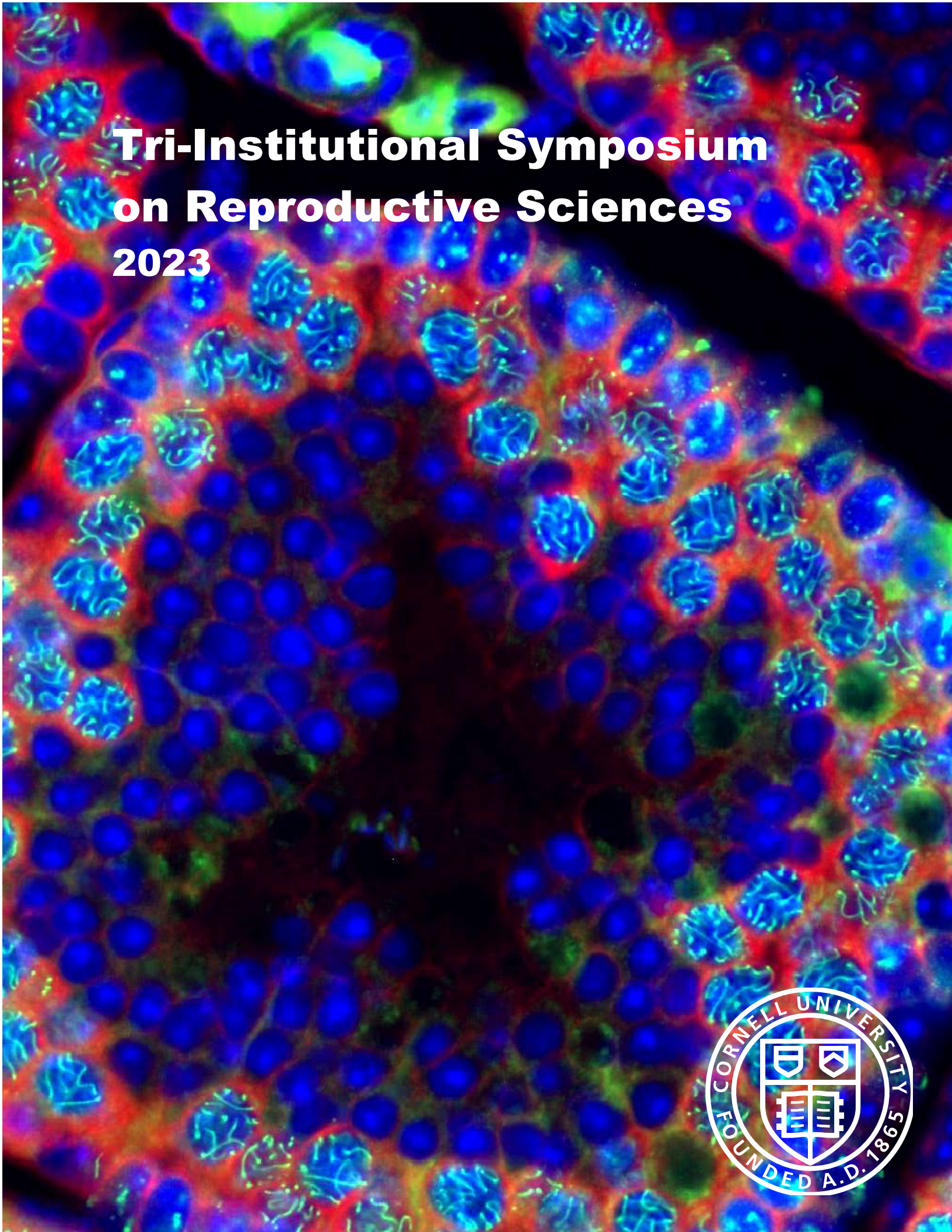
Tri-Institutional Symposium on Reproductive Sciences 2023



TRI-REPRO SYMPOSIUM

APRIL 27-28, 2023
CORNELL UNIVERSITY, COLLEGE OF VETERINARY MEDICINE, ITHACA NY

The Tri-Institutional Symposium on Reproductive Biology and Infertility is an annual regional reproductive biology meeting that alternates between Cornell University, the University of Pittsburgh, and the University of Pennsylvania.

SUPPORT PROVIDED BY:

- NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Tri-Institutional Symposium on Reproductive Biology and Infertility (Tri-Repro): R13 HD102144
- Center for Reproductive Genetics and Therapy; Magee-Womens Research Institute, University of Pittsburgh: P50 HD096723
- Center for Reproductive Genomics; Cornell University: P50 HD076210
- Penn Center for the Study of Epigenetics in Reproduction; University of Pennsylvania: P50 HD068157

University of Pittsburgh

Cornell University

Penn
University of Pennsylvania

MAGEE-WOMENS
RESEARCH INSTITUTE

Cornell Reproductive
Sciences Center

2023 Tri-Repro Organizing Committee

Paula Cohen, PhD.
Professor of Genetics
Cornell University

Kyle Orwig PhD.
Professor
Magee-Womens Research Institute

Marisa Bartolomei, PhD.
Professor
University of Pennsylvania

Trainees

Ian Wolff
Postdoctoral Fellow
Cornell University

Georgia Atkins
Graduate Student
University of Pittsburgh

Grace Lee
Graduate Student
University of Pennsylvania

Rui Huang
Graduate Student
Cornell University

Andre Caldeira-Brant
Postdoctoral Associate
Magee Womens Research Institute

Carolline Ascencao
Research Associate
Cornell University

Yahan Li
Postdoctoral Associate
University of Pennsylvania

Administrative Support provided by:

Jennifer Orwig – Research Manager, Magee-Womens Institute
James Bender – Administrative Assistant, Cornell University
Anya Samiljan - Administrative Assistant, Cornell University
Jane Lee – Administrative Manager, Cornell University

Symposium Agenda

All events will take place in The Yarnell Lecture Hall (LH4), LH5 and Takoda's Run (CVM 1st floor atrium)

Thursday April 27

7:30 – 8:30am ***Registration and Breakfast; Takoda's Run***

8:30 – 8:35am **Dr. Paula Cohen**, Cornell University, Welcome remarks

Session 1: Pregnancy and Embryo/Placenta

Moderators Rui Huang and Yahan Li

8:35-9:05am **Keynote Speaker: Dr. Heidi Stuhlmann**, Weill Cornell Medicine
Placental miR-126 and its Role in Fetal Programming

9:05-9:20am **Robin Skory**, Perelman School of Medicine, University of Pennsylvania
Embryo live-imaging reveals nuclear DNA shedding during blastocyst expansion and biopsy

9:20-9:35am **Emily Lopes**, University of Massachusetts Amherst
TRPV3 function contributes to zinc homeostasis and impacts early fertilization events in mice

Break: 9:35-9:45am

Session 2: Germline/Meiosis

Moderators Grace Lee and Carolline Ascencao

9:45-10:15am **Keynote Speaker: Dr. Yumi Kim**, John Hopkins University
The synaptonemal complex in C. elegans

10:15-10:30am **Jeffrey Vedanayagam**, Sloan Kettering Institute
Essential and recurrent roles for hairpin RNAs in silencing de novo sex chromosome conflict in Drosophila simulans

10:30-10:45am **Sarah Potgieter**, Rutgers University
ADAD1 is a novel translation regulator important for intracellular protein transport and sperm head shaping during spermiogenesis

10:45-11:00am **Stephanie Tanis**, Cornell University
Investigating Meiotic Prophase I as an Intervention Point for Male Contraceptive Targeting

11:00-11:15am **Victor Ruthig**, Weill Cornell Medicine
Alternative Splicing Alters the Female and Male Germ Cell Splice-Ome at Transitions Points in Fetal Germ Cell Development

Poster Session: 11:15am-1:30pm

Lunch at noon

1:30-1:45pm **Mitch Harancher**, University of Pittsburgh
An Approach to Address Iatrogenic Male Infertility and Prevent Disease Transmission in Mouse and Human Models of Sickle Cell Disease

1:45-2:15pm **Keynote Speaker: Dr. Colin Conine,** Perelman School of Medicine, University of Pennsylvania
The transmission of epigenetic information to offspring by sperm RNAs

Session 3: Reproductive Tract

Moderators Georgia Atkins and Andre Caldeira-Brant

2:15-2:45pm **Keynote Speaker: Dr. Humphrey Yao**, NIH
From oocyte-producing testis to blastocyst complementation: new frontier in deciphering sex differentiation

2:45-3:00pm **Ruixu Huang**, Dartmouth College
Mapping ovulation with spatiotemporal and single-cell resolution

3:00-3:15pm **Ana Martinez-Marchal**, University of Pittsburgh School of Medicine
SARS-CoV-2 modifies the testicular compartment and decreases the number of SSC upon acute COVID-19 infection

3:15-3:30pm **Rhasaan Bovell**, Cornell University
Relationship between serum Anti-Müllerian hormone concentration and ovarian stimulation response in cheetahs (Acinonyx jubatus)

Break 3:30-3:40pm

3:40-3:55pm **Jiyang Zhang**, Rutgers University
Targeting proprotein convertases and ovulation to develop non-hormonal contraceptives

3:55-4:25pm **Keynote Speaker: Dr. Athena Ren**, Cornell University
From gene regulation to physiology: Orchestration of time and space in mammalian ovulation.

4:25-4:30pm Poster Prizes

Stuart Moss Memorial Lecture

4:30-5:30pm **Dr. Francesca Duncan**, Northwestern University
Transforming ovarian aging into reproductive longevity

5:30-5:40pm **Dr. Paula Cohen**, Cornell University, Closing remarks

Reception 5:30-8:00pm Takoda's Run

Poster and talk prizes will be awarded at the reception

Friday April 28

The Yarnell Lecture Hall (LH4)

8:00 – 9:00am	***Breakfast: Takoda's Run and The Green Room for EIC/AIC participants***

Career Forum

Moderator Ian Wolff

9:00 – 11:00am	**Paula Cohen, PhD:** Professor and Associate Vice Provost for Life Sciences, Cornell University **Stephen Ward, PhD:** Program Officer, Bill & Melinda Gates Foundation **Daniel Goldberg, PhD:** Senior Program Officer, Bill & Melinda Gates Foundation **Mary Ann Handel, PhD:** Professor Emeritus, The Jackson Laboratory **Travis Kent, PhD:** Program Officer, NICHD Fertility and Infertility Branch **Heidi Stuhlmann, PhD:** Professor, Weill Cornell Medicine
11:00am	Hike to Newman's Overlook, Cornell Botanic Gardens for lunch

2023 Tri-Repro Symposium Biographies

Dr. Francesca Duncan, Northwestern University; *Stuart Moss Memorial Lecture*

Dr. Duncan earned her doctorate in Cell and Molecular Biology from the University of Pennsylvania. She completed two post-doctoral fellowships in reproductive science - one in the Department of Biology at the University of Pennsylvania and one in the Department of Obstetrics and Gynecology at Northwestern University. Her first independent faculty appointment was as an Assistant Professor in the Department of Anatomy and Cell Biology at the University of Kansas Medical Center. She joined Northwestern University as an Assistant Professor in the Department of Obstetrics and Gynecology in October 2017 and was promoted to Associate Professor with tenure in 2022. In addition to running an independent research laboratory, Dr. Duncan is the co-Director of the Center for Reproductive Science and Director of the Master of Science in Reproductive Science and Medicine program.

Heidi Stuhlmann, Weill Cornell Medicine; *Keynote Speaker and Career Panelist*

Dr. Stuhlmann joined Weill Cornell Medicine in 2006 and is a Professor of Cell and Developmental Biology and a Professor of Cell and Developmental Biology in Pediatrics. She has a doctorate in biology from the University of Hamburg, Germany, and she conducted her post-doctorate work at MIT / The Whitehead Institute for Biomedical Research and at Stanford University School of Medicine. She is the Co-Chair of the Cell and Developmental Biology PhD program in the Weill Cornell Graduate School of Medical Sciences and directs a T32-supported Training Program in Developmental and Stem Cell Biology. Her lab focuses on the molecular and genetic pathways that regulate vascular endothelial lineage specification, and on regulatory and epigenetic mechanisms in placental development and disease.

Dr. Yumi Kim, John Hopkins University; *Keynote Speaker*

Dr. Kim is an assistant professor in Biology whose research focuses on the molecular mechanisms that drive and coordinate meiotic chromosome dynamics. She received her Ph.D. from UCSD and did her postdoctoral work at UC Berkeley. Accurate chromosome segregation during meiosis is essential for the transmission of stable genomes from parent to offspring. Errors in this process lead to the production of cells with an abnormal number of chromosomes or aneuploidy, and this is a major cause of miscarriages and birth defects in humans such as Down syndrome. Our lab is interested in the molecular mechanisms that ensure faithful chromosome segregation during meiosis. By combining biochemical reconstitution and structural biology with cytological and genetic analysis in the nematode *C. elegans*, we address fundamental questions of how homolog pairing, synapsis, and crossover recombination are executed and coordinated during meiotic prophase.

Dr. Colin Conine, Perelman School of Medicine, University of Pennsylvania; *Keynote Speaker*

Dr. Colin Conine's research focuses on the functions of small RNAs in reproduction, inheritance, and development. He completed his PhD in the laboratory of Craig Mello at UMass Medical where he worked on the function of endogenous small RNA pathways regulating thermotolerant male fertility in *C. elegans*. Dr. Conine went on to complete his postdoctoral training in Oliver Rando's lab, where he was a Helen Hay Whitney Postdoctoral Fellow.

In January 2020, Colin joined the faculty at the University of Pennsylvania Perelman School of Medicine (Department of Genetics) and the Children's Hospital of Philadelphia (Division of Neonatology). His lab utilizes a combination of assisted-reproduction techniques paired with injection of RNAs or genetic ablation of small RNAs in the male germline, followed by single embryo genome-wide molecular techniques to determine the effect of sperm small RNAs on embryonic development and offspring phenotype.

Dr. Humphrey Yao, NIH; *Keynote Speaker*

Dr. Yao received his doctoral degree at the University of Illinois in Urbana-Champaign in 1999 and then completed his postdoctoral training at Duke University Medical Center in 2002. He became Assistant Professor in the Department of Comparative Biosciences at University of Illinois in Urbana-Champaign in 2003 and received tenure in 2009. Dr. Yao moved to NIEHS in 2010 and was promoted to Senior Investigator in 2018. Dr. Yao was the recipient of the Basal O'Connor Starter Research Award from March of Dimes Birth Defect Foundation, Pfizer Research Award, New Investigator Award for the Society for the Study of Reproduction, and Young Andrologist Award from the American Society of Andrology. He was also elected as the Chair for the 2018 Gordon Research Conference on Mammalian Reproduction.

Dr. Athena Ren, Cornell University; *Keynote Speaker*

Dr. Yi Athena Ren, Ph.D. is an assistant professor in the Department of Animal Science at Cornell University. Dr. Ren received her doctorate in reproductive physiology from Cornell University in Ithaca, NY, and continued her postgraduate training at Baylor College of Medicine in Houston, TX. Dr. Ren started her research program in 2019 focusing on understanding the molecular and cellular basis regulating mammalian ovulation. The cyclic changes in the ovary's structure and function in the reproductively active period, such as these occurring during ovulation, is an ode to the highly coordinated dance between the constructive and deconstructive forces of life: all have to be in the right place and at the right time, and all are hormonally regulated. The Ren laboratory aims to explore the ovary as a powerful and unique model for discovering fundamental mechanisms regulating dynamic tissue remodeling processes in physiological as well as pathological conditions. This knowledge will also provide insights into novel targets for infertility treatment and contraception design in women.

Dr. Paula Cohen, Cornell University; *Career Panelist*

Dr. Cohen, Professor and Associate Vice Provost for Life Sciences, Cornell University took a Postdoctoral position at the Albert Einstein College of Medicine, New York, where she focused on regulation of gonadal function in males and females, and in maturation of the hypothalamic-pituitary-gonadal axis. During this time, she became interested in germ cell biology and genome integrity, and transitioned into this area to study the roles of DNA repair proteins in mammalian meiosis. She joined the faculty of the Department of Genetics at Albert Einstein College in 2000, and then in 2004 was recruited to Cornell University, within the Department of Biomedical Sciences. In 2007, she was promoted to the rank of Associate Professor with indefinite tenure, and then was promoted to full Professor in 2013. In 2018, she became Associate Vice Provost for Life Sciences. In her own lab, Dr. Cohen has mentored 13 graduate students, 10 Postdoctoral fellows, 1 reproductive endocrinology fellow, and numerous undergraduates.

Dr. Stephen Ward, Bill & Melinda Gates Foundation; *Career Panelist*

Since 2009, Stephen Ward has served as a Program Officer within the Bill & Melinda Gates Foundation's Discovery & Translational Sciences team, managing a portfolio of programs related to early stage-drug discovery. His work has focused on developing new tools and novel biological approaches to rapidly accelerate discovery and development of new therapies, particularly in the areas of neglected tropical diseases, pediatric diarrheal disease, and family planning, and co-leads a program for the discovery of novel, non-hormonal contraceptive agents. Prior to joining the foundation, Stephen worked as a consultant, providing technical support for non-profit, government, and commercial programs, particularly focused on drug discovery projects for global health. Stephen received his BS in Biology from the University of Kentucky, a PhD in Molecular Microbiology from Washington University researching viral immune evasion strategies, and completed a post-doctoral research fellowship at the Harvard School of Public Health focused on the cell biology of cellular invasion of parasites.

Dr. Daniel Goldberg, Bill & Melinda Gates Foundation; *Career Panelist*

Dan comes to the Foundation with over 25 years of broad small molecule drug discovery and development experience as a medicinal chemist. Having worked in several major pharmaceutical and biotech companies he has experience in driving programs from concept to the clinic.

After completing his PhD in organic chemistry at Emory University and his post-doc at the University of Chicago, Dan joined Boehringer-Ingelheim Pharmaceuticals where he worked as a lab and project leader. He then moved to become a founding member of Karos Pharmaceuticals, a 9-person start-up company where he was the Director of medicinal chemistry and co-inventor for Rodatristat ethyl for the treatment of Pulmonary Arterial Hypertension- currently in Phase IIb clinical trials. Dan then moved west to Seattle as the Vice-President of drug discovery at Kineta, where he worked to enable several key partnerships with both Genentech and Pfizer Pharmaceuticals, before joining the Bill & Melinda Gates Foundation in 2020.

Currently, Dan co-leads the nonhormonal contraceptive initiative where he is utilizing his experience to discover and develop new, safe, and effective contraceptive options that respond to the needs of women and girls and empowers them to take charge of their own health and bring us closer to a more gender-equal world.

Dr. Mary Ann Handel, The Jackson Laboratory; *Career Panelist*

Dr. Handel, Professor Emeritus at The Jackson Laboratory received her Ph.D. from Kansas State University, followed by a Postdoctoral position at the Oak Ridge National Laboratory. My research has focused on discovery and screens for mouse genes required for fertility and their phenotypic characterization. I have been especially interested in mutations that reveal cell cycle regulation in mouse germ cells, with an emphasis on chromosome biology. Appropriate dynamics and behavior of chromosomes during meiosis (as well as mitosis) are essential to genetic integrity and reproductive success, and impact development of neoplasms and other conditions. I have exploited unbiased whole-genome ENU mutagenesis as a route to develop mouse models for studying fertility regulation, as a co-director of the NIH-supported Jackson Laboratory Reproductive Genomics Program. Overall, my research program has been aimed at discovering mechanisms that govern meiotic cell-cycle dynamics that are of broad-based significance beyond the biology of germ cells. In addition to my research endeavors, I have had leadership roles in many educational programs. These include being Director of the Graduate Program at the Jackson Laboratory, contributing to online education, and mentoring, over the years, the numerous undergraduate, graduate, and postdoctoral trainees who have contributed to my research program.

Travis Kent, PhD, NICHD Fertility and Infertility Branch; *Career Panelist*

Travis Kent, Ph.D., joined FIB as a program officer in April 2022. He manages the branch's male reproductive health program. Prior to joining FIB, he was a program officer in the Contraception Research Branch, focusing on novel contraceptive development, end-user behavior informing contraceptive design, and basic science aiding in contraceptive development. He started his career at NICHD in 2018 as an American Association for the Advancement of Science Science & Technology Policy Fellow, splitting his time between FIB and CRB.

Dr. Kent graduated from Washington State University with a Ph.D. in molecular biology from the laboratory of Dr. Mike Griswold, where he studied the impact of aberrant vitamin A metabolism on spermatogenesis. He completed his postdoctoral fellowship at the Jackson Laboratories with Dr. Mary Ann Handel, studying the role of testosterone in the exit of male germ cells from meiosis.

Talk Abstracts

Relationship between serum Anti-Müllerian hormone concentration and ovarian stimulation response in cheetahs (Acinonyx jubatus)

Rhasaan Bovell[1,2], Adrienne E. Crosier[3], Jennifer B. Nagashima[3], Jenny Santiestevan[3], Pierre Comizzoli[2], Ned J. Place[1]

[1]Department of Population Medicine & Diagnostic Sciences, Cornell University, Ithaca, NY, USA
[2]Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC 20008, USA
[3]Smithsonian Conservation Biology Institute, National Zoological Park, Front Royal, VA 22630, USA

Serum Anti-Müllerian hormone (AMH) concentration is used as a diagnostic marker in human reproductive medicine to predict how well a woman will respond to ovarian stimulation in preparation for assisted reproductive technologies (ARTs). Herein, we present preliminary data from two studies of zoo-housed cheetahs undergoing ovarian stimulation for various ARTs, with the principal objective of determining whether AMH has similar predictive value in this species. In study 1, blood samples were collected from six cheetahs within 1 month before ovarian stimulation for artificial insemination, and AMH levels were compared to counts of corpora lutea (CL) observed five days after the start of stimulation. Preliminary results indicate that more CLs tended to be observed among cheetahs with greater pre-stimulation AMH levels and suggest AMH >2 ng/mL is associated with favorable outcomes. In study 2, four cheetahs underwent ovarian stimulation to serve as oocyte donors for in vitro fertilization, while another four received the same regimen and served as embryo transfer recipients. AMH concentrations in these cheetahs were quantified using archived serum samples from the most recent date prior to stimulation, and then compared to the quantity, quality, and developmental competence of oocytes retrieved from donors, or the CL counts of recipients. Early results from these archived samples do not reveal a clear relationship between serum AMH and oocyte yield but do suggest associations among AMH and proportions of oocytes of high morphological quality or developmental competence among donors, as well as CL count among recipients. Of note, the combination of the oocyte donor and embryo recipient with the greatest AMH concentrations resulted in the only live birth ever reported, with a litter of two cubs. While preliminary, these results support the potential of pre-stimulation serum AMH concentration to predict the quality of the response to ovarian stimulation in cheetahs.

An Approach to Address Iatrogenic Male Infertility and Prevent Disease Transmission in Mouse and Human Models of Sickle Cell Disease

Mitchell R. Harancher, Kien Tran, Yi Sheng, Amanda Zielen, and Kyle E. Orwig

University of Pittsburgh School of Medicine

Sickle cell disease (SCD) is a crippling disease that significantly shortens lifespan and decreases quality of life. The only curative option for SCD is hematopoietic stem cell transplantation (HSCT). However, myeloablative conditioning prior to HSCT can cause infertility. SCD patients desire to have children, but also have concerns about passing the sickle cell trait to their offspring. The UPMC Fertility Preservation Program has cryopreserved testicular tissues for >70 young sickle cell patients to safeguard their future fertility. Spermatogonial stem cells (SSCs) in those tissues may be transplanted in the future to restore spermatogenesis in patient survivors. This creates an opportunity for targeted correction of the sickle cell mutation in patient SSCs, ex vivo, prior to transplantation to prevent transmission of the SCD trait to the next generation. Alternatively, the sickle cell mutation could be corrected in SCD patient-derived induced pluripotent stem cells (iPSCs) that gene corrected in vitro followed by differentiation into transplantable stem cells or sperm.

Sanger sequencing confirmed three candidate sgRNAs correctly targeted the SCD locus in the b-globin gene. Sanger sequencing also confirmed the SCD genotype in Towne's mice and human SCD patient iPSCs. Histology of Towne's mouse organs revealed the expected pathologies in the liver, spleen, and kidney. We established SSCs cultures from Towne's mice and derived iPSCs from a male patient with SCD. In ongoing experiments, the spermatogenic potential of Towne's SSCs will be tested by transplantation into infertile recipient male mice. Teratoma analysis and karyotyping will be used to validate SCD patient iPSCs. CRISPR/Cas9 gene editing with the validated sgRNAs will be used to correct the SCD mutation in Towne's SSCs and SCD patient-derived iPSCs.

Supported by UPMC, MWRI and NICHD HD096723.

Mapping ovulation with spatiotemporal and single-cell resolution

Ruixu Huang, Caroline Kratka, Cai McCann, Jack Nelson, Emily Zaniker, Luhan Tracy Zhou, Yiru Zhu, Daniela Russo, Hoi Chang Lee, Alex K. Shalek, Sami Farhi, Francesca E. Duncan, Brittany A. Goods

Dartmouth College

Ovulation is a dynamic process where the ovarian follicle undergoes a series of complex cellular, molecular, and physiological changes that lead to the release of a mature oocyte. To date, no studies have captured both the spatial and temporal aspects of these changes. These data, especially at single-cell resolution, can provide novel insights into the molecular mechanisms that regulate ovulatory processes. This is crucial for the development of non-hormonal contraceptives and to understand ovarian disease and fertility. Here, we performed an ovulation time course study with young mice and harvested their ovaries at 0hrs, 4hrs, and 12hrs after hyperstimulation by hCG injection. Nine total ovaries from three mice at three time points were isolated and used for Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH) to generate a single-cell spatial transcriptomic dataset. In parallel, contralateral ovaries were dissociated to perform single-cell RNA-sequencing (n = 6 mice total per timepoint). We identified 18 total cell clusters across the time course (n = 26,411 cells). Strikingly, we identify several clusters of cells that emerge dynamically over the course of ovulation. This includes two cumulus cell (CCs) clusters, with one population that arose 4 hours after hCG treatment and one that emerged 12 hours after hCG treatment. We also found a unique cluster of luteal cells at 12 hrs post hCG treatment. Trajectory analysis has revealed several novel genes that describe the differentiation of these time-dependent clusters. Finally, preliminary analyses of our spatial sequencing data suggest several genes that vary both temporally and spatially, revealing a set of transcripts that describe ovulation in a spatio-temporal manner. Taken together, this dataset comprehensively captures time-dependent and spatial changes in gene expression across the time course of ovulation. The unique and time-dependent CC clusters we identify here contain several possible non-hormonal contraceptive targets that warrant further study.

TRPV3 function contributes to zinc homeostasis and impacts early fertilization events in mice

Emily Lopes, Hiroki Akizawa, Ingrid Carvacho, Rafael Fissore

University of Massachusetts Amherst

Intracellular zinc ([Zn2+]i) levels in oocytes, unlike most somatic cells, are an order of magnitude higher than other transition metals such as iron and copper. During mammalian oocyte maturation, [Zn2+]i levels increase exponentially. To date, the transporter(s) and channel(s) that mediate this influx are not well characterized although two transporters, Zip6 and Zip10, have been reported to be involved. Upon fertilization, a Ca2+ wave is induced which triggers periodic releases of Zn2+ into the cell's extracellular environment (Zn2+ sparks). These sparks lower [Zn2+]i levels and facilitate the resumption of meiosis. Together, these findings suggest a role for Zn2+ pre- and post-fertilization. Interestingly, a member of the transient receptor channels, vanilloid subfamily 3 (TRPV3), known to mediate cation influx in mouse oocytes, displays exquisite functional expression that matches [Zn2+]i increase during maturation. We hypothesize that TRPV3, in oocytes, is involved in Zn2+ homeostasis. Using microfluorometry and confocal microscopy, we identified decreased levels of labile Zn2+ in MII oocytes (eggs) of Trpv3 KO mice (V3KO). We found that prior to maturation [Zn2+]i levels were similar between wildtype (WT) and V3KO oocytes. However, as maturation proceeded, labile Zn2+ concentrations increased in WT but not V3KO oocytes. Additionally, using electrophysiology we proved that TRPV3 can mediate Zn2+ influx in mouse eggs. Lastly, we examined how egg activation and subsequently Zn2+release were impacted in Trpv3 null eggs. Using FRET reporters, we show that the function of IP3R1, a major channel of Ca2+ release in mouse oocytes during fertilization, in V3KO eggs is compromised. Furthermore, we demonstrate that the pattern of Zn2+ release in these cells is abnormal after stimulation. Overall, TRPV3 is a pivotal contributor to Zn2+ homeostasis in mouse oocytes and eggs.

SARS-CoV-2 modifies the testicular compartment and decreases the number of SSC upon acute COVID-19 infection

Ana Martínez-Marchal, Yang Hu, Tianjiao Chu, Shruthi Shivkumar, Justin Bochter, Juan Pablo Morales, Timothy Robinette, Patrick Walsh, Marta Martín-Ruiz, Gretchen Rosado, Jimmaline Hardy, Maria Lopez-Panades, Andros Maldonado, Cristina Madrid, Lisa M. Barton, Eric J. Duval, Edana Stroberg, Subha Ghosh, Sanjay Mukhopadhyay, Kyle Orwig, Olivier Elemento, Guilherme Mattos Jardim Costa, Ignasi Roig, Miguel A. Brieño-Enríquez.

University of Pittsburgh School of Medicine

The new SARS-CoV-2 coronavirus arose in 2019, causing the Coronavirus disease 2019 (COVID-19), spreading worldwide with more than 757 million confirmed cases and over 6.8 million deaths. Even though COVID-19 has been reported to mostly cause a respiratory tract infection, other organs such as heart, liver, kidney, gastrointestinal tract, brain or skin are also affected. Interestingly, men are more susceptible to a higher severity and mortality due to COVID-19 infection than women. Differences in hormones could trigger different responses in both sexes, regulating the severity of the disease, the immune response, or the expression of COVID-19 entry factors. Men have a higher basal expression of ACE2 and TMPRSS2 in their gonads, specifically, Sertoli cells, Leydig cells, and spermatogonia have the highest expression of these entry factors in the testes, potentially being very permissive to the entry of the virus. Previous studies have shown the detrimental effects of different virus infections in the male genital system, including SARS-CoV (causing SARS disease), which has been shown to produce orchitis and alter the testicular compartment. There is a rising need to study if the gonadal function, and hence fertility, can be impaired upon SARS-CoV-2 infection. We analyzed autopsy testes samples of 31 COVID-19 diseased patients and compared them to 35 non-diseased controls. We observed an alteration of the testicular tissue, with an increase in immune infiltrates and thrombi in the interstitial tissue, and an increased cell death inside the tubules. We found that Sertoli cells (SOX9+) and spermatogonia stem cells (UTF1+) present an increase in DNA damage (ɣH2AX+) in COVID-19 samples, and strikingly, a 37% of spermatogonia stem cells are apoptotic in the virus infected samples. Furthermore, through snRNA-seq we deciphered the virus signature of infection in testes, revealing changes in cell populations and gene expression.

ADAD1 is a novel translation regulator important for intracellular protein transport and sperm head shaping during spermiogenesis

Sarah Potgieter, Christopher Eddy, Aditi Badrinath, and Elizabeth M. Snyder

Rutgers University

ADAD1 is a testis-specific RNA binding protein expressed in post-meiotic spermatids whose loss leads to severely defective sperm morphology and male infertility. Previous work has implicated ADAD1 in translation regulation however, the drivers of the Adad1 phenotype remain unclear. Detailed morphological analysis of Adad1 mutant sperm demonstrated defective DNA compaction and head shaping while RNA sequencing in Adad1 mutant testes revealed minimal transcriptome changes, suggesting ADAD1 acts downstream of transcription. Reduced ribosome association of transcripts and immunofluorescence of proteins encoded by translationally regulated transcripts, including AKAP4 and TNP1, revealed delayed translation in the absence of cell development delays indicating ADAD1 acts as a translational activator during spermiogenesis. Further analyses showed both TNP1 and AKAP4 displayed impaired subcellular localization suggesting abnormal protein transport in mutants. To better understand ADAD1's influence on spermatid intracellular protein transport, immunofluorescence in adult testes was used to define the formation and dissolution of the manchette, a protein transport microtubule network, across spermatid development. This analysis demonstrated abnormal manchette morphology along with delayed dissolution in mutant spermatids. Further, components of the LINC complex, which connects the manchette to the nuclear lamin, displayed delayed translation and/or localization in mutant spermatids. Together, these studies define the causative factors of the Adad1 phenotype and describe for the first time the underlying temporal regulation of the manchette and LINC complex during spermiogenesis. Ultimately, they reveal ADAD1 as a master regulator of manchette formation and dissolution, likely via translational regulation, and highlight the importance of the manchette and LINC complex in DNA compaction and head shaping during spermiogenesis.

Alternative Splicing Alters the Female and Male Germ Cell Splice-Ome at Transitions Points in Fetal Germ Cell Development

Andrew Yarilin and Victor A. Ruthig*

WCM

Alternative splicing by RNA binding proteins (RBPs) achieves broad protein diversity from a limited number of genes. In some developing tissues, shifts in the splice-ome act as developmental cues. The splice-ome is dynamic during adult spermatogenesis (spermatogonia, spermatocytes, spermatids). However, less is known about the fetal germ cell splice-ome. Recently, we identified a class of RBPs, including the splicing regulator Rbfox2, as differentially expressed between distinct pro-spermatogonia populations. These distinct populations are germ cells with higher (hi) or lower (lo) levels of the RBP DND1. Gene ontology analysis identified splicing as an enriched biological process in DND1-hi cells. Although DND1 is not a splicing regulator, our RNA immunoprecipitation data indicated DND1 regulates transcript expression of splicing regulators. Using our male germ cell transcriptome datasets, we analyzed differential transcript usage (DTU, splicing). DTU analysis found drastic splice-ome changes (1,121 genes) as pro-spermatogonia enter cell cycle arrest (E13.5 to E14.5). Many splice-ome changes at cell cycle arrest entry were related to chromatin regulation and cell cycle state. During arrest (E16.5 to E18.5) the splice-ome seems to stabilize (18 genes). However, the splice-ome during the arrest period is drastically different between DND1-hi and DND1-lo cells (2,185 genes E16.5, 1,188 genes E18.5). Many transcripts had dynamic changes in coding potential. We also previously showed that in the female and male germline expression of DND1 is negatively correlated with meiotic entry. Re-analyzing public RNA-seq datasets from fetal female germ cells identified switches in the female germ cell splice-ome at sex determination (121 genes) and a larger switch at meiotic entry (288 genes). Many of these meiotic switches in isoforms were specifically related to loss of coding potential and domain loss, especially open reading frames. Collectively, our data indicates female and male germ cells successfully differentiate during development partly through switches in alternative splicing.

Embryo live-imaging reveals nuclear DNA shedding during blastocyst expansion and biopsy

Robin M. Skory, Ana Domingo-Muelas, Adam A. Moverley, Goli Ardestani, Piotr Tetlak, Blake Hernandez, Eric A. Rhon-Calderon, Marisa S. Bartolomei, Denny Sakkas, Nicolas Plachta

UPENN

Assembling a blastocyst capable of implantation and in utero development relies on proper early patterning. In the mouse, genetic manipulation has enabled high resolution 3D imaging of the preimplantation embryo, revealing key changes in nuclear and cytoskeletal structure. Yet, live human embryo imaging has been restricted to brightfield in a single plane, limiting our knowledge of species differences. Here, we combine membrane-permeable dyes with resonant scanning and live-imaging to capture key events in human embryogenesis with new levels of spatial and temporal resolution. During blastocyst expansion, pressure from the growing cavity causes known changes in trophectoderm cell shape. In accordance with this morphogenesis, live imaging reveals that most nuclei adapt intact, whereas some nuclei undergo budding and form micronuclei. We show that this interphase process is distinct from chromosome segregation errors during mitosis and apoptosis-associated nuclear fragmentation. We also show that cells with a lower density of peri-nuclear keratins are more prone to undergo DNA shedding, and that keratin knockdown in the mouse results in higher rates of DNA shedding. Furthermore, to test the potential role of mechanical strain on nuclear shedding, we applied trophectoderm biopsy, a procedure widely performed for preimplantation genetic testing (PGT). We show that the addition of mechanical biopsy results in higher levels of nuclear budding in mouse and human embryos. Thus, we propose that DNA shedding is an additional mechanism generating mosaic aneuploidy in the embryo, in contrast to existing models solely based on chromosome segregation errors. Together, these results reveal distinct processes underlying human development and enables further investigation into the causes and consequences of aneuploidy in the embryo.

Investigating Meiotic Prophase I as an Intervention Point for Male Contraceptive Targeting

Stephanie Tanis, Leah E. Simon, Tegan Horan, Mercedes Carro, Amanda Touey-May, Connor Owens, Adriana K. Alexander, Jelena Lujic, Paula E. Cohen

Cornell University

Advances in contraceptive options have stalled since the advent of the pill over 70 years ago. A major obstacle in the search for a non-hormonal male contraceptive is the risk associated with non-reversibility when targeting developmental stages during spermatogenesis. To circumvent a host of downstream issues in spermatogenesis, we have chosen to target meiotic prophase I. However, it is unknown whether temporary cessation of prophase I will allow for normal resumption following a contraceptive block. The goal of the current study was to provide proof-of-principle showing that meiotic disruption could lead to a reversible cessation of spermatogenesis in male mice. In light of previous observations showing its critical role in meiotic progression, we chose to inhibit BRDT, the testis-specific member of the bromodomain and extra terminal family of proteins. BRDT acts as an essential regulator of prophase I by modulating many genes involved in critical meiotic processes, including meiotic sex chromosome inactivation (MSCI). Transcriptional silencing of the asynapsed XY chromosomes through MSCI is critical for meiotic progression, and MSCI disruption leads to spermatocyte death during mid-prophase I. Given this, we chose to focus on MSCI as an endpoint for BRDT inhibition as a contraceptive block. We injected male mice with a BRDT inhibitor for 8 weeks. Following treatment, we found considerable disruption of MSCI as evidenced by abnormal localization of components known to be critical for proper MSCI formation and a near ablation of post-meiotic cells. Further, scRNA-seq showed an increase of transcripts on the X chromosome. To profile the recovery of prophase I following a contraceptive block, we allowed mice six weeks of drug withdrawal and found only limited resumption of MSCI. These results suggest that extended time may be required for normal resumption of key meiotic processes and that mid-prophase I might not represent a suitable contraceptive timepoint.

Essential and recurrent roles for hairpin RNAs in silencing de novo sex chromosome conflict in Drosophila simulans

Jeffrey Vedanayagam, Marion Herbette, Holly Mudgett,
Ching-Jung Lin, Chun-Ming Lai, Caitlin McDonough-Goldstein,
Stephen Dorus, Benjamin Loppin, Colin Meiklejohn, Raphaëlle Dubruille and Eric C. Lai

Sloan Kettering Institute

Meiotic drive loci distort the normally equal segregation of alleles, which benefits their own transmission even in the face of severe fitness costs to their host organism. However, relatively little is known about the molecular identity of meiotic drivers, their strategies of action, and mechanisms that can suppress their activity. Here, we present data from the fruitfly *Drosophila simulans* that address these questions. We show that a family of *de novo*, protamine-derived X-linked selfish genes (the *Dox* gene family) is silenced by a pair of newly-emerged hairpin RNA (hpRNA) siRNA-class loci, *Nmy* and *Tmy*. In the *w[XD1]* genetic background, knockout of *nmy* derepresses *Dox* and *MDox* in testes and depletes male progeny, whereas knockout of *tmy* causes misexpression of PDox genes and renders males sterile. Importantly, genetic interactions between *nmy* and *tmy* mutant alleles reveal that *Tmy* also specifically maintains male progeny for normal sex-ratio. We show the *Dox* loci are functionally polymorphic within *D. simulans,* such that both *nmy*-associated sex-ratio bias and *tmy*-associated sterility can be rescued by wild-type X chromosomes bearing natural deletions in different *Dox* family genes. Finally, we provide experimental evidence for hpRNA-suppressed *Dox* family proteins, and in particular, PDox is detected on chromatin bridges in affected meiotic figures of tmy mutants. Altogether, these studies support a model in which protamine-derived drivers and hpRNA suppressors drive repeated cycles of sex chromosome conflict and resolution that shape genome evolution and the genetic control of male gametogenesis.

Targeting proprotein convertases and ovulation to develop non-hormonal contraceptives

Jiyang Zhang[1], Pawat Pattarawat[1], Inhyeok Hwang[1], Astha Adroja[1], Caroline Krattka[2], Francesca Duncan[2], Brittany Goods[3], Shuo Xiao[1]

[1]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University, Piscataway, NJ 08854
[2]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611
[3]Thayer School of Engineering, Dartmouth College, Hanover, NH 03755

Traditional female contraceptives are based on hormones such as progestin, which block ovulation but also cause undesired side effects, including hormone-related cancers, depression, stroke, and obesity. There is an urgent need to develop novel non-hormonal contraceptives. We previously performed single-mouse follicle RNA sequencing (RNA-seq) analysis to identify critical ovulatory genes and signaling using our established ex vivo ovulation system. The RNA-seq analysis revealed that Pcsk3 (Furin), Pcsk5, and Pcsk6, three members of proprotein convertase (PC) family, were continuously upregulated in follicular cells of ovulating follicles, suggesting their possible roles in contributing ovulation. Herein, we used the ex vivo ovulation system to test three PC inhibitors, including Furin Inhibitor I, Furin Inhibitor II, and Proprotein Convertase Inhibitor (PCI). All three PC inhibitors dose-dependently inhibited the rupture of ovulating follicles, with PCI having the most inhibitory potency. In addition, PCI did not affect follicle survival or hormonal secretion of estradiol (E2) and progesterone (P4). RT-qPCR results showed that PCI did not alter key genes or signaling pathways involved in follicle maturation, luteinization, and associated hormone synthesis and secretion. In situ zymography revealed that PCI inhibited gelatinase activation which critically regulates follicle rupture through proteolysis. Using a more complex ex vivo ovarian explant culture model, we were able to validate the inhibitory effect of PCI on follicle rupture. A preliminary in vivo experiment using a mouse superovulation model indicated that the treatment of PCI via intraperitoneal (IP) injection suppressed ovulation in vivo. Furthermore, the Genotype-Tissue Expression (GTEx) Portal database reveals that Pcsk5 has the most abundant expression level in human ovaries compared to all other tissues (e.g., uterus, breast, heart, and liver). Taken together, these results highlight the hormone-independent roles of PCs in regulating follicle rupture, suggesting an attractive druggable target for the development of non-hormonal contraceptives.

Poster Key

Click on any title to jump to abstract.

To return to this key from an abstract, click on the poster #

Poster Number	Primary Author Surname	Title
1	Abdelhady, A. et al.	Cryoprotectant and cooling-rate dependence of ice formation in bovine oocytes during cooling and warming probed by time-resolved X-ray diffraction.
2	Agyemang, J. et al.	Effects of exposure to bisphenol A(BPA) on maternal immune environment in pregnant mice
3	Akizawa, H. et al.	Physiological Zn2+ concentrations ensure robust fertilization-induced Ca2+ oscillations in mice.
4	Arroyo-Martinez, G. et al.	Roles of the 9-1-1 DNA damage response complexes in synapsis, DSB repair and silencing during mammalian meiosis
5	Arroyo-Salvo, C. et al.	Incubation conditions to improve equine sperm in vitro capacitation and fertilizing ability after ICSI
6	Arter, M. et al.	Characterizing the rapid molecular evolution of meiotic recombination proteins
7	Ascencao, C.F.R et al.	A Non-Canonical Role for TOPBP1 in XY Silencing is Essential for Meiotic Progression and Male Fertility
8	Atkins, G.R. et al.	Xenografting cryopreserved peripubertal human testicular tissue to mouse hosts
9	Ayers, M.C. et al.	Vitamin B6 Deficiency Perturbs Pregnancy Specific Chromatin Resulting in Gestational Diabetes
10	Baghaki, H. et al.	Does mesna (mercapto-ethanesulphonate sodium) protect ovarian reserve after cyclophosphamide treatment: an in vivo study with rats.
11	Balough, J.L. et al.	Defining ECM content and preservation in porcine vaginal tissue for use in developing ECM scaffolds that mimic the vaginal microenvironment
12	Ben-Shlomo, R. et al.	Considerable time is necessary for complete recovery of meiotic prophase I following disruption via BRDT inhibition
13	Bergman, K. et al.	Environmental contaminants modulate susceptibilities to gestational diabetes in mice
14	Blundon, J. et al.	Skp1 proteins are structural components of the synaptonemal complex in C. elegans
15	Boateng, R. et al.	The Jackson Laboratory Nonhormonal Contraceptive Model Development Program
16	Bradley, R.A. et al.	Post-translational modifications result in differential nuclear localization of CDK2 during male meiosis
17	Brown, N.C. et al.	Function and evolution of seminal fluid-derived odorant binding proteins in Drosophila
18	Caldeira-Brant, A. et al.	Effects of External Radiation Therapy for Prostate Carcinoma on Human Spermatogenesis

19	Carlisle, J.A. et al.	Evolution and Mechanisms of Female Factors Influencing Mating Plug Ejection in Drosophila Females
20	Carr, K. et al.	Characterization of the C-terminal region of MutS Homolog 5 (MSH5) reveals meiotic disruption in mouse meiosis
21	Carro, M. et al.	Argonaute-small RNA (AGO-smRNA) interactions drive sex chromosome silencing during meiosis
22	Chen, L. et al.	Perfluorononanoic acid (PFNA) concentration-dependently interferes with gonadotropin-dependent ovarian follicle maturation in an ex vivo folliculogenesis system
23	Cheron, S. et al.	Ovarian disrupting effects of organic matter extracts in real-world surface river waters
24	Chukrallah, L.G. et al.a	Two RNA binding proteins, ADAD2 and RNF17, interact to form novel meiotic germ cell granules required for male fertility
25	Cullen, S.M. et al.	The role of Khdc3 in epigenetic inheritance of obesity and metabolic disorders
26	Ding, X. et al.	Missense Variants in Human MLH1 and MLH3 Causing Premature Ovarian Insufficiency
27	Doctorovich, Y. et al.	Combing through Intrinsically Disordered Proteins to Find New Germline Genes
28	Dziubek, A. et al.	Impact of 3’ UTR variation on post-transcriptional regulation of spermatogenesis genes
29	Faheem Akhtar, M. et al.	Effect of summer months on testicular histoarchitecture and spermatogenesis in Dezhou donkey: A histological study
30	Galliou, J. et al.	Obesity Disrupts Preovulatory Ovarian Hemodynamics that are Correlated with Ovulation Rate
31	Gold, A. et al.	Uncovering the role of Polo-like kinase in regulating synaptonemal complex dynamics
32	Gopal, S. et al.	Investigating the role of NHR-49 in oocyte activation in C elegans
33	Gupta, N. et al.	TRPM7 is dynamically expressed in gametes and preimplantation embryos in mice
34	Hemphill, C. et al.	Epigenetic profile of hearts and brains from IVF offspring in a mouse model
35	Holmes, C. et al.	Antileukoproteinase: the whoa and go in neonatal foals and late gestation mares
36	Horan, T. et al.	Untangling unexpected functions of the FANCJ DNA helicase in mouse meiosis
37	Hu, Q. et al.	Identification of maternal effect genes altered by assisted reproduction, which lead to methylation errors in preimplantation embryos
38	Hua, S. et al.	H2A.Z Regulation of CTCF Binding and Higher-Order Chromatin Structure
39	Huang, R. et al.	The role of genomic maintenance during placental development

40	Hwang, I. et al.	Vitrification preserves follicular transcriptomic dynamics during ex vivo ovarian follicle maturation and ovulation
41	Kratka, C. et al.	Pharmacologic inhibition of proprotein convertases prevents cumulus expansion due to extracellular matrix instability
42	Li, Jing et al.	Naked Mole Rat Has2-expressing Mice: A Novel Mouse Model for Studying the Role of High Molecular Mass Hyaluronic Acid in Ovarian Aging and Reproductive Longevity
43	Li, Liangdao et al.	CRISPR Inhibition Screen Identifies Targets of Epigenetic Perturbations during PGC Development
44	Li, Tao et al.	ZMYM3 and PTIP associate ANKRD31 cooperatively ensuring high order chromatin structure and meiotic DNA break formation in the mouse pseudoautosomal region
45	Liu, Z. et al.	Transcription is not Sufficient for Methylation Establishment at H19/Igf2 Imprinting Control Region in the Male Germline
46	Loehr, A. et al.	Serum miRNAs as conserved biomarkers for the specific and sensitive detection of testicular germ cell tumors
47	Lu, M. et al.	Exploring the interplay between chromatin structure organization and meiotic DNA Double-Strand Break
48	Lujic,m J. et al.	A comprehensive analysis of major spatial transcriptomics platforms for identifying the most complete transcriptional atlas of the mammalian testis
49	Meng, F.W. et al.	Competition for H2A.Z sensitizes developing embryos to innate immune stimulation
50	Modzelewski, A. et al.	A mouse-specific retrotransposon drives a conserved Cdk2ap1 isoform essential for preimplantation development
51	Mohanty, G. et al.	Hyperpolarization induced acrosome reaction is mediated through the activation of GSK3 alpha in sperm head
52	Muhammad Faheem, A. et al.	Effect of summer months on testicular histoarchitecture and spermatogenesis in Dezhou donkey: A histological study
53	Munyoki, S. et al.	Timing is everything: Sex differences in the microbiome-gut-brain axis are time-of-day dependent
54	Nayyab, S. et al.	TSSK1 and TSSK2 are independently essential for male reproduction
55	Nguyen, D. et al.	Pre-Zygotic Genome Activation Heterochromatin Formation on Repetitive Elements is Essential for Precise Developmental Timing
56	N'tumba-byn, T. et al.	High-throughput screening of meiotic gene mutants through germ line stem cell transplantation
57	Pattarawat, P. et al.	A high-throughput ovulation screening platform to identify non-hormonal contraceptive candidates
58	Pirtz, M. et al.	Exploring normal and malignant mouse endometrium using scRNA-sequencing and spatial transcriptomics
59	Prasasya, R.D., et al.	TET1 Catalytic Activity is Required for Reprogramming of Imprinting Control Regions and Patterning of Sperm-Specific Hypomethylated Regions

60	Qin, Y. et al.	Investigating the Maternal Effects in Female-biased Embryonic Death Induced by Genomic Instability
61	Ralston, C.Q. et al.	USING SINGLE-CELL TRANSCRIPTOMICS TO IDENTIFY ELUSIVE CELL TYPES WITHIN THE HUMAN FALLOPIAN TUBE EPITHELIUM
62	Rhon-Calderon, E.A. et al.	Trophectoderm biopsy followed by vitrification after in vitro fertilization and embryo culture increases fetal and placental epigenetic dysregulation in a mouse model.
63	Safrai, M. et al.	Assessing the Potential of ChatGPT in Scientific Writing: A Rapid Production of Content with New Challenges
64	Safrai, M. et al.	Fertility preservation for young girls – When your zip code is more important than your diagnosis.
65	Simon, et al.	Generating an In Vitro Meiotic “Toolkit”
66	Smith-Raska, M.	Examining Small RNA Regulation in the Male Reproductive System with Single Cell RNA-Seq.
67	Tang, S. et al.	Membrane fusion in mammalian fertilization
68	Thomalla, J.M. et al.	TRPM's role in protein phosphorylation during Drosophila egg activation
69	Touey, A.E. et al.	Uncovering Novel Roles of Argonaute Proteins in Spermatogenesis and Early Embryonic Development
70	Voigt, A.L. et al.	New Insights Into Prepubertal Spermatogonial Stem Cell Development
71	Weber, W.D. et al.	Oocyte and Early Embryo Metabolism
72	Wolff, I.D. et al.	Phosphorylation of SKP1 regulates SKP1-Cullin-F-box (SCF) ubiquitin ligase complex assembly during mouse spermatogenesis
73	Wood, A. et al.	Elucidating the function of CNTD1 in crossover licensing in mammalian oogenesis
74	Zhan, T. et al.	Harmful algal bloom toxin microcystins interfere with gonadotropin-dependent ovarian follicle maturation and ovulation to result in poor female reproduction
75	Zhang, Ying et al.	Follicle-stimulating hormone at the threshold and excessive levels induces distinct ovarian responses and follicular transcriptomic profiling in an ex vivo murine folliculogenesis system
76	Zhang, Jingzhi et al.	Functions of H4K16ac and MOF in Bovine Embryogenesis
77	Zhang, Hanxue et al.	CCAAT/enhancer binding proteins alpha and beta selectively regulate gene expression by temporal and dose-dependent mechanisms in preovulatory ovarian follicles.
78	Zheng, S. et al.	Impacts of Early Life Social Experiences on Oxytocin Receptors in the Brain
79	Zhu, Y. et al.	Well-established nuisance compounds in the drug screening pipeline elicit distinct phenotypes during mouse in vitro oocyte maturation

Poster abstracts

Poster # 1

Cryoprotectant and cooling-rate dependence of ice formation in bovine oocytes during cooling and warming probed by time-resolved X-ray diffraction.

A.Abdelhady[a], S.H.Cheong[a], and R.E.Thorne[b]

[a] Department of Clinical Science, College of Veterinary Medicine, Cornell University, Ithaca NY
[b] Department of Physics, Cornell University, Ithaca NY USA

Post-thaw survival of oocytes depends on the amount, type, and grain size of intracellular ice formed. Ice formed during cooling depends on cryoprotective agent (CPA) concentration and cooling rate. Ice growth during warming also depends on ice formed during cooling and on the warming rate.
Objective: Determine the effects of cooling rates, and CPA concentrations on ice formation in bovine oocytes during both cooling and warming. The second objective is to determine the minimum CPA concentration required to achieve acceptable ice fraction and ice crystal sizes during the freeze/thaw cycle.
Methods: Bovine MII oocytes from slaughterhouse ovaries were cooled under various conditions (n=350, at least 10 oocytes per cooling speed and CPA concentration): at two cooling rates (~2000oC/min and >600,000oC/min using a NANUQ automated cryocooler); soaked with a standard CPA solution (15% dimethyl sulfoxide (DMSO), 15% ethylene glycol (EG)) at strengths between 100% and 40%, with constant (0.5 M) or decreasing sucrose concentration. X-ray diffraction and were recorded from oocytes at 100 K and then as oocytes were warmed.
Results: Ice grain sizes at higher CPA concentrations always remained modest, but larger grains developed when using lower CPA concentrations and slower cooling and warming rates. At the fastest cooling rate, oocytes could be reliably vitrified with only small grain ice on warming using CPA concentrations down to and including 6%DMSO, 6%EG, and 0.2M sucrose.
Conclusions: Time-resolved x-ray diffraction allows direct and detailed characterization of ice formation in oocytes during cooling and, more importantly, during warming. Improved cooling technologies allow large (~60%) reduction in CPA concentrations required to adequately inhibit ice formation.

Poster # 2

Effects of exposure to bisphenol A(BPA) on maternal immune environment in pregnant mice

J. Agyemang, J. Reed, P. Spinelli, and M. Susiarjo.

University of Rochester Medical Center (URMC)

Bisphenol A (BPA) is a ubiquitous environmental contaminant found in various consumer products. Exposure to BPA is associated with pregnancy loss in human and animal studies. One mechanism of pregnancy loss is aberrant maternal-fetal immune tolerance. Regulatory T cells (Treg) are critical moderators of maternal immune tolerance and their expansion during pregnancy downregulates pro-inflammatory signaling molecules. Our lab recently reported that exposure to BPA results in higher rates of hemorrhaging and resorption of allogeneic mouse conceptuses generated from mating of CBA female to C57BL/6 male mice. These changes were correlated with decreased Tregs number in maternal tissues from BPA exposed-CBA female mice relative to controls. The aim of this study is to test whether similar findings are observed in allogenic conceptuses generated from C57BL/6 females mated to CBA males, and syngeneic conceptuses from CBA females mated to CBA males. Conceptuses will be microscopically harvested, analyzed at embryonic day (E) E7.5 by two blinded individuals, and we will compare hemorrhaging rates in conceptuses from control and BPA-exposed mice. Hematoxylin and eosin (H&E) staining of decidual capsules will also be performed to study the histopathology of the hemorrhaging phenotype including the presence of mononuclear cell infiltration. Finally, adoptive transfer of Tregs will be performed to determine whether Tregs rescue pregnancy loss in mice exposed to BPA.

Poster # 3

Physiological Zn2+ concentrations ensure robust fertilization-induced Ca2+ oscillations in mice.

Hiroki Akizawa, Emily Lopes, Rafale Fissore

University of Massachusetts Amherst

Changes in the intracellular concentration of free calcium (Ca2+) underpin egg activation and initiation of development in all animal and plant species. In mammals, the type 1 inositol 1,4,5-triphosphate receptor (IP3R1) mediates the repetitive rises known as Ca2+ oscillations. More recent evidence shows that the intracellular concentration of zinc (Zn2+) exponentially increases during oocyte maturation in agreement with its essential roles during this process, metaphase II arrest, and egg activation, all conserved functions across species. It is nevertheless unclear if these vital cations for egg activation interplay during fertilization. Previously, we and others showed that Zn2+ depletion by addition of TPEN, a metal ion chelator, diminishes IP3R1 sensitivity, resulting in premature termination of Ca2+ oscillations after fertilization. In the present study, we further explored the IP3R1-mediated Ca2+ release following manipulation of Zn2+ levels. We found that Zn2+ insufficiency as well as Zn2+ overload greatly reduces Ca2+ release through IP3R1. To accomplish this, we used a genetically modified FRET sensor that allows specific sensing of Ca2+ in the endoplasmic reticulum (Ca2+ER) and combined it with a fluorescent dye to assess cytoplasmic Ca2+ to simultaneously measure Ca2+ levels in both compartments. Under the Zn2+-deficient conditions generated by TPEN, Ca2+ released from the ER induced by several agonists was significantly reduced. This effect was replicated using a double knockout mouse line deficient for two channels likely to transport Zn2+ and that lowered intracellular Zn2+ concentrations in eggs. On the opposite side, we increased Zn2+ levels by exposing eggs to pyrithione. Pyrithione dose-dependently inhibited the Ca2+ oscillations and hindered Ca2+ releasing mediated by IP3R1. Further, the inhibition of oscillations caused by lowering Zn2+ and TPEN could be rescued by elevating Zn2+ with pyrithione. These results suggest that Zn2+ levels in fertilizing eggs must be within a physiological range for optimal IP3R1 function and robust Ca2+ oscillations.

Poster # 4

Roles of the 9-1-1 DNA damage response complexes in synapsis, DSB repair and silencing during mammalian meiosis

Arroyo-Martinez, GA.[1], Pereira, C.[1], Lyndaker, A.[1], Smolka, M.[2], Weiss, RS.[1]

[1]Department of Biomedical Sciences, Cornell University, United States;
[2]Department of Molecular Biology and Genetics, Cornell University, United States

The defining events during meiosis start in prophase I, where programmed double-strand breaks (DSB) allow homologous chromosomes to synapse, crossover, and segregate accurately. Therefore, germ cells must implement DNA damage response (DDR) mechanisms to allow transmission of high-quality DNA to future generations. The heterotrimeric RAD9A-RAD1-HUS1 (9A-1-1) complex is a key player in the DDR. In somatic cells, the 9-1-1 complex 1) directly recruits DNA repair proteins to damage sites and 2) activates DDR signaling via interactions between the phosphorylated C-terminal tail of RAD9 and TOPBP1, resulting in ATR activation, which in turn is crucial for DNA repair, cell cycle regulation, and overall genome maintenance. Adding to the complexity, spermatocytes have subunit paralogs that form alternative 9-1-1 complexes (RAD9B-RAD1-HUS1 and RAD9B-RAD1-HUS1B). To study the 9-1-1 complexes in the germ line, we generated testis-specific Rad1 conditional knock-out (CKO) mice and observed severe asynapsis, compromised DSB repair, impaired meiotic silencing, and ATR signaling defects. Since Rad1 deletion disrupts clamp formation, the Rad1 CKO model does not differentiate between the signaling-dependent and independent roles of the 9-1-1 complexes in meiosis. To specifically understand the biological functions of 9-1-1 mediated ATR activation, we developed separation-of-function mutants with serine-to-alanine (SA) mutations in the C-terminal tail of RAD9A and RAD9B (Rad9A/9B SA) that disrupt RAD9-TOPBP1 interactions, perturbing ATR signaling. These mouse mutants were viable, whereas null mutations in Rad9a or Rad9b cause embryonic lethality. Rad9A/9B SA double mutant mice had reduced testis size, reduced sperm count, and seminiferous tubule defects, highlighting an important role for 9-1-1 mediated ATR activation in meiosis. Preliminary assessment of prophase I in Rad9A/9B SA mutant spermatocytes identified defects in synapsis and DNA damage repair. Current experiments aim to address the role of the 9-1-1 complexes in regulating meiotic silencing through ATR signaling. Together with phosphoproteomic analyses, these experiments are expected to shed light on how the 9-1-1 complexes can promote meiotic progression by facilitating DSB repair and meiotic silencing through ATR activation.

Poster # 5

Incubation conditions to improve equine sperm in vitro capacitation and fertilizing ability after ICSI

Arroyo-Salvo, C; Plaza, J; Río, S; Bogetti, E; Miragaya, M; Gambini, A; Perez-Martinez, S.

CEFYBO-CONICET, ARGENTINA

Intracytoplasmic sperm injection (ICSI) is currently the most reliable technique to produce in vitro equine embryos. Specific sperm culture conditions could improve the understanding of capacitation in this species and potentially increase the success of reproductive biotechnologies. The aim of this study was to evaluate the effect of a defined incubation medium on sperm motility, capacitation-associated events and oocyte activation after heterologous ICSI using pig eggs. Frozen-thawed sperm were incubated in non-capacitating (modified TALP medium, NC) and capacitating (modified TALP supplemented with 25 mM sodium bicarbonate and 1 mg/ml PVA, CAP) for 45 min at 38.5 °C in air. Sperm motility was evaluated by CASA system and the residues phosphorylated by PKA (pPKA) and tyrosine phosphorylation (pTyr) were evaluated by western blot. For heterologous ICSI, equine sperm cells were incubated for 45 min in NC or CAP medium, injected into matured pig oocytes and the presence of two pronuclei (2PN) was determined. Sperm incubation in CAP conditions showed an increase in total (25.6 % ± 5.5 vs 12.1 % ± 2.3) and progressive (23 % ± 5.2 vs 9.2 % ± 1.8) motility (P<0.05). Moreover, incubation in CAP conditions induced higher pPKA and pTyr levels. Interestingly, two parameters associated with hyperactivation, VCL (115.74 ± 11.4 vs 71.85 ± 9.1 µm/s) and ALH (1.16 ± 0.1 vs 0.75 ± 0.1 µm) were improved under CAP condition (P<0.05). Finally, sperm incubated under CAP conditions prior to ICSI, induced a higher proportion of 2PN than those incubated in NC conditions (76.4 % ± 7.28; 47.6 % ± 6.98, respectively) (P<0.05). These results confirm that incubation of sperm cells in our capacitation medium supports crucial events associated with sperm capacitation and improves fertilizing ability after ICSI. Future studies are needed to confirm these results in homologous ICSI and to elucidate the associated-signaling pathways involved.

Poster # 6

Characterizing the rapid molecular evolution of meiotic recombination proteins

Meret Arter, Scott Keeney

Memorial Sloan Kettering Cancer Center

Most sexually reproducing eukaryotes use meiosis, a specialized cell division program, to generate haploid and genetically distinct gametes. Most organisms use homologous recombination repair of self-inflicted DNA double-strand breaks into crossovers to segregate homologous chromosomes and achieve haploidisation of the parental genome. Robustness of the meiotic recombination process is vital to produce healthy offspring that inherit a complete and intact genome. Interestingly, despite homologous recombination being a well conserved pathway, many meiotic DNA repair proteins are evolving surprisingly fast. This fact has traditionally made it difficult to identify homologous proteins in distantly related species, but even in closely related species sequence conservation is low for many meiotic recombination proteins. The causes and functional consequences of rapid sequence divergence are not well understood. In this study, we set out to systematically characterize the molecular evolution of meiotic recombination proteins that are involved in DNA double-strand break formation, homologous recombination DNA repair and crossover formation. Our analyses in different lineages and on different evolutionary timescales using phylogenetic and population genomic approaches further confirm a remarkable lack of conservation for many key components of the recombination pathway, often in a lineage-specific manner. Unfortunately, most methods currently available are severely limited by the number of available sequences and they rarely allow for a robust distinction between models of reduced purifying selection or adaptive selection, especially at the level of individual codons. To overcome these limitations, we include functional knowledge and systematic comparisons of mitotic and meiotic paralogs together with structural predictions to identify specific residues and protein domains that diverge more rapidly than expected. Using a comparative and structure-aware approach, we can now characterize the divergence of the meiotic recombination machinery at unprecedented resolution.

Poster # 7

A Non-Canonical Role for TOPBP1 in XY Silencing is Essential for Meiotic Progression and Male Fertility

Carolline Fernanda Rodrigues Ascencao*, Jennie Sims*, Alexis Dziubek, William Comstock, Elizabeth Fogarthy, Jumana Badar, Raimundo Freire, John Schimenti, Andrew Grimson, Paula Cohen, Robert Weiss and Marcus Smolka

Cornell University

In mammals, the ability of males to cope with the limited synapsis of the X and Y chromosomes during meiosis I relies on the process of meiotic sex chromosome inactivation (MSCI). Components of the somatic DNA damage response machinery, including ATR, TOPBP1, MDC1 and BRCA1 play key roles in MSCI, although how they establish XY silencing remains incompletely understood. In particular, it remains unclear if and how DDR factors coordinate XY silencing with the formation of the sex body, a distinct phase-separated sub-nuclear structure formed during prophase I to house the unsynapsed XY bivalent. Here we report a Topbp1 mutant mouse (Topbp1B5/B5) with impaired XY silencing but grossly normal formation of sex body. While Topbp1B5/B5 mice are viable, without detectable somatic defects, males are completely infertile. Distinct from mice conditionally lacking ATR or TOPBP1 during meiosis, Topbp1B5/B5 males exhibit normal chromosome synapsis and canonical markers of DNA repair. ATR signaling is mostly intact in Topbp1B5/B5 spermatocytes, although specific ATR-dependent events are disrupted, including localization of the RNA:DNA helicase Senataxin to chromatin loops of the XY. Strikingly, Topbp1B5/B5 spermatocytes initiate MSCI although the completion of silencing is defective, with different genes in the X chromosome displaying distinct patterns of silencing de-regulation. These findings suggest a non-canonical role for the ATR-TOPBP1 signaling axis in establishing proper XY silencing via control of Senataxin and show that complete XY silencing is not a precondition for sex body formation and maintenance.

Poster # 8

Xenografting cryopreserved peripubertal human testicular tissue to mouse hosts

Georgia Rae Atkins BS, Meena Sukhwani PhD, Kyle E. Orwig PhD

University of Pittsburgh School of Medicine

Xenografting immature testicular tissues into castrated SCID mice has produced sperm and offspring from several mammalian species, but this has not been reported for human tissues. Prior studies from our lab determined that hCG is necessary to maintain monkey and human xenografts in mouse hosts. Human grafts transplanted under the skin of castrated SCID mice survive, grow, and produce testosterone, but unlike the experience with other species, the germ cells disappear over time. We hypothesized that the location of the graft and/or castration of recipient mouse will impact the survival and initiation of spermatogenesis from human immature testicular tissue. Male SCID mice had 2 grafts under the back skin and 2 grafts in the hindlimb muscle. Grafts were collected at 1 week, 2 weeks, 1 month, and 2 months. Mice were treated two times a week with 10 IU of hCG. Early results indicate that graft weight at the time of collection (reported as a percentage of starting weight) was higher under the back skin of non-castrated hosts (71.47±10.61) than the hindlimb of non-castrated hosts (45.78±4.09); or the back skin (58.99±8.99) and hindlimb of castrated hosts (52.94±7.96). We are replicating these experiments to determine if these differences are statistically significant, analyzing grafts to identify the presence/stage of germ cells, and measuring testosterone production.

Supported by NICHD HD100197.

Poster # 9

Vitamin B6 Deficiency Perturbs Pregnancy Specific Chromatin Resulting in Gestational Diabetes

Michael C. Ayers, Phil Spinelli, Martha Susiarjo, and Patrick J. Murphy

University of Rochester Medical Center

Pregnancy results in necessary changes to glucose metabolism to support the nutritional needs of the developing offspring. Initial increases in the mother's insulin resistance allow for ample glucose availability to the offspring. However, this also signals a compensatory proliferation of pancreatic beta islet cells to avoid the development of hyperglycemia in the mother. Gestational diabetes can be caused by nutritional deficiencies, including vitamin B6, a cofactor for serotonin synthesis required to signal proliferation. We sought to profile the changes in epigenetic marks in these cells to identify what changes underlie the reprogramming involved in islet proliferation. We performed CUT&Tag profiling of two epigenetic marks, the activating histone mark H3K4me3 and the repressive mark H3K27me3, on pancreatic beta islet cells in pregnant mice. Pregnancy results in a genome-wide increase in activating H3K4me3 at promoters. The changes in H3K27me3 in pregnancy are minor, so we propose that the activation of important genes through H3K4me3 changes is the major driver of pregnancy proliferation of islet cells. Impacted genes include developmental genes, possibly pointing to a reprogramming towards a more proliferative state reminiscent of embryonic development. Using a vitamin B6 deficient mouse model of gestational diabetes, we found that there is a large increase in the repressive H3K27me3 mark in pregnancy, as well as some decreases of H3K4me3. We are therefore currently investigating the hypothesis that the increases of H3K27me3 in the vitamin B6 deficient model inhibit the required increases of H3K4me3 at key loci.

Poster # 10

Does mesna (mercapto-ethanesulphonate sodium) protect ovarian reserve after cyclophosphamide treatment: an in vivo study with rats.

Hayriye Sema Baghaki, Cihan Kaya, Erdem Soztutar, Nilay Aksoy, Levent Yasar, Deniz Ekin, Murat Ekin

University of Pittsburgh School of Medicine

Purpose: This study sought to investigate the protective effect of 2-mercaptoethanol sodium sulfonate (mesna) on the ovarian reserve of rats treated with cyclophosphamide.

Method: Twenty-four adult female Wistar Albino rats equally divided into three groups. Group A (n = 8) received saline injections, Group B (n = 8) was given cyclophosphamide, and Group C (n = 8) was premedicated with mesna and given cyclophosphamide. Preoperative blood samples (1 ml) drawn before any procedures has done. All the groups underwent bilateral oophorectomy, and one milliliter of blood samples taken 24 hours after the surgery. The differences in the anti-Mullerian hormone (AMH) level between the pre and postoperative blood samples determined. The ovaries were morphologically evaluated based on features such as stromal edema, stromal hemorrhage, and follicular atresia. We also detected the AMH receptor expression level by immunohistochemical analysis. Finally, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis performed to assess the level of apoptosis.
Results: A significant increase noted in the AMH receptor expression level of cyclophosphamide only group compared with mesna and cyclophosphamide group (p: 0.007). In addition, there were significantly less atretic follicles in cyclophosphamide only group compared to mesna and cyclophosphamide group (p: 0.002). However, the TUNEL analysis revealed no significant difference between these two groups regarding apoptosis.
Conclusion: Mesna can prevent follicular atresia and increase the expression of AMH receptors in the follicles following acute cyclophosphamide toxicity.

Keywords: chemotherapy, cyclophosphamide, mesna, ovarian reserve.

Poster # 11

Defining ECM content and preservation in porcine vaginal tissue for use in developing ECM scaffolds that mimic the vaginal microenvironment

Julia L. Balough, Brittany Egnot, Marrisa Therriault, Bryan Brown, Pamela Moalli

University of Pittsburgh School of Medicine

The vagina is an elastic, fibromuscular tissue that undergoes dynamic changes in epithelial and connective tissue remodeling during the menstrual cycle and in response to birth and other injury. With age, vaginal tissue experiences atrophy and inflammation associated with the genitourinary syndrome of menopause. Vaginal fibroblasts remodel the extracellular matrix (ECM) in response to varied biomechanical environments. In turn, increased ECM stiffness can induce fibroblast differentiation into myofibroblasts. While myofibroblast differentiation is necessary for wound healing, myofibroblast accumulation via activation of anti-apoptotic pathways and collagen deposition induce pathologic fibrosis. The goal of this study is to determine the density of cellular and ECM components after decellularization in porcine vagina for future use as an ECM scaffold in an in vitro system to study vaginal fibroblast-ECM interactions. Core biopsies of 4-6 month old porcine vagina were taken and halved for direct comparison of intact versus decellularized tissue. Intact halves were lyophilized and weighed. The corresponding halves underwent a 5-day decellularization protocol using a combination of detergents and hypotonic water to induce cellular rupture. The ECM accounted for 13.4±0.8% and cellular content accounted for 7.53±1.00% of the total tissue mass. Studies are in progress to quantify the degree of cellular loss and preservation of collagen content after decellularization. Simultaneously, a trial of human vaginal fibroblast cells were seeded (100,000 cells/construct) into a reconstituted vaginal ECM gel (10mg/ml or 20mg/ml) and cultured for 5-days. Over the course of the culture, construct size and contraction were measured to determine how fibroblast cells adhere and generate force upon the ECM scaffold. Further, histologic analysis will be used to observe the depth of cell penetration into the gel, apoptosis and proliferation. Creation of an in vitro system that mimics the vaginal microenvironment is necessary to define cell-ECM interactions and for therapeutic discovery to address vaginal pathophysiology.

Poster # 12

Considerable time is necessary for complete recovery of meiotic prophase I following disruption via BRDT inhibition

Roni Ben-Shlomo, Stephanie Tanis, Leah E. Simon, Jelena Lujic, Paula E. Cohen

Cornell University

Current male contraception options such as condoms and vasectomies fall short in their effectiveness and reversibility, while hormonal options often cause undesired side effects. In search of an efficient, reversible, and non-hormonal alternative for males, we explored the cytogenetic effects of JQ1 in prophase I of meiosis. JQ1 is a small molecule inhibitor of BRDT, which is a critical regulator of meiosis during spermatogenesis. Adult male mice were administered JQ1 drug for 3 weeks to investigate if the meiotic disruption caused by JQ1 could be reversed upon withdrawal. Following the injection period, half of the treated mice were allowed to recover for 6 weeks before undergoing breeding analysis. Each mouse sired a minimum of 3 litters before being sacrificed for further analysis 20-30 weeks post treatment. Control mice were injected with vehicle. Analysis of prophase chromosome spreads from all test groups revealed disruption of meiotic markers such as RNA Polymerase II, γH2AX, and MLH1 following 3 weeks of JQ1 treatment. However, complete recovery of these markers was observed 20-30 weeks after cessation of treatment, indicating that the effects of JQ1 were reversible. In addition, there was no significant difference in mouse testis weights before and after injection, and the proportion of abnormal sperm to normal sperm was similar to the control group. Although the effects of JQ1 were reversible, complete recovery of meiosis required considerable time off the drug. Exploring the effects of other drugs in disrupting prophase I may assist in determining whether JQ1 itself is slow with recovery, or if perhaps an earlier time point within spermatogenesis should be assessed for contraceptive targeting. These studies provide proof-of-principle that pharmacological inhibition of meiosis could be a potential target for contraception, assuming appropriate accessibility to target cells in the testis and more rapid recovery of meiotic parameters.

Poster # 13

Environmental contaminants modulate susceptibilities to gestational diabetes in mice

Knickole Bergman, Ashley Fields, Phillip Spinelli, Clementina Mesaros, and Martha Susiarjo

University of Rochester

Gestational diabetes mellitus (GDM) is a form of diabetes that manifests during pregnancy and is characterized by high blood glucose levels and insulin resistance. GDM has a worldwide prevalence of 5-25.5% and is influenced by race, ethnicity, age, body composition, and screening and diagnostic criteria. People with GDM have an increased risk for miscarriage, premature birth, preeclampsia, and Cesarean section, as well as for developing type II diabetes following pregnancy. The developing fetus is also at risk for adverse health conditions, including increased birth weight, breathing problems, hypoglycemia, obesity, premature birth, and type II diabetes later in life. Published work has demonstrated that serotonin signaling plays a critical role in maternal pancreatic beta cell proliferation in pregnancy, and genetic and environmental factors that perturb serotonin signaling cause gestational diabetes in mice. Our lab has previously shown that maternal diet low in vitamin B6, a critical co-factor of serotonin synthesis, reduces beta cell proliferation in the pancreas and induces maternal hyperglycemia and glucose intolerance in mice. This observation suggests that factors influencing maternal vitamin B6 status during pregnancy modulates susceptibilities to gestational diabetes. Preliminary studies in our lab show that exposure to ubiquitous and persistent environmental contaminants such as Perfluorooctanoic Acid (PFOA) reduce levels of maternal vitamin B6 in the islets. Ongoing studies are performed to characterize the effects of gestational exposure to PFOA and bisphenol A (BPA) on maternal pancreatic islet morphology, serotonin signaling, and beta cell proliferation, as well as on metabolic phenotypes in pregnant mice. Elucidating the role of environmental exposure in pancreatic morphology and function is pertinent to understanding the disparate risks people have for developing GDM. These studies will contribute to new knowledge that benefits the health of mother and child.

Poster # 14

Skp1 proteins are structural components of the synaptonemal complex in C. elegans

Joshua Blundon, Brenda Cesar, Jung Woo Bae, Ivana Čavka, Jocelyn Haversat, Simone Köhler, and Yumi Kim

Johns Hopkins University

Faithful chromosome segregation during meiosis requires chromosomes to pair and recombine with their homologous partners during meiotic prophase I. In most eukaryotes, homologous chromosome alignment is reinforced by synapsis, a process defined by the assembly of the synaptonemal complex (SC), a tripartite protein structure that assembles between homologous chromosomes. The SC interacts with crossover-promoting factors and enables their diffusion and concentration along meiotic chromosomes, thereby regulating the number and distribution of crossovers. Recent evidence from diverse eukaryotes indicates that the Skp1-Cul1-F-box (SCF) E3 ubiquitin ligase regulates synapsis, although the mechanism of its action remains unclear. Here, we report that two paralogous Skp1-related proteins in C. elegans, SKR-1 and SKR-2, serve as structural components of the SC, independently of their canonical roles within the SCF complex. SKR-1 and SKR-2 associate with the other SC proteins and localize to the SC central region, constituting its central element. As recently shown for Dictyostelium Skp1, recombinant SKR-1 forms a dimer in vitro. Strikingly, mutating the dimer interface of SKR-1/2, without impairing the SCF activity, results in a complete failure in synapsis and crossover formation, which is indistinguishable from the phenotypes observed in worms lacking other SC proteins. Intriguingly, the Skp1 dimerization interface is predominantly hydrophobic and overlaps with the binding sites for Cul1 and F-box proteins. Thus, the dimerization of SKR-1/2 and SCF formation are mutually exclusive, providing a molecular basis for why SKR-1/2 cannot function as part of the SCF complex once incorporated into the SC. Together, our findings reveal a remarkable case of meiotic regulation where a highly conserved cell cycle regulator is repurposed as part of the essential meiotic scaffold and may provide a mechanism for coupling SC assembly and disassembly with cell cycle progression.

Poster # 15

The Jackson Laboratory Nonhormonal Contraceptive Model Development Program

Ruby Boateng, Christopher McCarty, Kristina Palmer, Robert Taft, Stephen A. Murray, Ewelina Bolcun-Filas, Robert E. Braun

The Jackson Laboratory

The Jackson Laboratory (JAX) is a world leader in mammalian genetics and human genomics research. JAX is a major partner in the International Mouse Phenotyping Consortium (IMPC) through the NIH-funded Knockout Mouse Project (KOMP). The goal of the IMPC is to functionally characterize, through reverse genetics and high throughput phenotyping, the human and mouse orthologous genome. To date, the consortium has generated and phenotyped > 9000 lines of mice. Among these, the IMPC has identified ~325 mouse knockout lines with infertility phenotypes, however the precise nature of the defects remains unknown for many of them. The JAX-NHC project focuses on the analysis of novel mouse models of infertility as an approach to expand our understanding of genes associated with infertility and identify novel nonhormonal contraceptive targets with the highest potential for translation. The goal of this project is to provide critical data and mouse lines that will enhance target prioritization for nonhormonal contraception and provide new tools and models for assessing biology and compound activity in the future. We are conducting deep reproductive profiling of 100 novel mouse lines with infertility phenotypes (50 female and 50 male) including gonadal histopathology, superovulation response, sperm quality analysis and IVF success rates. We will present our resources and reproductive phenotyping results for completed lines with the goal of sharing new knowledge and novel phenotypes with reproductive research community.

Poster # 16

Post-translational modifications result in differential nuclear localization of CDK2 during male meiosis

Rachel A. Bradley, Ian D. Wolff, Paula E. Cohen, & Stephen Gray

Cornell University

Meiotic prophase I is characterized by homologous chromosome pairing, synapsis, and crossover (CO) formation. Cyclin-dependent kinase 2 (CDK2) is a small serine/threonine kinase that drives cell cycle progression via phosphorylation of key targets. During prophase I of mouse spermatogenesis, CDK2 localizes to telomeres where it promotes stable telomeric and nuclear envelope interactions critical for homology search. Additionally, during pachynema, CDK2 localizes at interstitial sites which correspond to sites of double strand break repaired as COs, allowing for CDK2 to aid in promoting CO formation. CDK2 activity is tightly controlled by several mechanisms, including phosphorylation and dephosphorylation cycles. Specifically, CDK2 possesses an activating phosphorylation site (CDK2-pT160) and an inhibitory phosphorylation site (CDK2-pY15). CDK2-T160 phosphorylation was recently shown to be necessary for CO formation, however the exact localization of active/inactive CDK2 through meiotic prophase I is unknown. To assess localization of these differentially phosphorylated forms of CDK2 during meiotic prophase I, we used immunostaining of nuclear prophase I chromosome spreads with antibodies targeted against active CDK2-pT160 and inactive CDK2-pY15 relative to MLH3, a component of MutLγ which localizes to COs in pachynema. During pachynema, we observe colocalization of CDK2-pT160 and MLH3, further suggesting a role of active CDK2 in CO formation. Staining of testis sections and biochemical analysis of enriched prophase I sub-stages similarly show enrichment of active CDK2 during pachytene. As expected, inactive CDK2-pY15 did not co-localize substantially with MLH3. Additionally, CDK2-pY15 localizes at telomeres most abundantly in leptonema/zygonema. Together, these results demonstrate that CDK2-pT160 and CDK2-pY15 have distinct and unique localization patterns during prophase I of spermatogenesis. These observations help to increase our understanding of CDK2 activation cycles relative to its localization during meiosis. Understanding the spatial and temporal phosphorylation distribution of CDK2 may contribute to a greater understanding of the interplay of CO formation and meiotic cell cycle regulation.

Poster # 17

Function and evolution of seminal fluid-derived odorant binding proteins in Drosophila

Nora C. Brown, Benjamin Gordon, Caitlin E. McDonough-Goldstein, Snigdha Misra, Geoffrey D. Findlay, Andrew G. Clark, Mariana F. Wolfner

Cornell University

In Drosophila melanogaster and other insects, the seminal fluid proteins (SFPs) and male sex pheromones that enter the female with sperm during mating are essential for fertility and induce profound post-mating effects on female physiology and behavior. The SFPs in D. melanogaster and other taxa include several members of the large gene family known as odorant binding proteins (Obps). Previous work in Drosophila has shown that some Obp genes are highly expressed in the antennae and can mediate behavioral responses to odorants, potentially by binding and carrying these molecules to odorant receptors. These observations have led to the hypothesis that the seminal Obps might act as molecular carriers for pheromones or other compounds important for male fertility in the ejaculate, though functional evidence in any species is lacking. Here, we used RNAi and CRISPR/Cas9 generated mutants to test the role of the seven seminal Obps in D. melanogaster fertility and the post-mating response (PMR). We found that Obp56g is required for male fertility and the induction of the PMR, whereas the other six genes had no effect on fertility when mutated individually. Obp56g is expressed in the male's ejaculatory bulb, an important tissue in the reproductive tract that synthesizes components of the mating plug. We found males lacking Obp56g fail to form a mating plug in the mated female's reproductive tract, leading to ejaculate loss and reduced sperm storage. We also examined the evolutionary history of these seminal Obp genes, as several studies have documented rapid evolution and turnover of SFP genes across taxa. We found extensive lability in gene copy number and evidence of positive selection acting on two genes, Obp22a and Obp51a. Comparative RNAseq data from the male reproductive tract of multiple Drosophila species revealed that Obp56g shows high male reproductive tract expression only in species of the melanogaster and obscura groups, though conserved head expression in all species tested. Together, these functional and expression data suggest that Obp56g may have been co-opted for a reproductive function over evolutionary time.

Poster # 18

Effects of External Radiation Therapy for Prostate Carcinoma on Human Spermatogenesis

Andre Caldeira−Brant, PhD; Reginaldo Martelo, MD, PhD; Augusto Reis, MD, PhD; Edson Samesima, MD, PhD; Fernando Reis, MD, PhD; Fernanda Almeida DVM, PhD; Helio Chiarini−Garcia, PhD; Kyle Orwig, PhD

University of Pittsburgh School of Medicine

Radiation therapy (EBRT) is effective in treating prostate cancer (PC), but it may damage a patient's fertility due to scattered radiation. Patients are advised to wait for at least two years post-treatment before attempting to conceive because sperm defects have been reported within this period. However, it is not well understood how human testicular cells are affected and behave during this two-year window. Therefore, we aimed to evaluate the germs, Sertoli cells, and Leydig cells of adult patients diagnosed with PC and that underwent EBRT.

Five men with PC and that received EBRT were castrated ~20 months post-treatment (RT), due to cancer recurrence. Five other PC patients were castrated as the primary course of treatment, so no prior exposure to EBRT (NoT). Testicular biometrics, sex hormone levels, immunohistochemistry (IHC), and histological analysis were performed.

RT testes were smaller and lighter than NoT testes (p <0.05). FSH and LH were higher in RT than in NoT patients (p<0.01), but T levels were not different (p>0.05). While 100% of NoT seminiferous tubules contained VASA+ germ cells, only 9% of RT tubules contained VASA+ germ cells, with 70% of tubules exhibiting a Sertoli cell-only phenotype and 21% with a fibrotic phenotype. UTF1+ undifferentiated spermatogonia and SOX9+ Sertoli cells in RT patients had higher MCM7+ mitotic activity (p<0.001) and higher caspase-3+ apoptosis (p<0.01) than NoT patients. The Blood testis barrier protein, claudin-11, was unevenly organized in RT. Preliminary data shows a 2-fold decrease in INSL3+ Leydig cells in RT compared to NoT.

Scattered radiation resulting from EBRT impairs human spermatogenesis by affecting not only radiosensitive germ cells but also the somatic Sertoli cells, which are thought to be quiescent and therefore, radioresistant. At least 1-year post-EBRT, their numbers were significantly reduced, with higher proliferation and apoptosis than NoT patients, which triggered testicular atrophy.

Poster # 19

Evolution and Mechanisms of Female Factors Influencing Mating Plug Ejection in Drosophila Females

J. A. Carlisle, R. M. J. Craig, N. Brown, C. McDonough-Goldstein, B. Kim, M. Matera-Vatnick, D.S. Chen, A. Andrus, D. Cabello, A.G. Clark, M.F. Wolfner

Cornell University

Although it would seem that males and females both benefit by cooperating to maximize offspring number, competition between male and female reproductive strategies can lead to sexual conflict, which in turn can drive rapid diversification of genes mediating reproductive processes. While male genetic factors have been the main focus of study, it is becoming clear that female vertebrates and invertebrates also exert control over their reproductive outcomes. In Drosophila, female flies can manipulate the parental contributions of males by controlling the number of sperm stored from a mating. In Drosophila, the “mating plug” (MP), which forms in the uterus of the female via coagulation of ejaculated seminal proteins and female proteins, plays a key role in this process. Rapid ejection of the MP prevents sperm retention, while delayed ejection of the MP increases the number of sperm that can be stored. Using the Drosophila Genetic Reference Panel, we measured the mean times of mating plug ejection across many female genetic backgrounds. We then performed a genome wide association study to identify candidate genes that may influence mating plug ejection; we are beginning to test these, by using RNAi KD and CRISPR KOs to determine their role in mating plug ejection. Further, we have phylogenetically identified paralogous male and female proteins associated with mating plug composition, many of which are undergoing positive selection suggestive of opposing functions in MP ejection or formation.

Poster # 20

Characterization of the C-terminal region of MutS Homolog 5 (MSH5) reveals meiotic disruption in mouse meiosis

Ky'ara Carr*, Winfried Edelmann**, and Paula E. Cohen*

*Department of Biomedical Sciences, Cornell University, Ithaca, NY 14850
**Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

Meiotic recombination is a conserved hallmark during meiosis in sexually reproducing organisms. DNA double strand break (DSB) formation and their subsequent repair as crossovers (COs) are critical for homologous chromosome pairing and equal segregation at meiotic prophase I. Errors in these processes result in the formation of eggs and sperm bearing the wrong number of chromosomes, resulting in birth defects and miscarriages. In many organisms, at least two pathways, Class I and Class II, exist to ensure that appropriate CO numbers are achieved. DNA mismatch repair proteins MutS homologs 4/5 (MSH4/MSH5: MutSγ) are critical for further processing DSB events towards the Class I CO pathway. However, previous studies in our lab have shown that MutSγ accumulation in early prophase I in mouse far exceeds the total number of Class I COs, suggesting a role for this heterodimer beyond the class I pathway. Moreover, ATPase-deficient Msh5 mice revealed a loss of all COs across the genome, not just those processed under class I machinery. Thus, I hypothesize that MutSγ regulates crossover decisions between Class I and Class II through key protein interactions that coordinate different DNA repair activities. To test this hypothesis, our lab embarked on a structural approach to analyze MutSγ function. Utilizing a genetic approach, we identified a conserved C-terminal region of mammalian MSH5 with an essential role during male mouse meiosis. Mice lacking this domain displayed abnormal testicular architecture and sperm loss. Analysis of prophase I revealed defects in synapsis, DSB repair, and CO formation. To study the biochemical network associated with MSH5 role as a mediator between Class I and Class II CO events, we performed immunoprecipitation experiments followed by MS. Our studies will provide more insight on the role of MSH5 (and by extension, MSH4) during spermatogenesis, and shed light on the protein interactome of MSH5.

Poster # 21

Argonaute-small RNA (AGO-smRNA) interactions drive sex chromosome silencing during meiosis

Mercedes Carro[1,2], Amanda Touey May[1,2], Faraz Ahmed[3], Jennifer K Grenier[1,2], Andrew Grimson[1,4] and Paula Cohen[1,2]

[1] Cornell Reproductive Sciences Center (CoRe)
[2] Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University.
[3] Transcriptional Regulation & Expression Facility, Cornell Institute of Biotechnology
[4] Department of Molecular Biology and Genetics, College of Arts and Sciences, Cornell University

During pachynema of prophase I in male mice, the presence of unsynapsed sex chromatin triggers accumulation of repressive chromatin marks to the XY, establishing a silencing domain known as the sex body (SB). This process termed Meiotic Sex Chromosome Inactivation (MSCI), is initiated by an ATM-driven phosphorylation cascade, however the mechanisms behind XY silencing and its persistence throughout spermiogenesis are not fully understood. Our previous work demonstrated that Argonaute family members, AGO3 and AGO4, localize to the XY in pachynema, while loss of Ago4 alters expression of some XY transcripts, suggesting a role for AGOs in MSCI. AGOs are conventionally known by their role in posttranscriptional silencing in the cytoplasm, where they associate with smRNAs to target complementary mRNAs for cleavage. However, growing evidence supports roles for AGOs in transcriptional silencing in the nucleus. Our analysis of a triple Ago314 mouse line shows that mutant and heterozygous males have poor fertility with decreased testis size, reduced sperm counts, and increased apoptosis. Loss of these AGOs in meiosis results in mislocalization of SB markers, including γH2AX and TOPBP1. Moreover, mutant spermatocytes show infiltration of RNA pol II into the SB, coupled with a burst in expression of XY-linked genes, downregulation of prophase I specific genes (including Topbp1, Mdc1 and Sycp3), and premature expression of spermiogenesis genes (Acrv1, Izumo, and others). To identify specific smRNAs and proteins associated with disrupted MSCI, we performed immunoprecipitation with a tagged variant of AGO3. Protein interactors include many chromatin remodelers and transcriptional regulators. Excitingly, the major class of AGO3-associated smRNAs is encoded at a single retrotransposon-derived double-strand RNA hairpin on Chr4. Overall, our results demonstrate a nuclear role for AGOs in regulating transcriptional silencing during MSCI, and suggest involvement of a novel cluster of endogenous small-interfering RNAs in this process.

Poster # 22

Perfluorononanoic acid (PFNA) concentration-dependently interferes with gonadotropin-dependent ovarian follicle maturation in an ex vivo folliculogenesis system

Li Chen[1], Jiyang Zhang[1], Tingjie Zhan[1], Pawat Pattarawat[1], J. Julie Kim[2], Qiang Zhang[3], and Shuo Xiao[1]

[1]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University, Piscataway, NJ 08854;
[2]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611;
[3]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322

The per- and polyfluoroalkyl substances (PFAS) are widely used in consumer and industrial products. PFAS earn the name 'Forever Chemicals' due to their high persistence to environmental degradation and long half-lives in human bodies after absorption. Previous research revealed associations between exposure to PFAS and female ovarian disorders. However, nearly all studies focused on perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), two legacy PFAS that have been phased out. The ovarian impact of perfluorononanoic acid (PFNA), a newer PFAS have increasing environmental contamination levels, is unknown. Here, we used an in vitro 3D follicle culture model to investigate ovarian disrupting effects of PFNA. Immature mouse follicles were treated with 0, 0.1, 1, 10, and 100 µM PFNA for 6 days during follicle-stimulating hormone (FSH)-induced follicle maturation, a key ovarian function to sustain female reproductive cycles, ovulation, and fertility. PFNA concentration-dependently inhibited follicle growth, hormone secretion of estradiol and testosterone, and ovulation. RT-qPCR revealed that PFNA suppressed the expression of ovarian steroidogenic genes, including Star, Hsd3b1, and Hsd17b1, Follicles treated with the same concentrations and exposure window of PFNA were collected for single-follicle RNA sequencing and dose-response transcriptomic analysis. There were 500 differential expressed genes (DEGs) between PFNA and control groups. Gene ontology (GO) analysis revealed that these DEGs were associated with metabolism, ABC transporters, and ovarian steroidogenesis. We further used BMDExpress2 to determine the benchmark dose (BMD) of all 14346 genes. There 2528 genes with BMD < 100 µM and 282 genes with BMD < 0.1 µM. For example, several genes involved in ovarian steroidogenesis during follicle maturation, Lhcgr, Star, Cyp11a1, and Hsd17b1, had BMD of 87.7, 67.4, 60.1, and 84.7 µM, respectively. Together, our study demonstrates that environmentally relevant exposure levels of PFNA interfere with follicle maturation and associated ovarian functions, indicating its potential adverse impacts on women's reproductive health.

Poster # 23

Ovarian disrupting effects of organic matter extracts in real-world surface river waters

Samantha Cheron, Tinjie Zhan, Abigal Lazofsky, Hilly Yang, Qiang Zhang, Xueyuan Liu, Josephine Bonventre, Dan Millemann, Brian Buckley, and Shuo Xiao

Rutgers University

Organic contaminants are increasingly detected in surface waters worldwide, including the US. Many of them are endocrine disruptors, such as polychlorinated biphenyls (PCBs), per- and polyfluorinated substances (PFAS), and phthalates, which are primarily from municipal wastewater discharge, agricultural runoff, and naturally occurring toxins. The female reproductive impacts of such organic contaminant mixtures have been rarely evaluated. Herein, we investigated the effects of surface water organic extracts on the ovary, the female gonad. Eight surface water samples were collected from six sites of three New Jersey rivers, with two double-distilled lab water samples as the control. Organic matters were isolated via solid-phase extraction method. A 3D encapsulated in vitro follicle growth (eIVFG) model was applied to investigate the effects of water organic extracts on key ovarian functions. Immature mouse follicles were first treated with each water extract at a high concentration of 10-fold higher than the surface water concentration. The organic extracts of control waters (#1 and 2) and surface waters #3, 6, 9, and 10 did not affect follicle development, estradiol secretion, ovulation, and oocyte meiosis. However, the surface waters #4, 5, 7, and 8 significantly inhibited follicle growth and reduced post-ovulatory progesterone secretion, and the surface waters #4, 7, and 8 significantly inhibited ovulation. We next selected surface water #4 for in-depth analysis. The surface water #4 concentration-dependently (1-fold to 20-fold) inhibited follicle growth, ovulation, oocyte meiosis, and post-ovulatory progesterone secretion without inducing cytotoxicity. All treatment groups had comparable estradiol secretion, but the concentrations of 5-fold, 10-fold, and 20-fold had significantly increased testosterone secretion. These morphological and hormonal changes were consistent to the molecular changes examined by RT-qPCR. Together, we establish a reliable pipeline to evaluate reproductive impacts of surface waters, and the organic extracts of some surface river waters exhibit ovarian disrupting effects and may affect female reproduction.

Poster # 24

Two RNA binding proteins, ADAD2 and RNF17, interact to form novel meiotic germ cell granules required for male fertility

Chukrallah, Lauren G; Potgieter, Sarah; Gonzalez, Sheyla; Chueh, Lisa; Snyder, Elizabeth M.

Rutgers University

Mammalian male germ cell differentiation relies on complex RNA biogenesis events, many of which occur in non-membrane bound organelles termed RNA germ cell granules that are rich in RNA binding proteins (RBPs). Though known to be required for male germ cell differentiation, we understand little of the relationships between and functions of the numerous granule subtypes. ADAD2, a testis specific RBP, is required for normal male fertility and forms a poorly characterized granule in meiotic germ cells. This work aimed to define the role of ADAD2 granules in male germ cell differentiation and their relationship to other granules. Biochemical analyses identified RNF17, a testis specific RBP that forms meiotic male germ cell granules, as an ADAD2-interacting protein. Phenotypic analysis of Adad2 and Rnf17 mutants defined a shared and rare post-meiotic chromatin defect, suggesting shared biological roles. We further demonstrated ADAD2 and RNF17 are dependent on one another for granularization and together form a previously unstudied set of germ cell granules. Based on co-localization studies with well-characterized granule RBPs including DDX4 and PIWIL1, a subset of the ADAD2-RNF17 granules are likely components of the piRNA pathway in meiotic germ cells. In contrast, a second, morphologically distinct population of ADAD2-RNF17 co-localize with the translation regulator NANOS1 and form a unique cup-shaped structure with distinct protein subdomains. This cup shape appears to be driven, in part, by association with the endoplasmic reticulum. Lastly, a double Adad2-Rnf17 mutant model demonstrated loss of the ADAD2-RNF17 granules themselves, as opposed to loss of either ADAD2 or RNF17, is the likely driver of the Adad2 and Rnf17 mutant phenotypes. Together, this work identified a set of novel germ cell granules required for male fertility. These findings shed light on the relationship between germ cell granule pools and define new genetic approaches to their study.

Poster # 25

The role of Khdc3 in epigenetic inheritance of obesity and metabolic disorders

Sean M. Cullen, Liana Senaldi, Matthew Smith-Raska

Cornell University

Childhood obesity and associated metabolic disease is an ongoing public health epidemic. There is strong evidence that inherited factors contribute strongly to disease risk, but DNA-focused approaches have failed to explain much of this inheritance. We hypothesize that the mammalian germ cell gene Khdc3 is essential for integrating environmental signals, such as exposure to a high fat diet (HFD), into dynamic heritable changes in germ cell small RNAs, with important implications for the intergenerational inheritance of disease risk based on ancestral diet. Khdc3-null (KO) and wild type (WT) male mice were fed either a HFD or control diet (CD) for 8 weeks. Mice on the HFD gained significantly more weight than their control diet counterparts. Interestingly, KO mice on a HFD gained significantly more weight than WT mice on the HFD. Sperm small RNAs of KO mice on both the CD and HFD show significant dysregulation of small RNAs from multiple different classes including miRNA, piRNAs, and tRNA fragments. Mir135a-1 was found to be significantly upregulated in KO mice on both a HFD and CD; this miRNA has been associated with lipid and glucose metabolism. Furthermore, metabolic somatic tissues (liver, pancreas, adipose tissue) of wild type mice descended from Khdc3 mutant ancestors display evidence of metabolic dysregulation. More specifically, we observe transcriptional dysregulation of metabolic genes in these wild type mice descended from Khdc3 mutant ancestors, and are currently characterizing the phenotype of these mice as well as the mechanism of inheritance of these metabolic defects.

Poster # 26

Missense Variants in Human MLH1 and MLH3 Causing Premature Ovarian Insufficiency

Xinbao Ding and John C Schimenti

Cornell University

Primary ovarian insufficiency (POI) is an etiologically and clinically heterogeneous disorder caused by early depletion or non-functionality of the ovarian reserve. About 20-25% of cases are caused by genetic factors, with the causative genes primarily functioning in DNA damage repair, homologous recombination (HR), and meiosis. MLH1 and MLH3 play roles in DNA mismatch repair and regulation of meiotic crossing over, but roles in germ cell quality control or other forms of DNA repair have not been observed. By in silico analysis and CRISPR-Cas9 genome editing, we identified two missense variants of human MLH1/3 (i.e., MLH1 p.Asn64Ser and MLH3 p.Pro1262Ser) that result in follicle reduction in knock-in mouse models despite crossing over occurring at normal levels. Interestingly, Mlh1/3 mutant mice displayed a reduction in follicles of ~40% at 3-weeks of age, whereas HR defective Mcm9-/- females (MCM9 mutants cause POI in patients) exhibited over 90% follicle reduction. Further genetic study revealed that the DNA damage checkpoint kinase Chk2 is involved in the elimination of Mlh1/3 mutant germ cells, suggesting the presence of DNA damage in mutant PGCs and/or oocytes. In vitro differentiation experiments shown that Mlh1/3 are dispensable for PGC specification. By immunostaining chromosomal spreads of Mlh1/3 mutant spermatocytes, we identified the persistence of rH2AX flares on autosomes at late pachytene and diplotene stages, indicating the presence of DNA damage sites. These results unveiled a distinct mechanism of Mlh1/3 in germ cell quality control.

Poster # 27

Combing through Intrinsically Disordered Proteins to Find New Germline Genes

Yvan Doctorovich, Lic., William McFadden, MSc, Judith Yanowitz, PhD.

Magee-Womens Research Institute

Intrinsically disordered regions (IDRs) play critical functions in proteins without having a fixed structure. Unlike most proteins, which fold into specific conformations to carry out their functions, IDRs are flexible and dynamic, lacking a fixed structure even in the absence of molecular interactions. Despite this, IDRs have specific amino acid compositions and properties that are essential for protein function. At the same time, they have different evolutionary constraints on them because they need only retain change and not specific amino acid function. In this regard, they may appear to be rapidly evolving while in fact retaining function. Our lab has worked on several meiotic proteins that are highly disordered, leading us to consider whether this class of proteins may reveal novel germline functions.

To test this hypothesis, we compiled a comprehensive list of the highly disordered proteins in the worm model, Caenorhabditis elegans. We then knocked down these proteins individually using RNA interference (RNAi) and analyzed effects on fertility by examining the subjects' phenotypes in vivo over three generations and DAPI staining the adults to look for abnormalities ex vivo in the gonadal or embryonic development.

Thirty-three of the 118 genes analyzed caused a reproductive phenotype. This study highlights the significance of these understudied proteins and their potential importance in reproduction in C. elegans. It also emphasizes the importance of continued research into IDRs, which could lead to the discovery of novel targets for therapeutic intervention in human infertility.

Poster # 28

Impact of 3′ UTR variation on post-transcriptional regulation of spermatogenesis genes

Alexis Dziubek & Andrew Grimson

Department of Molecular Biology and Genetics, Cornell University, United States

Male germ cell development is a process that requires tight regulation in order to proceed correctly. When spermatogenesis is disrupted, mature spermatozoa may become defective or not be produced, causing infertility. As 5% of men are infertile and this percentage continues to rise, identifying the presently unknown factors in infertility is important, especially since many cases are idiopathic. One process which is important for proper spermatogenesis is post-transcriptional regulation, as many transcripts are sequestered before being translated. Also, transcription eventually shuts down during differentiation, making post-transcriptional events the sole means of gene regulation. The 3′ UTRs of genes play a major role in post-transcriptional regulation and contain binding sites for important regulatory factors and sites that control which 3′ UTR isoform is generated. Mutations in these sites are known to alter gene expression and play a role in cancer and other diseases, but the effect of population variation at these sites is not well understood. In order to investigate how genetic variation affects post-transcriptional regulation during spermatogenesis, we used a reporter system to test 3′ UTR variants for expression changes. To identify variants most likely to affect spermatogenesis, we took variants from public databases and selected a subset of them that were in genes known to cause spermatogenesis phenotypes, and that alter microRNA target sites, RNA binding protein motifs, or polyadenylation signals. We then generated full length 3′ UTR reporter constructs that contained either the reference or variant allele and tested their expression levels in germline derived cell lines for differences between variants. We identified several variants that altered expression, suggesting that variation in 3′ UTRs can play a role in heterogeneity of gene expression across the population and thereby potentially affect infertility or other diseases, either functioning as mendelian defects or by interacting with other variants and environmental factors.

Poster # 29

Effect of summer months on testicular histoarchitecture and spermatogenesis in Dezhou donkey: A histological study

Muhammad Faheem Akhtar and Wang Changfa

Liaocheng University

This study aims to illustrate testicular histoarchitecture and progression of spermatogenesis in Dezhou donkeys during summer months. For experiment, 8 male Dezhou donkeys having body weight 260 + 20 kg and aged 2 years were analyzed using microscopy and Jonhson's score. Based on acrosomal system, Dezhou donkey spermatogenesis comprised of XII stages. Various types of spermatogonia: A1, A2, A3, B1 and B2 were observed along with undifferentiated spermatogonia (Aund). Testicular histoarchitecture during spermatogenesis, seminiferous tubules diameter (ST), ST height ((µm), Luminal tubular diameter (µm), No. of ST/field, No. of germ cells along with normal Jonhson's score illustrated that stages of spermatogenesis and testicular histoarchitecture of adult Dezhou donkeys are not affected during summer months.

Poster # 30

Obesity Disrupts Preovulatory Ovarian Hemodynamics that are Correlated with Ovulation Rate

Justine M Galliou, Samantha R Greenspun, Rebecca M Williams, and Yi A Ren

Cornell

Over 65% of women with ovulation failure are obese, yet we do not fully understand how obesity affects ovulation. The preovulatory luteinizing hormone (LH) surge initiates a series of structural and functional changes in ovarian vasculature crucial for ovulation. As obesity negatively affects vascular function, we hypothesized that ovarian vascular function during the preovulatory period is dysregulated in obesity, contributing to impaired ovulation. To test this hypothesis, we applied color Doppler ultrasonography as a novel, non-invasive method to assess hemodynamics of the ovarian artery at time points spanning the preovulatory period in adult control and obese Agouti viable Yellow (AvY) mice that carry a mutation at the Agouti gene resulting in increased appetite. Super-ovulation with equine chorionic gonadotropin followed by human chorionic gonadotropin (hCG) was used to minimize neuroendocrine differences and synchronize ovulation. AvY mice ovulated significantly fewer oocytes than controls. Ovarian arterial flow velocity increased between 0h and 1h post-hCG in controls while staying constant in obese mice. To test whether this velocity increase is associated with ovulation outcome, we administered two doses of hCG to wild-type immature mice. The higher dose resulted in a larger velocity increase and a higher ovulation rate. These findings suggest that an increase in blood velocity between 0h and 1h post-hCG is important for ovulatory success and obesity disrupts this pattern. We also observed abnormal vessel diameter and flow rate (volume of blood flow/unit time) at later time points between hCG and ovulation. To probe the potential mechanisms of hemodynamic dysregulation in obese mice, we measured the transcript levels of genes regulating ovarian vascular function by qPCR. Genes within several vascular pathways, including the vasoconstriction regulator endothelin, were dysregulated. This study pinpoints that abnormal ovarian vascular function during the preovulatory period is a previously underappreciated contributor to impaired ovulation in obesity.

Poster # 31

Uncovering the role of Polo-like kinase in regulating synaptonemal complex dynamics

Ariel Gold, Matthew Hurlock, Yumi Kim

Johns Hopkins University

Successful chromosome segregation in meiosis requires pairing, synapsis, and crossover formation between homologous chromosomes during prophase I. Synapsis involves a zipper-like protein structure called the synaptonemal complex (SC) that forms between two paired homologs and functions as a scaffold for crossover recombination. Recent evidence shows that the SC has liquid crystalline properties, allowing for chromosome-wide signal transduction to regulate the number and distribution of crossovers. In C. elegans, SC materials form spherical aggregates in the absence of chromosome axes, called polycomplexes, and recruit factors required for crossover formation as a single focus, recapitulating the robust crossover control in normal meiosis. Despite the conserved structure of the SC, mechanisms governing its assembly and function in crossover control remain unknown. Using a strain lacking chromosome axes (htp-3) as an experimental platform, we demonstrate that Polo-like kinases (PLKs) control the number and shape of SC polycomplexes and their affinity to crossover factors. SC polycomplexes appear as multiple smaller aggregates in the absence of PLK-2, and the requirement for PLK-2 in coalescing SC aggregates depends on the presence of crossover factors. When both PLK-1 and PLK-2 are depleted, SC polycomplexes adopt aberrantly elongated shapes and no longer associate with crossover factors, suggesting that PLKs may confer the liquid-like properties to the SC and its affinity to crossover factors. Using phospho-specific antibodies, we further show that two paralogous SC components, SYP-5 and SYP-6, are robustly phosphorylated in a PLK-dependent manner at several conserved residues within their disordered C-terminal tails. Mutations preventing the phosphorylation of these sites on SYP-5/6 lead to a dramatic delay in synapsis, indicating that phosphorylation of SYP-5/6 is crucial for SC assembly. Here we will present our latest work investigating the role of PLK in regulating SC dynamics and crossover control.

Poster # 32

Investigating the role of NHR-49 in oocyte activation in C elegans

Sharada Gopal, Amaresh Chaturbedi, Sylvia Lee

Cornell University

Organisms need to maintain a delicate balance between their nutritional availability and reproductive needs. However, mechanisms that enable organisms to tune their reproduction in response to metabolic status are not fully understood. During sexual reproduction, signals from the sperm are necessary for oocytes to progress through meiosis. Sperm signals also trigger protein homeostasis mechanisms responsible for clearing protein aggregates within oocytes to prepare them for fertilization. The nematode worm C. elegans is a hermaphrodite capable of self fertilization. Specific gene mutations result in feminization of C. elegans, which lack sperms. In the feminized mutants, oocyte maturation is arrested and clearance of protein aggregates is disrupted. We found that NHR-49, a nuclear hormone receptor that is homologous to mammalian PPARa and a key regulator of fat metabolism and nutrition sensing, is necessary for sperm dependent meiotic resumption and proteostasis in oocytes. Loss of nhr-49 in feminized worms resulted in defective release of unfertilized oocytes. In summary, our research has uncovered a new function of NHR-49 in the germline.These findings suggest potential connections between nutritional regulation and reproductive processes.

Poster # 33

TRPM7 is dynamically expressed in gametes and preimplantation embryos in mice

Neha Gupta, Dominique Alfandari, Rafael Fissore

University of Massachusetts, Amherst

TRPM7 is a chanzyme with channel and kinase domains essential for embryo development in the mouse. However, the expression and role of the chanzyme in this process are unknown. To address these issues, we have synthesized a monoclonal antibody, DA5C7-TRPM7, in-house and used it in gametes and embryos. TRPM7 is expressed in sperm and eggs and displays stage-specific expression and localization in oocytes and embryos. In GV-stage oocytes, it is localized to the plasma membrane (PM), whereas in MII eggs displays cytosolic localization. In zygotes (PN), it remains cytosolic. In 2-cell embryos, despite retaining cytoplasmic staining, the distribution of TRPM7 changes, appearing forming regularly spaced clusters-like accumulations in the PM/cortex and the nucleus. Four-cell stage embryos showed larger PM/cortical clusters, increased nuclear localization, and poor cytoplasmic reactivity. As in the previous stage, eight-cell embryos displayed large PM/cortical clusters and nuclear distribution. TRPM7 reactivity seemed to wane at the morula stage, without distinct PM clusters but with cytoplasmic staining and nuclear localization. In the blastocyst stage, the reactivity rebounded, and the distribution of TRPM7 seemed somewhat dependent on the cell type. There was a significantly higher nuclear-to-cytoplasmic ratio of reactivity in TE than in ICM cells. The nuclear localization of TRPM7 suggests that the chanzyme undergoes cleavage, and the C-terminal kinase fragments enter the nucleus. Western blotting results also showed dynamic expression of TRPM7 in gametes and embryos and C-terminal Kinase fragments suggesting post-translation modifications, as described in somatic cells. Collectively, the dynamic expression and localization of TRPM7 throughout preimplantation development and the concurrence of PM and nuclear localizations in specific stages suggest TRPM7's vital role in embryo development and predict contributions of its channel and kinase domains. This knowledge is fundamental to elucidate the role of TRPM7 in fertilization and development and the role of its domains in gene expression and divalent cation homeostasis.

Poster # 34

Epigenetic profile of hearts and brains from IVF offspring in a mouse model

Cassidy N. Hemphill, Alexis Kaiser, Eric A. Rhon-Calderon, Laren Narapareddy, Marisa S. Bartolomei

UPENN

Assisted Reproductive Technologies (ART) are procedures that help achieve a successful pregnancy, with in vitro fertilization (IVF) being the most used ART today. While IVF procedures are safe, ART have been associated with adverse adult outcomes in the offspring, including cardiovascular, neurological, and metabolic irregularities. We hypothesized that IVF procedures alter DNA methylation patterns in the hearts and brains of ART offspring. To address this hypothesis, hearts and brains from naturally-conceived and IVF-offspring were collected at E18.5, 12 weeks, and 39 weeks. DNA methylation patterns at imprinting control regions (ICRs) were determined using targeted bisulfite sequencing and pyrosequencing, and gene expression was analyzed by RT-qPCR. In hearts, E18.5 IVF-offspring show significant changes in DNA methylation levels at IgDMR and Grb10 in both sexes, and a male-specific change in DNA methylation at Snrpn and Ncbr3'. At 12 weeks and 39 weeks, no changes in DNA methylation were observed. In brains from 39 weeks of age offspring, DNA methylation in the cerebellum at Ogg1, a gene involved in DNA repair, was significantly increased in IVF males compared to natural offspring. Expression of genes involved in brain aging were measured by RT-qPCR. We observed an upregulation of Ogg1, Hdac1, and Mecp2 in IVF offspring in all brain regions. Additionally, no changes in DNA methylation at ICRs were observed. Taken together, these results indicate that early changes in DNA methylation in IVF-offspring hearts are resolved later in life, and the brains of IVF-offspring might display an early aging phenotype.

Poster # 35

Antileukoproteinase: the whoa and go in neonatal foals and late gestation mares

Camille Holmes, Bettina Wagner

Cornell University

Antileukoproteinase (SLPI) is a pleiotropic protein produced systemically and at the mucosal surfaces, where it acts to maintain homeostasis. In mammals it can be detected at various locations, including the female genital tract (FRT), parotid glands, and breast. The most explored properties of SLPI are modulation of inflammation, inhibition of serine proteases, and antimicrobial action. These diverse roles establish SLPI as an important factor in a variety of biological processes. Here we developed novel monoclonal antibodies (mAbs) against equine SLPI and established a bead-based assay to quantify secretion. First, SLPI was measured in serum, saliva, and colostrum from healthy adult horses. SLPI secretion was significantly higher in colostrum (p<0.0001, 1695±1253ng/mL) than serum (22.72±60.50ng/mL), suggesting an involvement in reproduction and neonatal development. To follow this up, SLPI was measured in mare and foal pairs (n=18), from 0 days postpartum (0dpp) to 225dpp. This showed a peak in secretion on 0dpp in both mares and foals. Mares showed significantly higher secretion 0dpp and 2dpp, compared to normal healthy horses (p<0.0001). Mares rapidly decreased secretion, dropping within the normal range seen in healthy horses by 6dpp. Meanwhile, foals showed prolonged high secretion until 90dpp. Foal serum SLPI levels were significantly higher than their mares on 6dpp (p<0.0001), 14dpp (p<0.0001), 30dpp (p<0.0001), 60dpp (p=0.0059), and 90dpp (p=0.0007). Additionally, SLPI was measured in prepartum mares, to determine when upregulation first occurs. Retrospective analysis of samples taken within two weeks of parturition was used to quantify prepartum SLPI levels in serum. Paired samples (n=9) showed higher SLPI in mares prepartum compared to 1 week postpartum (p=0.0039). Together this suggests that SLPI plays an important role in parturition and early life, with high production seen in both prepartum mares and neonatal foals.

Poster # 36

Untangling unexpected functions of the FANCJ DNA helicase in mouse meiosis

Tegan Horan, Paula Cohen

Cornell University

Correct chromosome segregation in meiosis depends on crossovers, sites of physical DNA exchange between parental chromosomes. Although hundreds of programmed double strand breaks (DSBs) initiate meiotic recombination, only a small subset (~10% in mouse) resolve as crossovers. Nearly all crossovers are formed via the class I pathway, mediated by the MLH1/MLH3 heterodimer. Crossover number and placement are stringently controlled; however, it is unclear how recombination levels are established and maintained within the strict parameters observed in male mice. Our previous studies using a gene trap disruption suggested a role for the FANCJ (BRIP1) DNA helicase in meiotic recombination regulation. Mice homozygous for the disrupted FancJ allele showed elevated MLH1 foci and crossover number. In somatic contexts, FANCJ is known to interact with several key DNA repair proteins, including MLH1, BRCA1, and BLM. However, FANCJ's function in meiosis is poorly understood, and it remains unclear whether FANCJ's meiotic effects are due to interactions with MLH1 or BLM, or via an alternative mechanism. Recently, we produced a novel antibody in rabbits against mouse FANCJ, and used CRISP-Cas9 to tag the mouse endogenous FANCJ. We have also generated a full-gene deletion in mouse, allowing for a more precise analysis of the meiotic roles of FANCJ. Our data indicate FANCJ primarily localizes to chromosome cores in a pattern that is spatially and temporally separated from MLH1. Further, mice with a complete deletion of FancJ did not show any difference in MLH1 foci numbers, suggesting FANCJ is not essential for the class I crossover pathway. In our ongoing work, we have identified exciting FANCJ interactors in the germline that suggest not only functions in early DSB processing, but also roles outside crossover formation. These studies question previous assumptions of FANCJ function in the germline and reveal novel pathways for FANCJ that require further investigation.

Poster # 37

Identification of maternal effect genes altered by assisted reproduction, which lead to methylation errors in preimplantation embryos

Qidan Hu[1,2.3], Mellissa RW Mann[2,3](mannmr@mwri.magee.edu)

[1]School of Medicine, Tstinghua University, Beijing, China
[2]Magee-Womens Research Institute, Pittsburgh, PA USA
[3]Department of Obstetrics, Gynaecology and Reproductive Sciences, University of Pittsburgh School of Medicine, USA
University of Pittsburgh School of Medicine

Hypothesis:
Over the last decades, infertility has steadily risen and assisted reproductive technologies (ARTs) have become a standard medical intervention for infertility problems. However, ARTs are associated with imprinting disorders which can lead to severe consequences for growth and development of embryos. Here, we hypothesized ARTs alter crucial maternal-effect genes expression in oocytes, which are required in early embryos to maintain imprinted methylation.

Methods:
Maternal zinc finger protein (ZFP57) and DNA methyltransferase 1(DNMT1) are both involved with imprinting maintenance. First, we tried to find out the impact of ARTs on the cellular localization of these two candidates by employing immunohistochemistry at embryos derived from 4 groups: no ART, superovulation(SO), embryo culture(EC) and superovulation plus culture(SOEC). To further investigate the function of ZFP57 and DNMT1, we employed a protein depletion technology called TRIM-Away to degrade DNMT1 and ZFP57 respectively in embryos.

Results:
Confocal microscopy analysis showed ARTs led to ZFP57 and DNMT1 mislocalization in preimplantation embryos. For ART-derived embryos, ZFP57 had delayed nuclear import and had reduced ZFP57 perinucleolar rings in both trophectoderm and inner cell mass. Nucleoplasmic staining intensity of DNMT1 at 8-cell stage embryos was also decreased. TRIM-Away assay results demonstrated ZFP57 and DNMT1 reduction affects developmental rate of blastomeres.

Poster # 38

H2A.Z Regulation of CTCF Binding and Higher-Order Chromatin Structure

Shan Hua, Kristin Murphy, Patrick Murphy

University of Rochester

Maintaining proper higher-order chromatin structure is critical for regulating gene expression in cells. CTCF acts as an architectural protein in the genome, helping to maintain this structure by forming loop domains within the chromatin. These loop domains create insulated spatial neighborhoods that separate heterochromatic and euchromatic regions of the genome. In addition, CTCF can form boundaries that control the expression of imprinted genes and Hox genes, which are critical for organismal growth, and establishing the body plan of organisms, respectively. Dysregulation of CTCF can lead to improper gene expression or genome instability, which can cause various diseases, including cancer. However, the epigenetic regulation of CTCF binding is still poorly understood. Prior studies suggest that H2A.Z, a histone variant, might influence CTCF but it remains unknown whether it functions to promote or inhibit binding. Our research has found that H2A.Z enrichment is highly correlated with CTCF enrichment, and changes in CTCF binding occur at H2A.Z marked locations in the absence of ANP32E, the H2A.Z removal chaperone. These changes are also associated with imprinted genes and Hox genes dysregulation. Based on these findings, we hypothesize that H2A.Z might antagonize CTCF binding in the regulation of chromatin looping. To test this hypothesis, I will manipulate H2A.Z installation in mouse embryonic fibroblasts using CRISPR genome editing techniques and examine the genome-wide impacts on H2A.Z, CTCF, and chromatin conformation. By studying the fundamental mechanisms of the role of H2A.Z in higher-order chromatin maintenance, our project may provide insights into the development of various diseases, including cancer.

Poster # 39

The role of genomic maintenance during placental development

Rui Huang, Munisha Mumingjiang, and John Schimenti

Cornell University

During gestation, the placenta plays a crucial role in providing nutrients, and oxygen, removing waste, while also producing hormones, and acting as a barrier against microbial invasion. Abnormal placenta function can result in developmental defects and adverse pregnancy outcomes. Our laboratory is currently investigating the impacts of genomic instability (GIN) on placental development. GIN from chronic DNA replication stress (RS) can produce cytosolic DNA that activates the innate immune system, causing inflammation. Using a unique mouse model, our lab found that high levels of RS-induced GIN cause placental inflammation and female-biased embryonic lethality. We are now trying to understand why various cell types of the trophoblast lineage, which form the placenta, are impacted by GIN to cause placental defects. To address this, I plan to use a human and Trophoblast stem cell (TSC) culture system containing the GIN-causing mutations that cause placental RS.

I have successfully established both wild-type (wt) and GIN mouse TSCs (mTSCs) from this mouse model. Interestingly, the high-GIN mTSCs can only be derived under defined culture conditions that inhibit differentiation, whereas the wt mTSC can self-renew and proliferate under conventional culture conditions. More intriguingly, we observed that high-GIN mTSCs have a decreased cell proliferation rate, increased cell apoptosis, and elevated DNA damage. To elucidate the underlying mechanisms of these observations, I aim to use this platform to 1) understand how GIN and innate inflammation impact specific cell lineages during placental development, and 2) characterize the underlying genome maintenance mechanisms that render trophoblast derivatives to be sensitive to GIN.

Poster # 40

Vitrification preserves follicular transcriptomic dynamics during ex vivo ovarian follicle maturation and ovulation

Inhyeok Hwang[1], Astha Adroja[1], Jiyang Zhang[1], Yingzheng Wang[1,2], Daniela D. Russo[3,4,5], Qiang Zhang[6], Mary B. Zelinski[7,8], Alex K. Shalek[3,4,5], Brittany A. Goods[9], and Shuo Xiao[1]

[1]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University
[2]Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina
[3]Institute for Medical Engineering & Science, Department of Chemistry, and Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology
[4]Broad Institute, Harvard University & Massachusetts Institute of Technology
[5]The Ragon Institute of MGH, MIT and Harvard
[6]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University
[7]Division of Reproductive & Developmental Science, Oregon National Primate Research Center,
[8]Department of Obstetrics & Gynecology, Oregon Health & Science University
[9]Thayer School of Engineering, Dartmouth College

Vitrification is now widely used in cryopreserving gametes and embryos in assisted reproductive technology (ART) for fertility preservation and infertility treatment. However, whether vitrification preserves the viability and functionality of more complex ovarian follicles is largely unknown. We recently demonstrated that a closed vitrification system preserves follicle viability, and vitrified follicles exhibit comparable reproductive outcomes to fresh follicles in an in vitro 3D encapsulated follicle growth (eIVFG) system, including follicle maturation, hormone secretion, and ovulation. However, whether vitrification preserves molecular signatures of these follicular events remains elusive. Multi-layered secondary follicles isolated from 16-day-old CD-1 mouse ovaries were vitrified. With fresh follicles as the control, vitrified follicles were thawed and cultured with follicle-stimulating hormone (FSH) for 8 days in eIVFG. Grown preovulatory follicles were treated with human chorionic gonadotropin (hCG) to induce ovulation ex vivo. Both FSH-stimulated mature follicles on day 8 and hCG-induced ovulatory follicles at 0, 1, 4, and 8-hour were collected for single-follicle RNA-sequencing analysis. Principal component analysis (PCA) and Pearson's correlation analysis revealed that vitrified follicles had similar transcriptomic profiles to fresh follicles. There were 20-100 differentially expressed genes between vitrified and fresh follicles at various examined time points. None of those genes have been shown to be critical to gonadotropin-dependent folliculogenesis and ovulation, and no gene ontology (GO) terms or signaling pathways were significantly enriched. Moreover, many genes essential for follicle maturation (e.g., Cyp19a1, Amh, Inha, and Inhba) and ovulation (Areg, Has2, Pgr, and Ptgs2) had comparable expression abundance and dynamics between fresh and vitrified follicles. Together, these results demonstrate that vitrification preserves follicular transcriptomic profiling and dynamics during follicle maturation and ovulation; the integration of vitrification and eIVFG

provides a robust model for fertility preservation, conservation of endangered species, and establishing a high-content ovarian follicle biobank for studying ovarian biology, disease, toxicology, and novel contraception.

Poster # 41

Pharmacologic inhibition of proprotein convertases prevents cumulus expansion due to extracellular matrix instability

Caroline Kratka, Jiyang Zhang, Pawat Pattarawat, Luhan Tracy Zhou, Chanakarn Suebthawinkul, Hoi Chang Lee, Shuo Xiao, Francesca Duncan

Northwestern University

Proprotein convertases (PCs) cleave proteins into their functional forms. Using an ex vivo ovulation assay, we demonstrated that a PC inhibitor (PCI), which inhibits PCSK 3, 5, and 6, blocks follicular rupture without impacting progesterone secretion. Interestingly, PCI-treated follicles exhibit reduced expression of hyaluronan synthase 2, which regulates cumulus layer expansion through production of an extracellular matrix that is important for ovulation. Thus, we hypothesized that PCI treatment may block follicular rupture in part through direct effects on cumulus cells and the expansion process. To test this hypothesis, we collected cumulus oocyte complexes (COCs) from hyperstimulated female mice and performed in vitro maturation (IVM) in increasing doses of PCI (10 nM, 100 nM, 1 μM, 10 μM). Culture in DMSO alone served as a control. IVM was performed in a closed time-lapse incubator (EmbryoScope+™) to closely monitor the effect of PCI on oocyte meiotic progression and COC expansion. PCI treatment did not affect oocyte meiotic progression, as polar body extrusion rates were similar in gametes isolated from control and PCI-treated COCs. To rule out direct effects of PCI on the oocyte, we performed IVM on denuded oocytes. The duration of meiosis I was similar between control and PCI-treated oocytes, and there were no obvious spindle or chromosome abnormalities. However, PCI significantly impacted cumulus cell behavior by inducing a dose dependent loss of adherence to the complex by ~12 hours of IVM. A live-dead assay confirmed that PCI did not affect cumulus cell viability, and thus the observed phenotype was due to loss of structural integrity of the COC matrix. Preliminary findings suggest that PCSK5 is likely the PC that regulates cumulus expansion and is targeted by PCI. However, further studies are needed to validate this and identify substrates that could serve as non-hormonal contraceptive targets through regulation of cumulus expansion.

Poster # 42

Naked Mole Rat Has2-expressing Mice: A Novel Mouse Model for Studying the Role of High Molecular Mass Hyaluronic Acid in Ovarian Aging and Reproductive Longevity

Jing Li1,[2], Farners Amargant[2], Connor E. Owens[1], Cecilia E. Villanueva[3], Mariela Faykoo-Martinez[5], Melissa M. Holmes[5], Zhihui Zhang[6], Vera Gorbunova[6], Michele T. Pritchard[3,4], Ned J. Place[1], Francesca E. Duncan[2]

[1] Department of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY
[2] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL
[3] Department of Pharmacology, Toxicology and Therapeutics, and 4 Institute for Reproductive and Developmental Sciences, University of Kansas Medical Center, Kansas City, KS
[5] Department of Psychology, University of Toronto, Mississauga, ON
[6] Department of Biology, University of Rochester, Rochester, NY

The naked mole rat (NMR) is the longest-lived rodent that can live up to four decades, and NMR queens maintain their fertility throughout their lifespan. The NMR's resistance to cancer and remarkable lifespan is attributed, in part, to its ability to produce large amounts of very high molecular mass hyaluronan (vHMM-HA) via hyaluronan synthase 2 (Has 2). Transgenic mice expressing NMR Has2 (nmrHas2) have extended longevity and health span, but how the ovary is impacted in this model is unknown. In the mouse ovary, we showed previously that HA is an essential component of the ovarian stroma which regulates stiffness and decreases with age. Thus, we hypothesized that induced expression of nmrHas2 in the mouse ovary confers a beneficial effect on the ovarian microenvironment and reproductive outcomes. To test this hypothesis, we first examined HA and Has2 expression in the NMR ovary and then characterized ovaries from nmrHas2 and control mice. As visualized by a hyaluronan binding protein assay, HA localized throughout the NMR ovary and was 6-fold more abundant relative to the control mouse ovary. Using RNAscope™, we found that Has2 mRNA was expressed in the NMR ovary and was enriched in the growing follicle (especially the secondary follicle) and was >4 fold more within the oocyte compared to granulosa cells (GCs) in the secondary follicle. In comparison, in ovaries from nmrHas2 mice, nmrHas2 mRNA was expressed throughout the ovary but was enriched in somatic cells (GCs, luteinized GCs, theca cells, and stroma cells) relative to the oocyte. Within the secondary follicle, nmrHas2 was 3-fold more in the GCs compared to the oocyte. nmrHas2 transcripts were not detected in ovaries from non-nmrHas2 transgenic mice validating the specificity of the nmrHas2 probe. Studies comparing follicle numbers, ovarian HA and collagen content, and fertility between nmrHas2 and control mice are ongoing. Overall, this innovative mouse model will provide critical insight into the unique features of vHMM-HA in reproductive tissues and elucidate novel mechanisms of ovarian aging and longevity. (This work was supported by NSF Award 2005919)

Poster # 43

CRISPR Inhibition Screen Identifies Targets of Epigenetic Perturbations during PGC Development

Liangdao Li, Xinbao Ding, John Schimenti

Cornell University

Approximately 15% of couples at their reproductive ages are affected by infertility. Nearly half of the cases are idiopathic with unexplained causes. Infertility is a multifactorial disorder that are highly associated with both genetic and epigenetics changes due to environmental factors. However, how epigenetic changes affect the development of the Primordial germ cells (PGCs), the founding cells of both male and female germlines, thereby contributing to infertility is unknown. Here, we used CRISPR inhibition (CRISPRi) libraries targeting known epigenetic factors to identify indispensable epigenetic factors during PGC development. We have successfully constructed CRISPRi libraries harboring 701 genes targeting various epigenetic factors and co-factors. The integrity of the libraries was validated through Illumina sequencing, and the libraries were transduced into ESCs using lentiviral infection. Using in vitro differentiation of mouse embryonic stem cells (mESC) to mouse PGC-like cells (mPGCLC), we have successfully screened out the most negatively selected epigenetic candidates. Ncor2 (nuclear receptor corepressor 2), among the most negatively selected genes in PGCLCs, is part of a multi-subunit complex that facilitates histone deacetylases to downregulate transcriptional activities of targeted genes. RNA-seq data showed upregulation of Ncor2 expression during mPGCLC differentiation. We will further validate the role of Ncor2 in vitro using RNA inhibition (RNAi) and investigate histone deacetylation in PGC maintenance. Taken together, we have identified important epigenetic regulators during PGC development, and functional analysis on these regulators will be performed in the future.

Poster # 44

ZMYM3 and PTIP associate ANKRD31 cooperatively ensuring high order chromatin structure and meiotic DNA break formation in the mouse pseudoautosomal region

Tao Li and Scott Keeney

Memorial Sloan Kettering Cancer Center

Poster # 45

Transcription is not Sufficient for Methylation Establishment at H19/Igf2 Imprinting Control Region in the Male Germline

Zhengfeng Liu, Suhee Chang, Stella K. Hur, Rexxi D. Prysasya, and Marisa S. Bartolomei

UPENN

Genomic imprinting is a mammalian epigenetic mechanism that directs monoallelic parent-of-origin gene expression. It is primarily controlled by parental allele-specific DNA methylation at cis-regulatory elements called imprinting control regions (ICRs). DNA methylation of maternally methylated ICRs is established during oocyte growth by de novo DNMTs in a transcriptionally dependent manner. Paternally methylated ICRs gain methylation in prospermatogonia, but the mechanism of targeting remains unclear. One of the most well-characterized and conserved imprinted loci is the H19/IGF2 cluster. Paternally inherited methylation at human IC1(hIC1), the ICR for the H19/IGF2 cluster, ensures expression of the long non-coding RNA H19 from the maternal allele and expression of the fetal growth factor IGF2 from the paternal allele. Aberrant hIC1 methylation can cause the imprinting disorders Beckwith-Wiedemann syndrome and Silver-Russell syndrome. We previously developed a humanized mouse line by substituting the endogenous mouse H19/Igf2 ICR with the hIC1. Bisulfite sequencing of E17.5 H19+/hIC1 gonocytes suggested a failure of methylation establishment at hIC1. The goal of this work is to investigate why methylation establishment fails. We hypothesized that hIC1 fails to acquire DNA methylation in the mouse gonocytes due to lack of transcription across the locus. Using strand-specific RNA-seq, we detected bidirectional transcription through hIC1 during the period of methylation establishment. Previous studies suggested that at H19 ICR, de novo methylation of the paternal allele progresses faster than that of the maternal allele. To test the correlation between methylation establishment and transcription, we performed allelic RNA-seq of E17.5 gonocytes from mixed background mice. We observed similar level of transcription across both maternal and paternal copies of the H19 ICR. Together, our results indicate that transcription is not sufficient for the methylation establishment of paternally methylated ICRs in gonocytes. Our next experiments will assess whether an inappropriate chromatin environment prohibits de novo DNA methylation.

Poster # 46

Serum miRNAs as conserved biomarkers for the specific and sensitive detection of testicular germ cell tumors

Amanda R. Loehr, Ad J.M. Gillis, Dennis M. Timmerman, Michelle Liu, Melia Matthews, Jordana Bloom, Peter Nicholls, David Page, Andrew D. Miller, Leendert H.J. Looijenga, Robert S. Weiss

Cornell University

Testicular germ cells tumors (TGCTs) are the most common solid malignancy diagnosed in young men in the US. Current diagnostics for TGCTs include conventional serum protein markers, but these lack the sensitivity and specificity needed to serve as accurate markers of malignancy across all histologic TGCT subtypes. microRNAs (miRNAs) are small non-coding regulatory RNAs expressed by almost all cells in the body and can be used as biomarkers of many different diseases. In humans, miRNAs in the miR-371-373 cluster are highly expressed in the serum of TGCT patients and outperform existing serum protein markers for TGCT detection. We previously developed a genetically engineered mouse model featuring malignant mixed germ cell tumors consisting of pluripotent embryonal carcinoma (EC) and differentiated teratoma. Like the corresponding human malignancies, these murine cancers originate in utero and are highly sensitive to genotoxic chemotherapy. Here, we report that miRNAs in the mouse miR-290-295 cluster, homologs of human miR-371-373, were detectable in the serum of mice with malignant TGCTs but not in serum from mice with benign teratomas or tumor-free control mice. miR-291-293 were expressed and secreted specifically by pluripotent EC cells, and expression was lost following differentiation induced by the drug thioridazine. RNA sequencing analysis of EC and thioridazine-differentiated cells suggested that miR-290-295 mediated downregulation of target genes, including Cdkn1a and Lats2 encoding negative regulators of the G1/S transition, may be important for pluripotency maintenance and TGCT pathogenesis. Additionally, miR-291-293 expression was significantly higher in the serum of pregnant dams carrying tumor-bearing gPAK fetuses compared to that of control dams. These findings reveal that expression of the miR-290-295 cluster in mice and the miR-371-373 cluster in humans is a conserved feature of malignant TGCTs, which further validates the gPAK mouse as a representative model of the human disease. These data also suggest that serum miR-371-373 assays may be able to detect the presence of TGCTs in humans before clinical signs of the disease arise, possibly even before birth.

Poster # 47

Exploring the interplay between chromatin structure organization and meiotic DNA Double-Strand Break

Min Lu and Scott Keeney

MSKCC

Homologous recombination initiated by programmed double-strand breaks (DSBs) is essential for accurate chromosome segregations during meiosis. The evolutionarily conserved transesterase Spo11 catalyzes DSB formation along the chromosomes. After the catalysis, a tightly controlled process of repairing DSBs promotes chromosome pairing and segregation and increases the diversity of genetic information among haploid gametes. Drastic changes in chromosome structure accompany meiotic recombination. For example, dense arrays of chromatin loops emanating from linear, proteinaceous chromosome axes can act as a platform to recruit the DSB-forming machinery (e.g., Spo11 core complexes and accessory factors Rec114, Mei4, and Mer2). However, it is still largely unknown how the higher order architecture of meiotic chromosomes is coordinated with DSB formation and regulation. To address this question, we are employing the HiChIP method to detect and characterize meiosis-specific protein-directed genome architectures in Saccharomyces cerevisiae. HiChIP incorporates chromosome conformation capture (3C) techniques with chromatin immunoprecipitation (ChIP) sequencing. Using multiple chromosome-associated protein targets, we are sequencing HiChIP samples from different timepoints during meiosis to observe the dynamic structural changes. Our preliminary data showed striking patterns captured by anti-Rec114 HiChIP in yeast meiosis, and we plan to extend this assay to other DSB-related proteins such as Spo11 and the meiosis-specific cohesion subunit Rec8. We are also applying this assay to several mutant backgrounds in which the number, timing, and/or locations of DSBs are altered. We anticipate that HiChIP, together with the high-resolution DSB maps from Spo11-oligo sequencing, will provide insights into the interplay between DSB regulation and genome architecture.

Poster # 48

A comprehensive analysis of major spatial transcriptomics platforms for identifying the most complete transcriptional atlas of the mammalian testis

Jelena Lujic, David McKellar, Stephanie Tanis, Leah Simon, Iwijn De Vlaminck, Paula Cohen

Cornell University

While several high-resolution sequencing technologies have emerged over the past decade, only spatial transcriptomics (ST) offers the option of preserving critical spatial information for a given tissue. The emergence of multiple ST platforms, therefore, has begun to fill the missing pieces of the puzzle, providing an understanding of tissue architecture from the viewpoint of transcriptional profiles of individual cells. ST enables the recognition of cell-type tissue/organ composition, cell-to-cell organization and interaction as well as communication at the molecular level. Given its strict histological organization and delicate structure, the mammalian testis provides a unique opportunity for ST, along with several technical challenges. Further, published testicular scRNA-seq atlases do not provide information regarding the spatial transcriptomic patterns occurring during the dynamic process of spermatogenesis. Among the many commercial ST platforms that are currently available, we have chosen the three most commonly used due to their various advantages: multiplexed error robust FISH (MERFISH), Visium, and Slide-seq. Slide-seq and Visium both utilize slides with a fixed barcode location. With a 10µm spot diameter, Slide-seq overall provides the greatest cell capture resolution. This is advantageous, as significant events in the testis take place over a small distance (~20 µm). Further, we are now able to detect the full spectrum of RNAs using a recent method optimized for the Visium platform, as all other methods are currently limited to the capture of polyadenylated RNA transcripts (McKellar et al., 2022). Finally, MERFISH, an imaging-based FISH platform, allows us to select a specific list of target genes. This is advantageous for the testis, given the high transcriptional activity of certain cell types. As the emphasis of this study is on meiosis, a time- and location-sensitive process within spermatogenesis, we aim to understand which ST platform provides the best spatially-resolved atlas of these events.

Poster # 49

Competition for H2A.Z sensitizes developing embryos to innate immune stimulation

Fanju W Meng, Kristin E Murphy, Claire E Makowski, Patrick J Murphy

University of Rochester

The histone variant H2A.Z plays a critical role in early embryonic development, establishing transcriptional competency through chromatin regulation of developmental gene promoters and enhancers. We find this role to be compromised in zebrafish embryos during innate immune response. Stimulation of interferon response causes H2A.Z to exit developmental gene promoters, which undergo silencing, and to accumulate at a subset of evolutionarily young repetitive elements, including DNA transposons, LINEs, and LTRs. Remarkably, this epigenetic rewiring is greatly influenced by the abundance of total H2A.Z, and developmental consequences of interferon stimulation are mitigated by H2A.Z over-expression. Our study reveals that H2A.Z levels determine sensitivity to innate immune activation, and repetitive elements can function as a nuclear sink, imparting influence over total transcriptional output. These findings uncover general mechanisms whereby competition between counteractive biological processes underlies phenotypic outcomes.

Poster # 50

A mouse-specific retrotransposon drives a conserved Cdk2ap1 isoform essential for preimplantation development

Andrew Modzelewski, Wanqing Shao, Ting Wang, Davide Risso and Lin He

UPENN

Nearly 50% of all mammalian genomes originate from ancient viruses and mobile elements called retrotransposons. Over millions of years of co-evolution, most retrotransposons have been inactivated through mutation and constant epigenetic surveillance. Still, many retain regulatory and structural features that can influence nearby genes in cancer, auto-immunity, neurodegeneration, and aging cells. While silenced in most tissues, retrotransposon reactivation is a common phenomenon in preimplantation embryos. Indeed, their disruption results in embryonic lethality, suggesting unknown but essential functions. The repetitive nature of retrotransposons makes studying individual functions difficult, however comparative analysis of 8 mammalian preimplantation RNA-SEQ datasets reveal strikingly similar levels of dynamic retrotransposon family expression that are active during defined windows of time, sometimes spanning a single cell division. A subset of these splice with nearby protein coding genes to form hundreds of novel embryo and species-specific promoters, exons, and polyA sites. Using a highly efficient embryo editing method (CRISPR-EZ) I developed, I generated 5 retrotransposon deletions mouse lines, revealing the first essential retrotransposon in mammalian development. A mouse specific MT2B2 promoter transiently drives a truncated Cdk2ap1ΔN that promotes proliferation just prior to implantation. In contrast, the canonical Cdk2ap1 represses cell proliferation but expresses after implanting. The retrotransposon based MT2B2 promoter is essential, where deletion induces maternal and pup lethality in part by reduction of cell proliferation, impaired implantation, uterine crowding, and implantation into unsuitable uterine sites, reminiscent of the human pregnancy complication placenta previa. Surprisingly, Cdk2ap1ΔN is evolutionarily conserved in sequence and function, yet is driven by different promoters across mammals. The distinct Cdk2ap1ΔN expression strongly correlates with the duration of preimplantation in each species. Hence, retrotransposon reactivation is an aspect of normal biology, where species-specific transposon promoters can yield evolutionarily conserved protein isoforms, bestowing novel functions and species-specific expression to govern essential biological processes.

Poster # 51

New Insights Into Prepubertal Spermatogonial Stem Cell Development

Gayatri Mohanty, Bidur Paudel, Maria Gracia Gervasi, Darya Tourzani and Pablo Visconti

University of Massachusetts

Introduction and Objective:
Glycogen synthase kinase (GSK3) that exists in alpha and beta isoforms plays an essential role during sperm capacitation and fertilization. Spermatozoa gets exposed to the capacitating environment there is an efflux of cholesterol with albumin as its acceptor and influx of bicarbonate ions that induces the activation of cAMP-PKA signaling pathway. This induces hyperpolarization along with acrosome reaction. GSK3 also plays an important role in acrosomal exocytosis. The objective is to investigate the regulation of GSK3 isoforms during capacitation and acrosome reaction.
Methods: Caudal spermatozoa region from CD1 male mice were incubated in media that support or not capacitation. Alpha and beta isoform of GSK3 were visualized and its phosphoregulation was studied using key media components – bovine serum albumin (BSA) and bicarbonate. Hyperpolarized stated was induced with the addition of 1uM of Valinomycin and its antagonistic effect was studied through the use of potassium chloride at 70mM. While alkanization effect on phosphorylation status was studied through addition of NH4Cl. cAMP-PKA pathway was modulated through 50uM TDI1099- inhibitor of soluble adenyl cyclase or exogenous addition of dbcAMP and IBMX and the phosphorylation status was studied in terms of hyperpolarization and alkalinity.
Results: We observed the GSK3 alpha activation during capacitation localized predominantly to the head region. However, BSA increased phosphorylation, bicarbonate caused a dephosphorylation. Modulation of the cAMP-PKA axis through absence of bicarbonate or SAC inhibition or exogenous addition of dbCAMP and PKA generated a hyperpolarized state that caused an activation of GSK3a. With the induction of hyperpolarization through 1uM valinomycin or increase in alkalinity 40mM NH4Cl in the absence of bicarbonate generated an active GSK3a protein that was negated with potassium saturation. While GSK3 inhibitors blocked the acrosome reaction without affecting intracellular calcium.
Conclusion: These results indicate that the capacitation process is isoform specific with GSK3a playing an active role. Hyperpolarization or increased alkalinity induces a dephosphorylated GSK3a. This proves that the cAMP pathway can be bypassed for GSK3 activation while its inhibition blocks acrosomal exocytosis.

Poster # 52

Effect of summer months on testicular histoarchitecture and spermatogenesis in Dezhou donkey: A histological study

1. Muhammad Faheem Akhtar (first author)
2. Wang Changfa (Corresponding Author)

Liaocheng University

This study aims to illustrate testicular histoarchitecture and progression of spermatogenesis in Dezhou donkeys during summer months. For experiment, 8 male Dezhou donkeys having body weight 260 + 20 kg and aged 2 years were analyzed using microscopy and Jonhson's score. Based on acrosomal system, Dezhou donkey spermatogenesis comprised of XII stages. Various types of spermatogonia: A1, A2, A3, B1 and B2 were observed along with undifferentiated spermatogonia (Aund). Testicular histoarchitecture during spermatogenesis, seminiferous tubules diameter (ST), ST height ((µm), Luminal tubular diameter (µm), No. of ST/field, No. of germ cells along with normal Jonhson's score illustrated that stages of spermatogenesis and testicular histoarchitecture of adult Dezhou donkeys are not affected during summer months. To further reinforce our hypothesis, plasma hormone concentrations of Luteinizing Hormone (LH), Inhibin A, Inhibin B and Testosterone (T) was analyzed. Plasma inhibin A was positively correlated to inhibin B with few exceptions while LH was also positively correlated with plasma testosterone (T) depicting normal development of germ cells. We concluded that spermatogenesis and testicular histoarchitecture of Dezhou donkey are not affected by hot summer months.

Poster # 53

Timing is everything: Sex differences in the microbiome-gut-brain axis are time-of-day dependent

Sarah K. Munyoki, PhD[1-3], Julie P. Goff, MS[1-3], Steven J. Mullett, BS[4-5], Jennifer K. Burns PhD[7,8]
Aaron K. Jenkins PhD[7,8], Lauren DePoy PhD[7,8], Stacy G. Wendell,PhD[4-6],
Colleen A. McClung, PhD[7-9], Kathleen E. Morrison, PhD[10], Eldin Jašarević, PhD[1-3]

[1]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine,
[2]Department of Computational and Systems Biology, University of Pittsburgh School of Medicine,
[3]Magee-Womens Research Institute,
[4]Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine,
[5]Health Sciences Mass Spectrometry Core, University of Pittsburgh School of Medicine,
[6]Clinical Translational Science Institute, University of Pittsburgh School of Medicine,
[7]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA
[8]Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA
[9]Clinical and Translational Science Institute, University of Pittsburgh School of Medicine,
[10]Department of Psychology, West Virginia University, Morgantown, WV

Circadian rhythms orchestrate many essential homeostatic processes. Emerging evidence points to the gut microbiome and its metabolites as a novel class of circadian entrainment signals, but the molecular mechanisms by which diurnal rhythms in microbial metabolites contribute to host physiological processes remain unknown. Moreover, circadian rhythms are influenced by sex, and circadian disruption is often associated with sex-specific disease risk. Despite clear links between sex-specific processes and lifelong health trajectories, studies on circadian rhythms primarily use male mice or collapse both sexes into one experimental condition. We applied an integrated multi-Omics approach combining longitudinal analyses with dietary manipulation, metabolomics, transcriptomics, single-cell immunophenotyping, and machine learning to specifically examine the hypothesis that sex differences in the microbiome-gut-brain axis are time-of-day dependent.

We identified sex-specific differences in diurnal rhythms in the intestinal microbiota, the metabolites they produce, and the expression of host genes, with more pronounced effects in females. The magnitude of these sex differences varies by time of day. These time-of-day and sex-specific patterns were completely abolished in germ-free mice, suggesting that an intact microbiome is necessary for synchronizing sex differences across the microbiome-gut-brain axis. Further, transitioning mice to a high-fat low fiber diet abolished circadian rhythms in microbiota, metabolites, and host gene expression entrained by a chow diet. Consumption of the high-fat low-fiber diet generated new diurnal rhythms in the microbiota and host transcriptome. We show that circadian rhythms in the crosstalk between microbiota and their hosts are sex-specific and that diet plays an essential role in maintaining these differences.

Poster # 54

TSSK1 and TSSK2 are independently essential for male reproduction

Saman Nayyab, Maria Gracia Gervasi, Darya Tourzani, Wei Cui, Maria Eugenia Teves, Ana Maria Salicioni, Elizabeth Snyder, Pablo Visconti

UMass Amherst

In our previous studies, we have reported that members of the family of testis-specific serine/threonine kinases (TSSKs) are post-meiotically expressed in male germ cells and in mature mammalian sperm. The restricted post-meiotic expression of TSSKs as well as the importance of phosphorylation in signaling processes strongly suggests that TSSKs have an important role in germ cell differentiation and/or sperm function. This is further supported by published data that mouse TSSK6 knockout and TSSK1/2 double knockout (KO) lead to male sterility. The aim of our work focuses on individual TSSK1 and TSSK2 as potential candidates for male contraceptives. Using CRISPR/Cas9 technology, we were able to characterize reproductive features of single TSSK1 and TSSK2 KO and their effects on male fertility in mice. We conducted a fertility test to determine the sterility of these KO model organisms and show complete sterility of the knockout mice. In addition, sperm from those mice were unable to fertilize in vitro. Using Computer Aided Sperm Analysis (CASA), transmission and scanning electron microscopy, we showed that sperm from Tssk1 and Tssk2 knock out mice do not undergo hyperactivation and show abnormal morphology. We found that KO mice exhibited major abnormal sperm morphology by scanning electron microscopy (SEM) without observing changes in the testis or epididymis. Our data indicate that both TSSK1 and TSSK2 are needed for reproduction and can be used as targets for novel male contraceptives.

Poster # 55

Pre-Zygotic Genome Activation Heterochromatin Formation on Repetitive Elements is Essential for Precise Developmental Timing

Duy Nguyen, Patrick Murphy

University of Rochester

After fertilization, the maternal and paternal genomes come together and undergo epigenetic reprogramming to prepare for zygotic genome activation (ZGA) during the maternal-to-zygotic transition (MZT). Epigenetic mechanisms are crucial for ensuring that the initially transcriptionally silent zygotic genome becomes transcriptionally active at the precise developmental time point during MZT. However, few studies have investigated pre-ZGA transcriptional control of non-protein coding regions of the genome, such as repetitive elements. To investigate transcriptional control of repetitive elements, we treated zebrafish embryos with an epigenetic inhibitor of chromatin silencing factors G9a, which deposit H3K9me2, at fertilization. Strikingly, we observed significantly increased mortality and prolonged developmental delays in zebrafish embryos after only transient G9a inhibition during the first 30 minutes of development following fertilization. Our findings suggest that G9a-dependent heterochromatin formation may suppress pre-ZGA repetitive element transcription, which may be an essential aspect of precise early developmental timing control. We postulate that repetitive elements can regulate developmental gene expression by modulating the demand on the transcriptional machinery.

Poster # 56

High-throughput screening of meiotic gene mutants through germ line stem cell transplantation

Thierry N'tumba-byn, Scott Keeney

MSKCC

Most severe cases of infertility in men involve non-obstructive azoospermia (NOA). Meiosis is a crucial step in spermatogenesis, and mutations affecting the functions of meiotic genes are known to be the cause of some cases of NOA in humans. Therefore, methods to identify the role of specific genetic mutants in fertility issues and particularly in meiosis are needed. Culture systems supporting mammalian germ cell differentiation in-vitro have been reported, but it is unclear how faithfully or efficiently they recapitulate normal meiosis. Because of this, genetically engineered mouse models remain the gold standard for meiotic studies, but these are usually limited to one or a few mutations at a time and involve extensive breeding. This bottleneck makes it infeasible to examine large numbers of mutant alleles for a gene of interest. Here, we present a novel approach to overcome this bottleneck using parallelized genetic screening in germline stem cells. Cultures of mouse spermatogonial stem cells (SSCs) will be genetically modified in vitro to generate pools of clones with defined genetic changes. The SSC pools will then be transplanted into testes of recipient mice in which the endogenous germ cells have been depleted by treatment with busulfan. After engraftment of the transplanted SSCs, we evaluate which clones have altered meiotic potential. This innovative approach will allow us to test many genetic modifications in a single experiment. Preliminary data show that we can establish and maintain functional SSC cultures, manipulate them genetically to generate high complexity pools, transplant them at high density and observe their differentiation while maintaining high clonal complexity. This project will lay the foundation to identify novel genes fundamental not only to meiosis but to all the different steps of germ cell differentiation.

Poster # 57

A high-throughput ovulation screening platform to identify non-hormonal contraceptive candidates

Pawat Pattarawat, Jiyang Zhang, Wenlong Zhao, Tingjie Zhan, Xueyuan Liu, Eunchong Kim, and Shuo Xiao

Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University, Piscataway, NJ 08854

The need for non-hormonal female contraceptives is increasingly pressing, as hormone-based contraception can cause adverse side effects. There are currently few effective models that both recapitulate the hallmarks of ovarian follicle maturation and ovulation, the primary target of hormonal contraception, and possess scalability. We previously demonstrated that a 3D hydrogel encapsulated in vitro follicle growth (eIVFG) system faithfully recapitulates key ovarian functions, including gonadotropin-dependent ovarian follicle maturation, ovulation, luteinization, and associated hormone secretion. Here, we aimed to use eIVFG and other approaches to develop a high-throughput ovulation screening platform to identify compounds that can effectively block follicle rupture, a key step of ovulation, without interfering ovarian hormone secretion. Fully-grown preovulatory follicles from eIVFG were treated with 1.5 IU/mL Human Chorionic Gonadotropin (hCG) to induce ovulation ex vivo; meanwhile, follicles were co-treated with candidate compounds at 10 μM. The ovulation success was evaluated 15 hours post-hCG. Follicles with inhibited follicle rupture were cultured for an additional 48 hours to allow for luteinization and progesterone secretion. Utilizing this pipeline, we have screened a total of 426 compounds, including 152 compounds from the Cayman Chemical Epigenetic Library, 250 compounds from the Selleckchem Protease Inhibitor Library, and 24 compounds targeting established ovulatory molecules or signaling pathways. We identified 33 compounds that effectively blocked follicle rupture without affecting hormone secretion. These positive hits were selected for more advanced drug development activities such as dose-response exposure and molecular target identification. Furthermore, we used a closed vitrification method to cryopreserve individual immature mouse follicles to establish a high-content follicle biobank, which further increases the throughput of the ovulation screening platform. Together, with this robust high-throughput ovulation screening platform, we will screen larger-scaled compound libraries to identify non-hormonal contraceptive candidates.

Poster # 58

Exploring normal and malignant mouse endometrium using scRNA-sequencing and spatial transcriptomics

Matalin Pirtz, Lora H. Ellenson, Andrea Flesken-Nikitin, Benjamin D. Cosgrove, and Alexander Yu. Nikitin

Cornell University

Serous endometrial carcinoma (SEC) constitutes about 15% of endometrial cancers and is the most type aggressive and lethal type of uterine cancer. The cell of SEC origin remains insufficiently clarified. Malfunctions in stem cells have been connected to a variety of diseases, including cancer. Critically, the composition and function of resident endometrial epithelial stem cells remain insufficiently established. This gap in knowledge limits our understanding of SEC pathogenesis and development of prognostic and diagnostic methods and therapeutic modalities. Here, using single-cell RNA sequencing, we have established a single-cell transcriptomic atlas of the mouse endometrial epithelium. This approach, merged with spatial transcriptomic and immunostaining analyses, revealed that luminal epithelial cells consistently express Tacstd2 (encoding TROP2), while the glandular population express Foxa2. Our data suggest there are two stem cell pools in the endometrial epithelium that may distinctly contribute to the luminal or glandular populations, respectively. Moreover, we performed single-cell and spatial transcriptomic analyses of a mouse model we have generated to recapitulate endometrial carcinogenesis by conditionally inactivating Trp53 and Rb1 in Pax8-expressing cells. In this model, we observed that the luminal and glandular endometrial epithelial compartments became increasingly disorganized during cancer progression. Notably, TROP2-expressing cells were most associated with invasion in this mouse model. We also found no SEC developed 360 days after Trp53 and Rb1 conditional inactivation in glandular FOXA2-expressing cells. These results suggest that the luminal epithelial cells may represent the main cell of SEC origin. Our atlas of normal and mutant endometrial cells provides a valuable resource for further studies of endometrial biology and malignant transformation.

Poster # 59

TET1 Catalytic Activity is Required for Reprogramming of Imprinting Control Regions and Patterning of Sperm-Specific Hypomethylated Regions

Rexxi D. Prasasya, Blake A. Caldwell, Zhengfeng Liu, Songze Wu, Nicolae A. Leu, Johanna M. Fowler, Steven A. Cincotta, Diana J. Laird, Rahul M. Kohli, and Marisa S. Bartolomei

UPENN

DNA methylation erasure is an obligate process of mammalian primordial germ cell (PGC) reprogramming, where the germline fate is established from somatic cells in the epiblast. While the majority of the PGC genome demethylates through replication-coupled passive dilution, active demethylation has been shown to be required for complete reprogramming of subsets of loci, including imprinting control regions and gametogenesis related promoters. TET enzymes iteratively oxidize 5-methylcytosine (5mC) to generate 5-hyroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC) to facilitate active demethylation of the genome. Whether 5hmC is required to further promote replication-coupled passive dilution or whether 5fC/5caC generation is required to activate base excision repair mechanism during germline reprogramming remains unresolved due to the lack of genetic models to delineate the diverse activities of TET proteins. Here, we generated two mouse lines expressing catalytically inactive TET1 (Tet1-HxD) and TET1 that stalls oxidation at 5hmC (Tet1-V). We examined the resultant methylome of Tet1-/-, Tet1V/V, and Tet1HxD/HxD sperm, and showed that full length TET1V and TET1HxD can rescue the majority of hypermethylated regions of Tet1-/- sperm, thus demonstrating the relevance of TET1 extra-catalytic functions in achieving normo-methylation in the sperm genome. The presence of catalytically inactive TET1HxD resulted in distinct hypomethylated regions that were not observed with complete lack of TET1 or 5hmC stalling TET1. Imprinted regions require iterative oxidation of 5mC for complete reprogramming. We further reveal a broader class of hypermethylated regions in sperm of Tet1 mutant mice that are excluded from de novo methylation during male germline development and depend on TET oxidation for reprogramming. Genes associated with TET1-dependent differentially methylated regions (DMRs) are expressed throughout male gametogenesis, with candidate loci displaying the use of alternative promoters at these DMRs. Our study underscores the link between TET1-mediated demethylation during reprogramming and sperm methylome patterning.

Poster # 60

Investigating the Maternal Effects in Female-biased Embryonic Death Induced by Genomic Instability

Yiwen Qin, John Schimenti

Cornell

Mammalian development is a dynamic process that primarily depends on the genetic contribution of both parents. The pregnancy outcomes are also controlled by various environmental factors and genetic variations within the gametes such as macromolecules, organelles and epigenomes. Previously, our group discovered that genomic instability (GIN) caused by replication stress impacts embryonic developmental outcomes. A decrease in the level of minichromosome maintenance proteins (MCM) is observed in the mutant allele Mcm4Chaos3 (chromosome aberrations occurring spontaneously 3), which causes GIN in mice3. When more reduction of MCMs (MCM2, 6 or 7) is imposed on the mice homozygous for Mcm4Chaos3, there is a significant loss of female embryos starting from mid-gestation. This developmental defect is probably due to the inflammation in the placental tissue, likely originating from cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway. It was thought that the micronuclei trigger this pathway, as 7.5% of erythrocytes in Mcm4Chaos3/Chaos3 mice containing micronuclei compared to the 0.25% in their wildtype littermates. More importantly, the sex skewing depends on the maternal genotype, as swapping the dam genotype to relatively genomically -stable genotypes helped suppress the maternal-fetal inflammation and restored the ratio of born female to male mice back to 1:1. This phenomenon suggests that some unknown maternal factors contribute to the susceptibility of embryos to DNA damage in genomically-unstable dams. Mitochondria are the key modulators of p53-mediated apoptosis pathway which was shown to be activated under replication stress. Additionally, only oocytes' mitochondria are transmitted to the embryo. By understanding how maternal genomic instability leads to inflammation and eventually embryonic death, we will provide insights into the complex maternal-fetus interaction and identify factors contributing to the observed non-Mendelian inheritance.

Poster # 61

USING SINGLE-CELL TRANSCRIPTOMICS TO IDENTIFY ELUSIVE CELL TYPES WITHIN THE HUMAN FALLOPIAN TUBE EPITHELIUM

Coulter Q. Ralston, Alex Webster, Andrew K. Godwin, Lora H. Ellenson, Doris Mayr, Andrea Flesken-Nikitin, Benjamin D. Cosgrove, and Alexander Yu. Nikitin

Cornell University

The fallopian (uterine) tube epithelium has recently drawn attention as a likely place of origin for a large fraction of high-grade serous carcinomas (HGSC). Interestingly, both HGSC initiation and expression of stem-like features are more common in distal regions of the fallopian tube, suggesting a connection between the two. While differentiated types of epithelial cells within the distal fallopian tube have been described, the identity of distinct stem/progenitor cell population(s) and their cellular hierarchy are still debated. Furthermore, the heterogeneous stromal composition of the fallopian tube remains poorly elucidated. Here, we apply single-cell transcriptomics to six premenopausal human fallopian tube tissue samples to characterize the cells present within the distal region of the fallopian tube. We identified fourteen epithelial cell clusters expressing a variety of ciliated, secretory, and additional markers. Five clusters were FOXJ1+ ciliated cells and another five clusters were OVGP1+ secretory cells, which may reflect subtle differences within the ciliated and secretory clusters. There were also four undefined (UD) clusters that did not clearly express ciliated nor secretory features. Using previous reports, we identified the remaining UD clusters as UD1 expressing immunomodulatory genes, UD2 expressing pro-inflammatory genes, UD3 expressing quasi mesenchymal-related genes, and UD4 expressing stem-like genes associated with peg cells. Additional immunohistochemical analysis of fallopian tube sections confirmed cell type-specific expression patterns of many of these markers. Notably, PAX8 and KRT7 were observed in secretory cells, PROM1 (CD133) was observed in ciliated cells, and KRT5 was observed in peg cells. These findings provide the basis for identification and characterization of cell types and their differentiation states potentially associated with clinically distinct HGSC subtypes. In on-going work, we are using pseudotime analysis to infer a cellular hierarchy among the identified cell types within the distal fallopian tube.

Poster # 62

Trophectoderm biopsy followed by vitrification after in vitro fertilization and embryo culture increases fetal and placental epigenetic dysregulation in a mouse model.

Eric A. Rhon-Calderon, Marisa S. Bartolomei

UPENN

More than 9 million babies worldwide have been born through Assisted Reproductive Technologies (ART) and this number is expected to grow due to easy access to these procedures, increase successful pregnancies and postponing of childbearing. Trophectoderm biopsy for genetic testing (TEBx) is an ART procedure to test the presence of inherited genetic disorders and/or chromosome abnormalities. In addition to TEBx, major advances in the success of embryo cryopreservation through vitrification (Vit) have enabled the delay of embryo transfer. We hypothesized that mechanical stress induced during the biopsy followed up by vitrification exacerbates damage induced by IVF or TEBx alone. To address this, we biopsied blastocysts produced by IVF, vitrified, and transferred to recipient females. At E12.5, we examined placental morphology, and placental and fetal changes in DNA methylation. IVF+TEBx+Vit group exhibited no changes in mean placental weight. Mean fetal weight was significantly different compared to Naturals with no differences compared to other ART groups. Histological analysis of IVF+TEBx+Vit placentas revealed exacerbated differences in junctional and labyrinth zone compared to other ART groups. Immunohistochemistry for CD34 in placentas showed a decreased blood vessel density compared to Naturals and no differences with the other ART groups. Molecular analysis of placentas from the IVF+TEBx+Vit group showed a decrease of DNA methylation at ICRs compared to Naturals, with no differences for H19/Igf2, Kcnq1ot1 and Peg3 when compared to IVF and IVF+TEBx. Interestingly, IgDMR and Snrpn exhibited a larger decrease in DNA methylation when vitrification was added compared to the other ART groups and Naturals. Finally, embryonic DNA methylation at H19/Igf2, Kcnq1ot1, IgDMR and Snrpn was more affected when vitrification was added after TEBx compared to other ART groups, and significantly more reduced compared to Naturals. Taken together, we concluded that fetal and placental development is impacted by the addition of vitrification after trophectoderm biopsy.

Poster # 63

Assessing the Potential of ChatGPT in Scientific Writing: A Rapid Production of Content with New Challenges

Myriam Safrai, Kyle E. Orwig

University of Pittsburgh School of Medicine

Introduction: The release of the ChatGPT artificial intelligence chatbot in late 2022 resulted in millions of people from all over the world signing up to test it with questions on any subject. While there was enthusiasm surrounding its launch, controversy arose in academic fields regarding the accuracy of data and the potential for plagiarism.

Methods: ChatGPT was used to write a scientific article titled "Revolutionizing Reproductive Medicine: The Potential and Challenges of Artificial Intelligence in Fertility and Assisted Reproductive Techniques." It suggested the title, abstract, introduction, discussion, keywords, content for a table, and a letter to the editors. The authors assessed the final article for parameters such as accuracy of data, quality of writing, and potential for plagiarism.

Results: Responses to prompts took between 1 and 7 seconds, resulting in a rapid writing process. Broader topics resulted in more accurate answers. ChatGPT sometimes mentioned subjects in the introduction and discussion but not in the main article. This was resolved by adapting subsequent prompts to earlier subject mentions. While the English was good for most of the article, ChatGPT's references were entirely fictitious, misleading the readers.

Discussion: According to our findings, the new chatbot, ChatGPT, is technically capable of producing text for a scientific review on specific topics. Nonetheless, the lack of citations prevents it from doing so thoroughly. Furthermore, the human author must be well-versed in the field to assess generated responses properly and to avoid errors and missing information in responses that ChatGPT may generate. As a result, at this time, this technology cannot replace human scientific expertise. However, it has the potential to facilitate and accelerate the writing process if used judiciously, raising the need for individuals and scientific communities to adapt to create the most valuable form of this new technology while avoiding adverse implications.

Poster # 64

Fertility preservation for young girls – When your zip code is more important than your diagnosis.

Myriam Safrai, Amanda C. Zielen, Rachel Neelley, Lauren M. Wasser, Kyle E. Orwig

University of Pittsburgh School of Medicine

Introduction:
Prepubertal girls facing gonadotoxic treatments need ovarian tissue cryopreservation (OTC) for fertility preservation (FP). ASRM removed the experimental label for this procedure in 2012. However, the cost and socioeconomic status may affect a patient's access to OTC. This study evaluated the socioeconomic backgrounds of different populations based on their OTC access cost.
Material and Methods:
A retrospective analysis was conducted on prepubertal girls (<15 y.o.) who cryopreserved ovarian tissues with the UPMC fertility preservation program since 2011. Patients could cryopreserve ovarian tissue for free under an IRB approved research protocol (Free group, n=51) or by paying a fee for service (Pay group, n=123). Using zip codes, annual income was identified in the Census database and a Distressed Communities Index (DCI) was calculated. The DCI combines seven economic indicators to generate a single index score ranging from 0 (least distressed) to 100 (most distressed).
Results:
The age and diagnosis did not differ significantly between the Free and Pay groups. A higher percentage of the Pay group population had higher education (35±17% vs. 28±14%, p=<0.001), while the DCI was higher in the Free group population (57.001±27.25 vs. 36.45±28.87, p=<0.001) indicating a higher distress. The annual household income of the Free group (63,080.35±20,068.86) was lower than that of the Pay group (78,633.4±24,333.46, p<0.001). In the Free group, 63% of individuals belonged to the top two per-quartile DCI groups (indicating distress), while in the Pay group, only 37% did. The per capita of prepubertal girls receiving OTC for FP, was two times higher in the Free group than that in the Pay group (6.5 vs 3.5 per 100,000 individuals).
Conclusion:
The Free group population had comparatively more socioeconomic distress, but they had a higher per capita access to OTC for FP. We suspect the socioeconomic cost barrier to FP was reduced for patients in the Free group population because OTC service cost was free, allowing for greater access despite socioeconomic status. These data suggest that there is a potential financial disparity in access to FP care and this is exacerbated by the fact that FP services are rarely covered by insurance in the US.

Poster # 65

Generating an In Vitro Meiotic "Toolkit"

Leah E. Simon, Julieta Merlo, Stephanie Tanis, Jelena Lujic, Paula E. Cohen

Cornell University

Spermatogenesis is an exceptionally complex process, consisting of multiple transitions between fundamentally distinct developmental stages. Unsurprisingly, this process has been near impossible to recapitulate in vitro, and published methods have proven to be highly contentious. To establish a robust meiosis model for interrogating spermatogenesis and for translational research, my goal is to develop a toolkit of in vitro systems for the maintenance and differentiation of spermatogonia into meiosis-proficient spermatocytes. To accomplish this, 2D, 3D and organotypic culture strategies were applied to testicular cell suspensions or whole testis from prepubertal mice. Using these systems, we interrogated the roles of different factors that have been shown to be critical for spermatogonial differentiation, including retinoic acid, TGF-β, testosterone, and FSH. The 2D laminin-coated culture system allowed both proliferation of spermatogonial stem cells (SSC) and initiation of meiosis following short-term culture duration as evidenced by spermatogonia that form chains and synaptonemal complexes identified by SYCP3 immunofluorescence staining. Following 3D culturing techniques using hydrogels, spermatogonia formed clustered spheroids of cKIT-positive cells (differentiating spermatogonia marker) in long term culture (up to 20 days). In vitro meiotic progression was observed following whole testis culture for up to 6 days and was comparable to in vivo littermates, as evidenced by SYCP3 and γH2AX (identifying sex body) immunofluorescent staining of prophase chromosome spreads. Further characterization of meiotic initiation and progression, and methods for the optimization of cell recovery is currently underway. Thus, this work-in-progress aims to establish a toolkit of culture systems to provide the field with a versatile platform for SSC proliferation, differentiation, meiotic initiation, and progression. We hope to contribute to the design of an optimal system for experiments that is otherwise challenging to perform in vivo, and that is currently lacking within the community to routinely identify and assess suitable drug targets in vitro.

Poster # 66

Examining Small RNA Regulation in the Male Reproductive System with Single Cell RNA-Seq.

Smith-Raska, M.

Cornell University

Small RNAs such as microRNA, piwiRNA, and tRNA fragments are critical regulators of spermatogenesis; dysregulation of these molecules are associated with infertility. Expression of specific small RNAs has not been mapped to specific stages of spermatogenesis or to specific somatic cell types in a thorough and unbiased manner across all cells in the testes, because of the heterogenous nature of the testes and the difficulty in obtaining pure cell subpopulations.

We have leveraged single cell RNA-Seq to examine the gene expression patterns across all cell types in the testes, with a focus on genes that regulate small RNA expression and processing in both testicular somatic cells as well as cells in various stages of spermatogenesis. Flow cytometry was used to increase the representation of diploid somatic cells. The preliminary data reveals multiple cell types that are enriched for small RNA-processing processes. We note specific expression patterns of small RNA processing genes at specific stages of spermatogenesis, suggesting a complex coordination of small RNA expression and metabolism. We similarly find unique expression patterns of small RNA processing genes in the testicular somatic cells.

Single cell expression patterns in wild type testes were also compared with testes from mice deficient for the germ cell-specific gene Khdc3, which our preliminary data suggest control sperm small RNAs that have transgenerational effects on lipid and glucose metabolism.

Mapping of specific small RNAs to specific stages of spermatogenesis will be an important step in understanding how these molecules drive cases of infertility.

Poster # 67

Membrane fusion in mammalian fertilization

Shaogeng Tang, Yonggang Lu, Will M Skinner, Mrinmoy Sanyal, Polina V Lishko, Masahito Ikawa, Peter S Kim

Stanford University

Tmem95 encodes a sperm acrosomal membrane protein, whose knockout has a male-specific sterility phenotype in mice. Tmem95 knockout murine sperm can bind to, but do not fuse with, eggs. How TMEM95 plays a role in membrane fusion of sperm and eggs has remained elusive. Here, we utilize a sperm penetration assay as a model system to investigate the function of human TMEM95. We show that human TMEM95 binds to hamster egg membranes, providing evidence for a TMEM95 receptor on eggs. Using X-ray crystallography, we reveal an evolutionarily conserved, positively charged region of TMEM95 as a putative receptor-binding surface. Amino acid substitutions within this region of TMEM95 ablate egg-binding activity. We identify monoclonal antibodies against TMEM95 that reduce the number of human sperm fused with hamster eggs in sperm penetration assays. Strikingly, these antibodies do not block binding of sperm to eggs. Taken together, these results provide strong evidence for a specific, receptor-mediated interaction of sperm TMEM95 with eggs and suggest that this interaction may have a role in facilitating membrane fusion during fertilization.

Poster # 68

TRPM's role in protein phosphorylation during Drosophila egg activation

Jonathon M. Thomalla, Mariana F. Wolfner

Cornell University

To ensure successful initiation of embryonic development, a mature oocyte must turn on the programs needed for zygotic development. This developmental transition, called egg activation, occurs in the absence of transcription. Its events, including completion of stalled meiosis and translation of maternal mRNAs, require a rise in calcium levels in the oocyte. Phosphorylation states of hundreds of maternally-provisioned proteins change during egg activation, prompting the hypothesis that their phospho-states act as 'on' or 'off' switches for the events of egg activation. We previously showed that the calcium-activated phosphatase calcineurin, which is essential for egg activation, regulates a subset of phospho-changes, including cell cycle and translation regulators, linking the protein phosphorylation changes and their consequences to the calcium rise. The calcium rise in Drosophila oocytes begins with local calcium entry mediated by the TRPM cation channel, and spreads across the oocyte in a wave. To determine the extent of its effects on maternal proteins, we compared the phosphoproteomes of Trpm knockdown (KD) oocytes and activated, unfertilized eggs to those of controls. Surprisingly, our preliminary analysis of the mass spectrometry data indicates that many phospho-changes, including some shown to be calcineurin dependent, occur normally in TRPM KDs; for example, the translational regulatory protein Gnu and M-phase regulator Endos undergo their normal calcineurin-mediated dephosphorylation events. However, the APC-regulator Fzy does not undergo its normal calcineurin-mediated dephosphorylation event in activating TRPM KD eggs. We are exploring compensatory and temporal mechanisms that may explain the mild effect we see of TRPM KD on the phosphoproteome, with the goal of disentangling the players and timescale for egg activation and any 'fail safe' mechanism(s) that eggs utilize to ensure robust early development.

Poster # 69

Uncovering Novel Roles of Argonaute Proteins in Spermatogenesis and Early Embryonic Development

Amanda E. Touey, Maria de las Mercedes Carro, Faraz Ahmed, Jen K. Grenier, Andrew W. Grimson and Paula E. Cohen

Cornell University

AGO3 and AGO4 are RNA binding proteins that are found abundantly in the male germline and localize to pachytene spermatocytes during prophase I of meiosis. Specifically, these proteins are present in the sex body, a specialized sub domain of the nucleus housing the sex chromosomes. Analysis of a triple knockout mouse line (Ago413) has shown that loss of Ago1, Ago3 and Ago4 leads to impaired silencing of the X and Y chromosomes in pachytene spermatocytes as evident by aberrant localization of markers such as γH2AX and TOPBP1 and an influx of RNA Pol II within the sex body. Our data also show decreased sperm count and impaired motility along with decreased testis size in Ago413 heterozygous and homozygous mutant animals. Most curiously, mutant animals also show a distortion in the expected sex ratio of their offspring in favor of females and this bias is present as early as the blastocyst stage. Gene set enrichment analysis of RNA-sequencing data revealed significant upregulation of genes found on the X and Y chromosomes of Ago413 mutant spermatocytes, and that many of the upregulated genes on the Y chromosome are clustered within the sex reversal region (Sxr). In addition to these findings, our data also showed premature expression of spermatid associated genes and decreased expression of meiosis associated genes in Ago413 mutant spermatocytes. Additional analysis of smallRNA sequencing revealed a previously identified cluster of miRNAs was specifically deregulated in Ago413 mutant spermatocytes and round spermatids. This cluster, known as Fx-mir, is part of the Fragile X region and located within the Hstx2 locus on the X chromosome. Taken together, these results suggest a novel nuclear role for AGO3 and AGO4 during meiosis that is essential for proper silencing of sex chromosomes and downstream spermiogenesis events leading to equal sex ratios in the offspring.

Poster # 70

New Insights Into Prepubertal Spermatogonial Stem Cell Development

Anna Laura Voigt, Rkia Dardari, Nathalia de Lima e Martins Lara, Paula Cohen, Kyle E. Orwig, Ina Dobrinski

Cornell University

Spermatogonial stem cells (SSCs) are the basis of spermatogenesis and therefore male fertility. SSCs originate from bipotent primordial germ cells (PGCs) that colonize the developing male gonad as pre/prospermatogonia and subsequently mature into adult SSCs. Our understanding of the maturation characteristics including their temporal pattern from prepubertal spermatogonia to adult SSCs is just in its infancy. Using the porcine model, we discovered that spermatogonial maturation is largely extended into the prepubertal phase and characterized by a metabolic transition from high oxidative phosphorylation (OXPHOS) to anaerobic metabolism. Extensive metabolomic analyses of porcine spermatogonia showed that early prepubertal spermatogonia (1-week old pig) have high mitochondrial activity and respiration with a maximum capacity of 923.2 ± 9.54 pmol/min/cells vs 459.9 ±46.89 pmol/min/cells when compared to late prepubertal spermatogonia (8-week-old pig). In addition, immature spermatogonia preferentially consume pyruvate over glucose in vitro (1.23 ± 0.16 mM [pyruvate] vs 0.147 ±0.09 mM [glucose] decrease in media-concentration over 48 hrs) contrary to older stages when analyzed via mass spectrometry. Serial single RNA-sequencing (scRNA-Seq) analysis revealed that human spermatogonia exhibit a similar pattern, characterized by high expression of OXPHOS-associated genes that are downregulated by 11 years of age. A change in the expression of epigenetic modifiers in human prepubertal spermatogonia established that this metabolic transition might be associated with DNA de novo methylation as indicated by the expression of DNA-methyltransferase 3A (DNMT3A) beginning at 11 years and the subsequent and continued expression of the DNA maintenance methyltransferase 1 (DNMT1) starting at 13 years of age. This data shows that prepubertal spermatogonial maturation is characterized by metabolic and epigenetic maturation just before puberty. Further exploration of this epigenetic maturation will be pivotal in deciphering the developmental roadmap of SSC maturation and for advancing clinical application of prepubertal spermatogonia in fertility preservation.

Poster # 71

Oocyte and Early Embryo Metabolism

W.David Weber, Darya Tourzani, Kayla Grosso, Maria Gracia Gervasi & Pablo E. Visconti

UMass

As other cell types, oocytes and early embryos require energy in the form of ATP. As mammalian oocytes and embryos can only be obtained in limited numbers, it is difficult to investigate the importance of individual metabolites. Recently, mass spectrometry has become amenable for identification and semi-quantitation of metabolites in these cells. However, these methods are expensive and require specialized equipment and expertise. On the other hand, in almost all cell types, most of the ATP produced is used for protein synthesis; this feature has been used in cancer tissues to investigate energy metabolism pathways. Briefly, cells or tissues under investigation are incubated in the presence of puromycin. Cells are then fixed and stained with anti-puromycin antibodies. In the original reports, different types of cells are then analyzed for puromycin content using flow cytometry. To investigate the extent by which ATP was produced through glycolysis, oxidative phosphorylation or endogenous pathways (e.g. lipid oxidation). Parallel experiments are conducted using deoxy-glucose to block glycolysis and oligomycin to block oxidative phosphorylation, or both. Harringtonine, another protein synthesis inhibitor is used as a control for unspecific puromycin incorporation. Contrary to the use of metabolic flow analyzers such as Seahorse®, this method allows for the analysis of energy pathways in complex cell mixtures, and this method will work when the number of cells is not sufficient to have a workable signal to noise ratio. In the present work, we adapted the puromycin methodology to be used in oocytes and early embryos. Instead of flow cytometry we used quantitative immunofluorescence using confocal microscopy. Initial experiments were done using Metaphase II arrested oocytes and 8 hours post-fertilization embryos.

Poster # 72

Phosphorylation of SKP1 regulates SKP1-Cullin-F-box (SCF) ubiquitin ligase complex assembly during mouse spermatogenesis

Ian D. Wolff, Stephen Gray, Paula E. Cohen

Cornell University

Sexually reproducing animals generate gametes through the process of meiosis, a reductional cell division that yields haploid oocytes or sperm. Meiosis proceeds through two distinct stages: prophase I, when homologous chromosomes are physically tethered through the formation of crossovers, and M-phase, when homologs and sister chromatids are separated in the first and second meiotic divisions, respectively. Recently, the SCF (SKP1-Cullin-F-box) E3 ubiquitin ligase complex has been implicated in meiotic cell cycle progression in several organisms: a core SCF component, SKP1, is essential for meiotic recombination and the prophase I to M-phase transition, and SCF may be regulated by the crossover protein CNTD1 to promote downstream degradation of cell cycle inhibitors. SKP1 functions within the larger SCF complex bound to an F-box protein (FBP) to form the substrate recognition module that recruits a target protein for ubiquitination. To ensure this specificity, the mouse genome contains 74 FBP genes; each FBP binds a subset of target proteins, and temporal degradation of targets can be regulated through differential FBP gene expression. However, it remains unknown which FBPs are expressed throughout spermatogenesis, or how SKP1-FBP interactions are regulated to achieve stage-specific SCF complex assembly. In this work, we investigated the biochemical composition of the SCF complex during spermatogenesis in the mouse. We characterized a conserved phosphorylation event in the FBP interacting domain of SKP1 at T131 that is enriched in late spermatocytes. Analysis of previously published scRNA-seq data revealed that over half of the 74 FBP genes are expressed in late spermatocytes. We found that several expressed FBPs, including FBXO9 and FBXW7, require phosphorylation of T131 to interact with SKP1, suggesting that this conserved phosphorylation event is a key regulator in SCF assembly. Altogether, this work indicates that meiotic cell cycle progression may be driven through regulation of SCF complex assembly by post-translational modifications.

Poster # 73

Elucidating the function of CNTD1 in crossover licensing in mammalian oogenesis

Anna Wood, Rachel Bradley, Melissa Toldedo, Rania Ahmed, Ian Wolff, and Paula E. Cohen

Cornell University

In meiotic prophase I hundreds of double-strand breaks are induced to promote homologous recombination, resulting in the formation of non-crossovers (NCO) and crossovers (CO). However, only ~10% of these double-strand breaks results in COs. Only the latter events can resolve into the chiasmata that ensure tethering of homologous chromosomes right through until the first meiotic division. Thus, the appropriate maturation, distribution, and frequency of these crossover sites during prophase I ensures proper homolog segregation at the end of metaphase I. Errors in crossover designation and maturation can result in nondisjunction, leading to aneuploidy, germ cell death, birth defects, or infertility. Nondisjunction is disproportionately common to human oogenesis, but the factors underlying this unusually high rate of aneuploidy are poorly understood. Recent studies speculate that improper CO designation and maturation may be a cause. However, the majority of research on the mechanisms of mammalian CO formation has centered on spermatogenesis, leaving oocyte recombination dynamics largely understudied. Cyclin N-Terminal Domain Containing 1 (CNTD1) protein co-localizes with other essential CO proteins and is essential for designating sites for CO resolution in mouse spermatocytes. Spermatocytes lacking CNTD1 show normal prophase I progression, however they fail to accumulate the pro-CO heterodimer, MutL□, resulting in loss of >90% of chiasmata. Here, the mechanism of CNTD1 in CO designation and maturation will be explored in oocytes. Preliminary results recapitulate the lack of MutL□. Surprisingly, oocytes express the isoform of CNTD1, proposed to interact with CDK2, yet lack interstitial CDK2 at sites of COs. We will explore this exciting result and in doing so, we will highlight sex differences in CO designation and maturation, we may elucidate why oocytes are so error-prone.

Poster # 74

Harmful algal bloom toxin microcystins interfere with gonadotropin-dependent ovarian follicle maturation and ovulation to result in poor female reproduction

Tingjie Zhan[1], Yingzheng Wang[1,2], Pawat Pattarawat[1], Jiyang Zhang[1,3], Eunchong Kim[1], Delong Zhang[1], Saurabh Chatterjee[2], Ji-Yong Julie Kim[3], Geoffrey I. Scott[2], Qiang Zhang[4], and Shuo Xiao[1],*

[1]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University, Piscataway, NJ 08854;
[2]Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC 29208;
[3]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611;
[4]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322

Harmful algal blooms (HABs) are the excessive growth of cyanobacteria, which are referred to as "blue-green algae" in marine and freshwaters due to rising global temperatures. Humans are exposed to toxins released from HABs via contaminated drinking water and food. Microcystins (MCs) are the most common HAB toxins. It has been reported that women living in coastal regions with more HABs are at a higher risk of infertility and irregular menstrual cycles than women living inland, indicating the possible reproductive toxicity of HAB toxins. Here, we use an ex vivo 3D follicle culture system and in vivo mouse models to investigate ovarian disrupting effects of MCs. Immature mouse follicles were exposed to six common MC congeners (MC-LF, LY, LA, YR, RR, and LR) at 0.01-5 μM during follicle-stimulating hormone (FSH)-stimulated follicle maturation. MC-LF and MC-LY significantly inhibited follicle growth and estradiol secretion, and all six MCs concentration-dependently compromised follicle ovulation and reduced progesterone secretion. Mechanistic investigations revealed that MC-LR, the most common MC congener, significantly suppressed the expression of follicle maturation genes and inhibited protein phosphatase 1 (PP1) and associated PI3K/AKT/FOXO1 signaling in granulosa cells. We further used a mouse superovulation model to intraperitoneally treat mice with 10 μg/kg MC-LR during the follicle maturation window. MC-LR treated mice had significantly decreased ovulated oocytes and more unruptured follicles, confirming that MC-LR disrupts FSH-dependent follicle maturation to block ovulation. We then performed a more environmentally relevant exposure experiment by treating young adult female mice with 10 μg/kg MC-LR via daily oral gavage for 6 weeks. Compared to the control group, mice treated with MC-LR had 50% fewer corpus luteum, indicating defective follicle ovulation. Together, our study demonstrates that exposure to MCs, particularly MC-LR, interferes with follicle maturation by inhibiting the PP1/PI3K/AKT/FOXO1 signaling, which results in adverse female reproductive outcomes.

Poster # 75

Follicle-stimulating hormone at the threshold and excessive levels induces distinct ovarian responses and follicular transcriptomic profiling in an ex vivo murine folliculogenesis system

Ying Zhang[1], Tingjie Zhan[1], Eunchong Kim[1], Nataki Douglas[2], Qiang Zhang[3], and Shuo Xiao[1]

[1]Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Environmental and Occupational Health Sciences Institute (EOHSI), Rutgers University, Piscataway, NJ 08854;
[2]Department of Obstetrics, Gynecology and Women's Health, New Jersey Medical School, Rutgers University, Newark, NJ 07103;
[3]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322

In each human ovarian cycle, the rise of follicle-stimulating hormone (FSH) to a threshold level selects one follicle to become dominant and reach full maturation for ovulation. Ovarian stimulation in IVF clinics relies on supraphysiological levels of FSH to recruit multiple dominant follicles. Both insufficient and excessive FSH can cause poor female reproduction. While the concept of FSH threshold has been conceived for years, the underlying mechanisms remain elusive. Here, we used an encapsulated in vitro follicle growth (eVIFG) system to study effects of various concentrations of FSH on follicle maturation. Immature mouse follicles were cultured with 5 mIU/mL FSH for 4 days and then with 5, 10, 20, and 30 mIU/mL FSH from day 4 to 6. FSH at 10 mIU/mL began to increase secretion of estradiol (E2) but not progesterone (P4). RT-qPCR results showed that 10 mIU/mL FSH started to induce key follicle maturation genes, such as Cyp19a1, indicating that 10 mIU/mL FSH is at or slightly above the threshold to stimulate follicle maturation. FSH at 20/30 mIU/mL did not further promote E2 secretion nor Cyp19a1 expression; however, they significantly increased P4 and related steroidogenic genes of Star and Cyp11a1, recapitulating excessive FSH-induced premature luteinization. Follicles from day 4 of eVIFG and follicles treated with various concentrations of FSH on day 6 were collected for single-follicle RNA sequencing. Principal component analysis (PCA) separated follicles into distinct clusters, depending on FSH concentrations. Differentially expressed genes (DEGs) up-regulated by FSH were associated with Lipid metabolism, Steroidogenesis, Glycolysis. DEGs down-regulated by FSH were related to Cell cycle and DNA damage/repair. Together, our study demonstrates that FSH at threshold and excessive levels induce distinct ovarian responses and follicular transcriptomic profiling, which has multiple implications in studying ovarian biology, disease, and toxicology.

Poster # 76

Functions of H4K16ac and MOF in Bovine Embryogenesis

Jingzhi Zhang*, Chia-Kuan Wu*, Meihong Shi, Abdallah Abdelhady, Yoke Lee Lee, Soon Hon Cheong†, Ellie Duan†

Cornell University

Zygotic genome activation (ZGA) is an essential step in preparing embryos for cell differentiation and ensuring normal development. Many maternal factors are known to be involved in ZGA, such as pioneer transcription factors (TF) and histone modifiers. Males-Absent-On-the-First (MOF) is a known histone acetyltransferase of histone 4 Lysine 16 acetylation (H4K16ac), which plays a unique role in chromatin activation and promotes transcription. However, the mechanism by which MOF and H4K16ac establish euchromatin during bovine ZGA remains poorly understood. Further, it is unclear whether maternal H4K16ac can remain stably on embryonic genomes during reprogramming and if there is any biological significance underlying it. Here, we first examined the expression of Mof using online RNA-seq data (Graf et al., 2014) and validated the results via single embryo qPCR. Mof expression is highest in MII oocytes and gradually decreases after fertilization, indicating maternal inheritance. Mof expression was lowest at the 8-cell stage and increased from the 16-cell stage, consistent with zygotic transcription of Mof at bovine major ZGA (16-cell). Using Immunofluorescence (IF) staining, H4K16ac was found enriched in MII oocytes and zygotes. To test the indispensability of MOF, we are currently working on 2 treatments: siRNA and the MOF inhibitor MG149 to knock down or inhibit its function. For the siRNA strategy, MII oocytes were transfected for ~17 hrs and the knock-down efficiency was examined by qPCR of Mof mRNA. MG149 MOF inhibitor (10 nM) was supplemented to the zygotes culture medium after in vitro fertilization until they reached the blastocysts stage. The cleavage and developmental rates were similar between the MG149 group and the vehicle control group. Additional characterization of siRNA and MG149 treated embryos are underway. Overall, our results provide insights in the maternal origin of MOF and H4K16ac in bovine embryos, allowing continuing study of their functions during ZGA.

Poster # 77

CCAAT/enhancer binding proteins alpha and beta selectively regulate gene expression by temporal and dose-dependent mechanisms in preovulatory ovarian follicles.

Hanxue Zhang, Paul D. Soloway, Jimmy Dhillon, Bo Shui, Seoyeon Lee, Jennifer K Grenier, Paul R Munn, Rainer B Lanz, and Yi A Ren

Cornell University

CCAAT/enhancer binding proteins alpha and beta (C/EBPβ) are transcription factors induced by the preovulatory luteinizing hormone (LH) surge in ovarian granulosa cells. C/EBPα/β are indispensable for ovulation, but the underlying molecular mechanism is unclear. To address this knowledge gap, we compared two genetically modified mouse models in which the expression of Cebpa/b is manipulated to different levels at different time in granulosa cells by CRE-mediated recombination (Cyp19a1-Cre vs. Pgr-Cre). Super-ovulation of immature female mice was used with human chorionic gonadotropin (hCG) as the biological equivalent of the LH surge. Consistent with published data, Cyp19a1-Cre lead to blocked ovulation and undetectable C/EBPa/β levels after hCG. We found that Pgr-Cre lead to a 40% reduction of ovulation and a normal level of C/EBPa/β until at least 4h post-hCG/LH; interestingly, while the expression C/EBPβ doubled between 4-8h post-hCG/LH in wild-type mice, it stayed constant in mutants. These results indicate that C/EBPα/β in the first four hours post-hCG/LH are not sufficient and a further increase in C/EBPβ expression is required for normal ovulation. To investigate how different dosages and timing of action of C/EBPα/β regulate ovulation, we performed bulk RNA sequencing using granulosa cells from controls and the two mutants at a series of time points post-hCG/LH. We found that at early preovulatory stage, C/EBPα/β induced the expression of genes regulating extracellular matrix remodeling, epigenetic changes, and cell adhesion; surprisingly, increased expression of C/EBPβ between 4h-8h was required to inhibit the expression of genes of these same functional categories. In contrast, C/EBPα/β regulated vascular and inflammatory genes primarily via a dose-dependent mechanism. We further applied single-nuclear ATAC sequencing, which underscored transcription factors interacting with C/EBPα/β to regulate specific biological processes. Taken together, these findings reveal novel mechanisms of gene regulation by C/EBPα/β that are specific to the stage of ovulation induction and gene function.

Poster # 78

Impacts of Early Life Social Experiences on Oxytocin Receptors in the Brain

Susanna Zheng, George Prounis, Alexander G. Ophir

Cornell University

Early life social experiences have a profound impact on the development of the brain and behavior. Previous work has shown that prairie voles (Microtus ochrogaster) raised in a socially limited environment engaged in more social behaviors compared to those raised in a socially enriched environment. Furthermore, intranasal oxytocin has been therapeutically used in children, yet we know very little about how this might affect brain development. Oxytocin is a crucial neuropeptide involved in social behavior and parental care, and changes in early parental care has the potential to alter oxytocin receptors (OTR) throughout the brain. However, few studies have asked how different social experiences interact to alter brain phenotype. In this study, prairie voles were raised in either a socially limited condition (raised by a single mother and isolated after weaning) or raised in a socially enriched condition (raised biparentally and group housed after weaning). Subjects in each condition were also given either intranasal oxytocin or saline (PND 21-42). Finally, brains were collected, and we used autoradiography to quantify OTR throughout the brain. Results showed socially limited voles had significantly more OTR in the prefrontal cortex compared to socially enriched voles. Socially limited voles that were also given intranasal oxytocin had significantly more OTR in the anterior insular cortex than the rest of the groups. Finally, socially enriched voles that were given intranasal oxytocin had more OTR in the lateral septum compared to social enriched voles that were given saline. These results suggest that oxytocin receptors are generally robust and insensitive to early life experiences. However, three key brain regions (the prefrontal cortex, lateral septum, and anterior insular cortex) showed sensitivity to different aspects of social experience or intervention.

Poster # 79

Well-established nuisance compounds in the drug screening pipeline elicit distinct phenotypes during mouse in vitro oocyte maturation

Yiru Zhu, Caroline Kratka, Hoi Chang Lee, Jeffrey Pea, Richard Nelson, Francesca E. Duncan

Northwestern University

The rupture of the ovarian follicle at ovulation is essential to release the cumulus-oocyte complex (COC), making this biological process a target for the development of novel non-hormonal contraceptives. However, oocyte meiotic progression and cumulus cell layer expansion also occur at ovulation and could be targeted for contraceptive purposes. To this end, we recently established quantitative morphokinetic parameters of meiotic progression and cumulus expansion in mice using a closed time-lapse incubator (EmbryoScope+™) to enable screening compounds that disrupt this process. In the drug screening pipeline, nuisance compounds that have undesirable mechanisms of bioactivity can interfere with cellular assays. Therefore, the goal of this study was to fully characterize the impact of six well-established nuisance compounds on our complex phenotypic assay. We selected cycloheximide (CHX), digitonin, BVT-948 (BVT), rottlerin, NSC-663284 (NSC), and doxorubicin based on their specific mechanism of action as nuisance compounds. For this study, COCs were collected from gonadotropin-stimulated mice and either used directly or denuded oocytes were stripped from the COCs. Denuded oocytes or intact COCs were then matured in vitro in the presence of a nuisance compound (10μM) or vehicle alone (DMSO). Time-lapse images taken every 10 minutes across the time course of maturation were analyzed, and 24 total oocytes were evaluated per compound over three replicates. In vehicle-treated oocytes, >95% (23/24) were able to mature as evidenced by first polar body extrusion (PBE), and the average time to germinal vesicle breakdown (GVBD), time to PBE, and duration of meiosis I were 0.97± 0.04, 8.96 ± 0.16, and 7.99 ± 0.15 h, respectively. None of the oocytes matured in the nuisance compounds underwent PBE. Digitonin and rottlerin caused oocyte degeneration early (0.49± 0.02 h) and late (11.98 ± 0.33 h) during the maturation period, respectively. The remaining four compounds supported GVBD, and while CHX-treated oocytes had normal cellular morphology, the other compounds exhibited unique cytoplasmic features. COCs treated with BVT, NSC, CHX, and doxorubicin had compromised cumulus expansion compared with controls. Overall, integrating knowledge of how our phenotypic assays respond to nuisance compounds will enable a more robust hit triage during screening of potential non-hormonal female contraceptive candidates. This study was funded by the Bill & Melinda Gates Foundation (INV-003385).

Thank you for joining us in beautiful Ithaca, NY for the 2023 Tri-Repro Symposium!

See you in Pittsburgh in 2024!

University of Pittsburgh

Cornell University

Penn
University of Pennsylvania

MAGEE-WOMENS
RESEARCH INSTITUTE

Cornell Reproductive
Sciences Center