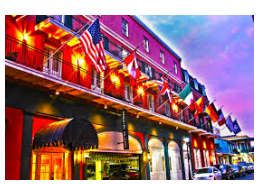
2016 ASA Basic Science Workshop

“ANDROmics: Genomics, Proteomics, and Epigenomics in understanding male reproduction”

New Orleans, Louisiana
Saturday, April 2, 2016
8:30:00a.m. - 4:30p.m.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Welcome from the Chairs

Welcome from the Chair of the Basic Science Workshop

Welcome to ASA and the 2016 Basic Science Workshop! I am incredibly excited to be a part of this amazing biennial addition to the ASA meeting program. The ASA Basic Science Workshop (BSW) acts as a platform for basic science andrology researchers at all stages of their career to gather and discuss techniques and tools important for their research. Over the years the BSW has covered a diverse range of topics from spermatogenic stage identification across species to RNA-sequencing analysis to electron microscopy. This year, we delve deeply into the "omics" of andrology, or "Andromics", and are thrilled to be hosting many excellent speakers with in depth understanding of cutting edge "omics" tools. These speakers run the gambit from newly minted PhDs to established pillars in their field and I am confident they will deliver thought provoking tools and concepts that you can apply to your research program. One of the primary goals of the BSW is to provide an opportunity for young researchers to share their expertise. The inaugural BSW, held in 2010, did exactly that for me. That workshop was my very first opportunity to give a talk at ASA and it was truly a transformative experience. The BSW was such a welcoming and invigorating experience it was a driving force behind my engagement in the ASA, a decision I am delighted with to this day. I sincerely hope you enjoy the 2016 BSW as much as I enjoyed the 2010 BSW and look forward to seeing how today influences your research in the future!

Elizabeth Snyder, PhD
Basic Science Workshop Chair

Welcome from the Program Chair of the 2016 Basic Science Workshop

Welcome to the new edition of the Basic Science Workshops. The theme of this year workshop is "*ANDROmics: Genomics, Proteomics, and Epigenomics in understanding male reproduction*" and we prepared an exciting program with leaders in the different fields of proteomics, epigenomics and genomics that will contribute greatly with the purpose of this workshops which is to share the novel techniques and strategies to answer research questions related to male reproduction. The andrology field is continuously evolving; it is then essential to look for new strategies to seek knowledge in order to design new diagnostic tools and therapies to help the infertile men. I strongly believe that our speakers will bring novel and state-of the-art technologies to help basic and clinical andrologists to accomplish the ultimate goal to assist infertile men to achieve fatherhood. I thank you very much all speakers that give their time to be part of this important event of our Society. I also thank you all who were involved to make this workshop a reality, particularly to Donna Rostamian, Executive Director at WJ Weiser & Associates, Inc., who tirelessly helped with the logistics to have a great meeting.

I truly hope that the 2016 Basic Science Workshop will be useful for your work and an enjoyable experience that will allow fruitful discussions, interactions and potential future collaborations.

Cristian O'Flaherty, DVM, PhD
2016 Basic Science Workshop Program Chair

2016 ASA Basic Science Workshop Committee

Elizabeth Snyder, PhD
Chair
Associate Research Scientist
The Jackson Laboratory
Bar Harbor, ME, USA

Cristian O'Flaherty, PhD
2016 BSW Program Chair
Associate Professor
McGill University
Montreal, Canada

Alan B. Diekman, PhD
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Professor
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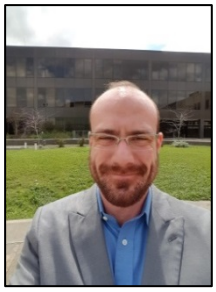
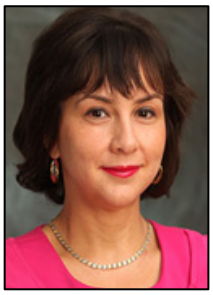
Fayetteville, AR, USA

Kate Loveland, PhD
Committee member
Professor and NHMRC Senior Research Fellow
Monash University
Clayton, Australia



Thomas Garcia, PhD
Committee member
Assistant Professor
University of Houston
Houston, TX, USA



Margarita Vigodner, DVM, PhD
Committee member
Associate Professor
Yeshiva University
New York, NY, USA

Sophie La Salle, PhD
Committee member
Assistant Professor
Midwestern University
Downers Grove, IL, USA

Program

8:30 a.m. – 8:35 a.m.	**Welcome and Opening remarks** Cristian O'Flaherty, DVM, PhD Program Chair McGill University -Research Institute-MUHC
8:35 a.m.-10:05 a.m.	**Session I – Proteomics and DNA integrity strategies** *Co-chairs: Alan Diekman, PhD and Martine Culty, PhD*
8:35 a.m. – 9:05 a.m.	**Sperm biomarkers for male infertility** Mark Baker, PhD University of Newcastle, Australia
9:05 a.m. – 9:35 a.m.	**The study of ion channels in sperm** Celia Santi, PhD Washington University
9:35 a.m. – 10:05 a.m.	**Assessment of sperm proteins and DNA integrity by flow cytometry** Elisabetta Baldi, PhD Florence University, Italy
10:05 a.m. - 10:25 a.m.	**Coffee Break**
10:25 a.m. - 12:05 a.m.	**Selected abstracts** *Co-Chairs: Mahmoud Aarabi, PhD and Timothy Jenkins, PhD*
10:25 a.m. - 10:45 a.m.	**Activation of NOTCH signaling in Sertoli Cells in vivo is dependent upon the presence of germ cells.** Thomas Garcia, PhD Division of Natural Sciences, School of Sciences and Computer Engineering University of Houston
10:45 a.m. - 11:05 a.m.	**3-Dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity *in vitro*** Hooman Sadri-Ardekani, PhD Wake Forest Baptist Medical Center
11:05 a.m. - 11:25 a.m.	**Paternal stress reprograms epigenetics marks in sperm and epididymis: implications for long-term transmission of experience** Jennifer Chan, Tracy Bale PhD University of Pennsylvania

Program

11:25 a.m. - 11:45 a.m.	**The Caenorhabditis elegans ortholog of human T-complex protein 11 (TCP11), M056.2, is necessary for male fertility** Matthew Marcello, PhD Pace University
11:45 a.m. - 12:05 a.m.	**Genomic studies in azoospermic and oligozoospermic males with male infertility** Alexander Yatsenko, PhD Magee-Womens Research Institute
12:05 p.m. - 1:00p.m.	**Lunch with Keynote Speaker** **Functional genomic approaches to define the stem cell state in male germ cells** Jon M. Oatley, PhD Washington State University *Introduced by Elizabeth Snyder, PhD*
1:00 p.m. – 4:20 p.m.	**Session II – Epigenetics, genome-scale tools and genetics models** *Co-chairs: Elizabetta Baldi, PhD and Mark Baker, PhD*
1:00 p.m. - 1:20 p.m.	**Assessing the sperm DNA methylome- past and future** Jacquetta Trasler, PhD McGill University, Canada
1:20 p.m. - 1:50 p.m.	**Whole genome sequencing-based approaches to assess the sperm DNA methylome** Mahmoud Aarabi, MD, PhD McGill University, Canada
1:50 p.m. - 2:20 p.m.	**Targeted and regional assessment of the sperm epigenome** Timothy Jenkins, PhD University of Utah
2:20 p.m. – 2:50 p.m.	**Coffee Break**
2:50 p.m. - 3:20 p.m.	**Genetic models in reproductive studies** Sophie La Salle, PhD Midwestern University

Program

2:20 p.m. - 3:50 p.m.	**Changing gene expression in isolated gonocytes** Martine Culty, PhD McGill University -Research Institute-MUHC
3:50 p.m. - 4:20 p.m.	**Reveal the physiological function of miRNAs in vivo** Wei Yan, PhD University of Nevada
4:20 p.m. - 4:30 p.m.	**Closing Remarks** Elizabeth Snyder, PhD BSW Chair The Jackson Laboratory

Invited speakers

Mark Baker, PhD
Associate Professor, University of Newcastle, Australia
Mark.Baker@newcastle.edu.au

Mark obtained his PhD from Monash University (2002) working within the cancer biology field. He was then recruited to Newcastle University by Laureate Prof. John Aitken to work on male gamete biology. During this time, he has established an active research program centered on the use proteomics to understand male infertility. He is now the Head of the Reproductive Proteomics Group that looks at understanding post-translational modifications that effect sperm function. Recently, Mark's group has had a growing interest in the causes of human male infertility. The quality of this work has been acknowledged through several awards including, NHMRC career development fellowships and the SRB RCRH Award for Excellence in Reproductive Biology Research (2015).

Invited speakers

Celia Santi, PhD
Associate Professor, Center for Women's Reproductive Sciences Research, Dept. of Obstetrics and Gynecology, Washington University School of Medicine
santic@wustl.edu

The interactions of the gametes involved in sexual reproduction represent a microcosm of molecular cell biology and physiology. My lab research focuses on those aspects of sexual reproduction that involve membrane excitability and control of membrane voltage in spermatozoa, which are essential for the sperm to fertilize the egg. In particular my work has been focusing in the role of potassium and calcium channels in sperm physiology, with special emphasis on the role that these channels play in sperm capacitation and the acrosome reaction. During development sperm mature in the epididymis where they remain quiescent. However, upon release from the epididymis they must undergo marked changes to increase their motility and capacity to fertilize the egg. These changes are labeled sperm capacitation and involve the acquisition of hyperactivated motility, and the ability to undergo the acrosome reaction, which entails a voltage and calcium-dependent exocytotic event. Ion channels in the sperm plasma membrane are essential molecules in the signaling processes that control sperm capacitation. As a PhD student in the National Autonomous University of Mexico [UNAM], I studied the role of calcium channels in sperm physiology. During my postdoctoral training in Dr. Terry Snutch's lab at the University of British Columbia (Canada) I carried out experiments studying the expression and modulation of low-voltage-activated calcium channels. As a Senior Scientist in Dr. Lawrence Salkoff's lab at Washington University I expanded my research to include molecular biology and electrophysiological experiments with the family of SLO potassium channels. As an Instructor I initiated an independent line of research combining my previous knowledge of the role of ion channels in sperm physiology and the expertise in molecular biology, genetics and biophysics that I acquired studying SLO family channels in the Salkoff lab. I focused my attention on one particular member of the SLO family of potassium channels (SLO3) which, because of its essential role in sperm physiology, is closest to my research interests. I established that the SLO3 channel is essential for sperm fertility and is a key determinant of sperm membrane potential. I determined that SLO3 knock-out (KO) mice are male infertile, do not hyperpolarize during capacitation, and have major defects in sperm motility and the acrosome reaction. Using the whole cell patch clamp technique I determined that SLO3 channels are responsible for the pH-dependent potassium current present in testicular sperm. In 2012 I become Assistant Professor and my laboratory has since laid the groundwork for understanding the role of the SLO3 K+ channel subfamily in sperm capacitation and the acrosome reaction. We have established the ionic basis that underlies the changes in membrane potential seen during sperm capacitation and we recently proposed a model that links the changes in membrane potential and pHi seen during capacitation with the calcium influx necessary for sperm hyperactivation. We are currently expanding our studies to bovine and human sperm to determine if the participation of SLO3 channels in sperm membrane potential control is conserved in mammals. In order to understand how sperm ion channels work and how they are modulated, we are using a combination of different techniques that include: comparative genomics, electrophysiological recordings in sperm and spermatogenic cells, heterologous expression of these ion channels and calcium imaging of sperm.

Invited speakers

Elisabetta Baldi, PhD
Associate Professor, Department of Experimental and Clinical Biomedical Sciences, Sexual Medicine and Andrology Unit, University of Florence, Italy
elisabetta.baldi@unifi.it

March 1979: Graduated in Biological Science in the University of Florence with 110/110 cum laude.
1979-1984: post-doctoral fellowship in the Institute of Clinical Pharmacology of the University of Florence.
April-May 1980: visiting scientist in the Laboratory of Biochemistry of the Imperial College of Science and Tachnology (London), directed by Dr. J. Hughes after winning a Wellcome Research Travel Grant for two months. During this visit she learns methods of biological assays.
March-April 1982: visiting fellow in the Laboratory of preclinical Pharmacology of the National Institute of Health (Washington, USA) directed by Prof. E. Costa to learn HPLC techniques.
November 1982: admitted to the post-graduate course in pharmacology at the University of Milan (Prof. F. Berti)
November 1984: Specialized in Pharmacology at the University of Milan (70/70).
1984: Intern in the Laboratory of Endocrinology of the University of Florence directed by Prof. M. Serio.
1988-1990: two years research fellowship in the Laboratory of Nephrology directed by Prof. M. Dunn at Case Western Reserve University (Cleveland, Ohio).
July 1990-2001: Researcher in the Andrology Unit of the University of Florence directed by prof. G. Forti.
January 2002: win a position of Researcher in the University of Florence.
May 2005 - Present: Associate Professor in Clinical Pathology (MED/05).

Scientific Awards:

- Win several National and International awards
- Invited speaker in more than 100 International and National Congress

Publications

- 147 publications with IF (69 in the last 5 years). Number of citations: 5827 (from Google Scholar), average citations/article: 31.3, H-index, 45 (contemporary: 19)

Actual fields of research:

- Sperm Biology: capacitation, acrosome reaction, motility. Nongenomic effects of steroid hormones. New predictive parameters of fertility status. Investigation on the origin and the consequences on reproduction of sperm DNA fragmentation. Role of the calcium channel CatSper in sperm function.
- Prostate cancer cell biology, study of the mechanisms that regulate invasion and development of androgen resistance.

Invited speakers

Jon M. Oatley, PhD
Director, Center for Reproductive Biology
Associate Professor, School of Molecular Biosciences
College of Veterinary Medicine
Washington State University
joatley@vetmed.wsu.edu

Jon M. Oatley, Ph.D. is the Director of the Center for Reproductive Biology and an Associate Professor in the School of Molecular Biosciences at Washington State University. Dr. Oatley obtained his Ph.D. from Washington State University in 2004, was a postdoctoral fellow in the laboratory of Dr. Ralph Brinster at the University of Pennsylvania, and began as an independent investigator in 2007. His research focuses on deciphering the mechanisms that regulate formation of the germline stem cell pool in mammalian testes during development and maintenance of the population in adulthood. Because the actions of the germline stem cell pool provide the foundation for continual spermatogenesis, Dr. Oatley's research is related directly to understanding fundamental processes that underpin male fertility. Dr. Oatley has authored more than 40 papers in top-tier journals in the fields of reproductive and developmental biology including PNAS, the Biology of Reproduction, Development, the Journal of Cell Science, PLoS Genetics, and Genes & Development. As an independent investigator, his research program has been funded by multiple grants from the National Institutes of Health and the United States Department of Agriculture. Dr. Oatley's honors include the Dean's Award for Outstanding Research from the College of Veterinary Medicine at Washington State University, the Baron Lecturer in Reproductive Biology Award from the University of Florida, the Young Andrologist Award from the American Society of Andrology, and the New Investigator Award from the Society for the Study of Reproduction. Dr. Oatley has served the profession as chair of the membership committee in the Society for the Study of Reproduction and as a current standing member in the CMIR study section of NIH.

Invited speakers

Jacquetta Trasler MD, PhD
James McGill Professor in Pediatrics, Human Genetics and Pharmacology & Therapeutics. McGill University
Senior Scientist, Developmental Genetics Laboratory, Montreal Children's Hospital and Research Institute, McGill University Health Centre, Canada
jacquetta.trasler@mcgill.ca

Dr. Jacquetta Trasler is a James McGill Professor in Pediatrics, Human Genetics and Pharmacology & Therapeutics at McGill University. She is a Senior Scientist and directs the Developmental Genetics Laboratory at the Research Institute of McGill University Health Centre (RI-MUHC). Dr. Trasler directed the McGill University M.D./Ph.D. Program from 1999-2007 and Pediatric Research at the RI-MUHC from 2007-2015 and has mentored a number of graduate, postdoctoral and clinical trainees. Her research interests focus on epigenetics and the molecular and developmental regulation of gene expression in the germline and early embryo. More specifically she studies DNA methylation and genomic imprinting and the molecular and cellular targets for drug effects on germ cells and embryos. Ongoing studies include effects of drugs, diet (folate) and assisted reproductive technologies on the epigenome of germ cells and embryos and the implications for intergenerational passage of epigenetic defects.

Invited speakers

Mahmoud Aarabi, MD, PhD
Postdoctoral Fellow, Department of Human Genetics, McGill University, and Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Canada
mahmoud.aarabi@mail.mcgill.ca

Mahmoud Aarabi MD PhD is a Reproductive Epigenomics Postdoctoral Fellow in the laboratory of Dr. Jacquetta Trasler at the Department of Human Genetics, McGill University, Montreal, Canada. Dr. Aarabi's research is focused on the impact of diet and genetic background of parents on their germ cells and offspring DNA methylome. Through collaborative study projects with several Canadian infertility clinics, he utilizes state-of-the-art next generation sequencing-based techniques to study the sperm epigenome. Dr. Aarabi has published 9 articles as the first author as well as 9 articles as the co-author in peer-reviewed journals. He has presented in numerous national and international conferences and received several awards and prizes for his research including: the Trainee Merit Award in the Annual Meeting of the American Society of Andrology and the Best Clinical Paper Award in the Annual Meeting of the Canadian Fertility & Andrology Society (2015), Lipshultz/Lamb Traveling Scholar Award from the Society for Male Reproduction and Andrology (2014) and the Professors' Prize for Outstanding Graduate Work at the Department of Biomedical & Molecular Sciences, Queen's University (2013).

Invited speakers

Timothy Jenkins, PhD
Assistant Research Professor, University of Utah
Tim.jenkins@hsc.utah.edu

I graduated with a BS in Physiology and Developmental Biology from Brigham Young University and performed my graduate work with Dr. Douglas Carrell at the University of Utah. I stayed at the University of Utah for a short post-doc as well as in the capacity of a research associate and was recently placed on the faculty as a research professor. My research interest centers on reproductive physiology and epigenetics. Specifically, I am interested in epigenetic and genetic marks in gametes that affect fertility, embryonic development, offspring phenotype and trans-generational inheritance. Of most interest to me are the sources and impacts of perturbations to the epigenetic program in sperm. My recent work has identified age-associated epigenetic alterations in the sperm that may affect phenotype in the offspring as well as the epigenetic assessment of large cohorts of patients undergoing fertility treatments.

Invited speakers

Sophie La Salle, PhD
Assistant Professor
Department of Biochemistry, Chicago College of Osteopathic Medicine, College of Dental Medicine – Illinois, Midwestern University
slasal@midwestern.edu

Sophie La Salle received a BSc in Biochemistry from the Université de Sherbrooke (2000) and a PhD in Pharmacology and Therapeutics from McGill University (2007). She completed her postdoctoral training in the Reproductive Biology group at The Jackson Laboratory (2006-2011). Since 2011, Dr. La Salle has been on the faculty of the Chicago College of Osteopathic Medicine and the College of Dental Medicine – Illinois at Midwestern University where she is currently an Assistant Professor in the Department of Biochemistry. She teaches in graduate courses for medical, dental and pharmacy students, as well as other healthcare-related programs.

Dr. La Salle's training has provided her with a foundation to study the genetic and epigenetic blueprint orchestrating progression of germ cells through gametogenesis. Her current research program is centered on deciphering the contributions of the vertebrate-specific gene *Spata22* (*spermatogenesis associated 22*) to the proper completion of meiotic prophase in male and female germ cells. She has also been involved in additional research projects where the mouse is used as a model to investigate the mechanisms of chlamydial-induced pathogenesis in the female reproductive tract, the role of fibrilin-1 in reproductive function, as well as the impact of diet-induced obesity on the reproductive system. Dr. La Salle is dedicated to training and preparing students for careers involving research. In the past three years, she has supervised the thesis work of eight Master of Biomedical Sciences students, and she has mentored a dozen medical fellows and graduate students in short-term research projects.

Invited speakers

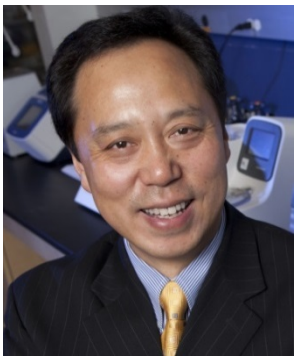
Martine Culty, PhD
Associate Professor, McGill University-Research Institute-MUHC, Canada
martine.culty@mcgill.ca

Current Position: Associate Professor, Departments of Medicine (Division of Endocrinology) and Pharmacology and Therapeutics, McGill University and Medical Scientist at the Research Institute of McGill University Health Center, Montreal, Canada
Education: B.S. (Animal Physiology) from the University Claude Bernard, Lyon, France; M.S. (Molecular & Cellular Biology) and Ph.D. (Molecular Chemistry; 1984) from Université Scientifique et Médicale de Grenoble, Grenoble, France. Postdoctoral fellowships (1984-1990): Biochemistry, McMaster University, Hamilton, Ontario, Canada; Endocrinology, Prince of Wales Hospital, University of New South Wales, Randwick; NSW Australia; Anatomy and Cell Biology, Georgetown University, Washington DC, USA.

Research Interests: My main research objectives are to understand the mechanisms regulating male germ cell development and how environmental disruptors can impact this process, focusing on neonatal gonocytes, precursors of the spermatogonial stem cells. While proper gonocyte development is a prerequisite for male fertility, the disruption of gonocyte differentiation is believed to be at the origin of testicular cancer. My work has advanced the understanding of gonocyte proliferation, differentiation, and responses to environmental endocrine disruptors by identifying a number of factors, genes and signaling pathways involved in these processes. Another major goal is to determine the impact of in utero exposure to environmental chemical mixtures on testis development and functions, using the rat model. These studies have unveiled differential short and long term effects of the phytoestrogen genistein and the phthalate DEHP, used alone or in combination at environmental relevant doses, identifying new target genes and pathways in testis. Additionally, I have contributed to the validation and selection of new innocuous plasticizers developed at McGill University.

Invited speakers

Wei Yan, MD, PhD
Professor, Department of Physiology and Cell Biology, University of Nevada School of Medicine
wyan@medicine.nevada.edu



Wei Yan received his MD from China Medical University in 1990 and PhD from University of Turku, Finland in 2000. After his post-doc training with Dr. Martin M. Matzuk at Baylor College of Medicine, he started his own lab at the University of Nevada School of Medicine in 2004. He is currently Professor of Physiology, Molecular and Cellular Biology. He received the 2009 Young Investigator Award of the Society for the Study of Reproduction (SSR), the 2012 Young Andrologist Award of the American Society of Andrology (ASA) and the 2013 Nevada Healthcare Hero Award for Research and Technology. He serves as serve as an Associate Editor for *Biology* of *Reproduction* and *Environmental Epigenetics*. His research addresses the molecular mechanism of gametogenesis and its application in fertility control. He has published >100 peer-reviewed research articles and book chapters. His lab is currently working on coding and noncoding genes that regulate spermatogenesis, and epigenetic contribution of gametes to fertilization, early embryonic development and adulthood health.

Sperm biomarkers for male infertility

Mark Baker, PhD
Associate Professor, University of Newcastle, Australia

Male infertility is a very common condition, with reports suggesting that one in 15 men of reproductive age are affected. The diagnosis of male-factor infertility is difficult and involves discounting female infertility through hormone measurements, pelvic examination and invasive laparoscopy. A semen profile analysis can suggest male infertility, if sperm counts are <15-20 million/ml, or <50% of sperm possess forward progressive motility (and < 25% rapidly progressive sperm) or <4% good morphology sperm. However, the diagnostic potential for a semen analysis is has been questioned by many reports (1-5). The treatment for male infertility often involves recourse to assisted reproductive technology (ART). Nationally we face a situation where 1 in 30 children born in Australia are the product of an assisted conception therapy that costs Australians approximately $600 million per annum to achieve success rates that hovers around 18%. We have used non-selected, sperm samples from a population of males attending infertility clinics with suspected infertility asthenozoospermic, teratozoospermic, asthenotetatozoospermic and normozoospermic idiopathic) and performed a quantitative proteomic analysis to see if any biomarkers were “common” to male infertility. Excitingly, several (42) biomarkers were significantly up or down regulated (minimum of 10-fold, $p<0.0001$) within this infertile population compared with control fertile donors. The discovery of these biomarkers constitutes the basis for a new, rapid and reliable approach towards developing a novel technique for the diagnosis of male infertility. Secondly, we are investigating the role of one of the major protein found to be more abundant within all infertile samples studied. This protein appears to be cleaved, and the peptide product produced has the ability to destroy sperm function. A novel model of male infertility will be presented and a possible approach for better sperm selection for ART presented.

References:

1. David G, et al. Male fertility potential in terms of semen quality: a review of the past, a study of the present. Fertil Steril 1979;31:103-16.
2. Smith KD, et al. Relation between indices of semen analysis and pregnancy rate in infertile couples. Fertil Steril 1977;28:1314-9.
3. Sobrero AJ, REhan NE. The semen of infertile men. II. Semen characteristics of 100 fertile men. Fertil STeril 1975;26:1048-56.
4. Zukerman Z, et al. Frequency distribution of sperm counts in fertile and infertile males. Fertil STeril 1977;28:1310-3.
5. Van Zyl JA, et al. Oligozoospermia: a seven-year survey of the incidence, chromosomal aberrations, treatment and pregnancy rate. Int J Fertil 1975;20:129-32.

The study of ion channels in sperm

Celia Santi, PhD
Center for Women's Reproductive Sciences Research, Dept. of Obstetrics and Gynecology, Washington University School of Medicine

Ion channels play a key role in determining sperm membrane potential, which affects essential aspects of sperm function like capacitation, hyperactivation and the acrosome reaction. By using a knockout mouse model we have recently demonstrated that the absence of a sperm-specific potassium channel known as SLO3 results in male infertility. This channel was cloned in 1998 in the Salkoff lab, and the study of this channel in heterologous systems showed that SLO3 is a high conductance potassium channel that activates at intracellular alkaline pH.

With the use of the patch clamp technique and voltage sensitive dyes we have found that this channel is present in the mature mouse sperm cell membrane and is responsible for the hyperpolarization associated with sperm capacitation. We further observed that SLO3 knock-out sperm are depolarized and have deficits both in hyperactivation and the acrosome reaction. These latter phenotypes are most likely due to a reduction in calcium influx. Our recent results using calcium sensitive dyes show that SLO3 regulates calcium entry through the sperm specific calcium channel CATSPER, possibly indirectly through a voltage-sensitive control of intracellular pH.

In summary: Using the SLO3 knock out mouse line and a variety of molecular, physiological, and genetic techniques we revealed voltage-sensitive processes that are essential for sperm capacitation, hyperactivation and the acrosome reaction; this information may impact the field of in-vitro fertilization and contraception.

References:

1. Santi CM, Butler A, Kuhn J, Wei A, Salkoff L (2009). Bovine and mouse SLO3 K+
2. channels: evolutionary divergence points to an RCK1 region of critical function. J. Biol. Chem, 284:21589-21598.
3. Santi CM, Martinez-Lopez P, de la Vega-Beltran JL, Butler A, Alisio A, Darszon A, Salkoff L (2010). The SLO3 sperm-specific potassium channel plays a vital role in male fertility. FEBS Lett. 584:1041-1046.
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5. channels and transporters in sperm function. Curr Top Dev Biol 102: 385-421.
6. Chavez, JC, dela Vega-Beltrán JL, Escoffier J, Visconti PE, Treviño CL, Darszon A, Salkoff L, Santi CM. (2013). Ion permeabilities in mouse sperm reveal an external trigger for SLO3-dependent hyperpolarization. PloS One 8(4): e60578.
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Assessment of sperm proteins and DNA integrity by flow cytometry

Elisabetta Baldi, PhD and Monica Muratori, PhD
Associate Professor, Department of Experimental and Clinical Biomedical Sciences, Sexual Medicine and Andrology Unit, University of Florence, Italy

Flow cytometry is now recognized as a valuable tool to study spermatozoa. It can measure physical as well as multicolor fluorescence properties spermatozoa by recording emission from single cells present in the suspension. It allows to study such properties in thousands of cells, rendering the results objective, highly repeatable and statistically solid. In some case, flow cytometry can be used to sort spermatozoa. However, when dealing with semen samples, the use of flow cytometry can be very tricky. Indeed, semen is a complex matrix containing, beside spermatozoa, immature germ cells, leukocytes, epithelial cells, apoptotic bodies (AB). All these elements may interfere with the flow cytometric analysis of spermatozoa and should be taken into account. Immature germ cells and other somatic cells can be easily eliminated from the analysis, whereas the same is not true for AB, anucleated elements of testicular origin present in variable amounts in semen from different subjects (high levels can be detected in oligozoospermia), with similar dimension as spermatozoa. In such a situation, it appears clear that the inclusion of AB in the analysis leads to incorrect results. In case of evaluation of sperm DNA fragmentation, for example, inclusion of AB leads to an underestimation of the sDF levels of the subject (Muratori et al, 2008). To eliminate apoptotic bodies from the cytometric analysis, a nuclear staining (such as propidium iodide or ypro-1) can be used as AB will result unstained. The use of nuclear staining allowed to distinguish two cytometric sperm populations with different biological and clinical characteristics. The strategies to evaluate sperm parameters by flow cytometry will be discussed.

References:

1. Baldi E, Luconi M, Krausz C, Forti G. Progesterone and spermatozoa: a long-lasting liaison comes to definition. Hum Reprod. 2011 26(11):2933-4.
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Mouse models incorporating fluorescent proteins to study signaling pathways in Sertoli cells *in vitro* and *in vivo*

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Since the late 1970s when the first transgenic mice were produced through injection of DNA into a single-cell mouse embryo, and then the late 1980s when gene knockouts were first created through embryonic stem cell manipulation *in vitro* followed by injection into blastocysts, the use of genetically modified mice in research has exploded at a fascinating pace. Now, it seems nearly every lab is utilizing mice that harbor DNA insertions, deletions, or substitutions to understand the basic biology of a disease process or signaling pathway in a tissue or organ system of interest. The use of cell- or promoter-specific fluorescent protein expression further expands the biologists' toolkit to allow *in vivo* or *ex vivo* study of specific cell populations or gene promoter activity. When coupled with additional experimental manipulation such as gene ablation, the biologist has an increasingly powerful tool to study a signaling pathway within a specific subpopulation of cells *in vivo* or *ex vivo*. In this talk, I will demonstrate several such applications of coupling cell-specific fluorescent protein expression, namely in Sertoli cells (through Amh-Cre), to either Sertoli cell-specific gene ablation; germ cell compartment ablation (*in vivo*) or addition (*in vitro*); or addition of a separate promoter-specific fluorescent protein reporter allele with distinct emission/excitation characteristics for labeling, isolation, and analysis of Sertoli cells.

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3-Dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity *in vitro*

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Current methods for determining gonadotoxicity of pharmaceutical compounds and environmental toxins rely heavily on *in vivo* functional assays in animals; however these assays cannot accurately predict toxicity in humans. Furthermore, existing *in vitro* two-dimensional culture of human testicular cells do not maintain the germ cell niche and the complex signaling interactions that existed *in vivo*. Therefore an effective *in vitro* model of human testis is desirable. We established a three-dimensional human testis organoid by combining isolated and propagated spermatogonial stem cells, Sertoli, Leydig cells and extracted human extracellular matrix. These 3D organoids maintained viability in culture, produced androgens, and went through cell differentiation from spermatogonia to post-meiotic germ cells. These organoids were frozen and thawed successfully and showed a dose-dependent response to gonadotoxic chemotherapeutic drugs. This three-dimensional human testis organoid model can be used for high throughput screening of drugs and environmental chemicals as well as study *in vitro* spermatogenesis.

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Paternal stress reprograms epigenetic marks in sperm and epididymis: Using mass spectrometry-based proteomics to identify global histone post-translational modifications

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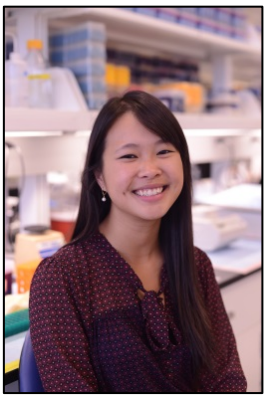

Epidemiological studies suggest that epigenetic inheritance of paternal experiences can influence disease risk in subsequent generations. Environmental exposures to famine, toxins or trauma in males have been linked with disease risk in offspring conceived decades following the paternal exposure, suggesting the environment can have lasting impact on the male germline. In our mouse model of paternal stress, stressed males sire offspring with a significantly altered stress response. Mechanistically, sperm analyses identified a significant increase in 9 microRNA (miRs) following paternal stress exposure. Zygote microinjection of these miRs recapitulated the offspring stress phenotype, providing a functional role for sperm miRs. Remarkably, preliminary data reveal that males bred 3 months following stress exposure still produce offspring with altered stress reactivity, suggesting lasting effects of experience on intergenerational transmission. To identify the upstream epigenetic mechanism that maintains stress-responsive miRs in sperm months after stress end, we used quantitative mass spectrometry–based proteomics to interrogate global histone post-translational marks (PTMs) in the caput epididymis, where miRs can be transcribed, packaged and released into the caput lumen via epididymosomes to fuse with maturing sperm. Histone PTMs can be dynamically modified and have lasting effects on gene expression, providing a mechanism by which stress-responsive miRs can be maintained in sperm. To understand an underlying histone code that may combinatorially regulate gene expression, we used the ensemble learning method Random Forest to classify histone PTMs differentially regulated in the caput of stressed males. These novel approaches convey a more complex understanding of global histone changes that can contribute to epigenetic reprogramming, and offer an exciting mechanism by which the environment can dynamically regulate sperm epigenetic marks.

Leveraging available molecular genetic tools to investigate the function of genes important for sperm function and fertility using *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* is a valuable tool for dissecting the molecular function of genes necessary for spermatogenesis and sperm function. *C. elegans* has a short reproductive life span (~3.5 days from birth to reproductive adult) and a plethora of readily available genetic tools. These characteristics, along with many others, make *C. elegans* an attractive model system for studying a gene of interest and dissecting its cellular function. We have been using *C. elegans* to analyze the function of the M05D6.2, the *C. elegans* ortholog of the human Tcp11 (t-complex protein 11) gene. In humans, Tcp11 is expressed in the testis and is suggested to be important for sperm function and fertility. We have begun to analyze M05D6.2 function using RNA interference (RNAi) to disrupt gene function in *C. elegans* males. We are also analyzing the fertility of *C. elegans* strains that contain missense mutations resulting in changes at conserved amino acid residues. This data will provide us with the experimental foundation to pursue the creation of additional transgenic *C. elegans* strains, including a strain expressing fluorescent M05D6.2 protein, a strain lacking any functional M05D6.2, and a strain containing a the same single nucleotide polymorphism (SNP) that was identified from infertile patient data. The data generated from these experiments will shed light on the function of this M05D6.2/Tcp11 and complement studies in humans and other model organisms. The research techniques outlined serve as a template to study any genes that are shared between *C. elegans* and humans and present an opportunity to understand important conserved cellular functions.

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Selected Abstracts

Genomic studies in azoospermic and oligozoospermic males with male infertility

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The genetic basis of non-obstructive azoospermia is unknown in vast majority of infertile men. To identify the genetic causes of azoospermia (AZ) we carried out comprehensive genome-wide screening via Comparative Genome Hybridization (CGH) array and massive Whole Exome Sequencing studies in population of well-defined infertile males with azoospermia. To detect genomic aberrations we utilized 400K whole-genome CGH array (Agilent). This platform is capable to detect deletions/ duplications (DNA copy numbers) and uniformly covers human genome with average resolution of 7 Kb/ probe. Using CGH we identified dozens of rare aberrations in infertile males. Among copy number variants detected, 1 small genomic deletion was selected on chromosome Xq13.2; it involves 3 exons of *TEX11*, identical in 2 azoospermic patients. It is located inside of X-linked *TEX11,* the testis-expressed 11 gene, which encodes a protein critical for male germ cell meiotic DNA recombination. *Tex11*-knockout male mice exhibit AZ with meiotic arrest (MA) at the pachytene stage. This loss causes a 79–amino acid in-frame deletion within the meiosis-specific conserved domain SPO22 of the TEX11 protein. Therefore, we assigned top priority to this a 99-kb hemizygous loss (deletion). To support significance of this finding, we performed full gene mutation screening via Sanger DNA sequencing in 289 azoospermic males and 384 controls. Our mutation screening study found five novel *TEX11* mutations in 287 AZ patients (total, 7/289, 2.4%): including three mutations in splicing cryptic sites, and two missense mutations affecting functional domains. All found mutations were absent in 384 normozoospermic controls (p=0.003). Importantly, majority, 5 *TEX11* mutations were found in 33 (15.2%) patients diagnosed with meiotic arrest and azoospermia (clinical histological form), resembling the *Tex11*-deficient mouse meiotic arrest phenotype. Immunohistochemical analysis showed specific cytoplasmic TEX11 protein expression in late spermatocytes, as well as in round and elongated spermatids, in normal human testes. In contrast, testes from azoospermic patients with *TEX11* mutations showed meiotic arrest and lacked TEX11 protein expression. We propose that hemizygous *TEX11* mutations on X chromosome are a frequent cause of meiotic arrest and nonobstructive azoospermia in infertile men.

To expand our search for AZ candidate genes we apply alternative approach using Whole Exome Sequencing in 100 patients with following search for hemizygous or recessive mutations in testis-specific and/or genes involved in male reproductive system. This approach includes study of sporadic unrelated AZ males, as well asfamilies with AZ patients. Currently a few studies with promising results are being performed.

Keynote Speaker

Functional genomic approaches to define the stem cell state in male germ cells

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Continual spermatogenesis relies on self-renewal of spermatogonial stem cells (SSCs) to sustain a foundational pool that arises in neonatal development from prospermatogonial precursors. Progenitor spermatogonia arise periodically from the SSC pool, transiently amplify in number, and then transition to a differentiating pathway. At present, the molecular mechanisms underpinning the stem cell state and thus the interface of the SSC to progenitor transition are undefined. Recently, we established that the SSC pool in mice is marked by expression of inhibitor of DNA binding 4 (ID4) and generated and *Id4-Gfp* reporter mouse line to study the population in more detail. Using this resource, we have found that the levels of ID4 expression are correlated to regenerative capacity and have been able to purify an SSC population based on intensity of *Id4-Gfp* transgene expression. Another resource for studying spermatogonia is primary cultures which can be readily established from various strains of mice. Within these cultures the majority of cells are progenitors and only ~10-20% of the population is SSCs which we have found to be marked by ID4 expression. In recent studies, we have devised a high throughput RNAi screening approach using primary cultures of spermatogonia established from *Id4-Gfp* transgenic mice to assess the influence of a variety of molecules on maintenance of the SSC pool based on altered dynamics of the GFP+ population. Outcomes of initial screens are yielding previously unidentified transcription factors that influence maintenance of the SSC pool in vitro and these are potentially also essential for maintenance of the population in vivo. This research that will be presented has been supported by grant HD061665 awarded to JMO from the National Institutes of Health.

Assessing the Sperm DNA methylome- Past and Future

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Epigenetic mechanisms including DNA methylation, covalent histone modifications, and noncoding RNAs, influence gene expression, and when perturbed, increase genomic instability and structural defects in heterochromatic domains. To date, DNA methylation is the most well characterized epigenetic modulator with essential functions in the germ line and embryo as well as in genomic imprinting. Present at 20 (mouse)- 30 (human) million sites in the genome, DNA methylation is found predominantly at the 5-position of cytosine residues within CpG dinucleotides, with approximately 60-80% of CpG containing cytosines being methylated. Patterns of DNA methylation in sperm differ markedly from those of somatic cells. DNA methylation patterns are for the most part erased in the primordial germ cells in the fetal gonad. In the male, following erasure, the major period of DNA methylation re-acquisition (*de novo* methylation) occurs before birth in male germ cells of the fetal testis; postnatally, the patterns must be maintained (maintenance methylation) during cell divisions that occur in spermatogonia. DNA methylation is remodeled on a minority of sequences as germ cells develop from spermatogonia to spermatocytes. At fertilization the oocyte and sperm contribute sex-specific patterns of methylation to the embryo. As embryos develop from the one cell to blastocyst stage, DNA methylation is lost at most CpG dinucleotides, with the exception of imprinted and some single-copy and repeat sequences. In animals and humans**,** several types of exposures (e.g. diet, cancer chemotherapy, endocrine disruptors, intracytoplasmic sperm injection) have been shown to alter sperm DNA methylation patterns and may result in growth abnormalities, altered gene expression or disease phenotypes. In this presentation we will provide an overview of the types of locus-specific and genome-wide techniques that have been used to assess alterations in male germ cell DNA methylation profiles. Promising emerging approaches such as single cell DNA methylation profiling and custom capture techniques will be discussed in the context of past and current technologies.

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Whole genome sequencing-based approaches to assess the sperm DNA methylome

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There is increasing evidence that interactions between parents' genes and exposures to environmental stressors not only affect their own health but can predispose their offspring to developmental defects, metabolic disorders, or other chronic diseases. Parental exposures may adversely affect the epigenetic marks, such as DNA methylation, which in turn control gene expression in cells. Alterations of DNA methylation in male germ cells may be transmitted to the embryo. Therefore, epigenetics and DNA methylation are at the center of current investigations about the impact of parental genes and environmental exposures on the offspring. The emerging state-of-the-art next generation sequencing (NGS)-based methods have enabled researchers to analyze millions of DNA methylation sites across the sperm genome. A range of techniques is currently available including whole genome bisulfite sequencing and enrichment/array-based approaches. Successful NGS-based assays involve four critical steps of sample/library preparation, sequencing, bioinformatics analysis and validation. Here, these steps will be reviewed with examples to demonstrate the required quality control measures. Also, currently available NGS-based techniques to study the sperm DNA methylome will be described and compared to understand the rationale for the choice of methods for different research objectives.

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Targeted and regional assessment of the sperm epigenome

Timothy Jenkins, PhD
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Sperm epigenome is a remarkably dynamic and intriguing landscape that requires unique scientific approaches to study. There are many attributes that can be assessed in sperm including DNA methylation and chromatin/nuclear protein composition. We will discuss the techniques used to study these marks with a specific focus on targeted assessment of areas of interest as opposed to genome wide analysis. Next generation sequencing techniques allow us to understand these epigenetic marks with greater detail than ever before. We will discuss the technology used and some of the bioinformatic approaches available to assess regional changes.

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Session II – Epigenetics, Genome-scale tools and genetic models

Genetic models in reproductive studies

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Our understanding of the genes and pathways required to support male germ cell development has been vastly broaden by the use of genetically-manipulated mouse models. In fact, *in vivo* models remain the gold standard to study reproductive function since appropriate systems consistently recapitulating spermatogenesis *in vitro* remain to be established. Here we will discuss various genetic approaches utilized to study spermatogenesis in the mouse, with an emphasis on recent technological advances allowing for fast and efficient genome modification. Genetic background and phenotype analysis considerations will also be broached, as well as genetic resources currently available to the research community.

Changing gene expression in isolated gonocytes

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Spermatogenesis relies on the formation in infancy of a pool of spermatogonial stem cells (SSCs) from their precursors, the transitional gonocytes (also called pro-/pre-spermatogonia). The term gonocyte encompass a succession of developmental phases, starting when germ cells become resident in the fetal gonad and ending with neonatal germ cells relocating to the basement membrane of the seminiferous tubules, where differentiation to either SSCs or first wave spermatogonial takes place. Gonocyte development comprises periods of cell proliferation, mitotic quiescence, DNA methylation, cell migration and differentiation, which are controlled mainly by the Sertoli cells in which they are embedded. Although the mechanisms and factors regulating some of these processes have been identified, many remain unknown. Various methodologies can be used to identify genes critical for gonocyte development and their function. While in vivo studies can provide information on the expression of specific genes, proteins and signaling pathways, and their potential disruption in knockout mouse models or toxicant exposure studies, they do not allow for mechanistic studies linking a pathway to a specific function. By preserving tissue organization and/or intercellular relationships, organ cultures and co-cultures are useful models to study the role of specific factors or the impact of toxicants. However, the fact that Sertoli cells produce many growth and differentiation factors makes it difficult or impossible to sort out effectors acting directly on gonocytes from those having Sertoli cells as primary target. For this reason, my laboratory has relied on the study of isolated gonocytes to determine mechanisms regulating neonatal gonocyte proliferation and differentiation. Here, I will describe methods used to overexpress or silence specific genes in isolated neonatal rat gonocytes, including transfection with lipofectamin reagents or by electroporation. Advantages and limitations of these approaches will be discussed.

Reveal the physiological function of miRNAs in vivo

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One miRNA can target hundreds of mRNAs, and one mRNA can be targeted by numerous miRNAs. This "one-to-multi" relationship determines that a lack of one or two particular miRNAs usually dose not cause discernable phenotype because of the compensatory effects. To reveal the physiological roles of miRNAs in vivo, one needs to inactivate a group of miRNAs that either possesses the same seed sequences or target the mRNAs that belong to the same pathways. In my talk, I will show you several examples where single miRNA knockout does not cause any developmental defects, whereas ablation of a cluster of miRNAs leads to severe phenotypes, thus revealing critical functions under physiological conditions [1-3]. Moreover, miRNA knockout *in vivo* allows for identification of their true targets.

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