2013 XXII\textsuperscript{nd} North American Testis Workshop

\textbf{"The Foundations of Male Fertility"}
April 10 – 13, 2013
Hyatt Regency San Antonio
San Antonio, Texas

\textbf{Chair}: William Wright, PhD
\textbf{Vice-Chair}: Jacquetta Trasler, PhD
Welcome to the XXII\textsuperscript{nd} North American Testis Workshop entitled, “The Foundations of Male Fertility.” Since 1972, this biannual meeting has brought together scientists from around the world to discuss the latest data on testicular physiology, biochemistry, molecular biology and cell biology. Traditions of this meeting include the open and constructive discussion of data, the encouragement of young scientists and the establishment of new and productive collaborations. We are confident that these traditions will continue at this year’s meeting.

A highly interactive and supportive program committee developed the program for this meeting. The members of this committee are: Leslie Heckert (U. Kansas), Marie-Claude Hofmann (MD Anderson Cancer Center), Kate Loveland (Monash Institute of Medical Research), Kyle Orwig (U. Pittsburgh), Stuart Moss (NICHD), Makoto Nagano (McGill University), David Page (MIT), Katja Teerds (U. Wageningen), Steve Ward (U. Hawaii) and Miles Wilkinson (U.C. San Diego). We are grateful for their enthusiasm and wise counsel. We also thank the organizations that provided funding for this meeting. These are: The Eunice Kennedy Shriver National Institute of Child Health & Human Development, the National Institute of Aging, the European Molecular Biology Organization and the Burroughs Wellcome Fund. This meeting could not occur without their financial support. We thank all of the speakers, all of whom responded enthusiastically to our invitation. We thank all of you who came to present your data in the poster sessions and to discuss the data of others. Your participation is what will make this meeting a success. Finally, we thank John McCarrey (U.T. Texas San Antonio) for organizing Thursday evening’s social event, Tejas Steakhouse and Rodeo.

Enjoy this meeting and the beautiful city of San Antonio.

Bill Wright
Chair of the Program Committee

Jacquetta Trasler
Vice-Chair of the Program Committee
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# Thank You

The organizers of the XXIInd 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.

- The Eunice Kennedy Shriver National Institute of Child Health and Human Development
- The Burroughs Wellcome Fund
- The National Institute of Aging
- The European Molecular Biology Organization
Faculty

**XXII\textsuperscript{nd} North American Testis Workshop**

*“The Foundations of Male Fertility”*  
*April 10 – 13, 2013*

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**James Amatruda, MD, PhD**  
UT Southwestern Medical Center

**John K. Amory, MD**  
University of Washington

**Mario Ascoli, PhD**  
The University of Iowa  
Dept. of Pharmacology

**Robert E. Braun, PhD**  
Jackson Laboratory

**Richard Freiman, PhD**  
Brown University  
Dept. of Molecular Biology

**Margaret Fuller**  
Stanford University  
School of Medicine  
Dept. of Developmental Biology

**Michael D. Griswold, PhD**  
Washington State University  
School of Molecular Biosciences

**Leslie L. Heckert, PhD**  
University of Kansas Medical Center  
Dept. of Physiology

**Scott Keeney, PhD**  
Memorial Sloan-Kettering Cancer Center

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**Sarah Kimmins, PhD**  
McGill University, Canada  
Dept. of Animal Science

**Dolores J. Lamb, PhD**  
Baylor College of Medicine  
Dept. of Urology

**Kate Loveland, PhD**  
Monash University, Australia  
Dept. of Biochemistry & Molecular Biology

**Jeffrey J. Lysiak, PhD**  
University of Virginia  
Dept. of Urology

**Erika Matunis**  
Johns Hopkins University  
School of Medicine  
Dept. of Cell Biology

**John Oatley**  
Washington State University  
School of Molecular Biosciences

**Kyle Orwig, PhD**  
University of Pittsburgh SOM

**David Page, PhD**  
Whitehead Institute for Biomedical Research, MIT

**Antione H.F.M. Peters**  
Friedrich Miescher Institute for Biomedical Research – Switzerland

**Renee R. Pera, PhD**  
Stanford University  
School of Medicine

**Jacquetta M. Trasler, MD, PhD**  
McGill University, Canada

**Kenneth S. K. Tung, MD**  
University of Virginia  
Dept of Pathology

**Christi A. Walter, PhD**  
University of Texas Health Science Center- San Antonio

**Monika A. Ward, MSc, PhD**  
Institute for Biogenesis Research  
University of Hawaii, Manoa

**William Wright, PhD**  
Johns Hopkins  
Bloomberg School of Public Health

**Shosei Yoshida**  
Division of Germ Cell Biology  
National Institute of Basic Biology, Japan

**David Zarkower, PhD**  
University of Minnesota
Program Schedule

**XXII\textsuperscript{nd} North American Testis Workshop**

*The Foundations of Male Fertility*

April 10 – 13, 2013

Chair: William Wright, PhD

Vice-Chair: Jacquetta Trasler, MD, PhD

\textit{Location: Regency East 1 – 3}

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**WEDNESDAY, APRIL 10, 2013**

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<td>Registration/Information Desk Open</td>
<td>Los Rios Foyer</td>
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<tr>
<td>7:00 p.m. – 7:15 p.m.</td>
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<td>Keynote Address</td>
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<td>8:15 p.m. – 9:45 p.m.</td>
<td>Testis Workshop Welcome Reception</td>
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**THURSDAY, APRIL 11, 2013**

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<tr>
<td>7:00 a.m. – 6:00 p.m.</td>
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<td>7:00 a.m. – 8:00 a.m.</td>
<td>Continental Breakfast</td>
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<td>8:00 a.m. – 8:45 a.m.</td>
<td>Benchmark Lecture</td>
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<td>8:45 a.m. – 8:50 a.m.</td>
<td>Introduction to Session I</td>
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**SESSION I: REGULATION AND RESTORATION OF FERTILITY IN MEN**

Chair: David Page, MD; Whitehead Institute, MIT

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<tr>
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<td>Introduction to Session I</td>
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8:50 a.m. – 9:25 a.m.  Retinoic Acid and Spermatogenesis in Man
John K. Amory, MD
University of Washington

9:25 a.m. – 10:00 a.m.  Constructing Human Sperm from Pluripotent Stem Cells
Renee A. Reijo Pera, PhD
Stanford University School of Medicine

10:00 a.m. – 10:25 a.m.  Break
Location: Regency East Foyer

10:25 a.m. – 11:00 a.m.  Genomic Causes of Male Infertility
Dolores J. Lamb, PhD
Baylor College of Medicine

11:00 a.m. – 11:15 a.m.  Short Talk – #52
Human Spermatogenic Failure Purges Deleterious Mutation Load from the Autosomes and Both Sex Chromosomes, Including the Gene DMRT1
Don Conrad, PhD
Washington University School of Medicine

11:15 a.m. – 11:30 a.m.  Short Talk – #101
Intact Full Length RNAs Are Well Preserved in Undamaged Sperm, Irrespective of Clinical Semen Parameters
Alexander Yatsenko, MD, PhD
Magee-Womens Research Institute

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

SESSION II: GENOME INTEGRITY
Chair: Jacquetta Trasler, MD, PhD; McGill University

1:00 p.m. – 1:05 p.m.  Introduction to Session II
Jacquetta Trasler, MD, PhD
McGill University

1:05 p.m. – 1:40 p.m.  Y Chromosome's Role in Maintenance of Sperm DNA Integrity
Monika A. Ward, PhD
Institute for Biogenesis Research
University of Hawai, Manoa

1:40 p.m. – 2:15 p.m.  The Father’s Age
Christie A. Walter, PhD
University of Texas at San Antonio

2:15 p.m. – 2:40 p.m.  Break
Location: Regency East Foyer

2:40 p.m. – 3:15 p.m.  Genetic and Genomic Approaches to Understand Testicular Cancer
James Amatruda, MD, PhD
UT Southwestern Medical Center
3:15 p.m. – 3:50 p.m.  **Controlling the Number and Distribution of Recombination Events in Mouse Meiosis**  
Scott Keeney, PhD  
Memorial Sloan-Kettering  
Cancer Center

3:50 p.m. – 5:50 p.m.  **Poster Session I**  
*Location: Regency West 4 – 6*

5:50 p.m.  **Buses leave for Social Event**  
*Location: Hotel Lobby*

7:00 p.m.  **Testis Workshop Social Event**  
*Location: Tejas Steakhouse & Rodeo*  
(not included in registration fee; tickets required)

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**FRIDAY, APRIL 12, 2013**

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

7:00 a.m. – 8:00 a.m.  **Continental Breakfast**  
*Location: Regency East Foyer*

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8:00 a.m. – 8:45 a.m.  **EMBO Young Investigator Lecture**  
Towards Understanding the Molecular Logic of Paternal Epigenetic Inheritance  
Antoine Peters, PhD  
Friedrich Miescher Institute for Biomedical Research, Switzerland

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**SESSION III: REGULATION OF GENE EXPRESSION BY TRANSCRIPTION FACTORS AND HISTONE MODIFICATIONS**  
Chair: Leslie Heckert, PhD; University of Kansas Medical Center

8:45 a.m. – 8:50 a.m.  **Introduction to Session III**  
Leslie Heckert, PhD  
University of Kansas Medical Center

8:50 a.m. – 9:25 a.m.  **TAF4b Regulates the Balance Between Spermatogonial Stem Cell Renewal and Differentiation**  
Richard Freiman, PhD  
Brown University

9:25 a.m. – 10:00 a.m.  **The Sperm Epigenome: It’s Role in Development and Paternal Disease Transmission**  
Sarah Kimmins, PhD  
McGill University
10:00 a.m. – 10:25 a.m.  Break  
*Location: Regency East Foyer*

10:25 a.m. – 11:00 a.m.  Regulation of Proliferation and Differentiation in the Spermatogonial Stem Cell Lineage  
Margaret T. Fuller, PhD  
Stanford University School of Medicine

11:00 a.m. – 11:15 a.m.  *Short Talk – #91*  
Sperm Histones Influence Early Embryonic Gene Expression  
Ralph G. Meyer, PhD  
University of Pennsylvania

11:15 a.m. – 11:30 a.m.  *Short Talk – #75*  
Stage-Specific Expression and Subcellular Localization of Long Interspersed Element Type 1 (LINE-1) Protein During Male Germ Cell Development  
(Abstract #75)  
Wenfeng An, PhD  
Washington State University

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

**SESSION IV: SOMATIC CELLS**

Chair: Kate Loveland, PhD; Monash University, Australia

1:00 p.m. – 1:05 p.m.  Introduction to Session IV  
Kate Loveland, PhD  
Monash University, Australia

1:05 p.m. – 1:40 p.m.  Find Me, Eat Me and I’m Full: The Clearance of Apoptotic Germ Cells  
Jeffrey J. Lysiak, PhD  
University of Virginia

1:40 p.m. – 2:15 p.m.  Functional Compartmentalization of Murine Germ Cells and Translocation of Syncytial Chains Across Sertoli Cell Tight Junctions  
Robert Braun, PhD  
The Jackson Laboratories

2:15 p.m. – 2:30 p.m.  *Short Talk – #40*  
Sertoli Cells Survive Xenotransplantation by Modulating the Macrophages at the Graft Site to be Regulatory Versus Cytotoxic  
Payal Mital  
Texas Tech University Health Sciences Center

2:30 p.m. – 2:55 p.m.  Break

2:55 p.m. – 3:30 p.m.  The MEK/ERK Cascade Regulates the Expression of All Genes Involved in Testosterone Synthesis in Leydig Cells  
Mario Ascoli, PhD  
The University of Iowa
3:30 p.m. – 4:05 p.m.  Regulatory T Cell-Dependent Tolerance to Non-Sequestered Male Meiotic 
Germ Cell Antigens  
Kenneth S. K. Tung, MD  
University of Virginia, Dept of Pathology

4:05 p.m. – 4:20 p.m.  Short Talk – #44  
MicroRNAs: Novel Androgen Responsive Transacting Factors in the Testis  
Subbarayalu Panneerdos, PhD  
University of Texas Health Science Center at San Antonio

4:20 p.m. – 4:35 p.m.  Short Talk – #71  
Genome Wide Mapping of DNA Breaks During Spermiogenesis  
Marie-Chantal Gregoire, MSc  
Université de Sherbrooke, Canada

4:35 p.m. – 6:35 p.m.  Poster Session II  
Location: Regency West 4 – 6

SATURDAY, APRIL 13, 2013

7:00 a.m. – 12:00 p.m.  Registration/Information Desk Open  
Location: Los Ríos Foyer

7:00 a.m. – 8:00 a.m.  Continental Breakfast  
Location: Regency East Foyer

SESSION V: STEM CELLS AND THEIR NICHE  
Chair: William Wright, PhD; Johns Hopkins University

8:00 a.m. – 8:05 a.m.  Introduction to Session V  
William Wright, PhD  
Johns Hopkins University

8:05 a.m. – 8:40 a.m.  Niche Microenvironment for the Mouse Spermatogenic Stem Cells  
Shosei Yoshida, MD, PhD  
National Institute of Basic Biology, Japan

8:40 a.m. – 9:15 a.m.  Molecular Regulation of Fate Determination in Male Germline Stem Cells  
Jon Oatley, PhD  
Washington State University

9:15 a.m. – 9:50 a.m.  Stem Cell Renewal in the Drosophila Testis  
Erika Matunis, PhD  
Johns Hopkins University School of Medicine
9:50 a.m. – 10:05 a.m.  
**Short Talk – #60**
Partial Overlap of SALL4 and PLZF Binding Sites in Spermatogonial Stem Cells Reveals Putative Shared and Distinct Functional Roles
Dawn Lovelace, BS, MS  
University of Texas at San Antonio

10:05 a.m. – 10:30 a.m.  
**Break**
*Location: Regency East Foyer*

10:30 a.m. – 11:05 a.m.  
Translating Spermatogonial Stem Cell Transplantation Toward the Clinic  
Kyle Orwig, PhD  
University of Pittsburgh

11:05 a.m. – 11:20 a.m.  
**Short Talk – #84**
Zinc Finger and TAL-Effecter Nuclease Mediated Gene Targeting in Mouse Spermatogonial Stem Cells  
Christina Dann, PhD  
Indiana University

11:20 a.m. – 11:45 a.m.  
**Short Talk – #104**
Aging is Associated with Altered Gene Expression and a Reduced Number and Quality of Spermatogonial Stem Cells  
Catriona Paul, PhD  
McGill University

11:45 a.m. – 11:55 a.m.  
Announcement of the 23rd North American Testis Workshop

12:00 p.m.  
Adjourn
**Keynote Address**

**MALES WITH MAJOR COMMITMENT ISSUES: DMRT1 AND SEXUAL DIFFERENTIATION**

Anna Minkina, Robin E. Lindeman, Clinton K. Matson, Anthony D. Krentz, Teng Zhang, Mark W. Murphy, Vivian J. Bardwell, David Zarkower  
U of Minnesota, Genetics, Cell Biology, and Development, Minneapolis, MN

DMRT1 is a conserved transcription factor required for vertebrate testis differentiation, acting in germ cells and Sertoli cells at multiple developmental stages. DMRT1 also controls primary sex determination in non-mammalian vertebrates. We have used conditional genetics and genomics to explore DMRT1 function in the mouse. A recent surprise was the discovery that DMRT1 actively maintains male fate in postnatal Sertoli cells preventing Foxl2 expression and transdifferentiation to granulosa cells, their female counterpart (1). Foxl2 and estrogen signaling act analogously role in the ovary, silencing Dmrt1 and blocking female-to-male transdifferentiation (2). These discoveries raise at least three important questions that will be addressed:

First, do postnatal sex maintenance and transdifferentiation involve the genes that determine sex in the fetal gonad? Mammalian sex is determined by a male network centered on Sox9 and FGF signaling, and a female network centered on Wnt/beta-catenin signaling. Using genetic approaches we find that sex maintenance and transdifferentiation involve a subset of sex determination components as well as other critical regulators.

Second, is DMRT1 sufficient to cause female-to-male transdifferentiation in the ovary? We used a conditional Dmrt1 expression cassette to show that ectopic DMRT1 strongly masculinizes the ovary. Apparently mammalian DMRT1 can determine male fate despite not performing that function for perhaps 300 million years, perhaps helping explain how DMRT1 could be recruited to this role multiple times in vertebrate evolution.

Third, why do fully differentiated post-mitotic testicular cells need a sex maintenance system? We will present evidence that gonadal supporting cells are exposed to signaling environments that are essential for gametogenesis but have potential to trigger sex reversal if the maintenance network is compromised.


**THURSDAY, APRIL 11, 2013**

8:00 a.m. – 8:45 a.m.

**Benchmark Lecture**

**MAKING WAVES WITH RETINOIC ACID**

Michael Griswold, Elizabeth Evans, Travis Kent, Minh–Han Tong, Cathryn Hogarth  
Washington State University

The action of vitamin A in the form of retinoic acid (RA) is essential for the progression of undifferentiated A spermatogonia into differentiating A1 spermatogonia and thus, initiating the cycle of the seminiferous epithelium. In the neonatal mouse (2dpp) testis the first wave of progression of A spermatogonia into A1 spermatogonia originates in patches along the tubule that are retinoic acid responsive and ultimately lead to asynchronous spermatogenesis. These results have led us to a model whereby the spermatogenic cycle is initiated by a pulse or gradient of RA that forms in regions of the tubules represented by the patches. This pulse of RA generated by the patches moves along the tubule as a result of continuous RA synthesis and degradation and the asynchronous spermatogenic wave is formed. If the formation of these differentiation patches in the neonatetestis is disrupted by exogenous RA or blocked by disrupting RA synthesis, spermatogenesis in the adult is no longer asynchronous. An excess of RA eliminates the patches and a deficiency of RA results in a block in the progression and an accumulation of A spermatogonia within the tubule. In either case, after recovery from excess RA or after reversal of the block, spermatogenesis recovers and proceeds in a synchronous manner. By utilizing WIN 18,446 to inhibit RA synthesis the progression of A spermatogonia into A1 spermatogonia is blocked and we can synchronize the first spermatogenic wave in neonates. This procedure results in the accumulation of A spermatogonia and when the block is released these undifferentiated spermatogonia progress synchronously through A1, A2, A3, A4, In, B spermatogonia to form preleptotene spermatocytes 8.6 days later. The synchronous development allows stage specific measurement of the RA pulse during the first wave and the expression levels of the genes required to move germ cells into meiotic prophase. We have shown that the expression of retinol dehydrogenase 10 (RDH10) is required for generating the RA pulse that components of the CYP26 family of P450 enzymes are involved in degrading the RA pulse and that expression of some genes required for meiosis is initiated early in the first wave. Supported by grants HD10808 and U54 42454 from NIH.
RETI NOIC ACID AND SPERMATOGENESIS IN MAN
John K. Amory MD, MPH
Professor of Medicine, University of Washington School of Medicine

In male rodents, Vitamin A (retinol) deficiency induces infertility due to a cessation of spermatogenesis. This occurs because Vitamin A’s active metabolite, retinoic acid, is required for spermatogonial differentiation, spermiogenesis and spermiation. Whether low concentrations of intratesticular retinoic acid contribute to infertility in humans is unknown. In a recent pilot study, we observed that relatively reduced concentrations of intratesticular 13-cis retinoic acid were associated with sub-normal sperm quality in humans. We are currently ascertaining if deficiencies of retinoic acid are present in the testicular tissue of infertile men. If populations of infertile men with low intratesticular concentrations of retinoic acid are identified, such men could be the focus of future studies of retinoic acid therapy on sperm production and fertility.

CONSTRUCTING HUMAN SPERM FROM PLURIPOTENT STEM CELLS
Renee A Reijo Pera, PhD
George D Smith Professor
Stanford University School of Medicine

Introduction: Human embryo development begins with the fusion of egg and sperm, a remodeling of the maternal and paternal pronuclei and a series of cleavage divisions. Subsequently, on Day 3, the embryonic genome is activated and the stage is set for a series of cell fate decisions that lead to formation of the distinct tissues of the blastocyst, the primary germ layers and the germ cell lineage. Our recent findings indicate that human embryo development is characterized by a complex pattern of gene expression with the vast majority of genes that are modulated being down-regulated. Moreover, we observed that the majority of genes that are expressed in early human preimplantation development are of unknown function/identity.

Objective: In order to probe gene function, and develop a potential platform for clinical intervention, we developed the tools necessary to examine one of the earliest decisions in human embryo development, namely the setting aside of cells in the early embryo to form the germ cells (egg and sperm) of the next generation.

Methods: We examined the ability of both human embryonic stem cells and induced pluripotent stem cells to form cells of the germ cell lineage in the presence and absence of exogenous expression of germ cell specific translational factors. We then used xenotransplantation to probe germ cell function.

Results: We have found that both human embryonic stem cells and human adult- and fetal-derived induced pluripotent stem cells can form the earliest germ cells and even meiotic male and female germ cells. Moreover, we have found that a family of translational factors regulates germ cell formation, maintenance and differentiation and can induce progression of germ cell development.

Conclusions: Recent results suggest that we can derive human gametes from stem cells in whole or in part with transplantation required for full maturation and development.
THURSDAY, APRIL 11, 2013
10:25 a.m. – 11:00 a.m.

GENOMIC CAUSES OF MALE INFERTILITY
Dolores J. Lamb, PhD, HCLD, Shuo Han, PhD and Mounia Louet, PhD
Center for Reproductive Medicine, Scott Department of Urology and Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

Congenital genitourinary defects, specifically cryptorchidism or failure of the testis to descend into the scrotum during development, are common in men and a well-recognized cause of male infertility. The incidence of non-obstructive azoospermia in men with unilateral cryptorchidism is 13% and among men with untreated bilateral cryptorchidism is 89% making cryptorchidism one of the most common causes of azoospermia. For men who undergo orchiopexy as a child, the risk of azoospermia declines to 46%. Array Comparative Genomic Hybridization (aCGH) was used to test the hypothesis that gene dosage changes underlie developmental defects of the urogenital tract causing azoospermia and aCGH can be used for gene discovery of novel genes required for normal male reproductive function. We identified de novo copy number gains on Xq28 in cryptorchid patients, as well as patients with hypospadias. The duplicated region encompassed a single gene, VAMP7. VAMP7 is a transmembrane protein belonging to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family localized in late endosomes and lysosomes. Human VAMP7 BAC transgenic mice recapitulated the human phenotype. The VAMP7 mice displayed undescended testes and had reduced penile length together with focal spermatogenic defects, diminished sperm motility and subfertility. Spermatogenic arrest and sloughing of germ cells at various stages of maturation was present. Vacuolization of Sertoli cells were evident within the affected seminiferous tubules. Large multimucleated round cells were present, some within the lumen of the tubule. Elevated levels of VAMP7 significantly reduced androgen receptor activity by trapping the nuclear receptor in the endosomal compartment and diminishing its in vivo binding to direct target genes. Overexpressed VAMP7 also drastically enhanced estrogen receptor transcriptional activity and up-regulated estrogen-responsive genes such as CYR61, CTGF and ATF3, all implicated in human hypospadias, a common masculinization disorder of external genitalia. Thus, increased gene dosage of VAMP7 interferes with both androgen and estrogen receptor actions in developing and postnatal genitourinary tissues and affects spermatogenesis, as well as the virilization of the reproductive tract translating into genitourinary birth defects in humans.

This study was supported in part by NIH grants K12 DK0083014 (KURe) and R01DK078121 from NIDDK to DJL.

THURSDAY, APRIL 11, 2013
1:05 p.m. – 1:40 p.m.

Y CHROMOSOME’S ROLE IN MAINTENANCE OF SPERM DNA INTEGRITY
Monika A. Ward, PhD
Institute for Biogenesis Research
University of Hawaii, Manoa

Packaging of the haploid genome into the sperm head requires extensive multi-step chromatin remodeling, ultimately resulting in a massive reduction in DNA supercoiling, disassembly of histones and their replacement with protamines, and specific modifications to the few histones that remain. Deficiencies in any of these processes can leave portions of DNA poorly compacted and/or harboring unrepaired DNA breaks, and are therefore associated with sperm DNA damage. In humans this is a risk factor for adverse clinical outcomes including poor fertilization, impaired embryo development, miscarriage, and an increased risk of morbidity in the offspring. Moreover, defects in the sperm epigenome, which is programmed during chromatin remodeling in the male germline, has been shown to adversely affect the resulting offspring. In spite of fundamental importance of chromatin remodeling very little is known about the regulation of this process, and the key players involved.

In recent years we have been working with mouse models with defined Y chromosome gene deficiencies. These mice have extensive deletions of the Y-specific (non-pairing) region of the Y chromosome long arm (NPYq), are infertile, and have sperm with severe headshape defects. We demonstrated that infertility of these mice is associated with sperm DNA damage and abnormal sperm chromatin packaging, implying involvement of NPYq encoded gene/s in chromatin remodeling during spermiogenesis. The NPYq region encodes multiple copies of at least 4 distinct genes, Ssty, Sly, Asty, Orly, which are exclusively expressed in spermatids implying speriogenic function. To identify which of the NPYq genes is involved in 'sperm DNA damage' phenotype, we characterized 'shSly mice', in which the function of NPYq encoded Sly has been disrupted by transgenically delivered siRNA. We have shown that these mice are infertile, with impaired chromatin packaging and DNA damage in sperm. This indicate that loss of SLY is responsible for the phenotype manifested in NPY deficient mice, and that SLY acts as a regulator of chromatin remodeling in spermiogenesis. Sly deficiency is associated with global derepression of sex chromosome genes and reduction of repressive histone modifications on postmeiotic sex chromatin. Thus, it is plausible that SLY plays a role in epigenetic reprogramming warranting maintenance of sperm DNA integrity via the regulation of expression of sex chromosome genes.

Supported by NIH P20RR024206 (P2) and HD072380.
THE FATHER’S AGE
Jamila R. Momand BS1, Guogang Xu MD, PhD1, and Kim Hildreth MS1, Christi A. Walter PhD1,2,3,4
1Department of Cellular & Structural Biology, 2Barshop Institute for Longevity and Aging Studies, 3Cancer Therapy and Research Center, University of Texas Health Science Center, 4Research, South Texas Veteran’s Health Care System

Introduction: Modern DNA sequencing was used recently to demonstrate that new mutations in the male germline occur as men age and drive the phenomenon known as the paternal age effect. Several dominant genetic disorders are associated with advanced paternal age, including Apert syndrome and achondroplasia. Autism spectrum disorders and schizophrenia, are also associated with a paternal age effect, such that the risk of offspring developing these disorders increases as the father’s age increases. Lifestyle changes over the last 30 – 40 years have resulted in a 30% increase in births to older fathers (≥35-years-old). While the impact of the paternal age effect is rising, the mechanism(s) eliciting this phenomenon remain unknown. We have utilized a mouse model exhibiting a paternal age effect to delineate the molecular mechanisms that culminate in a paternal age effect.

Objective: Previous studies with the mouse model have revealed a relationship between germline mutagenesis and diminished base excision repair activity due to reduced AP endonuclease 1 (APE1). The objectives were 1) to determine if transgenic expression of APE1 could rescue mice from the paternal age effect, and if so, 2) to delineate the causes of diminished APE1.

Methods: Male germ cells were obtained from lacI transgenic mice and bi-transgenic mice carrying hAPEX1 and the lacI mutation reporter transgenes, at young, middle and old ages. Spontaneous mutant frequencies were determined for germ cells using the lacI mutation assay. Western blot analyses were performed using germ cell protein extracts obtained from different aged mice to assess p53 activation and APE1 abundance.

Results: The mutant frequency was elevated in germ cells from old lacI mice, but the increase in mutant frequency did not occur in the hAPEX1, lacI bi-transgenic mice. APE1 abundance was reduced in germ cells from old mice and p53 activation was increased.

Conclusions: APE1 abundance is a major mediator of the paternal age effect in the mouse model. Activation of p53 increases with age in male germ cells and may be involved in eliciting reduced APE1 abundance.

GENETIC AND GENOMIC APPROACHES TO UNDERSTAND TESTICULAR CANCER
James F. Amatruda
Departments of Pediatrics, Molecular Biology and Internal Medicine
University of Texas Southwestern Medical Center, Dallas, TX USA

Testicular germ cell tumor (GCT) is the most common cancer in young men. The genes and pathways that contribute to the development of GCTs are not known, which is a serious impediment to the development of targeted therapy for this disease. To identify GCT candidate genes, we conducted a forward genetic screen in zebrafish, and identified a mutant with a high incidence of spontaneous testicular GCTs consisting of undifferentiated germ cells. Positional cloning of the mutant locus revealed an inactivating mutation in Bone Morphogenetic Protein (BMP) Receptor IB. We find evidence of impaired BMP signaling in the zebrafish GCTs, and altered expression level of BMP target genes. Extending these results through genetic crosses with other zebrafish BMP pathway mutants, we find that specific ligand-receptor interactions modulate the tumor incidence. In agreement with the zebrafish model, we find that undifferentiated human GCTs such as seminomas lack BMP signaling activity. We are also taking a genomic approach to identify additional molecular aberrations that may drive the development of human GCTs. Our results emphasize impaired differentiation as a possible oncogenic pathway and may foster the development of improved, targeted therapy of human GCTs.
CONTROLLING THE NUMBER AND DISTRIBUTION OF RECOMBINATION EVENTS IN MOUSE MEIOSIS
Julian Lange1, Liisa Kauppi1,4, Marco Barchi1,5, Frédéric Baudat3,6, Maria Jasin7 and Scott Keeney1,2
1Molecular Biology Program; 2Howard Hughes Medical Institute; 3Developmental Biology Program; Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY; 4University of Helsinki, Finland; 5University of Rome, Tor Vergata, Italy; 6Institute of Human Genetics, Montpellier, France

Meiosis is the specialized cell division that reduces the genome complement by half to generate gametes for sexual reproduction. During meiosis, homologous maternal and paternal chromosomes exchange genetic information through the process of homologous recombination, which is initiated with DNA double-strand breaks (DSBs) made by the SPO11 protein. Because every chromosome must receive a minimum number of DSBs to ensure proper pairing and segregation, attention has focused primarily on factors that support DSB formation. However, improperly repaired DSBs can cause meiotic arrest or mutation, thus having too many DSBs is likely as deleterious as having too few. Only a small fraction of SPO11 protein ever makes a DSB in yeast or mouse, and SPO11 and its accessory factors remain abundant long after most DSB formation ceases, implying the existence of mechanisms that restrain SPO11 activity to limit DSB numbers. We recently reported that the number of meiotic DSBs in mouse is controlled by ATM, a kinase activated by DNA damage to trigger checkpoint signaling and promote DSB repair (Lange et al., Nature 2011). We proposed that ATM restrains SPO11 via a negative feedback loop in which kinase activation by DSBs suppresses further DSB formation. Here, we further explore ATM-dependent DSB suppression by performing a high-resolution, genome-wide analysis of the number and distribution of breaks in spermatocytes from wild type and Atm−/− mice. In addition, we describe studies of male mice with lowered dosage of SPO11, which point to the existence of a novel feedback mechanism that links DSB number and distribution with inter-homolog interactions.

EMBO Young Investigator Lecture
TOWARDS UNDERSTANDING THE MOLECULAR LOGIC OF PATERNAL EPIGENETIC INHERITANCE
Antoine Peters, PhD
Friedrich Miescher Institute for Biomedical Research, Basal, Switzerland

Nucleosomes are the principal packaging units of chromatin and critical for gene regulation and genome stability. In mammals, a subset of nucleosomes fail to be replaced by protamines during spermatogenesis and are retained in mature spermatozoa providing opportunities for paternal epigenetic transmission. In humans, the remaining 10% localize at regulatory elements of genes. To assess evolutionary conservation and to dissect the molecular logic underlying nucleosome retention, we determined the genome wide nucleosome occupancy in mouse spermatids and in mature spermatozoa that only contain 1% residual histones. Our data indicate important roles of DNA sequence composition, DNA methylation, canonical and variant H3 histones and associated modifications in nucleosome retention versus eviction during the histone-to-protamine remodeling process in elongating spermatids and potentially in epigenetic inheritance by nucleosomes between generations.
TAF4B REGULATES THE BALANCE BETWEEN SPERMATOGONIAL STEM CELL RENEWAL AND DIFFERENTIATION
Lindsay A. Lovasco¹, Eric A. Gustafson¹, Kimberly A. Seymour¹, Dirk G. de Rooij², and Richard N. Freiman¹
¹Brown University, Department of Molecular and Cellular Biology and Biochemistry, Providence, RI; ²Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam AZ, The Netherlands

The long-term maintenance of mammalian spermatogenesis requires proper development of spermatogonial stem cells (SSCs) that can replenish the testis with germ cell progenitors throughout adult life. TAF4b is a gonadal-enriched component of the basal transcription factor complex, TFIID, and is required for long-term spermatogenesis. Deletion of TAF4b causes fertility loss and germ cell depletion in male mice by 11 weeks of age. Successful germ cell transplantation assays into adult TAF4b-deficient host testes suggests TAF4b has a germ cell autonomous function in SSC maintenance.

To elucidate the function of TAF4b in promoting long-term spermatogenesis, we have characterized the initial rounds of spermatogenic differentiation and renewal in the context of the TAF4b-deficient mouse testis. By quantifying germ cell numbers at birth, we observe a significant reduction in the initial postnatal germ cell pool in the TAF4b-deficient testes compared to controls, indicative of an embryonic defect in germ cell development. Numbers of spermatogonia remain significantly lower at the onset of the first round of spermatogenesis, which begins later in TAF4b-null testes compared to age-matched controls. Initial germ cell division at the basement membrane, initiation of meiosis and signatures of germ cell gene expression reflect this developmental delay. The distribution of GFRA1+ cells and increases in GDNF levels in the TAF4b-null testis resembles models of spermatogonial regeneration following germ cell damage, suggesting an attempt to recover from low initial germ cell numbers. While GFRA1+ spermatogonia are present predominantly as A single and A paired in wild type testes, TAF4b-deficient testes have abnormally long and clustered chains of GRFA1+ cells. Together these data indicate that TAF4b-deficient spermatogonial progenitor cells display a tendency for differentiation at the expense of self-renewal during the initial recovery period following reduced germ cell numbers at birth. Thus, the inability to maintain the male germline in the absence of TAF4b is most likely due to the exhaustion of the spermatogonial progenitor cell pool that cannot renew itself to sufficient levels during early waves of spermatogenesis.

THE SPERM EPGENOME: IT'S ROLE IN DEVELOPMENT AND PATERNAL DISEASE TRANSMISSION
Sarah Kimmins
McGill University, Department of Animal Science, Montreal, Canada

The phenotype of an individual is a consequence of complex interactions between the genome, epigenome, and the present and ancestral environments. Adverse paternal exposures can lead to altered offspring development and an increased risk for disease in later life. Epigenetic changes in gametes or their developing offspring have been suggested as a mechanism underlying altered phenotypic outcomes. Epigenetics refers to the heritable information transmitted by DNA methylation, the post-translational modification of histones and noncoding RNAs. The epigenome controls gene expression through its influence on chromatin structure. Sperm retain histones at highly specific regions that are associated with genes implicated in pre and post-implantation embryo development. Using ChIP-Seq and MeDIP-array we characterized the sperm epigenome in two mouse models with induced alterations to DNA and histone methylation. The first model was exposed to a folate deficient diet beginning in utero. Pregnancies sired by folate deficient males had increased embryo loss and offspring with birth defects. The sperm epigenome of folate deficient males showed changes in DNA methylation at genes associated with development and chronic diseases such as diabetes and cancer. The second model with an altered sperm epigenome was created by over-expression of a histone demethylase. Transgenic sperm had reduced histone H3 methylation at > 2000 genes. Offspring sired by transgenic fathers showed a range of developmental abnormalities and decreased survivability. Remarkably these effects persisted for several generations.
FRIDAY, APRIL 12, 2013
10:25 a.m. – 11:00 a.m.

REGULATION OF PROLIFERATION AND DIFFERENTIATION IN THE SPERMATOGONIAL STEM CELL LINEAGE
Margaret Fuller, Alexis Bailey, Megan Insco, Gonzalo Olivares, Chenggang Lu, Jongmin Kim, and Catherine Baker
Departments of Developmental Biology and Genetics, Stanford University School of Medicine, Stanford, CA 94305

A key regulatory point in the male germ line stem cell lineage is the switch from mitotic proliferation of spermatogonia to the spermatocyte program of cell growth, meiosis, and transcription of terminal differentiation genes. Here we show that a translational control cascade regulates the switch from mitosis to meiosis in the Drosophila male germ line stem cell lineage. In Drosophila testes, an oriented division of a male germ line stem cell at the testis apical tip normally produces a new stem cell and a gonialblast. The gonialblast becomes enclosed by two somatic cyst cells, then initiates exactly 4 rounds of mitotic transit amplifying (TA) divisions to produce a cyst of 16 interconnected germ cells that enter preregion of S phase and initiate spermatocyte growth in synchrony. The bag of marbles (bam) protein and its partner benign gonial cell neoplasm (bgcn) are required in male germ cells for spermatogonia to switch from TA cell proliferation to the spermatocyte program. Bam protein accumulates in 4-cell spermatogonial cysts, reaches a peak in 8-cell cysts and abruptly disappears in early 16-cell spermatocyte cysts, immediately after preregion of S phase. The number of TA divisions appears to be set by the accumulation of Bam protein to a critical threshold. The Drosophila TRIM-NHL tumor suppressor homolog and microRNA regulator Mei-P26 facilitates accumulation of the differentiation regulator Bam in TA cells. In turn, Bam and its binding partner Bgcn repress translation of mei-P26 in late TA cells via binding to the mei-P26 3'UTR. The switch from mitosis to meiosis sets up a spermatocyte specific transcription program that shapes the meiotic cell cycle by translational control, delaying production of cell cycle regulators cdc25 and cyclin B during meiotic prophase. The mitosis to meiosis switch also turns on expression of spermatocyte specific transcriptional regulators that program expression of spermatid differentiation genes and repress expression of somatic genes.

FRIDAY, APRIL 12, 2013
1:05 p.m. – 1:40 p.m.

FIND ME, EAT ME AND I’M FULL: THE CLEARANCE OF APOPTOTIC GERM CELLS
Jun Zhang1, Deaho Park3, Michael R. Elliot, Kodi S. Ravichandran1,3 and Jeffrey J. Lysiak1
Departments of Urology1, Microbiology2, and the Beirne B. Carter Center for Immunology Research3, University of Virginia, Charlottesville, Virginia

As part of developmental processes throughout life, we generate excess or superfluous cells. Of these, only a subset is deemed ‘fit’ for further maturation, while the rest undergo apoptosis. These apoptotic cells are quickly cleared by phagocytes. Spermatogenesis is an excellent example of this, during the development of germ cells, many excess cells are generated and only a subset of them mature further. Despite ongoing germ cell apoptosis, under normal steady-state conditions, very few apoptotic cells are detectable in the seminiferous epithelium suggesting an efficient corpse clearance mechanism. Studies of phagocytes in different tissues, including our studies in the testes, have identified several proteins involved in cell clearance, and we are just now beginning to understand how these proteins orchestrate cell clearance in vivo. Our work has uncovered a selective requirement for ELMO1 in Sertoli cell-mediated engulfment of apoptotic germ cells. Our current model is that ELMO1 and its partner DOCK1 function upstream of RAC1 in leading to reorganization of the Sertoli cell cytoskeleton during engulfment of apoptotic germ cells. The receptor BA11 recognizes phosphatidylserine (PtdSer) on apoptotic germ cells and recruits ELMO1 in this pathway. Interestingly, the mitochondrial inner membrane protein, UCP2, also influences the engulfment of apoptotic cells dependent upon this pathway. Using both general gene deletion and Sertoli cell specific gene deletion mice as well as a receptor blocking strategy we now report that inhibiting members of the ELMO engulfment module blocks the clearance of apoptotic germ cells and disrupts spermatogenesis. The receptor BA11 recognizes phosphatidylserine (PtdSer) on apoptotic germ cells and recruits ELMO1 in this pathway. Interestingly, the mitochondrial inner membrane protein, UCP2, also influences the engulfment of apoptotic cells dependent upon this pathway. Using both general gene deletion and Sertoli cell specific gene deletion mice as well as a receptor blocking strategy we now report that inhibiting members of the ELMO engulfment module blocks the clearance of apoptotic germ cells and disrupts spermatogenesis. In both Sertoli cell specific Rac1 deficient mice and Bait knockout mice significantly elevated numbers of apoptotic germ cells are seen and blockade of the Bait PtdSer interaction also leads to an increase in apoptotic germ cells. We now report that loss of Ucp2 limits the capacity of phagocytes to continually ingest apoptotic cells, while overexpression of Ucp2 provided a gain of function. Taken together, results demonstrate that members of the ELMO engulfment module are essential for the clearance of apoptotic germ cells by Sertoli cells and disruption of this pathway results in impaired spermatogenesis.
Spermatogenesis is a lengthy and highly specialized process of cellular differentiation that takes place within a tubular seminiferous epithelium in the testis. Extensive tight junctions between somatic Sertoli cells create one of the body’s tightest epithelial barriers and in doing so create separate functional compartments for diploid spermatogonia, in the basal compartment, and differentiating spermatocytes and spermatids, in the adluminal compartment. To become sperm, differentiating germ cells must cross this barrier without disrupting its functional integrity. To compound the challenge, germ cells are contained within cysts, which can be composed of hundreds of cells connected by intercellular bridges formed as a result of incomplete cytokinesis during proliferation of differentiating spermatogonia. Remarkably, these large clonal syncytial chains of germ cells are able to transit the Sertoli cell tight junctions (SCTJs) without compromising their functional integrity. Using confocal microscopy and genetically labeled cells we visualized the interplay of SCTJ components during the transit event. Consequently, we were able to determine that the germ cells accomplish this remarkable feat by becoming briefly enclosed within a network of transient compartments fully-bounded by old tight junctions on the adluminal side and new tight junctions on the basal side.

THE MEK/ERK CASCADE REGULATES THE EXPRESSION OF ALL GENES INVOLVED IN TESTOSTERONE SYNTHESIS IN LEYDIG CELLS
Mario Ascoli, PhD
Department of Pharmacology, Carver College of Medicine, The University of Iowa, Iowa City, IA

Objectives: Studies done in cell culture show that LH stimulates the ERK1/2 pathway and that this pathway is an important mediator of the proliferation and differentiation of Leydig cells. This study was designed to examine the importance of ERK1/2 on Leydig cell functions in vivo.

Methods: We conditionally deleted MEK1 and MEK2 (the kinases that phosphorylate ERK1/2) from Leydig cells by crossing Mek1f/f;Mek2-/- and Cyp17iCre mice (henceforth referred to as M-) and generated transgenic mice expressing a constitutively active form of Mek1 in Leydig cells using a transgene driven by the Cyp17 promoter (henceforth referred to as M+). For ease of identification, the M-, M+ and wild-type mice were also genetically manipulated to express the cherry tomato red fluorescent protein in Leydig cells.

Results: M- mice show recombination of the floxed Mek1 allele specifically in Leydig cells and a complete or substantial decrease in the phosphorylation of ERK1/2 when stimulated with EGF, Kit ligand, hCG or Bt2cAMP. They have normal body and testicular weight but they exhibit Leydig cell hypoplasia as judged by the presence of the fluorescent red Leydig cells. They also have low intratesticular testosterone, high serum LH and display several signs of hypoandrogenism. Surprisingly, the expression of all Leydig cell genes involved in testosterone synthesis (Star, Cyp11a1, Hsd3b6, Cyp17a1 and Hsd17b3) is reduced substantially in adult Leydig cells from M- mice. The expression of two orphan nuclear receptors (Nr4a1 and Nr5a1) that regulate the expression of many of these steroidogenic enzymes is unchanged or increased in Leydig cells of M- mice. Functionally, the reduced expression of Hsd17b3 may be limiting because testosterone synthesis is greatly reduced in primary cultures of Leydig cells from M- mice when stimulated with hCG, 22-hydroxycholesterol, pregnenolone, progesterone or androstenedione. M+ mice are just now beginning to be characterized but they clearly show specific expression of the constitutively active MEK1 in Leydig cells. We predict that they will display increased phosphorylation of ERK1/2, Leydig cell hyperplasia, hyperandrogenism, and increased capacity for testosterone synthesis.

Conclusions: The Leydig cells MEK/ERK cascade modulates androgen levels not only by impacting the density of Leydig cells in the testis but also by a coordinate regulation of all Leydig cell genes that participate in testosterone synthesis.
REGULATORY T CELL-DEPENDENT TOLERANCE TO NON-SEQUESTERED MALE MEIOTIC GERM CELL ANTIGENS


Depts. of Pathol and Microbiol., and Beirne Carter of Immunol., Dept. Intern Med. and Center of Regenerative Medicine and Immunity, University of Virginia, Charlottesville, VA; Cell Biology and Biochemistry, Texas Tech University HSC, Lubbock, TX; Center for Biomedical Research, Population Council, New York, NY; Dept. Molecular Biochem., Northwestern University, Evanston, IL

Autoantigen-specific CD4+ Foxp3+ regulatory T cells (Treg) need to interact with their cognate antigens in order to maintain their capacity to suppress autoreactive T cell response and maintain tolerance. The meiotic germ cell antigens (MGCA) are located behind the blood-testis barrier (BTB); thus the key question is whether (and how) they reach MGCA-specific Treg to maintain tolerance. In this study, we address the sequestration and tolerance status of two MGCA: lactate dehydrogenase 3 (LDH3) and zonadhesin (Zan). When total Treg were depleted from diphtheria toxin receptor-foxp3 transgenic mice by diphtherial toxin, they produced autoantibody against LDH3 but not to Zan. The EAO that followed is characterized by accumulation of immune complex and activated M1 macrophage in testis, and disruption of both BTB and testis function. In contrast, vasectomized mice that developed T cell-dependent EAO produced MGCA autoantibody that targeted Zan but not LDH3. The dichotomized antibody response suggests that 1) LDH3 is not sequestered and that its tolerance state is controlled by Treg; and 2) Zan is sequestered and it induces response when exposed in vasectomy. Indeed, intravenous injection of antibody into normal mice created LDH3-specific immune complexes but not Zan-specific immune complexes, located outside the BTB. Also, when immunized with mouse testis antigens, the response of wild-type female and ldh3-null male to LDH3 exceeded wild-type male. But they responded equally to Zan. The findings are confirmed in two protamine-ovalbumin (OVA) transgenic mice (OVA-hi and OVA-lo) that differed 41-folds in OVA expression. OVA egress was visible in OVA-hi but not OVA-lo mice; and OVA immunization led to EAO in OVA-lo but not in OVA-hi mice unless Treg were depleted. Finally, OVA and LDH3 were located in the residual bodies of normal Sertoli cells, maximally at stage IX of the spermatogenic cycle. We conclude that MGCA sequestration is selective and is not complete. While the non-sequestered MGCA exits seminiferous tubule possibly via the residual body and maintains Treg-dependent tolerance, the sequestered and non-tolerant MGCA is targeted when they are exposed in vasectomy.

NICHE MICROENVIRONMENT FOR THE MOUSE SPERMATOGENIC STEM CELLS

Yu Kitadate and Shosei Yoshida
Division of Germ Cell Biology, National Institute for Basic Biology, Okazaki, Japan

Niche microenvironment is considered as an indispensable component of the stem cell system that regulates the balanced stem cell self-renewal and differentiation. Drosophila testis harbors anatomically defined localized niche consisting of specialized somatic cells, namely the hub. In contrast, in mouse testis, seminiferous tubules show simple and uniform structure and do not exhibit specialized substructure that implies the defined niche. Spermatogenic stem cells (related to GFRα1+ spermatogonia) are scattered in the basal compartment of the tubules. So, mouse testis represents a niche microenvironment with facultative nature. Preceding studies have shown that stem spermatogonia are localized in a biased manner in the basal compartment to the area associated with the vasculature that surrounds the tubules. However, nature of this presumptive niche microenvironment has not been mostly unknown at a cellular and molecular level.

We have been challenging this issue by searching for genes expressed in somatic cells in proximity to blood vessels taking advantage of microdissection-cDNA microarray analyses combined with in situ hybridization screening. Our analysis has revealed that a particular somatic cell type, which is distributed in a vasculature-related pattern, express a set of genes that potentially regulate the stem cell functions such as secreted factors. In vitro, these cells support the cultured spermatogonial stem cells. In this workshop, we would like to discuss about the niche microenvironment and the stem cell regulation in mouse spermatogenesis, based on the function of the above-mentioned putative niche cells.
Continual spermatogenesis relies on the actions of an undifferentiated germ cell population comprised of spermatogonial stem cells (SSCs) and progenitor spermatogonia. Self-renewal by SSC maintains a foundational pool from which progenitor spermatogonia will arise and transiently amplify in number before committing to terminal differentiation. We have used the mouse as a model to investigate the role of inhibitor of DNA binding (ID) proteins in regulation of SSC functions. We discovered that ID2 and ID4 are expressed by undifferentiated spermatogonia and play an important role in regulating SSC self-renewal. Importantly, our findings suggest that ID4 is expressed by SSCs but not progenitor spermatogonia. We created a transgenic mouse line in which ID4 expressing cells are marked by GFP and we have used this model to investigate phenotypic, functional, and molecular characteristics of SSC and progenitor spermatogonia. Using functional transplantation analysis, we found that stem cell capacity for regeneration of spermatogenesis within the undifferentiated spermatogonial population is restricted to the ID4-GFP+ fraction. We have also utilized this mouse line to determine characteristics of the SSC population during postnatal development and steady-state spermatogenesis in adulthood. Using RNA-seq methodology with isolated ID4-GFP positive and ID4-GFP negative fractions of the undifferentiated spermatogonial population, we have begun to define molecular profiles that distinguish stem and progenitor components. In recent studies, we found that ID2 and ID4 interact with retinoblastoma protein (RB) in undifferentiated spermatogonia and have explored a role for RB in regulation of fate determination. Using conditional genetic ablation approaches, we discovered that RB is required in SSCs for self-renewal and maintenance of the germline. In contrast, RB is not required in progenitor spermatogonia for proliferation or differentiation but a subset of cells appear to lose lineage commitment reverting to a primitive state and undergoing carcinoma in situ (CIS) transformation. Using microarray analysis we discovered that loss of RB function results in markedly different transcriptome responses in SSCs compared to progenitor spermatogonia. Collectively, our findings have revealed that the molecular profiles and mechanisms controlling fate decisions are distinctly different in SSC and progenitor spermatogonia. This research was supported by grant HD061665 from the National Institutes of Health.

Stem cells often reside in specific local microenvironments, or niches, where signals from nearby cells and substrates promote stem cell maintenance. Spermatogonial stem cells in the testis of both vertebrates and invertebrates provide a lifetime supply of sperm, making this tissue an excellent model for studying stem cell biology. We have focused on the Drosophila testis, as it contains a morphologically distinct niche that can be probed with sophisticated genetic tools. The apex of each Drosophila testis contains a single niche comprised of a cluster of quiescent somatic cells called the hub, to which spermatogonial stem cells and somatic cyst stem cells (CySCs) adhere. We previously found that genetic ablation of spermatogonial stem cells prompts their differentiating progeny (called spermatogonia) to enter the niche and revert to functional spermatogonial stem cells. This was subsequently shown to occur in the mouse testis, underscoring its generality. Here, by genetically ablating CySCs, we find that hub cells are not only quiescent niche cells: they are also a reserve population of CySCs. Ablation of CySCs prompts hub cells to undergo an epithelial-to-mesenchymal transition via downregulation of Ecadherin. Delamination of cells from the hub is accompanied by a loss of hub cell quiescence. Hub cell proliferation is transient, however, and ceases when the niche is repopulated. The transdifferentiation of hub cells to CySCs requires the ablation of most or all CySCs, suggesting that CySCs normally actively signal to hub cells to maintain hub cell quiescence. Consistent with this hypothesis, hub cell transdifferentiation does not replenish CySCs that are lost during normal aging. Our work suggests that the ability of quiescent cells to transdifferentiate into stem cells in response to stem cell depletion may be a previously undiscovered feature of niches in general.
TRANSLATING SPERMATOGONIAL STEM CELL TRANSPLANTATION TOWARD THE CLINIC
Kyle Orwig, PhD
University of Pittsburgh

Spermatogonial stem cells (SSCs) maintain spermatogenesis and may have application for treating some cases of male infertility. We performed autologous and allogeneic SSC transplantations into the testes of 18 adult and 5 prepubertal recipient macaques that were rendered infertile with alkylating chemotherapy. After autologous transplant, the donor genotype from lentivirus-marked SSCs was evident in the ejaculated sperm of 9/12 adult and 3/5 prepubertal recipients after they reached maturity. Allogeneic transplant led to donor-recipient chimerism in sperm from 2/6 adult recipients. Ejaculated sperm from one recipient transplanted with allogeneic donor SSCs were injected into 85 rhesus oocytes via intracytoplasmic sperm injection. Eighty-one oocytes were fertilized, producing embryos ranging from four-cell to blastocyst with donor paternal origin confirmed in 7/81 embryos. This establishes a model for safety and technology development that will contribute to informed clinical translation.
8. **WITHDRAWN**

10. **WITHDRAWN**

12. **OXIDATIVE STRESS AND SPERM GENOME QUALITY IN INFERTILE MALE SMOKERS WITH VARICOCELE**
Monis Bilal Shamsi, MSc, Shweta Smitha Misro, MSc, Jhumur Pani, MSc, Dinesh Kumar, MBBS, Rima Dada, MD, PhD
Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Science, New Delhi, India

14. **ALTERED LONG NON-CODING RNA PROFILES IN THE TESTES OF PATIENTS WITH DIFFERENT TYPES OF NON-OBSTRUCTIVE AZOOSPERMIA**
Lv Mingrong, Tian Hui Graduate Students¹ and Sun Fei Post Docs²
¹Classmate; ²Teacher

16. **ROLE OF TUMOUR SUPPRESSOR NF2 ISOFORMS IN SPERM MATURATION**
Ansgar Zoch¹, Thomas Greither, PhD² and Helen Morrison, PhD¹
¹Leibniz Institute for Age Research – FLI Jena, Germany; ²Universitätsklinikum Halle, Germany

18. **ABERRANT EPIGENETIC CHANGES IN THE ABNORMALLY EXRESSED SEMINAL PLASMA MIRNAS (SP–MIRS) OF IDIOPATHIC NON–OBSTRUCTIVE AZOOSPERMIA**
Wei Wu, Yufeng Qin Doctor, Zheng Li Doctor, Jing Dong Doctor, Juncheng Dai Doctor, Xuejiang Guo Doctor, Yang Zhao Doctor, Yong Zhu Doctor, Wei Zhang Doctor, Jiahao Sha Doctor, Hongbing Shen Doctor, Yankai Xia Doctor, Zhibin Hu Doctor, Xinru Wang Doctor
Nanjing Medical University

20. **DECONTAMINATING HUMAN TESTICULAR CELLS FROM LEUKEMIC CELLS USING FLUORESCENCE-ACTIVATED CELL SORTING**
Hanna Valli¹, Serena Dovey², Meena Sukhwani², Brian Hermann³, Julia Donohue² and Kyle Orwig¹
¹Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh; ²Magee-Womens Research Institute, Pittsburgh; ³Department of Biology, University of Texas at San Antonio, San Antonio

24. **PROTEOMIC ANALYSIS OF SPERM ANTERIOR HEAD PLASMA MEMBRANE: UNFOLDING THE MOLECULAR COMPONENTS UNDERLYING SPERM–EGG INTERACTION**
Kessiri Kongmanas, MSc¹, Clarissa Sugeng, MSc¹, Puneet Souda, BSc, MBA², Kym Faull, PhD², Ken Kitajima, PhD³, John Aitken, PhD⁴, Trish Berger, PhD⁵, Julian Whitelegge, PhD⁵, Mark Baker, PhD⁶ and Nongnuj Tanphaichitr, PhD¹
¹Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada; ²Pasarow Mass Spectrometry Laboratory, University of California, Los Angeles, USA; ³Laboratory of Animal Cell Function, Bioscience and Biotechnology Center, Nagoya University, Japan; ⁴The ARC Centre of Excellence in Biotechnology and Development, School of Environmental and Life Sciences, University of Newcastle, New South Wales, Australia; ⁵Department of Animal Science, University of California, Davis
26. GENE COPY NUMBER INCREASE IN THE AZOOSPERMIA FACTOR C (AZFC) REGION AND ITS EFFECT ON SPERMATOGENIC IMPAIRMENT
Chuncheng Lu, PhD, Yankai Xia, PhD, Zhibin Hu, PhD, Xinru Wang, PhD

28. AN AGENT-BASED MODEL OF MOUSE SPERMATOGENIC CYCLES
Debjit Ray, Philip Pitts, Ping Ye, PhD
Washington State University

30. METABOLOMICS REVEALS NOVEL METABOLIC PATHWAYS IN RELATION TO REPRODUCTIVE TOXICITY OF BISPHENOL A
Minjian Chen, Shoulin Wang, Zuomin Zhou, Yankai Xia, Xinru Wang
State Key Laboratory of Reproductive Medicine, Institute of Toxicology, Nanjing Medical University

32. IS THERE ANY EFFECT OF INSULIN RESISTANCE ON MALE REPRODUCTIVE SYSTEM?
Ayhan Verit, Prof; Fatma Verit, Assoc Prof; Halil Oncel, MD; Halil Ciftci, Assoc Prof

34. INVESTIGATING THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN TESTICULAR MORPHOGENESIS USING PORCINE DE NOVO FORMED TESTICULAR TISSUE.
Camila Dores Ms, DVM, PhD Candidate, Ina Dobrinski DVM, MVSc, PhD, Dipl ACT
UCVM

36. ROLE FOR CHROMATIN MODIFYING PROTEIN SMC6 IN PERICENTROMERIC HETEROCHROMATIN DOMAINS DURING SPERMATOGONIAL DIFFERENTIATION AND MEIOSIS
Dideke E. Verver, Ans M.M. van Pelt, Sjoerd Repping, Geert Hamer
Center for Reproductive Medicine, Academic Medical Center/University of Amsterdam

38. LACKING OF SEIPIN IN TESTIS AFFECTS THE LIPID METABOLISM IN SPERMATIDS AND LEADS TO DEFECT OF SPERMATOGENESIS
Min Jiang

40. SERTOLI CELLS SURVIVE XENOTRANSPLANTATION BY MODULATING THE MACROPHAGES AT THE GRAFT SITE TO BE REGULATORY VERSUS CYTOTOXIC
Payal Mital, Kandis Wright, Jannette M. Dufour
Texas Tech University Health Sciences Center
Poster Session I

44. MICRORNAS: NOVEL ANDROGEN RESPONSIVE TRANSCONTING FACTORS IN THE TESTIS
Subbarayalu Panneerdoss, PhD, Yao-Fu Chang, PhD, Manjeet Rao, PhD
Greehey Children Cancer Research Institute, University of Texas Health Science Center at San Antonio

46. NEUTROPHIL AND MACROPHAGE INFILTRATION PRECEDES TESTICULAR GERM CELL APOPTOSIS AFTER MONO-(2-ETHYLHEXYL) PHTHALATE EXPOSURE IN AN AGE- AND SPECIES-DEPENDENT MANNER
Caitlin Murphy, BS, PhD, Angela Stermer, BS, John Richburg, BS, MS, PhD
Center for Molecular and Cellular Toxicology, College of Pharmacy, The University of Texas at Austin

48. IDENTIFYING RA–RESPONSIVE TRANSCRIPTS IN SERTOLI CELLS DURING SPERMATOGONIAL DIFFERENTIATION, DIVISION, AND THE ONSET OF MEIOSIS
Elizabeth Evans, MS, Cathryn Hogarth, PhD, Debra Mitchell, Michael Griswold, PhD
Washington State University

50. EVIDENCE THAT THE HOMEBOX TRANSCRIPTION FACTOR, RHOX10, PROMOTES SPERMATOGONIAL STEM CELL SELF-RENEWAL AND MEIOTIC PROGRESSION
Hye-Won Song, Ph D, Miles Wilkinson, PhD
Department of Reproductive Medicine, School of Medicine, University of California, San Diego

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Kenneth Aston, PhD1, Alexandra Lopes, PhD2, Emma Thompson, PhD3, Filipa Carvalho, PhD4, Amy Wilfert, BS5, Ni Huang, PhD5, Michiel Noordam, PhD5, Juncheng Dai, BS6, Avinash Ramu, MS7, Sergey Moskovtsev, MD, PhD8, Zhibin Hu, PhD8, Carole Ober, PhD9, Peter Schlegel, MD9, Douglas Carrell, PhD1 and Don Conrad, PhD8
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54. EFFECTS OF FADD PHOSPHORYLATION ON MEIOSIS IN MICE SPERMATOGENSES
Yan Zhang

56. FETAL CHEMOTHERAPY EXPOSURE INDUCES TESTICULAR CANCER
Gunapala Shetty, PhD, Ana Luiza Drumond, PhD, Paul Comish, MS, Connie Weng, MD, PhD, Angabin Matin, PhD, Marvin Meistrich, PhD
University of Texas MD Anderson Cancer Center
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Nicole Parker BS, Dolly Singh ScM, Hayley Falk BS, Benjamin Smith ScM, William Wright PhD
Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health

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Dawn Lovelace, Kazadi Mutoji, Brian Hermann
University of Texas San Antonio

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Jianqiang Bao, PhD, Wei Yan
University of Nevada Reno

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Enrique Sosa, MS, BS1, James M.A. Turner, MB, PhD2, Wei Yan, MD, PhD3 and John R. McCarrey, PhD1
1The University of Texas at San Antonio; 2Division of Stem Cell Biology and Developmental Genetics, Medical Research Council, National Institute for Medical Research, London, United Kingdom; 3University of Nevada School of Medicine, Reno, Nevada

66. ROLE OF UBIQUITIN LIGASE HUWE1 IN MODULATING SPERMATOGONIAL DEVELOPMENT IN THE TESTIS
Rohini Bose1, Ellis Fok2, Wenming Xu1, Martine Culty1, Hsiao-Chang Chan2, Antonio Iavarone2 and Simon Wing1
1Polypeptide Hormone Laboratory, Department of Medicine, McGill University and MUHC RI, Montreal, Quebec, Canada; 2Epithelial Cell Biology Research Centre, The Chinese University of Hong Kong, Hong Kong; 3SCU-CUHK Joint lab for Reproductive Medicine, West China Second University Hospital, Sichuan University, Chengdu, China; 4Department of Medicine, McGill University and MUHC RI, Montreal, Quebec, Canada; 5Institute for Cancer Genetics, Columbia University Medical Center, New York, NY

68. TDP-43 IS ESSENTIAL FOR SPERMATOGENESIS
Hari Prasad Osuru, PhD1, Craig Urekar1, Kwan Hee Kim, PhD2, Po-Min Chiang, PhD3, Philip Wong, PhD3 and Prabhakara Reddi1
1University of Virginia; 2Washington State University; 3Johns Hopkins University

70. THE HISTONE H3 DEMETHYLASE, KDM1 (LYSINE-SPECIFIC DEMETHYLASE 1) IS ESSENTIAL FOR THE ENTRY INTO MEIOSIS AND THE SURVIVAL OF SPERMATOGONIAL STEM CELLS.
Romain Lambrot, PhD1, Christine Lafleur, MSc1, Michael G. Rosenfeld, MD2 and Sarah Kimmins, PhD3
1McGill University, Department of Animal Science; 2Howard Hughes Medical Institute, University of California at San Diego School of Medicine; 3McGill University, Departments of Animal Science and Pharmacology and Therapeutics

72. UBE2K DEFICIENCY DISRUPTS MEIOSIS IN RATS AND THE DERIVATION OF RAT SPERMATOGONIAL LINES IN CULTURE
Jaideep Chaudhary, MS, Priscilla Jaichander, PhD, Gerardo A. Medrano, BS, Andrew E. Syvyk, PhD, Karen M. Chapman, BS, F. Kent Hamra, PhD
Department of Pharmacology, Cecil H. & Ida Green Center for Reproductive Biology Sciences, University of Texas Southwestern Medical Center, Dallas
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   Elizabeth Snyder PhD, Tongjun Gu PhD, Robert Braun PhD
   The Jackson Laboratory

76. **EFFECTS OF CAFFEINE AT DIFFERENT PERIODS OF INCUBATION IN SEMEN SAMPLES THAWED**
   Juliana Pariz PhD student¹, Camilla Miotti BSc², Patricia Pieri PhD¹ and Jorge Hallak Md, PhD¹
   ¹Division of Urology and Reproductive Toxicology Unit, Dept of Pathology – University of Sao Paulo Medical School and Androscience, High Complexity Andrology Laboratory and Male Reproductive Health Clinic; ²Division of Urology and Reproductive Toxicology Unit, Dept of Pathology – University of Sao Paulo Medical School

78. **ELUCIDATING THE ROLE OF UCH−L1 IN SPERMATOGENESIS**
   Whitney Alpbaugh BSc, Krysta Coyle BSc, Ina Dobrinski DVM, MVSc, PhD, DACT
   University of Calgary

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   Johanna Selvaratnam BSc, MSc, Catriona Paul PhD, Bernard Robaire PhD
   Department of Pharmacology and Therapeutics and of Obstetrics and Gynecology, McGill University, Montreal, QC, Canada

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   Suresh Ramaswamy, PhD¹, Hitomi Suzuki, PhD², Rachel Rosland¹, Aleksandar Rajkovic, MD, PhD¹ and Tony Plant, PhD¹
   ¹University of Pittsburgh School of Medicine; ²Magee Womens Research Institute

84. **ZINC FINGER AND TAL-EFFECTOR NUCLEASE MEDIATED GENE TARGETING IN MOUSE SPERMATOGONIAL STEM CELLS**
   Danielle Fanslow, Crystal Heim, Matthew Porteus, MD, PhD¹ and Christina Dann, PhD²
   ¹Stanford University; ²Indiana University

86. **CHARACTERIZATION OF SPERMATOGONIAL MARKERS IN THE MATURE TESTIS OF SCYLiorHinus CANICULA**
   Adrien Bosseboeuf, PhD student¹,2,3,4,5, Aude Gautier, PhD¹,2,3, Pierrick Auvray, PhD⁴,5 and Pascal Sourdaine, Pr¹,2,3
   ¹Normandie Univ, France; ²UCBN, BioMEA, F-14032 Caen, France; ³CNRS INEE FRE3484, F-14032 Caen, France; ⁴KELIA, France; ⁵Group CELLIS PHARMA, Parc Technopolitain Atalante Saint Malo, 35400 Saint Malo

88. **TRANSIENT HYPOTHYROIDISM AND TESTIS SIZE**
   K Teerds, PhD¹, Jaap Keijer, PhD¹ and Eddy Rijntjes, PhD²
   ¹Human and Animal Physiology, Department of Animal Sciences, Wageningen University; ²Institute for Experimental Endocrinology, Charité Universitätsmedizin Berlin, CVK, Augustenburger Platz 1, D-13353, Berlin
90. TESTIS EXPRESSED ACTIN-LIKE 7B (ACTL7B) IS REQUIRED FOR MOUSE SPERMATID MORPHOGENESIS, ACROSOME ATTACHMENT, AND FERTILITY
Tracy Clement, PhD¹, Chris Geyer, PhD², Garrett Warren³, William Willis, BS¹, Eugina Goulding¹ and Mitch Eddy, PhD¹
¹National Institute of Environmental Health Sciences; ²East Carolina University; ³North Carolina State University

92. ALTERNATE PROMOTER USAGE CONTRIBUTES TO THE BASAL AND FORSKOLIN−INDUCED EXPRESSION OF THE MOUSE NR4A1/NUR77 GENE IN MA−10 LEYDIG CELLS
Nicholas Robert, PhD¹, Luc Martin, PhD² and Jacques Tremblay, PhD¹
¹Laval University; ²University of Moncton

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Juan Hernandez, PhD¹, Sayeepriyadarshini Anakk, PhD² and David Moore, PhD¹
¹Baylor College of Medicine; ²University of Illinois at Urbana-Champaign

96. CLINICAL USEFULNESS OF DETERMINATION OF SEMINAL FRUCTOSE AND CITRIC ACID LEVELS IN INFERTILE PATIENTS WITH PROSTATOVESICULITIS DUE TO CHLAMYDIA TRACHOMATIS AND MYCOPLASMA SPP
Dr. Salomon Alvarez,¹, Dr. Benito Ramos¹, Dr. Alberto Niderhauser¹, Dr. Jesus Añer¹, Dr. Jose Javier Sanchez² and Dr. Guadalupe Gallegos¹
¹Medicine School, UANL; ²Medicine School, UAM

98. CHARACTERIZATION OF SPATA22, A NOVEL MAMMALIAN PROTEIN REQUIRED FOR MEIOTIC PROGRESSION IN MOUSE GERM CELLS
Jessica Landaiche, BS, Emily Hays, BS, Sophie La Salle, PhD
Midwestern University

100. EFFECT OF CHLAMYDIA TRACHOMATIS AND UREAPLASMA UREALYTICUM GENITOURINARY INFECTION TREATMENT ON SPERM MORPHOLOGY, DNA FRAGMENTATION AND MALE FERTILITY
Benito Ramos, PhD¹, Salomon Alvarez, PhD², Alberto Niderhauser, PhD², Jesus Añer, PhD², Jose Javier Sanchez, PhD³ and Maria Guadalupe Gallegos, PhD²
¹Medicine School; ²Medicine School, UANL; ³Medicine School, UAM

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Jason Kovac, Josephine Addai, Larry Lipshultz, Dolores Lamb
Baylor College of Medicine

106. RETINOIC ACID SIGNALING CELL-AUTONOMOUSLY DRIVES SPERMATOGENIAL DIFFERENTIATION
Ming-Han Tong, PhD, Mike Griswold, PhD
Washington State University
108. SPERMATOGONIAL BEHAVIOR IN RATS DURING RADIATION-INDUCED ARREST AND RECOVERY AFTER HORMONE SUPPRESSION
Amanda Albuquerque, Master Degree, Fernanda Almeida, PhD, Connie Weng, PhD, Gunapala Shetty, PhD, Marvin Mestrich, PhD and Helio Chiarini-Garcia, PhD
1Laboratory of Structural Biology and Reproduction, Federal University of Minas Gerais, Belo Horizonte, Brazil.; 2MD Anderson Cancer Center, University of Texas, Houston, TX

110. REGULATION OF THE PROLIFERATION AND DIFFERENTIATION OF ADULT LEYDIG STEM CELLS
Hana Odeh MSc, Colin Kleinguetl MSc, Jin-Yong Chung PhD, Barry Zirkin PhD, Haolin Chen PhD
Johns Hopkins Bloomberg School of Public Health
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Saeed Shokri1, Mahsa Kazemi2 and Zahra Arab Firouzjaei1
1Department of Anatomy, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran; 2Department of Physiology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

7. CELLULAR AND MOLECULAR MECHANISM OF MALE INFERTILITY IN THE ATHLETES THAT ABUSE ANABOLIC ANDROGENIC STEROIDS: APOPTOSIS IN SPERMATOGENIC CELLS, CASPASE 3 ACTIVITY AND THE GENERATION OF REACTIVE OXYGEN SPECIES (ROS) IN THE RAT MODEL
Azam Bayat1 and Saeed Shokri2
1Center of Health Services, Zanjan University of Medical Sciences, Zanjan, Iran; 2Department of Anatomy, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

9. THE TARGETING AND FUNCTIONS OF MIRNA-383 ARE MEDIATED BY FMRP DURING SPERMATOGENESIS
Hui Tian, Fei Sun, PhD, MD

11. OXIDATIVE STRESS AND SPERM DNA QUALITY IN MALE PARTNERS OF COUPLES EXPERIENCING RECURRENT IVF/ICSI FAILURE
Monis Bilal Shamshi, MSc, Shweta Smitha Misro, MSc, Kuldeep Mohanty, MSc, Rima Dada, MD, PhD
Laboratory for Molecular Reproduction and Genetics, Department of Anatomy, All India Institute of Medical Science, New Delhi, India

15. IDENTIFYING INTERACTING PROTEINS OF INDUCIBLE NITRIC OXIDE SYNTHASE IN HUMAN TESTIS
Mohammad Rashid, PhD, Kirti Tiwari, Karthik Prabhakara, Rexhina Alushi, Anamica Muruganandam, Barath Sivasankaran, Nikita Aware, Karthikeyan Thangavel, Jason Ettinger
University of Houston-Clear Lake

17. HISTOMETRICAL AND QUANTITATIVE PARAMETERS OF SPERMATOGENESIS IN PUBERAL PIGS
Rodrigo Castro, DVM, MSc1 and Eduardo Bustos-Obregón2
1Universidad de Chile; 2University of Chile, Medical School

21. ERBB4 IS ESSENTIAL AT PUBERTY FOR MAINTAINING ADULT MALE FERTILITY
Florence Naillat1, Ville Veikko lainen, PhD2, Raija Sormunen, PhD3, Ilkka Miinalainen, PhD3, Petra Sipilä, PhD4, Matti Poutanen, PhD5, Klaus Elenius, PhD5 and Seppo Vainio, PhD6
1Oulu Center for Cell Matrix Research, Biocenter Oulu; 2Department of Medical Biochemistry and Genetics, University of Turku; 3Electron Microscopy Unit, University of Oulu; 4Laboratory Animal Center, University of Helsinki; 5Department of Physiology, University of Turku; 6Oulu Center for Cell Matrix Research, Biocenter Oulu, Department of Medical Biochemistry and Molecular Biology, University of Oulu

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Swetasmita Mishra, MSc1, Kuldeep Mohanty, MSc2, Rajeev Kumar, MD2, Neena Malhotra, MD2 and Rima Dada, MD, PhD2
1All India Institute of Medical Sciences (AIIMS); 2AIIMS
25. EFFECT OF MOUSE SEXUAL MATURATION ON THE LEVELS AND CELLULAR CO–LOCALIZATION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN PRIMARY SERTOLI CELL CULTURES
Mahmoud Huleihel, PhD, Yael Abarbanel, BSc, Maram Azab, MSc, Esther Haber, PhD, Eitan Lunenfeld, MD
Ben-Gurion University

27. ESTABLISHMENT OF A PROTEOME PROFILE AND IDENTIFICATION OF MOLECULAR MARKERS EXPRESSED IN MOUSE SPERMATOGONIAL STEM CELLS
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29. POTENTIAL EARLY BIOMARKERS OF TESTICULAR TOXICITY IN RAT EPIDIDYMAL FLUID
Denise Sun-Lin, MS¹, David Seyler, PhD¹, April Paulman, PhD² and Holly Smith, BS¹
¹Eli Lilly and Company; ²Covance Laboratories

31. PROTEOMICS CHARACTERIZATION OF THE CYTOPLASMIC DROPLET OF EPIDIDYMAL SPERM
Louis Hermo, Catherine E. Au, Jeffrey Smirle, Charles E. Smith, Ali Fazel, Tommy Nilsson, John J.M. Bergeron
McGill University, Department of Anatomy and Cell Biology

33. A 1.1 MB SEGMENTAL DELETION ON THE X CHROMOSOME CAUSES MEIOTIC FAILURE IN MALE MICE
Jian Zhou, PhD¹, John McCarrey, PhD² and P. Jeremy Wang, MD, PhD¹
¹Center for Animal Transgenesis and Germ Cell Research, Department of Animal Biology, University of Pennsylvania School of Veterinary Medicine; ²Department of Biology, University of Texas at San Antonio

35. TESTICULAR GERM CELLS ACTIVATE INFLAMMATORY SIGNALLING PATHWAYS THROUGH TOLL-LIKE RECEPTORS AND STIMULATE CYTOKINE PRODUCTION
Julia Young, PhD¹, Julie Muir¹, Ashley Mansell, PhD² and Mark Hedger, PhD¹
¹Centre for Reproduction and Development, Monash Institute for Medical Research, Melbourne, VIC, Australia; ²Centre for Innate Immunity and Infectious Diseases, Monash Institute for Medical Research, Melbourne, VIC, Australia

37. SPERMATOGONIAL ACTIVITY IN THE TESTES OF PREPUBERTAL BOYS
Julie Hagen, BSc, Madelon van Wely, PhD, Andreas Meissner, MD, Annemieke de melker, PhD, Sjoerd Repping, PhD, Fulco van der Veen, MD, PhD, Ans van Pelt, PhD
CVV, AMC

39. CHARACTERIZATION OF UBIQUITIN CARBOXYL-TERMINAL HYDROLASE L1 IN MOUSE SPERMATOGONIA
Lin Tang, PhD¹, Alla Bondareva, MS³, Marie-Claude Hofmann, PhD² and Ina Dobrinski, DVM, PhD¹
¹Department of Comparative Biology & Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada; ²Department of Endocrine Neoplasia and Hormonal Disorders, University of Texas MD Anderson Cancer Center, Houston, TX

41. PURINERGIC ION CHANNELS IN MOUSE SPERMATOGONIA
David Fleck, MSc, Sophie Veitinger, PhD, Thomas Veitinger, PhD, Susanne Lipartowski, Corinna Engelhardt, Jennifer Spehr, PhD, Marc Spehr, PhD
RWTH Aachen University
43. PEAK RETINOIC ACID LEVELS COINCIDE WITH SPERMATOGONIAL DIFFERENTIATION DURING A SYNCHRONIZED FIRST SPERMATOGENIC WAVE
Cathryn Hogarth, PhD, Debra Mitchell, Samuel Arnold, Nina Isoherranen, PhD, Micheal Griswold, PhD
Washington State University; University of Washington

45. EFFECTS OF DIFFERENT METHODS OF CRYOPRESERVATION ON SPERM DNA INTEGRITY IN NORMOSPERMIC AND OLIGOASTHENOTERATOSPERMIC MEN
Yubaihe Shen, Geraldine Delbes, Marie-France Lusignan, Belen Herrero and Peter Chan
Department of Urology, McGill University Health Center; MUHC Reproductive Center, McGill University Health Center; Department of Urology and MUHC Reproductive Center, McGill University Health Center

47. AMYLOIDS ARE A CONSTITUENT OF THE SPERM ACROSOMAL MATRIX CORE
Benoit Guyonnet, PhD; Sandra Whelly, PhD; Gail A. Cornwall, PhD
Texas Tech University Health Sciences Center

49. GESTATIONAL EXPOSURE TO HIGH FAT DIET AND BISPHENOL A REPROGRAMS SPERMATOGENESIS IN RATS
Pheruza Tarapore, PhD, Ricky Leung, PhD, Vinothini Janakiram, PhD, Bin Ouyang, PhD, Shuk-mei Ho, PhD
University of Cincinnati

51. OPTIMIZED LENTIVIRAL VECTOR CARRYING INSULIN CDNA CAN STABLY IMPROVE DELIVERY OF INSULIN BY IMMUNE PRIVILEGED SERTOLI CELLS
Lea Ann Thompson, BS, MS, Matriculating, PhD, Gurvinder Kaur, PhD, Mithun Pasham, MS, and Jannette M. Dufour, PhD
TTUHSC; TTUHSC Post-Doc; TTUHSC Research Tech; TTUHSC Faculty

53. DOSE-DEPENDENT FUNCTIONS OF FGF9 IN PRIMORDIAL GERM CELL (PGC) DIFFERENTIATION
Ferhat Ulu, Hideki Sakamoto, PhD, Sung-Min Kim, PhD, Toshifumi Yokoyama, PhD, Yukiko Yamazaki, PhD
Institute for Biogenesis Research, John A. Burns School of Medicine, University of Hawaii

55. EXOME SEQUENCING IDENTIFIES DPY19L2 DELETION AND MUTATION AS A CAUSE OF GLOBOZOOSPERMIA AND REPAIRABLE BY ICSI
Yang Wen, graduate student, Xiaoyu Yang, Chenhui Ding, Min Jiang, Junchen Dai, Zhibin Hu, Jiahao Sha, Zuomin Zhou, Xuejiang Guo, Qi Zhou and Canquan Zhou
State Key Laboratory of Reproductive Medicine, Nanjing Medical University; Center for Reproductive Medicine and Department of Gynecology & Obstetrics, First Affiliated Hospital, Sun Yat-sen University; State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences

57. CAN TESTIS SPECIFIC PP1GAMMA2, ESSENTIAL FOR SPERMATOGENESIS AND MALE FERTILITY IN MAMMALS, BE REPLACED BY A UBIQUITOUS PP1 ISOFORM?
Tejasvi Dudiki, Nilam Sinha, PhD, Luis Korrodi-Gregório, PhD, Srinivasan Vijayaraghavan, PhD
Kent State University
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EPIDIDYMITS/ORCHTIIS RATIO DEMONSTRATES THE ROLE OF THE EPIDIDYMIS AS A BARRIER AND POWERFUL "BODY GUARD" AGAINST TESTICULAR INFECTIONS
Jorge Hallak, MD, PhD1; Andressa Ferrete, PhD, student2; Juliana Pariz, PhD3; Erick Silva, PhD4; Maria Christina Avellar, PhD4; Valter Cassao, MD5 and Patricia Pieri, PhD3
1Division of Urology and Reproductive Toxicology Unit, Dept of Pathology – University of Sao Paulo Medical School and Androscience, High Complexity Andrology Laboratory and Male Reproductive Health Clinic; 2University of Sao Paulo Medical School; 3Androscience: High Complexity Andrology Lab and Male Reproductive Health Clinic; 4Dept of Pharmacology Federal University of Sao Paulo; 5Division of Urology, University of Sao Paulo Medical School

61.
EVIDENCE THAT THE DNA LICENSING PROTEIN ORC2 BINDS SPERM ORIGINS AT NUCLEAR MATRIX ATTACHMENT SITES
Michael Ortega, BS, Joel Marh, BS, W. Steven Ward, PhD
Institute for Biogenesis Research

63.
POTENTIAL ROLE OF WNT SIGNALING IN MURINE SPERMATOGENESIS AND ITS MEDIATORS
Genevieve Kerr, Helen E Abud PhD, Julia C Young PhD, Katja Horvay PhD, Kate L Loveland PhD
Monash University

65.
THE AGEING MALE: THE IMPORTANCE OF ACTIVIN SIGNALING IN ADULT TESTIS GROWTH, DAILY SPERM PRODUCTION AND ENDOCRINE FUNCTION
Catherine Itman PhD1 and Kate Loveland PhD2
1University of Newcastle; 2Monash University

67.
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Gurvinder Kaur, Barrett Bowlin, Jannette Dufour

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CANNABIS SATIVA USE DECREASES TESTICULAR VOLUME, INCREASES PROLACTIN, INCREASES AROMATIZATION OF TESTOSTERONE TO ESTRADIOL AND ESTRONE AND HAS A NEGATIVE IMPACT IN SEMEN PARAMETERS AND SPERM QUALITY.
Jorge Hallak, MD, PhD1; Andressa Ferrete, PhD, student2; Juliana Pariz, PhD3; Paulo Saldiva, MD, PhD4; and Patricia Pieri, PhD3
1Division of Urology and Reproductive Toxicology Unit, Dept of Pathology – University of Sao Paulo Medical School and Androscience, High Complexity Andrology Laboratory and Male Reproductive Health Clinic; 2Division of Urology and Reproductive Toxicology Unit, Dept of Pathology – University of Sao Paulo Medical School; 3Androscience, High Complexity Andrology Laboratory and Male Reproductive Health Clinic; 4Dept of Pathology – University of Sao Paulo Medical School

71.
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Marie-Chantal Grégoire, MSc, Frédéric Leduc, PhD; Julien Massonneau, BSc; Olivier Simard, BSc; Rachade Hmamouchi, MSc; Isabelle Marois, MSc; Mélina Arguin, MSc; Martin Richter, PhD; Sébastien Rodrigue, PhD; Pierre-Étienne Jacques, PhD; Guylain Boissoneault, PhD
Université de Sherbrooke
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73. CHARACTERIZATION OF THE ROLE OF TUMOR NECROSIS FACTOR APOPTOSIS INDUCING LIGAND (TRAIL) IN SPERMATOGENESIS THROUGH THE EVALUATION OF TRAIL GENE–DEFICIENT MICE

Yichen Lin PhD, John Richburg PhD
University of Texas at Austin

75. STAGE-SPECIFIC EXPRESSION AND SUBCELLULAR LOCALIZATION OF LONG INTERSPERSED ELEMENT TYPE 1 (LINE-1) PROTEIN DURING MALE GERM CELL DEVELOPMENT

Simon Newkirk¹, Jun Yin¹, Cathryn Hogarth¹, Sandra Martin², Michael Griswold¹ and Wenfeng An¹
¹School of Molecular Biosciences and Center for Reproductive Biology, Washington State University, Pullman, WA; ²Department of Cell and Developmental Biology, University of Colorado School of Medicine, Aurora, CO

77. INDUCIBLE GENE REGULATION IN THE RAT GERMLINE

Priscilla Jaichander, PhD; Andrew E. Svyyk, PhD; Tetyana L. Svyyk, PhD; Gerardo A. Medrano, BS; Karen M. Chapman, BS; F. Kent Hamra, PhD
Department of Pharmacology, Cecil H. & Ida Green Center for Reproductive Biology Sciences, University of Texas Southwestern Medical Center, Dallas

79. PLATELET–DERIVED GROWTH FACTOR RECEPTORS: A ROLE IN NEONATAL GONOCYTE AND EMBRYONAL TERATOCARCINOMA CELL DEVELOPMENT

Gurpreet Manku¹,², Oli Sarkar¹,³, Annie Boisvert¹,³ and Martine Culty¹,⁴
¹The Research Institute of the McGill University Health Center; ²Department of Pharmacology & Therapeutics, McGill University; ³Department of Medicine, McGill University; ⁴Departments of Pharmacology & Therapeutics and Medicine, McGill University

81. THE LOCALIZATION OF RETINALDEHYDE DEHYDROGENASES IN THE POSTNATAL MURINE TESTIS

Travis Kent, Cathryn Hogarth, Ryan Evanoff, Debra Mitchell, Michael Griswold
School of Molecular Biosciences and the Center for Reproductive Biology

83. ESTABLISHMENT OF AN IN VITRO MODEL FOR FUNCTIONAL ANALYSIS OF THE SPERMATOGONIAL STEM CELLS NICHE IN SCYLIORHINUS CANICULA

Aude Gautier PhD¹,²,³, Adrien Bosseboeuf PhD student¹,²,³,⁴,⁵, Pierrick Auvray PhD⁵ and Pascal Sourdaine Professor¹,²,³
¹Normandie Univ, France; ²UCBN, BioMEA, F–14032 Caen, France; ³CNRS INEE FRE3484, F–14032 Caen, France; ⁴KELIA, France; ⁵Group CELLIS PHARMA, Parc Technopolitain Atalante Saint Malo, 35400 Saint Malo

85. OVER EXPRESSION OF THE HISTONE H3 DEMETHYLASE KDM1 IN SPERMATOGONIA ALTERS THE SPERM EPIGENOME, CAUSES ABNORMAL OFFSPRING DEVELOPMENT AND IS IMPLICATED IN TRANSGENERATIONAL EPIGENETIC INHERITANCE.

Keith Siklenka¹, Serap Erkek², Maren Godmann³, Romain Lambrot³, Christine Lafleur³, George Chountalos¹, Tamara Cohen³, Marilene Paquet⁴, Matthew Suderman⁵, Mike Hallett⁶, Antoine Peters⁶ and Sarah Kimmins¹
¹Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada; ²Friedrich Miescher Institute for Biomedical Research (FMI), CH-4058, Basel, Switzerland; ³Department of Animal Science, McGill University, Montreal, Canada; ⁴Comparative Medicine & Animal Resources Centre, McGill University, Montreal, Canada; ⁵McGill Centre for Bioinformatics, McGill University, Montreal, Canada; ⁶McGill University, Montreal, Canada.
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87. **CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF PIRNA−LIKE SMALL RNAs IN SOMATIC CELLS**
Nicole Ortogero, BS, MS¹, Grant Hennig, PhD¹, Dickson Luong, BS¹, Seungil Ro, PhD¹, Bhupal Bhetwal, PhD¹, John McCarrey, PhD² and Wei Yan, MD, PhD¹
¹University of Nevada, Reno; ²University of Texas, San Antonio

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Papia Chakraborty, PhD; Frank Buas, PhD¹; Benjamin Smith, PhD¹; Steve Eacker, PhD²; Manju Sharma, PhD¹ and Robert Braun, PhD¹
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¹University of Texas at San Antonio; ²University of Texas Health Science Center at San Antonio

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Alex Ridgeway, MS¹ and Dr. Dolores Lamb¹,²,³
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Paulo Campos-junior, MSc¹; Guilherme Costa, PhD¹; Gleide Avelar, PhD¹; Samyra Lacerda, PhD¹; Diva Guimaraes, PhD² and Luiz Franca¹
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Denise Archambeault, Penelope Bonnen¹, Anna Mielnik², Alexander Yatsenko¹,³, Dolores Lamb¹, Peter Schlegel² and Martin Matzuk¹
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Michelle Carmell, PhD¹; Kyomi Igarashi, BA¹; Dirk DeRooij, PhD¹; Yueh-Chiang, Hu, PhD¹; George Enders, PhD² and David Page, MD¹
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Monis Bilal Shamsi, MSc, Shweta Smitha Misro, MSc, Jhumur Pani, MSc, Dinesh Kumar, MBBS, Rima Dada, MD, PhD
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Introduction: Varicocele is the commonest surgically reversible cause of male infertility. Seminal oxidative stress (OS), characterized by increased reactive oxygen species (ROS) and/or reduced total antioxidant capacity (TAC) is reported to be associated with varicocele and infertility in general. A reduced testicular blood oxygenation in varicocele may enhance toxic effects of mutagenic and carcinogenic substances in tobacco smoke. The study was done to investigate the effects of smoking on seminal OS and sperm DNA quality in infertile males with varicocele.

Material and Methods: 57 infertile smokers with varicocele (Grade II or III), 34 non-smoking infertile males with varicocele (Grade II or III) and 40 non-smoker fertile controls were analyzed for ROS levels in semen (by chemiluminescence), TAC (by commercially available kit) and sperm DNA fragmentation index (DFI) (by comet assay). Twenty men from both groups of infertile patients were followed after varicocelectomy. ROS, TAC and sperm DNA damage were assessed in them at 1 and 6 months post-surgery.

Results: ROS levels and sperm DFI in infertile smokers with varicocele was significantly higher (p<0.05) as compared to non-smoking infertile males with varicocele and fertile controls. TAC levels in infertile group (smokers and non-smokers) was significantly lower (p<0.05) than controls. However, no significant difference (p>0.05) was observed in TAC levels between smoking and non-smoking infertile males. Significant decrease in ROS levels, was observed 1 month post-surgery, however improvement in sperm DNA quality was shown only after 6 months of surgery. In non-smoking infertile males the rate of decrease in ROS and sperm DFI was comparatively higher than the smoking infertile males.

Discussion: Till date it is believed that varicocele adversely affects spermatogenesis by testicular hypothermia. This study shows that one of the chief causes of sperm defects in varicocele is seminal OS and increased sperm DFI. Infertile men with varicocele exposed to cigarette smoke aggravate the already existing OS and experience higher sperm DFI. At least one full cycle of spermatogenesis (64 – 72 days) is needed before the beneficial effects of varicocelectomy are evident, therefore improvement of sperm DNA quality was observed only after 6 months of surgery. Findings of the study emphasize that in management of varicocele, cessation of smoking plays an important role in addition to surgical interventions.

14 ALTERED LONG NON-CODING RNA PROFILES IN THE TESTES OF PATIENTS WITH DIFFERENT TYPES OF NON-OBSTUCTIVE AZOOSPERMIA
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Introduction and Objectives: Long non-coding RNAs (lncRNAs) have been proposed to be key regulators of diverse biological processes. However, the expression patterns and functions of lncRNAs in human spermatogenesis remain largely unknown.

Methods: In this study, the expression profiles of lncRNAs in the testicular biopsies of patients with different types of non-obstructive azoospermia (NOA), including maturation arrest (MA) and hypospermatogenesis, were identified by lncRNA microarray. The altered lncRNA expressions including 679 down-regulated and 41 up-regulated in MA patients; 215 down-regulated and 32 up-regulated in patients with hypospermatogenesis, were detected. The down-regulation of the lncRNAs, HOTTIP and LINC00162, and the up-regulation of the lncRNA imsnRNA761, were further validated in both MA and hypospermatogenesis patients by using real-time PCR.

Results: In addition, knock-down of imsnRNA761 in NT2 cells (testicular embryonal carcinoma cell line) significantly altered mRNA expressions of several histone methyltransferases, DSB repair-related genes and cell cycle-related genes, such as EZH2, PRDM9, RAD51, etc., suggesting a role of imsnRNA761 in the regulation of cell destiny.

Conclusion: Here, we demonstrate the potential roles of lncRNAs in regulating human spermatogenesis in the first time.
**Poster Session I**

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**ROLE OF TUMOUR SUPPRESSOR NF2 ISOFORMS IN SPERM MATURATION**

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**Introduction:** Our lab studies the biology of the tumour suppressor NF2. We showed that the NF2 protein product Merlin inhibits small GTPases like Ras and Rac as part of its tumour suppressing activity.

**Methods:** In this study we concentrated on the role of the two major isoforms of Nf2, the mouse homologue of NF2. Characterisation of the expression pattern revealed expression of Nf2 in all organs tested with mostly equal expression of both isoforms. However, some organs such as the heart, muscle and liver expressed predominantly isoform II whereas the testis and spleen expressed almost exclusively isoform I. In order to further pin down specific roles of the Nf2 isoforms we analysed knockout animals specific for each isoform.

**Results:** Contrary to our expectations these animals did not develop any tumours indicating that both isoforms have tumour suppressor function. However, the isoform II animals showed defective fertility, as seen by a reduction in litter size. We could attribute this phenotype to the males and observed an increase in morphologically defective sperm in the knockouts. Interestingly this defect could also be seen in heterozygous animals, which were completely fertile. Both isoform knockouts showed sperm defects but the isoform I knockout compensated by increased (two-fold) sperm concentration. Further analysis by electron microscopy revealed numerous defective sperm heads, with either membrane disruption or small nuclei.

**Conclusion:** We therefore assume that the critical step for Nf2/Merlin activity in sperm maturation occurs in the testis during the massive morphological changes that occur on the way from round to elongated spermatids. These processes are commonly regulated by small GTPases such as Ras, Rac or Rho and therefore could be regulated by the tumour suppressor Merlin. To further address this question, we have started to analyse germ-cell specific knockouts of the whole Nf2 gene.

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**ABERRANT EPIGENETIC CHANGES IN THE ABNORMALLY EXPRESSED SEMINAL PLASMA MIRNAS (SP−MIRS) OF IDIOPATHIC NON−OBSTRUCTIVE AZOOSPERMIA**

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**Introduction:** Dysregulated expression of microRNAs (miRNAs) in various tissues has been associated with a variety of human diseases. MiRNAs have been proposed as potential biomarkers for the diagnosis of diseases such as cancer and diabetes.

**Methods:** In this study, we carried out miRNA expression profiling using seminal plasma to detect and identify characteristic patterns in non-obstructive azoospermia (NOA) and to investigate the molecular mechanisms of pathogenesis of NOA. We recruited 200 subjects and employed a strategy consisting of initial screening by TaqMan Low Density Array and further validation with TaqMan quantitative RT-PCR assay. Validation of the profiling results was conducted in two independent phases. In addition, the expression of the three validated seminal plasma miRNAs (sp-miRs; miR-141, miR-429 and miR-7-1-3p) were examined in testicular tissues of patients with NOA and of fertile controls.

**Results:** As a sensitive and specific biomarker, the profiling of the three identified sp-miRs provides a novel non-invasive, semen-based test for NOA diagnosis. The methylation status of the three sp-miRs were inversely associated with their expression patterns. In addition, we found that Rb1, Pik3r3, Cbl and Tgfβ2 were down-regulated by these sp-miRs. Given that sp-miRs show reproducible and stable expression levels, they are potentially novel noninvasive biomarkers for the diagnosis of NOA. We propose that the three sp-miRs may participate in a methylation-miRNA-gene network related to NOA development.

**Conclusion:** This work provides a foundation for interpretation of miRNA changes associated with pathogenesis of NOA and extends the current concepts of human NOA pathogenesis.
DECONTAMINATING HUMAN TESTICULAR CELLS FROM LEUKEMIC CELLS USING FLUORESCENCE-ACTIVATED CELL SORTING

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Introduction: Spermatogonial stem cells (SSCs) may have potential to treat some cases of male fertility, such as infertility that results from chemotherapy or radiation treatments for cancer or other conditions. For patients, it may be possible to cryopreserve SSCs prior to initiating gonadotoxic treatment and have those stem cells re-introduced into the testes at a later date to restore fertility. The proof-in-principle for this approach is already established in rodents, goats, sheep, dogs and monkeys. This preclinical safety and feasibility study was designed to identify cell surface antigens that could be used to isolate and enrich human SSCs and also removed occult malignant cells that might be present in the testicular tissues of cancer patients.

Methods: We used fluorescence-activated cell sorting (FACS), immunocytochemistry and a human-to-nude mouse xenotransplant assay to demonstrate that human spermatogonia express EpCAM (CD326), THY-1 (CD90) and a6-integrin (CD49f). The presence of spermatogonia in the EpCAM dim, THY-1 dim and a6-integrin+ fractions of human testis cell suspensions was confirmed by immunocytochemical staining for SALL4 and by xenotransplantation into nude mouse testes. In contrast, MOLT-4 acute lymphoblastic leukemia cells expressed the cell surface markers HLA-ABC and CD49e while TF-1a erythroleukemia cells expressed CD45 and CD49e, which were not expressed by human spermatogonia. Some leukemia cells also expressed a6-integrin and THY-1, but not EpCAM.

Results: Based on this information, we developed a combinatorial sorting strategy to separate MOLT-4 cells (EpCAM-/HLA-ABC+/CD49e+) from spermatogonia (EpCAM+/HLA-ABC-/CD49e-) in a contaminated human testis cell suspension. Human to nude mouse xenotransplantation revealed that the spermatogonial fraction was enriched 12-fold for spermatogonial stem cells and devoid of malignant contamination. This strategy was slightly modified to separate TF-1a cells (EpCAM-/CD45+/CD49e+) from spermatogonia in a contaminated human testis cell suspension.

Conclusion: Through a series of similar experiments, it may be possible to identify a broad panel of markers that can be generalized for decontamination of many cancer cell types including heterogeneous primary cancers from human testis cell suspensions.

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PROTEOMIC ANALYSIS OF SPERM ANTERIOR HEAD PLASMA MEMBRANE: UNFOLDING THE MOLECULAR COMPONENTS UNDERLYING SPERM–EGG INTERACTION
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Introduction: Capacitation is a process whereby mammalian sperm gain full ability to bind to the zona pellucida (ZP) and to fertilize the egg. This process involves reorganization of sperm plasma membrane components. The anterior sperm head plasma membrane (APM) is the site for ZP binding and APM vesicles with ZP affinity can be extracted from pig sperm by pressure at 650 psi. Characterization of APM components before and after capacitation would give a better understanding of how sperm acquire their full fertilizing ability.

Methods: Our proteomic analyses by quantitative mass spectrometry indeed revealed the presence of proteins with relevance to fertilization in both non-capacitated and capacitated APM vesicles; these include ZP binding proteins (i.e., SED1, zonadhesin, spermadhesins), cytoskeleton proteins (i.e., tubulins, actin) and chaperones (i.e., hsp 90α, chaperonin containing T-complex protein 1 (TCP-1)).

Results: However, these proteins were found with higher amounts in the capacitated APM samples. These proteomic results supported a current concept that conglomerates of ZP-binding molecules may exist in the APM as molecular complexes, as facilitated by chaperones and cytoskeleton proteins, so that they can function effectively in ZP binding and downstream signaling events. This concept was supported by the presence of high molecular weight (HMW) protein complexes (800 – 1200 kDa) in both non-capacitated and capacitated pig APM samples, as shown by blue native gel electrophoresis. However, far-western analysis revealed that only HMW protein complexes of the capacitated APM had affinity for biotinylated solubilized pig ZP. Corroborating this, proteomic analyses revealed increased levels of ZP-binding proteins (zonadhesin, SED1, SP-10) and unique presence of cytoskeleton proteins (tubulin α, β) and chaperones (TCP-1 subunits) in the capacitated HMW complexes. These results suggested that key ZP-binding molecules were brought together and coalesced into functional ZP-binding complexes on the capacitated sperm APM; similar to findings in somatic cells, the proper conformation and functioning of these APM complexes may be regulated by chaperones and cytoskeleton proteins. The HMW protein complex formation on capacitated sperm APM is likely essential for sperm-ZP interaction and possibly downstream signaling for fertilization. Therefore, a new perspective of using composition of APM protein complexes as a male fertility bio-index should be considered.

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GENE COPY NUMBER INCREASE IN THE AZOOSPERMIA FACTOR C (AZFC) REGION AND ITS EFFECT ON SPERMATOGENIC IMPAIRMENT
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Introduction: The azoospermia factor c (AZFc) region in the distal long arm of human Y chromosome features massive palindromes, and contains eight multicopy gene families which are proven or likely to encode proteins. The complete deletion of AZFc can cause spermatogenic impairment.

Methods: To determine the extent to which the individual gene underlying the phenotype of complete AZFc deletions in spermatogenesis, we conducted comprehensive molecular analysis (including Y chromosome haplogrouping, AZFc deletion typing and copy number quantifying) in 781 healthy controls and 654 idiopathic infertile men. Consistently, the b2/b3 partial deletion (b2/b3 deletion-only and deletion-duplication) was associated with spermatogenic impairment. Subsequently, we evaluated the influence of individual gene in subjects without partial AZFc deletions, which might be potential genetic modifiers in spermatogenesis.

Results: We found that the frequency of the DAZ and BPY2 duplication in the infertility were significantly higher than that in the fertility group. In combined analysis, compared with the combined pattern of all eight gene families with common level copies, both combined patterns of DAZ and BPY2 gene with abnormal level copies alone were associated with spermatogenic impairment. In addition, in Y chromosome haplogroup O1 (Y-hg-O1), the frequency of genomic duplications of all eight gene families was significantly higher in the case group than that in the control group.

Conclusion: Our findings demonstrated that the DAZ, BPY2 genes may be prominent players in spermatogenesis, while the others (excluding DAZ, BPY2) seem to modulate more subtly the program. Notably, AZFc rearrangements (especially genomic duplication) and the susceptible Y-hg (e.g Y-hg-O1) deserve comprehensive investigations to uncover spermatogenic roles of the AZFc region.
AN AGENT-BASED MODEL OF MOUSE SPERMATOGENIC CYCLES
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Introduction: The spermatogenic cycle describes the periodic development of male germ cells, which occurs every 8.6 days in the mouse. Each cycle can be divided into Stages I to XII based on well-defined associations of germ cells on seminiferous tubule cross-sections. The periodic patterning of germ cells results from multiple cellular events including differentiation, proliferation, apoptosis, and movement; disruption in these cellular events results in abnormal testicular morphology. However, the precise action of germ cells that leads to the emergence of different cellular patterns remains undefined.

Methods: We develop an agent-based model (ABM) to simulate the mouse spermatogenic cycle on a cross-section of seminiferous tubules. ABM is a suitable approach for studying dynamic systems in which individual heterogeneity and spatial interactions are important. The model depicts a tubule cross-section in a regular grid. Ten types of germ cells are included ranging from spermatogonia to spermatids. Kinetic parameters for differentiation, proliferation, apoptosis, and movement, are estimated from static and dynamic imaging and irradiation experiments.

Results: The dynamic global pattern of germ cell organization on a cross-section is achieved from the local, individual cellular behaviors. By manipulating cellular events either individually or collectively in silico, the model allows us to predict the causal events to the abnormal morphology observed in various genetic and environmental perturbations.

Conclusion: In summary, our model elaborates the temporal-spatial dynamics of germ cells, allowing us to trace individual cells as they change state and location. More importantly, the model provides a mechanistic understanding of how tissue morphology and sperm production are achieved. Our study may open new possibilities for manipulating cellular behaviors and interactions to restore the continual production of sperm.

Figure legend. A) ABM rules. B) Simulation results on a cross-section of seminiferous tubules at one time point. Germ cells of different types are color-coded.
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METABOLICOMICS REVEALS NOVEL METABOLIC PATHWAYS IN RELATION TO REPRODUCTIVE TOXICITY OF BISPHENOL A
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Introduction and Objectives: Male reproductive toxicity induced by bisphenol A (BPA) has been widely reported. Studying metabolites variation holds promise for the discovery of mechanisms linked to BPA reproductive toxic effects.

Methods: Ultra-high-performance liquid chromatography-tandem mass spectrometry was used to determine the BPA level in 325 human urine samples to validate its exposure in China. Male Sprague-Dawley rats were orally administered doses of BPA at the levels of 0, 0.0005, 50 mg/kg/d for 8 weeks. We used liquid chromatography-time-of-flight (TOF) and capillary electrophoresis (CE)-TOF metabolomics to discover, identify and analyze the variation of metabolites in testes and urine, respectively.

Results: Over 50% of urine samples contained detectable levels of BPA. In rat testes, by using multiple approaches, we identified linoleic acid (LA) and arachidonic acid (AA), two n-6 fatty acids, as potential testicular biomarkers. Decreased levels of LA, increased levels of AA and AA/LA ratio in testes were observed in exposed group. According to these suggestions, the levels of testicular antioxidant enzymes were detected. Testicular superoxide dismutase (SOD) declined significantly compared with that of the control, and the glutathione peroxidase (GSH-Px) and catalase (CAT) also showed decreasing trend in BPA treated group. In rat urine, 199 named metabolites were profiled in rat urine. Changes in metabolic pathways were observed including elevated nucleotide degradation.

Conclusions: Humans are widely exposed to BPA in China, and BPA exposure causes testicular n-6 fatty acid composition variation and decreased antioxidant capacity in rats. The metabolic profiling in urine indicates that BPA exposure caused changes in nucleotide metabolism in rats. Our results provided new insight into BPA-induced metabolic variation which may be related to its reproductive toxicity.

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IS THERE ANY EFFECT OF INSULIN RESISTANCE ON MALE REPRODUCTIVE SYSTEM?
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Objectives: To investigate the possible effect of insulin resistance (IR) on male reproductive system via evaluation of semen analysis, male sex hormones and serum lipid profiles, and testicular volume.

Methods: A total of 80 male patients who attended outpatient infertility clinic of Urology department of our University Hospital were enrolled in this prospective study. Body Mass Index, Testicular volume, the semen samples and serum hormone/Lipid profiles obtained from all subjects.

Results: The patients were divided into two groups as study and control according to the presence of IR. There were no statistical differences in terms of age, marriage period, testicular volume and BMI between the groups. There were no relationship between HOMA−IR and semen volume (r= −0.10, p=0.37), total sperm count (r=−0.09, p= 0.39), motility (r=−0.15, p=0.16) and morphology (r= −0.14, p=0.19). However, HOMA−IR was closely associated with hsCRP levels (r= 0.94, p<0.0001).

Conclusions: Despite of the documented strong inverse relationships between Diabetes Mellitus (DM) and male/female fertility, and also IR and female infertility via ovarian functions as in polycystic ovary syndrome, to our knowledge, there is no report about any influence of IR on male fertility. DM and metabolic syndrome (MetS) have a “Bad fame” on human fertility. Thus, IR automatically may be accused of causing detrimental effect on male infertility due to its hyperinsulinemic state and being one of the components for MetS. Interestingly, due to our preliminary results, we do not think any inverse direct correlation between IR and male reproductive functions.
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INVESTIGATING THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN TESTICULAR MORPHOGENESIS USING PORCINE DE NOVO FORMED TESTICULAR TISSUE.
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UCVM

Introduction and Objectives: Vascular Endothelial Growth Factor (VEGF) is known for its angiogenic function. In the testis, where no active angiogenesis occurs after birth, different isoforms are present. This cytokine is synthesized by cells that constitute the spermatogonial stem cell (SSC) niche. De novo formation of testis tissue from isolated testis cells is a tool for the study of morphogenesis, but spermatogenic efficiency in this model is currently lower than in vivo. The objective of this study was to examine two questions, first: Does VEGF supplementation improve vasculogenesis and the number of seminiferous tubules formed in the de novo morphogenesis bioassay, and second: Does VEGF enhance the number of tubules containing spermatogonia?

Methods: Testicular cells from one week old piglets were harvested by a two-step enzymatic digestion, and divided into two groups: Treated: 10x10^6 cells mixed in 100μl Matrigel reduced growth factors (MRGF) with 100ng/ml of VEGF165 and Control: 10x10^6 cells mixed in 100μl MRGF. Four cell pellets per animal (3 treated, 1 control) were grafted under the skin of 4 castrated immunocompromised mice each with grafts randomly assigned to injection sites (n=3 replicates). De novo formed tissue was recovered 12 weeks after transplantation; 35 out of 36 grafts were recovered from the treated group and 12 out of 12 from the control. Grafts were analyzed by dual immunohistochemistry for the detection of UCHL−1+ cells (spermatogonia) and smooth muscle actin + cells (blood vessels and the basement membrane of seminiferous tubules). Student t−test with p<0.05 was used to test for statistically significant differences between groups.

Results: The mean number of blood vessels or seminiferous tubules formed per graft did not differ between treated and control groups: 50.0±21.4 vs 49.8±18.7 and 78.2±56.4 vs 100.8±68.7, respectively. However, the percentage of seminiferous tubules containing spermatogonia was higher in the treated than in the control grafts, 18.6±12.5% vs 11.3±10.0% (p<0.005).

Conclusion: Contrary to what was expected, the addition of VEGF did not affect blood vessel or tubule formation. However, the increase of tubules containing spermatogonia could indicate an improvement in cell signaling which may guide spermatogonia to their niche in the newly formed seminiferous tubules. The addition of VEGF to cell pellets prior to grafting can enhance spermatogenic efficiency in testis tissue formed de novo from isolated testis cells.

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ROLE FOR CHROMATIN MODIFYING PROTEIN SMC6 IN PERICENTROMERIC HETEROCHROMATIN DOMAINS DURING SPERMATOGONIAL DIFFERENTIATION AND MEIOSIS
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Introduction: Spermatogonial differentiation, meiosis and subsequent spermiogenesis are characterized by the continuously ongoing, rapid and profound changes in composition and function of chromatin, the supra-molecular complex that packages, shapes and regulates the genome. Because proper chromatin architecture and dynamics also safeguard genomic stability, changes in the spatio-temporal organization of chromatin can lead to chromosomal aberrations or aneuploidies, initiate germ cell apoptosis or arrest, or, in the worst case, lead to congenital malformations in the offspring.

Methods: Chromatin structure and function are for a large part determined by the six members of the structural maintenance of chromosomes (SMC) protein family, which form three heterodimeric complexes: Smc1/3 (cohesin), Smc2/4 (condensin) and Smc5/6. Each complex plays distinct and important roles in chromatin dynamics, gene expression and differentiation. Of these, Smc5/6 is known to exhibit several diverse functions in maintenance of genomic integrity; including recombinational repair of DNA double-strand breaks, restarting collapsed replication forks and, interestingly, prevention of recombination in repetitive sequences like rDNA and pericentromeric heterochromatin.

Results: We find that, in mice, the Smc6 protein is absent from A-single, paired and aligned spermatogonia and is first expressed in differentiating spermatogonia that are irreversibly committed towards meiosis. Furthermore, Smc6 is strongly expressed during the first meiotic prophase and the two subsequent meiotic divisions, after which it gradually decreases in developing round spermatids. We observe that Smc6 functions as part of pericentromeric heterochromatin domains that already start to take shape during spermatogonial differentiation. During the first meiotic prophase these domains are thought to be required to prevent aberrant recombination events within and between pericentromeric regions.

Conclusion: We propose that Smc6 plays a key role in this process and thus prevents the accumulation of branched chromosome structures that would otherwise cause meiotic arrest and, consequently, male infertility.
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LACKING OF SEIPIN IN TESTIS AFFECTS THE LIPID METABOLISM IN SPERMATIDS AND LEADS TO DEFECT OF SPERMATOGENESIS
Min Jiang

Introduction: Seipin, the human Berardinelli-Seip congenital lipodystrophy 2 gene products has been associated with adipocyte differentiation, lipid droplet (LD) formation, and motor neuron development. It has high levels of expression in the testis, brain, and adipose tissues, while the role of seipin in testis is still unknown. In previous study, we find seipin-null mice with complete loss of adipose tissue, insulin resistance and absolutely male infertility.

Methods: Here, we used seipin-null mice (shorted for s-ko), seipin-germ cell-conditional knockout mice (shorted for s-gko) to study the role of seipin in testis spermatogenesis. Additionally, we used seipin-adipocyte cell-conditional knockout mice (s-ako) to study the effect of adipose tissue on testis. We find s-ako males with normal fertility and testis histology, while s-ko and s-gko males were infertility with normal serum hormone levels (FSH=LH=T=E2). The infertility due to lipid droplets accumulation in the round spermatids and elongated spermatids during spermiogenesis and affect the dynamic morphologic changes, especially nuclear condensation and elongation and intercellular bridges integrity with presence of multinucleated elongating spermatids and spermatids mass. At last the disorders led to a reduction in sperm counts and motility, high rate of abnormal sperm in cauda epididymis and infertility in the end.

Results: By the quantitative proteomics, we found that the lacking of seipin in testes lead to the lower expression of protein hormone−sensitive lipase(HSL/LIPE), which leading to the less hydrolysis of TAG or cholesterol ester and less free fatty acids in spermatids .The other proteins participated in fatty acid β-oxidation expressed highly as the feedback regulation ,and proteins which regulate the immune response, apoptosis and cell death expressed highly while proteins participate in spermatogenesis expressed lower than controls as the consequence of the defects.

Conclusion: In summary, seipin maintained the lipid metabolism in testis which maybe mediated though HSL and there were ectopic lipid droplets formation in spermatids when lacking seipin in testis. Meantime, the fertility of s-ako males suggested us that spermatogenesis didn’t need the white adipose tissue in mice.

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SERTOLI CELLS SURVIVE XENOTRANSPLANTATION BY MODULATING THE MACROPHAGES AT THE GRAFT SITE TO BE REGULATORY VERSUS CYTOTOXIC
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Introduction: Sertoli cells (SC) are immune-privileged cells that survive xenotransplantation long-term (>90 days) without any immunosuppression. Therefore, SC can be utilized to identify mechanisms of xenograft survival. In this study, SC and islets were used as a model to compare the immune response to immune-privileged and non-immune privileged cells, respectively.

Methods: Eleven million neonatal pig Sertoli cells (NPSC) or islets (NPI) were transplanted underneath the kidney capsule of Lewis rats, and graft bearing kidneys (n≥3) were collected at various time-points between days 1 – 20 post-transplantation. NPI were completely rejected within 6 days, whereas the NPSC survived throughout the study, as assessed by PCR for pig specific cytochrome oxidase II and immunohistochemistry (IHC) for GATA-4 as a marker for SC, and insulin for islets. The immune cell infiltration was compared between NPI and NPSC xenografts using IHC for presence of macrophages, T-cells, NK cells, granulocytes, and CD8 positive cells using cell specific markers. Results indicate that macrophages are the major immune cell type infiltrating the grafts, with delayed infiltration at the NPSC graft site. Fewer CD8 positive cells were present in the NPSC grafts compared to NPI grafts. Interestingly, the CD8 positive cells looked morphologically different than T-cells, and localized in the same area as macrophages. Double-immunostaining for CD8 and ED1 (Macrophage/monocyte marker) demonstrated CD8 positive macrophages were present in both the grafts, with fewer CD8 positive macrophages in the NPSC grafts than NPI grafts. The CD8 positive macrophages have a cytotoxic phenotype; and secrete TNFα, which can induce apoptosis in other cells. Therefore, the grafts were analyzed for apoptotic cells using TUNEL assay, consistent with the rejection of NPI, significantly more apoptotic cells were observed in the NPI grafts compared to NPSC grafts at days 4, 6 and 13 post-transplantation.

Results: The grafts were assessed for production of cytokines by IHC. Slight decrease in TNFα production at early time-points was observed in the NPSC grafts compared to NPI grafts. However, when compared to NPI grafts, the NPSC grafts showed an increased production of factors associated with regulatory macrophages, IL-10 and p-SMAD2 (marker for activated TGF-β).

Conclusion: Together these data suggest that the NPSC survive xenotransplantation by modulating the macrophages at the graft site to be regulatory versus cytotoxic.
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MICRORNAS: NOVEL ANDROGEN RESPONSIVE TRANSCONTING FACTORS IN THE TESTIS
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Introduction: Even after decades of research, the molecular basis for why spermatogenesis requires testosterone is not completely understood. This is in part because very few androgen-regulated genes have been identified so far. It is likely that many androgen-regulated genes in the testis don’t have typical androgen response elements, as they are not direct targets of AR, but instead respond indirectly to androgen via activation or suppression by other factors directly regulated by androgen. Recently, we have shown that one class of such androgen-regulated trans-acting factors in the testis is microRNAs (miR) that regulate the translation of target transcripts by binding to complementary sites in the mRNA. We have demonstrated that the expression of several of these miRs including miR-471 is regulated directly by androgen in the Sertoli cells. Using androgen suppression mouse models, we have shown that a number of miRs are up-regulated and their gene targets are down-regulated in the absence of androgen. Our long term goal is to understand the in vivo function and mechanism by which these androgen-responsive miRs regulate critical pathways essential for male fertility.

Methods: We generated transgenic mice expressing miR-471 driven by the Sertoli cell-specific promoter Rhox5Pp. We performed fertility, histological, germ cell apoptosis and phagocytosis assays to characterize reproducive phenotype of miR-471 mice.

Results: Our analyses of miR-471 transgenic mice reveal that Sertoli cell-specific overexpression of miR-471 resulted in severely compromised fertility due to increased germ cell apoptosis, defective Sertoli cell phagocytosis, multinucleated giant cells suggesting abortive meiosis in spermatocytes and extensive germ cell sloughing, a phenotype that correlates with impaired Sertoli cell-Sertoli cell/germ cell adhesion at the blood-test barrier. As several of the androgen responsive miRs are localized on the X-chromosome in a cluster, we believe that coordinated action of these miRs with each regulating discrete androgen-dependent steps is critical for proper germ cell development and differentiation.

Conclusions: Our study underscores the importance of a new class of regulatory molecules in testicular physiology. This is highly significant as miR-471 transgenic mice is the first mouse model to study the function of specific miRs in the Sertoli cells and miR-471 is first to be shown to be important for male fertility.

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NEUTROPHIL AND MACROPHAGE INFILTRATION PRECEDES TESTICULAR GERM CELL APOPTOSIS AFTER MONO-(2-ETHYLHEXYL) PHTHALATE EXPOSURE IN AN AGE- AND SPECIES-DEPENDENT MANNER
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Introduction: The testis is an immune privileged organ, however infectious and non-infectious inflammation of the testis has been shown to cause infertility. Phthalates are chemical plasticizers that are known Sertoli cell toxicants. Prepubertal rats exposed to a single high dose of Mono-(2-ethylhexyl) phthalate (MEHP) induces disruption of cytoskeleton and tight junctions in Sertoli cells followed by FasL-triggered apoptosis and/or detachment of germ cells. Despite that apoptosis itself does not instigate an inflammatory response, exposure to MEHP causes an increase in two pro-inflammatory cytokines; Tumor Necrosis Factor-alpha and Fas ligand.

Methods: Here we characterize the infiltration of immune cells into the MEHP-injured testis of Fisher F344 rats of varying ages (21, 28, 35 days old). Rats received a single high dose of MEHP (1g/kg, oral gavage) or vehicle (corn oil). Testes were collected at 12, 24, and 48 hours (h) after treatment. Interstitial cells were isolated from one testis, probed with antibodies against CD11b (neutrophil, macrophages, and dendritic cells), CD4 (T helper cells), and CD8 (cytotoxic T cells), and quantified by flow cytometry.

Results: At all time points and ages there were no differences in T cell populations. Pre- and peri-pubertal aged rats (21d and 28d, respectively) had a peak increase in the number of CD11b+ cells at 12h after treatment compared to age matched controls. In the 28d rats, CD11b+ cells began to decline by 24h, though still remained significantly elevated at 48h compared to controls. However, in 21d rats, CD11b+ cells quickly returned to control levels by 24h. The peak number of CD11b+ cells in 35d (pubertal) rats was delayed until 24h and remained significantly elevated at 48h; demonstrating differential responses of the testicular immune system at different stages of development. Histology and immunohistochemistry of rat testis confirmed the increase of neutrophils and macrophages (ED1+ cells), respectively. Interestingly, MEHP treated C57BL/6 mice showed no increase in CD11b+ cells at all ages (21d & 28d) and time points (12h, 24h, 48h). TUNEL staining in 28d rats showed a peak germ cell apoptosis at 24h (MEHP 98% ± 1.15 v Control 0.9% ±0.2 positive tubules, P<0.05). Taken together, these results suggest that inflammation peaks prior to apoptosis signifying a functional role of neutrophils and macrophages in the mechanism of MEHP-induced germ cell apoptosis in rats.
IDENTIFYING RA–RESPONSIVE TRANSCRIPTS IN SERTOLI CELLS DURING SPERMATOGONIAL DIFFERENTIATION, DIVISION, AND THE ONSET OF MEIOSIS
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Introduction: Spermatogenesis is the tightly regulated process in which spermatogonial stem cells differentiate to form mature spermatozoa. The active metabolite of vitamin A, retinoic acid (RA) is necessary for the maturation of spermatogonia and the proper entry of germ cells into meiosis during spermatogenesis. However, not all the genes that are RA-responsive during these processes have been identified.

Methods: Recent evidence indicates that RA activity within Sertoli cells is critical for the initial differentiation of spermatogonia and so to identify the RA-regulated transcriptome in Sertoli cells, we utilized RiboTag-floxed/Amh-cre animals to isolate Sertoli cell-specific ribosome-associated mRNA transcripts. To enrich for transcripts expressed during the initial differentiation of spermatogonia, neonatal animals were treated with a combination of the bis-(dichloroacetyl)-diamine (BDAD), WIN 18,446, which is known to block spermatogonial differentiation, and then injected with RA, thus synchronizing the first wave of spermatogenesis.

Results: Using microarray analysis, we identified 28 transcripts that are enriched in Sertoli cells and increased in expression at both 24 hours post-RA injection and 8 days post-injection, the two time points when spermatogonial differentiation takes place during WIN 18,446/RA generated synchrony. Functional annotation clustering revealed clusters associated with phosphate metabolic process (4 transcripts), golgi apparatus (4 transcripts), programmed cell death (3 transcripts), membrane (13 transcripts), and glycoprotein (10 transcripts). Interestingly, Rdh10, a retinoid metabolizing enzyme recently shown to be necessary for the normal progression of spermatogenesis, was one of the 28 candidate transcripts and will be the focus of future studies. These data provide a novel method of identifying actively translated transcripts in Sertoli cells during spermatogonial differentiation through the onset of meiosis.

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EVIDENCE THAT THE HOMEOBOX TRANSCRIPTION FACTOR, RHOX10, PROMOTES SPERMATOGONIAL STEM CELL SELF-RENEWAL AND MEIOTIC PROGRESSION
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Introduction: Homeobox genes encode well-studied transcription factors that regulate developmental events during embryogenesis, but whose functions in postnatal and adult development, including spermatogenesis, remain largely unknown. The X-linked reproductive homeobox (Rhox) gene cluster encodes homeobox genes that are good candidates to have roles in spermatogenesis. Most Rhox genes are selectively expressed in the reproductive tract and all are expressed in the testis. However, the only Rhox cluster gene with a known role in male reproduction is Rhox5, a Sertoli cell-expressed gene that we previously demonstrated promotes male germ cell survival and sperm motility. Here, we report the effect of targeted mutation of the Rhox10 gene, which we chose to mutate because it is expressed in germ cells very early in spermatogenesis.

Objectives: To determine whether Rhox10 has functions in spermatogenesis.

Methods: We characterized RHOX10 expression by quantitative-RT-PCR and immunohistological analyses. We generated Rhox10-null mice by gene targeting in ES cells and analyzed their phenotype with a variety of approaches.

Results: Using a RHOX10-specific antiserum that we generated, we found that RHOX10 is selectively expressed in gonocytes, spermatogonia, and early spermatocytes in mice. Analysis of Rhox10-null mice revealed that loss of Rhox10 elicits complex spermatogenic defects that worsened with age. During the first wave of spermatogenesis, Rhox10-null mice exhibited perturbed meiotic progression but had sufficiently normal spermatogenesis to generate nearly normal numbers of sperm. Subsequently, Rhox10-null mice exhibited progressively worse seminiferous tubule morphology, with many tubules either devoid of germ cells or only containing late-stage germ cells. This occurred by virtue of both reduced cell survival and reduced proliferation. By 5 months of age, Rhox10-null mice had testes ~30% of control size and sperm counts that were ~0.2% of controls. This progressive loss of germ cells is consistent with impaired spermatogonial stem cell self-renewal, but studies are ongoing to directly determine the biological basis for it.

Conclusions: Loss of Rhox10 causes defects in spermatocytes and spermatogonia during the first and subsequent waves of spermatogenesis, respectively. Given that Rhox10 encodes a transcription factor, we posit that it regulates transcriptional programs that drive these germ cell events.
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HUMAN SPERMATOGENIC FAILURE PURGES DELETERIOUS MUTATION LOAD FROM THE AUTOSOMES AND BOTH SEX CHROMOSOMES, INCLUDING THE GENE DMRT1
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Introduction and Objectives: Known genetic causes of non-obstructive azoospermia (NOA) include deletions in the azoospermia factor (AZF) regions of the Y chromosome, Klinefelter’s syndrome, and other cytogenetically visible chromosomal anomalies. Beyond these well-established causes, the genetic architecture of spermatogenic impairment is currently unknown. In this study, we test the hypothesis that additional “AZF-like” loci exist in the genome, either on the Y chromosome or elsewhere, and that, much like recent experience in the analysis of developmental disorders of childhood, causal submicroscopic deletions and duplications can be revealed in idiopathic cases by the appropriate use of genomic technology.

Methods: We used oligonucleotide arrays to measure single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) in 1302 men with idiopathic spermatogenic impairment and over 2870 controls. We performed statistical analyses on these data to search the genome for recurrent CNVs causing azoospermia, and to test for a global role of CNVs in spermatogenic failure.

Results: We estimate that each rare autosomal deletion detected in our study multiplicatively changes a man’s risk of disease by 6% (odds ratio (OR) 1.06 [1.03–1.1], p < 7 x 10−4), rare X-linked CNVs by 18% (OR 1.18 [1.09–1.27], p < 3 x 10−5) and rare Y-linked duplications by 40% (OR 1.40 [1.22–1.61], p < 2 x 10−6). We identified five patients with deletions of doublesex and mab-3 related transcription factor 1 (DMRT1), a gene on chromosome 9p24.3 that is orthologous to the putative sex determination locus of the avian ZW chromosome system. We found no such deletions in our 2870 controls or in 4519 controls from public databases.

Conclusions: The combined results indicate that DMRT1 loss-of-function mutations are a risk factor and potential genetic cause of human spermatogenic failure (frequency of 0.38% in 1302 cases and 0% in 7754 controls, p < 7 x 10−5). Our study also (1) shows that spermatogenic output is modulated by variation across the genome; (2) shows that some Y chromosome duplications apparently impair sperm production; (3) the CNV burden detectable in cases with spermatogenic failure is distinct from the burden reported in neuropsychiatric diseases such as autism and schizophrenia; (4) identifies other recurrent CNVs as potential causes of idiopathic azoospermia and generates hypotheses for directing future studies on the genetic basis of male infertility and IVF outcomes.

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EFFECTS OF FADD PHOSPHORYLATION ON MEIOSIS IN MICE SPERMATOGENESIS
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Introduction: FADD (FAS-associated death domain) is the key adaptor protein transmitting apoptotic signals mediated by the main death receptors (DRs). Besides being an essential instrument in cell death, FADD is also implicated in proliferation, cell cycle progression, tumor development, inflammation, innate immunity, and autophagy. The current study shows here FADD for the first time is probably associated with spermatogenesis of mice.

Methods: In order to study this specific role of FADD, we choose FADD−/− tgD mice (mice bearing the substitution of serine191 to aspartic acid) and the FADD +/− tgD mice (control) to carry out the experiments. The studies indicating that the FADD−/− tgD male mice are infertile completely and exhibit severe deficiencies in spermatogenesis.

Results: The convoluted seminiferous tubules of FADD−/− tgD mouse testis are obvious small. The lumen cell layers were decreased, and in which many of cells are developmental arrest. We further observed the markers in different periods of meiosis to study the process of meiosis. Compared to the control, pachytene spermatocyte autosome of FADD−/− tgD mice testis showed some continuing DNA double-strand break, and the expression of homologous recombination marker(mismatch repair protein MLH1) was decreased. In addition, our studies also indicated that FADD could interact with MLH1 and FADD phosphorylation affects the nuclear import of MLH1. Similar results were obtained in GC-2spdt(ts) cell lines.

Conclusion: Taken together, our results revealed a novel non-apoptosis function of FADD: regulation of spermatogenesis.
FETAL CHEMOTHERAPY EXPOSURE INDUCES TESTICULAR CANCER
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Introduction: Exposure of the primordial germ cells (PGCs) to environmental factors during fetal development has been cited as the cause of a 3-fold increase in testicular cancer in humans over the past 60 years. We recently reported (Shetty et al, PLoS One, 7:e32064, 2012) that treatment of pregnant mice from a strain genetically predisposed to testicular cancer (129.MOLF-Chr19 congenics (L1)) with an antiandrogen (flutamide) or an estrogen (DES) produced no increases in testis cancer incidence, but 2 doses of 0.8-Gy radiation increased the incidence of TGCT dramatically from 40% to 100% in the male offspring.

Methods: In the current study, pregnant L1 and 129 mice were treated with another DNA damaging agent, an alkylating chemical cyclophosphamide (CP), given as 2 injections of 7.5 mg/Kg, on days 10.5 and 11.5 of pregnancy. As expected CP caused some embryo mortality as only 20% of the vaginal plug positive mice produced progeny, compared to 42% in the untreated mice and also majority of the in utero exposed mice showed minor teratogenic effects. Interestingly, CP also increased the TGCT incidence in the male offspring to 25% in 129 and to 100% L1 mice, from the respective control values of 4% and 40%, implying that DNA damage is a common mechanism leading to testicular cancer induction. Further, although spermatogenesis was qualitatively normal in most tubules of CP-exposed offspring, testes weights were reduced to 70% of control with sporadically observed atrophic tubules, demonstrating some damage to spermatogenesis.

Results: The sensitivity to cancer induction by DNA-damaging agent at the critical stage, when the PGCs are being transformed from potentially pluripotent cells to cells committed to the spermatogenic lineage coincides with a period of global DNA demethylation and chromatin remodeling. We hypothesize that the DNA damage itself or the signaling pathways it activates interfere with these chromatin changes. Preliminary data showed that the germ cells in the irradiated testis stained more strongly for 5-methyl cytosine at E13.5 and 14.5 than did those of controls, suggesting the damage interferes with the global DNA demethylation.

Conclusion: This is the first proof of induction of testicular cancer by an external chemical agent and suggests that the male fetus of women exposed to DNA damaging agents during pregnancy might have an increased risk of developing testicular cancer, in addition to their likely having reduced spermatogenesis.

CHANGES IN STIMULATION BY GLIAL CELL LINE–DERIVED NEUROTROPHIC FACTOR (GDNF) HAVE RAPID EFFECTS ON THE REPLICATION OF STEM AND OTHER UNDIFFERENTIATED SPERMATOGONIA WITHIN A MATURE SEMINIFEROUS EPITHELIUM AND ON THE EXPRESSION BY THESE CELLS OF GFRα1
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Introduction: We have previously hypothesized that altered synthesis of GDNF in vivo by Sertoli cells is responsible for stage-specific changes in the replication and differentiation of spermatogonial stem cells. This predicts that experimentally altering GDNF stimulus to stem and other undifferentiated spermatogonia would affect their replication.

Methods: To test this prediction mature mouse seminiferous tubules were cultured for 1 to 3 days in the presence or absence of 250 pg/ml GDNF. During the last 24 hours replicating cells were metabolically labeled with 5-ethynyl-2'-deoxyuridine (EdU). Cells expressing the marker of stem and other undifferentiated spermatogonia, GFRα1, were then identified by immunocytochemistry. GFRα1+ A single, A paired and A aligned spermatogonia were enumerated as were EdU+ cells.

Results: Results demonstrated that after 3 days, the fraction of GFRα1+ cells that replicated was 2.24-fold higher with addition of GDNF. To test if GDNF also had such a rapid effect in vivo, we inhibited GDNF signaling for 3 days by use of a chemical-genetic approach. Twenty-four hours prior to tissue collection, the mature mice were injected with EdU (25 mg/kg body weight). Results showed that inhibition of GDNF singling reduced the fraction of GFRα1+ spermatogonia that replicated to 14% of controls. Additionally, confocal microscopy and image analysis demonstrated that this inhibition reduced GFRα1 expression per cell to 54% of controls. Numbers of GFRα1+ cells were also reduced. Cell loss could not be attributed to apoptosis, as there was no increase in numbers stem and other undifferentiated spermatogonia that expressed activated caspase 3. To determine if GFRα1+ cells were lost to differentiation, we initially stained tubules for the marker of spermatogonial stem cells, ZBTB16, and a marker for differentiated spermatogonia, ckit (ACK2 antibody). However, confocal microscopic analysis demonstrated that while no A single or A paired cells expressed ckit, 82% of the A aligned spermatogonia expressed both markers.

Conclusion: In summary, in the mature seminiferous epithelium, changes in GDNF signaling have rapid effects on the replication of stem and other undifferentiated spermatogonia and on their expression of GFRα1. These effects are consistent with the hypothesis that physiological changes in the expression of GDNF affect the replication and differentiation of spermatogonial stem cells in vivo.

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**PARTIAL OVERLAP OF SALL4 AND PLZF BINDING SITES IN SPERMATOGONIAL STEM CELLS REVEALS PUTATIVE shared AND DISTINCT FUNCTIONAL ROLES.**

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**Introduction:** Male fertility is dependent upon activity of spermatogonial stem cells (SSCs), which balance self-renewal and differentiation fate decisions to maintain spermatogenesis throughout adulthood. The molecular mechanisms that drive fate determination in SSCs, however, remain poorly understood. The transcription factors Sal-like 4 (SALL4) and zinc finger and BTB domain containing 16 (Zbtb16, aka: PLZF) are expressed by SSCs and may play essential roles in their self-renewal and differentiation. Recent evidence suggests SALL4 and PLZF physically interact, which may influence their activity in SSCs. Yet, few genomic targets of SALL4 and PLZF in SSC are known.

**Methods:** Thus, to begin elucidating the roles of SALL4 and PLZF in SSC fate determination, we defined the cistromes for each transcription factor by chromatin immunoprecipitation and high-throughput Illumina sequencing (ChIP-Seq). For this purpose, we derived three independent cultures of Thy-1+ spermatogonia, which contain SSCs, from 8 day-old DBA2 mouse pups, and used chromatin from each culture for ChIP-Seq.

**Results:** Transplantation to testes of W/Wv mice confirmed these cultures contained SSCs. ChIP-Seq identified 4,650 PLZF binding sites and 3,153 SALL4 binding sites found in all three samples. Of all the PLZF and SALL4 binding sites, 1372 (29.5% and 43.5%, respectively) were bound by both transcription factors. Gene ontology (GO) analysis of genes bound by SALL4 and/or PLZF using Ingenuity Pathway Analysis identified biological processes and pathways that were overrepresented in the cistromes. In particular, we found that PLZF bound an abundance of genes involved in protein ubiquination pathway, including some known to be restricted to undifferentiated spermatogonia. Protein kinase A signaling was identified as the top canonical pathway among genes bound by SALL4 alone and those bound by both SALL4 and PLZF.

**Conclusion:** These studies are the first to identify the complete regulatory repertoire of SALL4 and PLZF in SSCs and will help define the molecular mechanisms behind fate determination in these adult stem cells.

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**MIWI2 IS DISPENSABLE FOR POSTNATAL MALE GERM CELL DEVELOPMENT**

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**Introduction:** Murine male primordial germ cells undergo reprogramming characterized by genome-wide demethylation (E10.5 – 13.5) followed by remethylation (E14.5 – E16.5) during embryonic testicular development. Numerous transposable elements (TEs) can be transiently de-suppressed due to global demethylation, but are quickly re-silenced through the global remethylation. The piRNA pathway appears to be essential for TE remethylation because a global inactivation of Miwi2 leads to persistent TE expression, massive DNA damage and arrested spermatogenesis at the early meiotic phase.

**Objectives:** Given that the global remethylation occurs only between E14.5 – E16.5, it remains unknown whether the function Miwi2 is exclusively confined to this narrow time window, or Miwi2 may have other roles in male germ line development beyond the remethylation period because Miwi2 expression persists in prospermatogonia in postnatal testes.

**Methods:** To explore the potential post-remethylation role of Miwi2, we generated a Miwi2 flox mouse line and crossed the line with three male germline-specific Cre mouse lines (DDX4-Cre, Stra8-Cre and Prm-Cre) to inactivate Miwi2 in primordial germ cells (Ddx4-Cre with the Cre expression at E15.5), in prospermatogonia (Stra8-Cre with Cre expression at P3) and in elongating spermatids (Prm-Cre). We also crossed the Miwi2 floxed line with the EIIα-Cre line to obtain global MIWI2 knockout mice.

**Results:** All three male germ cell–specific Miwi2 conditional knockout mice exhibit normal growth. Testes of DDX4-Cre; MIWI2fl/Δ and EIIα-Cre; MIWI2fl/Δ mice displayed a phenotype similar to that in Miwi2 global knockouts (Miwi2−/−): spermatogenic arrest at the early zygotene stage. In contrast, no spermatogenic disruptions were observed in either Stra8-Cre; MIWI2fl/Δ or Prm-Cre; MIWI2fl/Δ mice.

**Conclusions:** Miwi2 is required for primordial germ cell development in fetal testes, but is dispensable for postnatal male germ cell development.
Introduction: MicroRNAs (miRNAs) are small, non-coding RNAs known to regulate gene expression at the post-transcriptional level, and potentially at the transcriptional level as well. Studies with gene knockouts have revealed that miRNAs are critically required for normal spermatogenesis and male fertility in mammals. In a previous collaborative study, we found that ~20% of testicular miRNAs map to the X-chromosome and that ~40% of these display testis-specific or testis-preferential expression. Surprisingly, real-time qPCR analysis further revealed an increase in the expression of a majority of the X-linked miRNA transcripts in spermatocytes, at a time when all 364 X-linked messenger RNA-encoding genes studied to date decline in expression due to Meiotic Sex Chromosome Inactivation (MSCI). We hypothesized that elevated levels of X-linked miRNA transcripts in primary spermatocytes are due to ongoing, active transcription of these genes, indicating escape of these X-linked miRNA genes from the repressive effects of MSCI.

Methods: To test this hypothesis, we have used immunofluorescence staining and RNA-Fluorescence In-Situ Hybridization (RNA-FISH) on spermatogenic cells from testes of adult mice.

Results: Our data confirm that while many autosomal mRNA genes remain transcriptionally active during meiosis, X-linked mRNA genes are consistently repressed by MSCI and many continue to be repressed by Post Meiotic Sex Chromatin (PMSC) in spermatids. Further, our data directly confirm three classes of X-linked miRNA genes – Type I which are subject to both MSCI and PMSC, Type II which escape MSCI but not PMSC, and Type III which escape both MSCI and PMSC (Fig. 1A,B). Lastly, preliminary three-dimensional analyses obtained by reconstructing confocal images of spermatocyte nuclei indicate that the type II and III X-linked miRNA genes that escape MSCI undergo de novo transcription only at the periphery of the heterochromatic XY body (Fig. 1C).

Conclusion: Further studies are underway to characterize the molecular mechanisms involved in escape of X-linked miRNA genes from MSCI or PMSC.

Figure 1. Visualizing de novo transcription during spermatogenesis. Immunofluorescence staining for gamma-H2AX (red) was used in combination with DAPI staining for cell nuclei (blue) to stage cells throughout spermatogenesis. Additionally, RNA-Fluorescence in situ hybridization for X-linked miRNAs (green) was performed to detect sites of ongoing transcription. (A) X-linked miRNA gene escaping MSCI in pachytene spermatocyte. (B) Expression of X-linked miRNA gene in round spermatids. (C) 3D reconstruction of confocal images of spermatocyte nuclei shows de novo transcription occurring at periphery of repressed chromatin. Scale bars: 30 μm (A,B) or 5 μm (C).
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ROLE OF UBIQUITIN LIGASE HUWE1 IN MODULATING SPERMATOGONIAL DEVELOPMENT IN THE TESTIS
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Introduction and Objectives: The ubiquitin (Ub) system plays important roles in spermatogenesis, examples being meiotic sex chromosome inactivation and the replacement of histones by protamines, but the precise mechanisms remain unclear. We previously identified and purified Huwe1 from the testis as an E3 Ub ligase that can polyubiquitinate histones. It has also been shown to be essential for normal differentiation of the cerebral cortex and cerebellum. Our objectives are to explore the roles of Huwe1 in ubiquitination/degradation of histones and/or germ cell development in the testis.

Methods: Germ cell specific knockout of this X-linked gene was obtained by mating transgenic male mice expressing Cre recombinase under the control of Ddx4 promoter with female mice carrying a floxed Huwe1 allele. Fertility of Cre-(WT) and Cre+(KO) mice was tested by mating them with normal CD1 females. Immunohistochemistry was used to assess changes in Ddx4 (germ cell marker), Ki67 (marker of proliferation), Stra8 (marker of spermatogonial differentiation) and TUNEL staining (marker of apoptosis). Q-PCR was used to look at various markers of self-renewal and differentiation of spermatogonia.

Results: The adult KO males were infertile with significantly smaller testes than the WT, abnormal testicular morphology with few Sertoli cells arranged haphazardly, increased Leydig cell density and barely any germ cells. At 6dpp, tubular morphology in the KO appeared normal and Ki-67 staining showed the presence of proliferating spermatogonia. However, there was significant down-regulation of self-renewal and differentiation markers such as Pou5f1/Ngn3 and Stra8/c-Kit respectively. The number of cells expressing Ddx4, a germ cell marker was reduced at 6dpp. There was no difference in apoptosis at the time point. At 15dpp, the KO testes showed absence of pachytene spermatocytes, no proliferation and loss of Stra8 expression.

Conclusions: Huwe1 inactivation early in the germ line led to two distinct anomalies in spermatogenesis. One, the developmental arrest of spermatogonia in the first wave and the other, depletion of the stem cell population that supports spermatogenesis during subsequent waves. Inactivation of Huwe1 at a later stage of spermatogenesis will be required to test its potential role in histone degradation.

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TDP-43 IS ESSENTIAL FOR SPERMATOGENESIS
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Introduction: TDP-43 (TAR DNA Binding Protein of 43 kDa) is an evolutionarily conserved ubiquitously expressed DNA/RNA binding protein. It was first identified as a transcriptional repressor of HIV1 gene transcription and subsequently shown to be a regulator of RNA splicing.

Methods: We cloned TDP-43 as a transcription factor binding to the promoter of the testis-specific acrv1 gene. TDP-43 is abundantly expressed in spermatocytes and round spermatids of the mouse and human testis. Using cell culture, biochemical, and in vivo methods including promoter mutation and occupancy studies we had previously shown that TDP-43 regulates round spermatid-specific transcription of the acrv1 gene. Based on data from the above candidate gene approach, we predicted that TDP-43 plays a global role in spermatogenesis. To test, we have generated mice with conditional deletion of TDP-43 in the postnatal testis using tardbp (gene symbol for TDP−43) floxed mice and the Stra8-iCre deleter strain. Stra8-iCre mediated deletion of floxed genes has been shown to occur at postnatal day 4 in the mouse testis which is ideal for deleting the TDP-43 gene prior to the onset of its expression in spermatocytes.

Results: TARDBP flox/null, Stra8 tg males were infertile whereas the control TARDP flox/wild-type, Stra8 tg mice were fertile thus indicating that TDP-43 is essential for spermatogenesis. Histology showed spermatogenesis maturation arrest during meiosis. We are currently trying to identify TDP43-target genes in testis and understand the mechanism by which TDP-43 regulates differentiation of the male germ line. Male factor infertility accounts for roughly half the number of infertility cases but the underlying causes are unknown.

Conclusion: Our work has shown for the first time that loss of function of TDP−43 could lead to male infertility.
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THE HISTONE H3 DEMETHYLASE, KDM1 (LYSINE–SPECIFIC DEMETHYLASE 1) IS ESSENTIAL FOR THE ENTRY INTO MEIOSIS AND THE SURVIVAL OF SPERMATOGONIAL STEM CELLS.
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Introduction: Spermatogenesis is a highly complex cell differentiation process fueled by spermatogonial stem cells (SSCs). The progression from a spermatogonial stem cell to a differentiating cell involves gene expression changes that are under epigenetic control. Epigenetic mechanisms governing gene expression involve histones and their modifiers which add and remove permissive or repressive marks from histone tails. The histone demethylase KDM1 removes gene activating methylation on histone H3 at lysine 4 (K4). As KDM1 is present in SSCs we hypothesized that it serves in the epigenetic regulation of SSCs biology.

Methods: To determine the function of KDM1 in SSCs we generated mice with a conditional knockout of KDM1 (cKO) specifically in spermatogonia. Analysis of the cKO revealed that KDM1 is essential for spermatogenesis, as adult cKO males were sterile and lacked germ cells. Testes were collected from cKOs at postnatal days (PND) corresponding to the appearance of spermatogonia (PND6) and meiotic cells (PND10).

Results: At PND6 spermatogonia were present in the cKO, however, at PND10 no meiotic cells were detected. Moreover from PND10 to 14 the number of spermatogonia in the cKO testes decreased dramatically with only rare germ cells remaining at PND14.

Conclusion: This analysis indicates that KDM1 is a master epigenetic regulator of SSCs and is required for SSCs survival and meiotic entry.

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UBE2K DEFICIENCY DISRUPTS MEIOSIS IN RATS AND THE DERIVATION OF RAT SPERMATOGONIAL LINES IN CULTURE
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Introduction: Ube2k encodes the ubiquitin conjugating enzyme E2k. We recently conducted a genetic screen for reproduction defects in rats and identified a recessive mutation in Ube2k linked to female and male sterility. Rats homozygous for the Ube2k gene trap mutation (Ube2k gt/gt) are smaller in body size and their spermatocytes arrest early in meiotic prophase I. Accordingly, UBE2k is widely expressed in germ and somatic cells of wildtype rats, but UBE2k expression is reduced >95% in tissues of Ube2k gt/gt rats.

Objective: Here, because Ube2k gt/gt rats maintain undifferentiated spermatogonia during adulthood, we tested the hypothesis that spermatogonial lines could be derived from primary cultures of Ube2k gt/gt rat testis cells. Successful derivation of such Ube2k gt/gt spermatogonial lines would facilitate genetic and biochemical experiments to determine how UBE2k functions to support spermatogenesis.

Methods: Highly pure fractions of type A spermatogonia were isolated from ~23 day old wildtype, Ube2k gt/wt and Ube2k gt/gt rat littermates by sequential selection of testis cells in culture on plastic, collagen, laminin and gelatin matrices. Isolated cells were plated onto feeder layers of irradiated, mouse embryonic fibroblasts in serum free medium formulated to selectively expand rat spermatogonial lines.

Results: Spermatogonial lines were successfully derived from testes of wildtype and Ube2k gt/wt rats, but not from Ube2k gt/gt rats (n=3 primary cultures/genotype). Interestingly, only colonies of somatic-like cells expanded from Ube2k gt/gt rat testes. Colonies of the somatic-like cells were never observed in cultures prepared from wildtype or Ube2k gt/wt rats.

Conclusion: Ube2k gt/gt rat testes contain factors sufficient to support proliferation of spermatogonia, but not meiosis. This indicates that spermatogonia from Ube2k gt/gt rats potentially harbor premeiotic defects that disrupt their proliferation in culture; and/or, spermatogonial proliferation in vitro is blocked in response to contaminating Ube2k gt/gt somatic testis cells. Thus, if such mutational effects are intrinsic to the germline, it remains possible that loss of UBE2k expression in spermatogonia promotes differentiation towards a somatic lineage.

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PUTATIVE DRIVING FACTORS IDENTIFIED BY HIGH THROUGHPUT RNA SEQUENCING FOR MALE INFERTILITY OBSERVED WITH MUTATION OF ADAD1
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Introduction: Adenosine deaminase domain containing (testis-specific) 1 (ADAD1, previously TENR) is observed exclusively in post-meiotic germ cells. Mutation of the endogenous Adad1 locus via transgene insertion (Adad1tm1Reb) results in male-specific infertility. ADAD1 contains a double stranded RNA binding motif (dsRBM) and an adenosine deaminase (AD) domain, similar to enzymes known to catalyze RNA editing events (ADARs). Unlike ADARs, ADAD1 contains a putative AD domain-inactivating amino acid change suggesting ADAD1 may be RNA editing inactive while still retaining dsRNA binding capacity. These observations give rise to the hypothesis that ADAD1 acts as a negative regulator of RNA editing in the testis.

Methods: High throughput RNA sequencing (HTRS) of Adad1Tg/Tg testes along with wildtype controls was selected to test this hypothesis. Prior to HTRS, the transgene was backcrossed into an alternative strain background (C57Bl6/J) to facilitate HTRS data analysis. HTRS analysis was conducted in order to determine the molecular factors giving rise to the observed infertility.

Results: Histological analysis demonstrated the 25 dpp Adad1Tg/Tg testis to have a similar cell composition as wildtype controls. Additionally, expression analysis of major post-meiotic transcripts demonstrated no significant differences between mutant and controls at this time point. HTRS-mediated analysis of gene expression, splicing, and RNA editing demonstrates unique impacts on the 25 dpp testis transcriptome with transgene mediated mutation of Adad1, supporting the notion of a role for ADAD1 in post-transcriptional processes. Future studies will dissect specific alterations to determine if they are causative to the observed infertility in Adad1tm1Reb/tm1Reb males.

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EFFECTS OF CAFFEINE AT DIFFERENT PERIODS OF INCUBATION IN SEMEN SAMPLES THAWED
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Introduction: Although semen cryopreservation in an effective method, able to fertilize an oocyte and generate a healthy child, the damage it can impair the structural and functional integrity of spermatozoa, as reduced motility. Caffeine (1,3,7-trimethylxanthine), a psychotropic drug, is present naturally, and as an additive in many foods and drugs. Coffee consumption was associated with increases in sperm concentration and motility, because it acts by inhibiting the enzyme phosphodiesterase, triggering an increase in the concentration of cyclic adenosine monophosphate (cAMP), resulting in increased energy metabolism of spermatozoa.

Objectives: To evaluate the effects of caffeine at different periods of incubation in semen samples thawed.

Methods: After collection by masturbation, twelve semen samples were analyzed according to WHO criteria, processed and cryopreserved with Test Yolk Buffer (1:1) in liquid nitrogen. The thawed samples were incubated with 1.5mM caffeine for 0, 5, 15, 30 and 60 minutes, followed by analysis of sperm motility and mitochondrial activity for 3,3‘-diaminobenzidine (DAB) method. We conducted paired t test of the means adopted and p <0.05.

Results: Samples incubated for 15 minutes showed increase of progressive and non-progressive motility compared to other periods of incubation, as well as reducing the percentage of immotile spermatozoa (p <0.05). Samples incubated for 5 minutes showed increase of sperm mitochondrial activity above 50%. Moreover, with incubation for 60 minutes, an increase of sperm mitochondrial activity below 50% (p <0.05) was observed.

Conclusions: Although cryosurvival rates are low after cryopreservation process, incubation with caffeine showed an increase in sperm motility, especially within 15 minutes, and presents increased mitochondrial activity within 5 minutes, suggesting that caffeine incubation may be an important tool to be applied in patients with poor semen quality undergoing infertility treatment.
ELUCIDATING THE ROLE OF UCH–L1 IN SPERMATOGENESIS
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Introduction: Lifelong male fertility relies on the spermatogonial stem cell population of the testis achieving a balance between self-renewal and differentiation. Ubiquitin carboxyl terminal hydrolase L1 (UCH–L1) is expressed exclusively in the nervous system, ovary and testis. The gad mouse, which expresses a truncated UCH–L1 protein, has been extensively studied for its neurodegenerative phenotype. Studies aimed at testicular characterization implicated UCH–L1 plays a role in spermatogenesis. The UCH–L1 knock out (UCH–L1 −/−) mouse has a stronger phenotype for the study of neurodegenerative disorders; however, the testicular phenotype of this model has not been described. This study aimed to characterize the testis and male fertility of the UCH–L1 −/− mouse to gain a better understanding of the role of UCH–L1 in spermatogenesis.

Methods: UCH–L1 −/− mice were compared to aged matched heterozygous (Het) and wild type (WT) controls at key time points during spermatogenesis (7, 17, 30, 60, 90 and 120 days old). Numbers of undifferentiated spermatogonia were compared based on immunohistochemistry in testicular tissue for expression of promyelocytic leukemia zinc finger protein (PLZF). Fertility of UCH–L1 −/− mice was assessed by comparatively breeding UCH–L1 −/− and WT males to WT females and assessing litter frequency and size.

Results: Body weight of UCH–L1 −/− males was significantly lower at 90 and 120 days old compared to both Het (p<0.0001 and p=0.0001 respectively) and WT (p<0.0001 and p=0.0043). Testis weight was not significantly different across genotypes at any age; however, when controlling for body weight, relative testis weight of UCH–L1 −/− mice was significantly higher than in Het and WT mice at 90 days (p=0.006 and 0.0017) and significantly higher than in Het mice at 120 days (p=0.0129).

Conclusion: This suggests that as body condition deteriorates in UCH–L1 −/− mice due to neurodegeneration, testis weight is unaffected. For samples analyzed to date (n= 3 testes/genotype), number of tubules containing PLZF positive cells as well as overall number of PLZF positive cells were not different across genotypes. UCH–L1 −/− males produced fewer litters compared to WT males; however, litter sizes were similar (8.8 n= 4 and 8.0 n=22, respectively). Reduced fertility in UCH–L1 −/− males appears to be associated with impaired ability to copulate due to neuromuscular deficits rather than overt testicular pathology.

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THE RESPONSES OF YOUNG VERSUS AGED ISOLATED GERM CELLS TO LONG–TERM IN–VITRO CULTURE
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Introduction: Males produce germ cells continually throughout life; however, the quality of these germ cells decreases with advancing age. Aging germ cells display a reduced capacity to respond to stress. Previous studies examined developing germ cells in the presence of the supporting Sertoli cells that have antioxidant defenses to protect the germ cells. This has limited our understanding of germ cell specific responses; thus there is little information on the responses of germ cells at specific stages of spermatogenesis. This study is designed to establish a novel model for culturing isolated germ cells, allowing for the study of antioxidant defenses within each germ cell type.

Methods: First, we examined the long-term in-vitro viability of isolated rat pachytene spermatocytes (PS) and round spermatids (RS) in the context of aging. We established a culture system from isolated germ cells, characterized the viability of isolated germ cells over–time in culture, and compared the viability of isolated and cultured germ cells from young and aged rats. Brown Norway rats were sacrificed at 4-mo (BNY) or 18-mo (BNA) of age, their testes decapsulated and digested, and germ cells separated using unit gravity sedimentation. Fractions of PS and RS ≥83% purity were pooled, seeded onto 96-well plates, and cultured for 48-hours (5% CO2, 32°C). Viability was assessed at various time points (T0-T48h) using trypan blue exclusion and LIVE/DEAD® Kit. RNA was extracted and gene expression analyzed using qRT-PCR.

Results: Viability remained constant (>87%) in PS until T21h (BNY: 89%; BNA: 87%), and in RS until T24h (BNY: 88%; BNA: 81%). These viabilities were in agreement with LIVE/DEAD data (age:ns; time; P<0.0001; interaction;ns). Gene expression changed over-time; Sod1 levels decreased in BNA vs. BNY PS at T4h (n=5, P<0.05). Ldhc (n=5, P<0.0001) and TP53rk (n=5, P<0.0001) in both BNA and BNY PS were significantly reduced at T13h & 21h.

Conclusion: These studies demonstrate that germ cells can be cultured following isolation without supporting cells and remain viable for 21 – 24 hours. Reduced Sod1 in BNA PS suggests vulnerability to oxidants. Reduced Ldhc and TP53rk suggest PS in-vitro development into RS-like germ cells and stress-related apoptotic activity, respectively; age does not seem to affect these two functional markers. This novel culture model will be an invaluable tool in studying germ cell responses. Supported by a grant from CIHR.
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INITIATION OF SPERMATOGONIAL DIFFERENTIATION AT THE TIME OF PUBERTY IN THE MONKEY IS ASSOCIATED WITH A TRANLOCATION OF SOHLH1 FROM THE CYTOPLASM TO THE NUCLEUS OF PRE-MEIOTIC GERM CELLS
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Introduction: Initiation of spermatogonial differentiation in higher primates occurs at puberty, and is driven by increased gonadotropin secretion at this stage of development. The seminiferous cords of the prepubertal monkey testis contain only Sertoli cells and undifferentiated Type A spermatogonia, but spermatogonial differentiation in the juvenile may be readily activated precociously by treatment with either LH or FSH. The cellular mechanisms that relay the gonadotropin dependent signal to differentiate, and molecular pathways that commit undifferentiated primates spermatogonia to the pathway of differentiation are poorly understood. Recent studies by one of our laboratories indicate that two germ cell specific basic helix-loop-helix transcription factors, Sohlh1 and Sohlh2 are necessary for spermatogonial differentiation in mice. The purpose of this study was to begin to examine the role of these transcription factors in regulating spermatogonial differentiation in primates.

Methods: To this end, immunofluorescence histochemistry using a purified anti-macaque polyclonal antibody raised in guinea pig (1:250) was employed to determine the expression of SOHLH1 in monkey testis during spontaneous puberty and during precocious puberty induced by exogenous gonadotropin stimulation. Using age, testis volume and circulating testosterone levels, monkeys were classified as mid-juvenile, late-juvenile, early-pubertal and mid-pubertal.

Results: In juvenile animals, SOHLH1 was predominantly observed in the cytoplasm of undifferentiated Type A spermatogonia. In contrast, puberty was associated with a progressive increase in nuclear location of spermatogonial SOHLH1 and by mid-puberty the cellular distribution of this transcription factor was similar to that in adult monkey testis. The cytoplasmic location of SOHLH1 in juveniles was highly associated with co-expression of GFRα1, but in mid-pubertal animals only 20% of the SOHLH1 positive spermatogonia expressed GFRα1. In the pubertal translocation of SOHLH1 to the nucleus was induced prematurely in juveniles by 11 days of pulsatile LH stimulation, either alone or in combination with FSH stimulation

Conclusion: Assuming that the action of LH on the juvenile testis is mediated by testosterone, the foregoing findings suggest that initiation of spermatogonial differentiation in the primate testis is associated with translocation of SOHLH1 into the nucleus of differentiating spermatogonia, and that this process is driven by androgen receptor signaling.

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ZINC FINGER AND TAL-EFFECTOR NUCLEASE MEDIATED GENE TARGETING IN MOUSE SPERMATOGONIAL STEM CELLS
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Introduction: Thousands of diseases are caused by a change in a single gene. Gene therapy is the process of treating such diseases by providing a correct form of the gene, ideally in stem/progenitors to the diseased cells. Targeted forms of gene therapy wherein specific, known changes are made to the genome offer clear advantages over early approaches wherein transgenes were introduced into the genome essentially at random. Gene targeting, or exchange of an exogenous DNA with the genome, relies on the cell’s homologous recombination machinery. The rate of homologous recombination is low but it can be stimulated by introducing a double-strand break in the genome. By introducing into the cell a nuclease that is engineered to cut near the gene of interest, together with a template for DNA repair, it is possible to stimulate gene correction by ~1 million-fold. Zinc finger and TAL–effector nucleases (ZFN & TALEN) are engineered to bind/cut a target DNA site. Our goal is to model the therapeutic process of correcting a gene in a patient’s stem cells ex vivo, followed by transplanting the corrected stem cells back into the patient.

Methods: We are using mouse spermatogonial stem cells (SSCs) as a model system. We generated spermatogonial stem cell lines (i.e. “GS cells”) from mice with a model mutant gene, namely GFP. Using flow cytometry to detect gene correction we have achieved both ZFN and TALEN mediated gene targeting in SSCs. Importantly, we could isolate and expand the rare targeted cells in culture, suggesting that the targeted cells maintained their capacity for self-renewal following genomic manipulation.

Results: Analysis of two resulting cell lines showed that the mutation/ZFN target sites were corrected with perfect accuracy; also, the cells still expressed many spermatogonial markers, suggesting they were phenotypically unaltered. We have data showing that following transplantation the gene-corrected stem cells can colonize in recipient testes; next we will test the function of the transplanted cells in restoring fertility. Applications of this approach include: generating transgenic animals, correcting gene defects in germ cells followed by transplantation to assure that progeny would not carry the mutant gene, and using gene corrected SSCs to generate pluripotent ES-like cells for subsequent autologous cell based therapies.

Conclusion: Our pursuit of technology for efficient gene targeting in SSCs represents an important first step toward the ambitious goal of SSC-based therapy.
CHARACTERIZATION OF SPERMATOGONIAL MARKERS IN THE MATURE TESTIS OF SCYLIORHINUS CANICULA

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Introduction: Spermatogonial stem cells (SSCs) self renewal and differentiation engagement are controlled by specific regulations in a closed microenvironment called niche and only poorly described. In drosophila and mammals niches, four mechanisms control the balance between self renewal and differentiation: the symmetric and asymmetric divisions, the reversion of differentiating spermatogonia and the loss of paired SSCs. The engagement in the differentiation way implies successive mitosis and the progressive acquisition of differentiated characters. In mammals, molecular characterization of the spermatogonial compartment shows that the Glial cell-Derived Neurotrophic Factor (GDNF) and the Promyelocytic Leukemia Zinc Finger (PLZF) are both SSC’s markers whereas C-kit is a marker of differentiated spermatogonia.

Objectives: Our purpose is to characterize the expression pattern of selected factors involved in SSC regulation in Vertebrates and identified in dogfish genome.

Methods: The phylogenetic position of the dogfish (Scyliorhinus canicula) as a chondrichtysan is an advantage to study the evolution of SSCs self-renewal and differentiation through vertebrates. Moreover, the dogfish testicular topology includes a cystic organization in a polarized testis with a linear spermatogenetic wave. This testicular area subdivision permits precise dissection and isolation of the germinative area including the potential SSCs niche. By this way, we showed that GDNF factor receptor α1 (GFRα1) and Pou2/pou5f1, both presented the same expression pattern than their orthologs. At the contrary, PLZF showed a less restrictive expression pattern in the dogfish with a strong transcripts level detected in SSCs but also in differentiated spermatogonia. Finally, new markers of differentiated spermatogonia were found in dogfish: Hmgb3 and Mcm6, both belonging to families involved in cell duplication.

Conclusion: To conclude, evolutionary conserved markers and new factors were characterized in the spermatogonial compartment in dogfish. Using those new markers, 3 spermatogonia subpopulations were defined: GFRα1+/Mcm6− SSC to AA14, GFRα1+/Mcm6+ AA18−32, and GFRα1−/Mcm6+ differentiated spermatogonia. Still in an evolutionary purpose, we currently develop a dogfish spermatogonial culture in order to evaluate the functional conservation of main niche regulators.

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Introduction: The role of thyroid hormones (TH) in testis development has mostly been studied by neonatal administration of propyl thiouracil (PTU), inducing hypothyroidism. This treatment results in a delay in Sertoli cells development, while Leydig cell development is reported to be arrested. Following cessation of the hypothyroid condition at the age of 26 days, Leydig cell growth and differentiation is initiated, leading to an approximate 70% increase in Leydig cell number in adulthood. Moreover, testicular weight and sperm output were increased by about 80%. In contrast, chronic dietary induced fetal–postnatal hypothyroidism, which also transiently affected Sertoli and Leydig cell development, did not result in an increase in testis weight in adulthood.

Objective: In the present study we have investigated whether the increase in testis weight in PTU treated rats is not due to hypothyroidism per se, but due to the cessation of the hypothyroid condition.

Materials and Methods: Hypothyroidism was induced in rats during fetal development by feeding dams an iodide-poor diet supplemented with 0.5% sodium perchlorate, or by administering 0.1% PTU to the drinking water of dams and new born rats. From the age of 28 days until sacrifice the rats were fed a euthyroid diet. A chronic dietary hypothyroid group and a group fed the euthyroid control diet from birth till sacrifice, served as controls.

Results: Up to the age of 28 days after birth THS levels in the PTU group were slightly higher compared to the dietary induced hypothyroid groups, and significantly higher compared to the euthyroid controls. Testis weight was significantly increased in both transient hypothyroid groups at the age of 100 days.

Conclusion: Hypothyroidism per se does not lead to an increase in testis weight in adulthood, but is a consequence of the cessation of the hypothyroid condition. We postulate that after discontinuation of the hypothyroid condition Sertoli cells undergo an additional wave of proliferation, leading to an increased testis weight in adulthood.
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TESTIS EXPRESSED ACTIN-LIKE 7B (ACTL7B) IS REQUIRED FOR MOUSE SPERMATID MORPHOGENESIS, ACROSOME ATTACHMENT, AND FERTILITY
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Introduction: Human male infertility is often associated with a high incidence of abnormally shaped sperm heads, suggesting that cytoskeletal regulation may be important for male fertility. The involvement of filamentous actin (F-actin) has been suggested for several aspects of spermatid differentiation, including acrosome formation and attachment to the nucleus, formation of tubulobulbar complexes, cytoplasmic removal, and spermiination. Although the structural components and morphological changes associated with spermiogenesis have been described in detail, relatively little is known about the mechanisms that drive these structural changes. Actin-like 7b (Actl7b) is an orphan actin-related protein (ARP) family member. Actl7b is an intronless gene expressed in spermatids and conserved in mammals. The objective of this study was to determine the functional relevance of Actl7b.

Methods: Immunohistochemistry and indirect immunofluorescence were utilized to investigate the expression of ACTL7B. It is localized in the cytoplasm of round and elongating spermatids and co-localizes with phalloidin labeled F-actin in or around the forming acrosome, suggesting a role in this process. Actl7b knockout mice were generated from targeted ES cells, obtained from the KnockOut Mouse Project (KOMP) Repository, in which the coding region was replaced with a LacZ reporter sequence.

Results: X-gal staining of tissues from heterozygous animals revealed that Actl7b is expressed in the testis and, unexpectedly, the brain. While Act7b knockout mice develop to adulthood and appear normal, breeding studies reveal that Actl7b knockout males mate and produce vaginal plugs, but are infertile. Testis and epididymal weights and sperm counts are lower in knockout males than in wild type males. Most of the knockout sperm are immotile, with less than three percent showing minimal flagellar movement. In addition, sperm heads are misshapen with a rounded appearance and electron microscopy studies demonstrate that acrosome attachment is disrupted.

Conclusion: These results indicate that Actl7b is required for spermatid morphogenesis, sperm integrity and motility, and male fertility. They also suggest that ACTL7B is either the F-actin recognized by phalloidin or is required for F-actin assembly in spermatids. This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

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ALTERNATE PROMOTER USAGE CONTRIBUTES TO THE BASAL AND FORSKOLIN-INDUCED EXPRESSION OF THE MOUSE NR4A1/NUR77 GENE IN MA–10 LEYDIG CELLS
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Introduction: NR4A1, also known as NUR77, is an orphan nuclear receptor expressed in several tissues including the testis, ovary and adrenal gland. In Leydig cells, NR4A1 has been reported to play pivotal roles by regulating the expression of several genes involved in steroidogenesis and male sex differentiation including Star, HSD3B2, and Insl3. In Leydig cells, steroid hormone production is stimulated by LH binding to its receptor (LHR) resulting in an increase in cAMP and cytoplasmic Ca2+ levels. Activation of the cAMP and Ca2+ signaling pathways lead to a rapid and robust activation of Nr4a1 expression, which occurs mainly at the transcriptional level and requires CaMKI kinase. The proximal NR4A1 promoter was found to contain distinct regions, each with several regulatory elements, responsible for basal (SP1, SP3) and hormone-induced (CREB, AP1, NF–κB) expression. However, the detailed molecular mechanisms controlling expression of this early responsive gene remain to be fully deciphered. Recent studies revealed that three distinct promoters control the expression of the human and mouse Nr4a1 gene in cardiac and adrenal cells. This leads to the production of three transcripts with alternative noncoding exon (ANE) 1: ANE1A produced from the most proximal promoter about 120 bp upstream of the ATG, ANE1B located about 9 kb upstream of the ATG, and ANE1C 12 kb upstream of the ATG. Thus we hypothesized that these distinct promoters might contribute to Nr4a1 expression in Leydig cells.

Methods: Using qPCR with ANE1-specific primers, we found that the three ANE are present in MA-10 Leydig cells indicating that the three Nr4a1 promoters are active in these cells. Treatment of MA-10 Leydig cells with Forskolin (Fsk) lead to a strong induction of ANE1A (23 fold) as previously characterized, but also of ANE1B (11 fold) while ANE1C was only weakly induced (2 fold). As previously reported for the proximal Nr4a1 promoter (renamed promoter 1A), we found that Fsk-mediated ANE1B induction also involves the Ca2+/CaMKI pathway.

Results: Using in silico promoter analysis, we found promoter 1A and 1B contain common regulatory elements such as CREB, NR2F2 and AP1 that might explain the cAMP responsiveness of these two promoters.

Conclusion: In conclusion, these data provide new insights into the mechanisms regulating Nr4a1 expression in MA-10 Leydig cells where promoter 1C ensures minimal NR4A1 levels whereas promoters 1A and 1B are responsible for the fine-tuning in response to a stimuli.

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A NOVEL EXTRAHEPATIC FUNCTION OF THE CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) IN THE TESTIS, FOR MAINTAINING NORMAL ANDROGEN LEVELS

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Introduction: The orphan nuclear receptor CAR has primarily been associated with hepatic detoxification of toxicant and endogenous compounds. CAR also affects intermediary metabolism and hepatocyte proliferation but its extrahepatic function has not been extensively elucidated. CAR expression in extrahepatic tissues is not as high as in the liver, and transcriptional function is thought to be the same as its hepatic function. We have found a novel role for CAR in the testis in maintaining androgen homeostasis. CARKO mice have a significant increase in testes weight (38%), and atrophy of the prostate and seminal vesicles; physiological changes associated with disruption in androgen homeostasis/function. We hypothesized that CARKO mice would have decreased androgen levels and that CAR would be associated with transcriptionally regulating genes involved in androgen production.

Methods: Serum testosterone and androstenedione levels were 50% lower in CAR KO mice, while estradiol and 17-hydroxyprogesterone levels were not altered. The detoxification genes: Cyp3a’s, Cyp2b’s, Cyp2c’s, Sult2a1, and Mrp transporters were not induced in the liver of CARKO mice, suggesting an extrahepatic mechanism for decreased androgen levels. We have rigorously tested the testicular expression of CAR.

Results: CAR was primarily found in Leydig islands by immunofluorescence, and CAR expression was detected in the TM3 Leydig cell line and primary Leydig cells with little expression in Sertoli cells and the TM4 Sertoli cell line. Steroidogenic enzymes Cyp17a1, Cyp19a1, and Srd5a1 were both down 2 fold in the liver, while Cyp17a1 and Srd5a1 were lower in testes of CARKO mice. Interestingly, Srd5a1 was almost undetectable in liver and testes of CARKO mice.

Conclusion: We are currently testing direct transcriptional regulation by Chromatin Immunoprecipitation assay (ChIP), of binding sites determined in silico. Activation of CAR by estradiol and inactivation by androstanol suggests CAR may respond to sex hormones as it does to other endogenous ligands, but regulate a unique set of genes in the extrahepatic tissues such as the testes. A novel role for CAR in the testes would also open the possibility for a new target of endocrine disrupting compounds, such as phthalates and alkylphenols known activators of CAR in the liver.

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CLINICAL USEFULNESS OF DETERMINATION OF SEMINAL FRUCTOSE AND CITRIC ACID LEVELS IN INFERTILE PATIENTS WITH PROSTATOVESICULITIS DUE TO CHLAMYDIA TRACHOMATIS AND MYCOPLASMA SPP

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Introduction: The role of Chlamydia trachomatis (Ct), Mycoplasma spp. (Myc) infections in inflammatory diseases of the prostate and seminal vesicles has been investigated for 30 years ago, and is still controversial, but it is now clear that the absence of leukocytes in seminal fluid does not exclude the possibility of prostatovesiculitis. The alteration of the viscosity and volume of ejaculate, are parameters that allow these infections suspect glandular and can be supplemented with the determination of seminal citric acid and fructose levels. Those were usually used to determine the normal function of these glands.

Objective: To establish whether levels of seminal fructose and citric acid are altered in association with genital infections caused by Ct and Myc.

Material and Methods: We studied 39 infertile patients in whom semen studies and specific microbiological tests for Ct and Myc were performed. The levels of citric acid and seminal fructose were determined by a spectrophotometric method after protein precipitation. A group of 5 healthy volunteers were studied as controls.

Results: In the group of patients, 84.6% were positive for Myc, 95% were positive for Ct, and 82% were positive for both microorganisms. The control group was negative for both microorganisms, in addition to normal levels of seminal fructose and citric acid. In the group of patients, fructose levels were abnormally low at 59%, and citric acid levels were low at 31% and 13% higher in patients. Patients with low seminal volume, seminal fructose showed lower levels (72.70%). 81% of cases with decreased seminal viscosity presented low seminal fructose. An important observation is that the levels of seminal fructose in patients infected with these bacteria are altered to below normal levels, compared with citric acid levels which are altered to both upper and lower levels. Extremely abnormal levels of these markers were observed in patients with positive culture for both bacteria.

Conclusions: Altered of seminal fructose and citric acid levels were more frequent observed in infertile men with infections caused by Ct and Myc than in the group of healthy men. The routine evaluation of semen parameters may be markers of prostate and seminal vesicle dysfunction, these determinations should be mandatory in cases of genital infections.
CHARACTERIZATION OF SPATA22, A NOVEL MAMMALIAN PROTEIN REQUIRED FOR MEIOTIC PROGRESSION IN MOUSE GERM CELLS
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Introduction: Identifying the cues that govern progression of germ cells through meiosis is critical to our understanding of the mechanisms that lead to the formation of healthy gametes. We have previously characterized the ENU-induced mouse mutation repro42, which causes both male and female infertility due to meiotic arrest. Recombination-based genetic fine mapping in combination with sequencing of candidate genes identified a nonsense mutation in Spata22 (spermatogenesis associated 22), a gene not previously known to play a role in gametogenesis.

Methods: Analysis of mutant repro42 surface-spread chromatin determined that arrest takes place at the transition between zygonema and pachynema during meiosis I, and that synaptonemal complex (SC) formation, synapsis, and repair of DNA double strand break are all impaired in the absence of SPATA22. Since the precise requirement for Spata22 during meiotic prophase remains elusive, we further characterized SPATA22 regulation and localization during male germ cell development in the mouse. A bioinformatics analysis predicted a number of phosphorylation sites along the SPATA22 sequence, but immunobloting analysis of testis protein extracts prepared in the presence or absence of phosphatase inhibitors uncovered no shift in molecular weight, suggesting SPATA22 is not phosphorylated. Previous immunohistochemical localization of SPATA22 in adult testis sections indicated SPATA22 is restricted to a specific population of meiotic germ cells.

Results: Analysis of surface-spread chromatin confirmed this and showed that SPATA22 is faintly detectable across the nucleus of leptotene spermatocytes, while it becomes more readily detectable in zygotene spermatocytes but remains mostly diffuse. However, by late zygonema numerous foci of SPATA22 are observed across the nucleus, and by pachynema SPATA22 becomes highly restricted to a few foci spread along the length of all SCs (5 – 8 foci per SC on average) in a manner similar to RPA1 (replication protein A1), a component loaded onto transitional nodules (TNs) following RAD51 and DMC1, two components of early nodules (ENs). Since we previously determined that both RAD51 and DMC1 are present in mutant repro42 spermatocytes, we assessed if the same held true for RPA1. RPA1 was undetectable in surface-spread chromatin prepared from mutant repro42 spermatocytes. Taken together, these data further support the role of SPATA22 in meiotic prophase and suggest it is involved following formation of ENs.
Effect of Chlamydia Trachomatis and Ureaplasma Urealyticum Genitourinary Infection Treatment on Sperm Morphology, DNA Fragmentation and Male Fertility

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Introduction: Previous studies in the population of the northeastern region of Mexico, demonstrated a high percentage of couples with diagnosis of unknown etiology infertility, suffer an internal genital infection by Chlamydia trachomatis(Ct) and Ureaplasma urealyticum (Uu). Bacteriospermia and leukocytespermia present at these subclinical infections diminish the quality of seminal fluid and sperm cells, but the implicit mechanisms in this pathology are not completely elucidated.

Objectives: Our group has been focused to the analysis of subcellular damage of the spermatozoa exposed to the coinfection by Ct and Uu. In the last decade it has been recognized the negative influence of the ROS in the integrity of the plasmatic membrane, the mitochondrial function and DNA fragmentation. To the sperm cell, these damages could explain the diminishing of the fertility capacity. The aim of this work was to obtain evidence of the mechanisms of spermatic damage associated to infection by Ct and Uu and to analyze the effect of a combined treatment with antibiotics and antioxidants.

Methods: We compared spermatic parameters, DNA fragmentation and sperm morphology before and after antibiotic treatments for Ct and Uu. We studied 70 infertile patients with seminal co-infection by Ct and Uu and 28 healthy and fertile men as a control group. Between the spermatic parameters that were significantly related with the infection we found a high percentage of abnormal spermatozoa and the abnormal pattern of chromatin dispersion (p<0.0001). Image 1

Results: The parameters modified after the treatment, due to their statistical significance were: the progressive linear movement, sperm with altered medium piece of the flagellum, acrosomal injuries: small and vacuolated acrosome and the chromatinic dispersion pattern of big halo. The observed improvement in the spermatic quality parameters and the infection control, was associated to the pregnancy in almost half the patients of this study.

Conclusion: The big chromatin dispersion halo, acrosome integrity and the normal structure of sperm falgellar middle piece, resulted of predictive parameters for the achievement of pregnancy, in patients with Chlamydia trachomatis and Ureaplasma urealyticum co-infections.
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DECREASED C-TERMINAL BINDING PROTEIN 2 EXPRESSION IN MEN WITH SERTOLI CELL ONLY SYNDROME
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Introduction: Spermatogenic failure in infertile men involves mechanisms that are not well understood. DNA repair systems are required during spermatogenesis to correct cellular insults or commit the cell to apoptosis. C-terminal binding protein (CTBP) is a transcriptional co-repressor that has been identified to play a role in preventing cellular apoptosis; however, its role in male infertility has never been assessed.

Methods: Tissues were obtained from men undergoing testis biopsy for non-obstructive azoospermia (NOA; n=16) & vasectomy controls (n=5). Gene-expression microarray (Agilent Sureprint G3) screened for genetic variations. Microarray data was evaluated with heatmaps, clustering and statistical analysis. Ingenuity Pathway Analysis (IPA) software using False Discovery Rates at 5% highlighted candidate genes and pathways involved. The presence of CTBP2 was assessed via PCR and qPCR. Immunostaining was performed on paraffin embedded testicular tissue.

Results: Microarray data followed by IPA analysis identified CTBP2 as a top transcription factor altered in NOA men. Genomic DNA was screened with PCR and exon regions common to all CTBP2 isoforms were present in the testicular tissue of both NOA and controls. qPCR found patients with hypo-spermatogenesis on biopsy to have a 1.3 fold increase in expression, maturation arrest to have a 1.1 fold increase. Those men with Sertoli Cell Only (SCO) Syndrome had a significantly decreased (0.7 fold) change in expression. Immunohistochemistry confirmed expression in all patients with correspondingly decreased levels in those with SCO.

Conclusions: Decreased levels of CTBP2 expression are found in men with SCO suggesting a contribution to male infertility.

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RETINOIC ACID SIGNALING CELL-AUTONOMOUSLY DRIVES SPERMATOGONIAL DIFFERENTIATION
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Introduction: Spermatogonial differentiation, the transition of A aligned spermatogonia into A1 spermatogonia, is an initial step of mammalian spermatogenesis, which is irreversibly committed toward gamete production. Retinoic acid (RA), the active metabolite of vitamin A is essential for the spermatogonial differentiation; however, it remains unclear whether RA signaling controls spermatogonial differentiation directly through spermatogonia or Sertoli cells or both. RA functions as a ligand through binding to and activating three RA receptor (RAR) isotypes, RARα, RARβ, and RARγ – that are widely expressed in the testis including spermatogonia. Germ cell−specific inactivation of individual Rars results in mild abnormalities. However, Rars are functionally redundant in cells, suggesting that other isotypes of Rars could compensate for the loss of an individual Rar. RaraT403, a truncated form of human RARα, inhibits endogenous RA−mediated transcription of target genes in a dose−dependent manner.

Materials: In the current study, we show that expression of RaraT403, a dominant−negative RARα (dnRARα) specifically in mouse undifferentiated spermatogonia impairs RA signaling in germ cells and cause a blockage of spermatogonial differentiation, similar to that seen in vitamin A−deficient mice.

Results: Accordingly, we find that dnRARα testes do not express STRA8 and KIT, markers for the undifferentiated spermatogonia.

Conclusion: In contrast, the number of Sertoli cells in dnRARα and control testes does not significantly differ. Taken together, these studies support the notion that RA signaling in vivo directly drives spermatogonial differentiation.

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Poster Session I

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SPERMATOGONIAL BEHAVIOR IN RATS DURING RADIATION-INDUCED ARREST AND RECOVERY AFTER HORMONE SUPPRESSION
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Introduction: Ionizing radiation has been shown to block spermatogenesis despite the presence of surviving stem cells in certain strains of rats, by blocking spermatogonial differentiation. The block is a result of damage to the somatic environment and is reversed when gonadotropins and testosterone are suppressed, but the mechanisms are still unknown.

Methods: The present study examined the spermatogonial differentiation kinetics and Sertoli cell factors that regulate spermatogonia after irradiation, during hormone suppression and after hormone suppression combined with Leydig cell elimination with ethane dimethane sulfonate. Stereological results showed that the number of Sertoli cells is unaffected by irradiation and immunohistochemical analysis showed that Sertoli cells still produced KIT-ligand (KITLG) and GDNF growth factors.

Results: High resolution light microscopy showed that only a few undifferentiated A spermatogonia survived after radiation. Very few of these cells did indeed differentiate to A1 spermatogonia and also expressed KIT receptor, demonstrating that the failure of differentiation was not a result of the absence of the KIT system. Hormone suppression resulted in an increase in undifferentiated A spermatogonia within 3 days, a gradual increase in KIT-positive spermatogonia, and differentiation mainly to A3 spermatogonia after 2 weeks. Elimination of Leydig cells in addition to hormone suppression enhanced the rate of spermatogenesis recovery indicating that there had been an inhibitory effect of a Leydig-cell factor on spermatogonial differentiation. KITLG protein expression did not change after hormone suppression indicating that it is not for a factor in the stimulation. However, GDNF increased steadily after hormone suppression.

Conclusion: Based on the concept that hormone suppression did not change Sertoli cell ultrastructure or its KITLG protein expression, and the higher GDNF expression is not expected to produce spermatogonial differentiation, the primary cause of block in spermatogonial development appears to be related to the Leydig cell products, and not to Sertoli cell factors such as KITLG or GDNF or the KIT receptor.

Funding: Financial Support: Capes, CNPq and Fapemig.

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REGULATION OF THE PROLIFERATION AND DIFFERENTIATION OF ADULT LEYDIG STEM CELLS
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Introduction: New Leydig cells appear after the adult Leydig cells are eliminated with ethane dimethanesulfonate (EDS). PDGFRα+ cells were purified from the testes after EDS. Depending upon culture conditions, these cells proliferated indefinitely or differentiated and produced testosterone (T). Seminiferous tubules were isolated from the interstitium. Culture of the tubules for one week resulted in a peak of cell division on the surface of the tubules, and then a return to basal division levels by week 2. With culture from weeks 2 – 4, 3βHD+ cells appeared on the surface of the tubules, and testosterone was detected in the culture medium. These results suggest that there are stem cells on the surfaces of the tubules that divide and then cease dividing, and that the products of the divisions then give rise to the newly formed adult Leydig cells.

Methods: To begin to identify how Leydig stem cells are regulated, we screened 35 factors or their signaling molecule modulators for their effects on the division or differentiation of the stem cells. The factors that were tested were selected based either on published results demonstrating their abilities to affect Leydig cell development, or on significant mRNA changes during the transition of Leydig stem to progenitor cells as seen by our array analysis. Each factor was tested for its effect on cell division or differentiation, using the cultured seminiferous tubules.

Results: Desert Hedgehog (DHH), PDGF-BB, FGF-2, activin, PDGF-AA, IL-1α, TGFα, IGF-1 and LIF had stimulatory effects on cell proliferation. Wnt, inhibit, PDGFA, DHH and Notch inhibition had positive effects on cell differentiation. TGFβ inhibited both cell division and differentiation.

Conclusion: The use of the seminiferous tubule culture system has promise of providing a powerful way to identify the elements of the stem Leydig cell niche and their functions despite the complexity of the tissue.
5.

PROTECTIVE EFFECTS OF MELATONIN ON THE TESTIS IN POST-STATUS EPILEPTICUS RATS FOLLOWING LITHIUM-PILOCARPIN INJECTION AS A MODEL OF TEMPORAL LOBE EPILEPSY (TLE)

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Introduction: Reproductive dysfunction is common among men with complex partial seizures of temporal lobe origin. The aim of this study was the assay of chronic treatment with melatonin during the latent phase of evolving TLE on the testis.

Methods: Eight groups of male Wistar rats were treated as follows: epileptic rats without any injection, solvent of melatonin as a vehicle (Sham); melatonin (5 mg/kg/daily) for 14 days and 60 days, melatonin (20 mg/kg/daily) for 14 days and 60 days, sedentary control without any injection (Control) and reverse injection of lithium-pilocarpine. Induction of Status Epilepticus caused significant decrease (p ≤ 0.05) in the sperm parameters.

Results: This experiment revealed that melatonin 5 mg for 60 days can significantly improve progressive motility of sperm (p ≤ 0.05). Moreover, melatonin 20 mg for 14 days and 5 mg for 60 days caused significant improvement in total motility of sperms. Furthermore, there was a significant reduction in total antioxidant (TA), superoxide dismutase (SOD) and catalase (CA) levels between epileptic rats. Melatonin 20mg for 60 days caused a significant increase in the levels of TA, SOD and CA. Melatonin 20mg for 14 days and 5 mg for 60 days showed a significant increase in SOD and CA, respectively.

Conclusion: It seems that chronic administration of low dose melatonin or semi chronic administration of high dose might be effective in ameliorating some consequences of Status Epilepticus on the sperm quality and antioxidant enzymes which can in turn affect male fertility.

7.

CELLULAR AND MOLECULAR MECHANISM OF MALE INFERTILITY IN THE ATHLETES THAT ABUSE ANABOLIC ANDROGENIC STEROIDS: APOPTOSIS IN SPERMATOGENIC CELLS, CASPASE 3 ACTIVITY AND THE GENERATION OF REACTIVE OXYGEN SPECIES (ROS) IN THE RAT MODEL

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Introduction: Anabolic steroids are used at high doses by athletes for improving athletic ability. It has been established high doses of anabolic steroids may increase testicular apoptosis. However, the effects of the combination of exercise and high dose of anabolic-androgenic steroids on testicular apoptosis and the generation of ROS are not known.

Methods: Five groups of Wistar rats were treated as follows for 8 weeks: solvent of nandrolone decanoate as a vehicle; nandrolone decanoate (10 mg/kg/weekly) (ND); running exercise by treadmill (1 hr/day, 5 days a week) (EX); combination of nandrolone decanoate and running exercise (ND-EX) and sedentary control without any injection or exercise (CO).

Results: Testis weight significantly decreased in ND-EX but sperm count reduced in ND and ND-EX and sperm motility decreased in all experimental groups relative to the vehicle and control groups (P≤0.05). Germ cell apoptosis and Caspase 3 activity was increased in all experimental groups but it was so much in the ND and ND-EX groups. The expression of FAS and FasL was not significant but the expression of Bax was increased and Bcl2 was decreased in the all experimental groups. It shows that germ cell apoptosis in this experiment is from mitochondrial pathway. The generation of ROS was elevated in all experimental groups especially in ND group (P≤0.05). Total Antioxidant was decreased in all experimental groups in comparison with CO group.

Conclusion: It concluded that running exercise seems to increase the extent of apoptotic changes caused by supraphysiological dose of nandrolone decanoate in rats, which can in turn affect fertility.
9. THE TARGETING AND FUNCTIONS OF MIRNA-383 ARE MEDIATED BY FMRP DURING SPERMATOGENESIS

Hui Tian, Fei Sun, PhD, MD

Introduction: Our previous studies have shown that microRNA-383 (miR-383) expression is downregulated in the testes of infertile men with maturation arrest (MA). Abnormal testicular miR-383 expression may potentiate the connections between male infertility and testicular germ cell tumors. However, the mechanisms underlying the targeting and functions of miR-383 during spermatogenesis remain unknown.

Methods: In this study, we found that fragile X mental retardation protein (FMRP) was associated with 88 miRNAs in mouse testis including miR-383. Knockdown of FMRP in NT2 (testicular embryonal carcinoma) cells enhanced miR-383-induced suppression of cell proliferation by decreasing the interaction between FMRP and miR-383 and then affecting miR-383 binding to the 3´UTR of its target genes, including IRF1 and Cyclin D1 both in vivo and in vitro.

Results: On the other hand, FMRP levels were also downregulated by overexpression of miR-383 in NT2 cells and GC1 (spermatogonia germ cell line). miR-383 targeted to Cyclin D1 directly, and then inhibited its downstream effectors, including p-pRb and E2F1, which ultimately resulted in decreased FMRP expression. Reduced miR-383 expression, dysregulated CDK4 expression (one of the downstream genes of miR-383) and increased DNA damage were also observed in the testes of Fmr1 knockout mice and of MA patients with a downregulation of FMRP.

Conclusion: A potential feedback loop between FMRP and miR-383 during spermatogenesis is proposed, and FMRP acts as a positive regulator of miRNA functions. Our data also indicate that dysregulation of the FMRP-miR-383 pathway may partially contribute to human spermatogenic failure with MA.

11. OXIDATIVE STRESS AND SPERM DNA QUALITY IN MALE PARTNERS OF COUPLES EXPERIENCING RECURRENT IVF/ICSI FAILURE

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Introduction: Sperm has dynamic and critical role in embryogenesis that extends beyond fertilization. Supraphysiological reactive oxygen species (ROS) levels damage both mitochondrial and nuclear genome. During embryonic development, nucleotide alterations if unrepaired are lethal to accurate transmission of genetic information and affect the embryonic viability and post natal health. Nicks/breaks and/or nucleotide modification caused by unregulated free radical concentration due to inefficient antioxidant machinery leads to sperm DNA damage. This study was planned to understand the association of seminal ROS, antioxidant capacity and sperm DNA damage in male partners of couples experiencing recurrent IVF/ICSI failure

Material and Methods: Male partners of 81 couples experiencing recurrent IVF/ICSI failure and 49 fertile controls were included in study. Semen was collected after 72 – 96 hours of sexual abstinence and analyzed according to WHO 2010 guidelines. ROS in neat semen was quantified by luminol induced chemiluminescence. TAC was assessed by ELISA using commercially available kit (Cayman Inc.). Sperm DNA damage and thereby DNA fragmentation index (DFI) was assessed by comet assay. Spearman correlation coefficient was used to test associations. P-value <0.05 was considered significant. Statistical analyses were performed using MedCalc trial version for Windows (MedCalc Software, Mariakerke, Belgium).

Results: The mean ROS levels in infertile men was significantly higher (p<0.05) as compared to controls. The mean DFI in infertile men was found to be 36.3, which is 2.6 times higher compared to average DFI in control men (13.5). The mean TAC levels in infertile men was significantly lower (p<0.05) as compared to controls. On applying spearman correlation analysis it observed that sperm DNA damage was positively correlated (r=0.736; p= 0.021) with ROS and negatively correlated (r= − 0.812; p=0.017) with TAC levels.

Conclusion: Seminal oxidative stress is a major cause of sperm DNA damage. DNA damage leads to recurrent spontaneous abortions, childhood cancers, genetic and epigenetic defects in offspring. Oxidative stress and sperm DNA damage assessment have better diagnostic and prognostic capabilities and should be included in diagnostic workup of patients experiencing IVF/ICSI failure.
15. IDENTIFYING INTERACTING PROTEINS OF INDUCIBLE NITRIC OXIDE SYNTHASE IN HUMAN TESTIS
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Introduction: Nitric Oxide (NO) is a signaling molecule that has pleiotropic regulatory effect and is important in various biological processes that include vasodilatation, neurotransmission, macrophage mediated immunity and carcinogenesis, etc. Studies with rats have reported that NO has both normal physiological effect on reproduction and inflammation-based infertility. NO has apparent capability to improve sperm viability and motility in both fertile and infertile individuals. However, NO has been implicated as a cause to obstructive azoospermia, one of the common causes of infertility. It has been reported that activation of inducible form of nitric oxide synthase (iNOS) led to age-related germ cell apoptosis, damage to seminiferous epithelium, and impairment of spermatogenesis in rats. Some studies also suggested NO mediate capacitation, hyper-activation and acrosome reaction that leads to fertilization. The enzyme iNOS, involved in the synthesis of NO, has been studied well in other tissues, and been found to be interacting with several putative proteins that may have regulatory functions. However, studies with iNOS in testis have not been done much.

Methods: We have employed Yeast Two-Hybrid (Y2H) assay to identify protein(s) that interact(s) with human iNOS by screening human testis cDNA library. Several iNOS protein fragments have been designed in our lab for construction of 'baits' in the Y2H assay.

Results: We have isolated about 200 'prey' cDNA clones till now that have shown strong interactions with the bait constructs.

Conclusion: DNA sequencing of the respective cDNA clones reveals some interesting proteins that are involved in acrosome function, cancer, proteasome, and some novel proteins.

17. HISTOMETRICAL AND QUANTITATIVE PARAMETERS OF SPERMATOGENESIS IN PUBERAL PIGS
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Introduction: There are some works published in this topic. In wild pigs there is an increase in testis size and seminiferous tubular diameter (Murta et al., 2012). In domestics pigs Ford and Wise (2011) reported increases of testicular diameter without other data are the aim of this work.

Material and Methods: Testes and epididymides of 8 domestic pigs (6 months old) were obtained from a local slaughterhouse. Samples of testis, head, body and tail of epididymis were processed by routine histological techniques, morphometry and cell quantification. Results were submitted to analysis of variance and Bonferroni test (p< 0.05).

Results: As expected, due to age homogeneity of the animals, testicular variables showed little dispersion. In epididymis from head to tail there is an increase of tubular an luminal diameters which is greater in cauda, known as the maturation segment in most mammals. Height of the epithelium decreases from cylindrical to cuboidal (Table 1), in correlation to the secretion or reabsorption of fluid by the epithelium, along the duct. In these same animals (Castro and Bustos, 2012) a good numbers of sperm were seen in sections of cauda. Cell count showed however, a low efficiency of the spermatogenic process (Table 2. CG/S index) suggesting germ cell loss during first meiotic prophase (apoptosis). Sertoli cells have already a fixed number (Castro and Bustos, 2012), though in wild boars this occurs later, at 7–8 months of age. In the present work elongated spermatids are seen at 6 months of age.

Conclusion: In domestics pigs at 6 months of age, testicular and epididymal histometric analyses showed that there are is sperm storage in cauda. Histological differential traits in the epididymis and Sertoly cells numbers estabilization at 6 months old, revealing the onset of puberty.

| Table 1. Histotemical parameters in testis and epididymis (μm) (X + SD) |
|----------------|----------------|----------------|
| Tubular diameter | Luminal diameter | Epithelial height |
| Testis | 267.55 ± 25.5 | 114.60 ± 24.44 | 64.90 ± 8.25 |
| Head | 291.58 ± 19.11 | 152.48 ± 6.06 | 49.38 ± 6.06 |
| Body | 419.38 ± 9.42 | 381.38 ± 4.91 | 47.61 ± 3.38 |
| Tail | 501.14 ± 9.77 | 461.33 ± 9.11 | 32.97 ± 7.84 |

n = 50 tubules per pig

| Table 2. Germ cells number/Sertoli cells in seminiferous tubules cross section |
|----------------|----------------|----------------|
| S | G | EPL | EPQ |
| N cells/tubule | 10.15 ± 3.06 | 65.83 ± 3.93 | 56.52 ± 4.41 | 50.15 ± 5.24 |
| CG/S index | --- | 6.48 ± 0.8 | 5.56 ± 0.6 | 4.94 ± 0.8 |

21.
ERBB4 IS ESSENTIAL AT PUBERTY FOR MAINTAINING ADULT MALE FERTILITY
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Introduction: ErbB family consists of four transmembrane receptor tyrosine kinases that mediate signals of the epidermal growth factor (EGF) family of ligands. ErbB receptors have important roles in embryonic development as well as in maintenance of adult tissues. Testis is one of the tissues where ErbB4 is most abundantly expressed. Its aberrant expression has been linked to germ cell tumors and, more specifically, to yolk sac tumors.

Objectives: Is ErbB4 pathway involved in male fertility?

Methods: Knock-out and knock-in mice, electron microscopy, optical projection tomography, immunohistochemistry, real time PCR.

Results: ErbB4 mRNA expression is first observed in the testis around embryonic day 14 in the germ and Sertoli cells. ErbB4 conditional knock-out mouse is generated by using MisCre mouse line which inactivates ErbB4 function specifically in the Sertoli cell lineage. The ErbB4 knock-out testes present several phenotypes after birth. These include structural alterations in cell adhesion and cell polarity of the seminiferous tubules which disturb the formation of the blood-testis barrier. Furthermore, the males were sub-fertile due to mis-localisation of the androgen receptor in the Leydig cells which induce low production of testosterone, and therefore the sperm production. When ErbB4 knock-out mice were mated with those expressing human ERBB4 in the Rosa26 Locus, the observed defects were rescued to a certain degree.

Conclusions: Taken together, these results suggest that ErbB4 signaling is involved in the regulation in the Sertoli cell polarity and thereby for male fertility by promoting maturation and maintenance of the testis, and its sperm production.

23.
EXPRESSION OF PARP1 IN INFERTILE MEN AND CORRELATION WITH DNA FRAGMENTATION INDEX
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Background: Maintaining genomic integrity in sperm is vital for birth of healthy offspring. Sperm DNA damage is associated with poor assisted reproductive technique (ART) outcome, birth of offspring with major congenital malformation, recurrent spontaneous abortions after assisted and spontaneous conception and even childhood cancers.

Purpose: This study was planned to analyze the expression levels of PARP1 in the sperm samples from both infertile (with normal semen parameters) and control (fertile) men and correlate with sperm DNA damage.

Methods: The study included both infertile men (n=30) and controls (n=17). The expression level of PARP1 was quantified by qPCR. For all samples sperm chromatin structure assay (SCSA) was performed and DNA fragmentation index (DFI) calculated.

Results: Relative quantification showed that the level of expression of PARP1 were significantly (P<0.0001) lower and percentage DFI significantly (P=0.0015) higher in sperm samples from infertile men compared to controls. There was a negative correlation between PARP1 levels and sperm DNA damage.

Conclusion: Abnormal expression of DNA repair enzymes explains for the persistence of DNA damage in sperm. PARP1 helps in recruitment of other downstream DNA repair genes. Thus optimal expression levels of this gene necessary for DNA repair. This may be the etiology of infertility or recurrent pre and post implantation loses following assisted reproduction in cases with idiopathic infertility. It is further being analyzed whether lower expression of PARP1 is secondary due to higher levels of methylation or sequence variations in the promoter region of this gene. Our study confirmed lower levels of PARP1 expression and high DFI in the sperm of infertile men compared to controls.
25.
EFFECT OF MOUSE SEXUAL MATURATION ON THE LEVELS AND CELLULAR CO–LOCALIZATION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN PRIMARY SERTOLI CELL CULTURES
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Introduction: Macrophage migration inhibitory factor (MIF) was originally identified as a cytokine that produced by activated T cells to inhibit macrophage migration. Leydig cells from adult rats were demonstrated to express MIF under physiological conditions. In previous study it was shown that Leydig cell-depleted testes continue to produce MIF, and suggested the existence of a mechanism of compensatory cytokine production involving the Sertoli cells. The aim of this study: is to examine the effect of mouse age on the capacity of Sertoli cell cultures to produce MIF.

Methods: Sexually Mature and immature (1 week – 12 weeks) BALB/c mice were sacrificed, and testes were immediately removed and decapsulated. Seminiferous tubules were enzymatically dissociated and the isolated tubular cells were fixed in cold methanol (for histological or immunofluorescence staining) or cultured for three days. After hypotonic shock, to remove residual germ cells, the cultures were grown in fresh media overnight. In the next day, media were replaced by fresh media with or without hormones (FSH and testosterone). After 4 – 48 hours of incubation, the supernatants (sups) were collected, and the cells were used for immunofluorescence staining and/or for RNA extraction. The levels of MIF in the sups were measured by enzyme-linked immunoassay (ELISA) using specific antibodies. RNA levels of MIF in Sertoli cell cultures were detected by real time PCR analysis using specific primers.

Results: Our results show that MIF expression in Sertoli cell cultures decreased with age at protein and RNA levels. The highest levels of MIF were detected in cultures of 1 week old mice. A significant decrease in MIF levels was observed in all other ages compared to 1 week old mice. Addition of hormones to the cultures of mouse Sertoli cells decreased their capacity to secrete MIF in most of the cases. MIF protein was specifically localized in the cytoplasm of Sertoli cells as examined by immunofluorescence staining.

Conclusion: Mouse primary Sertoli cell cultures constitutively produce MIF. MIF levels were decreased in Sertoli cell cultures in age dependent manner. This may suggest possible effect of Sertoli cell maturation in this process. Our results indicate possible involvement of hormones (FSH and testosterone) in the regulation of MIF production by Sertoli cell cultures under in vitro conditions. The physiological role of MIF in testicular function/spermatogenesis is not yet clear.

27.
ESTABLISHMENT OF A PROTEOME PROFILE AND IDENTIFICATION OF MOLECULAR MARKERS EXPRESSED IN MOUSE SPERMATOGONIAL STEM CELLS
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Introduction: Spermatogonial stem cells (SSCs) were undifferentiated cells to maintain spermatogenesis throughout the reproductive life of mammals. Although SSCs transplantation and SSCs culture provide a powerful tool to identify mechanisms regulating SSCs function, the precise signaling mechanisms governing SSCs self-renewal and the specific surface markers to purify SSCs remain to be further investigated.

Methods: In the present study, we established the steady SSCs culture according to Shinohara’s lab. Fertile progeny was produced after transplantation of culture SSCs into infertile mouse testes and red fluorescence can be stably and continuously transmitted to offspring. Next via advanced mass spectrometry and an optimized proteomics platform, we constructed the proteome profile with 682 proteins specific expressed in SSCs.

Results: Further bioinformatical analysis showed several known molecular regulated in SSCs were found in the list.

Conclusion: Those unknown proteins highly expressed in SSC provided adequate resources for us to explore the molecular mechanism of self-renewal and to search more specific surface markers of SSCs.
29. POTENTIAL EARLY BIOMARKERS OF TESTICULAR TOXICITY IN RAT EPIDIDYMAL FLUID

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Introduction: Preclinical evaluation of new drug compounds for target organ toxicity is an essential part of pharmaceutical drug development. Male reproductive toxicity is an important target organ to assess in this process. Currently, fertility testing as well as sperm count and tissue histology are the common means of identifying chemically-induced testicular injury in animals; and the translation of these results to humans is often unclear.

Objectives: Local injury within the testis is difficult to detect with blood-based biomarkers due to the limited movement of molecules across the blood-testis-barrier and potential dilution of biomarker by systemic circulation. The goal of this project was to identify testicular toxicity biomarkers from epididymal fluid with the intent that these early biomarkers could be used preclinically in early drug development for identification and mitigation of potential safety issues. Future investigations are planned to determine the translation of these biomarkers to clinical testing of semen.

Methods: Mature male Sprague-Dawely rats were treated with Carbendazim, a compound known to cause testicular injury. Epididymal fluid samples were collected post-mortem and analyzed by flow cytometry for spermatogenic germ cell morphometric alterations and changes in sperm membrane protein SP22 (sperm protein 22) levels. The epididymal fluid samples were also analyzed by immunoassay for IL-8 (Interleukin 8), TIMP-1 (tissue inhibitor of metalloproteinase 1), and MCP-1 (monocyte chemotactic protein-1). The biomarker responses were compared to histopathology and blood-based hormone biomarkers including Inhibin B and FSH (follicle-stimulating hormone).

Results: IL-8, TIMP-1 and MCP-1 and SP22 demonstrated dose responsive changes that correlated to minimal to slight microscopic findings in the seminiferous tubules and epididymis. Changes in SP22 were also observed earlier than histological observations, suggesting that SP22 may be an early sensitive biomarker of testicular toxicity.

Conclusion: Our results suggest that epididymal fluid biomarkers may be useful indicators of testicular toxicity for nonclinical safety testing early in the drug development process.

31. PROTEOMICS CHARACTERIZATION OF THE CYTOPLASMIC DROPLET OF EPIDIDYMAL SPERM

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Introduction: Spermatozoa of the epididymis of all mammalian species contain a cytoplasmic bulge along the flagellum called the cytoplasmic droplet (CD). This structure reveals an aggregate of flattened cisternal membranes, although not organized as a stack. We hypothesize that the CD may be relevant to sperm maturation and that the internal membranes of this structure are in part derived from the Golgi apparatus of germ cells of the testis.

Methods: To test this we determined the protein makeup of the isolated fractions of the CD of epididymal sperm (CDF) and compared this to the proteins of whole testis germ cell Golgi apparatus (TGF) using subcellular fractionation. The homogeneity of the fractions was assessed by marker protein enrichments and quantitative electron microscopy.

Results: Quantitative tandem mass spectrometry revealed that 765 proteins were shared between the two structures; these accounted for 71% of the total protein mass. Of the 765 proteins, a total of 62 proteins were examined by immunohistochemistry (IHC) in the testis and epididymis. Quantitatively, the most abundant CD proteins were those for glucose transport (glut3) and metabolism (hexokinase 1) and both were localized in situ to the CD by IHC. In addition, the CDF contained proteins characteristic of ER and Golgi apparatus both by proteomics and IHC of in situ epididymal sperm. By contrast, the TGF enriched in germ cell Golgi apparatus revealed that the most abundant protein as a novel putative glycosyl transferase (GL54D), as well as other established germ cell Golgi glycosyl transferases again verified by IHC in situ. Golgi proteins conserved between the CDF and TGF and verified by IHC included those proteins involved in structure and identity, including the P24 transmembrane proteins, the ATPase NSF, the GTP exchange factor-GBF1, and COPI coatamer proteins. However Golgi proteins related to glycosylation were not retained including GL54D and the germ cell Golgi specific mannosidase. While the internal membranes have a protein makeup characteristic of Golgi and ER, most of the functional proteins of the germ cell Golgi apparatus (GL54D, mannosidases, etc) are removed during spermiogenesis in the testis. The remaining Golgi proteins are related to membrane structure.

Conclusion: We conclude that the CD is a unique structure whose membrane components and molecular constituents work as a molecular machine to coordinate energy production coincident with the acquisition of sperm motility.
33. A 1.1 MB SEGMENTAL DELETION ON THE X CHROMOSOME CAUSES MEIOTIC FAILURE IN MALE MICE
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Introduction: The mammalian X chromosome contains a large number of multicopy genes that are expressed during spermatogenesis. The roles of these genes during germ cell development and the functional significance of gene multiplication remain mostly unexplored, as the presence of multi-copy gene families poses a challenge for genetic studies.

Methods: Here we report the deletion of a 1.1 Mb Nxf2−Nxf3 segment of the mouse X chromosome that is syntenic with the human Xq22.1 region and contains 20 genes that are predominantly expressed in testis and brain, including 3 members of the nuclear export factor gene family (Nxf2, Nxf3, and Nxf7) and 5 copies of preferentially expressed antigen in melanoma-like 3 (Pramel3).

Results: We have shown that germline-specific Cre/loxP-mediated deletion of this 1.1 Mb segment is efficient and causes defective chromosomal synopsis, meiotic arrest, and sterility in male mice.

Conclusion: Our results demonstrate that this 1.1 Mb region contains one or more novel X-linked factors that are essential for male meiosis. Moreover, this mouse mutant serves as an animal model for the human Xq22.1 deletion syndrome.

35. TESTICULAR GERM CELLS ACTIVATE INFLAMMATORY SIGNALLING PATHWAYS THROUGH TOLL-LIKE RECEPTORS AND STIMULATE CYTOKINE PRODUCTION
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Introduction: Spermatogenesis relies on communication between developing germ cells and supporting Sertoli cells, however the mechanisms by which these cells communicate are poorly understood. Cytokines, including tumour necrosis factor α (TNFα), interleukins (IL1α, IL1β, & IL6) and activin A are regulated by germ cell interactions throughout the cycle of the seminiferous epithelium. These inflammatory cytokines play an important role in spermatogenesis under normal physiological conditions. However, during inflammatory conditions cytokine synthesis can be up-regulated in the testis impairing normal spermatogenesis and steroidogenesis. Normally, production of inflammatory cytokines occurs through the Toll-like receptor (TLRs) pathways critical to the innate immunity. TLRs 1-6, which signal via adaptor proteins, MyD88 or TRIF, during innate immune responses to infection, have been identified in Sertoli cells. Moreover, our unpublished studies have shown that spermatogenic disruption occurs in MyD88-null mice, suggesting the involvement of TLR/MyD88 signalling in communication between the germ cells and Sertoli cells during normal spermatogenesis. The objective of this study was to evaluate the role of TLR signalling in testicular germ cell communication.

Methods: Intact spermatogenic cells were isolated from adult mice and separated into purified subsets representing three stages of their differentiation: round spermatids (RS), pachytene spermatocytes (P), and residual bodies (RB). Analyses of IL1α, IL1β, IL6, interferon1β and TNFα gene expression from primary mouse.

Results: Sertoli cells showed that germ cells modulate cytokine expression in Sertoli cells in vitro. RAW-ELAM macrophages, containing an NFkB-luciferase reporter, were significantly stimulated by RS, P and RB. Similarly, murine iMac macrophages secreted the cytokines, TNFα and IL6, when treated with RS, P and RB. P were significantly more effective than either RS or RB in both cell types. Germ cells did not stimulate cytokine production from iMac cell knockout lines lacking TLR2, TLR4 or MyD88, but were effective in cells lacking TLR3 or its unique adaptor protein, TRIF. Taken together, these data indicated that germ cells can activate TLR2/4 and MyD88 signalling in cells expressing these receptors.

Conclusion: In summary, this study shows that intact testicular germ cells activate TLR pathways to induce cytokine production, which may play important roles in regulating the seminiferous epithelium during normal spermatogenesis.
37. SPERMATOGONIAL ACTIVITY IN THE TESTES OF PREPUBERTAL BOYS
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CVV, AMC

Introduction and Objectives: Infertility is a frequent adverse effect of gonadotoxic cancer treatment in prepubertal boys. Preclinical research on in vitro propagation and autotransplantation of spermatogonial stem cells (SSCs) from cryopreserved testis biopsies has been initiated to open a way to future clinical interventions. For efficient transplantation later in life, after the cure of cancer, the required number of SSCs and subsequently the volume of the testis biopsy have not yet been determined. Our aim was to determine spermatogonial density during prepuberty so that an indication of the required biopsy volume for successful transplantation can be given.

Methods: For this purpose, a systematic review of the literature on spermatogonial density and the number of spermatogonia per tubular cross section in the prepubertal human testis was undertaken. A meta-regression of the data was performed to identify a general pattern of spermatogonial density during prepubertal testicular development.

Results: From a total of 125 papers, 18 studies were included in this analysis. Spermatogonial density and spermatogonial counts per tubular cross section showed a decline during the first three years after birth. In the following years up to puberty, a gradual increase was observed from the ages 3 till 5, ages 8 till 10 and from the age of 10 onwards.

Conclusion: Using the spermatogonial density for the corresponding age groups, the biopsy volumes of 0.13 cm³ between 1 and 7 years and 0.03 cm³ between 7 and 13 years of age should be sufficient to colonise all SSC niches in an adult testis after 64 days in vitro propagation and transplantation of SSCs from this biopsy.

39. CHARACTERIZATION OF UBIQUITIN CARBOXYL-TERMINAL HYDROLASE L1 IN MOUSE SPERMATOGONIA
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Introduction and Objectives: Spermatogenesis is a highly coordinated and complex process, which requires a fine balance between self-renewal and differentiation of Spermatogonial Stem Cells (SSCs) to support continuous sperm production. In the mammalian testis, SSCs represent a subset of undifferentiated Type A spermatogonia that are located at the basement membrane of the seminiferous tubules. The mechanisms that govern the fate decision of SSCs to maintain the stem cell identity versus to initiate differentiation remain largely unknown. Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is a deubiquitinating enzyme that is selectively expressed in the brain, testis and ovary. It has been implicated in neurological diseases as well as oncogenic processes. The objectives of this study were 1) to characterize the expression pattern of UCH-L1 in spermatogonia in vivo and in vitro by immunohistochemistry and RT-PCR; 2) to investigate the functions of UCH-L1 in spermatogonia by siRNA-mediated knock-down.

Methods: In the neonatal testis, UCH-L1 is detected in prospermatogonia. Later on, the expression of UCH-L1 is restricted to undifferentiated spermatogonia (Asingle, Apaired, Aaligned) that are associated with the basement membrane of the seminiferous tubules. UCH-L1 is rapidly down-regulated in differentiating spermatogonia and completely undetectable in spermatocytes and spermatids. UCH-L1 was abundantly expressed in the immortalized type A spermatogonial cell line C18-4 and was highly up-regulated in C18-4 cells undergoing mitosis. UCH-L1 was also detected in SSC clusters grown in vitro.

Results: Interestingly, we observed variable expression of UCH-L1 in daughter cells within a SSC cluster, similar to what we reported previously in vivo. This suggests that undifferentiated spermatogonia are heterogeneous at the molecular level and that variation in the UCH-L1 level may serve as a potential means of regulating the fate decision of spermatogonia. When UCH-L1 expression was down-regulated by 50nM UCH-L1siRNA to a level equivalent to 4% of normal UCH-L1 expression in C18-4 cells, cell proliferation was significantly inhibited (p<0.0001).

Conclusion: This result suggests that UCH-L1 likely regulates proliferation of undifferentiated spermatogonia. The mechanisms of the proliferation defect and transcriptional and post-transcriptional targets of UCH-L1 in undifferentiated spermatogonia are currently under investigation.

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**PURINERGIC ION CHANNELS IN MOUSE SPERMATOGONIA**  
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**Introduction:** Spermatogenesis is a fundamental and highly complex biological process that ensures male fertility. Spermatogonia are the precursors of all male germ cell stages. Their differentiation assures the lifelong production of mature sperm. However, few physiological details are known about testicular cell communication during spermatogenesis. As we and others have previously shown that Sertoli cells are able to communicate via ATP, we hypothesize a general role for purinergic signaling in the testis.

**Methods:** Using wildtype C57BL/6 mouse pups, we first developed a coculture of Sertoli cells and spermatogonia. Next, we investigated ATP-dependent signaling by whole-cell patch-clamp recordings from cultured spermatogonia. Involved ion channel subtypes of the P2X receptor family were then identified by pharmacological profiling and gene expression knockdown.

**Results:** Here, we report that cultured spermatogonia respond to extracellular ATP (1 – 100 µM). ATP-induced currents show fast activation and moderate desensitization. The current-voltage relationship reveals strong inward rectification. Current potentiation by ivermectin and inhibition by an acidic extracellular pH (6.3) indicate a functional role of P2X4 receptors. Interestingly, an increased ATP concentration (1 mM) activated an additional current with different kinetics, likely mediated by the low sensitivity P2X7 receptor. Knockdown of P2X4 expression significantly attenuated sensitivity to ATP concentrations ≤ 300 µM. Combined with molecular evidence, our results indicate that at least two different of P2X receptor subunits (P2X7 and P2X4) are functionally expressed in spermatogonia of young prepubertal mice. Downstream P2X receptor activation, a slowly activating calcium-dependent potassium current represents a functional antagonist of the depolarizing P2X2 receptors.

**Conclusion:** Together, these data represent a first important step towards a deeper understanding of cellular purinergic communication during spermatogenesis.

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**43.**

**PEAK RETINOIC ACID LEVELS COINCIDE WITH SPERMATOGONIAL DIFFERENTIATION DURING A SYNCHRONIZED FIRST SPERMATOGENIC WAVE**  
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**Introduction:** The active metabolite of vitamin A, retinoic acid (RA), is essential for spermatogenesis. Recent in situ hybridization and protein localization studies of the retinoid metabolizing and signaling machinery have correlated a rise and fall in RA levels with particular stages of the seminiferous epithelium. However, RA levels have yet to be accurately quantitated during the 8.6 day long murine spermatogenic cycle nor linked with specific differentiation steps during male germ cell development. The bis-(dichloroacetyl)-diamine, WIN 18,446, was recently shown to block the conversion of vitamin A to RA in the mammalian testis.

**Methods:** This current study investigated whether treatment of neonatal mice using a combination of WIN 18,446 and RA could drive a synchronous first wave of spermatogenesis and allow the investigation of RA levels during spermatogonial differentiation and meiotic onset. Oral treatment of 2 day old mice with WIN 18,446 for 7 consecutive days followed by an injection of RA 24 hours later (WIN 18,446/RA treatment) resulted in the simultaneous differentiation of almost all spermatogonia throughout the entire testis and synchronous spermatogenesis.

**Results:** Based on nuclear morphology, cell cycle analysis with BRDU and markers of spermatogonial differentiation (Stra8, Kit), the differentiating spermatogonia in WIN 18,446/RA-treated testes appeared to move through their five mitotic divisions in a uniform manner during the first cycle of the seminiferous epithelium, with testis tubules full of preleptotene spermatocytes and newly differentiating, STRA8-positive type A1 spermatogonia present 8 days post injection. Preliminary measurements of RA levels within testes collected from WIN 18,446/RA-treated animals that had been left to recover for either 24 hours, 4 days or 8 days post RA injection suggest that RA is synthesized every 8 days in the testis and coincides with the appearance of newly differentiating spermatogonia and preleptotene spermatocytes.

**Conclusion:** These data suggest that entry into the cycle of seminiferous epithelium, represented by the periodic differentiation of spermatogonia, is triggered by the tightly regulated rise and fall in RA levels along testis tubules and that transient treatment of mice with WIN 18,446/RA will be a useful tool for investigating stage-specific mechanisms during spermatogenesis.

**Funding:** This work was supported by the NIH Grants R01 HD10808 to MDG and U54 HD04245 NI.
45. EFFECTS OF DIFFERENT METHODS OF CRYOPRESERVATION ON SPERM DNA INTEGRITY IN NORMOSPERMIC AND OLIGOASTHENOTERATOSPERMIC MEN
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Introduction: Sperm Chromatin Structure Assay (SCSA®) is becoming a popular technique to evaluate sperm chromatin for clinical and research purpose. Measurement is commonly done in batch with samples that are cryopreserved with various methods. It is not clear if the method of freezing and storage affect the sperm chromatin structure. Our objective was to verify by SCSA® if different methods of cryopreservation and storage have an effect on human sperm chromatin structure compared to fresh samples.

Methods: Two categories of men, normospermia (N) (n=10) and oligoasthenoteratospermia (OAT) (n=4), were recruited. Raw basic semen parameters (motility, morphology and vitality) were analyzed according to WHO (2010) by CASA and by the VitalScreen kit (Somagen) and the sperm chromatin structure was assessed by SCSA®. Each sample was divided into four aliquots of 500 ul and processed as follow: 1) directly frozen in eppendorf at -80°C; 2) diluted (semen:extender ratio of 1:0.3) in Sperm Maintenance Medium (SMM; Irvine Scientific), cooled for 30 min at 4°C and frozen in eppendorf at -80°C; 3) diluted in SMM at a semen:extender ratio of 1:0.3; or 4) SpermFreezeTM (FertiPro N.V.) at a semen:extender ratio of 1:0.7. Each mixture from methods 3 & 4 was then transferred in a straw, suspended for 30 min in liquid nitrogen (LN) vapour and plunged into LN. After one month of storage, samples were thawed and analyzed for basic semen parameters and sperm chromatin structure using SCSA®.

Results: After direct freeze-thawing, sperm motility and vitality significantly decreased in N and OAT samples as compared to fresh semen. Freezing methods 2 and 4 preserved forward progressive motility of N and OAT samples; method 3 preserved forward motility of only N samples. The morphology was not affected by the freezing except for method 3 in N samples. The sperm chromatin structure, as measured by the DNA fragmentation index and the high DNA stainability, was not altered in frozen-thawed samples as compared to fresh semen in N and OAT.

Conclusions: Our results indicate that while the use of an extender is required to preserve basic semen parameters during cryopreservation, for research purposes with human sperm, the presence or absence of an extender and the various freezing methods used do not seem to affect the sperm chromatin structure. Further studies with other clinical categories of patients in a larger scale should be performed to confirm our findings.

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47. AMYLOIDS ARE A CONSTITUENT OF THE SPERM ACROSOMAL MATRIX CORE
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Introduction: Our laboratory has shown that the mouse sperm acrosomal matrix (AM) contains amyloids, that are self-aggregated proteins in a highly ordered cross β-sheet structure. Because amyloids are highly stable, detergent-resistant structures, we hypothesized that they form an AM core with which many of the soluble AM proteins associate.

Methods: In the present study experiments were undertaken to isolate and characterize the AM core. Purified AM were exposed to a two-step extraction to sequentially strip off soluble proteins. AM were first exposed to 1% SDS for 15min at 37°C followed by centrifugation at 42,000 X g to remove incompletely extracted AM and the supernatant centrifuged at 250,000 x g to pellet the extracted AM. The pellet was then extracted with 5% SDS or 70% formic acid for 15min at 37°C. From both conditions, detergent/acid-resistant structures were pelleted by ultracentrifugation suggesting the presence of a highly stable structure.

Results: Using conformation-dependent antibodies that recognize amyloids, we show that amyloids remain in the detergent and acid-resistant structures. However, in contrast to intact AM which are rich in the fibrillar forms of amyloid, the detergent/acid-resistant structures contained primarily oligomeric amyloid suggesting that the harsh treatment may be reversing the amyloid structures back to precursor forms. Examination of the 5% SDS resistance structures by reducing SDS-PAGE did not reveal any proteins by silver staining suggesting the AM core was not solubilized. However, incubation of the AM core in 7M urea/100 mM DTT prior to reducing SDS-PAGE allowed the detection of 8+ bands with molecular weights ranging between 15 kDa and 150 kDa with some proteins still remaining in the well which may be large MW proteins or complexes that were resistant to the solubilization conditions. To identify the proteins present in the AM core LC−MS/MS is currently being performed with the urea/DTT solubilized sample. Using immunofluorescence analysis, however, we show that the AM protein zonadhesin is present in the 5% SDS or 70% formic acid resistant structure suggesting it contributes to the formation of the AM core.

Conclusion: Taken together, our studies suggest that amyloids form the core structure of the AM and that several proteins (all or only some amyloidogenic) may contribute to its formation. The amyloid core may create a highly stable infrastructure for the differential release of acrosomal proteins during the acrosome reaction.

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49.
GESTATIONAL EXPOSURE TO HIGH FAT DIET AND BISPHENOL A REPROGRAMS SPERMATOGENESIS IN RATS
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Introduction: Increase in the incidence of male reproductive problems within the last few decades are too rapid to be explained by genetic factors alone, and suggest environment/lifestyle may play a significant role in their development. Since exposure to Bisphenol A (BPA) is ubiquitous and consumption of high-fat diets is deeply ingrained in our culture, prenatal exposure to both BPA and high levels of dietary fatty acids is a legitimate public health concern. Our objective was to study the relative contributions of high fat butter (HFB) diet and BPA on rat spermatogenesis.

Methods: We performed a pilot experiment, where Taconic Sprague-Dawley (SD) rats were exposed in utero to normal diet (AIN-93G-no-soy diet-16% kcal fat), HFB (HFB - 39% kcal fat), HFB plus 2.5, 25 (HFB/BPA25), 250 (HFB/BPA250), 2500 µg/kg bw per day BPA, and HFB plus 0.5µg/kg bw per day ethinyl estradiol diets.

Results: We found that adult males exposed in utero to HFB ± BPA had qualitatively normal spermatogenesis (Sp/sis) within the testis. However, when treated with testosterone (T) and 17β-estradiol (E2) for 20 weeks (which mimic the changed T:E2 ratios observed in aging males), the male rats exposed in utero to HFB ± BPA, and not the unexposed rats, exhibited impaired Sp/sis. Using hematoxylin and eosin staining, we found that on T+E2 treatment, 100% of HFB/BPA250 and 66% of HFB/BPA25, but not the unexposed rats; showed presence of >35% seminiferous tubules (STs) with Sp/sis arrested at Stage IX. STs were also examined for BRDT, Prm−1, H3S10phos, androgen receptor and aromatase Cyp19. Our results suggest a block either in spermatocyte differentiation into round spermatids, or in spermatid elongation. The HFB diet/T+E2 alone also induced an increase in impaired Sp/sis; however, the combined HFB/BPA250 showed a significance in relation to HFB alone.

Conclusion: We have thus shown that gestation is a critical window for dietary fatty acids–BPA interaction, that reprogram spermatogenesis, resulting in subtle changes in germ cell differentiation in adulthood. Moreover, these changes may go unnoticed, until critical secondary exposures occur, to unmask their effects. This study is of particular relevance since obese men show increased estrone and E2 levels, and stress, xeno−oestrogens, inflammatory cytokines and poor dietary choices up−regulate aromatase to increase intracellular E2.

51.
OPTIMIZED LENTIVIRAL VECTOR CARRYING INSULIN CDNA CAN STABLY IMPROVE DELIVERY OF INSULIN BY IMMUNE PRIVILEGED SERTOLI CELLS
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1TTUHSC; 2TTUHSC Post-Doc; 3TTUHSC Research Tech; 4TTUHSC Faculty

Introduction: Sertoli cells (SC) survive when transplanted across immunological barriers making them excellent targets as vehicles to deliver therapeutic proteins. Type 1 Diabetes is an autoimmune disease where the insulin producing beta-islet cells of the pancreas are destroyed. Current therapies sustain life, yet many complications still exist and replacement of beta-cell function is a major goal of research. Previously, SC transduced with adenovirus containing human insulin cDNA modified with furin cleavage sites, to allow for processing of proinsulin in non-endocrine cells, were able to restore normoglycemia in diabetic mice. However, the results were short lived due to the transient nature of the adenovirus.

Methods: This study utilized a lentivirus which incorporates DNA into the chromosome to transduce a SC line (MSC-1) with a furin-modified human insulin cDNA (LV-hl-MSC) to obtain stable, long-term production of insulin. MSC-1 cells were utilized since they maintain similar properties of the SC yet allow for more manipulation. LV-hl-MSC cultured in vitro stably produced insulin 11+ months post-transduction as shown by immunostaining and RT-PCR. In vivo, long term survival of LV-hl-MSC was confirmed by immunostaining for large T antigen of LV-hl-MSC allografts collected from diabetic BALB/c mice at 20 and 50 days. Insulin production in these grafts was verified by immunostaining and RT-PCR. However, due to low expression, normoglycemia was not restored. To increase expression and bioactivity of insulin, a second lentiviral vector was created with elements known to increase expression of the gene product and a furin- mouse insulin cDNA modified to increase bioactivity (LV-ml).

Results: It has been shown that 20 times the amount of human insulin compared to mouse insulin is required to lower blood glucose levels in mice. MSC-1 cells were transduced with LV-ml and then selected and sorted by flow cytometry for the highest expressing population. Insulin secretion in these cells, determined by ELISA, was 8.3 x10-8 ng/cell which is 8 times the amount of initial insulin secreted by L-hl-MSC. These cells will be transplanted in diabetic mice to test if the increase in expression and the alterations to increase bioactivity can stably lower blood glucose levels.

Conclusion: This study demonstrated that MSC-1 cells are able to stably express insulin long term in vitro and in vivo and expression levels can be increased in vitro.
Introduction and Objectives: Primordial Germ Cells (PGCs) are the precursors of gametes. For PGC male differentiation, (i) inhibition of meiosis and (ii) male-inducing factor(s) are essential. It was recently revealed that Fibroblast Growth Factor 9 (FGF9) is one of male-inducing factors.

Methods: Using isolated PGC culture system, we examined the function of FGF9 in PGC differentiation as follows: (1) how external FGF9 treatment affects PGC male differentiation, (2) what signal transduction pathways are activated by FGF9 treatment, and (3) effects of MAPK signaling pathways on PGC differentiation and proliferation.

Results: (1) We examined the effect of FGF9 on PGC male differentiation. XY PGCs at 11.5 and 12.5 dpc were cultured with various concentrations of FGF9 (0, 0.2, 1, 5, 25 and 100 ng/ml). We found low FGF9 treatment (0.2 ng/ml) increased expression of male specific markers (Dnmt3L and Nanos2) in PGCs. Conversely, high FGF9 treatment (25 ng/ml) suppressed these gene expressions compared with the control. Interestingly, high FGF9 treatment drastically stimulated PGC proliferation (40%) compared with the control and low FGF9 groups (5~10%). (2) Using Western blotting, we determined MAPK (ERK, p38, JNK) and AKT signaling pathways stimulated by low or high FGF9 treatment in XY PGCs. High FGF9 treatment significantly stimulated ERK signaling pathway in PGCs. In contrast, low FGF9 treatment enhanced p38 phosphorylation in the cells. (3) We investigated whether the ERK signaling pathway stimulated by high FGF9 regulates PGC proliferation. In the presence of both high FGF9 and an ERK inhibitor (U0126), PGC proliferation was suppressed, and Nanos2 expression was increased in XY PGCs. Next, we examined whether the p38 signaling pathway stimulated by low FGF9 treatment induces PGC male differentiation. In the presence of both low FGF9 and an p38 inhibitor (SB202190), Nanos2 expression was suppressed in XY PGCs at 11.5 and 12.5 dpc.

Conclusions: Our results suggest that (a) exogenous FGF9 has dose-dependent functions in XY PGC differentiation, (b) low FGF9 treatment stimulates p38 signaling pathway, especially α and β isoforms, in PGCs to promote PGC male differentiation, and (c) high FGF9 treatment activates ERK signaling pathway in PGCs to direct PGC proliferation, resulting in inhibition of PGC entry to either the male or female differentiation pathway.
EXOME SEQUENCING IDENTIFIES DPY19L2 DELETION AND MUTATION AS A CAUSE OF GLOBOZOOSPERMIA AND REPAIRABLE BY ICSI

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**Introduction:** Globozoospermia is a rare phenotype (incidence, 0.1%) causing male infertility with round-headed sperm and absence of an acrosomal cap. The acrosome is a giant vesicle which is required at the site of sperm-zonapellucida binding in the fertilization process. Men affected with globozoospermia are infertile because the deficiency of oocyte activation capacity but do not present chromosomal abnormalities.

**Objectives:** To improve our understanding of the genes involved in the pathogenesis of globozoospermia, we applied the whole-exome sequencing methods to identify genetic variants of the patients and their family members.

**Methods:** Using whole-exome sequencing of the patients and their healthy family members to find the patients only mutations. Later perform the PCR validation in the patients and controls we recruited latter to confirm the results of sequencing.

**Results:** We found a long deletion where DPY19L2 is located at this area of the patient (A-2:3) but not in his family members. PCR validation shows that five of the seven patients present with the deletion of DPY19L2. The patient (A-2:3) was treated with ICSI. After ICSI, 9 oocytes were successfully fertilized, 8 embryos developed to 6-8 cell stage on day 3, 2 of which were transferred into the uterus and resulted in a healthy girl (A-3:1). While we were analyzing the data, Harbuz reported that DPY19L2 causes male infertility and almost at the same time Isabelle Kosinski indicates that DPY19L2 deletion is the major cause of globozoospermia. We also found a new homozygous splice-site mutation on DPY19L2 splicing in a later collected patient (E-2:1). The insertion of the intronic sequences into the cDNA may cause a premature termination codon.

**Conclusions:** We demonstrated that DPY19L2 deletion is responsible for a majority of Chinese globozoospermia patients. And the homozygous splice-site mutation may damage the function of DPY19L2. It has the possibility for globozoospermia patients using intracytoplasmic sperm injection to obtain a healthy pregnancy.
57. CAN TESTIS SPECIFIC PP1GAMMA2, ESSENTIAL FOR SPERMATOGENESIS AND MALE FERTILITY IN MAMMALS, BE REPLACED BY A UBQUITOUS PP1 ISOFORM?
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Introduction: Serine/threonine phosphatase family constitutes PP1, PP2A, PP2B and PP2C. PP1 has four isoforms PP1α, PP1β, PP1γ1 and PP1γ2 encoded by three genes. The isoforms PP1γ1 and PP1γ2 are splice variants of Ppp1cc, which is comprised of 8 exons. PP1γ2 arises due to splicing of intron 7 that is retained in PP1γ1. These two isoforms are identical except for the 22 amino acid C-terminus tail of PP1γ2 expressed by exon 8. While PP1γ1 is ubiquitous in somatic cells including Sertoli cells, it is absent in developing male germ cells and spermatozoa. PP1γ2 is expressed at high levels exclusively in developing male germ cells. Mammalian spermatozoa contain PP1γ2 whereas non-mammalian spermatozoa contain PP1α or PP1γ1. The only phenotype observed in Ppp1cc knockout mice is male sterility due to impaired spermiogenesis. Recent transgenic rescue studies in our laboratory showed that PP1γ2, in the absence of PP1γ1, can restore spermatogenesis and fertility in Ppp1cc null mice (PLoS One. 2012; 7(10): e47623). Since PP1γ1 and PP1γ2 are identical in their catalytic properties, we examined if PP1γ1 could substitute for PP1γ2 in testis.

Methods: We generated four different transgenic PP1γ1 rescue mice lines. The first three transgenic lines were generated using cDNA that included a substantial or the entire portion of the 3' UTR of PP1γ1 mRNA. Results: Surprisingly, levels of transgenic PP1γ1 in testes of mice from all the three lines were low. Thus, there was no rescue of fertility in Ppp1cc null mice. Failure to achieve high levels of transgenic PP1γ1 expression led us to hypothesize that PP1γ1 mRNA containing intron 7, expressed in somatic cells, may be unstable in testicular germ cells. We made a fourth PP1γ1 transgenic rescue line with a cDNA construct, which mimicked PP1γ2 mRNA lacking intron 7. Mice from this rescue line expressed high testis levels of transgenic PP1γ1.

Conclusion: Results show that spermatogenesis was restored in the rescue mice and the males examined so far are sub-fertile. Our studies raise the intriguing possibility that PP1γ2 may have arisen in mammals to enable high levels of PP1 expression during spermatogenesis rather than for its unique biochemical properties.

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59. EPIDIDYMITS/ORCHTIIS RATIO DEMONSTRATES THE ROLE OF THE EPIDIDYMIS AS A BARRIER AND POWERFUL “BODY GUARD” AGAINST TESTICULAR INFECTIONS
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Introduction: Infections in the reproductive tract are among the most common diseases affecting male reproductive health. The mechanisms involved in the protection of the testis should be better understood as well as the role of epididymis in the process.

Objectives: To determine the ratio of epididymitis/orchitis and related factors and cofactors that determine each type of infection in a population of men looking for evaluation due to “testicular pain”.

Materials and Methods: In the “testicular pain” ambulatory and Androscience: Men’s Reproductive Health Clinic; 270 men aged 17 to 72 y.o. had a complete history, physical examination and complementary tests to determine the cause and factors associated with pain from 2008 to 2012.

Results: The epididymis was involved in the pathogenesis of “testicular pain” in 75/270 patients (27.7%), being 68 cases of epididymitis, 7 cases of orchiepididymitis (22/68 cases of painful epididymal cysts); orchitis as an isolated cause of “testicular pain” was only observed in 11/270 cases (4.0%) and had interesting cofactors: hernia repair (5 cases), cryptorchidism (2), retractile testis (2) cases and intermittent testicular torsion (1), trauma (1). Prostatitis was diagnosed in combination with epididymitis in 16 patients, but was never associated with orchitis. Interestingly, the presence of varicocele and history of vasectomy was not a significant cause of epididymitis and/or orchitis.

Conclusions: This objective study based on observation, history, physical examination and simple urine and semen tests demonstrated that the epididymis acts as physiological barrier against testicular infections and only when other cofactors disturb the imbalance of physiological functions associated with epididymial physiology the testis becomes involved in the the process of disease.
61.
EVIDENCE THAT THE DNA LICENSING PROTEIN ORC2 BINDS SPERM ORIGINS AT NUCLEAR MATRIX ATTACHMENT SITES
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Objectives: We have proposed that the sperm nuclear matrix provides critical information for the paternal DNA to function properly after fertilization. Here, we tested one potential function of this level of chromatin organization, the identification of DNA replication origins. How mammalian cells define origins remains a challenge in the field. It is known that ORC2 is the first protein to bind to DNA origins to initiate DNA replication licensing, the full loading of helicases at replication origins. But mammalian ORC2 binds to DNA non–specifically, so another mechanism must identify origins. In this study we tested the hypothesis that the attachment sites of DNA to the sperm nuclear matrix identify origins of replication.

Methods: We first treated oocytes with cyclohexamide (CHX) to determine whether ORC2 needed to be translated before licensing in early G1. Next, sperm nuclear halos, in which all the DNA is naked of proteins except where they attach to the nuclear matrix every 25 kb or so were injected into oocytes. Sperm halos cannot activate oocytes. We examined halo injected oocytes for 1, 2 and 4 hrs after injection after injection without activation. Oocytes were stained for ORC2 and for MCM7, one of the last licensing proteins to bind to DNA origins.

Results: We found that oocytes treated with CHX retain ORC2 for up to 6 hrs, suggesting that ORC2 translation is not required for the initial licensing events. When sperm halos were injected into oocytes, 3 to 5 large ORC2 foci appeared in central regions of the sperm nuclei by 2 hrs after injection of sperm halos. These regions appear at the same regions where centromeres have been shown to exist in mammalian sperm nuclei. No MCM7 was found in sperm halos unactivated oocytes.

Conclusions: The data suggest several possibilities for licensing in the zygote. First, they support, but do not yet prove, our hypothesis that the sperm nuclear matrix identifies origins of DNA replication, because ORC2 only bound to DNA near the matrix. Second, it suggests that ORC2 might initiate licensing in a progressive manner, starting at central regions of the chromosome and moving to neighboring origins after these are licensed (this model has recently been proposed for C. elegans). Finally, it suggests that the initial ORC2 binding does not require activation, but further progression to MCM7 binding, does.

63.
POTENTIAL ROLE OF WNT SIGNALING IN MURINE SPERMATOGENESIS AND ITS MEDIATORS
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Introduction: Male infertility is a worldwide health problem with increasing incidence. Disruptions in the development of male germ cells and their supporting somatic cells are considered to underpin many cases of idiopathic disease. We and others have established that testicular cell communication via the canonical Wnt signaling pathway is required for normal germ cell development. Although chronic deletion of β-catenin from Sertoli cells allows essentially normal spermatogenesis [1], disruption of APC in post-meiotic cells causes germ cell arrest [2].

Methods: We achieved acute disruption of Wnt signaling in adults by administration of beta-naphthoflavone to two mouse models to increase and decrease Wnt signaling levels, respectively: AhCre Apcflox/flox and AhCre β-catflox/flox. Both exhibited disrupted spermatogenesis with distinct but overlapping phenotypes apparent within 10 hours and also at 4 days. Pathway disruption in both models lead to rapid post-mitotic germ cell loss, apoptosis and perturbed Sertoli cell morphology with altered distribution of the blood testis barrier protein, connexin 43. We currently are generating VasaCre β-catflox/flox mice to investigate the intrinsic role of Wnt signaling in the germline, for comparison.

Results: Our transcriptional profiling of wild type postnatal mouse testes has identified a cohort of developmentally regulated Wnt pathway transcripts, encoding Wnt receptors (e.g. Fzd4) and ligands (e.g. Wnt3, Wnt3a, Wnt5b, Wnt7a and Wnt8b). Transcripts measured at reduced levels in AhCre β-catflox/flox mutant testes correspond to those predicted to localize in post-mitotic cells.

Conclusion: These findings demonstrate that ongoing control of Wnt signaling in germ cells is essential for adult spermatogenesis, and we have identified pathway components which may be required to support ongoing male infertility.

[1] Chang et al., Development 2008, 135;1875 – 85
Introduction: The growth factor activin A is essential for normal testis development. Activin drives Sertoli cell proliferation directly and indirectly, by promoting pituitary follicle stimulating hormone (FSH) synthesis, and regulates the pace of the first wave of spermatogenesis and timing of adult fertility. The transcription factor Smad3 is important for mediating these actions. The related inhibin antagonizes activin production and activity. Whereas progress has been made in understanding activin actions during development, little is known of the effects of chronically altered activin signaling in the ageing testis. To investigate this, we examined mice lacking one copy of the Inhibin alpha (Inha+) gene and one or both copies of Smad3.

Objective: To assess the impact of altered activin signaling on daily sperm production (DSP) and endocrine hormones in young and ageing mice.

Methods: Serum FSH, activin A and total inhibin, testicular activin A and inhibin, testis weight and DSP were measured at 8 and 26 weeks (n>5/genotype). Data were analyzed by t-test or Mann-Whitney with GraphPad Prism 5.

Results: In 8 and 26 week Inha+/- mice, serum FSH and serum and testicular activin A levels were not different to Inha+/+, but serum and testicular inhibin were reduced by half (P=0.01). Inha+/- testes were 10% larger than wildtype at 8 weeks (P=0.003) and DSP was 50% greater (P=0.004). By 26 weeks, Inha+/- testes were 20% larger (P=0.001) and DSP 35% greater (P=0.007) however Inha+/- testes did not grow during adulthood and by 26 weeks, DSP was 33% lower (P=0.009). Smad3+/- testes grew by 20% (P<0.001) from 8 to 26 weeks, but Smad3+/- testes did not, resulting in a 25% difference in testis weight between genotypes at 26 weeks (P<0.0001). In Smad3+/- mice, serum activin was twice that measured in wildtype mice (P=0.002) and testicular activin/g testis was 1.5-fold higher (P<0.01) at both 8 and 26 weeks. In contrast, serum and testicular inhibin levels were not different at 8 weeks but at 26 weeks, Smad3+/- serum inhibin was reduced by half (P=0.012) despite inhibin production/g testis being 1.45-fold higher (P=0.004). Where measured, Smad3+/- values were equivalent to wildtype or intermediate between Smad3+/+ and Smad3-/-.

Conclusion: Dysregulated activin signaling significantly impacts upon adult testis growth, DSP and the HPG axis, with Inha+/- and Smad3 mice proving valuable tools for understanding mechanisms underlying age-related endocrine disturbances.

67.

IMMUNE PRIVILEGED SERTOLI CELLS SURVIVE AS ALLOGRAFTS BY INDUCING AN ANTI-INFLAMMATORY ENVIRONMENT AND REGULATORY T CELLS

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Introduction: Immune privileged Sertoli cells (SC) survive long-term and protect co-grafted cells when transplanted as allo- or xenografts. However, their survival/protection mechanism remains unclear. The objective of this study was to investigate the immune privilege mechanism of SC. Previously we have demonstrated that allotransplanted SC survived whereas MSC-1 cells (a mouse Sertoli cell line), which lack some of the immunoprotective abilities associated with SC, were rejected when transplanted as allografts. Microarray analysis revealed that genes involved in inhibiting humoral and/or cell mediated immune response were upregulated in SC. Therefore, we hypothesized that SC survive as allografts by inhibiting humoral and/or cellular immune response.

Methods: Analysis of the SC or MSC-1 cell grafts and serum from the transplanted animals for humoral immune (antibody production and complement deposition) response showed no IgG production whereas an IgM response was generated against the grafted cells. Complement deposition was not detected in any of the grafts throughout the study suggesting inhibition of humoral immune response is not the main mechanism for SC survival as the results were similar between SC and MSC-1 cells. On the other hand, significant increase in apoptosis (cell mediated death) was observed in MSC-1 cell grafts while very few apoptotic cells were detected in SC grafts throughout the study.

Results: Analysis of the grafts for immune cell infiltration revealed that macrophages and CD4T cells infiltrated both sets of grafts, whereas little to no CD8T cells were detected in MSC-1 cell grafts. Furthermore, early anti-inflammatory environment (high IL10, low TNFα and low IL17) promoting regulatory T cells (Tregs) was detected in SC grafts compared to MSC-1 cell grafts. Tregs are important for maintaining tolerance at immune privileged sites, increasing graft survival and preventing autoimmunity. Therefore, we hypothesized that T cells detected in SC grafts could be of regulatory phenotype. To verify this, double immunoflorescence for Tregs (CD4+Foxp3+ cells and CD8+Foxp3+ cells) was performed on both sets of grafts. A large number of CD4+Foxp3+ and CD8+Foxp3+ Tregs were detected in SC grafts whereas Tregs were either absent or very few were detected in rejected MSC-1 cell grafts.

Conclusion: Overall, this led to the conclusion that SC survive as allografts by inhibiting apoptosis, creating an early anti-inflammatory environment and increasing the number of Tregs at the graft site.
69.

CANNABIS SATIVA USE DECREASES TESTICULAR VOLUME, INCREASES PROLACTIN, INCREASES AROMATIZATION OF TESTOSTERONE TO ESTRADIOL AND ESTRONE AND HAS A NEGATIVE IMPACT IN SEMEN PARAMETERS AND SPERM QUALITY.

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Introduction: Marijuana (Cannabis sativa) is the most used illicit drug in the world. Sadly and unfortunately, the war against drugs in the American Continent has been very unsuccessful and disappointing due to high demand, particularly in the United States as well as in Brazil, surrounded by countries who permit or do not want to look closely at this issue for obvious reasons of high profits and corruption. Therefore, decriminalizing and allowing self use and even growth of the marijuana plant for personal consumption has been taken into consideration and advocated by many countries. There is an urgent need for more information by the scientific community to counsel health professionals, politicians, the military and support awareness of the general public. It is a national security issue, for all Nations.

Objectives: To evaluate the effects of marijuana use in testicular function expressed by testis volume, hormonal levels and complete semen analysis and some sperm functional tests.

Material & Methods: Thirty-seven users (group A) who looked for andrology evaluation due to several reasons (male infertility, testis pain, sexual complains, infection, etc.) or just as part of a general check up and compare with 190 pre-vasectomy (group B) aged matched individuals as control were analysed.

Results: Testis volume in the control group was 21.7mL as compared to 14.3mL in the marijuana group (p<0.001), Prolactin levels were higher in group B (p<0.005), Testosterone/Estradiol + Estrone ratio is decreased (<10) in group B as compared to group A (p<0.001). Although FSH and LH did not reach statistical significant difference, they were much higher in group B than group A, even with higher prolactin levels. Seminal pH levels were higher in group B (p<0.001). Group B has decreased total motility, total progressive motility (p<0.001); both WHO (p=0.002) and Strict criteria morphologies (p<0.001) were severely affected. Antisperm antibodies were higher in group B.

Conclusions: Marijuana use has deleterious effects on testicular function, decreases testis volume, affects spermatogenesis, and alters steroids metabolism increasing aromatization and increases prolactin levels. Has an overall negative effect in male reproductive and sexual health. Marijuana should not be considered softly as a “recreational drug.”
71. \hspace{1cm} \textbf{GENOME WIDE MAPPING OF DNA BREAKS DURING SPERMIOGENESIS}

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\textbf{Introduction:} Spermiogenesis involves the most dramatic changes in cytoarchitecture known to the eukaryotic world. Nuclear compaction of spermatids by chromatin remodeling is accompanied by a large number of DNA strand breaks as we have shown by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). A significant proportion of double-strand breaks (DSB) have been detected both by neutral comet assay and pulse field gel electrophoresis. Since these DSB occur in haploid spermatids, they cannot rely on homologous recombination for reliable, templated repair but rather on DNA end-joining processes known to be error prone. Thus this becomes a potential threat to the genetic integrity of the male gamete. Mapping of these endogenous breaks will therefore allow us to better characterize the chromatin remodeling process and study the mutagenic potential of spermiogenesis. In order to elucidate the potential for DSB to induce genetic diversity, our first objective was to achieve genome-wide mapping of the endogenous DNA strand breaks in spermatids.

\textbf{Methods:} We first developed a fluorescence activated cell sorting (FACS) strategy to separate round (steps 1 – 9), elongating (steps 10–13) and elongated (steps 14 – 16) spermatids. We obtained three cells populations with purity ranging from 95 – 100% as well as a good yield given the amount of starting material. We then set up a new technique termed “damaged DNA immunoprecipitation” (dDIP) which allow us to capture DNA breaks on a genome-wide scale. The immunoprecipitated DNA fragments were paired-end sequenced using an Illumina platform and quality libraries were obtained for each cell population reaching over 20 million reads each. A bioinformatics analysis was performed on these libraries using a sliding window strategy and several clusters of break sites were observed. The presence of DNA breaks in these regions is confirmed by dDIP followed with real-time PCR as well as by terminal transferase-dependant PCR (TDPCR).

\textbf{Results:} The first genome-wide map of the endogenous DNA strand breaks in spermatids has been obtained. The identified hotspots loci should facilitate the study of the genetic instability associated with the chromatin remodeling and its contribution to the genetic landscape of the mature spermatozoa.

\textbf{Conclusion:} dDIP can also be useful in mapping DNA strand breaks or damages in a large number of contexts such as aging, meiosis or chemotherapy.

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73. \hspace{1cm} \textbf{CHARACTERIZATION OF THE ROLE OF TUMOR NECROSIS FACTOR APOPTOSIS INDUCING LIGAND (TRAIL) IN SPERMATOGENESIS THROUGH THE EVALUATION OF TRAIL GENE−DEFICIENT MICE}

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\textbf{Introduction:} TRAIL (TNFSF10/Apo2L) is a member of the tumor necrosis factor (TNF) superfamily of proteins and is expressed in human and rodent testis. TRAIL is known to induce apoptosis via binding to its receptors DR4 (TRAIL-R1/TNFRSF10A) or DR5 (TRAIL-R2/ TNFRSF10B) in humans, and TRAIL-R (MK/mDR5) in mice. The functional role of TRAIL in spermatogenesis is not known.

\textbf{Methods:} Here we utilize TRAIL−/− mice to evaluate the possible role of TRAIL in germ cell development by measuring testis weight, germ cell apoptosis, and spermatid head count at postnatal day (PND) 28 (peripubertal) or PND 56 (adult). TRAIL−/− mice have significantly reduced testis to body weight ratios as compared to wild-type C57 mice. Also, TRAIL−/− mice (PND 28) show a dramatic increase in the basal germ cell apoptotic index (A.I., 16.57%) as compared to the C57BL/6J wild-type strain (5.16%).

\textbf{Results:} The A.I. in adult C57 mice declined to 2 %, but remained elevated in adult TRAIL−/− mice (7.6 %); indicating a sustained high incidence of germ cell apoptosis. Analysis of the markers for the extrinsic and intrinsic apoptotic pathway, cleaved caspase 8 and cleaved caspase 9, respectively, adult TRAIL−/− mice showed more positive tubules than C57 mice by two fold in both markers. Spermatid head counts in adult TRAIL−/− mice were dramatically reduced by 39% compared with C57 mice, indicating these animals suffer a marked decline in the production of mature spermatozoa.

\textbf{Conclusion:} Taken together, these findings indicate that TRAIL is an important signaling molecule for maintaining germ cell homeostatis and functional spermatogenesis.
75. 
STAGE-SPECIFIC EXPRESSION AND SUBCELLULAR LOCALIZATION OF LONG INTERSPERSED ELEMENT TYPE 1 (LINE-1) PROTEIN DURING MALE GERM CELL DEVELOPMENT
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Introduction: Long interspersed elements type 1 (LINE-1s or L1s) are the most abundant autonomous retrotransposon in the human and mouse genomes. Its expression in germ cells is tightly regulated in order to prevent excessive insertional mutagenesis to the germ line. Nevertheless, it is evident that L1s have evolved mechanisms to maintain in the germ line a sufficient level of activity that allows themselves to perpetuate in mammalian genomes.

Methods: As a first step in understanding such mechanisms, we performed a detailed analysis of L1 expression during male germ cell development by immunofluorescence. Two distinct waves of L1 expression were identified in both inbred and outbred wild-type mice. The first wave is found in gonocytes of embryonic testes. The second wave is coincident with the onset of meiosis, in both prepuberal and adult testes.

Results: Profoundly, the expression of L1 ORF1 protein is stage-specific during spermatogenesis. Additionally, L1 ORF1 protein appears to colocalize with some unspecified subcellular structures, distinct from chromatid body, in late spermatocytes and round spermatids.

Conclusion: We speculate that the stage-specific expression and subcellular localization of L1 proteins may facilitate L1 retrotransposition. The potential intersection between L1 expression and spermiogenesis will also be discussed.

77. 
INDUCIBLE GENE REGULATION IN THE RAT GERMLINE
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Introduction: The laboratory rat represents the most widely studied non-human species in reproductive biology. And, though long absent from the genetic toolbox of scientists, novel and effective options for disrupting gene expression in rats are rapidly surfacing. In a preliminary report, recombinant spermatogonial cultures were used to produce a rat colony of >100 mutant animals enriched with distinct gene traps in protein coding genes. A random sample of 18 mutant rat strains from this colony was analyzed in a pilot screen for reproduction phenotypes. Inability to reproduce was linked to 67% of the founder mutations in which ~28% of total homozygous mutants were embryonic lethal (i.e. Exoc6b, Slc1a3, Slc35a3, Txndc13 Zmynd8) (Medrano G, et al, unpublished – Hamra Lab).

Objective: Due to the importance of the rat model in reproductive sciences, and the prevalence of embryonic lethality in rats harboring gene traps in protein coding genes, we sought to establish a system for inducible gene regulation in the rat germline.

Methods: Inducible forms of CRE recombinase (Cre-ERT2) have been successfully applied to help conditionally regulate gene expression in mouse testes. Thus, here, rat spermatogonial cultures on a Sprague Dawley background were genetically modified to express an inducible CRE recombinase (ERT2-Cre-ERT2) under control of a Deleted in Azoospermia-like (Dazl) promoter element. Selected spermatogonia were used as donor germelines to produce the respective transgenic rat strains.

Results: A panel of 8 rat strains harboring single copy DAZL-ERT2-Cre-ERT2 transgenes (tgGC-iCre1-8 rats) were generated. Genomic sites of transgene integration were defined for each strain. Initial evaluation of tgGC-iCre1 rats demonstrated >100-fold higher expression of iCre mRNA in testes compared to next highest signals detected in kidneys and ovaries (qPCR). Germ cells within testes of tgGC-iCre1 rats, and, spermatogonial cultures derived from tgGC-iCre1 rats robustly expressed reporter genes from inducible floxed alleles in their genetic backgrounds after treatment with 4-hydroxy-tamoxifen (4OHT). Recombined alleles were transmitted to progeny only from the 4OHT-treated tgGC-iCre1 parents.

Conclusion: The tgGC-iCre rat strains represent novel and effective recombination drivers for inducible genetic manipulations in the rat germline.

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PLATELET−DERIVED GROWTH FACTOR RECEPTORS: A ROLE IN NEONATAL GONOCYTE AND EMBRYONAL TERATOMACARCINOMA CELL DEVELOPMENT
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Introduction: Male reproduction depends on the proper development of neonatal gonocytes, the precursors of spermatogonial stem cells. Failure of this process might lead to infertility or testicular germ cell tumors. Understanding gonocyte development could provide clues on the origins of these pathologies. We have previously shown that gonocyte proliferation requires both platelet-derived growth factor (PDGF)-BB and 17β-estradiol, involving MEK1/2 activation.

Methods: We identified a variant form of PDGF receptor β (V1-PDGFβ) that was increased during retinoic acid (RA)-induced gonocyte differentiation. V1-PDGFβ was also expressed during the RA-induced differentiation of mouse F9 embryonal teratocarcinoma cells. F9 cells proliferate in response to PDGF-AA. They differentiate into parietal endoderm, but also express Stra8, a marker of premeiotic germ cells, in line with being at a stage prior to somatic-germ lineage specification. Our goal was to get insight on the role of PDGFRs in gonocyte and F9 cell differentiation.

Results: Northern blot analysis showed that neonatal testes express PDGFR α and β variants. RA increased the expression of PDGFRα and β variants in isolated gonocytes and F9 cells. The identity of PDGFRα variants in F9 cells was confirmed by mass spectrometry. Overexpression of V1-PDGFβ in gonocytes increased Stra8 expression and the percentage of differentiating gonocytes, but did not prevent cell proliferation. In both cell types, the inhibition of PDGFR tyrosine kinase activity reduced RA effects on the mRNA expression of differentiation markers, measured by quantitative PCR, suggesting that one or more PDGFR forms need to be activated. MEK1/2 was activated in RA-stimulated F9 cells and its inhibition blocked differentiation towards the somatic lineage, shown by Collagen IV and Laminin B1 expression, while increasing Stra8 mRNA levels. This suggests that MEK1/2 might act as a switch for lineage specification in F9 cells. In contrast, RA-induced gonocyte differentiation, assessed by Stra8 mRNA expression, was independent of MEK1/2, but was inhibited by blocking SRC, JAK2, and STAT5 activities, indicating their possible role in gonocyte differentiation.

Conclusion: These results suggest that neonatal gonocyte and F9 cell differentiation are regulated through crosstalks between RA and PDGFRs using different downstream pathways. It will be interesting to find whether these variants are similar to the PDGFRα variant mRNAs previously identified in testicular germ cell tumors.

81.
The Localization of Retinaldehyde Dehydrogenases in the Postnatal Murine Testis
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School of Molecular Biosciences and the Center for Reproductive Biology

Introduction: Spermatogenesis requires the tight coordination and regulation of vitamin A, known to be vital in several steps of the spermatogenic cycle, including spermatogonial differentiation, meiotic initiation, reorganization of the blood-testis barrier, spermiogenesis, and spermiation. Retinaldehyde dehydrogenase (RALDH) enzymes play a crucial role in vitamin A metabolism, catalyzing the conversion of retinaldehyde to retinoic acid (RA), the active form of vitamin A in mammalian cells. There have been several studies that investigate the localization of ALDH1A1, ALDH1A2, and ALDH1A3 within the testis, but the reports are conflicting. Additionally, there is another RALDH enzyme, ALDH8A1 that has been shown to both metabolize retinaldehyde and be present in the postnatal testes, but its localization has yet to be determined. The goal of this study was to provide comprehensive localization analysis of all four RALDH enzymes in the murine testis.

Methods: Microarray analysis of a wild type postnatal testis time course showed increasing transcript levels of Aldh1a2, decreasing levels of Aldh1a1, while Aldh1a3 remained at a steady, low level from birth to adulthood. Similar to Aldh1a2, quantitative PCR reveals an increase in Aldh8a1 testis transcript levels as the animal develops. Aldh8a1 transcript localization was conducted via in situ hybridization and preliminary data suggests that it is present within the Sertoli cell. Protein localization of ALDH8A1 and ALDH1A1 showed that both enzymes were present within the Sertoli cells of the adult testis. Importantly, ALDH8A1 is the only RALDH enzyme that we were able to detect via immunohistochemistry in spermatogonia.

Results: ALDH1A2 and ALDH1A3 were both present within germ cells, specifically spermatocytes and spermatids. Leydig cells appear to have robust expression of all four RALDH enzymes. Additionally immunoblots were conducted to verify antibody specificity.

Conclusion: These results together provide insight into how and where RA is synthesized within the murine testis and contribute to the overall understanding as to how RA metabolism and signaling is regulated in the mammalian testis.

Funding: This work was supported by the NIH Grant HD 10808.
Introduction:
Spermatogonial Stem Cells (SSCs) maintenance is crucial for the continuous production of gametes in males. The balance between auto-renewal and maintenance of these pluripotent cells and their engagement in a differentiation way is controlled by their specific environment, the SSCs niche. It can be restricted to a distinct structure like the hub in drosophila, or dispersed at the periphery of tubular epithelium like in mammals. Dogfish is a vertebrate model showing a polarized testis. The SSCs niche is restricted to a thin ventral band separated from the cystic spermatogenesis by a large testicular blood vessel. Spermatogonial sub-populations were defined previously in dogfish on the base of mammalian and new markers (pou2/pou5f1, gfrα1, mcm6). The present work aimed to establish an in vitro model for dogfish SSCs niche in order to test the functional conservation in vertebrates of mammalian key regulators of SSCs fate.

Methods:
Primary cultures of the germinative area of dogfish testis were first established. This germinative area contains single, paired and A aligned spermatogonia in a dense somatic tissue. This area was dissected and enzymatically dissociated before plating. Enrichment in spermatogonia was obtained by a panning procedure using gelatin-coated plates. Serum was restricted to limit the proliferation of somatic cells. Nevertheless, somatic cells were necessary for the maintenance of the germinal lineage as several enrichment steps reduced primary cultures maintenance.

Results:
In optimized conditions, the formation of colonies was observed after three weeks. PCNA immunolocalization and BrdU labeling revealed proliferative cells. The precise identification of cells constituting the germinative area and present in primary cultures is ongoing. C-kit positive spermatogonia were identified. Estrogen receptor α (ERα) positive cells were also detected. ERα localization on testis sections showed that it was a marker of somatic interstitial cells, in agreement with studies in other vertebrates. Finally, SSCs were identified in the colonies by detection of phosphatase alkaline activity.

Conclusion:
In conclusion, a primary culture model of the germinative area was developed for the first time in a chondrichtyan model and new markers of SSCs niche were identified. Due to the dogfish phylogenetic position, at the base of vertebrates, this in vitro model will be useful to evaluate the functional conservation of mammalian key regulators of the SSCs niche.

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POSTER SESSION II

85.
OVER EXPRESSION OF THE HISTONE H3 DEMETHYLASE KDM1 IN SPERMATOGONIA ALTERS THE SPERM EPIGENOME, CAUSES ABNORMAL OFFSPRING DEVELOPMENT AND IS IMPLICATED IN TRANSGENERATIONAL EPIGENETIC INHERITANCE.

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Introduction: Through epigenetics the paternal environment can influence health and development of generations to come, a process called epigenetic inheritance. The mechanisms underlying epigenetic inheritance are largely unknown but may involve transmission via the sperm epigenome.

Methods: In order to determine the role of the sperm epigenome in offspring development as well as the transmission of epigenetic mutations across generations we designed a transgenic mouse model with an altered sperm epigenome. Transgenic mice over-express, specifically in spermatagonia, the lysine specific demethylase 1 (KDM1), which removes methylation from histone H3 lysine 4 (H3-K4). H3-K4 methylation has previously been localized to genes in sperm implicated in embryonic development (Brykczynska et al, 2010; Hammoud et al, 2009).

Results: Characterization of the offspring sired by heterozygous KDM1 transgenics revealed reduced survivability and a range of developmental defects. Importantly, this phenotype was not limited to offspring sired by transgenic males. Descendants of transgenics that did not carry the transgene themselves also sired abnormal offspring. These non-transgenic descendants were examined over three generations. By the third generation, males, who had a transgenic great-grandfather, no longer sired abnormal offspring, which suggests reprogramming of the sperm epigenome. Analysis of the sperm epigenome of transgenic mice by chromatin immunoprecipitation followed by genome wide sequencing (ChIP-Seq) revealed specific reductions of H3K4me2 at >2000 genes including those implicated in embryo development.

Conclusion: The persistent abnormal development depicted by this model suggests for the first time that alterations to the sperm epigenome are implicated in transgenerational epigenetic inheritance.

87.
CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF PIRNA−LIKE SMALL RNAs IN SOMATIC CELLS

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Introduction: PIWI-interacting RNAs (piRNAs) are small noncoding RNAs that are exclusively expressed in germ cells and interact with the germ cell-specific PIWI proteins. During mammalian germ line development in the fetal testes, piRNAs are imperative for defending the genome against potential repetitive element invasion (e.g. transposons). They also play an essential role during spermatogenesis in adulthood. Several previous studies have reported piRNA-like small RNAs (piRNAs) expressed in somatic cell lineages, although the functional importance of somatic piRNAs has yet to be determined.

Methods: Our sequencing study identified hundreds of additional piRNAs from murine small intestine, testicular Sertoli cells, and interstitial cells of Cajal. Sequencing results confirm that piRNA characteristics (e.g. length, clustering, and nucleotide composition) are similar to canonical germ cell piRNAs. Moreover, piRNA populations tend to favor biogenesis of primary transcripts (5’ uracil) with no enrichment of secondary piRNAs (10th nucleotide adenine)- a characteristic of ping–pong repression of transposons. Within the three somatic small RNA libraries no trace of ping-pong complementarity was detected. In addition, piRNAs had more representation in pachytene related clusters in comparison to pre-pachytene related clusters. To further characterize piRNAs as a novel class of small RNAs, murine conditional knockouts of DICER and DROSHA, two proteins involved with known small RNA production, were generated and sequenced.

Results: Differential analysis of sequencing results revealed that piRNAs are present after knockout, implying independence of their biogenesis from these two proteins. Furthermore, we look at the effects of MIWI and MIW12 universal knockout on the biogenesis of piRNAs. This study confirms that piRNAs are a separate and distinct population of small RNAs that should no longer be ignored from somatic small RNA transcriptomes. These small RNAs are identical to germ cell primary piRNAs but are produced by a currently unidentified somatic cell biogenesis pathway.

Conclusion: Here we show that piRNAs are most similar to pachytene piRNAs, largely sharing sequence similarity and genomic location with non-ping-pong generated piRNAs. In addition, our data on expression, characteristics, and potential post-transcriptional targets among piRNAs is the first substantial step to elucidating the function and origin of these obscure small RNAs in somatic cells.
89.

**ANDROGEN RECEPTOR AND MAINTENANCE OF THE BLOOD TESTIS BARRIER**

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**Introduction:** Sertoli cell tight junctions (SCTJs) of the seminiferous epithelium help create a specialized microenvironment in the testis to aid differentiation and maturation of spermatocytes and sperm from spermatogonial stem cells. SCTJs must be chronically built and broken, with high fidelity, to allow the transmigration of preleptotene spermatocytes to ensure the continuous production of sperm. Impairment of androgen signaling is Sertoli cell prevented remodeling. The androgen receptor (AR) regulated tight junction protein encoding gene, Claudin 3 (CLDN3), localizes to newly forming SCTJs and is absent in Sertoli cell androgen receptor knockout (SCARKO) mice.

**Methods:** We tested the in vivo requirement of this putative AR effector gene and compared the cellular and molecular phenotypes to a novel SCARKO mouse model.

**Results:** Cldn3 null mice are fertile, show uninterrupted spermatogenesis and exhibit fully functional SCTJs based on imaging and small molecule tracer analyses. However, gene expression studies to identify additional AR regulated tight junction genes revealed additional tight junction components that represent potential redundant factors in Cldn3 mutants. Also, we identified a novel component of SCTJs, Claudin13 and a CLDN3 paralog and an AR regulated non-canonical tight junction protein 2 isoform that shows compensatory upregulation in Cldn3 mutant testes.

**Conclusion:** Our data suggest both compensatory and redundant mechanisms function during the AR dependent SCTJ remodeling and these mechanisms provide the necessary robustness to ensure long-term fertility.

91.

**SPERM HISTONES INFLUENCE EARLY EMBRYONIC GENE EXPRESSION**

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**Introduction and Objectives:** To achieve the extreme nuclear condensation necessary for sperm function, most histones are replaced with protamines during spermiogenesis. Mature sperm retain only a small fraction of core histones bound to DNA in distinct nuclear domains and specific genes, raising the possibility of an epigenetic relevance of sperm histones for gene expression after fertilization.

**Methods:** Using genome-wide analyses of two mouse models with impaired poly (ADP-ribose) (PAR) metabolism exhibiting aberrant retention of histones in mature sperm, we identified specific loci of altered sperm histone retention using promoter tiling arrays. Differential gene expression in individual 2-cell embryos, derived from such sperm and undergoing the first major wave of zygotic genome activation, was determined using RNA sequencing and microarrays.

**Results:** Strikingly, pairwise analyses revealed that a significant portion of genes differentially expressed in these embryos was also differentially histone-associated in sperm. The results also suggest that abnormal sperm histone retention, especially when being excessive, may occur at least in part randomly giving rise to sperm individual variability. Nevertheless, the findings unequivocally show that the packaging of the paternal genome influences reprogramming of gene expression in the early embryo that is essential for continued development and point to a potential epigenetic control mechanism. The results also implicate that PAR metabolism, which is an environmentally responsive pathway in humans, is a mediator of such epigenetic inheritance through the male germ line. We speculate that metabolic pathways other than PARsylation may also influence sperm histone patterning, providing a possible explanation how paternally inherited epigenetic disorders may occur.

**Funding:** This work was supported by grants from the NIH (HD48837 to RGM, HD022681 to RMS, and U54HD068157 support to RMS and RGM). This work was partially supported by an NIEHS funded Environmental Health Sciences Core Center grant P30–ES013508.
93.
IDENTIFICATION OF THE TARGETS OF SUMOYLATION IN TESTICULAR CELLS
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Introduction & Objectives: Sumoylation (a covalent modification by Small Ubiquitin-like Modifiers or SUMO proteins) has emerged as a critical regulatory event in cell function and has been implicated in various diseases; however, its role in reproduction is largely unknown. Identification of the target proteins for sumoylation is a critical step towards understanding its cellular functions. However, unlike somatic cells, in which hundreds of sumoylated proteins have been identified and studied, targets and regulation of SUMO in the testis are mostly uncharacterized. Because testicular tissue is complex and multicellular in nature, identification of cell-specific targets of sumoylation is required. Therefore, the objective for this research is to identify and initially characterize SUMO targets in specific cell types during spermatogenesis.

Methods: Mice were sacrificed and their testes were removed and de-capsulated. After two enzymatic digestions, the cells were separated using STAPUT technique based on a gravity sedimentation of the cells through a 2% – 4% BSA gradient. The collected fractions were analyzed using flow cytometry to determine their purity.

Results: Microscopic analysis using anti-SUMO antibodies confirmed the fraction purity and revealed that the pattern of sumoylation was unchanged after the STAPUT separation as compared to the freshly isolated cells. Highly optimized immunoprecipitation procedure for an enrichment of endogenous sumoylated proteins by using specific antibodies covalently cross-linked to the bead matrix, highly concentrated lysates and immediate inhibition of isopeptidases to prevent desumoylation, was followed by mass spectrometry protein identification.

Conclusion: The identified targets of sumoylation included proteins involved in regulation of transcription, metabolism and stress response.

95.
THE PHOSPHOGlycerate KINase GENE FAMILY – AN EXAMPLE OF EVOLUTION BY GENE DUPLICATION
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Introduction: Susumu Ohno proposed the principle of “Evolution by Gene Duplication,” by which one duplicate copy maintains the original gene function while the other diverses and undertakes new gene function(s) by acquiring key sequence changes. We postulate that the mammalian phosphoglycerate kinase (Pgtk) gene family exemplifies Ohno’s principle. The intronless, autosomal Pgtk2 gene derived from the intron-containing, X-linked Pgtk1 gene via retroposition. The Pgtk1 gene retained the original ubiquitous, constitutive expression pattern, but is subject to repression in meiotic and postmeiotic spermatogenic cells due to meiotic sex chromosome inactivation (MSCI) and postmeiotic sex chromatin (PMSC). Because the Pgtk2 gene is autosomal, it is not subject to MSCI or PMSC. Among the specialties this gene has evolved are 1) testis-specific expression, 2) post-transcriptional delay prior to translation of the transcript, and 3) sperm-specific localization of the encoded protein.

Objectives: Our objective was to identify key sequence changes that facilitated the evolution of these specialties in the Pgtk2 gene subsequent to the duplication event.

Methods: We used a bioinformatics approach to mine mammalian genome databases to identify such changes in the 5’ regulatory region (responsible for transcriptional regulation), the 3-UTR region (responsible for post-transcriptional regulation), and the coding sequence (responsible for protein functions). Among 11 eutherian species examined, we found that Pgtk1 promoters have conserved a CpG-island, a pair of GC-boxes, a pair of CAAT-boxes, and an NF-1 sequence, whereas Pgtk2 promoters have lost the CpG-island, retained a single GC-box and a single CAAT-box, and gained an enhancer sequence (E3/E4) with homology to an NF-1 element.

Results: We also found that Pgtk1 3’-UTRs have retained several conserved elements, but that the Pgtk2 3’-UTRs have evolved two unique polypyrimidine-tract binding-protein 2 elements not present in the Pgtk1 3’-UTRs. We have not yet identified sequences in the coding region related to sperm-specific localization. We are currently examining Pgtk genes from metatherian (opossum and tammar) and prototherian (platypus) species to discover more extensive evolutionary history of the divergence of the mammalian Pgtk promoters and 3’-UTRs.

Conclusion: Our results to date support Ohno’s theory of Evolution by Gene Duplication.
97. EVIDENCE OF GERMLINE EPI MUTATIONS ASSOCIATED WITH DIMINISHED MISMATCH REPAIR GENE EXPRESSION IN NON−OBSTRUCTED AZOOSPERMIC MEN
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Introduction: The ability to produce progeny is a required trait for the survival of all mammalian species and as such infertility is considered by nature to be a genetically lethal state. The advent of assisted reproductive techniques, specifically intracytoplasmic sperm injections (ICSI), has aided in achieving pregnancies in couples with severe male factor infertility. Currently, ICSI births account for a significant number of children worldwide. There are concerns, however, that the use of ICSI for men with non-obstructed azoospermia (NOA) may possess implications for the offspring ranging from birth defects to inherited systemic defects.

Methods: Preliminary studies within our lab have suggested that an intrinsic defect in DNA replication and repair via abnormal or absent expression of key mismatch repair (MMR) genes may be an underlying cause of NOA in some men. Therefore, the elucidation of the molecular basis of mismatch repair deficiencies in NOA men may better help assess the risk involved in ICSI and its consequences for their offspring. We hypothesize a possible roll of germline epigenetic alterations, namely DNA methylation, in NOA men with MMR deficiencies. DNA methylation has already been shown to downregulate key MMR genes in Hereditary Non-Polyposis Colon Cancer.

Results: Genome wide analysis of the DNA methylation signatures from testicular biopsies and blood of 26 NOA patients and 5 fertile controls was carried out using the Infinium HumanMethylation450 BeadChip from Illumina. In 4 of our NOA patients, we observed significantly aberrant DNA methylation within MSH5, a member of the mutS family of proteins that are involved in DNA mismatch repair and meiotic recombination. These patients also exhibited downregulation of MSH5 expression when measured by qPCR. We also observed increased resistance to DNA alkylating agent, N-Nitroso-N-methylurea (MNU), in our NOA patients, which is indicative of a defective MMR pathway.

Conclusion: Alterations in a genes expression, independent of gene mutations, may provide a novel mechanism in copying a disease phenotype when coupled with ICSI. If aberrant DNA methylation is present in both the somatic and testis tissue of NOA men and coupled with abnormal expression, this may indicate an epimutation that can be transmitted to potential offspring.

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99. SPAG11B/C EXPRESSION IN AN ORGANOTYPIC CULTURE OF RAT WOLFFIAN DUCT
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Introduction: The Wolffian duct (WD) is the embryonic precursor of the epididymis, which is essential for sperm maturation. Androgens are critical for WD development, exerting their earliest developmental effects on the epithelium via paracrine signals from the mesenchyme. Our previous studies have revealed that the C isoform of sperm-associated antigen 11B gene (SPAG11B/C) was mainly immunodetected in the mesenchyme of the developing rat WD, but more abundant in the epithelia of the postnatal epididymis. Furthermore, SPAG11B/C immunodistribution overlapped the androgen receptor immunodistribution in the developing WD. In order to gain insights into the role and regulation of SPAG11B/C during WD morphogenesis, here we have characterized a WD culture model to test the expression and regulation of SPAG11B/C by androgens in vitro.

Methods: WDs were collected from male Wistar rats at embryonic (E) ages E17.5 – E21.5. Some WDs at E17.5 were cultured for 3 – 5 days on cell inserts floating on serum-free medium (DEMEM/F12 1:1 vol/vol; 1% insulin-transferrin-selenium; 50 ug/mL ampicillin), and their development was evaluated under microscopy. Experimental groups included WDs co-cultured with testis, as well as WDs cultured in the presence or absence of exogenous testosterone (10 nM). The expression of SPAG11B/C and markers of cell proliferation and differentiation was assessed by immunofluorescence.

Results: WD development in vitro presented a similar temporal pattern to its previously reported elongation/coiling in vivo. Immunostaining for cell markers Ki67, phosphor-histone H3, pan-cytokeratin, and smooth muscle actin was observed both in WDs tested at E17.5 and those collected and cultured for 5 days in the presence of testosterone. Additionally, the elongation/coiling pattern of WDs cultured with testosterone mimicked that observed in co-culture with testis and was abrogated by androgen deprivation. SPAG11B/C-immunoreactivity was evident in the mesenchyme of WDs co-cultured with testis or incubated with testosterone, whereas it was reduced in WDs cultured in absence of either testis or testosterone for 3 days.

Conclusion: Collectively, our data confirmed the suitability of this WD culture system to study androgen action and to investigate putative factors implicated in epididymal morphogenesis. Moreover, we have shown the androgen-dependence of SPAG11B/C expression in cultured WD. Support: Fapesp, CNPq, CAPES, Fogarty International Center, NIH–NICHD 069654 (BTH).
INTACT FULL LENGTH RNAS ARE WELL PRESERVED IN UNDAMAGED SPERM, IRRESPECTIVE OF CLINICAL SEMEN PARAMETERS
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Introduction: Male infertility is a common and complex health condition. Despite recent increased research efforts, the majority of infertile males are currently diagnosed with descriptive semen deficiency or idiopathic (unknown) male infertility. For these reasons, sperm RNA can be a valuable biomarker of spermatogenesis expression and fertilizing quality of germ cells used in a fertility clinic. However, the utility of sperm RNA is hindered by its inconsistent quantity, quality, and purity in part due to the presence of somatic cells in semen, the absence of ribosomal RNAs, and the dense cytoplasm of sperm.

Methods: Here, we present a practical isolation method and evidence of intact, full length sperm RNA in an extensive study of 84 spermatozoal samples that vary in sperm concentration, morphology, motility, and somatic non-germ cell content in semen. We minimized degradation of RNA by limiting damage due to chemical exposure and maintaining ejaculates at human body temperature (37°C). We compared our improved sperm RNA isolation with current gradient isolation. We isolated RNA using our approach in 65 sperm specimens and 19 gradient specimens.

Results: Intact full length ribosomal 18S and 28S RNAs and mRNAs were identified in the majority of samples (83%, 54/65) isolated by our new methods. In contrast, intact RNAs were present in 27% (5/19) of sperm samples isolated via the known gradient approach. Microelectrophoretic analysis of sperm RNAs isolated by our method revealed relatively uniform high molecular fractions, including strong 18S and 28S rRNAs and long length mRNAs regardless of clinical diagnosis and amount of somatic cell content in semen. RT-PCR analysis of 16 genes expressed in somatic and germ cells (at different stages of spermatogenesis) further support the notion that mature sperm contain high quality intact mRNAs. Moreover, we performed RNA-seq of spermatozoa from normozoospermic men, which corroborated these findings.

Conclusion: Importantly, detected mRNAs represent genes expressed in pre-meiotic and post-meiotic sperm, and during early embryogenesis, thereby supporting the model that sperm RNA, rather than being residual material, have a vital role in sperm survival, fertilization, and early embryogenesis.
103.
PRODUCTION OF FERTILE SPERM FROM THE COLLARED PECCARY (TAYASSU TAJACU) TESTIS TISSUE AND CELL SUSPENSION XENOGRAFTS.
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Introduction: Because the collared peccary (Tayassu tajacu) has a peculiar Leydig cell cytoarchitecture, this species represents an unique mammalian model for investigating testis function. Testis xenograft is a fascinating approach in which testis tissue or isolated testis cells are able to develop after being xenografted under the back skin of an immunodeficient mouse. Therefore, this technique is a powerful approach for investigating, for instance, testis cell interactions and spermatogenesis.

Methods: In the present study, testis tissue and testicular cell suspension from immature collared peccaries (n=4; 3 months old) were xenografted in SCID mice (n=32). Complete spermatogenesis was observed six months after testis tissue xenograft. For testis cell suspension xenograft, the cells interacted and de novo testis morphogenesis was observed two months post-surgery and spermatogenesis was established only at eight months after grafting.

Results: Compared to cell suspension, a higher percentage of seminiferous tubules cross-sections with complete spermatogenesis was observed in the testis tissue xenografts (31.6 ± 5.4% vs. 9.4 ± 5.3%). In comparison to the back skin of non-grafted SCID mice, the functional analyses using Laser Doppler Scanner showed that the blood flow was 3-fold greater in testis tissue and cell suspension xenografts. Indicating that the collared peccary xenografts secreted physiological amounts of bioactive testosterone, the seminal vesicle weight of xenografted castrated mice was similar to non-grafted intact mice (p>0.05). Using ICSI technique it was observed that the sperm recovered from the collared peccary cell suspension grafts were able to produce diploid embryos. Showing that the collared peccary grafted-ICSI embryos were not generated from parthenogenesis, the evaluated activity of the paternal genome expression of neuronatin (NNAT) was observed in grafted-ICSI embryos. Confirming therefore that syngamy occurred in these embryos.

Conclusion: Taking together, our findings demonstrated for the first time de novo testis morphogenesis and complete spermatogenesis progression, with the production of fertile sperm, from a mammalian wild species xenografts. Therefore, besides using these powerful techniques in conservation programs, testis xenografts could also be employed for investigating testis cells interactions, morphogenesis and spermatogonial stem cells physiology and niche in the collared peccary. Such studies are currently being developed in our laboratory.
105.

WHOLE EXOME SEQUENCING AS A POTENTIAL DIAGNOSTIC TOOL IN MALE IDIOPATHIC INFERTILITY
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Introduction: Nearly 15% of couples worldwide struggle with infertility, with male-specific infertility as the root cause in nearly half of all cases. Despite this high prevalence in the general population, there remains a large proportion of infertile male patients (25 – 50%) for whom a definitive diagnosis cannot be attained. We hypothesized Next Generation whole exome sequencing is a powerful tool to discover underlying genetic causes of infertility in idiopathic patients.

Methods: As an initial study, we sequenced genomic DNA from 10 well-characterized infertile men displaying a meiotic maturation arrest phenotype upon testicular biopsy. Following read alignment and variant calling, single (SNPs) and multiple (MNP) nucleotide polymorphisms were compared to the dbSNP public database (National Center for Biotechnology Information). Additionally, population frequencies of our observed polymorphisms were obtained from the international 1000 Genomes Project database (years 2010 and 2011).

Results: Comparison to OMIM (Online Mendelian Inheritance in Man), MGI Mammalian Phenotype (Mouse Genome Informatics) databases identified over 200 male fertility-associated genes in which SNPs, MNP, insertions, and/or deletions were present in one or more of our patients. Since not all nucleotide changes alter protein coding or mRNA/protein processing, we utilized a variety of prediction tools to identify potentially deleterious variants. Through these analyses, we have obtained a cohort of candidate mutations which meet our criteria of (a) appearing in a gene with established roles in male fertility, (b) occurring at a low frequency in the general population, and (c) producing a potentially deleterious change to either mRNA or protein products from said gene. To eliminate false positives, mutations of interest have been confirmed via PCR amplification of patient DNA followed by Sanger sequencing. In addition, we are screening our libraries of normozoospermic and proven fertile male DNA to confirm our candidate mutations are indeed unique to infertile men.

Conclusion: We propose whole exome sequencing could serve as a valuable complement to existing diagnostic tests in the male infertility clinic. Furthermore, analysis of gene variants in infertile male patients may elucidate new targets for screening and treatment.

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107.

VEGFA REGULATES THE BIOLOGICAL ACTIVITY OF GERM CELLS IN BOVINE TESTIS TISSUE GRAFTS
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Introduction: Evidence suggests that VEGFA may regulate the biological activity of undifferentiated germ cells during testis development. The objective of the current study was to determine the effect of VEGFA treatment on germ cell homeostasis in bovine testis tissue.

Methods: To accomplish this goal, testis tissue from 8-week old Angus bulls (n>5) was obtained, treated with vehicle (0 µg) or VEGFA (0.5 or 1.0 µg), and subcutaneously grafted onto immunodeficient mice for 32-wks as a biological assay for germ cell homeostasis. All datasets were analyzed by ANOVA. Treatment did not affect the recovery rate (p>0.05) or ability of testis tissue grafts to grow (p>0.05) following removal from recipients as evidenced by graft weights when compared to controls. Morphometric analysis of spermatogonia and meiotic germ cell populations were used as an endpoint for evaluating the long-term effects of treatments on the subsequent development of germ cells.

Results: In comparison to controls (83.33%), testis tissue ectopically treated with 0.5 µg and 1.0 µg of VEGFA at the time of grafting significantly increased (p<0.05) germ cell survival as evidenced by 100% of testis tissue grafts containing spermatogonia following the grafting period. Interestingly, VEGFA treated tissues also contained significantly more (p=0.002) differentiating germ cells when compared to controls. In contrast to controls, an increased proportion (p<0.05) of seminiferous tubule cross-sections containing meiotic germ cells were observed in VEGFA treated tissues, a result likely due to increased germ cell survival. Radioimmunoassay and biological assay of vesicular gland weights indicated no effect (p>0.05) of treatment on testosterone production by testis tissue grafts.

Conclusion: Taken together, these findings support the hypothesis that VEGFA regulates the biological activity of undifferentiated germ cells. Future studies will focus on elucidating mechanisms responsible for these differences.
109. MOUSE GERM CELL NUCLEAR ANTIGEN GCNA1 DEFINES A NOVEL FAMILY OF GERM CELL SPECIFIC PROTEINS
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Introduction: An antibody recognizing the protein GCNA1 was produced in 1994 and has been widely used as a marker of mouse germ cells. It is one of the few markers known to be expressed coincident with the transition undergone by germ cells as they cease migration and enter the gonad. However, the antigen has never been identified.

Methods: Here we identify GCNA1 as a protein that is largely composed of highly acidic repeats and contains four distinct amino acid repeat classes. GCNA proteins from distant species contain a rapidly evolving acidic domain which is predicted to be highly disordered.

Results: Every species examined except for rodents also has a highly conserved uncharacterized domain. Proteins with this domain define a novel family of germ cell specific proteins.

Conclusion: GCNA mutants exhibit chromatin condensation abnormalities in prophase of meiosis, fail to spermiate, and are sterile.