dann, PhD)

Poster #56
DISTINCT PHENOTYPE OF SERTOLI CELLS LOCATED IN THE TRANSITION REGION BETWEEN THE SEMINIFEROUS TUBULES AND RETE TESTIS
Andre Figueiredo, Luiz Renato França and Guilherme Costa
(Presented by: Guilherme Costa)

Poster #57
THE SERINE/THEONINE KINASE 35 (STK35) ALLELE LOCUS ENCODES BOTH CODING AND NON−CODING RNAS AND IS REQUIRED FOR NORMAL FERTILITY
Yoichi Miyamoto, PhD[1], Penny Whiley, BSc[2], Hoey Goh, BSc[3], Chin Wong, BSc[3] and Kate Loveland, PhD[4]
[1]Laboratory of Nuclear Transport Dynamics, National Institute of Biomedical Innovation, Osaka, Japan; [2]Monash Institute of Medical Research− Prince Henry's Institute for Medical Research Institute, Clayton, Victoria, Australia; [3]Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, Clayton, Victoria, Australia; [4]Monash University/ MIMR−PHI Institute
(Presented by: Kate Loveland, PhD)

Poster #58
EFFECT OF GROWTH HORMONE ON TESTICULAR DYSFUNCTION INDUCED BY METHOTREXATE (MTX) IN RATS
Hamed Serati−Nouri, PhD
Drug Applied Research Center Tabriz University of Medical Sciences
(Presented by: Hamed Serati−Nouri, PhD)

Poster #59
THE TESTOSTERONE−MEDIATED BLOCK IN SPERMATOGONIAL DIFFERENTIATION AFTER CYTOTOXIC EXPOSURE: ROLE OF LEYDIG AND POSSIBLY SERTOLI CELLS
Gunapala Shetty, PhD[1] and Marvin Meistrich, PhD[2]
[1]University of Texas M.D. Anderson Cancer center; [2]University of Texas M.D. Anderson Cancer Center
(Presented by: Gunapala Shetty, PhD)

Poster Session II

Poster #60
CHROMATIN PATTERN AND STATUS OF GLOBAL DNA METHYLATION IN HUMAN SPERMATOZOA DNA PATTERN IN HUMAN SPERM VIA CMA3 AND 5−METHYL CYTOSINE STAINING
Jaleh Barzideh, MPhil in Medical Genetic
(Presented by: Jaleh Barzideh, MPhil in Medical Genetic)

Poster #61
DISSECTING GERM CELL METABOLISM THROUGH NETWORK MODELING
Leanne Whitmore, BS Biochemistry and Ping Ye, PhD Biochemistry
Washington State University
(Presented by: Leanne Whitmore, BS Biochemistry)

Poster #62
PROGRESSIVE DECLINE IN TESTICULAR FUNCTION IN LEPTIN-RECEPTOR-DEFICIENT (LEPR−DB/LB) OBESE MICE
Jennifer Long and Christopher Pearl
Western Michigan University
(Presented by: Christopher Pearl)

Poster #63
STEROIDOGENIC FATE OF LEYDIG CELLS THAT REPOPULATE THE TESTES OF AGED AND YOUNG BROWN NORWAY RATS AFTER IN VIVO ADMINISTRATION OF ETHANE DIMETHANESULFONATE (EDS)
Haolin Chen, PhD and Barry Zirkin, PhD
Johns Hopkins School of Public Health
(Presented by: Haolin Chen, PhD)

Poster #64
COACTIVATOR−ASSOCIATED ARGININE METHYLTRANSFERASE 1 ORCHESTRATES SPERMIOGENESIS AND IS ESSENTIAL FOR MALE FERTILITY IN MICE
Jianqiang Bao
University of Texas MD Anderson Cancer Center
(Presented by: Jianqiang Bao)

Poster #65
COMPARATIVE STUDY OF TWO MOUSE MUTANTS TO ELUCIDATE THE ROLE OF SLX4 DURING PROPHASE I OF MAMMALIAN MEIOSIS
Kadeine Campbell-Peterson, Kim Holloway, PhD and Paula Cohen, PhD
Cornell University
(Presented by: Kadeine Campbell-Peterson)

Poster #66
MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) SYNERGIZES WITH GDNF TO INDUCE MIGRATION OF SPERMATOGONIAL CELLS.
Mahmoud Huleihel, PhD[1], Dimitry Lan, MSc[1] and Eitan Lunenfeld, MD[2]
[1]Ben-Gurion University; [2]Soroka University Medical Center and Ben-Gurion University
(Presented by: Mahmoud Huleihel, PhD)

Poster #67
A GENE PARALOG PAIR THAT ACTS AS A MOLECULAR RHEOSTAT TO CONTROL MALE GERM CELL DEVELOPMENT
Eleen Shum, BS and Miles Wilkinson, PhD
UCSD
(Presented by: Miles Wilkinson, PhD)

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Poster Session I

Poster/Short Talk #1
IMPLICATIONS OF LIFETIME FOLATE DEFICIENCY AND SUPPLEMENTATION ON INTERGENERATIONAL HEALTH

Lundi Ly, BSc[1], Donovan Chan[2], Mylene Landry[2], Nathalie Behan[3], Amanda MacFarlane[3] and Jacquetta Trasler[4]
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(Presented by: Lundi Ly, BSc)

Introduction: The role of epigenetic modifications such as DNA methylation in developmental programs is undergoing continual research. New studies have shown that embryos are highly sensitive to signals from the gametes and the environment. Furthermore, during prenatal development that disrupt gamete epigenetic reprogramming, are further associated with adult disease and intergenerational effects. DNA methylation pattern acquisition in developing male germ cells during fetal growth requires adequate supply of methyl donors. The folate cycle is involved in the production of methyl groups necessary for methylation reactions. Previous studies showed that either postnatal folate deficiency (FD) or supplementation (FS) could alter the sperm epigenome. The main objective of this study was to determine if lifetime FS or FD induce an aberrant epigenetic landscape in germ cells detrimental to offspring health.
Methods: Female mice (F0; n=15) were placed on one of four amino acid controlled diets: a basal diet (FCD; 2mg folate/kg diet), a 20−fold folate supplemented diet (20FS), a 10− fold folate supplemented diet (10FS) or a 7−fold deficient diet (7FD). F0 females were mated to produce F1 litters whose germ cells were exposed to the folate diets through lifetime. F1 males were weaned onto their respective prenatal diets. F2 and F3 litters, unexposed to the folate treatments, were subsequently generated. Tissues and organs of interest were collected, and genome−wide DNA methylation analysis by reduced representation bisulfite sequencing (RRBS) was performed.
Results: Despite no apparent health effects in the F1 males, F2 litters derived from 7FD and 20FS exposed sperm are significantly smaller than FCD F2 litters at weaning. Preliminary analysis of RRBS results from F1 sperm (n = 5) demonstrate that perinatal exposure to 7FD, 10FS, and 20FS diets resulted in 153, 132 and 114 differentially methylated (DM) loci, respectively. Affected regions included intergenic, intron, exon, promoter, 5' and 3' UTR sequences. Ingenuity Pathway Analysis of associated genes from DM loci implicated various affected pathways such as those involved in embryo development and cell cycle regulation.
Conclusion: These results suggest that lifetime FD and FS can impact sperm development and offspring health. DNA methylation changes in the sperm following these lifetime exposures offer a potential mechanism of action.
(Supported by CIHR and CEEHRC).

Poster/Short Talk #2
THE RHOX10 HOMEOBOX TRANSCRIPTION FACTOR DRIVES THE INITIAL ESTABLISHMENT OF SPERMATOGONIAL STEM CELLS

Hye−Won Song, PhD[1], Kyle Orwig, PhD[2] and Miles Wilkinson, PhD[1]
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(Presented by: Hye−Won Song, PhD)

Introduction & Objective: The X-linked reproductive homeobox (Rhox) gene cluster encodes transcription factors selectively expressed in the male and female reproductive tracts. The only RHOX cluster family member with a known role in male reproduction is RHOX5, a Sertoli cell−expressed transcription factor that we previously demonstrated promotes male germ cell survival and sperm motility. To identify the function of the entire Rhox gene cluster, we inserted loxP sites at both ends of this ~700-kb gene cluster to conditionally delete all 33 Rhox genes.
Methods: Mouse gene targeting and recombineering, histological analysis, RNA-seq analysis, RNA interference, transfection, qPCR analysis.
Results: We found that conditional loss of the Rhox cluster in male germ cells causes two main defects: (i) a progressive decline in spermatogenesis after the first wave of spermatogenesis whose characteristics suggested a loss of spermatogonial stem cells (SSCs) and (ii) strongly reduced spermatid progression after the first wave of spermatogenesis. We elected to follow up on the putative SSC defect. We found that one Rhox cluster family member—Rhox10—is highly expressed in undifferentiated spermatogonia and thus was a good candidate to exert functions in SSCs. To test this, we generated Rhox10−null mice using standard gene targeting approaches. Histological analysis of these Rhox10−null mice showed that they had the same "progressive worsening" defect as Rhox cluster−null mice. Germ cell transplantation analysis directly demonstrated that postnatal Rhox10−null testes contained dramatically reduced number of SSCs. As further supporting evidence, marker analysis showed that these mutant mice had reduced numbers of germ cells expressing several SSC and undifferentiated spermatogonia markers, including PLZF, GFRα1, PAX7, and ID4. FACS

analysis of Id4−Gfp reporter mice revealed that the GFP−HI+ germ cell subset that normally emerges at P3 failed to appear at this time point in Rhox10−null mice. RNA−seq analysis performed on early postnatal Rhox10−null vs. control littermate testes revealed that many genes involved in SSC maintenance are positively regulated by RHOX10, some of which appear to be direct targets, based on ChIP and reporter analysis.
Conclusions: Our results support a model in which RHOX10 is a key upstream regulator of a transcriptional network promoting the initial establishment and maintenance of SSCs.

Poster/Short Talk #3
EVALUATING L1 TRANSGENES REGULATED BY THE ENDOGENOUS MOUSE L1 PROMOTER
Simon Newkirk[1], James Rosser[1] and Wenfeng An[2]
[1]Washington State University; [2]South Dakota State University
(Presented by: Wenfeng An)

Introduction: Long interspersed element type 1 (LINE−1, or L1) retrotransposons make up 17% of the human genome and are major drivers of genetic variation and genomic instability. Given the abundant and repetitive nature of endogenous L1s it is very difficult to identify accurately when and where new insertions take place. The objective of this study is to develop an L1 mouse model that represents the endogenous L1 activity to facilitate studies on mechanisms of L1 mobilization and its impact on the genome in animals.
Methods: We constructed multiple independent mouse lines through pronuclear microinjection of a single L1 transgene. To recapitulate transcriptional and posttranscriptional regulation of endogenous mouse L1s by the PIWI-interacting RNA (piRNA)/DNA methylation pathway, the L1 transgene contained the endogenous mouse L1 promoter. We investigated DNA methylation dynamics by bisulfite sequencing in fetal, neonatal and adult testes. Transgenic expression was determined by RT−PCR and RNA in situ hybridization.
Results: Among three mouse lines examined, we observed a clear correlation between the transgene copy number and DNA methylation profile during a time window when the germline genome is epigenetically remodeled. Consistent with RT−PCR data, RNA in situ results showed reduced transgene expression in adult mouse testes carrying high−copy transgenes. Therefore, the single−copy transgene is clearly preferred to represent endogenous L1s during embryonic and adult time points. We used droplet digital PCR to quantitatively determine the frequency of L1 retrotransposon during mouse development. Surprisingly, we found low but reproducible levels of de novo insertions not only in testis but also in several somatic tissues, a finding that is consistent with previous reports of low levels of expression in somatic tissues from endogenous human L1 elements. Currently, we are using this L1 model to quantify retrotransposition activities in piRNA pathway mutants.
Conclusion: Our work highlights the importance of using a single−copy L1 transgene in modeling endogenous L1 regulation and expression in mice. Further analysis will help to delineate the timing of L1 retrotransposition and to evaluate the role of L1−mediated genomic instability in piRNA−deficient animals.

Poster/Short Talk #4
OVEREXPRESSION OF ID4 ALTERS CELL CYCLE PROGRESSION AND TRANSITION FROM THE STEM CELL TO PROGENITOR STATE IN MOUSE SPERMATOGONIA
Qi-En Yang[1], Melissa Oatley[1], Fred Sablitzky[2] and Jon Oatley[1]
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(Presented by: Jon Oatley)

Introduction: Continual spermatogenesis relies on self-renewal of spermatogonial stem cells (SSCs) to maintain a foundational pool that arises in neonatal development from prospermatogonial precursors. Progenitor spermatogonia periodically arise from the SSC pool, transiently amplify in number, and then transition to a differentiating pathway. At present, the molecular mechanisms underpinning the SSC state are undefined. Recently, we established that the SSC pool in mice is marked by expression of inhibitor of DNA binding 4 (ID4).
Methods: To explore further the role of ID4, we generated a transgenic mouse line possessing an Id4 flox−stop−flox conditional overexpression transgene. These mice were crossed with Ddx4−cre transgenic mice to generate a model with ectopic expression of ID4 (ID4OE) beginning at embryonic day 15 in prospermatogonia of males and oogonia of females.
Results: At adulthood, female overexpression mice and control male littermates were fertile but ID4OE males were sterile. Testis weight of ID4OE males was reduced to only 20% of controls at postnatal day (PD) 60. Histological assessment of seminiferous tubules revealed that the majority contained Sertoli cells and a few undifferentiated spermatogonia at PD 60 for ID4OE mice but the spermatogonia were absent at PD 180. Total germ cell number during the neonatal period of PD 0−3 was not different between control and ID4OE males. Also, the number of proliferative germ cells at PD 1 and the number of germ cells that migrated from the center of seminiferous cords to the basement

membrane was not altered in ID4OE mice. In contrast, the number of proliferative spermatogonia and total number of undifferentiated spermatogonia at PD 3 was significantly reduced in ID4OE mice compared to controls. Also, the total number of SSCs (ID4−GFP+ spermatogonia) was found to be significantly reduced at PD 6−8. Furthermore, differentiating spermatogonia were not observed in ID4OE mice at any age examined but were evident in control mice beginning at PD 6.
Conclusion: Collectively, these findings indicate that transition from the prospermatogonial to postnatal SSC state occurs in the presence of constitutive ID4 expression but suppression is required for transition to the transiently amplifying progenitor state. In addition, these results suggest a novel role for ID4 in restricting cell cycle progression in spermatogonia. This research was supported by grant HD061665 awarded to JMO from the National Institutes of Health.

Poster/Short Talk #5
DIRECT GERMLINE EDITING IN SPERMATOGONIA ELIMINATES CRISPR/CAS9 CATALYZED ANIMAL MOSAICISM
Karen Chapman, BS[1], Gerardo Medrano, BS[1], Priscilla Jaichander, PhD[1], Jaideep Chaudhary, BS[1], Marcelo Nobrega, PhD[2], James Hotaling, MD[3], Carole Ober, PhD[2] and F. Kent Hamra, PhD[1]
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(Presented by: F. Kent Hamra, PhD)

Introduction: Organisms with targeted genomic modifications are rapidly produced by gene editing in embryos using CRISPR/Cas9 RNA-guided DNA endonuclease. CRISPR/Cas9 also effectively catalyzes heritable and non−heritable target allele variation in mosaic organisms produced from donor embryos. Consequently, months to years of additional breeding are required to establish “pure”, non−mosaic mutant pedigree in many applied species.
Methods: To bypass mosaic animal production, we used CRISPR/Cas9 to catalyze targeted genomic mutations in rat spermatogonial stem cell cultures. CRISPR/Cas9-modified spermatogonia regenerated spermatogenesis and displayed long term sperm forming potential following transplantation into rat testes. Targeted germline mutations in Epsti1 and Erbb3 were vertically transmitted from recipients to exclusively generate pure mutant progeny. Epsti1 mutant rats were produced without genetically selecting donor spermatogonia.
Results: Clonal enrichment of Erbb3-null germlines unmasked recessive spermatogenesis defects in spermatogonial culture that were buffered in recipients, yielding progeny isogenic at targeted alleles.
Conclusion: In each case, spermatogonial gene editing eliminated CRISPR/Cas9-catalyzed animal mosaicism.

Poster/Short Talk #6
THE ROLE OF PERITUBULAR MYOID (PM) CELLS IN THE REGULATION OF SPERMATOGONIAL STEM CELL (SSC) SELF-RENEWAL, PROLIFERATION AND DIFFERENTIATION IN THE TESTIS NICHE
Liang-Yu Chen, William Willis and Mitch Eddy
Gamete Biology Group/ RDBL/NIEHS / NIH
(Presented by: Liang-Yu Chen)

Introduction: Spermatogonial stem cells (SSC) sit at the periphery of the seminiferous tubule surrounded by Sertoli cells, peritubular myoid (PM) cells and the basement membrane of the tubule in a microenvironmental compartment referred to as the SSC niche. Testis niche is believed to serve the dual role of regulating SSC differentiation and proliferation and of maintaining the SSC population to continually self−renew and differentiate throughout a male’s reproductive lifespan. In vitro studies with isolated SSCs and in vivo loss and gain of function studies have shown that glial cell line−derived neurotrophic factor (GDNF) can stimulate SSC proliferation. Previously, we demonstrated that testosterone stimulates isolated PM cells to produce GDNF and maintain the ability of co−cultured SSCs to repopulate the testes of germ cell−depleted mice.
Methods: To define the role of GDNF produced by PM cells in vivo, we mated Myh11 Cre/+ and Gdnf f/f mice to generate PM cell−specific KO mice (cKO, Gdnf f/−Myh11 Cre/+).
Results: There were no differences in body weight and Sertoli cell numbers between WT (Gdnf f/+), HET (Gdnf f/+Myh11 Cre/+) and cKO mice, but testis size was reduced in cKO mice. In breeding experiments, cKO generated 0−2 litters and then became infertile. Spermatogenesis was observed in a few sporadic regions of seminiferous tubules at 12 weeks, even though GDNF was detected by immunohistochemistry in Sertoli cells.
Conclusion: These results illustrate that GDNF produced by PM cells serves a crucial role in SSC homeostasis. TUNEL staining determined there were not showed differences in apoptosis between WT and cKO mice. However, the numbers of ZBTB16 positive cells were significantly reduced in cKO compared to WT mice. This suggests that diminished GDNF secretion by PM cells disrupts SSC self−renewal in the niche. Sections of testes from 2−week−old cKO mice were labeled by KI67, ZBTB16, and KIT antibodies and the ZBTB16+ and KI67+ cells were not observed in some seminiferous tubules

but KIT+ cells, suggesting that SSCs were differentiating in those tubules. The expressions of marker genes for undifferentiated spermatogonia (Zbtb16, Bcl6b and Nanos2) were also decreased, while those for differentiation marker genes (Ngn3, Kit, Nanos3 and Spo11) were increased in 1 week old cKO testes. These results support the hypothesis that GDNF secreted by PM cells promotes self−renewal and the developmental fate of SSCs.
Funding: This research was supported by the Intramural Research Program of the NIH, NIEHS.

Poster/Short Talk #7
CHOLESTEROL TRAFFICKING FOR STEROID BIOSYNTHESIS IN MA−10 MOUSE TUMOR LEYDIG CELLS.
Sathvika Jagannathan, MSc[1], Seimia Chebbi, BSc[1], Francoise Hullin−Matsuda, PhD[2], Toshihide Kobayashi, PhD[2] and Vassilios Papadopoulos, PhD[1]
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(Presented by: Sathvika Jagannathan, MSc)

Introduction: The hormone-sensitive and rate-limiting step in steroid biosynthesis is the movement of cholesterol from intracellular sources to the inner mitochondrial membrane (IMM) where it is converted to pregnenolone by the cytochrome P450 side chain cleavage enzyme (CYP11A1). Despite the numerous studies carried out to analyze cholesterol trafficking in steroidogenesis, the exact source of cholesterol as well as the mechanism by which it is transported to IMM remains to be elucidated.
Methods: D4 is the fourth domain of perfringolysin O protein, which has the ability to bind with high affinity cholesterol−enriched membranes, i.e. containing cholesterol greater than 30 mol% of total lipid. A fluorescent mCherry−tagged D4 was used to visualize cholesterol trafficking in MA−10 mouse tumor Leydig cells.
Results: Confocal imaging microscopy showed that in D4−mCherry transfected MA−10 cells, fluorescence localizes at the plasma membrane, but upon 30−45 minutes treatment with the cAMP analog dibutyryl−cAMP (dbcAMP) a significant reduction in plasma membrane labeling was observed. Functional inhibitors of the steroidogenic acute regulatory protein (STAR), translocator protein (TSPO), voltage dependant anion channel (VDAC) and CYP11A1, proteins involved in cholesterol import into mitochondria and metabolism, blocked steroid formation, and slowed down the movement of D4−mCherry from the plasma membrane. Treatment with the substrate 22R−hydroxycholesterol, which results in maximal steroid formation, also slowed down the D4−mcherry movement, suggesting that elevated steroid formation acts as a feedback mechanism to control plasma membrane cholesterol release. Recombinant D4−GFP protein readily bound the outer leaflet of the plasma membrane and no reduction in fluorescence intensity was noticed even after 120 minutes of dbcAMP stimulation, indicating that cholesterol was trafficked from the inner leaflet of the plasma membrane. D4−mCherry also localized the late endosomes upon dbcAMP stimulation suggesting a route for the cholesterol from plasma membrane to mitochondria.
Conclusion: These data suggest that the bulk of the steroidogenic pool of cholesterol, mobilized by cAMP for acute steroidogenesis, likely originates from the inner leaflet of the plasma membrane.

Poster/Short Talk #8
REQUIREMENT FOR ADENOSINE DEAMINASE CONTAINING PROTEINS IN MALE GERM CELL DEVELOPMENT
Elizabeth Snyder, PhD, Anuj Srivastava, PhD and Robert Braun, PhD
The Jackson Laboratory
(Presented by: Elizabeth Snyder, PhD)

Introduction: Adenosine deaminase, RNA−specific (ADAR) proteins are the only known drivers of adenosine to inosine (A−to−I) RNA editing. Murine ADARs (encoded for by Adar, Adarb1, and Adarb2) contain two conserved domains: an adenosine deaminase (AD) domain, which catalyzes A to I conversion, and one or more double−stranded RNA binding motifs (dsRBM). While expression of Adarb1 and Adarb2 is confined to neural tissue, Adar is observed in a wider range of tissues, including the testis. In addition, the testis expresses two closely related AD domain-containing proteins, Adad1 and Adad2. Both carry amino acid substitutions in critical regions of the AD domain, suggesting they do not have catalytic activity, although this has not been formally proven. Both ADADs contain dsRBMs similar to those found in ADARs, implying they may bind a similar set of targets. Expression profiling in isolated testicular cell types, throughout testis development, and in germ cell ablated mutant models demonstrated both Adad1 and 2 are expressed exclusively in the meiotic and post−meiotic germ cell populations while Adar is expressed in germ and somatic cells.
Methods: The extent of RNA editing in the testis was determined by applying a computational pipeline to high throughput RNA sequence data of isolated testicular cell types. This analysis demonstrated A to I editing in both the germ line and soma, with a much higher number discovered in Sertoli cells as compared to germ cells. To address the functional role of RNA editing in the testis and the specific requirement of AD−domain containing proteins in male germ cell development,

we generated germ cell and Sertoli cell-specific knockout models of Adar, as well as CRISPR-induced mutant models of Adad1 and Adad2, respectively.
Results: Despite the occurrence of editing in both cell types, germ cell or Sertoli cell ablation of Adar had no appreciable impact on germ cell development. In contrast, mutation of either Adad1 or 2 resulted in male−specific sterility.
Conclusion: Tolerance for germ cell ADAR loss demonstrates ADAR−mediated editing is not essential for male fertility. However, the absolute requirement of both Adad1 and Adad2 for male fertility confirms a fundamental role of AD−domain containing proteins in germ cell development. Whether ADADs catalyze or regulate RNA−editing events in the germ line or have evolved essential functions outside of RNA editing is unknown. Current studies are aimed at distinguishing between these disparate hypotheses.

Poster/Short Talk #9
THE TESTICULAR TRANSCRIPTOME OF THE RHESUS MONKEY (MACACA MULATTA) ASSOCIATED WITH THE DECISION BY UNDIFFERENTIATED TYPE A SPERMATOGONIA TO COMMIT TO A PATHWAY OF DIFFERENTIATION
Suresh Ramaswamy, PhD[1], Gary Marshall, PhD[2], Seyedmehdi Nourashrafeddin, PhD[3], Rahil Sethi, PhD[4], Uma Chandran, PhD[4], William Walker, PhD[5] and Tony Plant, PhD[5]
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(Presented by: Suresh Ramaswamy, PhD)

Introduction: Juvenile primates are characterized by hypogonadotropism and testicular quiescence: seminiferous cords that contain only Sertoli cells and proliferating undifferentiated spermatogonia. Spermatogonia differentiation, however, can be initiated in juvenile testes with exogenous gonadotropin. We used this model to compare the testicular transcriptome associated with spermatogonia proliferation (vehicle treated) to that associated with spermatogonia proliferation and differentiation (LH/FSH treated).
Methods: Three juveniles were stimulated with a pulsatile infusion of LH and FSH for 48h, and 3 received vehicle. A bolus iv injection of BrdU was given 3h before castration at the end of the infusion. One testis was snap−frozen and stored at −80 for RNA−Seq.
Results: As expected, the testosterone response after 48h of LH stimulation was similar to that in adult males and, together with FSH stimulation, was associated with an increase in S−phase labeling in A pale (Ap), but not in A dark, spermatogonia. Moreover, the increase in S−phase labeling in Ap at 45h was not associated with either an increase in number of this cell type or in the appearance of differentiating B spermatogonia. The kinetics of spermatogenesis in the monkey, however, predicts that the progeny of the S−phase labeled Ap at 45h in the stimulated testis will include differentiating B spermatogonia. This being the case, 45h of LH/FSH stimulation is sufficient to activate genes that drive undifferentiated Ap to commit to differentiation, but is insufficient for the first generation of differentiating spermatogonia to be produced. We hypothesized that those genes that are differentially expressed in the gonadotropin−stimulated testes underlie the commitment of Ap to proceed down the path of differentiation. Ion Torrent RNA−Seq reads were aligned with TopHat2 using the rheMac2 reference genome, transcripts assembled and quantitated with Cufflinks and differentially expressed genes identified with Cuffdiff. Of the 30,000 detected transcripts, 787 were upregulated (>1.5 fold increase) by 48h of LH/FSH stimulation including STAR by 12−fold (confirmed by qPCR). In contrast, GFR−α1 was downregulated by LH/FSH (5−fold by qPCR), consistent with a recognized role of GFR−α1 in repressing differentiation.
Conclusion: In summary, we have established the first primate model to interrogate the transcriptional bases underlying the decision of undifferentiated spermatogonia to commit to the path of differentiation. Supported by NIH−R01HD072189.

Poster/Short Talk #10
TESTES SPECIFIC PROTEASE 50 (TSP50) MODULATES MALE FERTILITY
Carolina Jorgez, PhD, Nathan Wilken, BS, Alexander Pastuszak, MD, PhD and Dolores Lamb, PhD
Baylor College of Medicine
(Presented by: Carolina Jorgez, PhD)

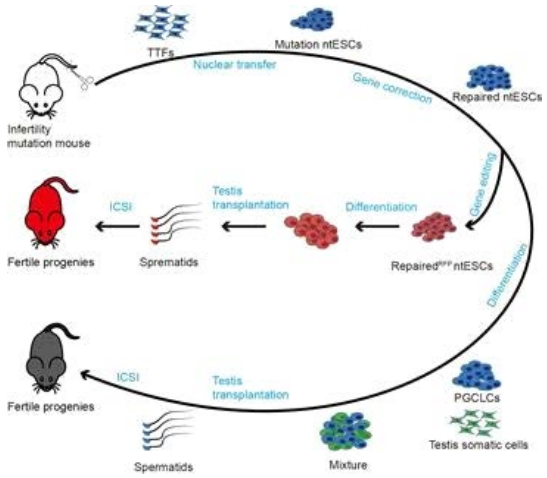
Introduction and Objectives: Despite advances in molecular diagnostics, the etiology of most male infertility remains unknown or undiagnosed. Recently, copy number variants (CNVs) that result in gene dosage changes have been implicated in male infertility. Here, we present data associating the testis−specific protease TSP50 (an evolutionarily

conserved testis−specific protease with p53 and Sp1 binding sites) with human male infertility. TSP50 is expressed in spermatocytes of humans, mice and rats at varying stages of spermatogenesis, but not in mature sperm. TSP50 does not have a known role in male fertility.
Methods: A cohort of 240 men including 122 with non−obstructive azoospermia (NOA) and normal karyotypes and Y-chromosome microdeletion assays, and 118 controls were evaluated for CNVs by array comparative genomic hybridization (aCGH) and/or qPCR using an assay specific for TSP50. A mutant mouse was generated using the CRISPR/Cas9 system to remove exon 2 of Tsp50, resulting in a premature stop codon and nonsense-mediated degradation.
Results: We identified a 58kb microdeletion at chromosome 3p21.31 encompassing TSP50 in 4 of 22 (18%) NOA men and in 0 of 10 controls. Validation by qPCR and analysis of 122 NOA men revealed 28 men (23%) with CNVs: 22 of whom had a TSP50 microdeletion, 6 of whom had no copies and 6 with microduplications (3 copies) of TSP50. Of 118 controls, 10 (8%) had a TSP50 microdeletion (1 copy of the gene). The absence of both copies or the presence of an extra copy of TSP50 was significantly different between cases and controls (p=0.029). All patients with TSP50 microduplications have maturation arrest (MA). Of the patients with no copies of TSP50, testis tissue was available for 2/6 and histologic evaluation demonstrated the presence of MA and hypospermatogenesis. Of the 16 infertile men with 1 copy of TSP50, 3 display MA, 2 men are Sertoli cell only (SCO) and the remainder of men have not undergone testis biopsy so the presence of histopathology is unknown.
Conclusions: Gene dosage changes in TSP50 that cause the loss or excess of TSP50 protein may underlie a previously unrecognized cause of infertility. The data suggests these CNVs may affect meiosis, primarily resulting in maturation arrest. TSP50 may play an essential role in spermatogenesis. Phenotypic analysis of the Tsp50 haploinsufficient or null mouse will further elucidate the role of TSP50 in testicular function.

Poster #11
GENERATION OF FERTILE OFFSPRING FROM KITW/KITWV MICE THROUGH DIFFERENTIATION OF GENE CORRECTED NUCLEAR TRANSFER EMBRYONIC STEM CELLS
Yan Yuan, PhD
(Presented by: Yan Yuan, PhD)

Introduction: Male infertility accounts for about half of human infertility. Genetic mutations could cause sperm defect leading to male infertility. Without functional gametes in the testes, patients could not produce progeny even with assisted reproduction technologies such as in vitro fertilization. It is a major challenge to restore the fertility of patients with azoospermia duo to genetic mutations.
Objective: In this study, using Kitw/Kitwv mouse model, we would want to investigate the feasibility of generating sperm from gamete deficient patients by combining the reprogramming and gene correcting technologies.
Methods: The Kitw/Kitwv mutation ntESCs were obtained from cloned embryos that created by nuclear transfer of Kitw/Kitwv somatic cells. For gene correction, mutation nuclear transfer embryonic stem cells (ntESCs) were co−−transfected with TALEN expression vectors and targeting vector. Then, drug−resistant clones were picked and removed the piggyBac−flanked selection cassette. The corrected ntESCs were further differentiated into primordial germ cell-like cells (PGCLCs) that purified by the surface markers of PGCs, SSEA1 and Integrin β3 after 6 days' differentiation followed the protocol described previously. The double positive PGCLCs were transplanted into the testis of busulfan treated 6- to 12-day old male mice. Haploid cells produced from the transplanted PGCLCs were isolated two months later, and used for intracytoplasmic sperm injection (ICSI).
Results: In this study, we derived embryonic stem cells from cloned embryos and corrected the gene mutation. By differentiation in vitro, the repaired ntESCs could generate functional PGCLCs which could generate functional spermatids.
Conclusions: Our experiments in mouse model will demonstrate that human male infertility patients with genetic mutations could be treated with reprogramming and cell therapy. Our study will explore a new path to rescue male infertility caused by genetic mutations.

Poster Session I



Poster #12
HORMONE INDUCED DIFFERENTIAL TRANSCRIPTOME ANALYSIS OF RAT SERTOLI CELLS DURING POSTNATAL TESTICULAR DEVELOPMENT
Indrashis Bhattacharya, PhD[1], Mukkesh Gautam, PhD[2], Bholashankar Pradhan, PhD[3] and Subeer Majumdar, PhD[3]
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(Presented by: Indrashis Bhattacharya, PhD)

Introduction and Objectives: Male infertility has become a global threat in the last decade. Sertoli cells (Sc) are the unique somatic cells in the testes that are the target of both Testosterone (T) and FSH thus regulate male fertility. Postnatal maturation of Sc in terms of appropriate hormonal responsiveness is prerequisite for the pubertal onset of spermatogenesis. However, lack of knowledge about the extent of hormone (both T and FSH) responsive gene expression during the different phases of Sc maturation, restricts our understanding about the molecular events necessary for sperm production.
Methods: Sc were isolated and cultured from immature (5 days old), maturing (12 days old) and mature (60 days old) rat testes. On day 4 of culture, Sc (of all three age groups) were stimulated with pulsatile FSH and T (in combination) treatment. The hormone induced differential gene expression data obtained from Sc of 12 days and 60 days of age were compared with that of Sc obtained from 5 days old rats using microarray technology. The array data was also revalidated further by QRT−PCR for some of the genes selected from all three age groups.
Results Obtained: Our data revealed that genes like Igfr1, Igfr2, Fgf9, Acvrl1, Bmpr1b, Tgfbr1 and Itga4, were upregulated in immature Sc. Ntf3, Nrg1, BDNF, SCF, GDNF and CXCl12 were upregulated in maturing Sc. Mature adult Sc were found to express genes involved in glucose metabolism, phagocytosis, and cytoskeleton structuring for the maintenance of spermatogenesis. The expression profiles of some of such genes like Aass, Unc5c, Ccl5, RoBo, Fat3, Tir, Wisp, Msln, Spz, Pwwp1 and Testin etc were validated by qPCR that authenticated the reliability of the array data further.
Conclusion: Taken together, this differential transcriptome data provide an important resource to reveal the molecular network of Sc maturation which is necessary to govern male germ cell differentiation, hence, will improve our current understanding of the etiology of some forms of male infertility.

Poster #13
CYSTEINE−RICH SECRETORY PROTEIN 2 (CRISP2) IS REQUIRED FOR FULL MALE FERTILITY
Shu Ly Lim, PhD, Duangporn Jamsai, PhD, Anne E. O'Connor, Bsc, Donna Merriner, Bsc and Moira O'Bryan, PhD
Department of Anatomy and Developmental Biology, Monash University, Vic, Australia
(Presented by: Shu Ly Lim, PhD)

Introduction: Cysteine-rich secretory protein (CRISP) 2 is a member of CRISP clade of the CAP superfamily. CRISP2 is highly testis-enriched compared to other CRISP proteins and localized to both the sperm acrosome and tail, suggesting a role in sperm function. With the exception of a proven ability of CRISP2 to regulated ryanodine receptor Ca2+ gating and their localization to the connecting piece of the tail, functional support for this hypothesis remains limited.

Methods: In order to definitively define the role of CRISP2 in male fertility we have generated two mouse models of CRISP2 dysfunction 1) a hypomorphic model of Crisp2 expression and 2) a germ cell knockout model.
Results: Using these models we have shown that the level Crisp2 expression directly affects male fertility. Crisp2 hypomorphic mice are sub-fertile. They have a reduced daily sperm output, poor sperm motility and a compromised ability to undergo the acrosome reaction.
Conclusion: In summary, our data support an important role for CRISP2 in ensuring normal sperm function.

Poster #14
DYNAMIC CHANGES IN THE EXPRESSION OF APOPTOSIS−RELATED GENES DURING RETINOIC ACID−INDUCED NEONATAL RAT GONOCYTE DIFFERENTIATION AND IN HUMAN TESTICULAR SEMINOMAS
Gurpreet Manku, PhD and Martine Culty, PhD
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(Presented by: Gurpreet Manku, PhD)

Introduction: Apoptosis is an integral part of the spermatogenic process, necessary to maintain a proper ratio of Sertoli to germ cell numbers and provide an adequate microenvironment to germ cells. Apoptosis may also represent a protective mechanism mediating the elimination of abnormal germ cells. Extensive apoptosis occurs between the first and second postnatal weeks, at the point when gonocytes, precursors of spermatogonial stem cells, should have migrated towards the basement membrane of the tubules and differentiated into spermatogonia. Although such an important process, the mechanisms regulating this process are not well understood. Gonocytes undergo phases of proliferation, migration, differentiation, and apoptosis which occur in a timely and closely regulated manner. Gonocytes failing to migrate and differentiate properly undergo apoptosis. Inadequate gonocyte differentiation has been suggested to lead to testicular germ cell tumor (TGCT) formation.
Methods: Here, we examined the expression levels of apoptosis-related genes during gonocyte differentiation by quantitative real−time PCR, identifying 48 pro- and anti-apoptotic genes increased by at least 2−fold in rat gonocytes induced to differentiate by retinoic acid, when compared to untreated gonocytes.
Results: Further analysis of the most highly expressed genes identified the pro-apototic genes Gadd45a and Cycs as upregulated in differentiating gonocytes and in spermatogonia compared to gonocytes. These genes were also significantly downregulated in seminomas, the most common type of TGCT, compared to normal human testicular tissues.
Conclusion: These results indicate that apoptosis−related genes are actively regulated during gonocyte differentiation. Moreover, the down-regulation of pro−apoptotic genes in seminomas suggests that they could represent new therapeutic targets in the treatment of TGCT.

Poster #15
IDENTIFICATION AND CHARACTERIZATION OF A BOVINE SPERM ACROSOMAL STRUCTURAL PROTEIN AND ITS MECHANISM OF INTERACTION WITH ACROSOMAL HYDROLASES
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(Presented by: Linda Smith, BS)

Introduction: Mammalian fertilization leads to the formation of a new organism when the exchange of genetic components of opposite gametes transpires. The mammalian sperm acrosome contains hydrolases, utilized in penetrating the egg investments. Our understanding of the functions of protein constituents of the outer acrosomal membrane-matrix complex (OMC) is limited. A highly purified OMC fraction isolated from bovine cauda sperm heads is comprised of 54, 50, 45, and 38−19kDa polypeptides. The objective of this study is to identify and to characterize the 45kDa (OMC45) polypeptide and to define its role in binding acrosomal hydrolases.
Methods: OMC45 polypeptide remains insoluble in the particulate fraction after high pH extraction. We isolated OMC45 polypeptide from the high pH insoluble fraction of OMC. Proteomic analysis of OMC45 by MALDI-TOF-TOF yielded 8 peptides that matched the NCBI database sequences of Tektin 3 (TEKT3). Using TEKT3 antibody, we performed the localization of OMC45 polypeptide and coimmunoprecipitation analysis to examine the immunological characterization of OMC45 polypeptide. Triton X-100-permeabilized cauda sperm exhibited intense staining of the acrosomal segment with anti-OMC45 and anti-TEKT3. Next, we tested the solubility properties of OMC45 polypeptide in RIPA buffer (RIPA =1% Triton X−100, 1% Na−deoxycholate, 0.1% SDS, 0.15M NaCl, proteases inhibitors in 10mM phosphate buffer, pH 7.2).
Results: Our data revealed that a portion of the OMC45 polypeptide was solubilized by RIPA buffer. An identical solubility pattern was found when the blot was stained with anti−TEKT3. The supernatant obtained after RIPA buffer extraction was subjected to co-immunoprecipitation analysis. A complete recovery of OMC45 polypeptide was observed in the anti-

OMC45 immunoprecipitation pellet. An identical blot stained with anti-TEKT3 exhibited the presence of TEKT3 polypeptide in the anti-OMC45 pellet. Our immunofluorescence and biochemical studies strongly confirm the proteomics identification of OMC45 polypeptide, that it exhibits a structural relationship to TEKT3. OMC45 glycoprotein possesses ~4kDa N-linked and ~4kDa O-linked oligosaccharides. Deglycosylated OMC45 revealed a significant reduction in both acrosin and N-acetylglucosaminidase (NAGA) binding in comparison to acrosin and NAGA binding to a native OMC45 polypeptide.
Conclusion: Our results suggest that oligosaccharides play an important role in hydrolase binding.
Funding: Supported by NIH SC3GM096875, NSF HBCU−UP1036257, and RISE Grant.

Poster #16
HISTONE H4K20 DEMETHYLASE REGULATES SPERMATOGENESIS
Charlie Degui Chen
(Presented by: Charlie Degui Chen)

Introduction: It is well known that the amount of heterochromatin increases with the differentiation of spermatogonia, however, the role of heterochromatin in this process is not defined. Because heterochromatin formation is regulated by methylation in histone H4 lysine 20 (H4K20me), we set out to identify histone demethylases for this heterochromatin mark.
Objectives: To identify a demethylase for H4K20 and examine its role in the differentiation of spermatogonia.
Methods: High-content cell−based screening of a cDNA library containing 4,500 nuclear proteins one by one, in vitro enzymatic assays, ChIP-seq coupled with RNA-seq, and gene knockout study.
Results: Ectopic expression of KDM9 led to a reduction in the global level of H4K20me1. ChIP-Seq experiments revealed that KDM9 demethylated H4K20me2 and H4K20me3 at specific genomic loci in vivo. In vitro, KDM9 specifically demethylated H4K20me1/2/3 and generated formaldehyde, and the enzymatic activity required Fe(II), α-ketoglutarate and ascorbic acid as cofactors. RNA-seq demonstrated that KDM9 regulated the transcription of repetitive elements, but not protein coding genes. KDM9 knockout blocked spermatogenesis in mice.
Conclusion: We identified a histone demethylase for H4K20 that regulates spermatogenesis. Since the protein sequence of the catalytic domain of KDM9 is different from LSD1 and JmjC domain-containing proteins, the two known classes of histone demethylases, this enzyme represents a new class of histone demethylase.

Poster #17
NOVEL ACTION OF FOLLICLE STIMULATING HORMONE (FSH) ON MOUSE TESTICULAR STEM CELLS
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(Presented by: Hiren Patel, MSc[1])

Introduction: FSH acts through its receptors on Sertoli cells in testis and granulosa cells in ovaries. Recently we deciphered a direct action of FSH on pluripotent very small embryonic-like stem cells (VSELs) and their immediate descendants ‘progenitors’ in adult ovary via FSH-R3. Similar pluripotent VSELs exist in adult testis along with spermatogonial stem cells (SSCs).
Objective: Present study was undertaken to decipher differential effects of FSH on mouse Sertoli and stem cells.
Methods: Experiment 1: Effect of pregnant mare serum gonadotropin (PMSG, FSH analog, 5 IU for 24 h) was studied on adult mouse testis by histology, immuno−localization (FSHR, OCT−4, and PCNA) and qRT−PCR for stem cell specific transcripts (Oct−4A, Oct−4, Sca−1, and Nanog), proliferation (Pcna) and FSHR transcripts (primers specific to exon 10 and 11 unique to Fsh−r1 and Fsh−r3 respectively). Experiment 2: Busulphan treated (BT, 25 mg/Kg) mice were used for further studies as it results in selective destruction of actively dividing cells (SSCs and germ cells) and sperm while relatively quiescent stem cells survive. PMSG (24h after six weeks of BT) effect was studied by above methods including flow cytometry on VSELs (<8 μm cells which are LIN−/CD45−/SCA−1+). Experiment 3: Studies were also undertaken on isolated populations of Sertoli and stem cells from BT testis. They were cultured in presence and absence of FSH (3 and 24 h) to delineate direct effects of FSH.
Results: Experiment 1: An increased population of cells with dark Hematoxylin stained nuclei; high nucleo−cytoplasmic ratio and positive for PCNA and OCT−4 was observed suggesting increased proliferation of the stem cells in response to PMSG treatment. Up−regulation of transcripts specific for stem cells, Pcna and Fshr (Fsh−r3 was highly up−regulated compared to Fsh−r1) was also observed. Experiment 2: PMSG treatment to BT testis resulted in up−regulation of specific transcripts for stem cells, Pcna and Fshr (Fsh−r3 was highly up−regulated compared to Fsh−r1). Flow cytometry analysis

showed that VSELs survive busulphan treatment (0.045±0.008%) and increase after PMSG (0.1±0.03%). Experiment 3: Fsh−r3 was predominantly up−regulated compared to Fsh−r1 along with Pcna in isolated stem cell culture from BT testis.
Conclusion: We show for the first time that besides Sertoli cells, stem cells (VSELs and SSCs) in adult testis express FSH receptors and respond directly to FSH possibly through alternatively spliced FSH−R3.

Poster #18
IMPORTANCE OF SOMATIC NICHE IN REGULATING TESTICULAR STEM CELLS DIFFERENTIATION INTO SPERM
Sandhya Anand, MSc, Kalpana Sriraman, PhD, Hiren Patel, MSc, Smita Bhutda, MSc and Deepa Bhartiya, PhD
National Institute for Research in Reproductive Health
(Presented by: Sandhya Anand, MSc)

Introduction: We have undertaken studies on busulphan treated (BT) mice testes to address fertility issues of cancer survivors. Very small embryonic−like stem cells (VSELs, <8µm, LIN−/CD45−/SCA−1+) exist as a sub-population amongst spermatogonial stem cells (SSCs) in adult mice testes and survive busulphan treatment (BTr) because of their quiescent nature (0.03±0.002% in normal versus 0.05+0.005% in BT testes by flow cytometry). Spermatogenesis is suppressed in chemoablated testes, possibly due to compromised somatic niche which is crucial for normal proliferation and differentiation of stem cells. Transplantation of healthy niche cells including Sertoli cells (SC) and mesenchymal cells through inter−tubular route restored spermatogenesis from surviving stem cells.
Objective: Present study aimed to understand the role played by transplanted SC and mesenchymal cells to restore spermatogenesis, in-depth analysis of niche in BT testis and in vitro stem cells−niche interaction to result in spermatogenesis.
Methods: SC and mesenchymal cells from GFP mouse were injected in testis of wild type BT mouse and studied on 1, 7, 14, 30 and 60 days post transplantation. To evaluate the effect of BTr on niche, SC from normal and BT testis were subjected to microarray studies. In addition, cells from BT testis were cultured for three weeks in SC conditioned medium with 10% FBS and 0.5 IU FSH.
Results: Transplanted GFP cells showed formation of neo−tubule like structures in the interstitium. Adjacent germ cells depleted native tubules showed gradual resumption of spermatogenesis from VSELs which survived chemotherapy. Microarray analysis showed up-regulation of 1835 genes and down-regulation of 1768 genes after BTr. Several signaling pathways including Wnt pathway (implicated in SC function) were affected. Up-regulation of Wnt4 and beta catenin was observed. During in vitro culture, SC from BT testis attached and provided support to the surviving stem cells which underwent proliferation, clonal expansion and differentiation into sperm within 3 weeks. Various stages of spermiogenesis were observed correlating with an increase in transcript levels of Pcna, Sca−1, Gfra, Prohibitin, Scp3 and Protamine.
Conclusions: Crucial role played by the somatic niche by secreting various factors in a paracrine manner to affect stem cells proliferation and differentiation is highlighted. Potential of VSELs to differentiate into sperm in presence of a supportive niche is also demonstrated.

Poster #19
DMRT1 IN SERTOLI CELLS REGULATES ADULT LEYDIG CELL MATURATION
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University of Kansas Medical Center
(Presented by: Leslie Heckert, PhD)

Introduction: DMRT1 is an evolutionarily conserved transcription factor that is essential to male fertility in mammals. While several past studies demonstrate the importance of DMRT1 to germ cell (GC) and Sertoli cell (SC) functions, there are currently no reports of its influence on the Leydig cells (LCs).
Methods and Results: Here, evaluation of Dmrt1−/−, Dmrt1−/− with a transgene that expresses DMRT1 only in SCs (Dmrt1−/−;tg37), and control mice showed that DMRT1 in SCs is important for adult LC development. At postnatal day 42 (P42), androgen deficiency was indicated in both Dmrt1−/− and Dmrt1−/−;tg37 mice by the absence of seminal vesicles (SVs), suggesting that LCs were compromised. Expression of the LC marker CYP11A1 revealed similar numbers of LCs in Dmrt1−deficient and control mice, suggesting that androgen deficiency was a result of defective LC function rather than LC loss. In animals older than P102, SVs were observed in 38% of the Dmrt1−/− and all of the Dmrt1−/−;tg37 mice, indicating androgen levels increased between P42 and >P105 and that DMRT1 in SCs significantly improved LC function. Semi−quantitative / quantitative RT−PCR and Western blot analyses for LC markers revealed that testes from P42 Dmrt1−/− and Dmrt1−/−;tg37 mice and older (>P12) Dmrt1−/− mice contained only immature LCs, while testes from older Dmrt1−/−;tg37 mice contained mature LCs, indicating that DMRT1 in SCs facilitates LC maturation. Finally, immunohistochemistry and Western blot analyses of Anti−Müllerian Hormone (AMH), a SC product that influences LC differentiation, revealed that AMH was absent from SCs of Dmrt1−/− mice but not from SCs of Dmrt1−/−;tg37 mice.

Conclusion: In all, the data show that DMRT1 acts indirectly to regulate adult LC maturation, with its primary influence occurring via its effects in SCs, and suggests that its regulation of AMH may play a role in this process.
Funding: Supported by NIH grant U54HD055763

Poster #20

RETINOIC ACID TRIGGERS C−KIT GENE EXPRESSION IN SPERMATOGONIAL STEM CELLS ENTAILING ACTIVE PARTICIPATION OF AN ENHANCEOSOME CONSTITUTED BETWEEN TRANSCRIPTION FACTOR BINDING SITES RARE, PU.1 AND ETS

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(Presented by: Swanand Koli, MSc)

Background: Regulation of c−kit gene during spermatogenesis remains largely unreciprocated.
Results: Retinoic acid (RA) regulates c-kit gene expression through discrete promoter regions.
Conclusion: Enhanceosome constituted between transcription factor binding sites RARE, PU.1 and ETS to RA response plays imperative role for c−kit gene activation.
Significance: RA induced c−kit gene regulation provide newer insights for understanding nuances between self−renewal and differentiation.
Restricted availability of retinoic acid in testicular milieu regulates transcriptional activity of c−kit (KIT, CD117) which concurrently determines the destiny of stem cells towards differentiation. We intended to decipher prevailing molecular machinery guiding RA responsiveness of c−kit gene using spermatogonial stem cells C18−4. We identified a novel retinoic acid response element (RARE) positioned at −989 nucleotides upstream of transcription start site (TSS) providing binding site for dimeric RA receptor i.e. retinoic acid receptor gamma (RARγ) and retinoic X receptor (RXR). RA treatment influenced c−kit promoter activity along with an endogenous c−kit expression in C18−4 cells. A comprehensive promoter deletion assay using pGL3B reporter system characterized regions spanning −271 bp and −1011bp upstream of TSS which function as minimal promoter and maximal promoter respectively. In silico analysis predicted region −1011 to +58 bp comprised of distal enhancer RARE and activators such as PU.1, Sp1 and four ETS tandem binding sites at proximal region. The gel retardation and ChIP assays clearly revealed interactions for RARγ, GABPα, PU.1 and Sp1 to their predicted consensus sequences, whereas GABPα occupied two out of four ETS binding sites within the c−kit promoter region. We propose that to RA response, an enhanceosome is orchestrated through scaffolding of CBP/p300 between RARE and elements of proximal promoter region, controlling germ line expression of c−kit gene. Our study outlines fundamental role played by RARγ along with other non−RAR transcription factors (PU.1, Sp1 & GABPα) in response to RA to regulate c−kit expression in spermatogonial stem cells.

Poster #21

DIFFERENTIAL EFFECTS OF COMBINED GENISTEIN AND DEHP ON TESTICULAR CELL LIPID HOMEOSTASIS AND STEROID PRODUCTION

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(Presented by: Steven Jones, MSc)

Introduction: Previous work in our laboratory demonstrated the ability of in utero exposure to a mixture of the phytoestrogen, Genistein (GEN), and plasticizer, DEHP, to induce long term alterations in gene and protein expression that are substantially different from individual exposures. Recent data identified fetal-type and adult Leydig cells and germ cells, as well as their progenitors as sensitive targets for low dose ED mixtures.
Methods: To further investigate the direct effects of GEN and DEHP and elucidate specific mechanisms of toxicity, MA−10 Leydig cells and isolated primary rat gonocytes were exposed in-vitro to varying concentrations of GEN and MEHP, the principle bioactive metabolite of DEHP.
Results: High−performance thin layer chromatography (HPTLC) demonstrated the ability of combined 10 μM GEN + MEHP, but not individual exposures, to increase levels of several Neutral Lipid (Cholesterol, Free Fatty Acid and Triglycerides) and Phospholipid (Sphingomyelin, Phosphatildylcholine, Phosphatidylethanolamine, Phosphatidylserine and Phosphatidylinositol) classes in MA−10 cells, indicating a generalized deregulation of lipid homeostasis. In contrast, combined 10 μM GEN + MEHP reduced neutral and phospholipid classes in primary gonocytes, suggesting a particular sensitivity of early spermatogonial stem cell progenitors.

Further investigation by qPCR analysis revealed concomitant alterations in MA−10 cholesterol (Hmgcoa) and phospholipid (Srebp1c, Fasn) mediator mRNAs, suggesting the possible involvement of upstream LXRα agonism. Interestingly, 10 μM combined GEN + MEHP also had a stimulatory effect (2 fold) on basal MA−10 progesterone production. Consistent with RIA results, the mRNA of steroidogenic and cholesterol mediators Star, Tspo and Cyp11a, Srb1 and Hsl (Lipe), as well as upstream nuclear receptors Nr2f2 (Coup−tfII) and Sf1 were all significantly increased uniquely in the mixture treatment group.
Conclusion: These results suggest a deregulation of testicular cell function in response to a combination of GEN + MEHP. Further research is underway to elucidate the origins of differential lipid alterations and concomitant bi-phasic stimulation of steroidogenesis. Taken more broadly, this research highlights the importance of assessing the impact of ED mixtures in multiple toxicological models across a range of environmentally relevant doses.

Poster #22
AN EMERGING ROLE FOR ACTIVIN B IN TESTICULAR FUNCTION AND PATHOLOGY
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(Presented by: Mark Hedger, PhD)

Introduction and objectives: Activin A plays a crucial role in testis development, spermatogenesis and steroidogenesis and immunity. It is constitutively produced in the adult testis, principally by Sertoli cells, where production is suppressed at the transcriptional level by follicle−stimulating hormone (FSH) and stimulated by mediators of inflammation, such as Toll−like receptor (TLR) ligands, interleukin−1 (IL1) and tumor necrosis factor (TNF). Elevated activin A is implicated as a cause of testicular disruption during inflammation. Activin B exhibits 65% homology with activin A and has similar actions, but also interacts with alternative activin receptor subunits. Since production and regulation of activin B in the testis has not been examined previously, this was investigated.
Methods: Adult rat and mouse testes homogenates, and medium collected from 18−20 day−old rat Sertoli cell cultures (2−48 h) were assayed for activin A and B using specific, validated two−site enzyme−linked immunosorbent assays. Expression of inhibin/activin subunit mRNA was measured by qRT−PCR in the cultured Sertoli cells.
Results: In comparison with several other tissues, including the anterior pituitary and immune tissues, the adult testis was a significant source of activin B − endogenous levels of activin B in the testis were similar to those of activin A. Activin B was constitutively produced by isolated Sertoli cells, but was undetectable in germ cells. In the Sertoli cell, activin B production was suppressed by FSH, acting via cAMP, even though activin βB subunit mRNA expression was increased. This was attributable to the fact that FSH also stimulates inhibin α−subunit production, which diverts the βB subunit towards formation of inhibin B. Activin B production was not stimulated by activation of inflammatory (TLR/IL1/TNF−mediated) pathways; in fact, βB subunit mRNA was inhibited by TLR/IL1/TNF activation. Furthermore, blocking MAP kinase signalling, which mediates stimulation of activin A during inflammation, increased activin B production by the Sertoli cell.
Conclusions: The data indicate that Sertoli cell production of both forms of activin is negatively regulated by FSH, albeit through entirely different mechanisms, and only activin A is increased by inflammatory stimuli. This suggests that both activins have a role in adult testicular function, but will play significantly different roles, particularly during testicular failure and inflammation.

Poster #23
XENOSCREENING OF IRRADIATION EFFECTS IN A PRIMATE TESTIS: MINIMIZING ANIMAL INPUT AND MAXIMIZING READOUT
Ina Tröndle, Joachim Wistuba, PhD, Anu David, PhD, Westernströer Birgit, PhD, Sandhowe Reinhild, Nicole Terwort, Nina Neuhaus, PhD, and Stefan Schlatt, PhD
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(Presented by: Ina Tröndle)

Introduction: Non-human primates are irreplaceable animal models for reproductive toxicity screening. Here we report an innovative monkey to mouse xenografting strategy potentiating physiological readouts under minimally invasive and limited input of animals. As model system we explore effects of irradiation on the primate testis elucidating a dose and cell specific response especially on stem cells.
Methods: One testis weighing 997.3 mg was removed from a 3-year old prepubertal cynomolgus monkey, decapsulated and dissected into 122 fragments which were randomly assigned to three groups (N=40) and subjected in vitro to 0, 1 and 4 Gy of irradiation. For pre-grafting control 17 fragments were fixed and 9 were snap−frozen in liquid nitrogen. The

remaining grafts were injected subcutaneously into twelve castrated nude mice (4 recipients per group with 8 grafts) which were maintained for 4 months when approx. 75% of grafts were recovered and either fixed and embedded in paraffin (15 grafts per group) or snap−frozen for subsequent analysis by RT2−Profiler PCR−Arrays (Grafts analyzed: pre-grafting = 3; 0 Gy = 7; 1 Gy = 5; 4 Gy = 7).
Results: Histological analysis revealed a dose dependent depletion of spermatogonia per seminiferous tubule and a decline of tubules containing spermatocytes. PCR-arrays showed that germ cell specific markers declined significantly while Sertoli- and Leydig cell markers remained unchanged demonstrating a dose and germ cell specific effect of irradiation.
Conclusion: This novel xenografting strategy provides an innovative screening tool generating valid endpoints with input of only a single monkey testis. The PCR marker panel provided a unique distinction of germ and somatic cell specific effects. Despite of orchidectomies and injections no invasive treatments were performed in any animal. This powerful approach is applicable to other organs with special emphasis on stem cell driven processes.

Poster #24
HUMAN INFERTILITY AND GENE EXPRESSION
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(Presented by: Rajan Dighe, PhD)

Introduction: Spermatogenesis is a very complex process of differentiation occurring in a unique milieu present in the seminiferous epithelium. However, the differential gene expression accompanying this process, its regulation and correlation to human infertilty have not been clearly established due paucity of the human testicular tissues, particularly the controls, and lack of appropriate culture conditions for germ cell differentiation in vitro. In the present study, an attempt has been made to characterize human infertility and correlate it to the differential gene expression pattern of the testicular germ cells.
Methods: The testicular biopsy samples from infertile patients were subjected to flow−cytometric analysis to determine the germ cell pattern. The patients diagnosed as obstructive azoospermia (Group I and II) revealed presence of diploid, tetraploid and haploid cells with an additional population of the late spermatids clearly indicating complete spermatogenesis.
Results: Several patients showed incomplete spermatogenesis, with some exhibiting diploid and tetraploid germ cells (Group III), but not the haploid germ cells suggesting arrest at the meiotic stage, while some patients exhibited only diploid cells (Group IV) indicating arrest at the pre−meiotic stage. The RT−PCR analysis for various markers specific for different testicular cells revealed that the Group I and II expressed the Sertoli cell markers FSHR, SCF and the Leydig cell markers LHR, 3β HSD and 17β HSD, spermatogonial marker cKit, tetraploid markers Cyclin A1, LDH−C and the haploid specific marker Protamine 1. The Group III patients did not express Protamine 1 and showed differential expression of LDH−C and Cyclin A1 while the Group IV did not express Protamine 1, LDH−C and Cyclin A1.
Conclusion: These data suggest arrest of spermatogenesis at different stages. These analyses are being followed by microarray analysis to identify differential gene expression and attempts will be made to correlate the gene expression pattern to the arrest of spermatogenesis and infertility.

Poster #25
CONTRAST ENHANCED ULTRASOUND CONFIRMS TESTICULAR CIRCULATION TO BE HAMPERED BY DISTURBED VASCULARIZATION IN 41, XXY* MICE, A MODEL FOR KLINEFELTER SYNDROME
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(Presented by: Stefan Schlatt, Prof, Dr)

Introduction: Testosterone deficiency in Klinefelter syndrome (KS) was thought to result from disturbed Leydig cell (LC) function. However, in a KS mouse model (41,XXY*), LCs were found to be hyperplastic and hyper-reactive. Furthermore,

intratesticular testosterone (ITT) concentrations were comparable to controls with a normal karyotype. We confirmed this in a cohort of patients, excluding insufficient ITT levels as the cause of hypogonadism. It was reported that arteries in KS patients are altered and circulation impaired. Changes in testicular vascularization were also found previously in the 41,XXY* mouse model. We hypothesize disturbed testicular blood supply might be involved in the endocrine phenotype.
Methods: We therefore performed a study in which an enhanced ultrasound based analysis of the testicular blood flow rate in 41,XXY* mice was conducted. Adult male 41,XXY* (n=5) and littermate mice (n=6) underwent ultrasound analyses with the Non-Targeted Contrast Agent Vevo MicroMarker. The agent containing gas filled micro−bubbles was administered intravenously for lower body perfusion. After initial perfusion, micro−bubbles were destroyed by high ultrasound pressure and the reperfusion period was documented and analysed. In parallel, electrocardiograms (ECGs) were taken. Afterwards mice were sacrificed and testes removed for histological analysis of the vascularization.
Results: Whilst ECGs did not reveal differences in heart function between the groups, the reperfusion time for testes was significantly increased in 41,XXY* mice (XXY* 28.8± 1.69s; XY* 19.9± 2.8s) Testes of 41,XXY* mice (XXY* 4.6 ± 0.10mm²; XY* 11.1 ± 0.34mm²) and the area covered by blood vessels (XXY* 0.025 ± 0.003mm²; XY* 0.040 ± 0.002mm²) were significantly smaller as revealed by histology.
Conclusion: Conclusively, our data indicate an impaired blood flow in testes of males with a supernumerary X chromosome, which might contribute to the endocrine phenotype of KS.

Poster #26
DNA METHYLATION AND CONSERVED GERM CELL EXPRESSION OF DAZ FAMILY GENES
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Nanjing Medical University
(Presented by: Chenwang Zhang, BS)

Introduction: The RNA−binding protein BOULE and DAZL, members of highly conserved DAZ family, play important roles in animal germ cell development, however the mechanisms that regulate their germ cell specific expression in different species including human remain unknown.
Methods and Results: We have examined expression of Boule and Dazl among different tissues in mouse and found that Boule and Dazl are only expressed in gonads. Examining DNA methylation difference of both genes between the testis and somatic tissues, we found that the promoters of Boule and Dazl is hypomethylated in testis where they are expressed and hypermethylated in somatic tissues where they do not express. The methylation pattern also changes during the first wave of spermatogenesis, corresponding to their expression change. We extended our investigation into other species such as human, pig, chicken and even zebra fish. Similar correlation is present in all these animals examined. Finally removal of DNA methylation in somatic cell line(NIH3T3) led to expression of Dazl.
Conclusion: Together our results provided insight into the role of methylation in regulating tissue specific expression of highly conserved germ cell core components.

Poster #27
EFFECT OF A TEMPORARY INHIBITION OF GDNF SIGNALING ON THE NUMBERS AND REPLICATION OF GFRA1+ AS, APR AND AAL SPERMATOGONIA
Nicole Parker, BS and William Wright, PhD
Johns Hopkins Bloomberg School of Public Health
(Presented by: Nicole Parker, BS)

Introduction: The testicular histology of some men with nonobstructive azospermia (NOA) suggests they have lost significant numbers of spermatogonial stem cells (SSCs) and their immediate progeny, progenitor spermatogonia (PS). Experimental manipulations of mice with loss and then restoration of numbers of these cells are able to reveal mechanisms responsible for restoring the cells and insight to the dysfunction in those cases of NOA. Data indicate that glial cell line-derived neurotrophic factor (GDNF) plays an essential role in SSC and PS maintenance. Previously we have found using a chemical−genetic approach when GDNF signaling is temporarily inhibited for 3 days, replication of GFRα1+ A single (As), A paired (Apr), and A aligned (Aal) spermatogonia was significantly reduced from controls. We have used this approach to test the hypothesis that in mice where GDNF signaling is temporarily inhibited and then restored, the partial loss of SSCs and PS is followed by increased replication of these cells and simultaneous rebuilding of their numbers. We further hypothesized that this recovery is correlated with increased transcriptional expression of GDNF.
Methods: To test these hypotheses, we inhibited GDNF signaling for 9 days and testes were collected on days 10 to 28 of the experiment. One day prior to collection, mice were injected with the thymidine analogue, 5−ethynyl−2'−deoxyuridine (EdU) to label replicating cells. We then determined the numbers of GFRα1+ As, Apr and Aal spermatogonia, the fraction replicating, and the message levels of GDNF.

Results: Measurement of GFRα1+ cells/mm2 following treatment showed significant GFRα1+ cell loss and then recovery over time. Day 10 showed an average 11−fold decrease in cell numbers across all cell types and day 28 only a 1.8 fold decrease from controls. Surprisingly the replication of GFRα1+ As spermatogonia on day 10 had a 2.2 fold increase in respect to controls (p= 0.06), the opposite of previous data that showed inhibition for 3 days reduced replication. The results also show a 4.1, 1.6, and 1.5 fold increase in replication of As, Apr, and Aal respectively at day 14. To determine if this increase was correlated to increased expression of GDNF mRNA, we measured the transcript at each time point. GDNF mRNA levels at all time points were similar to controls.
Conclusion: As predicted there is an increase in number and replication of SSCs and PSs but it is not correlated with increased transcriptional expression of GDNF.
Funding: Supported by (R01HD074542−01).

Poster #28
MULTICELLULAR HUMAN TESTICULAR ORGANOID: A NOVEL IN VITRO GERM CELL AND TESTICULAR TOXICITY MODEL
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(Presented by: Samuel Pendergraft, MS)

Introduction: Mammalian spermatogenesis is regulated through paracrine and endocrine activity, cell signaling, and local control mechanisms. These highly specific signaling interactions are effectively absent upon placing testicular cells into two−dimensional primary culture. The specific changes that occur between key cell types and involved spermatogenesis signaling pathways during primary culture remain to be elucidated. However, current protocols to produce mature germ cells in vitro are inefficient and are limited in supporting post−meiotic cells. In order to address these limitations we have developed a 3−dimensional (3D) testis organoid in vitro by combining stem cell and tissue engineering approaches. This model can be utilized as a means to evaluate gonadotoxic agents, and as a means to address critical deficiencies in our understanding of basic human spermatogenesis.
Objectives: The overall goal of this study is to establish, characterize, and culture a multicellular, 3D, human testis organoid and to assess its functionality and spermatogenic capacity over time.
Methods: Development of our model system consisted of (1) Identification and analysis of specific cellular components necessary for use in our 3D culture method, (2) Establishment of basic design parameters, culture conditions, and (3) Characterization of human testicular organoids using live cell imaging, immunofluorescence, immunohistochemistry, cell type and stage−specific gene expression, and viability assays.
Results: Human Spermatogonial stem cells (SSCs), Sertoli, and Leydig cells were isolated, characterized, and expanded from tissue obtained through the National Disease Research Interchange (Philadelphia, PA, USA). These cell types were integrated successfully into 3D organoids and maintained viability as determined by ATP and Live/Dead assays for over 4 weeks in culture. Gene expression within these multicellular human testis organoids was measured over time for cell and stage−specific markers including UCHL1, DAZL, VASA, SYCP3, SPO11, PRM1, ACROSIN, SOX9, GATA4, INSL3, AND HSD3B.
Conclusion: Testicular in vitro organoids were successfully generated using isolated human SSC, Sertoli, and Leydig cells and maintained long term. Future directions include optimizing the spermatogenic capacity of the organoids and evaluating their use as a novel testicular toxicity model.
Funding: AFIRM II, Award No. W81XWH−13−2−0052. NIH grant 5U42RR006042 and Erret−Fisher Foundation grant GTS 3679.

Poster #29
THE UBIQUITIN LIGASE HUWE1 IS REQUIRED FOR ESTABLISHMENT AND MAINTENANCE OF SPERMATOGONIA
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(Presented by: Ellis Fok, PhD)

Introduction: Spermatogonia are a heterogenous population of cells that include spermatogonia stem cells (SSC) and committed progenitors. The mechanisms underlying the establishment and maintenance of spermatogonia remain largely unknown. Our previous studies identified an E3 ubiquitin ligase, Huwe1, and demonstrated its cytoplasm−nucleus shuttle during the transition from gonocyte to spermatogonia.
Methods: In the present study, we inactivated Huwe1 specifically in primordial germ cells (Huwe1−/Y) to explore the role of Huwe1 in spermatogonia. Huwe1−/Y mice were infertile. Immunostaining of germ cell markers, Ddx4 and Tra98, in different developmental stages (3, 6, 8 and 15 dpp) of Huwe1−/Y testes revealed a progressive degeneration of germ cells from 6 dpp onwards with absence of germ cells in the adult.
Results: BrdU incorporation assay and immunostaining of gonocyte marker, FoxO1, showed a significant decrease in the mitotic re−entry of gonocytes and their transition to spermatogonia in 3 dpp Huwe1−/Y testes. Realtime PCR showed decreased expression of both undifferentiated (Gfra1, Plzf and Ngn3) and differentiating spermatogonia (c−Kit, Stra8 and Dazl) markers in 6 dpp Huwe1−/Y testes. Knockout of Huwe1 in primary SSC culture by tamoxifen−inducible Cre recombinase (CreERT2) or in C18−4 spermatogonial cell line by CRISPR/Cas9 both resulted in cell degeneration, indicating that Huwe1 is required for maintenance of spermatogonia. Germ cell degeneration after knockout of Huwe1 in vitro and in vivo was associated with increased cell size, G2/M cell cycle arrest, increased γH2Ax level and its ubiquitination, indicating increased DNA damage response. Taken together, loss of Huwe1 perturbs the establishment and maintenance of spermatogonia in the first wave of spermatogenesis through persistent or hyper−activation of the DNA damage response. To investigate the involvement of Huwe1 in human male infertility, we genotyped 27 different SNPs in the Huwe1 gene region of patients (204) with non−obstructive azoospermia (NOA) and fertile controls (247). Three SNPs were significantly associated with NOA and one of these was located in the promoter region 672 bp upstream of the transcriptional start site.
Conclusion: Thus Huwe1 is essential for establishment and maintenance of spermatogonia and mutations in this gene may cause human azoospermia. Further studies are being undertaken to decipher the mechanism(s) underlying the control of DNA damage response by Huwe1 in spermatogonia.

Poster #30 – WITHDRAWN

Poster #31
INACTIVATION OF UBIQUITIN LIGASE HUWE1 PERTURBS SPERMATOGONIAL DIFFERENTIATION AND TRANSITION TO MEIOSIS
Rohini Bose, MSc[1], Ellis Fok, PhD[1], Kai Sheng, BSc[1], Wenming Xu, PhD[2], Hsiao Chang Chan, PhD[3], Antonio Iavarone, MD[4], Anna Lasorella, MD[4] and Simon S. Wing, MD[1]
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(Presented by: Rohini Bose, MSc)

Introduction: Spermatogenesis involves crucial, highly regulated transitions between developmental programs such as mitotic proliferation, meiosis and differentiation. How these switches are regulated remains to be delineated. The ubiquitin proteasome system plays a significant role in protein turnover and cellular remodeling and may be involved in these transitions in spermatogenesis. We previously identified the ubiquitin ligase Huwe1 in the testis and showed that it shuttles from the cytoplasm to nucleus as gonocytes transition to spermatogonia. In addition inactivating Huwe1 in gonocytes results in a delay in their mitotic re−entry and leads to spermatogonial depletion. Here we examined the role of Huwe1 in spermatogonial differentiation, meiotic entry and progression.
Methods: We inactivated it in differentiating spermatogonia by expressing Cre recombinase using the Stra8 promoter. Huwe1−/Y males (KO) were subfertile siring 33% smaller litters compared to the Huwe1 flox/Y (WT). The average testes weight of adult KO was only 30% of the WT with sperm concentration being 76% lower. Morphological analysis of adult testis revealed a heterogeneous phenotype with some tubules displaying complete loss of spermatids with fewer differentiating spermatogonia and spermatocytes, while other tubules showed presence of some spermatids. TUNEL assay showed increased levels of apoptosis in a majority of the tubules. To investigate phase-specific defects, we used

quantitative real time PCR to study genes of spermatogonial differentiation (Stra8, cKit), early meiosis (Spo11, Dmc1) and meiotic sex chromosome inactivation (Chic, Hprt, Ube1x, Atp7A, Gla, Ube1y, Rbmy).
Results: Stra8, Spo11 and Dmc1 were significantly down regulated in the KO with a significant up regulation of cKit and no change in others. In addition, chromosome analysis using SCP3 or γH2AX (markers of meiotic progression) of surface spreads prepared from 28 day−old mice revealed severe degeneration of spermatocytes in the KO (2.4% WT vs. 53.3% KO) with the percentage of zygotene and pachytene spermatocytes falling in the KO by 90% and 95% respectively. Currently, I am using WIN 18,446−retinoic acid to synchronize spermatogenesis in KO males to achieve a synchronized inactivation of Huwe1 in differentiating spermatogonia and to understand the underlying mechanism of the observed phenotype.
Conclusions: Collectively, my results indicate that Huwe1 is essential for spermatogonial differentiation and entry into meiosis.

Poster #32 – WITHDRAWN

Poster #33
SUPPLEMENTATION WITH HIGH DOSE FOLATE AFFECTS THE SPERM EPIGENOME IN MEN PRESENTING WITH INFERTILITY
Mahmoud Aarabi, MD, PhD[1], Maria C. San Gabriel, PhD[2,3], Donovan Chan, PhD[4], Armand Zini, MD[2,3] and Jacquetta Trasler, MD, PhD[5]
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(Presented by: Mahmoud Aarabi, MD, PhD)

Introduction: Supplementation with high doses of folic acid is widely used in clinics to improve the sperm parameters of infertile men. While dietary folate is a major source of methyl groups for epigenetic processes such as DNA methylation, little is known about the impact of high dose folate on the sperm epigenome and whether alterations can be transmitted to the offspring.
Materials and Methods: To address the epigenetic consequences of excess folate supplementation, semen and blood samples were collected from 30 men with idiopathic infertility who received 5mg/day of folic acid for 6 months at the McGill University Reproductive Centre and the Clinique OVO. Folate and hormone levels, semen parameters and the sperm epigenome were investigated before and after treatment. Germ line−specific differentially methylated regions of the imprinted genes H19, DLK1/GTL2, SNRPN, KCNQ1OT1, PLAGL1 and MEST were screened in sperm by pyrosequencing. A next generation sequencing−based method, reduced representation bisulfite sequencing (RRBS), was utilized to study over 3 million DNA methylation sites across the sperm epigenome. RRBS was also used to study the sperm epigenome in Balb/C mice (n=7) treated with high dose (20−fold control) or control folate diets for 12 months.
Results: Blood folate levels increased significantly following the supplementation period (P<.0001). Sperm parameters and blood homocysteine, vitamin B12 and hormones remained unchanged. Neither infertility nor excess folate affected the methylation levels of imprinted loci. Interestingly, preliminary analysis of RRBS revealed slight but significant loss of methylation across genic and intergenic regions of sperm DNA in both human and mouse although some specific sites demonstrated gain of methylation. Ingenuity Pathway Analysis of differentially methylated sites suggested changes in methylation of genes involved in pathways related to cancer and developmental disorders.
Conclusion: Six month of folate supplementation in infertile men increases blood folate levels while sperm parameters and DNA methylation at imprinted loci remain unchanged. Unexpected loss of methylation across the sperm epigenome suggests the involvement of other factors such as folate metabolic pathways. We are now performing in−depth analysis as well as validation of the RRBS findings. Supported by Canadian Institutes of Health Research (CIHR)

Poster Session II

Poster #34
KINETICS OF STEM CELL DIVISIONS IN PRIMARY CULTURES OF UNDIFFERENTIATED SPERMATOGONIA
Aileen Helsel and Jon Oatley
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(Presented by: Aileen Helsel)

Introduction: Self-renewal of spermatogonial stem cells (SSCs) provides the basis for establishment and maintenance of a foundational pool that is required for continuity of the spermatogenic lineage. During steady-state conditions, progenitor spermatogonia arise from the SSC pool at periodic intervals and then transiently amplify in number before transitioning to a differentiating state. At present, the kinetics of SSC divisions that provide for maintenance of the pool via self−renewal and generation of initial progenitor spermatogonia are undefined. Addressing this gap in knowledge requires observation of cell divisions in real time which is difficult with in vivo experimentation. Although unavailable for humans, methodology for the maintenance of a primary population of undifferentiated spermatogonia consisting of SSCs and progenitor spermatogonia is available for mice, which provides a platform to conduct long-term imaging of cell division kinetics. However, until recently, distinguishing the spermatogonial subtypes via live imaging was not possible thus limiting the ability to critically assess division kinetics of the SSC pool specifically. In previous studies, we established that expression of the helix−loop−helix protein inhibitor of DNA binding 4 (ID4) is a distinguishing feature of SSCs and generated a transgenic mouse model in which ID4 expressing cells are marked by GFP.
Methods: Here, we conducted live imaging analysis of primary cultures established from Id4−Gfp transgenic mice over periods of 3−6 days to critically assess division kinetics of both SSCs and progenitor spermatogonia.
Results: First, we discovered that the percentage of total spermatogonia that was ID4−GFP+/SSCs increased by ~2−fold during the analysis period, beginning at ~8% and increasing to ~18% by 72 hours. Second, on average ~20% of the ID4−GFP+/SSC population underwent a division during this period and based on this observation we calculated the average division rate as ~15 days. Third, ~80% of divisions by ID4−GFP+/SSC cells yielded two ID4−GFP+ daughters; whereas, ~20% produced only one ID4−GFP+ daughter.
Conclusion: These initial observations of SSC behaviors in primary cultures of spermatogonia suggest that maintenance of the SSC pool occurs primarily through symmetric divisions at an intrinsically controlled interval. This research was supported by grant HD061665 awarded to JMO from the NICHD.

Poster #35
MAINTENANCE OF HUMAN SPERMATOGONIAL STEM CELLS ON LAMININ
Hanna Valli, PhD and Kyle Orwig, PhD
University of Pittsburgh School of Medicine
(Presented by: Hanna Valli, PhD)

Introduction: Spermatogonial stem cells (SSCs) may have application for treating some cases of infertility, for example infertility that results from gonadotoxic therapies for cancer and other conditions. Several centers worldwide are cryopreserving testicular tissue for prepubertal boys before initiation of gonadotoxic therapies (chemotherapy, radiation). However, biopsies obtained from prepubertal boys are likely to contain few stem cells, raising questions about the feasibility of downstream applications, such as SSC transplantation. Several groups have reported culturing human SSCs in the presence of testicular somatic cells, (Sadri−Ardekani et al 2009, 2011), mouse fibroblasts (Wu et al., 2009), human ESC−derived fibroblast like cells (Chen et al., 2009), Sertoli cells (Nowroozi et al., 2011; Liu et al., 2011; Mirzapour et al., 2012), matrigel (Kokkinaki et al., 2011) and gelatin (He et al., 2010).
Methods: Here we utilized a multifactor sorting strategy (EPCAM+/HLA−ABC−/CD49e−) to enrich human SSCs and reduce testicular somatic cell contamination. Sorted cells were plated on mouse embryonic fibroblasts (STO and C166) and laminin. Ubiquitin Carboxyl−Terminal Esterase L1 (UCHL1) immunocytochemistry was used to track survival of human undifferentiated spermatogonia. In all conditions cells were plated (~4 x 10^5 cells/cm2) with mouse serum−free medium (mSFM) containing 10ng/mL of glial cell−line derived neurotrophic factor (GDNF) and 1ng/mL of basic fibroblast growth factor (bFGF). After 8 days in culture, cells from one well were stained with UCHL1 and cells from another well were passaged.
Results: The staining results show that recovery of UCHL1 positive cells was similar in all 3 conditions (36% on laminin, 35% on STOs and 41% on C166). Under these conditions, cells grew in clusters and testicular somatic cells were few in number and did not overgrow the SSCs. After passaging, the morphology of the clusters was the best in laminin cultures, with cluster frequency and size declining in the STO and C166 cultures.
Conclusion: Here, we show for the first time that human SSCs can be maintained on laminin coated dishes for a short period of time. We continue to monitor the maintenance of human spermatogonia on laminin and transplant studies to support the ICC data are forthcoming. This work was supported by NIH grants HD076412, HD075795 the US−Israel Binational Science Foundation and Magee−Womens Research Institute.

Poster Session II

Poster #36 – WITHDRAWN

Poster #37
STUDY OF RNA BIOMARKERS OF NORMAL SPERMIOGENESIS IN NORMAL SEMEN AND SPERM VIA TRANSCRIPTOME ANALYSIS.
Alexander Yatsenko, MD, PhD[1], Archana Kishore, PhD[1], Andrew Georgiadis, BS[1], Randy Beadling, BS[1], Etta Volk[2], Joseph Sanfilippo, MD[3], Thomas Jaffe, MD[4], James Lyons−Weiler, PhD[5] and Tamanna Sultana, PhD[5]
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(Presented by: Alexander Yatsenko, MD, PhD)

Introduction: Previous studies have indicated that mature human sperm contains a complex population of RNAs that have been implicated in past and coming events such as spermatogenesis, fertilization, and possibly early embryonic development. Recent attempts were made to identify those RNAs associated with good fertility and good sperm quality. Here, we report the transcriptome analysis of normal semen and sperm based on total RNA−seq study.
Methods: Equimolar RNA from five semen and 6 mature sperm samples were studied. To increase efficiency of total RNA sequencing analysis we excluded ribosomal RNA via rRNA reduction. Preliminary, our RNA seq analysis detected a total of ~19,900 and ~17,00 genes in 2 RNA samples. After normalization, we identified 16,898 transcripts that were common to both samples. Using more stringent criteria with average sequence read coverage of >1, we identified ~10,000 transcripts and 5,000 transcripts in two samples, where ~4,500 transcripts were shared between the two samples. We classified these transcripts as protein coding, non−coding and pseudogenes. To reveal the functional annotation and pathways of these genes, all protein coding genes were subjected to Ingenuity Pathway Analysis and PANTHER analysis. This annotation resulted in 14 major categories, including DNA replication/repair, gene expression regulation, post−transcriptional regulation, post−translational modification, cellular maintenance, cellular structure, molecular transport, cell movement, cell signaling, cell cycle regulation, apoptosis, metabolism, spermatogenesis, and embryogenesis.
Results: Based on number of genes involved, IPA identified the top pathways including, translation regulation, cellular growth, cell cycle, DNA repair, apoptosis and transcription regulation. A number of novel transcripts were also identified in this study, however their role in spermatogenesis remain to be explored.
Conclusion: Our study suggests the presence of important sperm RNAs that could serve as informative biomarkers of male germ cell quality and potentially predict fertilization and early embryonic development outcome in IVF/ICSI procedures.

Poster #38
REQUIREMENT FOR MOV10L1 RNA HELICASE ACTIVITY IN THE PROCESSING OF PIRNA PRECURSORS
Qi Fu[1], Anastassios Vourekas, PhD[1], Ke Zheng, PhD[2], Erica Goode and P. Jeremy Wang, PhD[1]
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(Presented by: Qi Fu)

Introduction and Objectives: PIWI−interacting RNAs (piRNAs) are a class of small non−coding RNAs highly expressed in the germlines of many species from C. elegans to mammalian species. They most notably protect the integrity of the germline genome through transcriptional and post−transcriptional silencing of active transposable elements (TEs) during germ cell development. piRNA biogenesis is tightly coupled to TE silencing. In mammals, the RNA transcripts of active TEs are targeted into the piRNA processing pathway as piRNA precursors for degradation and biogenesis of primary piRNAs. Primary piRNAs then enter the ping−pong amplification loop to generate secondary piRNAs with complementary sequences. Finally, secondary piRNAs are believed to guide their PIWI proteins to transcriptionally silence active TEs in the nucleus. However, the mechanisms underlying piRNA biogenesis remain unclear.
Results: Moloney Leukemia Virus 10−like 1 (MOV10L1) is a testes−specific RNA helicase required for piRNA biogenesis. Mov10l1−deficient males are viable but infertile, due to meiotic arrest. Disruption of Mov10l1 leads to the accumulation of piRNA precursors. Our CLIP−seq results reveal that MOV10L1 directly associates with piRNA precursors. The majority of MOV10L1−bound RNAs map to piRNA hotspots, but do not represent mature piRNA sequences. MOV10L1 binds to RNA near regions with high secondary structure potential. Because RNA helicases recognize and resolve secondary structures, we hypothesize that MOV10L1 processes piRNA precursors through its RNA helicase activity. I designed two Mov10l1 knock−in mouse models containing conserved point mutations in the ATP binding and ATP hydrolysis sites of the MOV10L1 RNA helicase domain. Male Mov10l1 homozygous knock−in mice exhibit meiotic arrest, mislocalization of piRNA pathway proteins, a derepression of the LINE1 TE, and a lack of piRNAs associated with MILI. In conclusion, MOV10L1 RNA helicase activity is required for piRNA biogenesis. In the future, I plan to determine if MOV10L1 RNA

helicase activity is required for the processing of piRNA precursors by measuring the levels of piRNA precursors in Mov10l1 knock−in mice.
Conclusions: Our previous and current studies demonstrate that MOV10L1 is a master regulator of piRNA biogenesis in mammals. As an essential germ cell−specific gene, mutations in human Mov10l1 are expected to cause male infertility. Thus, MOV10L1 may be a molecular target for male contraception.

Poster #39
INHIBITION OF RALDH ENZYMES ALTERS TESTICULAR RA AVAILABILITY AND AFFECTS MULTIPLE STEPS OF SPERM PRODUCTION.
Travis Kent[1], Sam Arnold[2], Rachael Fasnacht[1], Ross Rowsey[1], Debra Mitchell[1], Cathryn Hogarth[1], Pat Hunt[1], Nina Isoherranen[2] and Michael Griswold[1]
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(Presented by: Travis Kent)

Introduction and Objectives: Retinoic acid (RA) has been implicated in playing vital roles for many spermatogenic processes, including spermatogonial differentiation, blood−testis barrier (BTB) reorganization, meiotic initiation, and spermiation. Inhibition of the RA synthesis enzymes is currently being investigated as a potential target for male contraceptives. However, neither the kinetics of these inhibitors, nor the effects of ALDH inhibition on various tissues of the adult mouse are known.
Methods: Male adult mice (3 to 5 months of age, N = 3 per timepoint) were treated with WIN 18,446, a potent RALDH inhibitor, for either 1 or 8 days and subsequently euthanized at various time points between 0 and 24 hours after the final treatment. Retinoid levels were then quantified within various organs utilizing a novel LC/MS/MS approach. A thorough histological analysis was performed on testicular cross−sections from adult animals treated for 8 days with WIN 18,446 to investigate misregulation of spermatogonial differentiation and spermiation. Finally, animals treated for 12 days with WIN 18,446 were analyzed for any misregulation occurring during meiosis.
Results obtained: Testicular RA levels and RALDH activity were found to drop within 30 minutes of treatment. Retinaldehyde levels were shown to be 200−300% higher in WIN 18,446 treated animals when compared to controls. Examination of the both the liver and kidney, however, revealed no significant decrease in RA levels after 1 or 8 days of WIN 18,446 treatment, indicating that inhibition of RALDH enzymes could be a viable contraceptive target. In the WIN 18,446 treated animals, there was a significant loss of STRA8−positive preleptotene spermatocytes, indicating misregulation of spermatogonial differentiation. There were also misaligned elongated spermatids and a significant increase in BTB permeability in the WIN 18,446 treated animals. There was a slight increase in the number of recombination events in animals treated with WIN 18,446, but no significant difference in distribution of those events across the synaptonemal complexes. However, animals treated with WIN 18,446 displayed a significant increase in both major and minor meiotic defects.
Conclusions: Together these data show that lowered testicular RA levels are likely responsible for misregulation of multiple processes throughout spermatogenesis.
Funding: This work was supported by the NIH Grants R01 HD10808, T32GM083864, and U54 HD04245.

Poster #40
NORMAL SPERM HEAD MORPHOGENESIS REQUIRES ADP−RIBOSYLTRANSFERASE 11 (PARP11) IN MICE
Mirella Meyer-Ficca, PhD[1], Motomasa Ihara, MD, PhD[2], Jessica Bader, BS[1], N. Adrian Leu, BS[3], Sascha Beneke, PhD[4] and Ralph G. Meyer, PhD[1]
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(Presented by: Mirella Meyer-Ficca, PhD)

Introduction: Sperm are highly differentiated cells characterized by their species-specific nuclear shapes and extremely condensed chromatin. Abnormal head shapes represent a form of teratozoospermia that can impair fertilization capacity. We show that poly(ADP−ribose) polymerase−11 (ARTD11 / PARP11), a member of the ADP−ribosyltransferase family, is expressed preferentially in round spermatids and spermatids undergoing nuclear condensation and differentiation.
Furthermore, deletion of the Parp11 gene in mice results in teratozoospermia and male infertility, due to the formation of abnormally shaped, fertilization−incompetent sperm, despite normal testis weights and sperm counts. Female Parp11−/− mice are normal and fertile. At the subcellular level, PARP11−deficient elongating spermatids reveal structural defects in the nuclear envelope and chromatin detachment associated with abnormal nuclear shaping, indicating functional relevance of PARP11 for nuclear envelope stability and nuclear reorganization during spermiogenesis.
Results: In vitro, PARP11 exhibits mono(ADP−ribosyl)ation activity with the ability to ADP−ribosylate itself. In transfected somatic cells, PARP11 co−localizes with nuclear pore components such as NUP153. Site directed mutagenesis of the

transfected cDNA indicated that amino acids Y77, Q86 and R95 in the N−terminal WWE domain, as well as the catalytic domain are essential for colocalization of PARP11 with the nuclear envelope, but catalytic activity of the protein is not required for colocalization with NUP153.
Conclusion: This study demonstrates that PARP11 is a novel enzyme important for proper sperm head shaping and identifies it as a potential factor involved in idiopathic teratozoospermia in human patients.
Funding: We gratefully acknowledge support by grants from the National Institutes of Health (NIH R01 HD48857 and U54HD068157) and the Utah Agricultural Experiment Station, Utah State University (UTA01166) to RGM.

Poster #41
THE FIRST WAVE OF MURINE SPERMATOGENESIS RELIES ON AN INTACT RAR SIGNALING MECHANISM
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[1]Washington State University; [2]SIBCB
(Presented by: Cathryn Hogarth, PhD)

Introduction: The retinoic acid receptor (RAR) signaling pathway is essential for proper spermatogenesis. Conditional mutations rendering either the RARï• ¡ or RARï□§ isoforms unable to bind either retinoic acid (RA) or their co−activators have helped shape our understanding of cell−specific responses to RA within the testis. However, in both of these mutants, functional redundancy between isoforms appears to be occurring as the testes display a heterogeneous phenotype, with spermatogenesis proceeding normally in some tubules.
Methods: To overcome this, we utilized a conditional transgenic mouse model to overexpress a dominant negative form of human RARï• ¡, which sequesters all the RARs and RXRs but cannot be activated by RA, thereby blocking all RAR and/or RXR signaling.
Results: Driving the expression of this transgene in postnatal male germ cells, via the Stra8iCre line, resulted in severely reduced differentiation of spermatogonia at the onset of spermatogenesis and an accumulation of ZBTB16−positive cells, indicating that activation of RAR by RA in germ cells is essential for the first wave of spermatogenesis. Conversely, significantly more STRA8−positive spermatogonia, indicative of premature differentiation, were observed in juvenile mice expressing the transgene specifically within Sertoli cells suggesting that intact RAR signaling is required for the appropriate timing of RA synthesis. In addition, vacuoles began to form within the seminiferous epithelium as early as 15 days post partum (dpp) in the testes of these animals, with sloughing of advanced germ cells occurring from 20 dpp. Analysis of the mutant testes at 60 and 80 dpp revealed seminiferous tubules displaying very few or no germ cells and staining for SOX9 indicated that the Sertoli cell nuclei had lifted off the basement membrane and were placed randomly throughout the seminiferous epithelium. Comparison of these phenotypes with the published single receptor knockout data revealed that complete ablation of RAR signaling within either germ or Sertoli cells yielded significantly more and stronger testicular defects than those induced by mutating any single RAR alone.
Conclusion: In addition, these results imply that RAR signaling in germ and Sertoli cells is a critical control mechanism driving the proper differentiation of spermatogonia and is likely essential for both establishment and maintenance of the blood−testis−barrier.
Funding: This work was supported by the NICHD grant R01 10808 grant awarded to MDG.

Poster #42
CHARACTERIZING THE SPERMATOGONIAL RESPONSE TO RETINOIC ACID DURING THE INITIATION OF SPERMATOGENESIS IN THE NEONATAL MURINE TESTIS.
Kellie O'Rourke, Cathryn Hogarth, Debra Mitchell, Jon Oatley and Michael Griswold
(Presented by: Kellie O'Rourke)

Introduction: Vitamin A, in the form of retinoic acid (RA), is essential for spermatogonial differentiation. However, the direct effects of RA on spermatogonia in the neonatal testis and whether RA influences the establishment of the spermatogonial stem cell (SSC) pool have yet to be fully examined.
Methods: This study utilized our published WIN 18,446/RA treatment protocol to manipulate RA levels within the neonatal testis, enriching for either undifferentiated spermatogonia and SSCs or simultaneously differentiating spermatogonia. The objective of this study was to investigate whether manipulating RA levels within the testis altered the development of the SSC pool. Whole mount immunofluorescence was performed on testis tubules isolated from WIN 18,446 only (N=5) and WIN 18,446/RA−treated neonatal mice allowed to recover for either 4 or 12 hour intervals following RA injection (N=3 for each time point).
Results: Only markers of undifferentiated spermatogonia were present in germ cells within testes of WIN 18,446 only−treated mice, confirming that RA is essential for the first A to A1 transition. Testis tubules isolated from mice exposed to WIN 18,446/RA contained significantly more STRA8− and fewer GFRα−1−positive cells when compared to WIN 18,446−treated only tubules. In addition, comparison of WIN 18,446 only and vehicle control treated animals

demonstrated that spermatogonia containing a SSC marker (ID4−GFP) are significantly more abundant in treated animals, similar to what was observed in wild−type testes before the SSC pool is normally established. ID4−GFP−positive cells were largely present as A single spermatogonia in 9 dpp age match non−treated controls (N=3). Preliminary transplantation analysis demonstrated that testes from WIN 18,446 only treated animals possess an increased number of SSCs when compared to controls.
Conclusion: This observation implies that lack of RA during neonatal development leads to an increase in the size of the SSC pool while blocking the differentiation of spermatogonia.
Funding: This work was supported by NIH Grant R01 HD10808 to MDG.

Poster #43
CLASSICAL RA SIGNALING IS NECESSARY IN LEYDIG CELLS FOR NORMAL SPERMATOGENESIS.
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Washington State University
(Presented by: Estela Arciniega)

Introduction: 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 preliminary data, supported by a small number of publications, suggest an inverse relationship between the expression of enzymes involved in either testicular androgen or RA biosynthesis, especially when either testosterone or RA is present in excess. In addition, Leydig cells contain the machinery necessary for RA biosynthesis. Our objective was to determine whether Leydig cells require the classical RA signaling mechanism.
Methods: To test this, we utilized a transgenic mouse line that expresses a dominant negative form of RA receptor alpha (RAR−DN) and the Leydig cell−specific Cre mouse line, Cyp17iCre, to generate male mice with Leydig cells unable to perform classical RA signaling.
Results: Interestingly, morphological analysis of 30, 60, and 90 dpp RAR−DN−Flox/Cyp17iCre−positive mice revealed that the testes of these animals display spermatogenic errors consistent with pachytene spermatocyte apoptosis, 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 dpp mutant testes. Immunohistochemistry localizing STRA8 within the RAR−DN−Flox/Cyp17iCre−positive testes demonstrated that normal spermatogonial differentiation was occurring. In addition, qPCR measurements showed decreased levels of steroidogenic enzyme transcripts in the 90 dpp mutant testes.
Conclusion: These data imply that the classical RA signaling mechanism is needed in Leydig cells for the normal production of testosterone and therefore proper spermatogenesis. Future studies will involve measuring serum and testicular testosterone levels in RAR−DN−Flox/Cyp17iCre−positive mice and examining the maintenance of the blood testis barrier, meiosis, Sertoli−spermatid adhesion and sperm release, processes known to be regulated by both vitamin A and testosterone.

Poster #44
HOW DO HISTONES CONVEY INFORMATION BETWEEN GENERATIONS?
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(Presented by: Ralph Meyer, PhD)

Introduction: In mature sperm, most, but not all, histones have been replaced by protamines during spermiogenesis and the DNA is highly condensed. The small fraction of retained histones appears to be selectively bound to DNA in distinct nuclear domains including gene coding regions. This finding led to the proposal that sperm histones have an important role in regulating gene expression in the early embryo. Recently, a link between histone association with genes in sperm and their subsequent expression in 2−cell embryos was demonstrated by perturbing poly(ADP−ribose) (PAR) metabolism during spermiogenesis in mice. PAR metabolism in spermatids is mediated by PARP and PARG enzymes with specific functions in epigenetic gene regulation and chromatin reorganization.
Methods and Results: Inhibition of PARP activity, using either a genetic mouse model or pharmacological approach, led to aberrant retention of histones in sperm. Genome−wide histone mapping in mature sperm showed that defined genes were affected by abnormal histone positioning due to PAR inhibition during spermiogenesis. Transcript frequencies of the

affected genes were either elevated or reduced in 2−cell embryos generated with such sperm, depending on the individual genes affected by abnormal histone occupancy in sperm.
Conclusion: These results provoke the hypothesis that the presence or absence of nucleosomes and the activating or silencing nature of their histone modifications provide a connection between their expression profile in early embryos and histone modification status in the sperm. This hypothesis and potential mechanisms will be further discussed in this presentation.

Poster #45 – Switched to Short Talk/Poster #2

Poster #46
CYCLOPHOSPHAMIDE (CPA) TREATMENT ALTERS THE EXPRESSION OF MEMBERS OF THE ZIP FAMILY ZINC TRANSPORTERS IN PACHYTENE SPERMATOCYTES.
Anne Marie Downey[1], Barbara Hales, PhD[2] and Bernard Robaire, PhD[3]
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(Presented by: Anne Marie Downey)

Introduction: Previous studies have shown that paternal exposure to CPA, a chemotherapeutic agent and immunosuppressant, causes DNA damage and oxidative stress in the testis and has detrimental effects on sperm quality and progeny outcome. How CPA affects the developing germ cells and how they respond to this insult remain unresolved. The objective of this study was to test the hypothesis that CPA affects gene expression in pachytene spermatocytes and round spermatids, the meiotic and post−meiotic germ cells.
Methods: Adult male Sprague-Dawley rats were gavaged daily with saline or CPA (6 mg/kg) for 4 weeks; pachytene spermatocytes and round spermatids were collected by unit gravity sedimentation of testicular cells.
Results: Using whole genome gene expression microarrays we have identified differentially expressed genes in both cell types. Interestingly, transcripts for two members of the ZIP family of metal ion transporters (ZIP5 and ZIP14), which play an important role in supplying zinc to cells, were up−regulated over 1.5 fold in pachytene spermatocytes after CPA treatment. Further analysis revealed that transcripts for two more ZIP transporters (ZIP6 and ZIP13) were also significantly up−regulated. The expression of ZIP transporters remained unchanged in round spermatids. PCR validation confirmed the microarray results. Preliminary protein analysis also indicated an increased expression of the corresponding proteins.
Conclusion: These results suggest that zinc uptake is increased in pachytene spermatocytes in response to CPA treatment. As zinc plays an important role in antioxidant activity, enhanced uptake may reflect an increased demand for zinc in response to elevated oxidative stress following CPA treatment. Zinc is also an important trace element in spermatogenesis, particularly for proper compaction of chromatin. The poor sperm chromatin quality previously observed after CPA treatment may, in part, be due to altered zinc homeostasis in the germ cells.
Funding: This study is supported by CIHR.

Poster #47
PATERNAL USE OF TOBACCO, ALCOHOL AND EXPOSURE TO PESTICIDES MAY DAMAGE SPERM CHROMATIN STRUCTURE: ROLE IN CHILDHOOD CANCER − RETINOBLASTOMA (NON-FAMILIAL SPORADIC)
Shiv Basant Kumar, MSc[1], Bhavna Chawla, MD[2] and Rima Dada, MD, PhD[1]
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(Presented by: Shiv Basant Kumar, MSc)

Introduction: Life style habits adopted by father play an important role in maintaining the sperm DNA health. This study was planned with aim to analyze the effect of paternal life style habits (smoking, alcohol and pesticides exposure) and its effect on sperm DNA damage as a possible etiological factor for non-familial sporadic retinoblastoma in children.
Methods: A total of 56 cases of retinoblastoma (Non−familial, No somatic mutation in father or mother) and 50 controls (father of healthy children with in 2 years of age) were recruited at a tertiary referral centre, India. Semen samples were collected from the fathers and analyzed for semen parameters and biological markers as Sperm Chromatin Structure Assay, 8−hydroxy−2'−deoxyguanosine (8−OHdG), Telomere length and Reactive Oxidative Stress (ROS) levels were assessed. Logistic binary regression was used to compute the odds ratios (OR) of various life style habits of father for Retinoblastoma.
Results: Seminal mean ROS levels were significantly higher [$p=0.0079$] in fathers of Rb patients compared to controls. There was significant increase in Mean DFI [$p<0.001$], 8−OHdG level [$p<0.01$] compared to controls. Relative sperm

mean telomere length (T/S) of cases was shorter as compared to controls (p =0.459). We observed OR [95%CI] for smokers, alcohol users and pesticides exposed was [10.0(2.9−34.45; p<0.001)], 3.5(1.01−12.16; p=0.037)] and [7.292(2.13−24.92; p<0.001)] respectively.
Conclusion: As results shows, smoking, pesticides exposure and alcohol intake adversely affect Sperm chromatin structure and the health of offspring. In this study we found 76% of fathers had increased ROS levels and 72% had increased DFI. Oxidative damage leads to accumulation of mutagenic bases and shortening of telomeres. To our knowledge, this is the first in vivo observation using the ROS, DFI and 8−OHdG for genotoxic potential for men who smoke and drink and pesticides exposed.

Poster #48
HUMANIN ANALOGUE (HNG) PREVENTS MALE GERM CELL APOPTOSIS AND ATTENUATES WHITE CELL SUPPRESSION INDUCED BY TEMOZOLOMIDE IN SEVERE COMBINED IMMUNO−DEFICIENCY (SCID) MICE BEARING MEDULLOBLASTOMA
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LA BioMed Research Institute & Harbor−UCLA Medical Center
(Presented by: Yue Jia, MD, PhD)

Introduction and Objectives: Onco−infertility is a major concern of cancer survivors. White blood cell (WBC) suppression during chemotherapy is associated with infections causing significant morbidity and limiting use of chemotherapy. Humanin is a broad spectrum anti−apoptotic peptide which has been shown by our group to decrease chemotherapy−induced male germ cell apoptosis while enhancing therapeutic anti−tumor effect. In this study, we investigated whether HNG (a potent analogue of humanin) will prevent chemotherapy (Temozolomide, TMZ, an alkylating agent used clinically to treat brain tumors) induced male germ cell apoptosis and WBC suppression in SCID mice bearing subcutaneous implants of human medulloblastoma.
Methods: DAOY human pediatric medulloblastoma cells (1.0x108cells/animal) were injected subcutaneously into the right flank of 7−week old male SCID mice. After 3 weeks to allow tumor growth, each group of mice (n=4 to 5/group) received one of the following treatments: 1) vehicle (control); 2) HNG intra−peritoneal (IP) injection (HNG, 5mg/Kg BW/day, 3 days pretreatment + 5 days treatment); 3) TMZ IP injection (TMZ, 50mg/Kg BW/day X 5 days); 4) TMZ and HNG IP injections (TMZ+HNG). Mice were sacrificed 24h after last injection, subcutaneous tumors were dissected and weighed, complete blood counts were obtained by an automated cell counter (VetScanHM2); and male germ cell apoptosis was assessed by TUNEL staining and quantified (Apoptosis Index, AI, number of apoptotic germ cell/Sertoli cell).
Results: TMZ markedly suppressed tumor growth; addition of HNG did not alter the marked suppression of tumor growth by TMZ. HNG alone had no effect on male germ cell apoptosis, but significantly prevented TMZ−induced apoptosis in both middle stages (TMZ 0.058±0.006, TMZ+HNG 0.023±0.003, p<0.05) and early+late stages (TMZ 0.116±0.009, TMZ+HNG 0.060±0.010, p<0.05). In addition, HNG significantly attenuated TMZ suppressed total WBC (TMZ 0.43±0.05x106/ml, TMZ+HNG 0.90±0.16x106/ml, p<0.05) and granulocyte cell count (TMZ 0.34±0.04x106/ml, TMZ+HNG 0.76±0.12x106/ml, p<0.05).
Conclusions: We conclude that the anti−apoptotic peptide HNG ameliorated TMZ−induced germ cell apoptosis and white blood cell suppression without affecting the chemotherapeutic effect on transplanted medulloblastoma tumor in an immunocompromised mouse model. Our data suggest that HN and its analogues may be used as supportive therapy to attenuate granulocyte suppression and preserve fertility.

Poster #49
COMPARISON OF GDNF MRNA EXPRESSION IN TESTES OF FERTILE AND INFERTILE MEN AND FERTILE MICE.
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(Presented by: Dolly Singh, BS)

Introduction: Normal spermatogonial stem cells (SSCs) renewal and differentiation is essential for maintaining spermatogenesis in the adult testis. SSCs are a subset of undifferentiated spermatogonia and have the ability to self−renew or give rise to progenitor spermatogonia. Glial cell line−derived neurotropic factor (GDNF), secreted by Sertoli cells, has been shown to be one of the growth factors required for SSCs regulation in mice (1, PMID: 22232066; 2, PMID: 25165119). Importantly, GDNF is also expressed in human testes, raising the possibility that this growth factor plays a

role in male infertility (3, PMID: 11732574). The objective of this study was to compare testicular GDNF mRNA in testes of men with normal spermatogenesis, and infertile men with either maturation arrest (MA) or Sertoli Cell Only syndrome (SCO), which results from loss of SSCs. Additionally, we compared GDNF mRNA expression in testes of fertile mice and men to gain insight into whether the mouse was a good model for studying GDNF expression in humans.
Methods: Clusterin mRNA was measured as a control and data were normalized to 18S rRNA and subjected to one−way ANOVA. Human GDNF mRNA was measured by digital PCR. Human and mouse clusterin mRNA's, mouse GDNF mRNA &18S mRNA were measured using Taqman assays. From each sample of RNA, cDNA was synthesized and was used across all assays.
Results: GDNF mRNA levels varied significantly among the normal, MA and NOA patients (n=3 per group; $p<0.05$). Expression of GDNF in human testes decreased by 57% in SCO men and increased 32% in MA testes when compared with normal testes. This increase in GDNF mRNA levels in MA patients can be due to a decrease in testicular area causing a higher ratio of Sertoli cells /gram tissue vs. normal men. Clusterin mRNA levels were 37% and 75% higher in MA and SCO testes vs. the normal population, respectively ($p=0.06$). GDNF mRNA levels in testes of fertile men and mice were identical, while clusterin mRNA levels in normal human testes were 3% of mouse levels.
Conclusion: The low levels of GDNF expression in SCO men may reflect an intrinsic testicular defect that could have contributed to limiting SSCs. If these levels can be raised, the testicular environment may be able to support spermatogenesis in the presence of limited SSCs, potentially restoring fertility in a subset of infertile men.
Funding: Supported by 5R01HD074542−04.

Poster #50
OXIDATIVE STRESS: A DIAGNOSTIC APPROACH TOWARDS VARICOCELE
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(Presented by: Arozia Moazzam, MD, PhD)

Introduction: Varicocele, vascular lesions of the pampiniform plexus, is one of the most controversial issues in the field of andrology, regarding its diagnosis and management. Earlier studies suggest that males with varicocele are more at risk of spermatogenic dysfunction, hence affecting fertility. The mechanism by which varicocele affects fertility and spermatogenesis is still unknown. Reactive oxygen species (ROS) in the semen have both, physiological and pathological role in male fertility. Increased oxidative stress affects fertility by causing damage to sperm membranes, proteins, and DNA. Seminal oxidative stress is thus emerging as an essential step in the diagnosis and prognosis of subfertile males approaching the infertility clinics.
Objectives: The aim of the study was to evaluate (i) levels of reactive oxygen species (ROS) in semen (ii) the total antioxidant capacity (TAC) of the seminal plasma (iii) relationship between levels of ROS and TAC in males coming for subfertility evaluation, diagnosed clinically with or without varicocele.
Methods: Our study population consisted of 115 normal healthy fertile males, 121 sub fertile Semen analysis was performed using the WHO (1999) Criteria, and Strict Tygerberg Criteria. ROS in the semen was assessed employing the Chemiluminescence Assay. Total antioxidants in the seminal plasma were measured using the TAC Assay.
Results: A significantly raised ($p<0.001$) levels of ROS, with significantly ($p<0.001$) decreased total anti−oxidant capacity levels were seen in the varicocele negative and varicocele positive subjects as compared to the healthy fertile controls. ROS levels showed a significant ($p<0.05$) negative correlation with TAC levels in the varicocele positive subjects.
Conclusion: The present study has enlightened the important relationship between oxidative stress and subfertility in males with varicocele. Reduced fertility seen in varicocele maybe a consequence of increased oxidative stress. Even though it is evident that oxidative stress is involved in the pathogenesis of varicocele, yet different aspects still need to be investigated.

Poster #51
IN VITRO SEMINIFEROUS TUBULE FORMATION FROM HUMAN ISOLATED TESTIS CELLS
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(Presented by: Gleide Avelar, PhD)

Introduction: In the past few years many techniques have been developed to allow the spermatozoa formation in vitro. Among these approaches, the conventional testicular cells culture resulted in complete spermatogenesis in Danio rerio and elongated spermatids in humans. However, in none of them seminiferous tubules profiles were identified. These seminiferous tubules developed in vitro have been considered as an important source for testis tissue expansion and gamete production ex vivo using, for instance, xenotransplantation technique.

Objectives: Investigate the ability of fresh adult human testicular cells to form seminiferous tubules in vitro.
Methods: Normal human adult testicular tissue was enzymatically digested with hyaluronidase, collagenase and trypsin. The obtained isolated testis cells were cultured in supplemented StemPro media (Life Technologies) containing GDNF, FGF, EGF, LIF, and the hormones estradiol and progesterone. The culture medium was changed twice a week. In the 23rd day of culture aggregated elongated profiles, measuring approximately 1.0 cm in length and 0.1−0.3 mm width, were observed. These structures were fixed in 4% paraformaldehyde and embedded in paraffin. The obtained histological sections were tested using MAGE−A4 primary antibody and counterstained with Hematoxylin.
Results: In the outermost layer of these structures, light microscopy evaluation showed the presence of elongated cells with the morphology similar to peritubular myoid cells that were associated with a basement membrane surrounding a rudimentary seminiferous epithelium; in which groups of MAGE−A4 positive cells (germ cells) and supporting cells were observed. In some regions, the MAGE−A4 positive-cells, resembling the spermatogonia, were located close to the basal membrane. Also, a lumen was noted in some of these structures.
Conclusions: We demonstrated that, in a culture system considered of medium complexity, isolated adult human testicular cells are able to reorganize and form seminiferous tubules-like structures. Although still incipient, the biotechnological potential of this approach is enormous and could be used, for instance, for human fertility preservation and treatment, genetic modification of germ cells.

Poster #52
SUMOYLATION REGULATES G2/MI TRANSITION IN MEIOTIC SPERMATOCYTES
Yuxuan Xiao, PhD[1], Benjamin Lucas, PhD[1], Tania Schiff, BSc[1] and Margarita Vigodner, PhD[2]
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(Presented by: Margarita Vigodner, PhD)

Introduction: We have recently performed cell−specific identification of sumoylated proteins from freshly purified meiotic spermatocytes and primary spermatids. Hundred and twenty proteins were uniquely identified in the spermatocyte and/or spermatid fractions.
Objective: Given the important role for several SUMO targets in meiosis (such as Top2A, SYNPs, CDK1 and other kinases), we examined whether sumoylation is important for the meiotic prophase I to metaphase I transition.
Methods: The G2/MI transition was induced in vitro by treating highly purified pachytene spermatocyte fraction with the phosphatase inhibitor Okadaic Acid (OA) with and without addition of a sumoylation inhibitor (Ginkgolic acid, GA).
Results: In a control culture, OA treatment induced condensation of chromosomes and the G2/MI transition. In contrast, the addition of GA prevented chromosome condensation and G2/MI transition. The use of specific markers confirmed the arrest of the meiotic prophase around the diplotene stage, although de-synapsis was initiated in some cells. Neither disassembly of the synaptonemal complex nor H3Ser10 phosphorylation were detectable in GA-treated spermatocytes. Control spermatocytes showed massive H3Ser10 phosphorylation and full disassembly of the SC. Western blot analysis confirmed the inhibition of sumoylation after the addition of GA to spermatocytes compared to control (DMSO) cultures. Moreover, these cells exhibited decreased tyrosine phosphorylation of several proteins upon the inhibition of sumoylation.
Conclusions: sumoylation is required for G2/M transition in meiotic spermatocytes in vitro; there is growing evidence that phosphorylation and sumoylation interact at multiple levels during meiotic prophase.

Poster #53 – WITHDRAWN

Poster #54
SUBPOPULATIONS OF NEONATAL MOUSE UNDIFFERENTIATED SPERMATOGONIA DEFINED BY BI−MODALLY EXPRESSED GENES
Thu Nguyen[1], Kazadi N. Mutoji, PhD[1], Christopher B. Geyer, PhD[2], Jon M. Oatley, PhD[3], John R. McCarrey, PhD[1] and Brian P. Hermann, PhD[1]
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(Presented by: Brian P. Hermann, PhD)

Introduction: Spermatogenesis and male fertility are dependent upon formation of a pool of spermatogonial stem cells (SSCs) in the mammalian testis. SSCs arise from prospermatogonia, but only some prospermatogonia form the foundational SSC pool, suggesting there are subtypes of undifferentiated germ cells with discrete molecular signatures that predispose distinct fates. We recently used single−cell gene expression approaches to demonstrate considerable heterogeneity in mRNA and protein abundance of a panel of germ cell and stem cell genes in the P6 mouse testis. The results of that study supported the concept that multiple spermatogonial subtypes are present in the neonatal mouse testis which may represent functionally distinct spermatogenic cell types. Twenty−seven of the 172 genes examined exhibited

bimodal mRNA levels among Id4−eGFP+ spermatogonia (i.e., present/absent), suggesting these genes mark two or more discrete cell subpopulations of Id4−eGFP+ undifferentiated spermatogonia.
Methods: To address this possibility, we performed a series of experiments to determine whether these genes are co−expressed at the mRNA and protein levels, and thus, whether they mark discrete populations of spermatogonia. Flow cytometry and fluorescence-activated cell sorting (FACS) with antibodies against cell surface proteins found among the bimodal genes were used to sub-fractionate P6 Id4−eGFP+ spermatogonia.
Results: Flow cytometry co-staining for three cell surface markers found among the bimodal transcripts (TSPAN8, EPHA2 and PVR) demonstrated colocalization in 27.1 ± 12.1% of Id4−eGFP+ cells (n=4). In addition, mRNA levels for a panel of bimodal genes measured using qRT−PCR were largely enriched among TSPAN8−high and EPHA2-high sorted subpopulations of P6 Id4−eGFP+ spermatogonia.
Conclusion: These results suggest that the genes which exhibit bimodal transcript abundance patterns among individual spermatogonia mark phenotypically−distinct subpopulations of P6 Id4−eGFP+ spermatogonia. This further supports our contention that the gene expression heterogeneity we recently reported reflects the existence of distinct subtypes of undifferentiated spermatogonia with differing functional capacities, including stem and progenitor spermatogonia fates.
Funding: This study was supported by NIH grants HD062687 (BPH), HD061665 (JMO), HD072552 (CBG), and GM092334 (JRM), NSF grant 1337513 (BPH), the Max and Minnie Tomerlin Voelcker Fund, the Kerr Foundation, the Kleberg Foundation, and The University of Texas at San Antonio.

Poster #55
REGULATION OF LEVELS OF OCT4 PROTEIN IN MOUSE SPERMATOGENESIS
Yu Zheng, Jennifer Balke and Christina Dann, PhD
Indiana University
(Presented by: Christina Dann, PhD)

Introduction: OCT4, officially named POU5F1, is a POU−type homeodomain−containing transcription factor. In the early embryo and in embryonic stem (ES) cells OCT4 functions as a dose−dependent regulator of cell fate with only a 50% change in OCT4 levels, either up or down, resulting in loss of pluripotency. Our lab has focused on the function of OCT4 in spermatogonial stem cells (SSCs), where we found it to be required for self renewal. Interestingly, OCT4 expression in SSCs is much lower than in ES cells. Furthermore, OCT4 is readily detected in spermatogonia of juvenile testes but only rarely detected in adult. While OCT4 is well known to be transcriptionally regulated, the relatively low levels of OCT4 in SSCs can be explained only in part by transcriptional control. For instance, a transcriptional reporter transgene containing the Oct4 promoter drives expression of GFP throughout spermatogonia, only a subset of which contain endogenous OCT4 protein.
Methods: To begin to investigate the mechanisms regulating OCT4 protein expression we used two flow cytometery based approaches to isolate spermatogonia at various stages of development and then performed quantitative RT−PCR and Western.
Results: We found that adult undifferentiated spermatogonia (e.g. Oct4−GFP+/Kit−) contained lower levels of OCT4 protein compared to juvenile spermatogonia despite having similar levels of OCT4 mRNA. As the data supported the possibility of a post−transcriptional mechanism for OCT4 regulation, we investigated the idea that OCT4 stability was controlled by the post−translational modification, ubiquitination. OCT4 protein levels were quantified following treatment with the proteasome inhibitor, MG132. MG132 treatment of undifferentiated spermatogonia isolated from testes, or in vitro cultures of SSCs/progenitors (germline stem or “GS”cells), led to higher OCT4 levels suggesting that OCT4 can be ubiquitinated in spermatogonia. We are currently directly testing whether ubiquitinated OCT4 is present in spermatogonia.
Conclusion: The data support the hypothesis that OCT4 protein levels in spermatogonia are defined by multiple mechanisms including possibly ubiquitination. Tight regulation of OCT4 during spermatogenesis could be required to ensure the appropriate amount is present to direct self renewal or permit differentiation. Furthermore, given the link between OCT4 overexpression in germ cell tumors it is likely that dampening of OCT4 levels during spermatogenesis is important to prevent tumorigenesis.

Poster #56
DISTINCT PHENOTYPE OF SERTOLI CELLS LOCATED IN THE TRANSITION REGION BETWEEN THE SEMINIFEROUS TUBULES AND RETE TESTIS
Andre Figueiredo, Luiz Renato França and Guilherme Costa
(Presented by: Guilherme Costa)

Introduction: The region that connects the seminiferous tubules (ST) to the rete testis, known as the transition region (TR) or transitional zone, is an area of the testis that has not yet been well characterized. Therefore, its morphofunctional importance for spermatogenesis and testis function as a whole is not yet completely understood. Particularly, the peculiar

characteristics of the TR, such as the presence of morphologically modified Sertoli cells (SC) and the distinct immunomorphological aspects of this region, as well as the surrounding interstitium, motivated us to investigate if those cells are phenotypically different (i.e. express different factors or molecules) from SC located in other ST area.
Objectives: In the present study we have evaluated several important parameters related to the SC proliferation/differentiation and the expression of the monocyte chemo−attractant protein 1 receptor (CCR2) along the seminiferous tubules (ST), in the TR, and in the connection between seminiferous tubules and the TR (Cx) in prepubertal and adult Wistar rats.
Material and Methods: Using immunostaining, the cell proliferation was investigated through Ki−67 and BrdU, while p27, GATA−4, androgen receptor (AR) expressions were studied in order to evaluate the SC differentiation status. CCR2 expression was assessed in order to evaluate the interaction between the immune cells and SC.
Results: Unlike what is established in the literature, in which is considered that SC stop proliferating in vivo at 2−3 weeks of age in rats, we found Ki−67 and BrdU positive SC at 36 and 120 days−old. However those mitotic SC were present only in the TR. Regarding the SC maturation, at both ages and different from the other areas, we observed SC located in the TR that did not express AR or GATA−4. Also, in comparison to the Cx and ST, in general a lower AR expression was found in SC located in the TR. Furthermore, in older rats, the SC present in the TR showed a higher CCR2 expression. This elevated CCR2 expression could be involved in the induction of self−antigens tolerance or with cell proliferation, as observed in prostate cancer.
Conclusion: Taken together, these findings suggest a distinct behavior/function of SCs located in TR, as well lead us to hypothesize the presence of transiently amplifying SC subpopulation in this region.
Financial support: FAPEMIG and CNPq

Poster #57
THE SERINE/THEONINE KINASE 35 (STK35) ALLELE LOCUS ENCODES BOTH CODING AND NON−CODING RNAS AND IS REQUIRED FOR NORMAL FERTILITY
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(Presented by: Kate Loveland, PhD)

Introduction: The importin α family of proteins are key mediators of nucleocytoplasmic transport. Although they are usually cytoplasmic, two importin αs, α2 and α4, are nuclear−localized in spermatocytes and spermatids in human and mouse testes. We recently identified the STK35 gene as one of two up−regulated targets of nuclear importin α2 and α4 proteins in HeLa cells exposed to hydrogen peroxide [1] and thus sought to assess the potential relationship between nuclear importins and STK35 in post−mitotic male germ cells. Serine/threonine kinase 35 (STK35) was also identified as CLP36−interacting kinase 1 (Clik1) with autophosphorylation activity. It exhibits highest adult human tissue transcript levels in testis [2] and is upregulated in certain cancers [3]. The STK35L1 isoform contains a 133 a.a. N−terminal extension that can interact with nuclear actin, while STK35L1 was implicated in cycle progression maintenance and endothelial cell migration in human cell lines [4].
Methods and Results: We identified several transcripts originating from the Stk35 in mouse testis, including a new lncRNA. In situ hybridization analyses revealed that each transcript is present in different male germ cell types, indicating they are coordinately regulated. We developed an Stk35 knockout (KO) mouse with a genetic deletion encompassing both coding and non-coding RNAs. Viable KO mice were infrequent in both sexes; adult survivors had lower testis and ovary weights, and both male and female gonads had drastically reduced germ cell numbers. Somatic cell types appeared unaffected, indicating that this allele contributes specifically to germ cell viability.
Conclusion: These findings demonstrate that the genomic region encoding the Stk35 allele contributes to normal germ cell development, and we propose that a key function of importin α proteins is to regulate its activity. Our ongoing studies address Stk35 function in cellular stress responses, spermatogenesis and normal development, building on the growing understanding from our earlier finding that the nuclear-localized importin α4 protein can protect germ cells from cellular stress [5].

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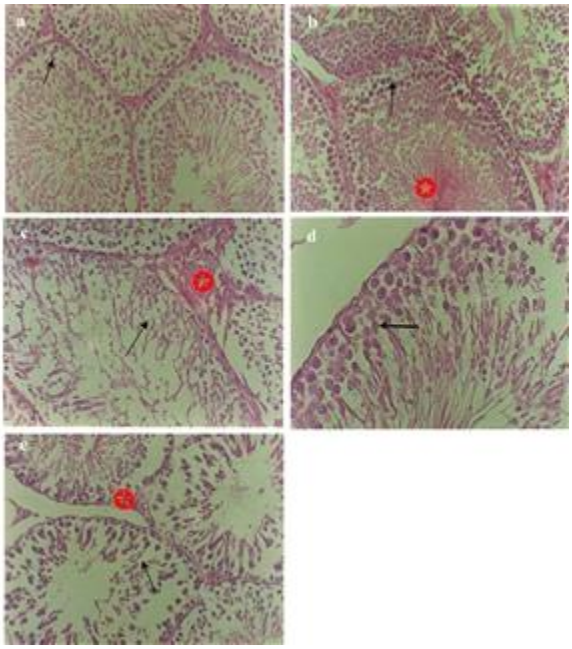
Poster Session II

Poster #58
EFFECT OF GROWTH HORMONE ON TESTICULAR DYSFUNCTION INDUCED BY METHOTREXATE (MTX) IN RATS
Hamed Serati−Nouri, PhD
Drug Applied Research Center Tabriz University of Medical Sciences
(Presented by: Hamed Serati−Nouri, PhD)

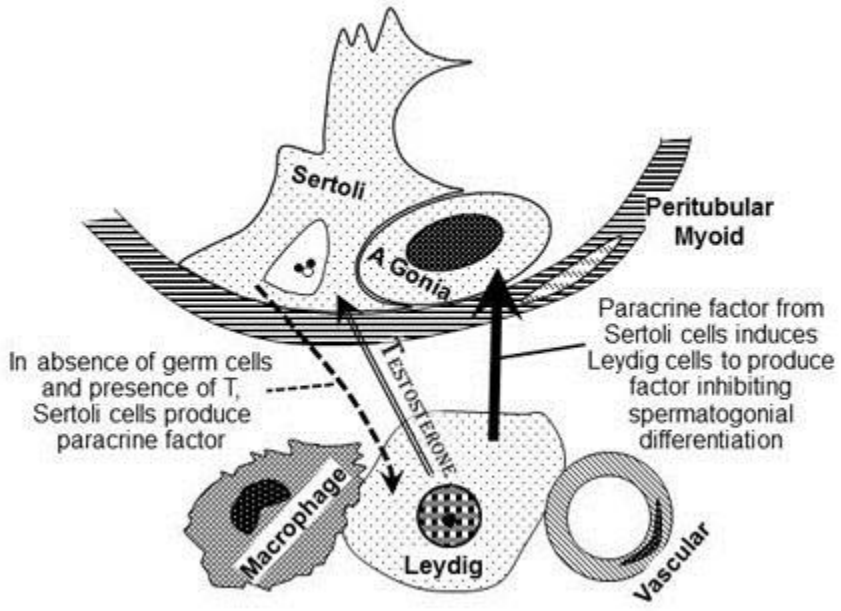
Introduction & objective: Methotrexate (MTX) is a chemotherapeutic agent causing defective oogenesis and spermatogenesis. This study was performed to assess the role of human growth hormone (GH) on testis recovery after treatment with MTX.
Methods: Forty male Wistar rats were selected and randomly divided into four groups (n = 10): control (vehicle), GH group (0.3 mg kg)1 GH for 28 days, IP), MTX group (MTX 1 mg kg)1 week)1 for 4 weeks, IP) and GH/MTX group (0.3 mg kg)1 GH for 28 day plus 1 mg kg)1 week)1 MTX for 4 weeks, IP). On days 14 and 28, five rats from each group were killed, testes of rats of all groups were removed, spermatozoa were collected from epididymis and then prepared for analysis.
Results: MTX caused significant increase in interstitial tissue and capsular thickness and decrease of testicular and body weight ($P < 0.05$). Moreover, it caused significant decline in seminiferous tubule diameter and epithelium thickness ($P < 0.05$). There was no obvious change in morphometrical parameters between MTX/GH and control groups. In MTX group, sperm parameters decreased significantly ($P < 0.05$). Administration of GH plus MTX reduced the effects of MTX on sperm parameters and testosterone concentration.
Conclusions: These results suggested that GH had a protective effect on almost all destructive effects caused by MTX in rat testes and thus improved sperm parameters.



Poster #59
THE TESTOSTERONE−MEDIATED BLOCK IN SPERMATOGONIAL DIFFERENTIATION AFTER CYTOTOXIC EXPOSURE: ROLE OF LEYDIG AND POSSIBLY SERTOLI CELLS
Gunapala Shetty, PhD[1] and Marvin Meistrich, PhD[2]
[1]University of Texas M.D. Anderson Cancer center; [2]University of Texas M.D. Anderson Cancer Center
(Presented by: Gunapala Shetty, PhD)

Introduction: In rats, depletion of differentiated germ cells by cytotoxic agents (radiation, procarbazine, busulfan, hexanedione) can completely block spermatogonial differentiation. In all such models testosterone (T) suppression restores spermatogonial differentiation, which can be maintained if T is subsequently restored. Transplantation studies with irradiated rats showed that the block in differentiation was a not due to damage to the spermatogonia but rather alteration of the somatic environment. It had been puzzling why after irradiation, it took 8 weeks for the differentiation block to reach a high level and why it took 6 weeks of androgen ablation for differentiation to resume in most tubules.
Methods: Using ethane dimethane sulfonate (EDS) to specifically eliminate mature Leydig cells (LCs) in irradiated rat testes with a complete block in spermatogonial differentiation, we restored differentiation in 24% of tubules 2 weeks later.

Results: When the EDS was combined with androgen ablation spermatogonial differentiation occurred in >70% of tubules at 2 weeks.
Conclusion: These results show that LCs are the source of an inhibitory factor blocking spermatogonial differentiation.
The common factor for induction of the block by different toxicants is the elimination of differentiated germ cells. Only Sertoli cells are in direct contact with germ cells and its gene expression is known to change when germ cells are depleted. We propose that the gradual germ cell disappearance after irradiation upregulates expression of a T−dependent paracrine factor made by Sertoli cells that activates the Leydig cells to produce the inhibitory factors. This can explain why suppression of T gradually reinitiates the differentiation of spermatogonia while the Leydig cell loss rapidly restores it. T suppression results in sustained reappearance of pachytene spermatocytes and should restore normal gene expression by Sertoli cells. Hence the paracrine factor that stimulates Leydig cells to produce the inhibitory factor is no longer produced even when T is restored, and both spermatogonial differentiation and the completion of spermatogenesis can then proceed. Microarray analyses of genes expressed in specific testis cells may elucidate the identity of these factors.



Poster #60
CHROMATIN PATTERN AND STATUS OF GLOBAL DNA METHYLATION IN HUMAN SPERMATOZOA
DNA PATTERN IN HUMAN SPERM VIA CMA3 AND 5−METHYL CYTOSINE STAINING
Jaleh Barzideh, MPhil in Medical Genetic
(Presented by: Jaleh Barzideh, MPhil in Medical Genetic)

Introduction: Chromatin compaction and methylation status are biomarkers to detect the pattern and quality of sperm DNA.
Objectives and Methods: The purpose of this study is to compare two criteria of sperm chromatin compaction and global methylation status in relation to the functional quality of human spemratozoa.
Results: The confocal microscopy and flowcytometry showed the Immunocytofluorescent pattern of ChromomycinA3 (CMA3) staining and the 5−methyl cytosine, sequencely. The CMA3positivity level showed a quality relation dependency ($p<0.0001$), also significant correlation ($P<0.05$) with the flowcytometry level of global methylation. Moreover initial confocal microscopy finding from CMA3 stained head of sperm demonstrated the spatial pattern of chromatids.
Conclusion: Overall the results of this study support the concept that perfect spermatozoa collected from the high density Percoll fraction possesses higher compaction related to hypomethylated nuclear DNA. Interestingly the results of ChromomycinA3 assay demonstrated spatial geometry of chromatids in the sperm head. Also the direct significant correlation of CMA3 positivity with methylation status suggesting that during the development of spermatozoa, failure to chromatin compaction is associated with more extensive methylation of sperm DNA in poor quality of spermatozoa.

Poster Session II

Poster #61
DISSECTING GERM CELL METABOLISM THROUGH NETWORK MODELING
Leanne Whitmore, BS Biochemistry and Ping Ye, PhD Biochemistry
Washington State University
(Presented by: Leanne Whitmore, BS Biochemistry)

Introduction: Metabolic pathways are increasingly postulated to be vital in programming cell fate, including stemness, differentiation, proliferation, and apoptosis. The commitment to meiosis is a critical fate decision for mammalian germ cells, and requires a metabolic derivative of vitamin A, retinoic acid (RA). Recent evidence showed that a pulse of RA is generated in the germ cell of male mice thereby triggering meiotic commitment. However, enzymes and reactions that regulate this RA pulse have yet to be identified.
Methods: We developed a mouse germ cell−specific metabolic network with a curated vitamin A pathway. Using this network, we implemented the computational approach flux balance analysis throughout the initial wave of spermatogenesis to elucidate important reactions and enzymes for the generation and degradation of RA.
Results: Our results indicated that primary RA sources in the germ cell include RA import from the extracellular region, release of RA from binding proteins, and metabolism of retinal to RA. Further, in silico knockouts of genes and reactions in the vitamin A pathway predicted that deletion of Lipe, hormone−sensitive lipase, disrupts the RA pulse thereby causing spermatogenic defects. Examination of other metabolic pathways revealed that the citric acid cycle is the most active pathway. In addition, we discovered that fatty acid synthesis/oxidation are the primary energy sources in the germ cell.
Conclusion: These findings enhanced our understanding of the metabolic control of germ cell differentiation and will help guide future experiments to improve reproductive health.

Poster #62
PROGRESSIVE DECLINE IN TESTICULAR FUNCTION IN LEPTIN-RECEPTOR-DEFICIENT (LEPR−DB/LB) OBESE MICE
Jennifer Long and Christopher Pearl
Western Michigan University
(Presented by: Christopher Pearl)

Introduction: Genetically modified mice lacking the hormone leptin (ob/ob) or leptin receptor (db/db) are reported to be subfertile and/or infertile, but there is limited information about testicular function (sperm and hormone production) and the progression of infertility. This study was designed to investigate testicular function in a leptin—receptor-deficient obese mouse model (the POUND mouse; Lepr−db/lb; Charles River).
Methods: Male POUND mice were compared to wild−type c57 mice at 8 and 16 weeks of age.
Results: Paired testis weight was not significantly different at 8 or 16 weeks of age. Sertoli cells were counted after GATA4 immunostaining using the physical dissector method; Sertoli cell counts were similar between c57 and POUND mice at both ages. Sperm production was assessed based on counts of homogenization and detergent−resistant spermatids. At 8 weeks of age, the number of elongated spermatids, sperm per milligram of testis and daily sperm production were comparable between POUND and wild-type mice. However, at 16 weeks of age, the number of elongated spermatids, sperm per milligram of testis, and daily sperm production were all significantly reduced in POUND mice compared to wild-type c57 mice. Total sperm per epididymis and sperm per milligram epididymis followed a similar pattern as testicular sperm with comparable numbers at 8 weeks of age, and significant reductions in POUND mice at 16 weeks of age. Serum testosterone, LH and FSH concentrations were determined by ELISA. At both 8 and 16 weeks of age, serum FSH and testosterone were significantly reduced in POUND mice. Mean serum LH was not significantly different between wild-type and POUND mice but was highly variably at 16 weeks of age with some animals exhibiting very high LH concentrations, and others below assay detection.
Conclusion: Collectively, these data suggest that testicular sperm production is reduced in POUND mice at 16 weeks of age leading to extremely low numbers of sperm in the epididymis. Thus, subfertility/infertility in these animals can be attributed, at least in part, to low sperm counts. Sperm production appears to be reduced as a result of diminished spermatogenic efficiency of the seminiferous tubule since testis size and sertoli cell numbers were not affected. Interestingly, sperm production was similar between POUND and c57 mice at 8 weeks suggesting that fertility problems in POUND mice result from a progressive loss of testicular function after initiation of spermatogenesis.

Poster Session II

Poster #63
STEROIDOGENIC FATE OF LEYDIG CELLS THAT REPOPULATE THE TESTES OF AGED AND YOUNG BROWN NORWAY RATS AFTER IN VIVO ADMINISTRATION OF ETHANE DIMETHANESULFONATE (EDS)
Haolin Chen, PhD and Barry Zirkin, PhD
Johns Hopkins School of Public Health
(Presented by: Haolin Chen, PhD)

Introduction: The capacity of Brown Norway rat Leydig cells to produce testosterone decreases with aging. We have reported that a single injection of ethane dimethanesulfonate (EDS) depletes the steroidogenically hypofunctional Leydig cells from the testes of old rats; that 10 weeks thereafter a new generation of Leydig cells repopulates the old testes; and that the ability of the new Leydig cells to produce testosterone is equivalent to testosterone production by young rat Leydig cells (Chen et al., 1996). Our objective herein was to compare the steroidogenic fate of the Leydig new cells that repopulate the testes of old rats after EDS.
Methods: Young (3 month-old) and old (18 month-old) rats were injected with EDS to eliminate existing Leydig cells. Ten and 30 weeks thereafter, the ability of the newly formed Leydig cells isolated from young and old rats to produce testosterone in response to LH were compared.
Results: Ten weeks after EDS, testosterone production by Leydig cells from young and old rats was equivalent, whereas in age−matched controls testosterone production was significantly higher by young Leydig cells. At 10 weeks post EDS, the ability of the repopulated cells to produce cAMP in response to LH, and their P450scc activity, were consistent with the ability of the cells to produce testosterone. Thirty weeks after EDS, the ability of the Leydig cells isolated from young rats to produce testosterone had not diminished. However, in contrast, testosterone production by Leydig cells from the old EDS-treated rats was reduced significantly from the 10-week level.
Conclusion: These results indicate that although the Leydig cells restored to young and old rats after EDS initially produced testosterone at equivalently high levels, the cells in the old testes did not maintain this ability. This suggests that the stem cells from which new populations of Leydig cells are derived differ between young and old testes; and/or that factors intrinsic or extrinsic to the new Leydig cells in old testes are responsible for reductions in testosterone and thus for Leydig cell aging.
Funding: Supported by NIH grant R37 AG021092

Poster #64
COACTIVATOR−ASSOCIATED ARGININE METHYLTRANSFERASE 1 ORCHESTRATES SPERMIOGENESIS AND IS ESSENTIAL FOR MALE FERTILITY IN MICE
Jianqiang Bao
University of Texas MD Anderson Cancer Center
(Presented by: Jianqiang Bao)

Introduction: It has been well−studied that histone lysine methylation-mediated epigenetic modifications are involved in a wide range of developmental processes during male germ cell development in mice, such as reprogramming, transposon repression and meiosis. By contrast, biological functions of arginine methylation, which is catalyzed by the protein arginine methyltransferase family (PRMT), are less studied in the germ cell field although a growing body of evidence demonstrate that arginine methylation in histone or non−histone proteins is also involved a variety of biological processes, including cell proliferation, differentiation, apoptosis and epigenetic reprogramming etc.
Methods: In this study, we employed Cre−Loxp strategy to successfully inactivate Coactivator-associated arginine methyltransferase 1 (Carm1), one member of PRMT family, by crossing germline-specific Cre deletor line (Stra8−cre) with floxed Carm1(Carm1fl/fl) mouse line.
Results: In the conditional Carm1 knockout (Stra8−cre; Carm1fl/Δ) male mice, spermiogenesis was severely disrupted, leading to the significant reduction of the total number of elongating and elongated spermatids in the testes. As a result, the number and the motility of mature sperm recovered in the cauda epididymis significantly declined in the knockout testis as compared to those in the WT mice. A great amount of spermatids were prematurely released to the cauda while majority of mature sperm observed exhibit severe head abnormalities (Teratozoospermia), such as small or mis−shapen heads, crooked/bent heads and acephalic sperm.
Conclusion: Mechanistically, we hypothesized that there is a defect in the histone-to-protamine transition in the elongating/elongated spermatids in the absence of arginine methylation-mediated signaling pathway in the Carm1 knockout germ cells. This study represents, to our knowledge, the first study demonstrating the critical roles of arginine methylation during spermatogenesis.

Poster Session II

Poster #65
COMPARATIVE STUDY OF TWO MOUSE MUTANTS TO ELUCIDATE THE ROLE OF SLX4 DURING PROPHASE I OF MAMMALIAN MEIOSIS
Kadeine Campbell-Peterson, Kim Holloway, PhD and Paula Cohen, PhD
Cornell University
(Presented by: Kadeine Campbell-Peterson)

Sexually reproducing organisms rely on the exchange of genetic material that occurs during the first stage of meiosis I, prophase I, to prevent deleterious mutations from accumulating in their offspring. The defining feature of prophase I is the formation of a multitude of double strand breaks (DSBs) along the chromosome which aid in the search necessary for homolog pairing. A finite number of breakage events result in the exchange of genetic material between homologous chromosomes known as crossovers (COs). Previous studies in mouse have indicated that COs are formed via repair of DSBs through two distinct pathways: the major class I pathway (involving the MutS/MutL heterodimers of MSH4/5 and MLH1/3, respectively) and the minor MUS81−mediated class II pathway. MLH1 mutants are infertile and retain about 10% of observed COs which are presumed to arise from the MUS81−controlled pathway. By contrast, mice devoid of MUS81 are fertile with normal CO counts and have elevated MLH1 foci suggestive of some level of crosstalk between the two pathways. Importantly, mutants in SLX4, a multi−domain scaffold protein functioning within several DNA repair pathways, phenocopies the MUS81 mutant, indicating a role for SLX4 in meiotic prophase I in the mouse. Thus, like MUS81 homozygous mutants, males bearing a genetrap insertion in SLX4 exhibit decreased testis weight, accompanied by decreased germ cell numbers and increased TUNEL−positive germ cells relative to wildtype littermates. Additionally, MLH1 foci counts at prophase I were elevated in the genetrap mouse, while chiasmata numbers remain constant. These results support our hypothesis that SLX4 participates in a crosstalk between genetic components of the class I and class II CO pathways. To further investigate the role of a complete loss of SLX4, my studies are aimed at identifying key binding partners for SLX4 specifically in the context of prophase I. Several candidate interactors have been well documented in the literature, including the aforementioned MUS81, SLX1, and components of the DNA mismatch repair (MMR) pathway, which include MLH1 and MLH3. My studies demonstrate a role for specific interactions in mediating the function of SLX4 in prophase I crossover control. Importantly, studies of the Slx1 homozygous mutant animals reveal no role for SLX1−SLX4 interactions in these events. Taken together, my studies further elucidate the role of SLX4 and its binding partners in prophase I events in mammals.

Poster #66
MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) SYNERGIZES WITH GDNF TO INDUCE MIGRATION OF SPERMATOGONIAL CELLS.
Mahmoud Huleihel, PhD[1], Dimitry Lan, MSc[1] and Eitan Lunenfeld, MD[2]
[1]Ben-Gurion University; [2]Soroka University Medical Center and Ben-Gurion University
(Presented by: Mahmoud Huleihel, PhD)

Introduction: Macrophage migration inhibitory factor (MIF) was originally identified as a cytokine that produced by activated T cells to inhibit macrophage migration. MIF is expressed by a variety of cells and tissue including eosinophils, epithelial cells, endothelial cells and macrophages. Leydig cells from adult rats were demonstrated to express MIF under physiological conditions. Leydig cell−depleted testes produced MIF, and suggested Sertoli cells for this source. We and other have shown that glial cell line−derived nerve growth factor (GDNF) could induce spermatogonial cell migration. Recently, we have shown that MIF is produced by Sertoli cells (in mouse testicular tissue and primary cultured Sertoli cells) under normal conditions (in preparation for publication).
Objectives: To evaluate the effect of MIF on the capacity of GDNF to induce migration of mouse isolated spermatogonial cells in vitro.
Methods: Seminiferous tubules from 7-day-old mice were used to isolate GFR−alpha1 positive cells by MiniMACS. The migration assay was performed using Transwell in 24-well dishes. GFR−alpha1 positive cells suspended in serum−free medium were placed in the upper chamber, and recombinant (r)−MIFF (0.01,0.1,1,5,10,50 and 100 pg/ml), rGDNF [10 pg/ml; gave optimal migration effect on spermatogonial cell (SPG) migration] or combination of rGDNF (10 pg/ml) and MIF (10,50 or 100 pg/ml) were placed in the lower chamber. The plates were incubated for 5 hours at 370 C in 5% CO2. Migrated cells, on the underside of the filters were examined and counted under a microscope following fixation and staining with crystal violet.
Results: Addition of MIF in Transwell system showed a significant increase in the migration of SPG in a dose−dependent manner, when optimal effect was examined at 1 and 5 pg/ml. A decrease in MIF migration effect was shown in a dose−dependent manner at 10,50 and 100 pg/ml. At 100 pg/ml, MIF significantly decreased SPG migration compared to control (without MIF). GDNF significantly increased SPG migration. Addition of MIF (10 or 100 pg/ml) to GDNF did not

affect the capacity of GDNF to induce SPG. However, addition of MIF (50 pg/ml) to GDNF was significantly increased SPG migration compared to GDNF or MIF alone. This effect was in a synergistic manner.
Conclusions: Here we show for the first time that MIF and GDNF are synergizing to induce SPG migration. This study may suggest a novel role for MIF in the regulation of spermatogenesis.

Poster #67
A GENE PARALOG PAIR THAT ACTS AS A MOLECULAR RHEOSTAT TO CONTROL MALE GERM CELL DEVELOPMENT
Eleen Shum, BS and Miles Wilkinson, PhD
UCSD
(Presented by: Miles Wilkinson, PhD)

Introduction & Objective: Gene duplication is a major evolutionary force that drives the generation and adaptation of species. This process generates a back−up gene copy and provides an opportunity for divergence to encode new functions. Here, we report a gene duplication event that occurred at the dawn of the vertebrate lineage that yielded a different outcome – functional antagonism. One product of this duplication event – UPF3B – is a well−studied X−linked gene in mammals that is critical for nonsense−mediated RNA decay (NMD), a highly conserved RNA degradation pathway that promotes the decay of a subset of mRNAs. Its autosomal counterpart – UPF3A – encodes an enigmatic protein highly expressed in the testes previously shown to have only trace NMD activity when assessed using an artificial tethering assay. Our goal in this study was to identify the function of UPF3A.
Methods: Mouse gene targeting, RNA−seq analysis, RNA interference, transfection, co−immunoprecipitation analysis, reporter analysis, qPCR analysis.
Results: Using knockdown and knockout approaches in vitro and in vivo, respectively, we discovered that UPF3A is actually a potent NMD inhibitor that acts by sequestering the key NMD factor, UPF2, from the molecular complex that forms on mRNAs destined to be degraded by NMD. To examine the biological function of UPF3A, we generated Upf3a−floxed mice. Since we found that UPF3A is most highly expressed in spermatocytes, we conditionally ablated Upf3a in male germ cells use Stra8−Cre mice and observed perturbed progression through meiosis and dramatically reduced sperm count. Genome−wide studies identified a large set of transcripts stabilized by UPF3A.
Conclusions: Together, these data supported a model in which is UPF3A is essential to antagonize NMD in order to stabilize mRNAs encoding proteins that promote meiosis and spermatocyte survival. UPF3A action is counterbalanced by its paralog, UPF3B, which is a NMD activator that we previously showed strongly destabilizes UPF3A protein (Chan et al. Nature Stuct Mol Biol 16:747). Our results suggest that the UPF3A and UPF3B paralogs evolved to oppose each other so that, together, they can serve as a molecular rheostat to globally control the stability of specific mRNAs during development, including in gametogenesis.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