UMSRS

UPPER MIDWEST SUMMIT FOR REPRODUCTIVE SCIENCE

OCTOBER 14, 2024

IHOTEL & ILLINOIS CONFERENCE CENTER
1900 S 1ST ST, CHAMPAIGN, IL 61820

SYMPOSIUM OVERVIEW
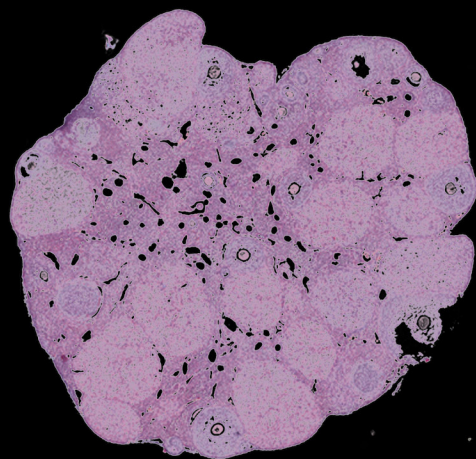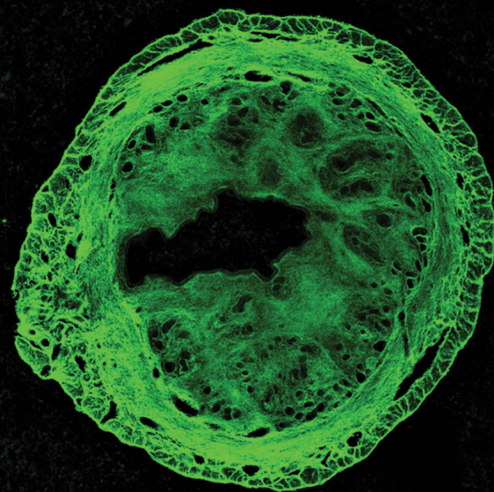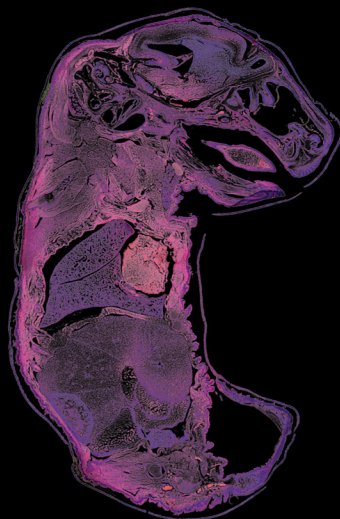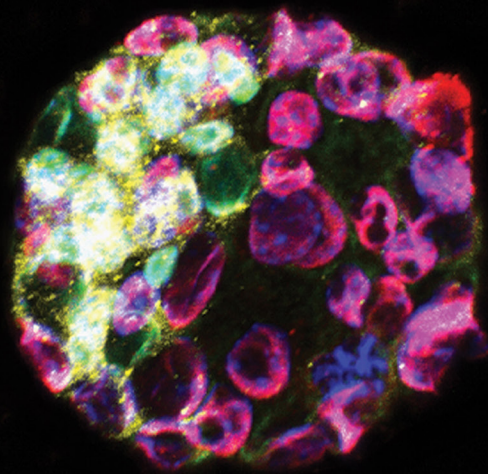
7:00 – 8:00 am	Registration and Continental Breakfast
8:00 – 8:15 am	Welcome and Opening Remarks
8:15 – 8:40 am	Alumnus Speaker: Irene O. Aninye, Ph.D.
8:40 – 9:25 am	**Oral Session I (3 talks)**
9:25 – 9:50 am	Alumnus Speaker: Ye Yuan, Ph.D.
9:50 – 10:10 am	Break
10:10 – 11:10 am	A.V. Nalbandov Memorial Lecture: Francesco J. DeMayo, Ph.D.
11:10 – 11:55 am	**Oral Session II (3 talks)**
11:55 – 1:00 pm	Lunch
1:00 – 1:25 pm	Alumnus Speaker: Jaeyon Kim, Ph.D.
1:25 – 2:10 pm	**Oral Session III (3 talks)**
2:10 – 2:55 pm	Coffee Reception and Poster Presentations I (odd numbered posters)
2:55 – 3:45pm	Coffee Reception and Poster Presentations II (even numbered posters)
3:45 – 4:10 pm	Alumnus Speaker: Genoa Warner, Ph.D.
4:10 – 5:10 pm	Janice Bahr lecture: Carmen J. William, M.D. Ph.D.
5:10 – 5:25 pm	Student Awards
5:25 – 5:30pm	Closing Remarks

UPPER MIDWEST SUMMIT FOR REPRODUCTIVE SCIENCE

Alma welcomes you...to the Upper Midwest Summit for Reproductive Science (UMSRS)-2024 at the University of Illinois at Urbana-Champaign. What once was known as the Minisymposium, which was conceived by Northwestern University, has evolved into a rotating, statewide annual symposium that continues to provide an opportunity to celebrate the strong heritage in the reproductive sciences research of Illinois (and neighboring states). The main goals of the UMSRS are to foster the exchange of scientific information in the reproductive sciences, to facilitate the career development of the next generation of reproductive scientists in Illinois (and surrounding states), and to establish a promising future for reproductive sciences research in the state of Illinois (and the Midwest).

The UMSRS also pays tribute to pioneer 'giants', such as Drs. Andy Nalbandov, Neena Schwartz, and Jack Gorski who have pushed Illinois to the forefront of reproductive sciences, and a new generation of scientists has continued this legacy by succeeding in the field of reproductive sciences. Many also serve as leaders in key professional societies such as the Society for the Study of Reproduction, the Endocrine Society, the Society for Gynecologic Investigation, and the American Society for Reproductive Medicine. ISRS provides an opportunity to recognize and celebrate the accomplishments of these scientists and the development of their trainees.

The student organizing committee includes trainee representatives from the University of Illinois at Urbana-Champaign, University of Illinois at Chicago, Northwestern University, Purdue University and DePaul University. The trainees worked together to organize the meeting, which highlights the outstanding research being done in Illinois and other institutions in the Midwest. We look forward to a great event! Once again, welcome to the University of Illinois at Urbana-Champaign!

The University of Illinois reproductive biology program has a large, internationally recognized group of faculties whose work focuses on various aspects of reproductive biology, early embryonic development, and endocrine physiology. Many of our laboratories investigate basic molecular and cellular mechanisms as well as carry out translational studies using whole animal and in vitro models. We are quite diverse in our research interests and are located in various departments on the campus of the University of Illinois. Many of the faculty are also members of thematic programs on campus such as the NIH Reproductive and Developmental Toxicology Training Grant, the Neuroscience Program, and the Division of Nutritional Sciences.

The University of Illinois is proud to present UMSRS-2024, which features Dr. Francesco J. DeMayo as the A.V. Nalbandov Memorial keynote speaker and Dr. Carmen J. Williams who will deliver the Dr. Janice Bahr Lecture, as well as oral and poster presentations by graduate students and postdoctoral fellows.

A.V. NALBANDOV MEMORIAL LECTURESHIP

Professor Andrew Vladimir Nalbandov (1912-1986) was internationally recognized in the field of reproduction and endocrinology and served the University of Illinois from 1940-1977. "Andy", as he was known to friends and colleagues, obtained an M.S. at Oklahoma State University and a Ph.D. at the University of Wisconsin. In 1940, Andy took his first academic position at the University of Illinois in the Department of Animal Science as an instructor of Genetics. At the time of his retirement, he was a professor of Animal Physiology, Physiology, and Zoology.

The contributions of Andy to the scientific community and to the field of reproductive biology are numerous. Andy did extensive work investigating the causes of sterility and embryonic mortality in pigs, the factors that influenced estrous cycles in sheep, and the mechanisms that controlled the induction of ovulation in cattle, sheep, pigs, rabbits, and poultry. Perhaps one of Andy's greatest contributions to the field of reproduction was to help found the Society for the Study of Reproduction, where he also served as its President. These contributions justly earned him leadership positions in the scientific community and numerous awards. Among them were the D.Sc. honoris causa from the Technical University of Munich, the Marshall Medal from the Society of Fertility in Great Britain, the Distinguished Leadership Award in Endocrinology from the Endocrine Society, and the Hartman Award from the Society for the Study of Reproduction.

To honor Andy's legacy, friends and colleagues established the "A.V. Nalbandov Memorial Lectureship", which provides funding for an annual lecture by a distinguished scientist in the field of reproductive sciences. This year, Francesco J. DeMayo, Ph.D., Chief and Senior Principal Investigator at the Reproductive and Developmental Biology Laboratory in the National Institute of Environmental Health Sciences will deliver the keynote address.

A.V. NALBANDOV LECTURESHIP RECIPIENT IN REPRODUCTIVE SCIENCE


Francesco J. DeMayo, Ph.D.

Chief and Senior Principal Investigator, Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences

Dr. DeMayo is a Chief and Senior Principal Investigator of the Reproductive and Developmental Biology Laboratory at the National Institute of Environmental Health Sciences. He is also an Adjunct Professor in the Department of Molecular and Cellular Biology at Baylor College of Medicine. Dr. DeMayo received his B.S. in General Studies at Cornell University and his M.S. and Ph.D. in Physiology at Michigan State University. He completed postdoctoral training at Baylor College of Medicine and continued to the rank of Cullen-Duncan-McAshan Endowed Chair in Cancer Research, and Professor of Molecular and Cellular Biology and Pediatrics.

Dr. DeMayo is a distinguished scientist renowned for his contributions in the field of reproductive biology and endocrinology. Utilizing genetically engineered mouse models, human samples, and advanced techniques, his research has focused on determining the molecular mechanisms regulating uterine receptivity and function. Dr. DeMayo's research has identified the paracrine signaling pathways that regulate uterine epithelial-stroma crosstalk in controlling uterine epithelial cell proliferation and receptivity for embryo attachment. Further findings include determining the role of nuclear receptors and transcription factors governing differentiation of human endometrial stroma cells during pregnancy, the role of progesterone in the regulation of myometrial function, along with discoveries related to reproductive-related diseases and how environmental exposures may impact reproductive function.

Throughout his impressive career, Dr, DeMayo received numerous awards and excelled in his academic service. To name a few, at Baylor College he was honored with Gordon Cain Professorship in the Department of Molecular and Cellular Biology, the Dan L. Duncan Professorship in Dan L. Duncan Cancer Center, the Cullen-Duncan-McAshan Endowed Chair in Cancer Research in the Department of Molecular and Cellular Biology, and the Michael E DeBakey Research Award. Dr. DeMayo also served as an executive board member and president of the Society for the Study of Reproduction (SSR). Recently, he was recognized as an SSR Distinguished Fellow and received the Carl G. Hartman Award. Notably, Dr. DeMayo co-founded the Mammalian Reproduction
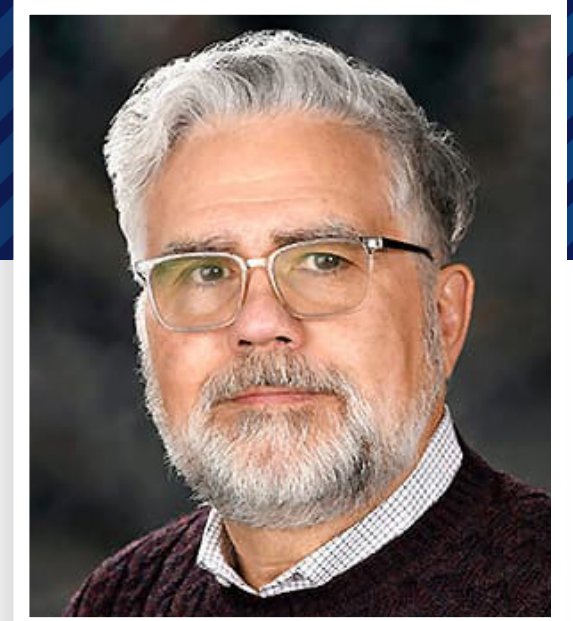
Gordon Research Conference. Throughout his career, he has mentored many undergraduate and graduate students, and postdoctoral fellows. He has served on various journal editorial boards such as Molecular Endocrinology, Biology of Reproduction, and NIH review panels.

For his outstanding research and seminal contributions to the field of Reproductive Sciences we are excited to have Dr. DeMayo as the A.V. Nalbandov Memorial Lectureship Awardee.

DR. JANICE BAHR LECTURESHIP

Dr. Janice Bahr is Professor Emerita of Physiology, Dept. of Animal Sciences and Molecular and Integrative Physiology. She received a B.A. from Viterbo College, La Crosse, WI, a M.S. and a Ph.D. from the University of Illinois. After completing her Ph.D., she was appointed as an Assistant Professor in the Department of Animal Sciences with cross-appointment in Molecular and Integrative Physiology in 1974, and she was promoted to Full Professor in 1983. She was an Associative Vice Chancellor for Research from 1996-2002. Dr. Bahr established a widely recognized research program in reproductive endocrinology with specific emphasis in ovarian function and secondary emphasis in male reproduction. Her research group consisted of numerous outstanding graduate students and postdoctoral fellows who were attracted to Dr. Bahr's research group because she gave students the freedom to propose the research question they wanted to investigate and select the appropriate animal model ranging from fish to mammals. Dr. Bahr was specifically recognized for her outstanding mentoring of graduate students. She received awards from the Department of Animal Sciences, College of ACES, and the University of Illinois. She was the first recipient of the SSR Trainee Mentor Award in 2007. Dr. Bahr was the first woman recipient of the prestigious L.E.Casida Award in 2017, a national award given by the Association of Animal Sciences and SSR. She was very involved in SSR for which she received the SSR Distinguished Award in 1997. She was President of SSR from 1993-1994. Dr. Bahr and her research group were active participants in the University of Illinois, Urbana-Champaign Reproductive Physiology Program, established over 60 years ago. To support this program, Dr. Bahr made a significant financial donation to the University of Illinois, Urbana-Champaign Foundation and established the Janice M. Bahr Reproductive Biology Lectureship.

JANICE BAHR REPRODUCTIVE BIOLOGY LECTURE


Carmen J. Williams, M.D., Ph.D.

Senior Investigator, Reproductive Medicine Group, Reproductive & Developmental Biology Laboratory, National Institute of Environmental Health Sciences

Carmen J. Williams, MD, PhD, is a highly distinguished figure in reproductive medicine and developmental biology, with an illustrious career that spans over three decades. As the Senior Investigator of the Reproductive Medicine Group at the National Institute of Environmental Health Sciences (NIEHS), Dr. Williams has significantly advanced our understanding of the intricate biological processes involved in fertilization and early mammalian development. She also serves as the Deputy Chief of the Reproductive & Developmental Biology Laboratory, leading critical research initiatives focused on the environmental influences on reproductive health.

Dr. Williams has earned widespread recognition for her pioneering research and mentorship. In 2022, she was elected as a Fellow of the American Association for the Advancement of Science (AAAS), a prestigious honor awarded for her distinguished contributions to reproductive and developmental biology. Her election to AAAS is a testament to her groundbreaking work in elucidating mechanisms underlying fertilization, polyspermy block, and early embryo development.

Dr. Williams was awarded the National Institutes of Health (NIH) Director's Ruth L. Kirschstein Mentoring Award in 2023, recognizing her exceptional commitment to mentoring early-career scientists and fostering diversity in biomedical research. That same year, she was honored as the NIEHS Mentor of the Year, a reflection of her dedication to nurturing the next generation of scientific leaders.

Dr. Williams' influence extends beyond her laboratory work. She has been a sought-after keynote speaker at prestigious conferences and academic institutions globally. Her prominent lectures include the Roy Hertz Memorial Lecture at the NIH C. Everett Koop Memorial Symposium on Women's Health in 2014, and the Stuart Moss Memorial Lecture at the Tri-Repro Reproductive Biology Meeting at the University of Pennsylvania in 2022. In 2024, she was invited for the Epigenetics Section Seminar Series, Babraham Institute, Cambridge, UK, and at the 29th Annual Texas Forum for Reproductive Sciences, UT Southwestern Medical Center, Dallas, TX, further cementing her role as a thought leader in the field.
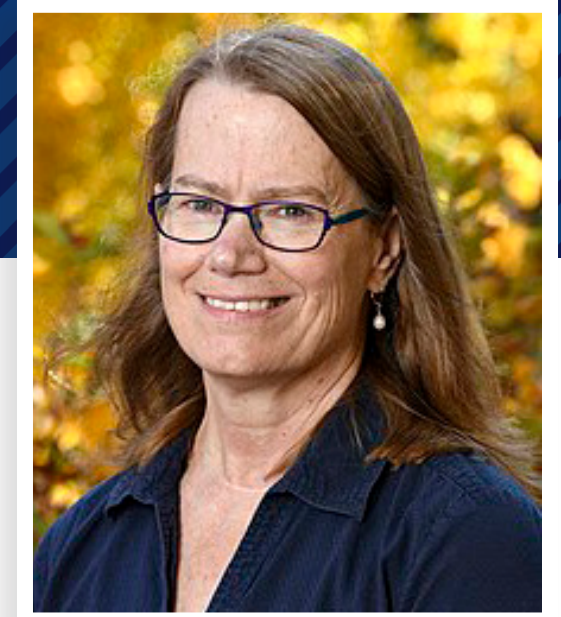
Dr. Williams has also demonstrated leadership in her field through her editorial and advisory roles. She is currently the Chair of the Scientific Advisory Board for the Frontiers in Reproduction course at the Marine Biological Laboratories, and serves on multiple NIH committees, including the NIH Women Scientists Advisors. Her editorial contributions include serving as an Associate Editor for Biology of Reproduction and PLOS Biology.

Among her numerous accomplishments, Dr. Williams holds several patents, including one for the use of epithelial membrane protein 2 (EMP2) in treating lung disorders, showcasing her interdisciplinary impact. She has also co-authored a vast number of influential peer-reviewed publications, many of which have been selected as NIEHS "Papers of the Month," further highlighting her exceptional research output and impact.

With her unparalleled expertise, extensive contributions to reproductive biology, and dedication to mentoring future scientists, Dr. Carmen J. Williams stands as an exemplary keynote speaker, offering invaluable insights into the future of reproductive and developmental biology research. Because of her accomplishments and contributions to the field of Reproductive Sciences, Dr. Carmen J. Williams will deliver the Keynote Lecture at the 11th Annual Upper Midwest Summit for Reproductive Science, and she will receive the Janice M. Bahr Reproductive Biology Lectureship Award.

Irene O. Aninye, Ph.D.

Chief Science Officer at Society for Women's Health Research

Ph.D. advisor: Dr. David J. Shapiro

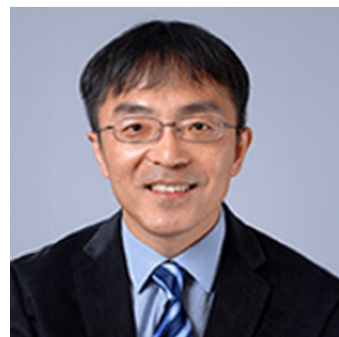**"*From Bench to Boardroom: A Journey through Science, Equity, and Leadership*"**

Ye Yuan, Ph.D.

Research Director, Colorado Center for Reproductive Medicine

Ph.D. advisor: Drs. Rebecca Krisher and Matthew Wheeler

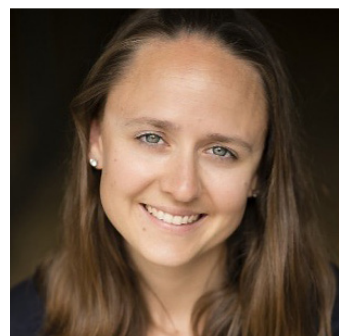**"*Translational Research in human ART*"**

Jaeyon Kim, DVM, Ph.D.

Associate Professor of Biochemistry and Molecular Biology at Indiana University School of Medicine

Ph.D. advisor: Dr. Milan K. Bagchi

"*Lessons from a former trainee*"

Genoa Warner, Ph.D.

Assistant Professor of Chemistry and Environmental Science at New Jersey Institute of Technology

Postdoctoral advisor: Dr. Jodi A. Flaws

"*Safer chemical design: avoiding endocrine disruption*"

7:00 AM **REGISTRATION AND CONTINENTAL BREAKFAST**

8:00 AM **WELCOMING REMARKS: MILAN K. BAGCHI, PH.D.**
Deborah Paul Endowed Professor and Director, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign

8:15 AM **UNIVERSITY OF ILLINOIS ALUMNI SPEAKER: IRENE O. ANINYE, PH.D.**
Introduced by: ***Kavyashree (Kavya) Basavaraju***, *University of Illinois at Champaign-Urbana*

Title: "*From Bench to Boardroom: A Journey through Science, Equity, and Leadership*"

ORAL SESSION 1: OVARIAN AND UTERINE DEVELOPMENT
Session Moderators: **Sameeksha Bhaye**, University of Illinois at Champaign-Urbana and **Shams Mowafak Saad**, Purdue University

8:40 AM **T1 A COMPREHENSIVE GENE RANKING STRATEGY FOR PRIORITIZING OVARIAN TARGETS FOR NON-HORMONAL CONTRACEPTIVE DRUG DISCOVERY FROM TRANSCRIPTOMIC DATASETS**

Jeffrey Pea [1*], *Ruixu Huang* [2*], *Rutuja Phatate* [2], *Olha Kholod* [2], *Zhanel Nugmanova* [2], *Caroline E. Kratka* [1], *Emily J. Zaniker* [1], *Yiru Zhu* [1], *Anna E. Heinrich* [1], *Jiyang Zhang* [3], *Hoi Chang Lee* [1], *Shuo Xiao* [3], *Brittany A. Goods* [2†], *Francesca E. Duncan* [1†]

[1] *Northwestern University,* [2] *Dartmouth College, and* [3] *Rutgers University*

8:55 AM **T2 INFLUENCE OF GUT MICROBIOME AND DI-ISONONYL PHTHALATE (DINP) EXPOSURE ON UTERINE DEVELOPMENT IN MICE**

Sarah Ibrahim, Jacqui Ngyuen, Vanessa Salgado, Shah Tauseef Bashir, Karen Chiu, Jodi A. Flaws, Romana A. Nowak

University of Illinois at Urbana-Champaign

9:10 AM   T3 **OVOPATH: A COMMUNITY-SUPPORTED MACHINE-LEARNING ALGORITHM TO ENHANCE OVARIAN FOLLICLE STAGING AND IMPROVE FOLLICLE HEALTH CLASSIFICATIONS**

Hannah McDowell [1], *Sofia Granados Aparici* [2], *Isaac Vieco Martí* [2], *Rosa Noguera* [2], *Monica M. Laronda* [1]

[1] *Northwestern University and* [2] *University of Valencia-INCLIVA, Valencia*

9:25 AM   **UNIVERSITY OF ILLINOIS ALUMNI SPEAKER: YE YUAN, PH.D.**
Introduced by: **Kavyashree (Kavya) Basavaraju**, *University of Illinois at Urbana-Champaign*

Title: *"Translational Research in human ART"*

9:50 AM   **REFRESHMENT BREAK**

10:10 AM   **A.V. NALBANDOV LECTURESHIP IN REPRODUCTIVE SCIENCE AND UMSRS KEYNOTE SPEAKER: FRANCESCO J. DEMAYO, PH.D.**
Introduced by: **Sameeksha Bhaye**, *University of Illinois at Urbana-Champaign*

Title: *"Understanding the Role and Regulation of the Progesterone Receptor in Pregnancy"*

ORAL SESSION 2: SPERM AND FERTILITY
Session Moderators: **Janavi Thayagraj**, *Northwestern University* and **Jacob Beal**, *University of Illinois at Urbana-Champaign*

11:10 AM   T4 **MODULATION OF SPERM FUNCTION BY BACTERIAL VAGINOSIS (BV) TOXINS: LIPOPOLYSACCHARIDE AND VAGINOLYSIN**

Shweta Bhagwat [1], *Juan Ferreira* [1], *Ronald McCarthy* [1], *Leila Asadi* [1], *Ping Li* [1], *Ariana Gaydon* [1], *Alexander Zhang* [1], *Christy Armstrong* [2], *Andrea Verhagen* [2], *Sydney Morrill* [2], *Vaka Reddy* [1], *Lloyd Robinson* [1], *Warren G. Lewis* [2], *Amanda L. Lewis* [2], *Celia M. Santi* [1]*

[1] *Washington University in Saint Louis and* [2] *University of California San Diego*

11:25 AM   T5 **KNOCKOUT OF PKDREJ, A CANDIDATE OVIDUCT RECEPTOR ON SPERM, RESULTS IN MALE INFERTILITY**

Kankanit Doungkamchan [1], *Leonardo Molina* [1], *Caroline Lucas* [2], *Kadirvel Govindasamy* [1], *Kevin Wells* [2], *Kristin Whitworth* [2], *Randall Prather* [2], *and David Miller* [1]

[1] *University of Illinois at Urbana-Champaign and* [2] *University of Missouri at Columbia, Columbia*

11:40 AM   T6 **GUT MICROBIOTA CHANGES RAPIDLY IN RESPONSE TO SUPPRESSION OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS AND DIRECTLY IMPACTS REPRODUCTIVE PARAMETERS**

Anna Clapp Organski [1], *Anjali Reddivari* [2] *Joan S. Jorgensen* [3], *William G. Schrage* [3], *J Alex Pasternak* [1], *Tzu-Wen L. Cross* [1]

[1] *Purdue University,* [2] *Northwestern University,* [3] *University of Wisconsin Madison*

11:55 PM   **LUNCH BREAK**

1:00 PM   **UNIVERSITY OF ILLINOIS ALUMNI SPEAKER: JAEYON KIM, PH.D.**
Introduced by: **Jacob Beal**, *University of Illinois at Urbana-Champaign*

Title: *"Lessons from a former trainee"*

ORAL SESSION 3: PLACENTAL AND ENDOMETRIAL BIOLOGY
Session Moderators: **Cary Brandolino**, *DePaul University* and **Ritwik (Rick) Shukla**, *University of Illinois at Urbana-Champaign*

1:25 PM   T7 **HUMAN EXTRA-EMBRYONIC TROPHOBLAST CELLS SECRETE EXTRACELLULAR VESICLES TO COMMUNICATE WITH MATERNAL CELLS DURING PLACENTA DEVELOPMENT**

Xiangning Song, Jacob R. Beal, Indrani C. Bagchi, and Milan K. Bagchi

University of Illinois at Urbana-Champaign

1:40 PM   T8 **DECIDUALIZATION ALTERS THE MICROSTRUCTURE AND MECHANICS OF THE MURINE ENDOMETRIUM**

C.S. Bastías [1], K. Sandoval [1], S. Bhaye [1], S. Chen [1], D.O. Duele [2], Y. Kulkarni [1], M. Dean [1], C.M. Luetkemeyer [1]

[1] University of Illinois at Urbana-Champaign and [2] University of Colorado Boulder

1:55 PM   T9 **PROGESTERONE-RESPONSIVE UTERINE TISSUE-RESIDENT NATURAL KILLER CELLS**

Bruna K. Tatematsu and Dorothy K. Sojka

Loyola University at Chicago

2:10 PM   **COFFEE RECEPTION AND POSTER SESSION I** (Odd Numbered Abstracts)

2:55 PM   **COFFEE RECEPTION AND POSTER SESSION II** (Even Numbered Abstracts)

3:45 PM   **UNIVERSITY OF ILLINOIS ALUMNI SPEAKER: GENOA WARNER, PH.D.**
Introduced by: **Jacob Beal**, *University of Illinois at Urbana-Champaign*

Title: *"Safer chemical design: avoiding endocrine disruption"*

4:10 PM   **JANICE BAHR REPRODUCTIVE BIOLOGY LECTURESHIP AND UMSRS KEYNOTE SPEAKER: CARMEN J. WILLIAM, M.D. PH.D.**
Introduced by: **Ritwik (Rick) Shukla**, *University of Illinois at Urbana-Champaign*

Title: *"Diverted trajectories: Developmental estrogen exposure alters adult cell fate and function"*

5:10 PM   **AWARDS FOR POSTERS AND ORAL PRESENTATIONS**

5:25 PM   **CLOSING REMARKS: ARPITA S. BHURKE, PH.D.**
Post-Doctoral Fellow at the Institute of Genomic Biology, University of Illinois at Urbana-Champaign

ORAL SESSION 1:
Ovarian and Uterine Development

T1: **A COMPREHENSIVE GENE RANKING STRATEGY FOR PRIORITIZING OVARIAN TARGETS FOR NON-HORMONAL CONTRACEPTIVE DRUG DISCOVERY FROM TRANSCRIPTOMIC DATASETS**

Jeffrey Pea[1]*, Ruixu Huang[2]*, Rutuja Phatate[2], Olha Kholod[2], Zhanel Nugmanova[2], Caroline E. Kratka[1], Emily J. Zaniker[1], Yiru Zhu[1], Anna E. Heinrich[1], Jiyang Zhang[3], Hoi Chang Lee[1], Shuo Xiao[3], Brittany A. Goods[2†], Francesca E. Duncan[1†]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[2] Thayer School of Engineering, Dartmouth College, Hanover, NH, USA
[3] Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, USA

* Co-first author, † Co-corresponding author

Despite the prevalent use of hormonal contraception globally, they are not ideal for many women due to substantial side effects, including nausea and migraines with aura, with up to 40% women discontinuing their use within the first year. Consequently, it remains critical to develop novel targets for non-hormonal contraception. In the era of omics technologies, numerous transcriptomic datasets have been generated which provide a detailed molecular perspective of ovarian biology, including follicle development and ovulation. However, there is currently no pipeline tailored to leverage these datasets for non-hormonal contraceptive discovery, which requires high safety thresholds to prevent detrimental fertility and off-target outcomes. Therefore, we developed a comprehensive ranking strategy that integrates reproductive, safety, and translational considerations in the prioritization of gene targets for non-hormonal contraception from existing transcriptomic datasets. In our two-step approach, we first generated a scoring matrix combining fields systematically collected across various resources, including human homology (Ensembl), mouse tissue specificity (CITDBase), temporal expression patterns, and safety considerations in mice (MGI). This matrix also incorporated a gene flaglist for processes undesirable for targeting, including development and upstream hormone regulation. In our second step, datasets were reviewed manually against documented reproductive phenotypes, safety considerations in humans, and human ovarian cell specificity. We applied this strategy to three recently published transcriptomic datasets focused on the ovulation time course using mouse ex vivo and in vivo systems. From 47,667 genes, this strategy resulted in an initial list of 142 genes (following scoring matrix) leading to a shortlist of 21 genes (following manual review). Top targets with high composite scores and low safety concerns, such as *Zpld1* and *Tchhl1*, are being used for functional analysis using an *in vivo* AAV system. Taken together, our integrated scoring strategy represents an advancement in computational approaches to apply specifically for women's health and contraceptive discovery.

T2: **INFLUENCE OF GUT MICROBIOME AND DI-ISONONYL PHTHALATE (DINP) EXPOSURE ON UTERINE DEVELOPMENT IN MICE**

Sarah Ibrahim[1], Jacqui Ngyuen[2], Vanessa Salgado[1], Shah Tauseef Bashir[1], Karen Chiu[3], Jodi A. Flaws[3], Romana A. Nowak[1]

[1]Department of Animal Sciences, College of Animal Consumer and Environmental Sciences; [2]Department of Molecular and Cellular Biology, College of Liberal Arts and Sciences; [3]Department of Comparative Biosciences, College of Veterinary Medicine at the University of Illinois Urbana-Champaign (UIUC), Urbana, USA

Diisononyl phthalate (DiNP) is a high-molecular weight phthalate used to make plastics more flexible and durable. DiNP usage is increasing as it is replacing another similar phthalate, di(2-ethylhexyl) phthalate (DEHP). However, DiNP exposure has been shown to have negative effects on female reproductive health due to its endocrine disrupting chemical properties. Ingestion is the most common route of DiNP exposure, making the gastrointestinal tract and its metabolizing and hormone secreting gut-microbiome directly exposed to DiNP. The gut-microbiome has been shown to influence female reproductive health and conditions. This study examined the effect of absence and presence of a gut microbiome on uterine development as well as the effects of acute DiNP exposure either in the absence or presence of a gut microbiome on uterine development. Over a 3-day period, 18 female C57Bl/6 germ-free (-microbiome) one-month old mice were orally dosed with either sterile PBS (n=8) to remain germ free (-microbiome) or dosed with colon contents (n=10) to develop a gut-microbiome (+microbiome). This was followed by a 10-day period where half of the -microbiome and +microbiome mice were orally dosed with corn oil and half were orally dosed with 200 μg/kg/day of DiNP. Mice used as a control group were specific pathogen free (SPF) (born with microbiome). Mice were euthanized in diestrus. Uteri were collected, processed, and fixed in paraffin for histological analyses. Tissue sections were stained with hematoxylin and eosin for morphological measurements. Immunohistochemistry was done for specific markers: estrogen receptor alpha (ESR-1), FOXA2 (uterine gland marker), Ki67 (proliferation), and alpha smooth muscle actin (smooth muscle cells). Slides were scanned and imaged using NDP.scan 3.2.15 software and Hamamatsu NanoZoomer 2.0 HT. Statistical analyses were performed using GraphPad Prism 9.4.0 and significance levels were set at $p<0.05$. Uterine development appeared to be markedly delayed by the initial absence of a gut-microbiome in all germ-free mice. In comparison to the control specific pathogen free mice, there was a significant reduction in uterine size, stroma area, and numbers of uterine glands in all the treatment groups. Analyses of myometrium thickness showed that the two myometrial layers were substantially underdeveloped in all of the germ-free mice, regardless of whether a microbiome was established subsequently in the treatment protocol. Proliferation of endometrial stromal cells was reduced in the germ-free mice. Notably, the number of uterine glands was greatly reduced in all the germ-free mice groups though ESR-1 and FOXA2 expression appeared normal. DiNP treatment had no significant effects on these parameters. Peripheral estradiol levels did not differ between groups and the ovaries had similar numbers of follicles at the different stages of development. These results reveal that the initial absence of the gut-microbiome has a crucial effect on uterine development, resulting in smaller uterine size, a thinner, underdeveloped myometrium, and many fewer uterine glands. Whether establishing a microbiome in germ free mice at an earlier age might overcome the initial negative impact of no microbiome deserve further investigation. Although DiNP exposure had no effect in either the presence or absence of a gut-microbiome, this suggests examining alternative pathways that DiNP exposure can affect uterine development.

T3: **OVOPATH: A COMMUNITY-SUPPORTED MACHINE-LEARNING ALGORITHM TO ENHANCE OVARIAN FOLLICLE STAGING AND IMPROVE FOLLICLE HEALTH CLASSIFICATIONS**

Hannah McDowell[1,2], Sofia Granados Aparici[4,5], Isaac Vieco Martí[4,5], Rosa Noguera[4,5], Monica M. Laronda[1,2,3]

[1] **Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois**
[2] **Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, Illinois**
[3] **Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois**
[4] **Cancer CIBER (CIBERONC), Madrid**
[5] **Pathology Department, Medical School, University of Valencia-INCLIVA, Valencia**

The ovarian follicle is made up of supporting granulosa (GCs) and theca cells (TCs) encompassing an oocyte. Ovarian follicles develop through a series of growth stages that correspond with oocyte growth and morphological changes of surrounding GC and TCs. Growth phases have historically included primordial follicle (PMF), primary, secondary, and antral founded on histological sections. However, follicles transitioning from primordial to primary stages are not currently classified even though once activated, PMFs display key morphological changes. This is primarily due to the lack of standardized methods for classifying, counting, and evaluating follicles in ovarian sections. Here we have developed a semi-automated pipeline of analysis to classify and assess follicle features based on key morphological parameters using QuPath, termed "OvoPath". We aim to present this work at UMSRS to invite individuals to utilize OvoPath, thereby improving the pipeline with additional samples.

To develop this tool, we used human ovarian biopsy H&E-stained images from patients undergoing ovarian tissue cryopreservation (OTC) at Lurie Children's Hospital. Using a supervised approach, follicles were classified then segmentation of the whole follicle area, individual GCs nuclei, and oocyte was performed using manual and automatic QuPath annotation tools. Extraction of feature data including size, shape, and distribution was performed to generate threshold values that represent independent follicular stages. Then, a tunable script was generated using Groovy programming language to automatically classify follicles and provide valuable digital assessments of GC and oocyte information.

We aimed to assess the utility of this tool by investigating follicle features in patients who have or have not been exposed to low levels of alkylating chemotherapy. We evaluated ovarian punch biopsies from 29 OTC patients (0.53-22.81 years, cyclophosphamide equivalent dose (CED) 0-8g/m^2). Assessing follicle count per mm^2 of tissue within each follicular stage resulted in no significant differences between individuals with CED > 0mg/m^2 (exposed) and CED = 0mg/m^2 (unexposed). However, exposed pre-pubertal patients have significantly smaller primordial follicles (PMFs) (1430 vs. 1539 +/- 30.76 μm^2) and significantly fewer GCs per PMF (8.95 vs. 11.61 +/-0.31 GCs/PMF) and transitional follicles (TFs) (10.28 vs. 13.08 +/- 0.27 GCs/TF) compared to unexposed pre-pubertal patients. Interestingly, exposed post-pubertal patients have significantly larger primordial follicles (1512 vs. 1416 +/- 39.95 μm^2), and like pre-pubertal patients, significantly fewer GCs per PMF (9.47 vs. 10.36 +/-0.42 GCs/PMF) and TFs (11.19 vs. 12.13 +/- 0.36 GCs/TF) compared to unexposed post-pubertal patients. Therefore, by utilizing

OvoPath we can assess more granular details of each follicle and observe significant differences between cohorts. OvoPath reveals previously overlooked morphometric features that may indicate reduced follicle health and quality. These features may reveal the impact of acute chemotherapy on follicles that would not otherwise be identified with follicle stage counts alone. Further orthogonal validation of markers of apoptosis and activation will be performed using these samples to correlate these data with follicle health. Altogether, we aim to provide the reproductive community with an accessible, unbiased, and standardized method to assess follicle features to better understand the impact of diseases or exogenous conditions on follicle quality.

ORAL SESSION 2:
Sperm and Fertility

T4: **MODULATION OF SPERM FUNCTION BY BACTERIAL VAGINOSIS (BV) TOXINS: LIPOPOLYSACCHARIDE AND VAGINOLYSIN**

Shweta Bhagwat[1], Juan Ferreira,[1] Ronald McCarthy,[1] Leila Asadi,[1] Ping Li,[1] Ariana Gaydon,[1] Alexander Zhang,[1] Christy Armstrong,[2] Andrea Verhagen,[2] Sydney Morrill,[2] Vaka Reddy,[1] Lloyd Robinson,[1] Warren G. Lewis,[2] Amanda L. Lewis,[2] Celia M. Santi.[1]*

[1] Washington University in Saint Louis, [2] University of California San Diego, * Corresponding Author

Abstract: Bacterial vaginosis (BV) is a prevalent condition affecting reproductive-aged women worldwide. It involves an imbalance in the vaginal microbiome and is characterized by an increase in anaerobic microorganisms such as *Gardnerella vaginalis* and *Prevotella bivia*, along with their respective toxins: Vaginolysin (VLY) and lipopolysaccharide (LPS). It is also associated with various adverse reproductive outcomes, including infertility, however, the mechanisms by which BV causes infertility are largely unknown. We hypothesize that the toxins produced by BV-associated bacteria impair sperm function and cause infertility. To test our hypothesis, we focussed on monitoring the physiological changes that sperm undergo to prepare for fertilization. Towards this we assessed sperm motility and hyperactivation using computer-assisted sperm analysis, viability by Hoechst staining, acrosome reaction (AR) in Acr-eGFP transgenic mice and human sperm, as well as intracellular calcium ($[Ca^{2+}]i$) dynamics by single-cell analysis using Fluo-4 AM dye, in response to LPS and VLY. In addition to this, we investigated the effect of LPS on changes in the ($[Ca^{2+}]i$) in CatSper KO mouse sperm, and in the presence of toll-like receptor 4 (TLR4) antagonist in *wild-type* (WT) mouse sperm, shedding light on the potential pathways mediating the observed effects. In mice and human sperm, we showed that LPS and VLY induced a significant increase in the percentage of hyperactivated sperm, during the early minutes of incubation only under capacitating (CAP) conditions, followed by a decrease in sperm motility, AR, and hyperactivation, on prolonged exposure to the toxins. AR was severely affected in the presence of both the toxins. Additionally, we demonstrate the efficacy of polymyxin B in inhibiting LPS-induced sperm hyperactivation, highlighting the specificity of LPS in modulating sperm function. Sperm ($[Ca^{2+}]i$) imaging demonstrated a significant and irreversible increase in Fluo-4 fluorescence upon acute application of LPS and VLY, particularly in CAP sperm. Moreover, these toxins elicited a rapid upsurge in ($[Ca^{2+}]i$) exclusively in CAP sperm. Furthermore, we observed that LPS-induced ($[Ca^{2+}]i$) increase was mediated by the TLR4 receptor, dependent on external calcium and independent of the CatSper ion channel. Prolonged exposure with LPS and VLY significantly reduced mice sperm fertilization ability during *in vitro* fertilization (IVF). Overall, this study summarizes valuable insights into the complex interplay between BV-associated pathogens and sperm physiology, with profound implications for reproductive health and fertility.

T5: KNOCKOUT OF PKDREJ, A CANDIDATE OVIDUCT RECEPTOR ON SPERM, RESULTS IN MALE INFERTILITY

Kankanit Doungkamchan[1], Leonardo Molina[1], Caroline Lucas[2], Kadirvel Govindasamy[1], Kevin Wells[2], Kristin Whitworth[2], Randall Prather[2], and David Miller[1]

[1] Department of Animal Sciences and Institute of Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, United States
[2] National Swine Resource and Research Center, University of Missouri at Columbia, Columbia, MO, United States

Abstract: PKDREJ, a homolog of the sea urchin receptor for egg jelly, is a member of the polycystin-1 gene family and is expressed only in the spermatogenic lineage. Previous studies found that mouse sperm lacking PKDREJ exhibited normal morphology and motility. The mutant sperm also had reduced fertility that was only observed in competition with wild-type sperm due to slower capacitation and delayed entry into the cumulus-oocyte complex. We have found that PKDREJ is also a candidate receptor for oviduct glycans containing Lewis X (Le^x) trisaccharide and multi-antennary 6-sialylated motifs. These glycans form a functional sperm reservoir in the porcine oviduct. In this study, we investigated the role of PKDREJ in the formation of the sperm reservoir and fertility by generating PKDREJ-knockout boars using the CRISPR/Cas9 system. We confirmed the genotype of the male founders using DNA extracted from ear notches and sperm. We identified 2 male founders for further study that were mosaics with multiple indels resulting in truncation of PKDREJ protein and no wild-type alleles. Western blots of sperm protein from the 2 founders demonstrated a deficiency in PKDREJ. Immunofluorescence showed a reduction in PKDREJ on the head of sperm from these founder males. The motility and morphology of sperm from the PKDREJ-knockouts were indistinguishable from wild-type sperm. Sperm lacking PKDREJ did not show any reduction in binding to oviduct spheroids or zona pellucida when compared to wild-type sperm. A fertility trial using artificial insemination (AI) was conducted. The pregnancy status for each female was assessed by collecting the uteri and fetuses 30 days after AI. No pregnancies were observed using semen from PKDREJ-knockout boars (0% pregnancy rate) compared to 65% pregnancy rate in the wild-type group (P < 0.05, n=20). A competitive insemination fertility trial inseminating an equal number of sperm from wild-type and PKDREJ-knockout boars into 7 females yielded only offspring from wild-type boars; there were no offspring from PKDREJ-knockout boars. Thus, PKDREJ-knockout sperm exhibited severely compromised fertility. We conclude that PKDREJ is crucial for porcine male fertility following artificial insemination.

T6: GUT MICROBIOTA CHANGES RAPIDLY IN RESPONSE TO SUPPRESSION OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS AND DIRECTLY IMPACTS REPRODUCTIVE PARAMETERS

Anna Clapp Organski[1], Anjali Reddivari[2] Joan S. Jorgensen[3], William G. Schrage[4], J Alex Pasternak[5], Tzu-Wen L. Cross[1]

[1]Department of Nutrition Science, Purdue University, West Lafayette, IN, 47907
[2] Department of Electrical and Computer Engineering, Purdue University, West Lafayette, IN, 47907
[3] Department of Comparative Biosciences, University of Wisconsin, Madison, WI, 53706
[4] Department of Kinesiology, University of Wisconsin, Madison, WI, 53706
[5]Department of Animal Sciences, Purdue University, West Lafayette, IN, 47907

Our research team has identified an interaction between the gut microbiome and the hypothalamic-pituitary-gonadal (HPG) axis. Specifically, the gut microbiome may respond to changes in gonadotropins and influence gonadal production of sex hormones. Herein, we suppressed the HPG axis using a gonadotropin-releasing hormone (GnRH) antagonist to further elucidate this interaction between the gut microbiome and the HPG axis. We aimed to assess (1) the alterations of the gut microbiome in response to rapid suppression of the HPG axis in conventionally raised mice that harbor a natural microbiome and (2) the direct impact of the resulting gut microbiome on sex hormone homeostasis and reproductive parameters using gnotobiotic mice via fecal microbiome transplant (FMT). HYPOTHESIS: We hypothesized that suppression of the HPG axis using a GnRH antagonist will rapidly perturb the gut microbiota in both sexes and these gut microbiotas can alter sex hormone production and reproductive phenotypes once transplanted into gnotobiotic mice. METHODS: C57BL/6J male and female conventionally raised mice (n=10/group) received weekly IP injections of Degarelix (GnRH antagonist) or saline (control) for 4 weeks. Testicular and uterine weights were measured at euthanasia to confirm treatment efficacy. Longitudinal changes in the gut microbiota were assessed using 16S rRNA gene sequencing of fecal samples collected every two days during the first week after treatment initiation and weekly thereafter. To assess the gut microbiome-directed physiological impact, fecal samples collected from these mice were used to colonize sex-matched germ-free C57BL/6J recipient mice (n=7-10/group) via FMT. In male FMT recipients, sperm count, testicular weight, and intra-testicular testosterone and dihydrotestosterone levels were assessed. In female FMT recipients, mice were euthanized in diestrus, and uterine weight and intra-ovarian estradiol levels were measured. RESULTS: As expected, Degarelix treatment resulted in significantly lower testicular and uterine weight in conventionally raised mice. Longitudinal assessment of the fecal microbiota in these conventionally raised mice showed that Degarelix treatment induced significant shifts in the fecal microbiota four days after the initiation of treatment, whereas changes in females occurred later at 2 weeks after treatment initiation. When these gut microbiomes were transplanted into germ-free male recipients, Degarelix-associated microbiota did not induce changes in sperm count, testicular weight, or intratesticular dihydrotestosterone when compared to control microbiota. However, recipients of Degarelix-associated microbiota tended to have lower intratesticular testosterone compared to those who received the control microbiota. Female FMT recipients of Degarelix-associated microbiota had significantly greater uterine weight and intra-ovarian estradiol compared to those who received the control microbiota. CONCLUSION: Suppression of the HPG axis using GnRH antagonist Degarelix led to rapid perturbation of the gut microbiota in both male and female mice. However, sex differences may exist in the interaction between the gut microbiota and the HPG axis resulting in the gut microbiome affecting reproductive parameters in a dimorphic fashion between male and female mice. The highly modifiable nature of the gut microbiota makes it an ideal target for mitigating sex-hormone-sensitive diseases and may be used to develop novel therapeutics.

ORAL SESSION 3:
Placental and Endometrial Biology

T7: HUMAN EXTRA-EMBRYONIC TROPHOBLAST CELLS SECRETE EXTRACELLULAR VESICLES TO COMMUNICATE WITH MATERNAL CELLS DURING PLACENTA DEVELOPMENT

Xiangning Song[1], Jacob R. Beal[1], Indrani C. Bagchi[2], and Milan K. Bagchi[1]

Departments of [1]Molecular and Integrative Physiology, and [2]Comparative Biosciences, University of Illinois, Urbana-Champaign, Urbana, IL.

During the establishment of pregnancy in humans, bidirectional maternal-fetal communication occurs between the endometrial and extra-embryonic trophoblast cells, and it is essential for the proper development of the placenta. We have recently reported that human endometrial decidual cells secrete membrane-enclosed extracellular vesicles (EVs), which carry a variety of molecular cargo that promote the differentiation of trophoblast stem (TS) cells into extravillous trophoblast (EVT) cells. The EVT cells migrate through the endometrium to remodel uterine spiral arteries to increase placental blood flow. However, the precise paracrine mechanisms by which the EVT cells communicate with the endometrial cells to execute these functional changes remain unknown. We utilized an *in vitro* human trophoblast stem cell differentiation system to demonstrate that the TS cells secrete EVs into conditioned media as they differentiate to the EVT lineage. Interestingly, compared to normoxic conditions, we found that hypoxia facilitates EV secretion by the EVT cells during trophoblast differentiation. Consistent with this finding, we observed a significant increase in hypoxia-inducible transcription factor HIF2α expression in EVT cells. Knockdown of HIF2a transcripts in EVT cells leads to marked downregulation of EV secretion by these cells. Mass spectrometry analysis of EVT-derived EVs revealed that they carry several protein cargoes that potentially mediate crucial paracrine mechanisms that regulate the remodeling of endometrium to accommodate the invading trophoblasts, and functionally modify arterial cells. Collectively, our findings support the concept that bidirectional EV secretion occurs at the maternal-fetal interface. Altered secretion of EVs by human EVT during pregnancy-related disorders can potentially affect decidual tissue remodeling and uterine spiral artery modification, which are critical events for the establishment of a functional placenta.

T8: DECIDUALIZATION ALTERS THE MICROSTRUCTURE AND MECHANICS OF THE MURINE ENDOMETRIUM

C.S. Bastías[1], K. Sandoval[2], S. Bhaye[3], S. Chen[3], D.O. Duele[4], Y. Kulkarni[1], M. Dean[2,3], C.M. Luetkemeyer[1]

[1]Department of Mechanical Science and Engineering, University of Illinois Urbana-Champaign, Urbana, IL,
[2]Division of Nutritional Science, University of Illinois Urbana-Champaign, Urbana IL,
[3]Deparment of Animal Sciences, University of Illinois Urbana-Champaign, Urbana IL,
[4]Paul M. Rady Department of Mechanical Engineering, University of Colorado Boulder, Boulder CO

Although endometrial dysfunction is a major cause of female infertility, we currently lack diagnostic biomarkers and therapeutic targets for the condition. A successful pregnancy requires proper decidualization, a process characterized by thickening of the endometrium and extreme extracellular matrix remodeling. Because tissue stiffness is largely determined by the composition and organization of the extracellular matrix, impaired and healthy decidualization are likely to affect the mechanical properties of the endometrium in different ways. In fact, one study on humans found that decidualized endometrium is stiffer than undecidualized endometrium. Thus, we aim to develop mechanics-based biomarkers (i.e., stiffness measures associated with fiber alignment, density, or thickness) for decidualization, which could lead to novel non-invasive diagnostic tools and therapeutic targets for endometrium-related infertility. This study aims to evaluate changes in the stiffness and collagen microstructure caused by decidualization in the healthy endometrium of a murine model. We hypothesized that decidualization of the murine endometrium will increase tissue stiffness, similar to humans, with more aligned and thicker collagen fibers compared to undecidualized endometrium.

Wild-type C57BL/6 (n=4; 8 weeks old) were unilaterally ovariectomized. After a 14-day recuperation period, the mice were mated with male mice. The presence of vaginal plugs denoted embryonic day 1. Mice were euthanized on day 8. Uterine horns were cut length-wise along the mesometrial pole. Embryos were removed, and a section of each uterine horn was stained for 2 hours at 4°C in a solution of Ghost Dye (tags all free amine groups). The antimesometrial side of the uterus was indented under the weight of a glass microsphere, and images were taken before and after indentation with an upright confocal microscope. Tissue stiffness was determined from the images using the Hertzian contact model, which relates the contact area and pressure between the spherical indenter. Second harmonic generation was used to evaluate changes in the collagen microstructure. A custom MATLAB code employing OrientationJ (Image J) was used to determine fiber orientation and alignment.

Decidualization altered the mechanical properties and microstructure of the endometrium. Decidualized endometrium was stiffer than undecidualized endometrium, with average indentation moduli (i.e., stiffness) of 740 ± 340 Pa and 147 ± 90 Pa, respectively. Second harmonic generation image analysis determined that the mean coherency (i.e., degree of alignment) of collagen fibers in decidualized endometrium was larger than in undecidualized samples (0.39 and 0.34, respectively). However, the volume fraction (the relative collagen density) was slightly larger for the undecidualized samples: 0.12 and 0.09, respectively.

Our findings suggest that decidualization causes microstructural changes such as increased collagen alignment which increases the stiffness of the endometrium. This suggests that collagen remodeling and associated changes in tissue mechanical function are important components of a healthy pregnancy and may be useful as biomarkers for uterine infertility. This is the first study to investigate the effects of decidualization on both endometrium mechanics and collagen microstructure; however, a larger sample size is needed to determine significance. Future work will aim to understand whether the stiffness of the endometrium changes in models of impaired decidualization.

T9: PROGESTERONE-RESPONSIVE UTERINE TISSUE-RESIDENT NATURAL KILLER CELLS

Bruna K. Tatematsu[1] and Dorothy K. Sojka[1].

[1]Department of Microbiology and Immunology, Loyola University Chicago: Health Sciences Division, Maywood, IL, United States, 60153

Innate lymphoid cells (ILC) are important in mucosal sites for tissue homeostasis, repair and provide immunity against local pathogens. Emerging evidence suggests that ILCs can respond to environmental cues, such as sex hormones, and modify the functional outcome. A subset of ILC, called tissue-resident natural killer (trNK) cells, compose 70% of the immune cells in the nonpregnant murine uterus. However, the signals regulating the function of these cells in the uterine tissue remain unknown. Here, to distinguish cells in circulation from those residing in the tissue, we intravascularly labeled immune cells in intact C57/Bl6 WT and ovariectomized (OVX) mice supplemented with steroid hormones, and analyzed uterine trNK cells by high-dimensional flow cytometry. We found that trNK cells are absent in the prepubescent uteri, appear at puberty, fluctuate during the female reproductive lifespan, and decline in reproductively aged mice. Mice OVX at three weeks (sexually immature) and analyzed at seven weeks (sexually mature) did not have trNK cells in the uterus, unless progesterone was administered. OVX or intact mice treated with progestin had expansion of uterine trNK cells, not observed in distal sites such as the spleen, mammary gland, ovaries and oviduct. Such expansion was abrogated with RU486, a progesterone antagonist. Moreover, mice lacking progesterone receptor on NK cells, revealed a missing subset of uterine trNK cells. Our studies suggest that progesterone regulates the presence of trNK cells in the uterine tissue. Further understanding of how progesterone modulates uterine trNK cells can help us improve hormone therapy for women worldwide.

POSTERS

Poster #	Name	Poster Title
1	Adarsh Ram	ROLE OF IRX3 IN THE UTERUS DURING EARLY PREGNANCY
2	Md Hasanur Alam	OOCYTE MICROINJECTION: APPROACH TO STUDY TOXICOLOGY AT LOW-DOSE AND INDIVIDUAL CELL LEVEL
3	Mohamed Ali	VITAMIN D ANALOG DOXERCALCIFEROL INHIBITS INFLAMMATION AND FIBROSIS IN HUMAN UTERINE FIBROIDS USING 3D ORGANOID MODEL
4	Nourhan Ali	DIMINISHED AUTOPHAGY ACTIVATION IS A CRUCIAL FACTOR FOR WEAKENED DNA REPAIR IN FULL GROWN
5	Farners Amargant i Riera	PIEZO1 AND PIEZO2 MIGHT MEDIATE THECA CELLS MECHANOSENSITIVITY
6	Tomiris Atazhanova	HUMAN OVARIAN STIFFNESS MEASURED BY SHEAR WAVE ELASTOGRAPHY (SWE) AS A NON-INVASIVE BIOMARKER OF REPRODUCTIVE AGING
7	Kavyashree Basavaraju	UNCOVERING THE DISTINCT ROLES OF NELF-B AND TDP-43 IN MALE MEIOSIS THROUGH SCRNA-SEQ ANALYSIS
8	Kannan Athilakshmi	THE RNA-BINDING PROTEIN HNRNP I/PTBP1 REGULATES EMBRYO TRANSPORT AND UTERINE RECEPTIVITY DURING EARLY PREGNANCY IN THE MOUSE
9	Jacob Beal	EXPOSURE TO PHTHALATE INDUCES ABNORMAL DNA METHYLATION PATTERNS IN HUMAN ENDOMETRIAL STROMAL CELLS IMPAIRING ESTROGENIC REGULATION OF EXTRACELLULAR VESICLE SIGNALING
10	Gabriela Berniac	INVESTIGATING HOW MICROTOPOGRAPHY-INDUCED MORPHOLOGICAL CHANGES AFFECT GRANULOSA CELL TRANSCRIPTION AND OVARIAN HORMONE PRODUCTION
11	Sameeksha Bhaye	CHARACTERIZATION OF A DIET-INDUCED OBESE MOUSE MODELS WITH REDUCED FERTILITY
12	Cary Brandolino	EFFECT OF VITAMIN A ENRICHMENT ON OVARIAN FOLLICLE DEVELOPMENT IN WILD TYPE AND MTA TRANSGENIC MICE

Poster #	Name	Poster Title
13	Mary Bunnell	TRANSITION OF ESR2-LINEAGE CELLS TO ESR1-EXPRESSING CELLS IN THE HYPOTHALAMIC-PITUITARY-OVARIAN AXIS: INSIGHTS FROM A NOVEL TRANSGENIC MOUSE MODEL
14	Kerem Catalbas	HYPOTHALAMIC AGRP NEURONS REGULATE THE HYPERPHAGIA OF LACTATION
15	Sijie Chen	INVESTIGATING THE SIGNIFICANCE AND MECHANISMS OF RNA SOLUBILITY PHASE TRANSITION DURING THE OOCYTE-TO-EMBRYO TRANSITION IN VERTEBRATES
16	Teresa Chou	QUANTITATIVE MODELING TO CHARACTERIZE MATERNAL INFLAMMATORY RESPONSE OF HISTOLOGIC CHORIOAMNIONITIS IN PLACENTAL MEMBRANES
17	Harun Cingoz	UNDERSTANDING THE IMPACT OF OBESITY ON METHYLATION CHANGES IN ENDOMETRIAL CARCINOMA
18	Aubrey Converse	THE OVARIAN MACROPHAGE-DERIVED MULTINUCLEATED GIANT CELL TRANSCRIPTOME REVEALS UNIQUE MOLECULAR PROFILES
19	Charlesda Costa	CO-EXPOSURE TO TRIBUTYLTIN AND A HIGH-REFINED CARBOHYDRATE DIET LEADS TO ABNORMAL FERTILITY, PLACENTA, PREGNANCY OUTCOMES, AND OFFSPRING METABOLIC COMPLICATIONS IN FEMALE RATS
20	Angela Dean	PHTHALATES ALTER THE EXPRESSION OF GENES ASSOCIATED WITH THE ARYLHYDROCARBON RECEPTOR PATHWAY IN THE MOUSE OVARY
21	Flávia Caroline Farias Dos Santos	SUBACUTE EXPOSURE TO A MIXTURE OF CONTAMINANTS (TRIBUTYLTIN + CADMIUM + MERCURY) LEADS TO REPRODUCTIVE COMPLICATIONS IN FEMALE RATS
22	Pooja Devrukhkar	SPATIAL TRANSCRIPTOMIC MAPPING OF P16INK4A POSITIVE REGIONS REVEALS A SENESCENT SIGNATURE IN THE NATIVE POSTMENOPAUSAL HUMAN OVARY.
23	Hao-Chun Fan	THE ROLE OF SIGLECS IN SELECTIVE SPERM CLEARANCE WITHIN THE PORCINE OVIDUCT
24	Juan Ferreira	SLO2.1/NALCN FUNCTIONAL COMPLEX ACTIVITY IN MOUSE MYOMETRIAL SMOOTH MUSCLE CELLS DURING PREGNANCY.
25	Audrey Fields	IODOACETIC ACID ALTERS THE EXPRESSION OF OXIDATIVE STRESS AND APOPTOSIS GENES IN THE MOUSE OVARY

Poster #	Name	Poster Title
26	Endia Fletcher	THE EFFECTS OF PRENATAL EXPOSURE TO AN ENVIRONMENTALLY RELEVANT PHTHALATE MIXTURE ON OVARIAN GENE EXPRESSION IN F1 AND F3 FEMALE MICE
27	Sundus (Sunny) Ghuneim	CO-EXPOSURE TO MONO(2-ETHYLHEXYL) PHTHALATE AND ELEVATED TEMPERATURE INHIBITS MOUSE ANTRAL FOLLICLE GROWTH AND STEROIDOGENESIS IN VITRO
28	Lauren Haky	TARGETING GALANIN RECEPTOR 3 TO BLOCK OOCYTE MEIOTIC MATURATION FOR NON-HORMONAL CONTRACEPTIVE DEVELOPMENT
29	Prianka Hashim	CGAS-STING PATHWAY ACTIVATION IN OVARIAN SECONDARY FOLLICLES COMPROMISES FOLLICLE SURVIVAL, GROWTH, AND OOCYTE QUALITY
30	Emily Hayes	LOSS OF SIK3 IN GRANULOSA CELLS DISRUPTS DOWNSTREAM FSH SIGNALING PATHWAYS AND METABOLISM
31	Anna Heinrich	IN VIVO OVULATION KINETICS ARE DISRUPTED WITH ADVANCED REPRODUCTIVE AGE
32	Alaina Houghton-Chamberlain	IRX3 AND IRX5 FOSTER HEALTHY OOCYTE-PRE-GRANULOSA CELL INTERACTIONS DURING GERMLINE CYST BREAKDOWN AND PRIMORDIAL FOLLICLE FORMATION
33	Zane Inman	DI(2-ETHYHEXYL) PHTHALATE INCREASES EXPRESSION OF SEVERAL OXIDATIVE STRESS MARKERS IN THE MOUSE OVARY
34	Irene Infancy Joseph	ROLE OF TDP-43 IN POSTNATAL DIFFERENTIATION OF EPIDIDYMIS AND EPIDIDYMAL MATURATION OF SPERM
35	Sasha Kakkassery	ULTRA-STRUCTURAL ANALYSIS OF SYNAPTONEMAL-COMPLEX STRUCTURE USING DNA-PAINT MINFLUX NANOSCOPY
36	Abby Keisker	EFFECT OF CYP26 INHIBITION ON OVULATION IN MICE
37	Shivi Khurana	NELF-B IN SERTOLI CELLS IS CRUCIAL FOR THE INITIATION AND MAINTENANCE OF MEIOSIS IN GERM CELLS IN MICE
38	Hyunho Kim	THERAPEUTIC POTENTIAL OF HUMAN CATHELICIDN LL-37 STIMULATED UC-MSC EXOSOME FOR TREATING POLYCYSTIC OVARY SYNDROME

Poster #	Name	Poster Title
39	Jingjing Kipp	IMPACT OF RETINOIC ACID RECEPTOR ALPHA CONDITIONAL KNOCKOUT ON OVARIAN FOLLICLE DEVELOPMENT IN THE MOUSE
40	Stav Kramer	THE EFFECTS OF A COMBINATION OF WESTERN DIET AND PHTHALATE EXPOSURE ON INFLAMMATORY MARKERS IN THE OVARY
41	Hana Kubo	MULTIPLE HUMAN INDUCED PLURIPOTENT STEM CELL LINES DIFFERENTIATED WITH A LIGAND-BASED APPROACH RECAPITULATE GRANULOSA CELL DEVELOPMENT AND EXPRESS STEROIDOGENIC PATHWAY GENES
42	Katie Land	EXPOSURE TO PHTHALATES DISRUPTS OVULATORY LUTEINIZING HORMONE/CHORIOGONADOTROPIN RECEPTOR SIGNALING AND DECREASES DOWNSTREAM PROSTAGLANDIN PRODUCTION IN HUMAN GRANULOSA CELLS IN VITRO
43	Hoi Chang Lee	SPERM SAFE: MAXIMIZING THE PRESERVATION AND RECOVERY OF LIMITED NUMBERS OF MALE GERM CELLS
44	Jie Li	THE EFFECTS OF SHORT-TERM AND CHRONIC DAILY PROPYLPARABEN EXPOSURE ON ESTROUS CYCLICITY AND FERTILITY IN MICE
45	Farzana Liakath Ali	INTRA-TESTICULAR ADMINISTRATION OF SERTOLI-PRIMED EXOSOMES BEFORE CHEMOTHERAPY PROTECTS SPERMATOGONIAL STEM CELLS IN PREPUBERTAL MICE: A NEW HOPE FOR FERTILITY PRESERVATION
46	Ilka Lorenzo	REVEALING THE MECHANISMS OF ACENTROSOMAL SPINDLE STABILITY IN MAMMALIAN OOCYTES
47	Meng Ma	REMODELING OF THE ENDOPLASMIC RETICULUM (ER) DURING EARLY EMBRYO DEVELOPMENT
48	Humberto Magalhaes	ENDOCRINOLOGICAL AND EMBRYO-ASSOCIATED PARAMETERS IN PPID MARES TREATED WITH PERGOLIDE AND METFORMIN
49	Allison McCarthy	SIGNALING THAT MEDIATES THE MAMMALIAN EGG'S MEMBRANE BLOCK TO POLYSPERMY: ANALYSES OF CALMODULIN, CALCIUM/CALMODULIN-DEPENDENT KINASE II, AND A CAMKII SUBSTRATE
50	Ross McNally	THE ROLE OF HORMONES AND OXIDATIVE STRESS IN LEIOMYOMA DEVELOPMENTAND SURVIVAL

Poster #	Name	Poster Title
51	Supipi Mirihagalle	TRANSIENT SCROTAL HYPERTHERMIA AFFECTS PORCINE EPIDIDYMAL EXTRACELLULAR VESICLES AND SPERM
52	Leonardo Molina	SIGLECS IN THE PORCINE OVIDUCT AND SIALYLATED LIGANDS ON SPERM: POTENTIAL ROLE IN THE SPERM RESERVOIR
53	Courtney Murphy	BIOMECHANICS AS A NOVEL NON-INVASIVE BIOMARKER OF OOCYTE MEIOTIC POTENTIAL
54	Muhammad Ashir Nabeel	REGULATION OF LACTATE SECRETION BY PROGESTERONE IN BOVINE UTERINE EPITHELIAL CELLS AND BASIGIN/LACTATE TRANSPORTER EXPRESSION IN PRE-IMPLANTATION BOVINE EMBRYOS
55	Lujain Nachbat	SPERM BINDING TO GLYCANS IN THE STORAGE RESERVOIR AS A MECHANISM OF REPRODUCTIVE ISOLATION
56	Natalia Obrochta	TARGETING MEIS2 IN PLATINUM RESISTANT OVARIAN CANCER
57	Ji-Eun Oh	TGFB1-INDUCED NONCANONICAL SIGNALING MODULATES THE SWITCH FROM ESR1 TO ESR2 DURING GRANULOSA CELL DIFFERENTIATION IN THE DEVEOPING OVARY
58	Yinka Ojo	PHTHALATE- INDUCED PITUITARY INFLAMMATION LEADS TO EARLY FEMALE REPRODUCTIVE AGING
59	Jadesola Oladosu	THE MOUSE OVARY HAS THE CAPACITY TO CONVERT THE NEONICOTINOID PESTICIDE IMIDACLOPRID TO TOXIC METABOLITES IN VIVO
60	Mervat Omran	DRUG REPURPOSING SCREENING AND MECHANISM ANALYSIS USING THREE- DIMENSIONAL HUMAN STEM CELLS DERIVED ORGANOIDS
61	Shravya Pant	DEFINING THE EFFECTS OF LOW-DOSE ACUTE CHEMOTHERAPY ON THE OVARIAN MICROENVIRONMENT IN PRE-PUBERTAL AND ADOLESCENT PATIENTS
62	Lyda Yuliana Parra Forero	3D APPROXIMATION OF THE EXPRESSION OF LINEAGE-SPECIFIC MARKERS OF INNER CELL MASS (SOX2, OCT4) AND TROPHECTODERM (CDX2) IN MOUSE BLASTOCYSTS
63	Natalia Pascuali	THE OBESOGENIC CHEMICAL TRIBUTYLTIN INDUCES DYSLIPIDEMIA IN PRIMARY HUMAN OVARIAN THECA CELLS
64	Tanya Pattnaik	THE ROLE OF DOPAMINE IN FEEDING BEHAVIOR DURING LACTATION IN MICE

Poster #	Name	Poster Title
65	Madeline Perry	AGE-ASSOCIATED OVARIAN MACROPHAGE-DERIVED MULTINUCLEATED GIANT CELLS SHARE TRANSCRIPTOMIC SIGNATURES WITH REGRESSING CORPORA LUTEA
66	Sara Pietroforte	INCREASED AGE-RELATED OVARIAN STIFFNESS IMPAIRS FOLLICLE DEVELOPMENT AND OOCYTE QUALITY BY MODULATING FOLLICLES' TRANSCRIPTOME
67	Makenzie Plough	THE MECHANOTRANSDUCER YAP1 IS HIGHLY EXPRESSED IN GROWING FOLLICLES AND ITS ACTIVITY IS REGULATED BY THE AGE-ASSOCIATED INCREASE IN OVARIAN STIFFNESS
68	Ingrid Possa Paranhos	NEUROANATOMICAL AND TRANSCRIPTIONAL ANALYSIS OF HYPOTHALAMIC RESPONSE TO LACTATION
69	Reza Rajabi-Toustani	MINFLUX NANOSCOPY REVEALS ULTRA-FINE STRUCTURE OF AXIAL AND CENTRAL ELEMENTS IN SYNAPTONEMAL COMPLEX
70	Alisha Ramos	A PHENOTYPIC SCREENING ASSAY IDENTIFIES NOVEL INHIBITORS TARGETING WEE2 KINASE ACTIVITY TO BLOCK EGG ACTIVATION FOR NON-HORMONAL CONTRACEPTION
71	Shams Saad	CHARACTERIZING THE EXPRESSION AND FUNCTION OF THE ACTIN-BINDING PROTEIN NEXILIN IN MAMMALIAN OOCYTES
72	Adira Safar	LONG-TERM DIETARY EXPOSURE TO AN ENVIRONMENTALLY RELEVANT PHTHALATE MIXTURE AFFECTS OVARIAN AGING MARKERS
73	Natasha Salpeter	THE FEASIBILITY OF ADENO-ASSOCIATED VIRUS-MEDIATED TRANSDUCTION IN HUMAN PRIMARY GRANULOSA CELLS
74	Kassandra Sandoval	CHARACTERIZING EXTRACELLULAR VESICLES FROM THE BOVINE UTERINE EPITHELIUM
75	Ramses Santacruz-Marquez	LONG-TERM DIETARY EXPOSURE TO DI(2-ETHYLHEXYL) PHTHALATE INDUCES INFLAMMATION AND FIBROSIS IN THE OVARY
76	Anika Schipma	THROUGH THE TROUGH: ENGINEERING 3D TESTICULAR TUBULES TO SUPPORT SPERMATOGENESIS IN VITRO
77	Taylor Seaton	LONG-TERM DIETARY EXPOSURE TO PHTHALATES ALTERS EXPRESSION OF OXIDATIVE STRESS MARKERS IN THE OVARY

Poster #	Name	Poster Title
78	Arpna Sharma	HYPERPLASTIC OVARIAN STROMAL CELLS EXPRESS GENES ASSOCIATED TO TUMOR PROGRESSION: A CASE STUDY
79	Ritwik Shukla	LONG-TERM DIETARY EXPOSURE OF MICE TO A MIXTURE OF PHTHALATES CAUSES DYSREGULATION OF ESTROGEN RECEPTOR SIGNALING AND LEADS TO ENDOMETRIAL HYPERPLASIA
80	Janavi Thyagraj	INNOVATION FOR INSUFFICIENCY: PRECURSOR TO AN IN VITRO ASSAY TO PREDICT ADRENAL CORTEX FUNCTION USING HUMAN INDUCED PLURIPOTENT STEM CELLS
81	Swarna Tisha	EFFECT OF LONG-TERM PROPYLPARABEN EXPOSURE ON COLLAGEN DEPOSITION AND GENE EXPRESSION IN MURINE OVIDUCT
82	Uyen Tran	DETERMINING BIOCOMPATIBILITY OF 3D PRINTING RESINS AND EFFECT OF PARYLENE-C COATING FOR APPLICATIONS IN COMPLEX REPRODUCTIVE TISSUE CULTURES
83	Yuexi Wang	PTBP1 MEDIATES SERTOLI CELL ACTIN CYTOSKELETON ORGANIZATION BY REGULATING ALTERNATIVE SPLICING OF ACTIN REGULATORS
84	Anita Waye	CHEMICAL MIXTURE DYSREGULATES PLACENTAL CELL SIGNALING PATHWAYS, HORMONE SECRETION, AND ANTIANGIOGENIC PROPERTIES IN TERM HUMAN PLACENTAS
85	Shuangqi Wang	MEIOTIC SILENCING VIA ASYNAPSIS AND GEL-LIKE PHASE SEPARATION
86	Brittney Williams	EXPOSURE TO AN ENVIRONMENTALLY RELEVANT PHTHALATE MIXTURE ALTERS LEVELS OF OVULATORY ANGIOGENIC FACTORS AND RECEPTORS IN MOUSE GRANULOSA AND ENDOTHELIAL CELLS IN VITRO
87	William Xu	EXPRESSION OF SEX HORMONE RECEPTORS IN LEVATOR ANI MUSCLE TISSUE FROM PATIENTS WITH PELVIC ORGAN PROLAPSE
88	Qiwei Yang	DECODING PSEUDOURIDINE, THE EMERGING ROLE OF DYSKERIN PSEUDOURIDINE SYNTHASE 1 IN UTERINE FIBROIDS
89	Tianming You	ROLE OF PROGESTERONE RECEPTOR AS A DOWNSTREAM MEDIATOR OF ESTROGEN-INDUCED MUSCLE FIBROSIS IN MOUSE MODELS OF INGUINAL HERNIA

Poster #	Name	Poster Title
90	Emily Zaniker	A NOVEL 3D-PRINTED AGAROSE MICROMOLD SUPPORTS SCAFFOLD-FREE MOUSE EX VIVO FOLLICLE GROWTH, OVULATION, AND LUTEINIZATION
91	Pedram Zarei	EFFECTS OF POSTPARTUM ACETYLSALICYLIC ACID ADMINISTRATION AND CALCIUM SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE AND CULLING IN HOLSTEIN DAIRY COWS
92	Luhan (Tracy) Zhou	ANOMALOUS HIGH TEMPERATURE EXPOSURE EFFECTS ON MOUSE WEIGHT AND OVARIAN DEVELOPMENT
93	Yiru Zhu	A COMPLEX PHENOTYPIC SCREENING ASSAY OF OOCYTE MEIOTIC MATURATION IDENTIFIES NOVEL TARGETS FOR NON-HORMONAL CONTRACEPTIVE DISCOVERY
94	Azna Zuberi	THERAPEUTIC TARGETING OF THE TRYPTOPHAN-KYNURENINE-ARYL HYDROCARBON RECEPTOR PATHWAY WITH APIGENIN IN MED12-MUTANT LEIOMYOMA CELLS

POSTER ABSTRACTS

P1: ROLE OF IRX3 IN THE UTERUS DURING EARLY PREGNANCY

Adarsh Ram [1], Athilakshmi Kannan[1], Alaina Houghton-Chamberlain[3], Milan K. Bagchi[2], Joan S. Jorgensen[3], and Indrani C. Bagchi[1]

Departments of Comparative Biosciences [1], Mol & Integrative Physiology [2], University of Illinois at Urbana-Champaign, Urbana, IL, Department of Comparative Biosciences [3], University of Wisconsin-Madison, Madison, WI

Two major factors contributing to female infertility are the premature loss of the ovarian reserve and early implantation/placentation failure. Previous studies have shown that canonical Wnt/β-catenin signaling directly stimulates the transcription of the members of the Iroquois B (IrxB) homeodomain cluster genes, specifically *Irx3* and *Irx5*, crucial for oocyte and follicle survival within the developing ovary. Upon ablation of *Irx3* in female mice, the follicles fail to mature and ultimately die due to unorganized gap junction assembly and inadequate oocyte-granulosa cell contacts. Interestingly, our recent studies have shown that *Irx3* is induced in the uterus during early pregnancy as endometrial stromal cells undergo differentiation to form decidual cells in a process known as decidualization. We and others have previously reported that BMP2, a morphogen belonging to the TGFβ superfamily, and its downstream target Wnt4 regulate uterine decidualization during early pregnancy. Employing a primary culture system in which undifferentiated stromal cells isolated from pregnant mouse uteri are subjected to decidualization in vitro, we found that *Irx3* was significantly upregulated 12h after the induction of Wnt4 in response to exogenous BMP2. Consistent with this observation, we found that siRNA-mediated silencing of β-catenin expression in these cells led to a dramatic reduction in the expression of IRX3, indicating that it functions downstream of *Wnt4*-induced β-catenin signaling in the uterus during decidualization. Although IRX3 functions as a transcription factor, a significant portion of this protein is intriguingly localized in the cytoplasm of endometrial stromal cells, with punctate dots organized in a donut-shaped appearance, suggesting IRX3's presence on the surface of cytoplasmic vesicles. Further studies using proximity ligase assays indicated the interaction of IRX3 with Septin 7, a GTP-binding protein that plays a crucial role in various cellular processes, including vesicular trafficking. Indeed, the pattern of Septin 7 expression overlaps with IRX3 expression in the uterus during early pregnancy, raising the possibility that cytoplasmic IRX3 interacts with cytoskeletal proteins as they orchestrate vesicular exocytosis. Further studies will investigate whether IRX3 acts as a dual-function protein to regulate cell-cell dynamics, similar to β-catenin, which has a well-established role in both nuclear and cytoskeletal cell programming.

P2: OOCYTE MICROINJECTION: APPROACH TO STUDY TOXICOLOGY AT LOW-DOSE AND INDIVIDUAL CELL LEVEL

Md Hasanur Alam [1], Huanyu Qiao [1]

[1] Department of Comparative Biosciences, University of Illinois at Urbana Champaign, Urbana, IL.

Abstract : Iodoacetic acid (IAA) is a water disinfection byproduct formed through reactions between oxidizing disinfectants like chlorine and naturally occurring iodide in water. IAA is ubiquitous and can be found in treated water at levels up to 2.18 μg/L. Exposure to IAA at 5 μM in culture medium has been shown to interfere with mouse oocyte maturation by elevating reactive oxygen species (ROS) levels, disrupting spindle assembly, inducing DNA damage, and causing meiotic arrest. However, the precise amount of IAA that actually enters the oocytes during culture remains unclear. In this study, we used microinjection technology to inject precise amounts of IAA directly into oocytes to examine its effects. Cumulus oocyte complexes (COCs) were collected for microinjection. We first measured the volume of the oocytes and the volume of injected IAA, allowing us to calculate the exact amount of IAA injected into the oocytes. The COCs were then cultured for 6 hours. After removing the cumulus cells, the oocytes were examined for meiotic progression and overall health. The germinal vesicle breakdown (GVBD) rate was significantly higher (P < 0.01) in the water-injected control group compared to the IAA-injected groups (0.25, 0.5, 1, and 2 μM). Conversely, the proportion of unhealthy oocytes was significantly higher (P < 0.01) in IAA-injected groups (0.25, 0.5, 1, and 2 μM) compared to the control group. The denuded oocytes were further cultured for 18 hours to assess meiotic progress. The polar body extrusion rate was significantly higher (P < 0.01) in the water-injected control group than the IAA-injected groups (0.25, 0.5, 1, and 2 μM). Whereas, the proportion of unhealthy oocytes was again significantly higher (P < 0.05) in the IAA injected groups. IAA injection into oocytes led to the formation of defective polar bodies with the area of the polar body significantly reduced (P < 0.01) in the 0.25, 0.5, and 1 μM IAA-injected groups than the control group. IAA microinjection increased the ROS levels in oocytes in a dose-dependent manner, indicating low-dose IAA (< 5 μM) could also induce oxidative stress in oocytes. Our results indicate that environmentally relevant low-dose IAA interferes with mouse oocyte maturation by elevating ROS levels and disrupting polar-body extrusion.

P3: VITAMIN D ANALOG DOXERCALCIFEROL INHIBITS INFLAMMATION AND FIBROSIS IN HUMAN UTERINE FIBROIDS USING 3D ORGANOID MODEL

Mohamed Ali [1], Mervat Omran [1], Ayman Al-Hendy [1]

[1] Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL. USA.

Uterine Fibroids (UFs) are the most common benign tumors in reproductive-age women, with current treatment options primarily being surgical. Given the association between vitamin D deficiency and UF risk, exploring the Vitamin D receptor (VDR) agonist Doxercalciferol (DCL) as a potential therapeutic option for UFs is of significant interest. Our team was the first to develop a 3D organoid model for human UF tissues derived from corresponding stem cells (SCs) in vitro. This model preserves key aspects of tissue scaffolding, enabling SCs to undergo appropriate differentiation and proliferation in response to an estrogen-mimicking microenvironment, closely resembling in vivo conditions. In this study, we investigated the potential anti-UF effects of the VDR analog DCL using 3D UF organoids, compared to untreated controls. DCL exhibited dose-dependent antiproliferative effects on human UF cells in both 2D and 3D culture conditions. The MTT cell viability assay revealed that DCL, at concentrations of 100 and 1000 nM, significantly inhibited the proliferation of 2D-cultured UF cell line (HuLM) as well as UFSCs isolated from two patients after 3 days of treatment. Furthermore, 3D UF organoids treated with 100 nM DCL showed a reduction in fibrosis, as indicated by Mason trichrome staining, underscoring the antifibrotic effects of DCL. Subsequent analysis of the UF organoid secretome, following 100 nM DCL treatment, using Multiplex ELISA, demonstrated a significant decrease in the secretion of the profibrotic cytokine transforming growth factor beta (TGF-β1), as well as angiogenic cytokines VEGF-A and PDGF AB/BB. Additionally, DCL treatment (100 nM for 12 days) of UF organoids from two patients led to a significant reduction in the secretion of inflammatory cytokines IL-6, IL-8, TNF-α, and IFN-γ, as well as a downregulation of TNF-α and NF-kB (p65) expression, as confirmed by IHC staining. In conclusion, our studies demonstrate the promising anti-UF effects of DCL, suggesting it as a safe, non-hormonal, long-term, and cost-effective therapeutic alternative to Vitamin D3 for UFs. Notably, DCL offers a lower risk of hypercalcemia compared to therapeutic doses of Vitamin D3, making it a potentially advantageous clinical option pending further research. This study was supported by NIH funding and SEUD research grant.

P4: DIMINISHED AUTOPHAGY ACTIVATION IS A CRUCIAL FACTOR FOR WEAKENED DNA REPAIR IN FULL GROWN

Nourhan Nashat Ali [1,2#], Fei Sun [1#], Daniela Londoño-Vásquez [1], Constantine A. Simintiras [3], Huanyu Qiao [4], M. Sofia Ortega [5], Yuksel Agca [6], Masashi M. Takahashi [7], Rocio M. Rivera [1], Andrew M. Kelleher [8], Peter Sutovsky [1,8], Amanda L. Patterson [1,8], Ahmed Z. Balboula [1*]

[1] Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA
[2] Department of Physiology, Faculty of Veterinary Medicine, Assiut University, Assiut 71515, Egypt
[3] School of Animal Sciences, Agricultural Center, Louisiana State University, Baton Rouge, LA 70803, USA
[4] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL 61802, USA
[5] Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA.
[6] Department of Veterinary Pathobiology, University of Missouri, Columbia, MO 65211, USA
[7] Research Faculty of Agriculture, Hokkaido University, Hokkaido 060-0809, Japan.
[8] Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, MO, 65211, USA

#Equal Contribution

*Corresponding author: Ahmed Z. Balboula, University of Missouri, 163B Animal Sciences Research Center, Columbia, MO 65211, USA, abalboula@missouri.edu

DNA damage repair (DDR) machinery is not robust in full-grown mammalian oocytes. The underlying mechanisms of this weakened DDR are unknown. We show that DDR is dysfunctional in oocytes, leading to aneuploidy. Our data reveal that oocyte failure to repair damaged DNA is due to the inability of DDR proteins to access altered "closed" chromatin conformation in DNA-damaged oocytes. Our data also demonstrate that, unlike somatic cells, mouse and porcine oocytes fail to activate autophagy in response to DNA double-strand breaks, which is the cause of altered chromatin conformation and inefficient DDR. Importantly, autophagy activity is further reduced in maternally aged oocytes (which harbor severe DNA damage) and its induction rescues DDR function in maternally aged oocytes. Our findings provide evidence that reduced autophagy contributes to weakened DDR in oocytes, especially in those from aged females, offering scope to improve assisted reproductive therapy in women.

P5: PIEZO1 AND PIEZO2 MIGHT MEDIATE THECA CELLS MECHANOSENSITIVITY

Farners Amargant i Riera [1]

[1] Department of Obstetrics and Gynecology and Center for Reproductive Health Sciences (CRepHS). Washington University in St. Louis, School of Medicine, St. Louis, MO.

Follicles are the functional unit of the ovary and are formed by an oocyte surrounded by granulosa cells. During folliculogenesis, follicles are exposed to different spatio-temporal mechanical properties within the ovary. Primordial follicles (quiescent follicles) reside in the ovarian cortex, hypothesized to be stiffer to maintain follicle quiescence. Once follicles are activated and start growing, they move to the internal region of the ovary, known as medulla, which offers a softer, more permissive environment for follicle expansion. Our lab discovered that follicles are highly mechanosensitive. In vitro culture of follicles in alginate gels of increased stiffness triggers a fibroinflammatory environment in the follicle, resulting in a decline in follicle growth and quality. Therefore, mechanical cues of the ovary are novel regulators of follicular fate and quality. However, a key question in the field is which cell type in the follicle senses the mechanical properties of the ovary and what molecular pathways transform these mechanical inputs into chemical signals. The Piezo family channels, consisting of Piezo1 and Piezo2, are mechanosensitive ion channels that play an essential role in detecting and transmitting mechanical signals to the cells in multiple tissues, such as bone, cartilage and lungs. Thus, we hypothesized that Piezo channels might be the main mechanosensitive channels of the follicle. We first evaluated RNA expression levels of Piezo1 and Piezo2 in ovaries isolated from CB6F1 mice (N=5, 6-12weeks). Piezo1 is expressed in all the follicle cells, but it is especially enriched in the theca cell layer. In contrast, Piezo2 expression is mostly in the theca cells and the corpora lutea, with minimal RNA expression in the oocyte and granulosa cells. These results show that Piezo channels are present in the follicle and are primarily found in the theca cells, a cellular structure that might stretch to allow follicles to expand. We then focused our analysis on Piezo1. Immunohistochemical analysis confirmed Piezo1 localization in theca cells (N=5 mice). To investigate Piezo1's role in theca cells, we performed ovarian stromal cell isolation and culture (N=10 mice). Stromal cells were incubated with Yoda1, a Piezo1 agonist, at different concentrations (5, 10 and 20 mm, and 1% DMSO (diluent)) for 72h. We then measured whether Yoda1 activated Piezo1 by measuring intracellular calcium. Stromal cells were incubated with Fura-2, a calcium fluorescent marker (calcium ion level is proportional to the ratio 340/380). Control cells had a maximum 340/380 ratio of 0.470, while Yoda1-incubated cells had a maximum 340/380 ratio of 0.80, demonstrating that Piezo1 is present in our stromal cell culture and that Yoda1 can induce Piezo1 opening and activation. Altogether, these results show that Piezo family channels are highly enriched in the theca cell layer where, upon activation, they allow calcium to enter. Future work will investigate whether Piezo channels confer strain-dependent mechanosensitivity to follicles and what the contribution of each Piezo channel is in follicle mechanosensing. This project has been supported by the start-up funds from the Washington University in St. Louis, School of Medicine to F.A.R.

P6: HUMAN OVARIAN STIFFNESS MEASURED BY SHEAR WAVE ELASTOGRAPHY (SWE) AS A NON-INVASIVE BIOMARKER OF REPRODUCTIVE AGING

Tomiris Atazhanova MD, MSc [1], Emily J. Zaniker BA [1], Alexis Trofimchuk MS [1], Lydia Hughes MD [1], Man Zhang MD, PhD [2], Elnur Babayev MD, MSc [1], Francesca E. Duncan Ph.D [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611, USA
[2] Department of Radiology, Michigan Medicine, University of Michigan, Ann Arbor, MI 48109, USA

The human ovary is one of the first organs to age. The mammalian ovarian microenvironment or parenchyma becomes fibro-inflammatory and stiff with age, which negatively impacts follicle development, ovulation, and gamete quality. Human ovarian stiffness may, therefore, be a biomarker of reproductive aging. The objective of this study was to use shear wave elastography (SWE) to evaluate ovarian stiffness and its association with reproductive aging parameters.

We developed a standard operating procedure to evaluate human ovarian stiffness with ultrasound-based SWE. At the time of routine diagnostic ultrasound as part of the evaluation for infertility or fertility preservation, SWE measurements were obtained in high-confidence regions (3-12 measurements/ovary) using the GE Logic Fortis ultrasound system. Additional variables collected for each participant included age, body mass index (BMI), antral follicle count (AFC), and levels of follicle stimulating hormone (FSH) and anti-Mullerian hormone (AMH). Simple correlation and multiple linear regression analyses were performed.

To date, SWE measurements and clinical data have been obtained from 38 participants ranging in age from 24 to 47 years (mean age: 34.5 ± 4.9 years). Key ovarian reserve markers (AMH, AFC, FSH) exhibited expected correlations as general population. No clear trends were observed between BMI and other parameters. Ovarian stiffness (mean, median, and max) tended to increase with age. The strongest positive trend was observed between age and maximum stiffness ($p = 0.06$, $r = 0.31$). Multiple linear regression analysis did not show statistically significant association between maximum stiffness and other parameters.

SWE can be used to measure the stiffness of the human ovarian parenchyma. Trends suggest associations between ovarian stiffness, age, and reproductive aging markers. Studies are ongoing to increase the sample size and correlate the ovarian stiffness measurements with outcomes of medically assisted reproduction.

P7: UNCOVERING THE DISTINCT ROLES OF NELF-B AND TDP-43 IN MALE MEIOSIS THROUGH SCRNA-SEQ ANALYSIS

Kavyashree Basavaraju [1], Emily Kaye [2], Karen Adelman [2], and Prabhakara Reddi [1]

[1]Department of Comparative Biosciences, University of Illinois at Urbana Champaign, Urbana, IL, 61802, USA. [2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115, USA

Male germ cell development progresses from spermatogonial stem cells to spermatozoa, majorly through three developmental stages: Spermatogonia (SG) undergo mitosis and differentiation, spermatocytes (SC) undergo meiosis to form haploid round spermatids (RS), and RS then differentiate into mature spermatozoa with acrosome and flagellum formation. Germ cells transition through developmental stages in the seminiferous epithelium, requiring precise spatiotemporal gene regulation. However, the transcriptional mechanisms controlling this gene expression remain poorly understood. Understanding this complete process is critical since any defects will typically result in infertility, which affects at least 30 million men globally.

Transcriptional regulation involves transcription factors that bind DNA, splicing factors that process RNA, and RNA Polymerase II (Pol II) pausing, which fine-tunes gene expression. Transactive response DNA binding protein of 43 kDa (TDP-43) and negative elongation factor complex member B (NELF-B) are critical in transcriptional regulation as TDP-43 regulates transcription, RNA processing, and mRNA stability, while NELF-B induces Pol II pausing. Their coordinated roles are essential for precise gene expression; accurate transcriptional control is vital for proper cell differentiation. Our study found that TDP-43 loss causes meiotic arrest and subfertility, while NELF-B loss disrupts spermatogenesis, suggesting similar defects caused by these regulators.

We investigated transcriptional changes in germ cells lacking NELF-B or TDP-43 using 10X single-cell RNA-seq (scRNA-seq) on whole testis samples from PND24 mice, where all germ cell types (SG, SC, RS) are present, and defects were observed histologically in cKO animals. scRNA-seq reads were mapped using 10x Genomics Cell Ranger and processed with Seurat to identify the cell clusters. Cell types were identified using previously defined marker genes. Cell counts were normalized by the number of mice per genotype. Differentially expressed genes (DEGs) in SG and early meiotic cells were identified via pseudobulk analysis using DESeq2, and functional enrichment analysis of the DEGs was determined by ClusterProfiler.

scRNA-seq analysis identified 23 clusters representing 24,269 cells, consolidated into ten cell types, with control (n = 11,170), NELF-B cKO (n = 6,404), and TDP-43 cKO (n = 6,695). Loss of NELF-B or TDP-43 significantly reduced SC and RS germ cells, while SG and somatic cells remained unaffected. NELF-B loss gradually impacted prophase I, whereas TDP-43 loss severely affected the pachytene stage, aligning with its high expression and role in synapsis and homologous recombination. Differential expression analysis revealed 207 DEGs in NELF-B cKO (evenly up and downregulated) and 842 DEGs in TDP-43 cKO (mostly downregulated). Downregulated genes were enriched with spermatid development and motility, indicating that NELF-B or TDP-43 loss impairs gene expression essential for spermatogenesis.

Overall, Loss of NELF-B disrupts male germ cell maturation, with TDP-43 loss worsening these defects. Interestingly, despite similar histological defects and meiotic failure in TDP-43 and NELF-B cKO mice, more genes are affected by the loss of TDP-43, likely due to its multiple roles in transcription regulation, RNA processing and stability, suggesting that TDP-43 plays a more critical role in spermatogenesis than NELF-B. Our findings highlight the distinct roles of NELF-B and TDP-43 in spermatogenesis, offering insights into male infertility and potential therapeutic targets.

P8: THE RNA-BINDING PROTEIN HNRNP I/PTBP1 REGULATES EMBRYO TRANSPORT AND UTERINE RECEPTIVITY DURING EARLY PREGNANCY IN THE MOUSE.

Athilakshmi Kannan [1], Wenyan Mei [1], Milan K. Bagchi [2], Indrani C. Bagchi [1]

Departments of Comparative Biosciences [1], Mol & Integrative Physiology [2], University of Illinois at Urbana-Champaign, Urbana, IL

Polypyrimidine tract binding protein 1 (PTBP1), an RNA-binding protein belonging to the heterogeneous nuclear ribonucleoprotein (hnRNP) family, plays an important role in alternative splicing, localization, translation, and stability of a subset of mRNAs. Our recent studies indicated a marked upregulation of PTBP1 in the uterus during early pregnancy. To elucidate the function of PTBP1 in the uterus, we utilized the Cre-Lox technology using progesterone receptor (PR)-driven Cre and generated conditional knockout mice ($Ptbp1$ [d/d]) in which Ptbp1 gene is deleted in tissues expressing PR. We carried out a six-month breeding study to test fertility. Oviducts and uteri are collected from day 4 of pregnant control and $Ptbp1$ [d/d] mice for analysis. Six-month breeding study demonstrated that $Ptbp1$ mutant females are severely sub fertile. To investigate the cause of infertility in $Ptbp1$ [d/d] females, we examined their ovarian function and acquisition of uterine receptivity to embryo implantation. Our studies revealed that ovulation was not affected in the absence of PTBP1. To further investigate, we flushed the uterine lumens of control and mutant mice on day 4 of pregnancy to retrieve blastocysts. While uterine flushing of the control mice had 8-12 blastocysts, we found only a few viable blastocysts in the uterine flushing of the mutant mice, raising the possibility that the transport of blastocysts from the oviduct into the uterus is compromised in the absence of PTBP1. Further studies revealed that cell-specific ablation of Ptbp1 from oviductal smooth muscle cells and epithelium of the isthmus led to the retention of developing embryos in the ampullary isthmus junction of the Ptbp1-null females. Collectively, these studies revealed that the RNA-binding protein PTBP1 regulates embryo transport through the oviduct and could be related to female infertility conditions like tubal pregnancy.

P9: EXPOSURE TO PHTHALATE INDUCES ABNORMAL DNA METHYLATION PATTERNS IN HUMAN ENDOMETRIAL STROMAL CELLS IMPAIRING ESTROGENIC REGULATION OF EXTRACELLULAR VESICLE SIGNALING

Jacob R. Beal [1], Arpita Bhurke [2], Jodi Flaws [2], Indrani C. Bagchi [2], Milan K. Bagchi [1]

[1] Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign
[2] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign

Di(2-ethylhexyl) phthalate (DEHP), a known endocrine disrupting chemical, is a plasticizer found in many common consumer products. High levels of DEHP exposure have been linked to adverse pregnancy outcomes, yet little is known about how it affects human uterine functions. We previously reported that the transcription factor hypoxia-inducible factor 2 alpha (HIF2α) promotes the expression of Rab27b, which controls the secretion of extracellular vesicles (EVs) that facilitate communication between multiple cell types within the pregnant uterus, ensuring reproductive success. In this study, we examine whether exposure to DEHP, or its primary metabolite mono(2-ethylhexyl) phthalate (MEHP), disrupts the critical process regulated by HIF2α. We demonstrated that, in in vitro culture, exposure of differentiating primary human endometrial stromal cells (HESC) to an environmentally relevant concentration (1 µg/mL) of DEHP or MEHP markedly reduces the expression of HIF2α without affecting the differentiation of these cells. We observed a concomitant decrease in Rab27b expression and consequent reduction in EV secretion from HESC. Our previous studies showed that estrogen acting via estrogen receptor alpha (ERα) regulates HIF2α gene expression in HESC. Interestingly, we found that DEHP or MEHP exposure disrupts estrogenic regulation of the HIF2α/Rab27b signaling pathway. ERα was no longer able to bind to the HIF2α regulatory region following phthalate treatment, and epigenetic analysis suggested that this may be due to changes in the methylation status of nearby CpG islands. We also discovered that abnormal DNA methylation was likely due to increased expression of DNA methyltransferase 1 (DNMT1) gene in response to phthalate exposure. DNA hypermethylation in response to phthalate exposure has been previously observed in certain cell and tissue types. Here, we provide a mechanism by which phthalates may induce DNA hypermethylation within endometrial cells and report that this change in methylation pattern has promoter-specific consequences, in this case disrupting estrogenic regulation of EV secretion during HESC differentiation, which would affect reproductive functions.

P10: INVESTIGATING HOW MICROTOPOGRAPHY-INDUCED MORPHOLOGICAL CHANGES AFFECT GRANULOSA CELL TRANSCRIPTION AND OVARIAN HORMONE PRODUCTION.

Gabriela Berniac [1-5]; Hana Kubo [1-4]; Xinlong Wang [5]; Guillermo A. Ameer [5]; Monica M. Laronda [1-4]

[1] Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2] Fertility & Hormone Preservation & Restoration Program, Department of Surgery, Ann & Robert H. Lurie Children's Hospital of Chicago; [3] Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago; [4] Department of Obstetrics & Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [5] Department of Biomedical Engineering, McCormick School of Engineering, Northwestern University, Evanston, IL

As of 2022, premature ovarian insufficiency (POI) is estimated to affect around 3.5% of women globally, characterized by a loss in ovarian function and hormonal dysregulation in individuals under the age of 40. One population group that is particularly affected is pediatric cancer survivors, of which there are estimated to be over 495,000 in the United States as of 2020. This is because cancer treatments involving surgery, radiation, and alkylating chemotherapies have been shown to impair ovarian function, leading to POI. In addition to infertility, individuals with POI face a greater lifetime risk of osteoporosis, cardiovascular disease and neurocognitive disorders due to reduced ovarian hormones. While current hormone replacement therapies can alleviate symptoms, they do not replicate dynamic hormone regulation, lack essential hormones and are not tolerated by many individuals. Granulosa cells (GCs) are critical hormone-producing cells that are specific to the ovary and surround the growing oocyte. During each stage of folliculogenesis, GCs undergo significant functional and morphological changes as they produce ovarian peptide and steroid hormones that act on most organ systems. Previous research has demonstrated that altering cellular shape via microtopographical cues using bioengineered micropillar structures resulted in transcriptional reprogramming of human mesenchymal stem cells through cytoskeletal reorganization and nuclear deformation. Therefore, this project aims to induce morphological changes in primary bovine GCs in vitro using micropillar structures to mimic the morphological changes that occur to GCs during folliculogenesis in vivo. We hypothesize that micropillar structures will cause transcriptional reprogramming in GCs to increase ovarian hormone production through the induction of morphological changes via microtopographical cues. Micropillar structures are fabricated using methacrylated poly(octomethylene citrate) via contact printing. This allows control over the size, shape, and spacing of the micropillars to tailor them to the specific cell type and desired deformations in the x, y, and z planes. Additionally, a primary GC isolation procedure has been developed ensuring that a majority of cells express FOXL2, a GC cell marker identifiable via ICC/IF. Baseline culture model conditions for GCs have also been characterized by measuring estradiol levels via an ELISA over a seven-day period. Furthermore, preliminary data thus far suggest a significant increase in estradiol production in GCs with induced morphological changes, although further work is necessary to confirm these results. Cytoskeletal reorganization will be identified by staining F-actin, while nuclear deformation will be quantified by calculating the nuclear shape index, where a ratio closer to 1 indicates circularity and lack of nuclear deformation. Results thus far indicate increased nuclear deformation in GCs with induced morphological changes, suggesting that transcriptional reprogramming is likely occurring. This transcriptional reprogramming can be confirmed by using RT-qPCR and ICC/IF to identify expected gene and protein expression changes, focusing on FOXL2, along with CYP19A1 and HSD17B1, which encode enzymes crucial for hormone conversion and are expected to increase post-change in morphology. We anticipate that the knowledge obtained from this project will contribute towards developing a cell-based solution for regenerating ovarian hormone production in POI patients.

P11: CHARACTERIZATION OF A DIET-INDUCED OBESE MOUSE MODELS WITH REDUCED FERTILITY

Sameeksha Bhaye [1], Kassandra Sandoval [2], Chanaka Rabel [1], Malia Danielle Berg [1], Bruce Southey [1], Kelley Swanson [1,2] and Matthew Dean [1,2]

[1] Department of Animal Sciences, [2] Division of Nutritional Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Obesity is a chronic illness impacting men and women globally. It is linked with diseases like insulin resistance, diabetes mellitus, and subfertility. Studies indicate that women with higher BMI undergoing IVF or embryo transfer had reduced implantation and live birth rates. However, diet induce obese (DIO) mouse models have inconsistent phenotypes, and many do not report a decline in fertility as seen in obese women. Thus, our objective was to develop a DIO mouse model that mimics the changes observed in obese women.

Eight-week-old mice were randomly divided into three groups and fed diets containing 10% (low-fat diet, LFD), 45% (high-fat diet, HFD), or 60% (very high-fat diet, VHFD) kcal from fat for 18 weeks. Weight was monitored weekly, and significant weight gain was observed in both the HFD and VHFD group by the 7th week of feeding. During the 5th, 10th, and 15th week of feeding, mice were fasted overnight, and an intraperitoneal glucose tolerance test was performed. The VHFD group's glucose intolerance increased from the 5th week to 10th and 15th week. Whereas the HFD group was glucose intolerant during the 10th and 15th week. In the 18th week, a group of mice were sacrificed in proestrus post fasting for 5-6 hours. High-fat diet groups had higher adiposity and serum leptin concentration relative to LFD mice. However, there was no difference in serum insulin concentrations among the three groups. Compared to lean mice, serum cholesterol peaks had a significant rise in both high-fat diet groups. Whereas serum glycerol-3-phosphate peaks were only significantly higher in VHFD mice. Metabolites like 2,3-dihydroxybutanoic acid, dodecanoic acid, inosine, and pyrrole-2-carboxylic acid were detected in the serum of both high-fat diets but not the LFD group. In contrast, allantoin and hydroxy-3-methylglutaric acid were only detected in VHFD group's serum. Compared to LFD and HFD mice, liver triglyceride concentration was increased only in HFD group. Glycogen concentration was reduced in both HFD and VHFD groups relative to the lean mice.

Other mice from this cohort were housed with males for 60 days to compare fertility. Observance of the vaginal plug was considered day 0.5 of pregnancy and mice were sacrificed on day 18.5. If there was no plug after 60 days of co-housing, the mouse was considered infertile. HFD and VHFD groups showed a 50% and 60% reduction in fertility compared to the LFD group respectively. In mice that did mate, there was no change in litter size, but there was a significant reduction in fetal weight in both the HFD and VHFD mice. There was no change in the placental mass in all the groups. Placental efficiency was significantly reduced in the VHFD group compared to the LFD group.

In summary, we developed a DIO mouse model that mimics some features of obese women such as metabolic dysfunction, reduced fertility, and intrauterine growth restriction. Our lab's focus is to understand glycogen metabolism in the uterus, and I will further investigate how obesity affects it.

P12: EFFECT OF VITAMIN A ENRICHMENT ON OVARIAN FOLLICLE DEVELOPMENT IN WILD TYPE AND MTA TRANSGENIC MICE

C.L Brandolino [1], L. Alaverdyan1, J.L. Kipp [1]

[1] Department of Biology, DePaul University, Chicago, IL

Retinoic acid (RA) is a modulator of reproductive functions with well-studied roles in embryogenesis and sperm development. This molecule is essential to our health, and it is obtained through the consumption and subsequent metabolism of vitamin A. Previous reports by our laboratory have identified the RA signaling pathway as a primary activin target in the ovary. However, little is known about how RA and activin interaction may impact ovarian follicle development. To better elucidate the interaction and function of these two signaling pathways in the ovary, we fed a vitamin A-enriched (VAE) diet to a subfertile, activin-deficient transgenic mouse line (MTα transgenic mice). Ovarian follicle counting of 19-day-old mice showed an increase in the total number of follicles in both VAE-treated MTα transgenic mice and normal littermates (NLM) as compared to control diet mice. Similarly, at 7 weeks, VAE increased the total follicle numbers in both NLM and MTα transgenic mice. However, there was a striking difference in how this increase manifested for each genotype. On the control diet, MTα transgenic mice showed an accumulation of follicles at the early stages with decreased progression to later stages. Treatment with the VAE diet reversed this hindrance in the MTα transgenic mice, allowing follicle development to progress to later stages. VAE-treated NLM showed higher atretic follicles and decreased corpora lutea (CL), suggesting that a balanced level of RA in the body is critical for proper ovarian follicle development. Surprisingly, VAE-treated MTα transgenic mice did not show increased atretic follicles or decreased CL, suggesting vitamin A was able to overcome some defects in ovarian follicle development in these mice without causing adverse effects as seen in the NLM. Additionally, we observed that at one year of age, under the control diet, the uterus weight of MTα transgenic mice was significantly lower than that of the NLM, while VAE treatment was able to bring the uterus weight back to normal. These data suggest that vitamin A can partially overcome the ovarian and uterine defects in the activin-signaling-compromised MTα transgenic mice. The evidence provided by this study further demonstrates an interaction between activin and RA signaling pathways and the importance of this interaction in the ovary. Supported by DePaul URC grant and FSRG grant to JK.

P13: ETRANSITION OF ESR2-LINEAGE CELLS TO ESR1-EXPRESSING CELLS IN THE HYPOTHALAMIC-PITUITARY-OVARIAN AXIS: INSIGHTS FROM A NOVEL TRANSGENIC MOUSE MODEL.

Mary Bunnell [1], Ji-Eun Oh [1], Po-Ching Patrick Lin [1], CheMyong Jay Ko [1]

[1] Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

Estradiol is a critical sex steroid hormone that plays an essential role in the development and function of the hypothalamic-pituitary-ovarian (HPO) axis. Its actions are predominantly mediated by two nuclear receptors with opposing functions: estrogen receptor alpha (ESR1) and estrogen receptor beta (ESR2). While ESR1 primarily drives the key actions of estradiol, ESR2 often moderates these effects. In adult female mice, the expression patterns of ESR1 and ESR2 are well-defined within the HPO axis, with each receptor subtype typically showing mutually exclusive expression. Specifically, ESR1 is expressed in Kiss1 neurons in the hypothalamus, gonadotropes in the pituitary, and theca cells and ovarian surface epithelial cells in the ovary, whereas ESR2 is expressed in GnRH neurons in the hypothalamus, gonadotropes in the pituitary, and granulosa cells in the ovary. This study investigates the hypothesis that ESR2-lineage cells can transition to ESR1-expressing cells within the HPO axis in females. To explore this, we generated a novel transgenic mouse line in which the *Esr1* gene is selectively deleted in cells expressing Esr2 (Esr2-Esr1KO mice). We then assessed the reproductive phenotype of these mice, hypothesizing that the deletion of Esr1 would result in impaired fertility. Indeed, female Esr2-Esr1KO mice were infertile, failing to produce any litters when paired with proven male breeders (0% fertility vs. 100% in wild-type controls; $p < 0.0001$). Histological analysis revealed that Esr2-Esr1KO ovaries were smaller than those of wild-type controls and displayed abnormal features, including follicular cysts, disrupted cumulus-oocyte complexes, numerous atretic follicles, and a lack of corpora lutea, with only a few healthy follicles present. Despite responding to superovulation induction with PMSG and hCG, Esr2-Esr1KO mice released fewer oocytes compared to wild-type controls (3.47 ± 1.46 vs. 16.2 ± 4.2 in WT, $p = 0.0008$). These findings suggest that while the intra-ovarian ovulatory machinery remains functional, gonadotropin secretion is compromised in the Esr2-Esr1KO mice in which ESR1 expression was lost in cells derived from ESR2 lineages. Overall, this study suggests that some ESR1-expressing cells within the HPO axis may originate from ESR2-lineage cells. Further investigation is warranted to determine whether ESR1 expression is specifically lost in Kiss1 neurons or gonadotropes.

P14: HYPOTHALAMIC AGRP NEURONS REGULATE THE HYPERPHAGIA OF LACTATION

Kerem Catalbas [1], Tanya Pattnaik [1], Samuel Congdon [1], Christina Nelson [1], Lara C. Villano [1], Patrick Sweeney [1]

[1] Department of Molecular and Integrative Physiology, Neuroscience Program, University of Illinois Urbana-Champaign

The lactational period is associated with profound hyperphagia to meet the energy demands of nursing. These changes are crucial for the long-term metabolic health of both the mother and offspring, as both overfeeding and underfeeding during lactation increase the subsequent risk of the mother and child developing metabolic and psychiatric disorders later in life. Despite its significance, the behavioral mechanisms and neural circuitry mediating lactational hyperphagia remain incompletely understood. In this study we investigated the role of hypothalamic agouti-related peptide (AgRP) neurons in regulating the hyperphagia observed during lactation.

Using home cage feeding devices, we characterized the feeding behavior of lactating mice, observing increased meal size, altered circadian feeding patterns, and reduced sensitivity to gut-brain satiety signals. We employed chemogenetic, immunohistochemical, and *in vivo* imaging approaches to examine the activity of AgRP neurons during lactation. Our results indicate that AgRP neurons exhibit increased sensitivity to negative energy balance and are directly activated by the lactational state. Chemogenetic inhibition of AgRP neurons significantly reduced feeding in lactating mice, underscoring their critical role in mediating lactational hyperphagia.

These findings reveal that lactation alters multiple components of feeding behavior and position AgRP neurons as pivotal regulators of this process. Understanding the neural mechanisms underlying lactational hyperphagia is essential for addressing metabolic health issues in postpartum mothers and their children.

P15: INVESTIGATING THE SIGNIFICANCE AND MECHANISMS OF RNA SOLUBILITY PHASE TRANSITION DURING THE OOCYTE-TO-EMBRYO TRANSITION IN VERTEBRATES

Sijie Chen [1], Meng Ma [1], Jing Yang [1]

[1] Department of Comparative Biosciences, University of Illinois at Urbana Champaign, Urbana, IL

In vertebrate animals, the transition from a prophase I arrested oocyte to a totipotent embryo is one of the most dramatic events in development. This oocyte-to-embryo transition (OET) involves a series of events, including meiotic oocyte maturation, ovulation, fertilization, and zygotic genome activation (ZGA), which occur sequentially in the absence of de novo transcription. To precisely control these stages of transcriptional silencing, oocytes and early embryos rely on maternal gene products, such as maternal mRNAs and proteins, which are synthesized during oogenesis and deposited in the oocyte cytoplasm. However, the precise mechanisms by which these maternal factors are remodeled to orchestrate the OET remain largely unclear. Recently, we demonstrated a novel post-transcriptional remodeling process, where mRNAs undergo the solubility phase transition during vertebrate OET. The mRNAs that bind with protein condensates are found in the Triton X-100-resistant pellet fraction, characterized as "insoluble," while those present in the supernatant fraction are termed "soluble". From the RNA-seq of each fraction, a significant proportion of RNAs are initially in a soluble state in fully grown oocytes but then shift to the insoluble fraction during Xenopus oocyte maturation and zebrafish oocyte-to-embryo transition (OET), identifying this process as a soluble-to-insoluble phase transition (S-I). We also identified another group of RNAs that undergo an insoluble-to-soluble phase transition (I-S).

While the S-I phase transition is correlated with the maternal mRNA decay, which is essential for the proper progression of early embryonic development, the I-S phase transition is correlated with the germplasm remodeling, which is essential for the gamete development. In this study, we aim to unveil the molecular mechanisms that underlie these phase transitions, and how they are regulated, to provide new insights into post-transcriptional regulation during development and reproduction. Supported by R35 grant.

P16: QUANTITATIVE MODELING TO CHARACTERIZE MATERNAL INFLAMMATORY RESPONSE OF HISTOLOGIC CHORIOAMNIONITIS IN PLACENTAL MEMBRANES

Teresa Chou [1], Karolina J. Senkow [1], Megan B. Nguyen [1], Payal V. Patel [1], Kirtana Sandepudi [1], Lee A. Cooper [1], Jeffery A. Goldstein [1]

[1] Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL

Purpose: The placental membranes are a key barrier to fetal and uterine infection. Inflammation of the membranes, diagnosed as maternal inflammatory response (MIR) or alternatively as acute chorioamnionitis, is associated with adverse maternal-fetal outcomes. MIR is staged 1-3 by the depth of maternal neutrophil infiltration, with higher stages indicating more hazardous inflammation. However, the diagnosis relies upon subjective evaluation and has not been deeply characterized. The goal of this work is to develop a machine learning cell classifier for 8 major cell types of the placental membrane (amniocyte, decidual cell, chorionic stromal cell, endothelial cell, trophoblast, neutrophil, lymphocyte, and macrophage) and quantify inflammation in MIR1-2.

Methods: H&E-stained placental membrane slides were digitized. To build a placental membrane cell classifier, a convolutional neural network was trained on a dataset of hand-annotated and machine learning-identified cells. Overall cell class-level metrics were calculated. The model was applied to 20 control, 20 MIR1, and 23 MIR2 placental membrane cases. MIR cell composition and neutrophil distribution were assessed via density and Ripley's cross K-function. Clinical data, including Apgar score, maximum maternal temperature, and maternal WBC count, were compared to neutrophil density and distribution.

Results: The classification model achieved a test-set accuracy of 0.845, Matthew's Correlation Coefficient of 0.814, and F1-score of 0.843, with high precision and recall for amniocytes, decidual cells, endothelial cells, and trophoblasts. Using this model to classify 53,073 cells from healthy and MIR1-2 placental membranes, we recapitulated prior histologic definitions of MIR, where MIR1-2 cases have higher neutrophil density and fewer decidual cells and trophoblasts, and neutrophils colocalize heavily around decidual cells in healthy placental membranes and around trophoblasts in MIR1. In addition, we provide new evidence that neutrophil density impacts distribution in MIR, and neutrophil colocalization with trophoblasts correlates with maximum maternal temperature during labor.

Conclusions: This paper introduces cell classification into the placental membranes and quantifies cell composition and neutrophil spatial distributions in MIR. Our data agrees with prior knowledge of MIR and provides evidence for a quantitative phenotype of neutrophils that correlates with low-grade fever, a nuanced symptom of systemic inflammation. This work aids future studies seeking to understand the pathophysiology behind chorioamnionitis and pushes the field forward to improving maternal-fetal outcomes.

P17: UNDERSTANDING THE IMPACT OF OBESITY ON METHYLATION CHANGES IN ENDOMETRIAL CARCINOMA

Harun Cingoz [1] and J. Julie Kim [1]

[1] Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Endometrial cancer (EC) is the most common gynecological cancer in women, with incidence rising due to increasing obesity rates. Obesity not only elevates EC risk but also contributes to its progression through chronic inflammation, insulin resistance, and hyperinsulinemia. Studies have shown that diet and overall body weight can alter DNA methylation patterns, potentially leading to the dysregulation of genes involved in cell growth and differentiation. We hypothesized that obesity leads to hypomethylation in EC by decreasing the expression of DNMT3B, a key enzyme in de novo DNA methylation. Using The Cancer Genome Atlas (TCGA) tumor data, we analyzed DNA methylation profiles and DNMT expression levels in 233 endometrioid EC patients categorized as obese (BMI > 30) or lean (BMI < 25). In vitro validation was performed using Ishikawa cell spheroids co-cultured with fat spheroids. Patients were also stratified into quartiles based on DNMT3B expression levels for further methylation analysis. Obese EC patients showed 615 differentially methylated CpG sites, with 70% being hypomethylated. These sites were enriched in EC related pathways such as TGF-beta, PI3K/AKT/mTOR, and WNT beta-catenin signaling. DNMT3B expression was significantly reduced in obese patients' tumors and in Ishikawa spheroids cocultured with fat spheroids. Patients with low DNMT3B expression regardless of their obesity status exhibited 6518 differentially methylated CpG sites, 70% being hypomethylated, in pathways similar to those in obese patients. A comparative analysis revealed 160 genes commonly differentially methylated in both obese and DNMT3B-low groups. Principal Component Analysis based on the 615 DMRs clustered 233 EC patients into two clusters. This clustering identified two obese patient groups: one with low levels of DNMT3B and another with higher DNMT3B expression. The group with higher DNMT3B levels had clinical outcomes similar to those of lean patients but exhibited a higher recurrence rate than any other group. Identifying these groups based on obesity-specific DMRs and DNMT3B expression could provide insights into how obesity-related methylation changes affect cancer progression and recurrence.

P18: THE OVARIAN MACROPHAGE-DERIVED MULTINUCLEATED GIANT CELL TRANSCRIPTOME REVEALS UNIQUE MOLECULAR PROFILES

Aubrey Converse[1], Shweta S. Dipali[1], Madeline J. Perry[1], Francesa E. Duncan[1], Michele T. Pritchard[2,3]

[1]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; [2]Department of Pharmacology, Toxicology & Therapeutics and 3Institute for Reproductive and Developmental Sciences, University of Kansas Medical Center.

Female reproductive aging is characterized by a decline in oocyte quantity and quality as well as increased chronic inflammation and fibrosis in the ovarian microenvironment. While fibroinflammaging occurs in various tissues, its onset in the ovary precedes non-reproductive tissues. Application of single-cell transcriptomics to the ovary has revealed shifts in immune cell populations that contribute to ovarian inflammaging. However, due to technical limitations of capturing large cells, these methods have overlooked a highly age-dependent macrophage population, macrophage-derived multinucleated giant cells (MNGCs). MNGCs are highly penetrant in ovaries of reproductively old mice but completely absent in young ovaries. MNGCs are associated with chronic inflammatory pathologies in other tissues including granulomas and the foreign body response, but little is known about ovarian MNGCs. Therefore, we defined the specific molecular signature of MNGCs using laser capture microdissection and RNA sequencing. The top 50 expressed MNGC genes were predominantly involved in iron homeostasis, antioxidant activity, cell and protein degradation, metabolism, and immune processes. When compared to normal (single-nucleus) ovarian macrophages, MNGCs exhibit upregulation of genes associated with adaptive immunity-related gene ontology (GO) pathways, suggesting cross-talk between these macrophages and adaptive immune cells. Furthermore, GO pathways related to developmental processes were down regulated in MNGCs compared to normal ovarian macrophages, suggesting MNGCs are terminally differentiated. Discreet differences in genes involved in degradation, immune, and metabolic processes between MNGCs and normal ovarian macrophages indicate MNGCs have unique functions in the ovary apart from that of other macrophages. Of interest, components of the T-cell co-receptor CD3 were uniquely expressed in MNGCs. Upon validation, CD3 expressing cells were found within the giant cell complex, indicating that MNGCs reside within broader immune complexes that also contain lymphocytes. The co-localization of T-cells and macrophages in these giant cell complexes and the adaptive immune processes highlighted by their transcriptome indicate these unique immune complexes may be similar to granulomas observed in other inflammatory pathologies and likely play a significant role in modulating inflammaging of the aging ovary. Current work is underway to investigate the drivers of MNGC formation and their impact on the ovarian microenvironment.

P19: CO-EXPOSURE TO TRIBUTYLTIN AND A HIGH-REFINED CARBOHYDRATE DIET LEADS TO ABNORMAL FERTILITY, PLACENTA, PREGNANCY OUTCOMES, AND OFFSPRING METABOLIC COMPLICATIONS IN FEMALE RATS.

C.S. da Costa [1], J.F.Z. de Oliveira [1], M.A.G. Doro [1], J.B. Graceli[1]

[1]Department of Morphology, Federal University of Espírito Santo, Vitoria, Brazil

Tributyltin (TBT) is a persistent contaminant with adverse effects on reproductive health. Concurrently, the global surge in high-refined carbohydrate diet (HCD)-induced obesity raises concerns about metabolic and reproductive complications and its impact on pregnancy. While studies individually explore the consequences of TBT and HCD exposure, the combined effects on gestation, placental function, and fetal metabolism remain unexplored. Thus, this study aimed to test the hypothesis that co-exposure to TBT and HCD impair placenta morphophysiology and fetal metabolism. Female rats were divided into: 1) Control rats treated daily with a vehicle (0.4 % ethanol, via gavage) and fed with standard laboratory chow Socil (RD); 2) TBT rats treated daily with TBT (100 ng/kg/day, via gavage) and fed with RD; 3) HCD rats that were fed with HCD (~30 % refined sugars) 4) Rats that were treated daily with TBT and feed with HCD diet for 15 days. And then mated overnight by pairing with the control male. The mating confirmation was done in the morning by the presence of sperm in the vaginal smear (gestational day (GD) 0.5). On GD 19.5, females were anesthetized with ketamine and xylazine (90 mg/kg and 4.5 mg/kg, ip) and euthanized by cardiac puncture. The uterus was harvested, from which the fetuses and placentas were obtained. The sex, number and weight were recorded. Pregnant serum, liver, and fetuses were collected for metabolic evaluations. Placentas were separated by sex and collected for histology and molecular assessments. All the protocols were approved by the Animal Use Ethics Committee of the Federal University of Espírito Santo (N° 01/2021). Comparisons between the groups were performed using one- or two-way ANOVA, followed by Tukey's multiple comparison test. All data are reported as the mean ± SEM. HCD-TBT females increased body weight gain during exposure 9, 12 and 15 days. However, during the pregnancy HCD-TBT rats presented reduced gestational weight gain in the GD 6, 9 and 12 and an abnormal glucose tolerance compared to control. Laparotomy evaluation allowed to observe a reduced implantation efficiency and increased pre-implantation loss in HCD-TBT rats. Female HCD-TBT fetuses reduced liver weight and elevated liver cholesterol content. Male HCD-TBT fetuses increased glucose, reduced HDL levels and reduced liver weight. In both sexes HCD-TBT fetuses, the placenta reduced diameter. Female placenta reduced total and basal layer thickness, increased glycogen cell clusters, and high GLUT1 protein expression. Male HCD-TBT placenta increased mTOR protein expression, reduced labyrinth layer thickness, and increased glycogen cell clusters. Female HCD-TBT placenta increased collagen deposition. Together, these data suggest that the co-exposure of TBT plus HCD induces pregnancy metabolic abnormalities. Moreover, fetal and placental morphology and metabolic changes were also observed, but with some sexual dimorphism features.

P20: PHTHALATES ALTER THE EXPRESSION OF GENES ASSOCIATED WITH THE ARYL HYDROCARBON RECEPTOR PATHWAY IN THE MOUSE OVARY

Angela E. Dean [1], Ramsés Santacruz-Márquez [1], Mary J. Laws [1], Jodi A. Flaws [1]

[1] Department of Comparative Biosciences, University of Illinois Urbana-Champaign

Phthalates such as diethyl (2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP) are chemicals commonly used as plasticizers and are known endocrine disrupting chemicals (EDCs). Exposure to these chemicals over a person's lifetime is a concern, especially as women age. Most women reach menopause around the age of 51. However, other factors such as exposure to EDCs can influence the age at menopause. Recently, we have shown that EDCs known as phthalates alter aryl hydrocarbon receptor (AHR) signaling in ovarian cells. However, it is not known how phthalates affect AHR signaling in the ovary and throughout reproductive aging *in vivo*. The AHR is a transcription factor that is a member of the Per/ARNT/Sim- basic helix-loop-helix (bHLH) family, which plays a role in ovarian follicle development and function. Once a ligand is bound, the AHR heterodimerizes in the nucleus with ARNT to promote transcription of its downstream targets such as CYP1A1 and CYP1B1. We hypothesized that phthalate exposure alters expression of genes associated with the AHR signaling pathway in the ovary throughout reproductive aging. We treated adult female CD-1 mice with varying doses (0ppm, 0.15ppm, 1.5ppm, or 1500ppm) of phthalates (DEHP, DiNP, or a phthalate mixture) in the diet for 1, 6, and 12 months. The 0.15ppm and 1.5ppm doses correspond to human daily exposure and occupational exposure, respectively. We also included a high dose (1500ppm) to examine the dose effect. The phthalate mixture (Mix) contained DEHP, DiNP, diethyl phthalate (DEP), dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), and benzyl butyl phthalate (BzBP). At each time point, ovaries were collected, and we examined expression of genes associated with AHR signaling using qPCR. Treatment with 1.5ppm and 1500ppm of the Mix at 12 months reduced expression of *Ahr* in the ovary, while DEHP and DiNP alone did not alter *Ahr* expression at this time point. Furthermore, none of the phthalates changed expression of *Ahr* at 1 and 6 months compared to controls. At 1 month, 0.15ppm and 1500ppm DiNP and 1.5ppm and 1500ppm Mix exposures increased expression of Arnt compared to controls. DEHP, DiNP, and Mix at 6 and 12 months did not change the expression of Arnt compared to controls. At 1 month, 1.5ppm Mix increased expression of *Cyp1a1* in the ovary compared to controls, as did 1500ppm DEHP at 6 months. At 12 months, exposure to 1.5 ppm and 1500ppm DiNP reduced expression of *Cyp1a1* compared to controls. Treatment with the different phthalates did not alter expression of *Cyp1b1*. Overall, the tested phthalates affected the expression of genes in the AHR pathway in the ovary. However, the different phthalates influenced changes in gene expression at varying time points and doses.

P21: SUBACUTE EXPOSURE TO A MIXTURE OF CONTAMINANTS (TRIBUTYLTIN + CADMIUM + MERCURY) LEADS TO REPRODUCTIVE COMPLICATIONS IN FEMALE RATS

F.C.F. Dos Santos [1] and J.B. Graceli [1]

[1] Department of Morphology, Federal University of Espirito Santo, 290440-090, Vitória, Brazil.

In a real-world environment, we are exposed to a complex mixture of contaminants, such as heavy metals, fertilizers, and pesticides. Tributyltin (TBT), cadmium (Cd), and mercury (Hg) contamination have been reported simultaneously in different ecosystems, including in seafood used for human intake. These chemicals individually cause reproductive complications. However, the reproductive consequences of exposure to a mixture of TBT plus Cd plus Hg (MIX) are not well known. Thus, this study aimed to test the hypothesis that MIX exposure alters reproductive parameters in female rats. Adult female Wistar rats (12 weeks old, ± 230g, n=5-10 per group) were exposed to a mixture of contaminants, such as TBT (100ng/kg/day), Cd (100 mg/L), and Hg (1st dose 4.6 mg/kg, subsequent dose 0.07 mg/kg/day) for 15 days. All protocols were approved by CEUA-UFES (04/2023). Estrous cyclicity, hormone levels, ovarian and uterine histomorphometry were assessed. The data was shown as mean ± SEM. For Gaussian and non-Gaussian data, the Student's t and the MannWhitney tests were used for statistical analysis, respectively. MIX rats spend less time in the estrous phase (p<0.05). We observed a borderline increase in serum estrogen levels and an increase in FSH levels in MIX rats compared to controls (p=0.08 and p<0.05, respectively). MIX exposure led to ovarian abnormalities, such as the presence of lipid droplets and hyperemia inside of corpora lutea, and the presence of inflammatory cells in ovary stroma. Specifically, a reduction in corpora lutea number and an increase in preantral follicles number were observed in MIX rats (p<0.05 and p<0.05, respectively). An increase in the preantral /antral follicles ratio was observed in MIX rats compared to the control (p<0.05). In addition, the presence of mast cells increased in ovarian of MIX rats (p<0.05). Uterine irregularities, such as hyperemia in the endometrium, the presence of vacuolization, and apoptosis in the luminal and glandular epithelium (GE) were observed in MIX rats. Compared to controls, an increase in uterine and myometrium areas were observed in MIX rats (p<0.05 and p<0.05). The presence of hyperplastic glands and inflammatory cells inside of glands was observed in the endometrium of MIX rats. A reduction in the number and height of GE was observed in MIX rats compared to the control (p<0.05 and p<0.05). The uterine mucus gland score was lower in MIX rats (p>0.05). An area of uterine gland lumen increased in MIX rats (p<0.05). After ovariectomy (OVX), a reduction in uterus weight was observed in OVX-control and OVXMIX rats compared to respective intact groups, respectively (p<0.05 and p<0.05). Uterus weight after estrogen replacement (E2) in both OVX-control and OVX-MIX rats was similar to weights observed in intact rats of their respective groups (p>0.05). We didn't observe a reduction in body weight in OVX-E2-MIX rats compared to intact-MIX rats (p>0.05). Together, these data suggest that the mixture of MIX contaminants induces reproductive abnormalities in female rats, perhaps affecting neuroendocrine control and fertility, which will be analyzed in the future.

P22: SPATIAL TRANSCRIPTOMIC MAPPING OF P16INK4A POSITIVE REGIONS REVEALS A SENESCENT SIGNATURE IN THE NATIVE POSTMENOPAUSAL HUMAN OVARY.

Pooja Devrukhkar [1], Mark A. Watson [2], Natalia Murad [2], Hannah Anvari [1], Tommy Tran [2], Elisheva Shanes [3], Mary Ellen G. Pavone [1], David Furman [2], Simon Melov [2], Birgit Schilling [2], Francesca E. Duncan [1-2]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, U.S.A., [2] Buck Institute for Research on Aging, Novato, CA 94945, U.S.A., [3] Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, U.S.A.

The human ovary is one of the first organs to age and exhibit an early onset of functional decline affecting reproductive longevity and overall health. Fibroinflammation, a hallmark of the aging ovarian microenvironment is consistent with presence of senescent cells in this tissue, but bona fide senescent cells have not yet been characterized or mapped in the human ovary. We performed immunohistochemistry for p16[INK4a], a canonical marker of cellular senescence across tissues, on human ovarian tissue (N=48 participants; 52-84 years old). p16[INK4a] expression localized to small clusters within the stroma and was enriched in structures including vessels, cysts, and the ovarian surface epithelium (OSE). p16[INK4a] positive regions were typically found where markers of macrophages, fibroblasts, and myofibroblasts were also expressed. Overall, there was a low level of p16[INK4a] expression within the tissue but there was a trend towards increased expression and greater heterogeneity with age. Given the distinct localization of p16[INK4a] positive regions, we hypothesized that spatially mapping the transcriptomic signature of p16[INK4a] positive regions would inform a signature of cellular senescence in the native ovary. We thus performed Digital Spatial Profiling (DSP) with GeoMx using ovarian tissue from an 80-year-old participant. We stained alternate tissue sections for p16INK4a to map positive regions onto sequential slides for spatial profiling. A total of 95 regions of interest (ROIs) were annotated, including p16-positive and -negative ROIs, ovarian regions (cortex and medulla), and ovarian structures (vessels, cysts, OSE, and stroma). Downstream analyses revealed a distinct signature specific to p16[INK4a] positive ROIs, consisting of ~30 genes, including known senescent markers LAMB1, TIMP3, and SERPINE1. Cortex and medulla p16-positive and -negative ROIs clustered apart, suggesting regional differences in the senescence signature. In an unsupervised manner, this signature could be spatially mapped back to tissues in this participant as well as two other participants (67 and 71 years old) with tight correlation to p16[INK4a] positive annotated ROIs. We thus revealed an ovary-specific transcriptome of p16-positive regions that allows us to identify region-specific cellular senescence signatures, will define new potential molecular markers of senescent cells, and broaden our understanding of the tissue microenvironment of cellular senescence.

P23: THE ROLE OF SIGLECS IN SELECTIVE SPERM CLEARANCE WITHIN THE PORCINE OVIDUCT

Hao-Chun Fan [1], Leonardo Molina [1], David J. Miller [1], Adrienne M. Antonson [1].

[1] Department of Animal Sciences, University of Illinois at Urbana Champaign, Urbana, IL.

During mammalian fertilization, most of the sperm that invade the uterus trigger leukocyte infiltration and are subsequently cleared. In contrast, sperm in the oviduct does not trigger this phagocytic response. Nonetheless, the oviduct maintains its capacity to respond to pathogens. The formation of this unique immune microenvironment in the oviduct is not fully understood but might be related to the glycocalyx on the sperm surface. Notably, the sialic acid on the glycocalyx of the sperm surface and the sialic acid-binding immunoglobulin-like lectins (Siglecs) on the epithelial cells of the female reproductive tract have been shown to influence the clearance of sperm in the uterus. Our preliminary research has also identified the presence of numerous inhibitory Siglecs (Siglecs-2, -3, -5, -10, and -11) in the epithelial cells of the oviduct. Based on these findings, we hypothesize that when sperm attaches to the uterine epithelium, the sialic acids on the sperm surface interact with immune-activating Siglecs on epithelial cells. This interaction triggers downstream signaling, producing cytokines and chemokines that activate and attract peripheral blood mononuclear cells (PBMCs) to clear the sperm. In contrast, in the oviduct, sperm may activate immune-inhibiting Siglecs, reducing the production of PBMC-activating factors. To test this hypothesis, we successfully established a primary epithelial cell culture model of the oviduct using an air-liquid interface cell culture system. In the next step, we will add PBMCs to the liquid side to observe the response of PBMCs to epithelial cell stimulation in this co-culture model. Additionally, by treating sperm with different enzymes, such as neuraminidase or sialyltransferases, and adding them to the air side, we can examine whether the PBMC response is mediated by the involvement of Siglecs on the epithelial cells. This study might provide new insights into the mechanisms underlying selective sperm clearance and the immune privilege of the oviduct, potentially uncovering novel therapeutic targets for reproductive health.

P24: SLO2.1/NALCN FUNCTIONAL COMPLEX ACTIVITY IN MOUSE MYOMETRIAL SMOOTH MUSCLE CELLS DURING PREGNANCY.

Juan J. Ferreira * and Lindsey N. Kent *, Ronald McCarthy, Alice Butler, Xiaofeng Ma, Nikita Peramsetty, Chinwendu Amazu, Alexander Zhang, Grace C. Whitter, Sarah K. England** and Celia M. Santi**.

Department of Animal Sciences, University of Illinois at Urbana Champaign, Urbana, IL.

Throughout pregnancy, the myometrial smooth muscle cell resting membrane potential shows a consistent increasing depolarization to aid in the transition of the uterus from a quiescence to a contractile state. However, the underlying regulating mechanisms remain unknown. Building upon our recent work describing the role of the Na^+-activated K^+ channel (SLO2.1) and the Non-Selective Na^+ Leak Channel (NALCN) forming the SLO2.1/NALCN complex in human myometrial smooth muscle cells (hMSMCs) at the end of pregnancy and regulating hMSMCs contractility at the end of pregnancy. Briefly, Na+ ions entering through NALCN activates SLO2.1, facilitates potassium efflux, leads to subsequent membrane hyperpolarization (cells becoming more negative) and a reduction in contractility. Conversely, decreased SLO2.1/NALCN activity results in reduced potassium efflux, what causes membrane depolarization, promotes $Ca2^+$ influx via voltage-dependent calcium channels, and increases uterine contractility.

Here we provided comprehensive insights into the role of the SLO2.1/NALCN complex in regulating mouse myometrial smooth muscle cell (mMSMC) activity across pregnancy. We observed dynamic changes in the expression of *Slo2.1* and *Nalcn* during pregnancy, both showing higher expression in non-pregnant and early gestational mice, declining towards term. Functional studies revealed that SLO2.1 channels mediate a significant portion of the K^+ current in mMSMCs, particularly in non-pregnant and early pregnancy. Importantly, the activation of SLO2.1 by Na^+ influx through NALCN leads to membrane hyperpolarization in early pregnancy stages, contributing to the maintenance of uterine quiescence. However, this effect diminishes in later pregnancy stages, correlating with reduced SLO2.1/NALCN activity. Moreover, we found that the NALCN/SLO2.1 complex regulates intracellular $Ca2^+$ responses, with its activity being more pronounced in non-pregnant and early pregnancy stages, highlighting its crucial role in modulating myometrial contractility throughout gestation. These findings shed light on the intricate mechanisms underlying uterine function during pregnancy and could open avenues for targeted pharmacological interventions in pregnancy-related complications.

P25: IODOACETIC ACID ALTERS THE EXPRESSION OF OXIDATIVE STRESS AND APOPTOSIS GENES IN THE MOUSE OVARY

Audrey Fields [1], Andressa Varella Gonsioroski [2], and Jodi Flaws [1]
Department of Comparative Biosciences and Division of Nutritional Sciences

[1] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL
[2] Veterinary Diagnostic Laboratory, Pathobiology and Diagnostic Investigation Department, Michigan State University

The incorporation of water disinfectants into the main water supply has significantly decreased the presence of waterborne diseases such as cholera. However, the interaction between disinfectants and organic material generates water disinfection byproducts (DBPs). Iodoacetic acid (IAA) is one unregulated DBP and has been reported as a reproductive toxicant. Our previous studies show that IAA inhibits follicle growth and alters the expression of steroidogenic factors in vitro, but its effects on the expression of oxidative stress markers are unknown. Thus, this study tested the hypothesis that IAA exposure alters gene expression of oxidative stress markers in the mouse ovary. To test this hypothesis, adult CD-1 mice were dosed with either water (control) or IAA (0.5 mg/L; 10 mg/L; 100 mg/L; 500 mg/L) in their drinking water for 35 days. Following this, ovaries were collected when the mice were in diestrus, RNA was extracted, reverse transcribed, and subjected to quantitative polymerase chain reaction (qPCR) to analyze the expression of glutathione markers (*Gpx1, Gpx2, Gsr, Gss, Gsta1, Gstm1, Gsto1, Gstp1 Gstt1*), superoxide dismutase markers (*Sod1, Sod2*), and apoptosis markers (*Bax, Bcl2*), and catalase. IAA exposure decreased expression of the apoptosis marker *Bcl2* in the 0.5 mg/L, 10 mg/L, 100 mg/L, and 500 mg/L treatment groups compared to control and decreased the expression of *Cat* in the 0.5 gm/L, 10 mg/L, 100 mg/L and 500 mg/L treatment groups compared to control. In addition, IAA significantly decreased the expression of the superoxide oxidative stress marker *Sod1* in the 0.5 gm/L, 10 mg/L and 100 mg/L IAA treatment groups compared to control but did not affect the expression of *Sod2* compared to control. IAA exposure decreased expression of glutathione oxidative stress markers: *Gss* in the 0.5 mg/L and 500 mg/L treatment groups compared to control, *Gsta1* in the 0.5 mg/L and 10 mg/L treatment groups compared to control, *Gstp1* in the 10 mg/L treatment group compared to control, and *Gstt1* in the 0.5 mg/L, 10 mg/L, and 500 mg/L treatment groups compared to control. IAA exposure did not alter the glutathione markers *Gpx1, Gpx2,* or *Gsr* compared to control. IAA exposure increased expression of the apoptosis marker *Bax* in the 10 mg/L, 100 mg/L, and 500 mg/L treatment groups compared to control. IAA exposure increased expression of glutathione marker *Gstm1* in the 10 mg/L, 100 mg/L, and 500 mg/L treatment groups compared to control. These data indicate that IAA exposure alters oxidative stress gene expression in the mouse ovary. Supported by NIH R21 ESO28963.

P26: THE EFFECTS OF PRENATAL EXPOSURE TO AN ENVIRONMENTALLY RELEVANT PHTHALATE MIXTURE ON OVARIAN GENE EXPRESSION IN F1 AND F3 FEMALE MICE

Endia Fletcher [1], Mary J. Laws [1], Jodi A. Flaws

[1] Department of Comparative Biosciences, University of Illinois, Urbana, IL

Phthalates are a family of synthetic chemical compounds used as plasticizers and solvents. Phthalates are commonly detected in the environment as mixtures in building materials, medical equipment, and personal care products. Although virtually 100% of the U.S. population has measurable exposure to several types of phthalates, little research has been done to understand the transgenerational effects of prenatal exposure to phthalate mixtures on reproductive health in female offspring. Thus, this study tested the hypothesis that prenatal exposure to an environmentally relevant phthalate mixture affects cytokines expressed in the ovaries from the F1 and F3 generations. To test this hypothesis, pregnant CD-1 dams were dosed orally with vehicle control (corn oil) or four different doses of phthalate mixture (20 μg/kg/day, 200 μg/kg/day, 200 mg/kg/day, 500 mg/kg/day). The pregnant dams were allowed to deliver pups naturally and selected ovaries from the F1 female offspring were collected at PND 21, PND 60, and 6 months. The remaining F1 female offspring were mated with non-treated CD-1 male mice to produce the F2 generation and F2 females were mated with non-treated CD-1 male mice to produce the F3 generation. Ovaries were collected from the F3 generation on PND 60. RNA was isolated from the F1 and F3 ovaries and used in qPCR reactions to quantify expression of selected cytokine genes: interleukin1β (Il-1β), interleukin 4 (Il4), interleukin 6 (Il6), and interleukin 10 (Il10). Sera were also collected to measure C-reactive protein (CRP) levels, a reliable marker of inflammation. The results indicate that prenatal exposure to the phthalate mixture at 500 mg/kg/day decreased the expression of Il-1β in the F1 ovaries at PND 60 compared to controls. Exposure to the mixture at 20 μg/kg/day also decreased the expression of Il-1β, Il4, and Il10 in F1 ovaries compared to control at 6 months. The results also indicate that in the F3 generation at PND 60, the mixture increased the expression of Il-1β at 200 mg/kg/day, Il10 at 20 μg/kg/day and 500 mg/kg/day, and Il6 at 20 μg/kg/day and 200 μg/kg/day compared to controls. Lastly, the sera analysis indicated a significant increase in CRP levels at 200 μg/kg/day of the mixture in the F1 generation at PND 21. Collectively, these data suggest that the phthalate mixture may interfere with the immune system of F1 and F3 offspring in the ovary long after initial exposure. This research is supported by NIH R01ES032163 and NIH Diversity Supplement R01ES032163S.

P27: TCO-EXPOSURE TO MONO(2-ETHYLHEXYL) PHTHALATE AND ELEVATED TEMPERATURE INHIBITS MOUSE ANTRAL FOLLICLE GROWTH AND STEROIDOGENESIS *IN VITRO*

Sundus Ghuneim [1]; Gretchen Ruschman [2]; Caroline Harper [2]; Madison Wilson [2]; Patrick Hannon [1,2]

[1] Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, United States
[2] Department of Obstetrics and Gynecology, University of Kentucky, Lexington, KY, United States

Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer found in common consumer goods, such as household items, food packaging, and medical devices. DEHP is rapidly metabolized upon ingestion to mono(2-ethylhexyl) phthalate (MEHP), which is bioactive and known to exert endocrine-disrupting toxicities. Due to the extensive use of DEHP in consumer goods, humans are ubiquitously exposed and are vulnerable to endocrine and reproductive dysfunction by MEHP. Studies have shown that MEHP exposure disrupts the essential ovarian processes of antral follicle growth and steroidogenesis. Simultaneously, humans are ubiquitously exposed to non-chemical stressors, such as rising global temperatures, and studies have shown that exposure to elevated temperatures can impair female reproductive health. Given the progression of climate change and the unavoidable exposure to both increasing heat and DEHP, this study used an *in vitro* follicle culture system to test the hypothesis that exposure to high temperature (HT) will exacerbate the negative effects of MEHP exposure on antral follicle growth and steroidogenesis. Antral follicles were isolated from 4-5-week-old CD-1 mice and cultured for 96 hours in media containing follicle-stimulating hormone at a control temperature (CT; 37°C) or high temperature (HT; 42°C; 8hr/day). Follicles were treated with vehicle control (dimethyl sulfoxide; DMSO) or MEHP (0.2-20μg/ml) in both temperature groups for the 96-hour culture period. Separate incubators were used for the CT and HT treatment groups, in which all HT treatments occurred for 8 hours per 24-hour period to model daytime, occupational exposure to high temperatures. Follicular growth was measured every 24 hours via inverted microscopy. Follicles and media were collected at 24 and 96 hours for steroidogenic gene expression analyses and sex steroid hormone measurements, respectively (n=3-7 with 5-10 follicles/group/replicate, p ≤ 0.05). Antral follicle growth was decreased by HT+DMSO treatment compared to the CT+DMSO control group at 48, 72, and 96hr. Further, antral follicle growth was decreased by HT+MEHP compared to CT+DMSO (48-96hr) and even when compared to certain doses' CT+MEHP dose equivalents (72-96hr). While progesterone and testosterone levels remained unchanged, estradiol levels were decreased at 96hr by CT+MEHP and HT+DMSO compared to CT+DMSO, indicating decreases in estradiol levels induced by MEHP alone and HT alone. However, estradiol levels at 96hr were also decreased by HT+MEHP compared to CT+DMSO and compared to certain CT+MEHP dose equivalents, indicating a potential exacerbation of MEHP-induced decreases in estradiol levels by HT treatment. No changes were observed in mRNA levels of *Star*, *Hsd3b1*, or *Cyp17a1*. However, mRNA levels of *Cyp11a1* (96hr), *Hsd17b1* (24hr), and *Cyp19a1* (96hr) were decreased by HT+DMSO and all doses of HT+MEHP compared to CT+DMSO. These findings suggest that exposures to HT and MEHP inhibit antral follicle growth and disrupt steroidogenesis, where the observed decreases in steroidogenic mRNA levels correlate to the decreases in estradiol levels. Further, these findings establish for the first time that HT may exacerbate MEHP-induced effects on ovarian steroidogenesis. Thus, combined exposure to these ubiquitous chemical and non-chemical stressors may act synergistically to potentiate ovarian dysfunction. S

P28: TARGETING GALANIN RECEPTOR 3 TO BLOCK OOCYTE MEIOTIC MATURATION FOR NON-HORMONAL CONTRACEPTIVE DEVELOPMENT

Lauren R. Haky [1], Jeffrey Pea1, Yiru Zhu [1], Hoi Chang Lee [1], Alisha Ramos [1], John Proudfoot [2], Francesca E. Duncan [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[2] Ovarian Contraceptive Discovery Initiative (Independent Consultant)

Hormonal contraceptives are highly effective and extensively used but have a variety of side effects, underscoring the need for novel non-hormonal alternatives. Oocyte meiotic maturation, involving germinal vesicle (GV) breakdown and polar body extrusion, is required to generate a fertilizable gamete. Inhibiting this biological process could be a potent non-hormonal contraceptive target. We, therefore, screened 818 compounds from the SelleckChem Bioactives Library in a phenotypic screening assay of oocyte meiotic maturation and identified a potent hit compound, maintaining oocytes arrested at prophase I in the GV-intact stage in 100% of oocytes. Oocytes incubated without hit compound extruded polar bodies. This compound was annotated to target Galanin Receptor 3 (GalR3), a transmembrane GPCR that, when stimulated, inactivates adenylyl cyclase 3 (ADCY3) and limits intracellular cAMP production. High levels of oocyte cAMP maintain meiotic arrest. We expect GalR3 stimulation induces meiosis by inactivating ADCY3 and decreasing cAMP levels, whereas GalR3 inhibition promotes ADCY3 activity, maintains high cAMP levels, and prevents meiotic resumption. In validation studies, we demonstrated that GalR3 is expressed in oocytes. Hit compound meiotic arrest was highly reproducible with internal library frozen and independent vendor fresh stocks and showed a potent dose-dependent response to hit compound (IC50: 84.35nM). Hit compound maintained prolonged meiotic arrest for at least 90 hours demonstrating the long-term efficacy. Moreover, the block in meiotic progression was reversible; following compound washout, oocytes resumed meiosis, extruded polar bodies, and exhibited normal spindle morphology. Structural analogs of the hit compound and additional GalR3-specific inhibitors resulted in either full or partial inhibition of meiotic progression, supporting GalR3 as the actual target and providing critical structure activity relationship information. Studies are ongoing to confirm the biological function of GalR3 during oocyte meiotic maturation.

P29: CGAS-STING PATHWAY ACTIVATION IN OVARIAN SECONDARY FOLLICLES COMPROMISES FOLLICLE SURVIVAL, GROWTH, AND OOCYTE QUALITY

Prianka H. Hashim [1], Aubrey K. Converse [1], Fatimah Aljubran [2], Jennifer L. Gerton [2], Francesca E. Duncan [1]

[1] Department of Obstetrics & Gynecology, Northwestern University, Chicago, IL 60611
[2] Stowers Institute for Medical Research, Kansas City, MO 64110

The follicle is the functional unit of the ovary comprised of an oocyte surrounded by somatic granulosa and theca cells. Individuals are born with a finite number of follicles, indicating that mechanisms which remove poor-quality follicles to ensure fertilization of only high-quality oocytes are essential. In multiple cell types, the cGAS-STING pathway serves as a surveillance mechanism. cGAS (cyclic GMP-AMP synthase) is a cytosolic double-stranded DNA (dsDNA) sensor which, upon activation forms a second messenger (2'3'-cGAMP), activates STING (Stimulator of Interferon Genes) and initiates an inflammatory response. We hypothesize that the cGAS-STING pathway functions as a surveillance mechanism in follicles to detect and respond to dsDNA in oocytes leading to inflammation and removal of poor quality follicles. We examined the expression of cGAS and STING in ovarian follicles, and then utilized an ex vivo follicle growth system to determine whether treatment with Q-VD-OPH & ABT-737, known to induce release of mitochondrial DNA into the cytosol, activated the cGAS-STING pathway and impacted follicle survival, growth, and oocyte quality. Using RNA in situ hybridization and immunocytochemistry, we demonstrated that cGAS expression is enriched in the oocyte, whereas STING expression is enriched in granulosa and theca cells. cGAS expression in the oocyte increased significantly between the primary-to-secondary follicle transition. To assess the function of the cGAS-STING pathway, early secondary follicles were treated with Q-VD-OPH & ABT-737 or vehicle control. Short-term treatment was non-toxic to follicles as assessed by live-dead staining and resulted in pathway activation as measured by significantly increased expression of phospho-IRF3, phospho-TBK1, and phospho-STING compared to controls. To assess whether follicles produced a cGAS-dependent inflammatory signature in response to treatment, follicles from cGAS wild type or knockout mice treated with Q-VD-OPH & ABT-737 or vehicle control were submitted for RNASeq. Wild-type treated follicles produced an interferon-dependent inflammatory response that was not replicated in treated cGAS KO follicles. Long-term 10-day treatment of follicles encapsulated and cultured in alginate hydrogels exhibited decreased follicle survival and growth in addition to increased oocyte atresia compared to controls. These results demonstrate that the cGAS-STING pathway machinery is expressed in early secondary ovarian follicles and is responsive to pharmacologic activation. Additionally, long-term treatment compromises follicle quality suggesting that the cGAS-STING pathway may function as an active surveillance mechanism to regulate folliculogenesis.

P30: LOSS OF SIK3 IN GRANULOSA CELLS DISRUPTS DOWNSTREAM FSH SIGNALING PATHWAYS AND METABOLISM

Emily T. Hayes [1] and Carlos Stocco [1]

[1] Department of Physiology and Biophysics, School of Medicine, University of Illinois at Chicago, Chicago, IL 60612

The salt-inducible kinases (SIKs) are serine/threonine kinases and recently discovered regulators of female fertility. Previously, our group showed that SIK3 global knockout mice are infertile with ovaries that lack antral follicles. Further, we demonstrated that knocking down SIK3 in the granulosa cells of the ovarian follicle in vivo results in subfertility, follicle growth defects, increased apoptosis, and decreased steroidogenic gene expression in the granulosa cells. However, the mechanism by which SIK3 regulates follicle growth, survival, and function in the granulosa cells is unknown. To begin to determine direct and indirect targets of SIK3 in response to short FSH stimulation, we performed a phosphoproteomic screen. To do this, we isolated and cultured granulosa cells from preantral follicles of SIK3$^{flox/flox}$ mice. We then knocked down SIK3 in vitro via adenovirus-mediated Cre recombination for 48 hours. Thereafter, cells were treated with 150 ng/ml recombinant FSH for 45 minutes. Lysates were subjected to Western blot to confirm knockdown and FSH stimulation. A 60% knockdown of SIK3 protein expression was achieved, and both samples showed robust pAkt expression, indicative of FSH stimulation. Thus, lysates were then used in a commercial phospho antibody array. From a total of 584 phosphosites measured, we found 30 targets that were >1.5-fold greater in SIK3 knockdown cells versus control and 40 targets that were <0.70-fold less in SIK3 knockdown cells. Enrichment analysis of these targets to identify the most overrepresented functional annotation terms revealed significant association with "PI3K Akt signaling," "EGF EGFR signaling," "Ras signaling," "ErbB signaling," "Hippo signaling regulation," "Focal adhesion," "Leptin signaling," and "Insulin signaling." Interestingly, the 3rd-most downregulated phosphorylation site was serine 794 of insulin receptor substrate-1 (IRS-1), which contains the SIK consensus sequence and thus points to a plausible direct target of SIK3 in granulosa cells. This data points to SIK3 being involved in both signaling pathways downstream of FSH and metabolism in granulosa cells, possibly serving as a critical connector between fertility and metabolism. Supported by R01HD097202 grant.

P31: IN VIVO OVULATION KINETICS ARE DISRUPTED WITH ADVANCED REPRODUCTIVE AGE

Anna Heinrich [1], Caroline E. Kratka [1], Emily J. Zaniker [1], Jeffrey Pea [1], Luhan T. Zhou [1], Hoi Chang Lee [1], Emmett Kelly [1], Alisha Ramos [1], Yiru Zhu [1], Elnur Babayev [1], Francesca E. Duncan [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Ovulation refers to the process by which a mature metaphase II (MII)-arrested egg within a cumulus-oocyte-complex (COC) is released from a large antral follicle and is essential for fertilization. We previously demonstrated that fewer eggs are ovulated and corpora lutea formed in mice of advanced reproductive age compared to young counterparts following superovulation due to an increase in failed ovulatory events, including unruptured luteinized follicles and large antral follicles that did not rupture. To further understand the underpinnings of the age-dependent decrease in ovulatory capacity, we performed an in vivo time course of ovulation in reproductively young (6-12 week old) and old (12-14 month old) mice and compared the kinetics of ovulation. To do this, we hyperstimulated young and old mice and collected one ovary and oviduct from each animal for histologic analysis and the contralateral ovary and oviduct for COC collection at 0, 4, 8, 11, 12, or 13h post-hCG. Fewer COCs were collected from reproductively old mice at all time points examined. The kinetics of ovulation was also altered, with the majority (51.6%) of COCs having ovulated at 12h in young mice compared to only 7.3% in old mice. Moreover, in response to the ovulatory cues, there were age-dependent changes in the follicular response. Relative to young controls, follicles from reproductively old mice did not accumulate as much follicular fluid and expand to the same terminal size, did not undergo thinning of the mural granulosa cell layer in preparaticaemmon for follicular rupture, and did not have COCs that expanded to the same degree or contain mature MII-arrested eggs. To distinguish whether there was an age-dependent delay or impairment in ovulation, we performed an additional extended time course (0, 13, 16, and 20h post-hCG) in reproductively young and old mice. While few COCs (7.9%) from reproductively old mice ovulated by 13h, the majority (85%) of COCs in young ovaries had ovulated by this point. However by 16h, most (68.8%) COCs from reproductively old mice had ovulated. These results demonstrate that the kinetics of ovulation are delayed with advanced reproductive age. Transcriptomic studies are ongoing to determine the underlying molecular mechanisms.

P32: IRX3 AND IRX5 FOSTER HEALTHY OOCYTE-PRE-GRANULOSA CELL INTERACTIONS DURING GERMLINE CYST BREAKDOWN AND PRIMORDIAL FOLLICLE FORMATION

Alaina Houghton-Chamberlain [1], Lauren Lansing [1], Linda Wang [1], Joan S. Jorgensen [1]

[1] Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, USA

The foundation of the ovarian reserve occurs during fetal development in mammals and across animal phyla. Initially, oocytes exist in clusters called germline cysts. Late in gestation and shortly after birth in mice, germline cyst breakdown occurs, which includes a massive transfer of cytoplasmic cargo, including organelles such as Golgi, from donor oocytes to neighboring oocytes via intercellular bridges. Donor oocytes die while acceptor oocytes become surrounded by pre-granulosa cells to form primordial follicles. Previously, we discovered that expression of two Iroquois family homeobox transcription factors, IRX3 and IRX5, expanded from ovarian pre-granulosa cells to include the oocyte during germline cyst breakdown. As homeobox transcription factors, IRX3 and IRX5 are typically localized to the nucleus of cells; however, we were surprised to detect IRX3 in oocyte cytoplasm concentrated towards oocyte-oocyte interacting interfaces. Our previous studies using genetically modified mouse models demonstrated that IRX3 and IRX5 direct oocyte—pre-granulosa cell interactions critical to establishing robust primordial follicles that are equipped to transition into mature follicles with healthy oocytes. Therefore, based on these data, we hypothesize that IRX3 and IRX5 foster healthy follicle cell interactions by optimizing cytoplasmic cargo accumulation within acceptor oocytes via interactions with oocyte cytoskeleton components during cyst breakdown and primordial follicle formation. To test our hypothesis, we examined oocyte morphology within ovaries from *Irx3-Irx5^{EGFP}; Irx3-Irx5^{EGFP}* double knockout (DKO) compared to wild type mice. Because DKO embryos die by embryonic day 15 (E15), we collected ovaries at E14.5, cultured them for 5 days to allow time for germline cyst breakdown and primordial follicle formation, and then processed them for histology. Results showed that although there were no differences in the total number of oocytes or oocyte size between the two groups, immunofluorescent imaging showed that DKO ovaries had a reduced proportion of oocytes with Golgi rings that also presented in abnormal shapes and loci when compared to wild type ovaries. Next, to understand the cytoplasmic function of IRX3, we conducted co-immunoprecipitations (n=3) and mass spectrometry to identify binding partners within wild type ovaries at postnatal day 0 or birth (P0) when germline cyst breakdown and primordial formation is active. Mass spectrometry identified 10 interacting proteins, 4 nuclear and 6 cytoplasmic. Of the cytoplasmic proteins, 5 belonged to the septin family, SEPTIN 7, 11, 2, 9 and 8. Septins heterodimerize in complexes to form a range of structures that coordinate cell division, cell compartmentalization, and polarity maintenance. Double-label immunofluorescent staining and Proximity Ligation Assays (PLA) in P0 ovary sections confirmed co-localization of IRX3 and SEPTIN7 in the cytoplasm of oocytes. Additional studies are underway to validate other septin proteins. Altogether, our results suggest an integral relationship between IRX3 and IRX5 with the oocyte cytoskeleton, in particular SEPTIN 7, to optimize intracellular machinery organization and facilitate oocyte—pre-granulosa cell assembly into healthy primordial follicles that survive into adulthood.

P33: DI(2-ETHYHEXYL) PHTHALATE INCREASES EXPRESSION OF SEVERAL OXIDATIVE STRESS MARKERS IN THE MOUSE OVAR

Zane C. Inman [1], Endia Fletcher [2], Justin Huff [2], Taylor Seaton [2], Mary J. Laws [3], Jodi A. Flaws [3]

[1] Department of Comparative Biosciences, University of Illinois Urbana-Champaign, Urbana, Illinois

Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in a wide range of consumer products including building materials, food and beverage containers, and children's toys. This is concerning because DEHP can leach out of products and enter the human body. Furthermore, DEHP has been shown to exhibit endocrine disrupting abilities after both short-term and long-term exposures. However, little is known about the effects of DEHP on the ovary. Thus, this study tested the hypothesis that DEHP alters gene expression in the mouse ovary. To test this hypothesis, female CD-1 mice aged 39-40 days were orally dosed with either vehicle control (corn oil) or DEHP (20µg/kg/day - 200mg/kg/day) for 10 days. The ovaries from these mice were then collected immediately post-dosing and subjected to gene expression analysis for regulators of oxidative stress. The results show that DEHP exposure at both 200 µg/kg/day and 200 mg/kg/day significantly increased ovarian expression of superoxide dismutase 1 (*Sod1*), catalase (*Cat*), glutathione peroxidase 1 (*Gpx*), glutathione S-transferase kappa 1 (*Gstk1*), and glutathione S-transferase alpha 1 (*Gsta1*). DEHP exposure at all doses significantly increased ovarian expression of superoxide dismutase 2 (*Sod2*), glutathione S-transferase theta-1 (*Gstt1*), and glutathione S-transferase theta-2 (*Gstt2*). Additionally, DEHP exposure at 20 µg/kg/day significantly increased ovarian expression of glutathione peroxidase 2 (*Gpx2*). DEHP exposure at 200 µg/kg/day significantly increased ovarian expression of glutathione S-transferase pi 1 (*Gstp1*) and DEHP exposure at 200 mg/kg/day significantly increased ovarian expression of glutathione S-transferase zeta 1 (*Gstz1*). Finally, DEHP exposure at all doses significantly decreased ovarian expression of glutathione synthetase (*Gss*). All of the selected genes are related to the regulation of oxidative stress in the body. *Sod1* and *Sod2* are genes that encode the enzyme superoxide dismutase, an enzyme that breaks down superoxide radicals in the body. Similarly, *Cat* is a gene that encodes the enzyme catalase, an enzyme responsible for breaking down hydrogen peroxide in the body. *Gpx, Gpx2, Gss, Gsta1, Gstp1, Gstk1, Gstt1, Gstt2,* and *Gstz1* are all responsible for the production and transfer of glutathione, another prominent antioxidant in the body. Together, these data suggest that DEHP exposure for 10 days affects oxidative stress pathways in the mouse ovary.

P34: ROLE OF TDP-43 IN POSTNATAL DIFFERENTIATION OF EPIDIDYMIS AND EPIDIDYMAL MATURATION OF SPERM

Irene Infancy Joseph[1] **and Prabhakara Reddi**[1]

[1]**Department of Comparative Biosciences, UIUC.**

TAR DNA/RNA-binding protein of 43 kDa (TDP-43) is a ubiquitously expressed and evolutionarily conserved protein with roles in gene transcription and RNA splicing. Aberrant expression of TDP-43 has been identified in sperm from infertile men. Knockout in mouse spermatogonia and Sertoli cells led to meiotic arrest and loss of blood-tests-barrier, respectively, leading to infertility. The current project aims to understand TDP-43's involvement in epididymal function and sperm maturation.

Squash preparations of seminiferous tubules and immunofluorescence analysis with C-terminal and N-terminal TDP-43 antibodies showed TDP-43 expression in the nuclei of Step 1-8 spermatids. Expression decreased notably in Step 9-10 spermatids, localizing to the manchette region. TDP-43 was also found in the centriole of approximately 85% of Step 10-16 spermatids, suggesting a role in sperm morphogenesis. In contrast, only 20% of caudal sperm showed centriole staining, indicating potential modifications during epididymal transit.

Caudal sperm revealed significant TDP-43 localization in the acrosome and post-acrosomal regions of the sperm head, the latter not seen in testicular sperm. Additionally, 98% of caudal sperm exhibited tail staining, contrasting with 60% observed in testicular sperm. This suggests gain and relocalization of TDP-43 in sperm during epididymal transit. Immunohistochemistry demonstrated TDP-43 expression in the principal, basal, clear, and myoid cells of the Initial Segment (IS), Caput, Corpus, and Cauda of the epididymis. TDP-43 was identified in the epididymosomes, which deliver proteins to the sperm during epididymal maturation.

To address the requirement of TDP-43, we employed Defb41-iCre-mediated deletion of TDP-43 specifically in the principal cells of the IS and caput. Immunohistochemistry at postnatal day 35 (PND 35) confirmed successful TDP-43 deletion in IS and Caput. The height of the epididymal epithelium appeared reduced (n=1). Notably, the cauda of cKO mice exhibited abnormalities in coiling and morphology (n=1). This disruption may reflect functional deficiencies of the IS and Caput and alterations in paracrine signaling influencing postnatal differentiation of cauda.

Analysis of adult cKO mice (3 months) revealed that TDP-43 was absent in the principal cells of IS and Caput, but present in clear, basal, and myoid cells. Immunofluorescence of caudal sperm from cKO mice demonstrated reduced levels of TDP-43 in the acrosome, post-acrosomal region, and tail of caudal sperm, suggesting that sperm acquire TDP-43 mainly in the proximal epididymis.

Our findings highlight TDP-43's critical role in epididymal function and sperm maturation. Future work using markers including Izumo and CRISP2 will determine if loss of TDP-43 in the proximal epididymis disrupted the overall sperm maturation process. CASA analysis and fertility trials will determine the impact on sperm motility and fertilizing ability, respectively.

P35: ULTRA-STRUCTURAL ANALYSIS OF SYNAPTONEMAL-COMPLEX STRUCTURE USING DNA-PAINT MINFLUX NANOSCOPY

Sasha Kakkassery[1]**, Reza Rajabi-Toustani**[1,2]**, Dr. Huanyu Qiao**[1,2]**.**

[1]**Department of Comparative Biosciences**
[2]**Carl R. Woese Institute for Genomic Biology**

The synaptonemal complex (SC) is a railroad-like protein complex that bridges homologous chromosomes during prophase I of meiosis. The axial elements (AEs), including SYCP3, form the two side rails of the SC and are connected by transverse filaments, SYCP1. The central element (CE) of the SC, including SIX6OS1, lays on top of the SYCP1, and further stabilizes the connection between the two AEs. The SC forms between homologous chromosomes in wild-type spermatocytes but between sister chromatids in Rec8-/- mutants. By studying SC remodeling at the crossover sites, we aim to better understand the roles of the SC in crossover formation. Using Minimal Photon Flux (MINFLUX) nanoscopy to compare Rec8-/- mutants and wild type, we demonstrated that the Rec8-/- mutation results in a decrease in SYCP3 and SIX6OS1 thickness due to sister-chromatid pairing in Rec8-/- spermatocytes, as opposed to homologous-chromosome pairing in wild type. Despite the difference in thickness between the mutant and wild type being established, the number of layers that constitute the AEs and the organization of chromatin loops by AEs are yet to be determined.

Edu, an analog of thymidine, is used to label the chromatin loops of newly synthesized sister chromatids, allowing visualization of the interaction between sister chromatids and AEs. Conventional microscopy and super-resolution microscopies, such as SIM, STED, STORM, and PALM, can achieve resolutions of 10-100 nm, but visualizing molecular interactions and conformations at the molecular scale (~1 nm) remains challenging. MINFLUX nanoscopy, with its ability to detect structures at 1-2 nm resolution, offers insights into the chromatin-loop organization and axis structures. By combining MINFLUX with sequentially multiplexed DNA-based labeling (DNA-PAINT), MINFLUX nanoscopy can efficiently detect multiple antigens/target proteins simultaneously. Thus, using SYCP3 and EdU immunostaining, DNA-PAINT MIFLUX provides an opportunity to elucidate intricate chromosome structures along the SC, particularly at crossover sites.

We found that the layers of the AE vary from one end of the chromosome to the other, and the chromatin loops of the two sister chromatids mingle at AEs and do not have distinct layers within the AEs. In addition, the two AEs of the two homologous chromosomes fuse at the crossover sites to stabilize the DNA recombination during diplonema.

In summary, this DNA-PAINT MINFLUX study provides a brand new view of meiotic chromosome structure and its function in terms of synapsis and recombination.

P36: EFFECT OF CYP26 INHIBITION ON OVULATION IN MICE

Abby Keisker [1] and Jingjing Kipp [1]

[1] Department of Biological Sciences, DePaul University, Chicago, IL

The active metabolite of vitamin A, retinoic acid, is vital to several physiological functions and is most notably associated with vision and organ development. Its role in the male reproductive system is well understood, yet studies on female reproduction are lacking. The ovaries express retinoic acid receptors and enzymes that are crucial in stereognosis and oogenesis. Recent studies suggest that retinoic acid increases serum concentrations of estradiol and progesterone. Our previous studies have shown that both retinoic acid and R115866 increase granulosa cell proliferation. R115866 is a Cyp26b1 inhibitor that blocks retinoic acid metabolism and increases endogenous retinoic acid levels. Studies by others have also suggested that retinoic acid or vitamin A improves oocyte maturation and embryo survival in domestic animals. Based on these findings, we hypothesize that retinoic acid may play a critical role in ovulation. To test this hypothesis, we examined the effect of R115866 injection on the outcomes of superovulation in mice. Female mice were injected daily with vehicle (control) or R115866 for seven days starting at day 15 or day 44, Superovulation was then induced with PMSG/hCG followed by oocyte collection. The numbers of oocytes collected in the day 15 groups were 7.4 +/- 2.1 (control) and 15.7+/- 1.2 (R115866); the numbers of oocytes collected in the day 44 groups were 6 +/- 2.5 (control) and 17.6 +/- 2.5 (R116866), suggesting increased ovulation by elevated endogenous retinoic acid levels. We also observed an increase in cyst formation in the mice treated with R115866 in both age groups. Overall, our results suggest that retinoic acid plays an important role in ovulation. Currently, we are investigating the underlying mechanisms. Funded by DePaul USRP grant to AK and FSRG grant to JK.

P37: NELF-B IN SERTOLI CELLS IS CRUCIAL FOR THE INITIATION AND MAINTENANCE OF MEIOSIS IN GERM CELLS IN MICE.

Shivi Khurana [1], Helena D. Zomer [1] and Prabhakara P. Reddi [1]

[1] Department of Comparative Biosciences, University of Illinois UrbanaChampaign, Urbana, IL, 61802, USA

Recruitment of RNA polymerase II (Pol II) to the promoter is essential for transcription. Negative elongation factor (NELF) associates with Pol II in the promoter-proximal region and inhibits transcription elongation, called Pol II pausing. NELF-B which forms the core of the NELF protein complex, is expressed in the testis. Based on the fact that Sertoli cells express genes in a stage-specific manner during the cycle of the seminiferous epithelium, we hypothesized that pausing might play a crucial role in Sertoli cell function. We conditionally knocked out (cKO) the NELF-B gene in mouse Sertoli cells (SCs) using Amh-Cre. Mice were evaluated at young (postnatal day 21, PND 21) and adult (3–10 months old) age. cKO mice of all age groups showed a significant (P < 0.01 by t-test) decrease in testicular weight. Histopathological analysis revealed that testicular atrophy started at PND 21 and persisted in adults. We found a reduction in seminiferous tubule diameter, vacuoles within the seminiferous epithelium, and an increase in the luminal apoptotic cells. Consistent with this, the adult cKO males were infertile with low sperm count and morphological defects. SCs are in close contact with germ cells and play a pivotal role in the progression of spermatogenesis. Therefore, to quantitatively assess the germ cell populations impacted due to the loss of NELF-B in SCs, immunostaining was performed using γH2AX and SP-10 antibodies. At PND 21, NELF-B cKO testes indicated an increase in Type B spermatogonia and a decrease in Leptotene/Zygotene and Pachytene spermatocytes as well as round spermatids. Fifty tubules per testis cross-section were counted for each wild type (n=3) and cKO (n=3) and analyzed by t-test. Overall, we observed that meiosis was severely impacted, potentially due to impaired mitosis to meiosis transition. This defect may stem from the SCs driven Retinoic Acid (RA) pathway, which is critical for the transition of mitotically dividing Type B spermatogonia to prophase I of meiosis I. To investigate this mechanism, NELF-B was knocked down using small interfering RNA (siRNA) in the TM4 Sertoli cell line. Knockdown efficiency was validated by RT-qPCR and Western blot, revealing a 70% decrease in NELF-B expression. Furthermore, RT-qPCR analysis of TM4-NELF-B knockdown cells showed a significant reduction (n=3, P < 0.05 by t-test) in the mRNA expression of Sertoli cell specific RA pathway genes, including CRbp1 and Aldh1A1. These findings are consistent with RTqPCR analysis of primary Sertoli cells isolated from cKO mice (n=1). Therefore, our data suggests that NELF-B may functionally impact the execution of RA signalling for the initiation and maintenance of meiosis in germ cells. Thus, the absence of NELF-B in Sertoli cells led to defects in the onset and progression of meiosis. Future work will characterize the crosstalk between Sertoli and germ cells impacted by the loss of NELF-B. Together, these results identify NELF-B as an essential regulator of spermatogenesis and male fertility.

P38: THERAPEUTIC POTENTIAL OF HUMAN CATHELICIDN LL-37 STIMULATED UC-MSC EXOSOME FOR TREATING POLYCYSTIC OVARY SYNDROME

Hyunho Kim [1], Hang-soo Park [1], Mohammad Mousaei Ghasroldasht [1], Farzana Liakath Ali [1], Beckman Analea [1], Nina Hafner [1], Ayman Al-Hendy [1]

[1] Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL 60637, USA.

Introduction: Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, diagnosed with the presence of hyperandrogenism, oligo-anovulation, and polycystic ovarian morphology. Currently, no medication directly treats PCOS, so current treatment is limited to managing symptoms commonly associated with PCOS, such as hormonal imbalance, infertility, and metabolic syndrome. Previous studies found that treatment with extracellular vesicles(EV) derived from human mesenchymal stem cells (MSC) improves reproductive and metabolic parameters in the Letrozole-induced PCOS model. Analyzing the MSC effect on the *in vitro* PCOS model and human PCOS theca cells showed the improved phenotypes were mediated by the anti-inflammatory factor Interleukin-10(IL-10), which has immune-suppressive and anti-inflammatory effects. Antibacterial peptide LL37, the only member of the human cathelicidin family, is a unique peptide that can regulate innate immune and inflammation responses like IL-10. This work aimed to investigate whether LL37 can improve the efficacy of mesenchymal stem cell(MCS) derived EV therapy on an *in vitro* PCOS model.

Methods: Umbilical-derived MSC(UC-MSC) was treated with LL-37(10 μg/mL). Anti-inflammatory factor IL-10 was measured in MSC, and the results were compared before and after treatment with LL-37. EVs were isolated from UC-MSC in conditioned media. Androgen-producing human adrenocortical carcinoma (H295R) cells were used as an *in vitro* PCOS model. H295R cells were divided into a control group, a group treated with naïve exosomes, or a group treated with LL-37 stimulated exosomes. Cell proliferation was measured by counting cells. Androgenic production was measured through steroidogenic gene expression with quantitative real-time polymerase chain reaction(qRT-PCR) and testosterone-level enzyme-linked immunosorbent assay(ELISA).

Results: The IL level of LL-37 stimulated MSC(119.7 ± 14.24pg/ml) was statistically significantly higher than the IL-10 level of untreated MSC(231.6 ± 11.87 pg/ml). H295R cells treated with LL-37 EV(4.5±0.6×106 cells) had fewer cells than untreated H295R cells(5.6±0.5×106 cells), suggesting slower cell proliferation. LL-37 stimulated EV group had significantly lower Steroidogenesis-related gene (Cyp11a1: 0.82 ± 0.03 fold, Cyp17a1: 0.73 ± 0.04 fold, Dennd1a: 0.81 ± 0.04 fold) than naïve exosome group(Cyp11a1: 1.0 ± 0.03 fold, Cyp17a1: 1.1 ± 0.04 fold, Dennd1a: 0.95 ± 0.03 fold). LL-37 exosome group(2.6 ± 0.05 ng/ml) had statistically significant lower testosterone compared to control(5.6 ± 0.05 ng/ml) and lower testosterone compared to naïve exosome group(3.7 ± 0.78 ng/ml).

Conclusion: LL-37 improves UC-MSC EV's anti-inflammatory and inhibitory effect of androgen production on the *in vitro* PCOS Model. LL-37 enhanced UC-MSC in H295R cells shows promising results and therapeutic potential to treat PCOS, consistent with previous studies investigating stem cell therapy on PCOS. Further studies are warranted to confirm the findings in animal and human samples.

P39: IMPACT OF RETINOIC ACID RECEPTOR ALPHA CONDITIONAL KNOCKOUT ON OVARIAN FOLLICLE DEVELOPMENT IN THE MOUSE

Zlata Bogin 1, Audrey Urbanowski [1], Abby Keisker [1], Carl White [2], Jingjing Kipp [1]

[1] DePaul University, Chicago, IL
[2] Rosalind Franklin University of Medicine & Science, North Chicago, IL, USA

The ovaries are the primary endocrine and reproductive organs in females. The ovarian follicle is the basic structural and functional unit of the ovary. When there is abnormal development in ovarian follicles, infertility and reproductive diseases such as polycystic ovarian syndrome (PCOS), premature ovarian failure (POF), and ovarian cancers may occur. Retinoic acid (RA), a biologically active derivative of vitamin A, is a signaling molecule that plays a vital role in various physiological processes, including embryonic development, tissue differentiation, and reproduction. We have shown that RA stimulates granulosa cell proliferation through a cell signaling cascade involving Retinoic Acid Receptors (RARs), which have three isoforms alpha, beta, and gamma. In this study, we characterized a new retinoic acid receptor alpha conditional knockout (RARA cKO) mouse model with RARA deleted in granulosa cells during embryonic development. The body weight and uterus weight of the RARA cKO mice were decreased at week 7 but returned to normal at week 15 and 12 months. The animals were either sub-fertile or infertile with phenotypic evidence of POF. In addition, a variety of ovarian pathologies were observed, including the presence of hemosiderin, increased cyst formation, and abnormal follicle counts. Overall, this study provides evidence of the involvement of retinoic acid signaling, mediated by RARA, in ovarian development and fertility. This study will help improve our understanding of the prevention and treatment of ovarian diseases. Supported by an interinstitutional research grant to JK and CW, a DePaul Graduate Student Research Grant to ZB and a URC grant to JK.

P40: THE EFFECTS OF A COMBINATION OF WESTERN DIET AND PHTHALATE EXPOSURE ON INFLAMMATORY MARKERS IN THE OVARY

Stav Kramer [1], Zach Bomstein [2], Ashley Deviney [1], Mary Laws [1], Kimberly Allred [2], Clinton Allred [2], Jodi A. Flaws [1]

[1] Department of Comparative Biosciences, University of Illinois at Urbana Champaign, Urbana, IL.
[2] University of North Carolina Greensboro, NC.

Phthalates are a diverse group of chemicals commonly used in a wide range of products, including food packaging, children's toys, and plastic containers. These compounds have been shown to negatively impact female reproductive health, but their effects on the ovary in conjunction with a western diet—characterized by high fat and processed foods prevalent in the United States—are not well understood. This study investigated how the combined exposure to a western diet and phthalates affects inflammatory processes in the ovary, with a focus on expression of immune function markers (Il-1α, Il-1β,Il-1r1, Il1r2, Il-4, Il-6, Il-10, and Tnf-α). To do so, female C57BL/6 mice were divided into six experimental groups: control diet with vehicle, control diet with di(2ethylhexyl) phthalate (DEHP), control diet with diisononyl phthalate (DiNP), western diet with vehicle, western diet with DEHP, and western diet with DiNP. RNA was extracted from the ovaries of these mice to measure the expression levels of specific immune-related genes. The results indicate that DiNP exposure significantly increased in Il-1β expression in the ovary, irrespective of whether the mice were on a vehicle or western diet. Furthermore, DiNP exposure significantly decreased Il-1r2 expression in the ovary in mice on a control diet and mice on a western diet. In contrast, control diet with DEHP, control diet with DiNP, western diet with vehicle, western diet with DEHP, and western diet with DiNP did not significantly affect expression of Il-1α, Il-1r1, Il-4, Il-6, Il-10, and Tnf-α compared to control diet with vehicle. Overall, these findings indicate that DiNP exposure alters the expression of some inflammatory markers in the mouse ovary. These findings also indicate that western diet may not influence the expression of the selected immune markers in the mouse ovary. Supported by NIH R01 ES034112.

P41: MULTIPLE HUMAN INDUCED PLURIPOTENT STEM CELL LINES DIFFERENTIATED WITH A LIGAND-BASED APPROACH RECAPITULATE GRANULOSA CELL DEVELOPMENT AND EXPRESS STEROIDOGENIC PATHWAY GENES.

Hana Kubo [1-4]; Lina Lu [5,6]; Ruli Gao [5,6]; Monica M. Laronda [1-4]

[1] Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2] Fertility & Hormone Preservation & Restoration Program, Department of Surgery, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; [3] Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; [4] Department of Obstetrics & Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [5] Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL; [6] Center for Cancer Genomics, Robert H. Lurie Cancer Center, Northwestern University, Chicago, IL

Premature ovarian insufficiency (POI) is defined as the loss of ovarian function prior to the age of 40. Due to the loss of hormone production, POI patients suffer from health issues such as osteoporosis, increased risk of cardiovascular disease, and psychological distress. Current hormone therapies prescribed to patients do not restore the full ovarian hormonal milieu, do not respond to signals from the pituitary resulting in lack of cyclicity, and the doses are given at supraphysiologic and static. To improve on these current therapies, we have generated a monolayer ligand-based differentiation protocol for human induced pluripotent stem cells (hiPSCs) to generate granulosa-like cells (GLCs). These hiPSC-derived GLCs could become the foundation for a personalized cell-based hormone therapy that is comprehensive, responsive, and dynamic. We treated hiPSCs with WNT-agonist CHIR99021 treatment for 2 days, followed by a combination treatment of BMP4, FST, and DKK1-inhibitor, gallocyanine, for 3 days. We have tested our protocol on three hiPSC lines. Expression of pluripotency marker *POU5F1* was reduced during differentiation, while expression of the intermediate mesoderm markers *PAX2, LHX1*, and *WT1* and bipotential gonad marker *GATA4* peaked at day 2. Peak expression of the bipotential gonad marker *NR5A1* occurred at day 5. Interestingly, *FOXL2* expression peaked at day 2 and drops by day 5. In one cell line, FOXL2 protein peaks at day 2 by Jess Western while there is the highest percentage of FOXL2-expressing cells at day 5 by immunofluorescence. Additionally, *CYP19A1* and *HSD17B1* along with *AMH* are induced during this differentiation protocol, suggesting these GLCs can be steroidogenic and produce peptide hormones. scRNA-seq analysis of the GLCs with integration of multiple primary human granulosa cell datasets demonstrated that a small subset of the heterogeneous population generated following this GLC protocol cluster with prepubertal ovarian granulosa cells rather than fetal or adult granulosa cells, suggesting that the cells generated in this protocol are in a hormonally immature state. Future directions include continuing to optimize culture conditions to enrich for the GLCs within our culture and to mature the GLCs for maximized hormone production. Put together, the data suggests that cells differentiated with our protocol show promise as a foundation for a personalized cell-based hormone therapy. *This work is supported by the NIH/NICHD R01HD104683 (MML, HK), HuBMAP U01HD110336 (MML, RG, HK, LL), and Gesualdo Family Research Scholar (MML) and NIH/NCI training grant T32 CA009560 (HK).*

P42: EXPOSURE TO PHTHALATES DISRUPTS OVULATORY LUTEINIZING HORMONE/ CHORIOGONADOTROPIN RECEPTOR SIGNALING AND DECREASES DOWNSTREAM PROSTAGLANDIN PRODUCTION IN HUMAN GRANULOSA CELLS *IN VITRO*

Katie Land [1-2]; Hong Xu [2]; James Akin [3]; Patrick Hannon [1,2]

[1] Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, United States; [2] Department of Obstetrics and Gynecology, University of Kentucky, Lexington, KY, United States; [3] Bluegrass Fertility Center, Lexington, KY, United States

Humans are ubiquitously exposed to phthalates, which are widely used chemicals that disrupt female reproductive health. Phthalates are a family of solvents and plasticizers found in commonly used products including cosmetics, food/beverage containers, medical tubing, and car upholstery, among other sources. Phthalates undergo rapid metabolism in the body, and these metabolites can target the ovary. Due to their classification as endocrine-disrupting chemicals, exposure to phthalates and their active metabolites could impair ovulation. To best mimic human exposure, this study used an environmentally relevant phthalate metabolite mixture (MPTmix), which was derived from urinary phthalate measurements in pregnant women. The ovulatory process is initiated by an endogenous surge of luteinizing hormone (LH) or clinical treatment with human chorionic gonadotropin (hCG, a potent LH analogue). LH/hCG binding to its receptor (LHCGR) on ovarian granulosa cells promptly activates various intracellular signaling cascades, including pathways mediated by protein kinase A (PKA), protein kinase B (AKT), and extracellular signal-regulated kinase 1/2 (ERK1/2). Additionally, it is postulated that ERK1/2 signaling drives the production of prostaglandin E_2 (PGE_2) via transcription of *PTGS2*, a prostaglandin synthase. Increases in PGE_2 are vital for the inflammatory changes that occur during ovulation, including cumulus-oocyte complex expansion, follicle rupture, and angiogenesis. We hypothesized that MPTmix exposure would disrupt essential signaling cascades involved in ovulation (PKA, AKT, ERK1/2), alter mRNA levels of *PTGS2* and enzymatic activity of *PTGS2* protein, and decrease PGE2 levels. Granulosa-lutein cells from follicular aspirates of women undergoing *in vitro* fertilization were acclimated in culture to regain LH/hCG responsiveness. Prior to hCG treatment, cells were exposed for 48hr to vehicle control (dimethylsulfoxide, DMSO) or varying doses of the MPTmix (1-500μg/ml). Following treatment ± hCG and ± MPTmix, cells and media were collected at 0, 0.5, 6, 12, 24, or 36hr to measure the levels of cAMP (upstream of PKA), p-PKA, p-AKT, p-ERK1/2, PTGS2 mRNA, PTGS2 activity, and PGE2 levels (n=3-11; p≤0.05). Treatment with hCG alone rapidly (0.5hr post-treatment) increased cAMP, p-PKA, p-AKT, and p-ERK1/2 levels when compared to DMSO. However, treatment with hCG+MPTmix 500μg/ml decreased cAMP, p-PKA, and p-ERK1/2 levels relative to the hCG ovulatory control group at 0.5hr. Similarly, hCG treatment increased PTGS2 mRNA levels and PTGS2 activity at all timepoints when compared to DMSO alone. Treatment with hCG+MPTmix decreased PTGS2 mRNA levels at 6hr (500μg/ml), 12hr (1, 10, 500μg/ml), 24hr (all doses), and 36hr (100, 500μg/ml) when compared to hCG. Additionally, hCG+MPTmix treatment decreased PTGS2 activity at 24hr (all doses) and 36hr (1, 100, 500μg/ml) relative to hCG. The decreases in PTGS2 activity correlate with the 24hr and 36hr MPTmix-induced decreases (all doses) in PGE2 levels compared to hCG. These data demonstrate that phthalate exposure alters requisite ovulatory signaling cascades, and has further downstream inhibitory effects on mediators that drive functional changes leading to oocyte release. These findings in human granulosa cells exposed to an environmentally relevant mixture of phthalates suggest that phthalates may impair the ovulatory process in women. Supported by R01ES033767, R00ES028748, P30ES026529.

P43: SPERM SAFE: MAXIMIZING THE PRESERVATION AND RECOVERY OF LIMITED NUMBERS OF MALE GERM CELLS

Hoi Chang Lee [1], Drew Klemann [2], Tahlia Smith [2], Francesca E. Duncan [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL
[2] Center for Reproductive Science, Masters of Reproductive Science & Medicine Program Northwestern University, Chicago, IL

While life preserving, cancer treatments such as radiation, chemotherapy, and surgery can threaten fertility in men, women, and children. Fertility preservation strategies for males are simpler than for females because large numbers of sperm are present in the ejaculate of healthy males, and it is relatively straightforward to obtain and freeze sperm. However, fertility preservation in males with conditions of extremely low numbers of germ cells is more complex. Fortunately, with intracytoplasmic sperm injection (ICSI), fertility can technically be restored with only a single sperm. Previous studies have reported that the zona pellucida (ZP) protects the oocyte, as well as discovered that the mouse ZP can store low numbers of mouse sperm for optimal recovery and viability after freezing. By drilling a hole into the ZP and removing the cytoplasm followed by decellularization, the ZP became our "sperm safe." We reported that decellularized(de-celled) mouse ZPs can be used as a mouse sperm safe for the reliable storage and recovery of functional sperm. We hypothesize that de-celled ZPs from porcine and bovine eggs can also be used as a "sperm safe" for the reliable storage and recovery of functional sperm that can be used for ICSI. For the procedure of this project, we isolated bovine and porcine ZPs, loaded mouse or human sperm into the sperm safe, frozen and thawed the ZPs, and recovered sperm from the sperm safe derived ICSI. Porcine ovaries were able to obtain 1.6 times (9.4 bovine COCs vs 15.2 porcine COCs per ovary) more COCs per ovary compared to bovine ovaries. For this reason, we decided to use porcine ovaries for this project. The number of sperm safe recovered after the freeze-thaw was 34 out of 35 for fresh and 35 out of 37 for de-celled ZPs. In addition, the number of sperm recovered after the freeze-thaw was 96 out of 175 for fresh and 81 out of 165 for de-celled. No significant differences were found in sperm recovery between fresh and de-celled sperm safes. To assess the structure of the de-celled ZP, we collected and processed ZP samples to perform scanning electron microscopy and transmission electron microscopy. The de-celled porcine ZP are ideal naturally occurring biomaterial for the preservation and recovery of small numbers of sperm. It has a good potential model for clinical applications in cases of azoospermia or low sperm counts but further studies are required to optimize a protocol.

P44: THE EFFECTS OF SHORT-TERM AND CHRONIC DAILY PROPYLPARABEN EXPOSURE ON ESTROUS CYCLICITY AND FERTILITY IN MICE

Jie Li [1], Swarna Pal Tisha [1], and Ayelet Ziv-Gal [1]

[1] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois

Parabens are a group of chemicals widely used as preservatives in cosmetics, prescription medications, and personal care products. Evidence suggests that parabens have weak estrogenic endocrine-disrupting properties. This is concerning because altered signaling of reproductive-related hormones can have marked effects on estrous cyclicity and fertility outcomes in females. The effects of propylparaben, one of the most commonly used parabens, on mouse estrous cyclicity and fertility are unknown. Here we hypothesized that propylparaben at low levels of exposure can negatively affect estrous cyclicity and fertility outcomes in mice. Thus, adult female CD-1 mice were treated with propylparaben at 0, 2, 20, and 200 µg/kg/day over the course of one, three, and six months. Estrous cyclicity was evaluated over three weeks prior to the end of each dosing period to compare cycle lengths and numbers, and time spent in each estrous stage. Fertility studies were conducted after 6 months of chronic propylparaben exposure (n=7-8 per group). Analysis of variance of cyclicity outcomes (cycle length in days and numbers of cycles in 21 days) was performed with a linear mixed model for repeated measures. The model included treatment as a fixed effect and mouse as a random effect to account for repeated measures on the same mouse. The effect of treatment on the marginal means of the outcome variables was compared using Fisher's Least Significant Difference test. Overall, significant statistical differences were limited, likely due to relatively high biological variability between groups and small numbers of mice per group (n=6-9/group). The mean (±SEM) 'numbers of cycles' at 3 months differed between groups (p=0.055) and was higher in the control group (2.11±0.27) compared to the 2 µg/kg/day (1.27±0.32; p=0.055) and the 200 µg/kg/day (1.13±0.33; p=0.031). Further, we observed reduced neonatal survival along with a higher incidence of pups' death per litter following propylparaben exposure. Specifically, percentages of total live pups/total pups were 91% (controls), 90% (2µg/kg), 77% (20 µg/kg), and 83% (200µg/kg). Incidences of dead pups were 3/7 (controls), 6/8 (2µg/kg), 5/8 (20 µg/kg), and 2/8 (200µg/kg). Yet, statistical analysis of fertility outcomes did not result in significant differences between the groups, including averages of pup's weight (1.66gr-1.71gr), litter size (10-12), or number of dead pups per litter. Overall, our finding suggests that environmentally relevant propylparaben exposure had a limited impact on estrous cyclicity and fertility in female mice.

P45: INTRA-TESTICULAR ADMINISTRATION OF SERTOLI-PRIMED EXOSOMES BEFORE CHEMOTHERAPY PROTECTS SPERMATOGONIAL STEM CELLS IN PREPUBERTAL MICE: A NEW HOPE FOR FERTILITY PRESERVATION

Farzana Liakath Ali [1], Omer Raheem [1,2], Mervat Omran [1], Hang-Soo Park [1], Analea Beckman [1], Mohammad Mousaei [1], Ayman Al-Hendy [1]

[1] Department of Obstetrics and Gynecology, The University of Chicago, Chicago, IL 60637, USA
[2] Department of Surgery, University of Chicago, Chicago, IL 60637, USA

INTRODUCTION: The fertility preservation of pre-pubertal boys undergoing chemotherapy remains experimental. This study aims to leverage the regenerative effect of Sertoli-Primed human-umbilical cord-derived MSCs exosomes (SP-exo) as a fertility preservation approach in a prepubescent mouse model.

METHODS: Four-week-old C57/BL6 male mice were divided into control, chemotherapy (CTX), Single, multiple TS-Exo injection, and sham control (eight mice in each group). Testicular Dysfunction (TD) was established in all mice except the control group by intraperitoneal injection of 100mg/kg cyclophosphamide and 20mg/kg Busulfan. The fertility preservation group (single injection) received 10 µL of 1.5 X109 SP-exos by intra-testicular injection 24 hrs before CTX. The multiple injection group received one injection before 24 hrs. of CTX and two after 24 hrs. and 48 hrs. of CTX. The sham control received 10 µL of sterile saline. After puberty (8 weeks), mice were bred for three cycles. Pregnancy rate, number of pups, testicular histology, immunohistochemistry, immunofluorescence, serum hormone analysis, Q.RT-PCR, western blot, and electron microscopy were performed to analyze the effect of SP-exos on the preservation of Spermatogonial Stem cell (SSC) niche. A one-way ANOVA with a post hoc test with Bonferroni correction was used for statistical analysis.

RESULTS: Fertility preservation groups (both single and multiple) that received SP-Exo group showed an 85% pregnancy rate through all three cycles of breeding whereas the CTX group showed 15% pregnancy only at the third cycle. The sham control had no pregnancy. Among the single and multiple groups of Exo injection, the total number of pups was significantly higher in the multiple exosome injection group (p<0.01). Histology and TUNEL assay confirmed anti-apoptotic morphology in SP-Exo treated groups. IHC and Immunofluorescence indicated preservation of the Spermatogonial stem cell (SSC) niche in multiple SP-Exo groups compared to CTX and single SP-Exo injection. Testosterone levels were significantly higher in the exosome pre-treated group compared to the CTX group (p<0.05) whereas the reverse was observed with FSH and LH levels. Both molecular and protein analysis confirmed the anti-apoptotic effects exerted by the exosomes through the downregulation of p38MAPK/ERK and AKT signaling pathways. Electron microscopy confirmed the preservation of mitochondrial architecture and peroxidation in both single and multiple SP-Exo injected groups.

CONCLUSION: Pre-treatment of SP-Exos preserved the SSC in both single and multiple injection groups indicating that the presence of SP-Exo helped in homing and prevented cellular and DNA damage caused by cytotoxic poly chemotherapy drugs. The only current option includes freezing of testicular tissue from prepubertal boys before chemotherapy and there is a huge unmet need for fertility preservation in pediatric cancer survivors. Our preclinical findings warrant further validation and encourage pediatric oncologists to consider exosomes as a novel cell-free therapeutic option to preserve germ cell niche before chemotherapy, increasing fertility chances.

P46: **REVEALING THE MECHANISMS OF ACENTROSOMAL SPINDLE STABILITY IN MAMMALIAN OOCYTES**

Ilka Lorenzo [1] and Sadie Wignall [1]

[1] Interdisciplinary Biological Science (IBiS) program Molecular Bioscience and Center of Reproductive Science and Medicine, Northwestern University, Evanston IL

Sexual reproduction relies on meiosis, a specialized form of cell division that involves one round of DNA replication followed by two rounds of chromosome segregation, resulting in haploid gametes1. Unlike mitosis, meiosis is notably susceptible to chromosomal segregation errors, particularly in oocytes. Oocyte meiosis presents unique challenges due to the absence of centrosomes, which are critical for regulating spindle assembly and stability during cell division. Mammalian oocytes are naturally prone to high rates of aneuploidy, with reported incidences ranging from 20-30% in young adults (under 35 years) to over 50% in women older than 35 years5,6. This high incidence of aneuploidy poses significant public health concerns, as it is associated with infertility, pregnancy complications such as miscarriage, and genetic disorders like Down Syndrome6,7,10. The underlying mechanisms that make mammalian oocytes prone to errors remain poorly understood. Recent studies have identified a unique phenomenon in mammalian spindles that may contribute to the high rates of aneuploidy. At metaphase II (M2), after the formation of bipolar spindles, some oocytes undergo a dynamic process where their spindles collapse and reform multiple times while awaiting fertilization. This phenomenon appears unique to mammals, with a notably high occurrence in humans 10,7,12 . The high dynamism during M2 may increase the risk of spindle multipolarity and monopolarity, kinetochore attachment errors, chromosome misalignment, and ultimately, chromosomal mis-segregation and aneuploidy. Our research group has identified a pool of proteins crucial for acentrosomal spindle stability in C. elegans oocytes, including Kinesin-5, Kinesin-12, Dynein, ZYG-8, and ZYG-9 1,2,4,11 . This project extends these findings to mammalian systems, focusing on murine and porcine models. We aim to characterize the role of these proteins in stabilizing the acentrosomal spindle during M2 arrest and maintaining chromosomal segregation integrity during anaphase II (A2). We are currently using ATP hydrolysis inhibitors to disrupt the function of different motor proteins and assess spindle and segregation defects in both mammalian systems 13,14. We use fixed imaging techniques to identify phenotypical abnormalities, which help suggest the functions of these proteins during M2 and A2. Our preliminary data indicate a key role for Kinesin-12 in stabilizing the acentrosomal spindle and ensuring proper chromosomal segregation. Inhibition of this protein leads to severe abnormalities in the spindle and chromosomes, including elongated, split, and presence of dual spindles, ultimately preventing the progression of meiosis. This is the first time Kinesin-12 has been characterized in mammalian oocytes M2 and our findings indicated that Kinesin-12 acts as a primary force during M2 in mammalian oocytes. Additionally, we are characterizing the functions of Dynein and Kinesin-5 in spindle stability. While multiple studies have demonstrated the importance of these antagonistic proteins during spindle assembly, there is limited research on their roles in spindle stabilization3,9. Our findings reveal these proteins' roles in maintaining pole integrity, chromosomal alignment, and the localization of acentrosomal microtubule organizing centers (aMTOCs) in murine oocytes. This study aims to (1) provide comparative data across C. elegans, murine, and porcine spindle mechanisms and (2) identify key proteins involved in maintaining spindle integrity and chromosomal segregation.

P47: **REMODELING OF THE ENDOPLASMIC RETICULUM (ER) DURING EARLY EMBRYO DEVELOPMENT**

Meng Ma [1], Sijie Chen [1], Jing Yang [1,2]

[1] Department of Comparative Biosciences, [2] Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL

The oocyte-to-embryo transition (OET) plays vital roles in reproduction. During the OET, the dormant oocyte transitions to a rapidly growing embryo with specific cell lineages, which facilitates different cell fate determination and differentiation. It has been known that remodeling of cellular organelles, like proteosome, mitochondria and the endoplasmic reticulum (ER), happened during OET. However, there is a limited understanding how cellular machineries are remodeled to orchestrate the OET. Our work has uncovered that ER morphology undergo sheet-to-tubule remodeling during oocyte maturation in Xenopus, which is crucial for asymmetric localization of maternal RNA. Further analysis revealed ER exhibits lineage-specific abundance and morphology in Xenopus embryos. To address whether ER remodeling during lineage differentiation could influence cell fate determination, we have developed a novel screening strategy in Xenopus to identify potential ER regulators during oocyte maturation. CRACR2B was identified, which can induce plasma membrane-ER association in both oocytes and mature eggs. Overexpression of CRACR2B in Xenopus embryos caused various patterning defects, including delayed gastrulation and malformation of the head and shortened anterior-posterior axis in the tadpole stage. To verify the effect of CRACR2B causing ER remodeling defects on embryo patterning, we developed an optogenetic tool, CIBN-CAAX/RRBP1-CRY2, to mimic the function of CRACR2B, which can induce the same ER remodeling defects, plasma membrane-ER association. We expect to see the same patterning defects using this tool. Overall, our studies suggest that ER remodeling might alter cell fates determination during the OET. Supported by R35GM131810.

P48: ENDOCRINOLOGICAL AND EMBRYO-ASSOCIATED PARAMETERS IN PPID MARES TREATED WITH PERGOLIDE AND METFORMIN.

H. Magalhaes [1], I. Canisso [1]

[1] Department of Veterinary Medicine, University of Illinois at Urbana Champaign, Urbana, IL.

Twenty-two Quarter horse mares aged 16 to 23 years old (18.4 ± 4.73 years) were enrolled in the study. The study was carried out during two foaling seasons (2022-2023 and 2023-2024) involving client-owned animals presented for breeding. Mares received a free choice mix of alfalfa grass hay, 1 kg/per animal/day of concentrate, and water ad libitum. All the mares were evaluated by the same operator and were bred by the same stallion in both seasons. All the stallions had proven fertility with multiple viable foals. The study compared the endocrinological panel and embryo-associated parameters from PPID/metabolic mares (ACTH >30 pg/mL, Nov- July) before and after treatment. All the mares underwent one breeding season as a control and, in the subsequent breeding season, the mares were treated with pergolide (1 mg/day, PO) and metformin (3 g/day, PO). Blood was collected once for control and treated mares (Cortisol, ACTH, and, T4). To evaluate the glycaemic curve (glucose and, insulin) mares had blood collected at hour 0 (12 hrs fasting period) then 60 and 90 min (post-prandial period) after administration of 120 mL of corn syrup (Karo®). The mares were ultrasound daily until the detection of a 35 mm follicle for induction. The data was analyzed by Mixed Model and expressed in mean ± SD and, significance was set as $P < 0.05$. The mean ± SD for ACTH, cortisol, T4, Insulin, and Glucose were: 36 ± 11.6, 16.5 ± 3.3 pg/ml; 50.4 ± 4.1, 38.4 ± 6.8 ng/mL; 22.2 ± 2.5, 25.2 ± 4.02 ng/mL; 20.1 ± 15.3, 25.1 ± 18.1 UI/dL; 120.5 ± 28.9 and, 111.5 ± 13.3 mg/dL for control and treated mares, respectively. There was an effect of the group for ACTH, T4, and cortisol ($P < 0.05$), and an effect of the group and time for Glucose (p <0.05). No interaction group: time was appreciated. Control mares had an ACTH two-fold higher than treated mares. The overall recovery embryo/flush was 0.15 ± 0.4 and, 0.73 ± 0.5 for the control and treated group (P < 0.05). The overall embryo recovery rate per oocyte retrieved, fertilization rate, and, embryo development rate for ICSI mares were: 0.17 ± 0.1, 0.27 ± 0.1 embryos; 0.28± 0.2, 0.61 ± 0.15 fertilized oocytes; 0.70 ± 0.34 and, 0.55 ± 0.2 embryos for control vs treated mares. Treated mares presented a superior embryo recovery rate per oocyte retrieved than control mares (P < 0.05). The overall embryo for ICSI and ET was significant for the control mares but not for treated mares (P > 0.05). There was no difference in the follicle's diameter at 24h after induction for control vs. treated mares for ET and ICSI (P < 0.05). In conclusion, the embryo recovery for ET was 3-fold higher in treated mares while ICSI mares presented a superior embryo rate per oocyte retrieval and fertilization rate than control mares.

P49: SIGNALING THAT MEDIATES THE MAMMALIAN EGG'S MEMBRANE BLOCK TO POLYSPERMY: ANALYSES OF CALMODULIN, CALCIUM/CALMODULIN-DEPENDENT KINASE II, AND A CAMKII SUBSTRATE

Allison McCarthy [1], Nicole Branca [1], Agnes Doszpoly [2], Tamara Kinzer-Ursem [2], Janice Evans [1]

[1] Department of Biological Sciences and [2] Weldon School of Biomedical Engineering, Purdue University

The egg-to-embryo transition is mediated by increased cytosolic calcium, with events of this key developmental event including the completion of meiosis, progression to embryonic mitosis, and the establishment of the blocks to polyspermy. Prevention of polyspermic fertilization is essential for reproductive success, as embryos fertilized by multiple sperm nearly always die early in embryogenesis. The blocks to polyspermy in mammalian eggs take place at the level of the egg coat and the egg plasma membrane. The focus of our work is elucidating the signaling pathways that lead to establishment of the membrane block to polyspermy. Increased cytosolic calcium facilitates the establishment of the membrane block to polyspermy, and thus we have been investigating the best-understood calcium-dependent effector molecule, calcium/calmodulin-dependent kinase II (CaMKII). It is known that the gamma isoform of CaMKII mediates multiple events of the egg-to-embryo transition, including that expression of a constitutively-active form of CaMKIIγ (CA-CaMKIIγ) leads to parthenogenetic egg activation. However, we have found that CA-CaMKIIγ-activated eggs' membrane are still receptive to sperm, indicating that this increase in CaMKIIγ activity is not sufficient for membrane block establishment, even though these eggs exit from metaphase II arrest and progress to embryonic interphase. This demonstration that CaMKII is not sufficient for membrane block establishment prompted testing the hypotheses that CaMKII is necessary for membrane block establishment. The small molecule KN-93 has used as an inhibitor of CaMKII for more than three decades, although more recent findings show that it inhibits CaMKII by binding to CaM, thus having the likelihood of inhibiting multiple CaM-dependent pathways. We observe that KN-93-treated eggs that are inseminated in IVF assays to assess membrane block establishment (i.e., with the zona pellucida removed and with controlled insemination conditions) become highly polyspermic. KN-93 has appeared to be a more robust effect on membrane block establishment than we have previously observed with an inhibitor that inhibits CaMKII by blocking a regulatory site in the kinase domain (called the T site), raising the possibility that other Ca^{2+}/CaM-dependent, non-CaMKII activities play a role in membrane block establishment. To evaluate this in more depth, we are undertaking studies with the CaM inhibitor KN-93 and a next-generation ATP-competitive CaMKII inhibitor. We also are examining candidate downstream effectors of CaMKII, and have demonstrated that oocytes and eggs express the CaMKII substrate Tiam1 (T-lymphoma invasion and metastasis). Tiam1 is a guanine nucleotide exchange factors that activate the small G-proteins Rac1 and Cdc42, which places Tiam1 as a potential mediator of membrane block establishment and other events occurring in the egg cortex during the egg-to-embryo transition. Future experiments will undertake reverse genetics depletion of CaMKIIγ and Tiam1, and assessing effects on the events of the egg-to-embryo transition. These studies will elucidate the factors and pathways that contribute to the change in the egg membrane's receptivity to sperm after fertilization.

P50: THE ROLE OF HORMONES AND OXIDATIVE STRESS IN LEIOMYOMA DEVELOPMENT AND SURVIVAL

Ross McNally [1], Huma Asif [1], Jian-Jun Wei [1,2], J. Julie Kim [1]

[1] Departments of 1 Obstetrics and Gynecology and [2] Pathology, Northwestern University, Chicago, IL, USA

Uterine leiomyomas (ULM), non-cancerous tumors of the myometrium (MM), occur in 77% of women of reproductive age and are the leading cause of hysterectomies in the US. Treatment options are limited, with surgery being most common. Thus, a new and effective intervention is desperately needed. We have previously demonstrated a key role of oxidative stress in ULMs in promoting senescence and cell death. Given the important role of hormones in the pathophysiology of ULM we aimed to elucidate the interaction between hormones and oxidative stress on ULM. In this study, we investigated the interaction of hormones with oxidative stress in ULMs, through the assessment of cell metabolism, senescence, and the transcriptome. ULM tissues were collected from consented patients and fibroid cells treated with hormones (E2 and R5020), hormone antagonists (Fulvestrant and Onapristone), and paraquat, an inducer of superoxide anions. In the presence of paraquat, expression of p21, a marker of senescence increased. Addition of hormones resulted in the attenuation of p21 expression in the paraquat treated cell lines. To identify additional genes impacted by paraquat and hormones, ULM cultured as three-dimensional spheroids, treated with paraquat, hormones, and antagonists alone or in combination, were subjected to RNA-seq. RNAseq analysis revealed that combined treatment with hormones, antagonists, and paraquat resulted in the differential expression of 3309 genes (FDR Adj p value, $p \leq 0.05$) compared to control. Pathway analysis indicated an enrichment of metabolic associated genes linked to oxidative phosphorylation and glycolysis in ULM spheroids treated with hormones, both paraquat and hormones, or the combination of hormones, paraquat, and antagonists. To further understand the role of hormones in metabolic functions, ULM cells were treated with PQ, hormones, and hormone antagonists were subjected to the metabolic assay, Seahorse; significant changes in ULM mitochondrial function were observed as a result. All treatment groups caused a decrease in maximal respiration and spare respiratory capacity, whereas an increase in proton leak was observed with paraquat, as determined via measurement of oxygen consumption rate (OCR). However, glycolysis, glycolytic capacity, and glycolytic reserve remained largely unchanged since the extracellular acidification rate (ECAR) was not altered when ULM cells were exposed to these treatments. In summary, our results demonstrate that hormones play an important role in the context of high oxidative stress, promoting survival of ULM. Supported by NCI R01CA254367.

P51: TRANSIENT SCROTAL HYPERTHERMIA AFFECTS PORCINE EPIDIDYMAL EXTRACELLULAR VESICLES AND SPERM

Supipi Mirihagalle [1], Kankanit Doungkamchan [1], David Miller [1]

[1] Department of Animal Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana-Champaign

Elevated environmental temperatures reduce reproductive performance in mammals. In swine, the scrotum needs to be maintained 2-8°C below abdominal temperature to produce high-quality semen. The mechanisms of how scrotal hyperthermia affects sperm production are not clear. In preliminary experiments, insulated and control sacks were attached to the scrotum of 3 boar littermate pairs for 48 hr to elevate the scrotal skin temperature by 3°C and semen was collected before and 3 times/week after the insulation removal. Based on proteomic analysis of collected sperm, the abundance of 4 proteins (SPATA18, TOMM34, VDAC1, and GPX4) was increased 2-5 fold by scrotal hyperthermia compared to pre-insulation abundance as early as 6 days after insulation removal and before significant deterioration of sperm motility and morphology were observed. Sperm collected 6 days after insulation removal were in the epididymis during scrotal insulation, suggesting that epididymal maturation was affected by the scrotal hyperthermia but there are few studies of the heat sensitivity of the epididymis. Epididymal extracellular vesicles (EVs) fuse with sperm and add proteins as they move through the epididymis, allowing sperm to gain the ability to be motile and fertile. All 4 proteins that are elevated following hyperthermia are transmembrane proteins and may be secreted by the epididymis as EVs and added to sperm. Therefore, we hypothesized that the change in sperm protein profile following hyperthermia may be due to heat stress on the epididymis, affecting the secretion of EVs, causing alterations in EV protein content, or affecting the fusion ability of EVs to sperm. We collected caput and cauda epididymal fluid and isolated EVs by ultracentrifugation. The average diameter of the caput and cauda EVs was 133.1±47.3 nm and 105.3±40.5 nm and they contained the EV marker proteins CD81 and CD9. After characterizing the EVs, we performed western blots for EV and epididymal sperm protein lysates and found that all 4 proteins that were elevated following hyperthermia are present in EVs and sperm collected from both that caput and cauda epididymis. This suggests that those 4 proteins are added to the sperm via epididymal EVs. Quantitative analysis of the EV and sperm proteins showed no significant difference in the abundance of the 4 proteins in caput vs cauda epididymis. A mixture of caput and cauda EVs were labeled with FM4-64FX dye and incubated with caput sperm to observe EV-sperm fusion in-vitro which resulted in EV fusion to sperm head and mid-piece area. To understand the effects of scrotal hyperthermia on epididymal EVs and sperm, a scrotal insulation experiments was carried out and epididymal fluid and sperm were collected at 3 days post insulation. According to preliminary data analysis, there are effects of hyperthermia on EV and sperm proteome. Future work will include two additional scrotal hyperthermia experiments to analyze the effects of heat on epididymal EVs and sperm 0 and 6 days post insulation removal to obtain a broad understanding of the effects of hyperthermia on epididymal EVs and sperm. This work is supported by USDA.

P52: SIGLECS IN THE PORCINE OVIDUCT AND SIALYLATED LIGANDS ON SPERM: POTENTIAL ROLE IN THE SPERM RESERVOIR

Leonardo M. Molina [1]; Hao-Chun Fan [1]; Lauren E. Pepi [2]; Asif Shajahan [2]; Parastoo Azadi [2]; Daniel B. McKim [1]; Adrienne M. Antonson [1]; David J. Miller [1]

[1] Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801
[2] Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602

During mammalian insemination, seminal fluid and sperm elicit myeloid cell infiltration that contributes to the elimination of sperm in the uterus. However, unlike the uterus, invading sperm do not trigger phagocytic responses in the oviduct in the absence of dysfunction or disease states. Thus, the oviduct possesses a distinct immunological microenvironment that innately tolerates sperm while maintaining the capacity to respond to pathogens. This unique physiology is not currently understood, and elucidating the underlying mechanisms may fundamentally advance the field of reproductive immunophysiology and identify novel mechanisms contributing to reproductive dysfunction and disease states. It has been suggested that sperm glycocalyx contributes to innate oviductal tolerance, but the cell and molecular mechanisms are not understood. The current investigation focused on the role of sialic acid-containing glycoconjugates on sperm and their potential to elicit innate tolerance via cognate sialic acid-binding immunoglobulin-type lectins (Siglecs) expressed in the oviduct. Consistent with this, gene expression analysis identified eight Siglecs expressed in the porcine lower oviduct, five of which are immune inhibitory (Siglecs-2, -3, -5, -10, and -11). Mass spectrometry analysis of porcine sperm revealed the presence of a mixture of α2,3 and α2,6 linked sialic acids with α2,3-linked sialic acids being the predominant linkage type. Of the detected glycans, several sialic acid-containing glycoconjugates were identified as potential ligands for Siglecs, including O-linked glycans: NeuAc1GalNAc1, NeuGc1GalNAc1, NeuAc2Gal1GalNAc1 and glycolipids: NeuAc2Gal1GalNAc1Gal1Glc1, Fuc1Gal1GalNAc1NeuAc1Gal1Glc1. Sperm lectin staining revealed the presence of these sialoglycans in the apical region of the sperm head. Primary oviduct epithelial cells cultured in an air-liquid interface system were chosen as a model to study the oviduct. This allowed us to mimic the oviduct physiology closely and study the effects of sperm sialic acids on the oviduct's immune response via qPCR. These discoveries demonstrate broad expression of oviductal inhibitory Siglecs and glycolipidomic identification of potential cognate sialoglycan ligands on sperm that spatially co-localize. This reveals a sperm-sialoglycan and oviductal-Siglec axis that may contribute to the distinct immunophysiology of the oviduct fundamentally required for undisrupted reproduction in mammals. This research was supported by NIH R24GM137782 to PA, 1R21HD111954-01A1 to DM and AA, and NIH 1F31HD108959 to LM.

P53: BIOMECHANICS AS A NOVEL NON-INVASIVE BIOMARKER OF OOCYTE MEIOTIC POTENTIAL

Courtney A. Murphy [1], Matthew R. Bersi [2], Farners Amargant i Riera [1]

[1] Department of Obstetrics & Gynecology, Washington University in St. Louis School of Medicine, St. Louis, MO
[2] Department of Mechanical Engineering and Material Science, Washington University in St. Louis, St. Louis, MO

In vitro fertilization (IVF) often results in a high discard rate of immature germinal vesicle (GV) oocytes, with up to 52.5% discarded in women over 40. Though rarely done in IVF labs due to time and cost, methods are available to mature GV oocytes to meiotic stage II (MII) oocytes with success rates of 41.2% to 82%. By selecting for the highest quality GVs, we could increase this rate to nearly 100%, thus making it a viable option for IVF clinics. Several groups have shown that oocyte biomechanical properties are important indicators of oocyte quality as they modulate spindle meiotic movement to the cortex and cell division fidelity. Therefore, the objective of this work is to develop a non-invasive system based on oocyte mechanobiology to rapidly predict the maturation potential of GV oocytes. By identifying high-quality GV oocytes that can mature to MII stage, the system could increase IVF success rates and provide better fertility options. To determine the mechanical properties of single oocytes, we are using a micropipette aspiration system. Using this system, we can estimate the mechanical properties of single cells by determining the distance traveled by these cells inside the glass micropipette in response to specific aspiration pressures. As a proof-of-concept, we measured the biomechanical properties of mice GV oocytes. To do so, CB6F1 mice (6-8 weeks old, N=2 mice) were injected with PMSG, and GV oocytes were collected 45-46h post-injection. Oocytes were mechanically stripped, removing the surrounding cumulus cells. Using an Elastic model, we found that the Zona Pellucida (ZP) of mouse oocytes has a stiffness of 4.36±1.17 kPa while the oocyte stiffness value was 3.06±0.67 kPa. Since we want to correlate the mechanical properties of each oocyte with its maturation capacity, we next determined the maturation rates of oocytes cultured individually (n=27 oocytes) or in a group (n=46 oocytes). The maturation rates of oocytes cultured individually were significantly higher than those cultured in groups (individual, 88.9%, group 47.8%, p<0.002). Overall, our data show that a micropipette aspiration system can effectively determine the mechanical properties of single oocytes and that individual maturation cultures do not affect oocyte maturation rates. Studies to validate that oocyte aspiration does not impact maturation potential are underway. In parallel, we have also set up a maturation protocol for human GV oocytes in our laboratory. GV oocytes are obtained from the WashU Fertility & Reproductive Medicine Center and transported to our lab within 2 hours after retrieval. Oocyte maturation is performed for 46 to 48h. Our preliminary data shows that 34% of the GV oocytes mature to MII, 33% arrest at MI and 33% fail to mature. Future directions are focused on testing the mechanical properties of human GV oocytes and correlating these values with maturation rates as well as cellular and molecular markers of oocyte quality.

P54: REGULATION OF LACTATE SECRETION BY PROGESTERONE IN BOVINE UTERINE EPITHELIAL CELLS AND BASIGIN/LACTATE TRANSPORTER EXPRESSION IN PRE-IMPLANTATION BOVINE EMBRYOS

Muhammad Ashir Nabeel [1], Lyda Yuliana P. Forero [1], Matthew J. Dean [1], Romana A. Nowak [1]

[1] Department of Animal Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois, Urbana, IL, USA

Purpose: Lactate is produced through anaerobic glycolysis in early pregnancy. It is an essential energy source for developing embryos and preparing the uterine environment for embryo implantation. Lactate is transported across the cell membrane through monocarboxylate transports (MCTs). Basigin (BSG), a transmembrane glycoprotein, interacts with MCTs and facilitates their proper expression, localization, and function on the cell membrane. Our objectives were to investigate regulation of lactate secretion by progesterone (P4) in a bovine uterine epithelial (BUTE) cell line and the expression of BSG and lactate transporters in pre-implantation embryos.

Methods: BUTE cells were treated with 0, 30, 300, and 3000 nM of P4 for 24 hours. The conditioned medium was collected after 24 hours, and a lactate assay was carried out to quantify lactate secretion. The expression of BSG and the monocarboxylate transporters MCT1 and MCT4 in pre-implantation embryos at 2-cell, 4-cell, 8-cell, morula, blastocyst, hatching blastocyst, and hatched blastocyst stages were determined by immunofluorescence (IF) staining.

Results: Lactate secretion by BUTE cells was upregulated in response to 3000 nM P4. This hormone-dependent regulation of lactate secretion indicates that lactate may serve as an essential metabolic substrate or signaling molecule supporting the uterine environment necessary for embryo implantation and development. We identified the expression of BSG as well as MCT1 and MCT4 across multiple stages of pre-implantation embryo development, including the 2-cell, 4-cell, 8-cell, morula, blastocyst, hatching blastocyst, and hatched blastocyst stages. The identification of BSG, MCT1, and MCT4 across the various pre-implantation stages suggests that these proteins can facilitate the transport of lactate and other metabolic substrates essential for the energy needs of developing embryos.

Conclusion: Our study provides insight into the regulation of lactate secretion by BUTE cells, highlighting the impact of increasing P4 on lactate secretion, which occurs during the implantation window. The expression of BSG and MCTs at the various stages of pre-implantation embryo development supports that these are essential for meeting the metabolic needs of the developing embryo.

Future Directions: We will investigate the regulation of MCT1 and MCT4 in BUTE cells, the effects of lactate on the development and metabolism of bovine embryos, and the effect of lactate on uterine receptivity. Keywords: Lactate, Progesterone, Basigin, Monocarboxylate, transporters, Immunofluorescence.

P55: SPERM BINDING TO GLYCANS IN THE STORAGE RESERVOIR AS A MECHANISM OF REPRODUCTIVE ISOLATION

Lujain Nachbat [1], David Miller [1], Mark Hauber [1], Al Roca [1], Jason Herrick[2], Gustavo CaetanoAnolles [1], Carlo Unverzagt [3]

[1] Department of Animal Sciences, University of Illinois, [2] Omaha Henry Doorly Zoo, [3] Department of Bioorganic Chemistry, University of Bayreuth

Reproductive isolation is a key concept in evolutionary biology, encompassing barriers that prevent interbreeding among species. These barriers are classified into premating, postmatingprezygotic (PMPZ), and postzygotic mechanisms. While premating isolation involves traits that prevent mating, PMPZ barriers, which occur after mating but before fertilization, have been less studied. Current research suggests that PMPZ isolation may involve incompatibility between sperm and the female reproductive tract, particularly within the sperm storage reservoir. Our hypothesis posits that sperm binding to unique glycans in the female reproductive tract contributes to PMPZ isolation. We base this on preliminary findings from mammals and studies in Drosophila, which indicate that sperm binding to specific carbohydrate structures (glycans) could influence reproductive isolation. To test this hypothesis, we will examine the glycanbinding patterns of sperm from felids and finches—species with different hybridization capabilities. The project will utilize a comprehensive, transdisciplinary approach. Felids offer a model system due to their well-documented genomic information and varying hybridization success, while finches provide an opportunity to study avian reproductive mechanisms. Specifically, we will use zebra finches (Taeniopygia guttata) and owl finches (Stizoptera bichenovii) to investigate interspecific differences in sperm glycan-binding patterns. In felids, we will analyze sperm from multiple species to understand glycan-binding specificity and its correlation with reproductive isolation. Our methodology includes using large-scale glycan arrays to identify sperm binding patterns to various glycans. Preliminary results suggest distinct glycan-binding specificities among different mammalian species, such as differences in sialylated and galactoside glycans. By comparing these patterns across species with and without evidence of hybridization, we aim to determine if glycan-binding specificity aligns with reproductive isolation mechanisms. This study will provide novel insights into the molecular mechanisms of sperm storage and reproductive isolation, expanding our understanding of species evolution and glycan recognition systems. The research has potential implications for evolutionary biology, reproductive science, and glycobiology, and may inform future studies and funding opportunities. Financial support for this study will be sought from NSF, NIH, and other institutions focused on evolutionary and molecular sciences. The proposed budget is $60,000 over two years, covering glycan arrays, semen shipping, graduate student salary, microscopy, and animal care. This project also aligns with opportunities for collaborative grants, such as the NSF-DFG Lead Agency Program.

P56: TARGETING MEIS2 IN PLATINUM RESISTANT OVARIAN CANCER

Natalia Obrochta [1], Vanessa Hernandez [2], Yinu Wang [2*], Daniela Matei [2*]

[1] Department of Neurobiology, Northwestern University, Evanston, IL.
[2] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL

Background: Ovarian cancer (OC) has the highest mortality rate among gynecological cancers. Relapse of the disease accompanied by chemoresistance is common; however, the biological causes are unknown. MEIS2 is a transcription factor (TF) found in normal stem cells that regulates genes responsible for cell proliferation, differentiation, and survival. Here, I examined the role of MEIS2 in regulating stemness features that promote chemoresistance of OC, and the impact of a MEIS2 blockade on OC initiation and progression.

Methods: Paired isogenic OC cell lines (OVCAR5, OVCAR4 and COV362 platinum sensitive (Pt-S) and resistant (Pt-R)) were used. The effects of MEIS2 inhibition (by using a pharmacological inhibitor MEIS2i and biological knockdown (KD)) were assessed in OC cells through colony formation, cell proliferation, flow cytometry for analysis of ALDH+ cancer stem cell (CSC) populations, and detecting stemness gene expression using q-RT-PCR and Western Blotting.

Results: MEIS2 expression levels were increased in Pt-R vs. Pt-S OC cells at mRNA and protein levels. Treatment with MEIS2i ($500nM$-$1\mu M$) reduced the number of colonies in OC cells by at least 2 fold change ($p<0.001$); however, the proliferation abilities of these cells were not diminished. The higher dosage of MEIS2i (2.5-$5uM$) inhibited this process. Intriguingly, OC cells (OVCAR4/5, COV362) treated with MEIS2i ($250nM$-$1uM$, 48 hours) decreased ALDH+ CSCs population by at least ~2 fold change ($p<0.05$), and inhibited the expression of stemness associated genes ALDH1A1, ALDH1A3, and Sox2 at the transcriptional ($p<0.05$) and translational level, compared to control cells. Knocking down MEIS2 in OVCAR5 cells decreased the CSC population from 29.4% to 14.3% ($p<0.05$) and inhibited stemness gene expression ($p<0.05$).

Conclusions: Blocking MEIS2 in OC cells inhibit stemness traits that contribute to chemotherapy resistance and tumor relapse, suggesting MEIS2 as a potential new treatment target. Supported by Friends of Prentice Grant (SP0081288).

P57: TGFB1-INDUCED NONCANONICAL SIGNALING MODULATES THE SWITCH FROM ESR1 TO ESR2 DURING GRANULOSA CELL DIFFERENTIATION IN THE DEVEOPING OVARY

Ji-Eun Oh [1], Chanjin Park [1], Po-Ching Lin [1], Sherry Zhou [1], and CheMyong J. Ko [1]

[1] Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL 61802, USA.

Granulosa cells (GCs), expressing ESR2, originate from surface epithelial cells, expressing ESR1, during perinatal development, yet the mechanisms governing the cessation of ESR1 expression and the induction of ESR2 remain unknown. This study hypothesizes that transforming growth factor beta 1 (TGFβ1) orchestrates this process by downregulating ESR1 and upregulating ESR2 expression in differentiating GCs. Single-cell RNA sequencing of ovaries from embryonic day 16.5 and postnatal days 7 and 14 revealed that endothelial cells express TGFβ1, while GC lineage cells express TGFβ receptors. Spatial analysis of differentiating GCs (FOXL2+), endothelial cells (PECAM1+), and TGFβ1 in perinatal ovaries showed that TGFβ1-immunoreactivity (ir) is concentrated around blood vessels, particularly in the medullary region at embryonic days 16.5 and 18.5 ($P<0.001$, One-way ANOVA). By postnatal day 4, when primordial follicle formation is largely complete and GCs begin expressing ESR2, TGFβ1-ir had spread into the ovarian cortex. Notably, regions with high medullary TGFβ1-ir exhibited lower ESR1 expression compared to the cortex. To assess TGFβ1's role in the transition from ESR1 to ESR2 expression, embryonic ovaries were cultured with TGFβ1, with or without LY-2157299, a TGFβ receptor I inhibitor. RT-PCR results demonstrated that TGFβ1 significantly decreased Esr1 expression (0.6-fold, $P < 0.05$) and increased Esr2 expression (13-fold, $P < 0.05$), effects that were negated by LY-2157299 co-administration. Further analysis showed that TGFβ1 mediates ER expression via noncanonical signaling pathways. Inhibition of Smad3 phosphorylation with SIS3 did not prevent TGFβ1-induced Esr2 expression, while co-treatment with LY-294002, a PI3K inhibitor, abolished the changes in ER expression. These findings suggest that TGFβ1 drives the shift from ESR1 to ESR2 through a PI3K/AKT-dependent noncanonical pathway, bypassing canonical Smad2/3 signaling. In conclusion, the developing ovary undergoes a shift in estrogen receptor expression from ESR1 to ESR2, orchestrated by noncanonical TGFβ1 signaling pathways, particularly those involving PI3K/AKT, rather than the canonical Smad2/3 pathway.
This project is supported by NIH HD094296 grant.

P58: **PHTHALATE- INDUCED PITUITARY INFLAMMATION LEADS TO EARLY FEMALE REPRODUCTIVE AGING.**

Y.V. Ojo [1], K.E. Weis [1], M. Laws [2], R. Santa Cruz Marquez [2], J.A. Flaws [2] and L.T. Raetzman [1]

[1] Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, IL, USA.
[2] Department of Comparative Biosciences, University of Illinois College of Veterinary Medicine, Urbana-Champaign, IL, USA.

Reproductive aging in humans is accelerated by phthalates, a group of ubiquitous endocrine-disrupting chemicals. However, how phthalates accelerate aging in the hypothalamic-pituitary-gonadal axis is unknown. We focused on the pituitary gland, the source of the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). We show proinflammatory cytokine Il1b and Fshb mRNA expression increased with aging in the pituitary. Therefore, we hypothesized that the common phthalates di-(2-ethyhexyl) phthalate (DEHP) and diisononyl phthalate (DINP) modulates inflammation in the pituitary, contributing to early reproductive aging. To address this, female CD-1 mice were orally dosed with corn oil or increasing concentrations of DEHP and DINP for 10 days. Pituitary tissue was collected immediately after dosing or after 15 months and analyzed by RT-qPCR. We found that acute phthalate exposure did not alter *Fshb* and *Lhb* mRNA expression. However, acute phthalate exposure decreased *Il1b* and increased *Tnf* mRNA levels, suggesting an inflammatory imbalance. At 15 months, DINP exposure led to increased *Lhb* and repressed *Nlrp3* mRNA levels. Next, we investigated the impact of phthalates and the proinflammatory stimulus LPS on inflammation and gonadotropin gene expression directly at the pituitary, using dissociated cultures. Both MEHP and LPS decreased Fshb mRNA relative to control but did not impact *Lhb* expression. LPS exposure caused induction of *Il1b* and *Tnf* mRNA levels, however MEHP repressed the induction of Il1b by LPS and increased *Tnf*. Together, these findings suggest that phthalate exposure alters the mRNA expression of inflammatory markers and gonadotropins in the pituitary, which could accelerate reproductive aging. Supported by NIEHS grant R01 ES034112 and Toxicology Scholar Award from the University of Illinois Urbana-Champaign.

P59: **THE MOUSE OVARY HAS THE CAPACITY TO CONVERT THE NEONICOTINOID PESTICIDE IMIDACLOPRID TO TOXIC METABOLITES *IN VIVO***

Jadesola I. Oladosu [1], Vasiliki E. Mourikes [1], Colin Lee [1], Megan Mahoney [1], Jodi A. Flaws [1]

[1] Department of Comparative Biosciences, University of Illinois College of Veterinary Medicine, Urbana-Champaign, IL, USA.

Imidacloprid (IMI) belongs to a class of neuro-active insecticides called neonicotinoids. Neonicotinoid pesticides are nicotine derivatives that target nicotinic acetylcholine receptors (nAChRs) in the nervous system. IMI is used in large scale agricultural systems, sold for private residential use, and is found in veterinary pharmaceuticals. Because IMI is more water soluble than other insecticides, there is tremendous potential for environmental accumulation and chronic exposure of non-target species. IMI can be converted to desnitro-imidacloprid (DNI) by the liver through phase I biotransformation; both compounds act as an agonist for mammalian nAChRs, with DNI having a significantly higher affinity than IMI for nAChRs. A previous study showed that IMI is converted to toxic metabolites in vitro. However, it was not known whether the whole ovary contains the metabolic enzymes required to convert IMI to toxic metabolites. Thus, this study tested the hypothesis that the ovary contains the enzymes required to metabolize IMI. To test this hypothesis, mice (6 weeks of age) were dosed orally with either vehicle control (dimethyl sulfoxide), a low dose of IMI (0.5 mg/kg body weight), or high dose of IMI (5.7 mg IMI/kg body weight for 30 days. After dosing, the ovaries were collected and ovarian RNA was used for qPCR reactions to quantify gene expression of metabolic enzymes associated with IMI metabolism. The expression of the following six genes was measured: aldehyde oxidase 1 (*Aox1*), aldehyde oxidase 2 (*Aox2*), aldehyde oxidase 3 (*Aox3*), cytochrome P450 family 2 subfamily D member 22 (*Cyp2d22*), cytochrome P450 family 2 subfamily E member 1 (*Cyp2e1*), and cytochrome P450 family 4 subfamily F member 18 (*Cyp4f18*). These genes were selected because they are known to convert IMI to toxic metabolites such as DNI in the liver. The results indicate that the ovaries expressed all six genes. However, exposure to both low and high doses of IMI did not significantly increase expression of *Aox1, Aox2, Aox3, Cy2d22*, or *Cyp4f18* compared to control. Interestingly, low dose IMI significantly increased the expression of *Cyp2e1* compared with control. These data indicate that mouse ovaries metabolize IMI and that IMI can induce ovarian *Cyp2e1* expression at a low dose, with IMI following a non-monotonic dose response curve. *Cyp2e1* is important in IMI metabolism because it reduces IMI to DNI and other downstream metabolites. Collectively, these data suggest that the mouse ovary has the capacity to metabolize IMI to DNI and other downstream metabolites. Supported by NIH F30 ES033914 and NIH R01 ES028861.

P60: DRUG REPURPOSING SCREENING AND MECHANISM ANALYSIS USING THREE-DIMENSIONAL HUMAN STEM CELLS DERIVED ORGANOIDS

Mervat.M. Omran [1,2], Somayeh Vafaei [1], Samar Alkarit [1], Farzana Liakath Ali [1], Qiwei Yang [1], Mohamed Ali [1], Ayman Al-Hendy [1]

[1] Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL 60637, USA. [2] Cancer Biology Department, National Cancer Institute - Cairo University, 11769 Cairo, Egypt.

Background: Uterine fibroids (UFs) are the most important benign monoclonal neoplastic threat to women's health worldwide, with a prevalence of up to 80% in premenopausal women, which can cause heavy menstrual bleeding, pain, and infertility. With no long-term non-invasive anti-UFs treatment option currently available, deeper insights regarding tumor etiology are the key for developing newer therapies. The aim of this study was to evaluate Drug repurposing screening and mechanism analysis on at-risk myometrium (fibroid-containing uteri, MyoF) as well as fibroid myometrium (UF) stem cells (SCs) using 3D organoid system.

Methodology: MMSCs, isolated from MyoN, UF or MyoF, were embedded with Matrigel, as a scaffold, in MESENCULT organoid specific media in a V-bottom plates, specifically Akura™ 96 Spheroid Microplates (InSphero). After proliferation over 7 days, cultured organoids was exposed to either Vitamin D3(100nM) , Doxercalcifrol (100nM) or Vitamin D3 (100nM) or Vitamin D3 and EGCG (10uM) combination or estradiol (E2, 10 ng/ml), progesterone (P4, 10 ng/ml) or their combinations for 48 h. Cell viability assayed using CellTiter-Glo 3D Cell Viability Assay. The apoptosis was assayed using RealTime-Glo Annexin V Apoptosis and Necrosis. Paraffin-embedded organoid blocks were prepared, and Immunohistochemistry (IHC) for different markers was conducted. Mechanical properties of MMSCs derived organoids were tested with a single indentation protocol using a Piuma nanoindenter (Optics11, Amsterdam, Netherlands).

Results: Our result showing significant decrease in cell viability of UF organoids treated by both Vitamin D3 or Doxercalcifrol (P=0.05), in addition to, significant increase in apoptosis (P=0.05, 0.001), respectively. This finding was confirmed using IHC staining which showing significant decrease in proliferative marker PCNA accompanied by decrease in antiapoptotic marker Bcl-2(P=0.05 , 0.01), respectively. The Young's modulus of at risk organoids (MyoF) treated with Vitamin D3 or Vitamin D3 and EGCG combination is were significantly less stiffer (one -fold decrease, 1.5 fold decrease) than untreated organoids(P=0.0001) ., which confirm the role of mechano-transduction as a mechanistic explanation in uterine fibroid treatment options. For MyoF , UF derived organoids, There is marked visible significant increase in Estrogen receptor antigen expression (ER) in both Estradiol and combination treated group relative to control groups (14.34%,9.64%) (P=0.001, 0.01) and (15.6%,14.3%) (P=0.01, 0.05), respectively.

Conclusion: We found that Vitamin D3, Doxercalciferol, and EGCG exhibited effective antifibroid effects. Our study offers an innovative approach for drug discovery, and the representative mechanistic features of drug responses provide valuable resources for developing novel clinical treatments for UF.

Keywords: Vitamin D3, Doxercalciferol, EGCG, 3D organoids, UF, MyoF

P61: DEFINING THE EFFECTS OF LOW-DOSE ACUTE CHEMOTHERAPY ON THE OVARIAN MICROENVIRONMENT IN PRE-PUBERTAL AND ADOLESCENT PATIENTS

Shravya Pant [1,2], Hannah B. McDowell [1,2], Monica M. Laronda [1,2]

[1] Department of Pediatrics, Division of Endocrinology and Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University
[2] Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA.

Survival rates for children and adolescents diagnosed with cancer are >85% following advancements in anticancer therapeutics. However, treatments, particularly alkylating chemotherapy, can result in infertility and hormonal dysregulation as they are often gonadotoxic. Currently, ovarian tissue cryopreservation (OTC) is the only fertility preservation option for pre-pubertal females, wherein tissue containing primordial follicles (PMFs) is cryopreserved. Patients can later retransplant their tissue via ovarian tissue transplantation (OTT), restoring fertility and endocrine function. While some OTT patients have children, hormone functionality lasts only ~2-5 years due to a massive depletion of PMFs post-transplantation, leaving room for improvement in this methodology. We hypothesize that prior exposure to low levels of alkylating chemotherapy may impact how ovarian vasculature supports PMF development post-transplantation. We aimed to study the impact of exposure to alkylating agents on the ovarian microenvironment and supporting vasculature using 31 ovarian biopsies from OTC patients from Lurie Children's Hospital (0.53-22.81 years, cyclophosphamide equivalent dose (CED) 0-8g/m²). While all patients received a CED lower than what is considered high risk for developing premature ovarian insufficiency, we sought to investigate acute effects on the ovarian microenvironment. Here we describe differences in ovarian vasculature and follicle characteristics following exposure to alkylating agents.

There were significantly more abnormal vessels in pre- (0.44 +/-0.12, 0.15 +/-0.06 vessels/μm²) and post-pubertal patients (0.72 +/-0.20, 0.13 +/-0.09 vessels/mm²) exposed to alkylating chemotherapy. No significant increase in collagen density was identified following acute treatment, although post-pubertal patients had greater collagen density (0.28, 0.63 +/-0.09 fibrotic intensity/total intensity). Immunohistochemistry analysis of 23 samples was conducted using an endothelial glycan-specific dye, anti-CD34, and anti-alpha-smooth muscle actin to assess differences in vessel maturity. There were more CD34+ immature vessels in the exposed pre-pubertal cohort compared to non-exposed (2.14 +/-0.66, 0.79 +/-0.67 vessels/mm²). In contrast, exposed post-pubertal patients had fewer CD34+ vessels (0.63 +/-0.37, 1.48 vessels/mm²) and fewer endothelial cells (120.3 +/-22.12, 185.4 +/-31.88 count/mm²) compared to non-exposed. These results suggest that exposed post-pubertal patients are differentially affected, consistent with previous research suggesting post-pubertal patients may be more susceptible to fertility-related damage.

In addition to ovarian vasculature, we assessed follicle stages and granulosa cell (GC) counts to investigate potential differences in quiescence following exposure to alkylating agents. Follicle count per area for each stage was not significantly different in exposed vs non-exposed patients. However, transitional follicles (TFs) in exposed pre-pubertal patients were significantly smaller, and PMF and TFs contained significantly fewer GCs per follicle when compared to non-exposed counterparts. Exposed post-pubertal patients also showed a significant reduction in the number of GCs per PMF and TF. Impending assessment of activation and apoptosis/DNA damage via immunohistochemistry will strengthen these findings and expand our understanding of acute effects of alkylating agents on follicle health and quality. These data may suggest alterations to the current transplantation methods to improve fertility and hormone restoration for cancer survivors.

P62: **3D APPROXIMATION OF THE EXPRESSION OF LINEAGE-SPECIFIC MARKERS OF INNER CELL MASS (SOX2, OCT4) AND TROPHECTODERM (CDX2) IN MOUSE BLASTOCYSTS.**

L.Y. Parra-Forero [1], R.A. Nowak [1]

[1] Department of Animal Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois, Urbana, IL, USA.

The study of embryo structure in real dimensions is important to develop predictive methods of implantation in embryos produced *in vivo* or *in vitro* and even designed *in silico*. The integration of biological strategies with the bioinformatics tools could help to achieve this aim. Numerous biological events have not yet been elucidated such as the study of embryo development, polarization and exclusion of blastomeres, cell-cell communication and differentiation of inner cell mass into epiblast and primitive endoderm. We have shown the preliminary results of the development of a non-invasive structural graphical model to evaluate the expression of markers usually used to investigate the inner cell mass (SOX2 and OCT4) and trophectoderm (CDX2) development in mouse blastocysts. We showed quantitative data such as area (um2), ellipticity (oblate), volume (um3), minimum and maximum intensity, of blastomere morphology and structure inside a mouse blastocyst in a three-dimensional study using Imaris 10.1 software. We obtained a spatial approximation of the surfaces of the blastomere that allowed us to identify and count them, obtaining single-cell data, determining the place of evaluation and the stage of development of the blastocyst. These advances could be applied to a large number of experiments in embryology and basic science, as they could help to understand processes such as chromatin conformational changes and different protein expression patterns in blastomeres without biopsies. The expectations of these models are high, however, the substantial amount of numerical data may require the use of Interpretable Machine Learning (IML) and Deep Learning (DL), since the interpretation of a single variable could lead to errors and premeditated conclusions. Further studies are needed to determine the algorithms necessary for the creation and refinement of the model, however, our data will serve as a precedent for the advancement of these technologies in the future. Supported by Grant from NIHR21 ES026388.

P63: **THE OBESOGENIC CHEMICAL TRIBUTYLTIN INDUCES DYSLIPIDEMIA IN PRIMARY HUMAN OVARIAN THECA CELLS.**

Natalia Pascuali [1], Yong Pu [1], Sarah Pearl [2], Denny Martin [2], Allison Sutton [3], Ariella Shikanov [3], Almudena Veiga-Lopez [1]

[1] University of Illinois at Chicago
[2] Sparrow Hospital
[3] University of Michigan

Background and Purpose: Exposure to obesogenic chemicals leads to increased adipogenesis and adipose tissue accumulation. In particular, the widely used organotin tributyltin (TBT) has been shown to interfere with pathways controlling lipid metabolism and induce lipid accumulation in non-adipose tissues, including the ovary. We have previously reported that organotins dysregulate cholesterol trafficking in ovarian theca cells, but, whether they also exert lipogenic effects in ovarian cells and their underlying mechanisms remains unexplored. We aimed to investigate if environmentally relevant exposures to organotins (TBT, or dibutyltin (DBT)) dysregulate mouse ovarian steroidogenesis, impair oocyte maturation, and result in neutral lipid accumulation and lipid-related transcript expression in cumulus cells and pre-implantation embryos. We also tested the effect of TBT on human theca cell lipid composition and cytokine secretion.

Methods: Organotypic mouse ovary cultures (PND21) were exposed to vehicle (0.1% DMSO) or TBT-chloride (10 or 50 ng/ml) for 8 to 11 days. Conditioned media was collected, and ovaries were fixed, paraffin-embedded and stained with H&E. Estradiol and progesterone concentrations were measured by ELISA, while histology slides were used for follicle counts. In addition, murine oocytes and fertilized embryos were exposed to TBT during in vitro maturation (20 h) and in vitro culture (4 days), after which oocyte maturation and lipid accumulation was assessed, along with lipid homeostasis-related gene expression in cumulus cells and blastocysts. Lastly, primary theca cells derived from human ovaries were exposed to TBT or DBT (both at 50 ng/ml) and their lipidome evaluated by untargeted liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), and their cytokine secretion by bead-based multiplex assays.

Results: There were no significant differences in the murine follicle counts or progesterone concentrations after TBT exposure. However, from days 6 to 11 of exposure, secreted estradiol levels were higher in mouse ovaries exposed to 50 ng/ml of TBT. Expression of genes involved in lipogenesis and fatty acid synthesis were upregulated in cumulus cells and blastocysts exposed to TBT. However, TBT did not impact the rates of oocyte maturation. Over 140 lipids, including increased TAGs and DAGs and reduced galactosyl-ceramides, cholesterol esters, cardiolipins, and sphingomyelins, were dysregulated in TBT-exposed human theca cells. Both DBT and TBT affected cytokine levels, such as TNF-α and IL-9 in human theca cells.

Conclusions: TBT induced dyslipidemia in mouse ovaries and primary human theca cells, which may be responsible for some of the TBT-induced fertility dysregulations reported in rodent models of TBT exposure.

P64: THE ROLE OF DOPAMINE IN FEEDING BEHAVIOR DURING LACTATION IN MICE

Tanya Pattnaik [1], Lara Villano [1], Samuel Congdon [1], Christina Nelson [1], Patrick Sweeney [1]

[1] Department of Molecular and Integrative Physiology, University of Illinois, Urbana-Champaign

Lactation is a significant energetic challenge and critical period that impacts both maternal and offspring health. Lactating mammals drastically increase their food intake to produce milk and generate heat for their offspring. However, the behavioral, molecular, and neural circuit mechanisms mediating the increased feeding associated with lactation are not well understood. Here, we characterized the feeding microstructure and motivation to seek food in mice during lactation using home cage feeding devices (Feeding Experimentation devices; FED3). Lactating mice had altered meal structure and increased food seeking, with an increased preference for palatable food. Since dopaminergic pathways are critical for the regulation of food-seeking and palatable food intake, we utilized pharmacological and imaging approaches to characterize changes in dopaminergic pathways during lactation in mice. Pharmacological inhibition of dopamine receptors potently reduced meal size and food-seeking behavior in lactating mice, normalizing these behaviors to the levels observed in non-lactating animals. Dopamine receptor antagonism also reduced palatable food intake in lactating mice. Next, to characterize functional changes in dopamine pathways during lactation, we utilized *in vivo* fiber photometry to measure changes in nucleus accumbens dopamine transmission in non-lactating and lactating mice during feeding. We observed that lactating mice had a higher dopamine release in response to palatable food consumption than non-lactating female mice, suggesting that increased dopamine levels contribute to enhanced hedonic feeding during lactation. Overall, our study demonstrates that lactating mice have increased food motivation and hedonic feeding behavior, which is accompanied by altered dopamine transmission. The findings are crucial for advancing our understanding of disorders that lie at the intersection of metabolism and reproduction.

P65: AGE-ASSOCIATED OVARIAN MACROPHAGE-DERIVED MULTINUCLEATED GIANT CELLS SHARE TRANSCRIPTOMIC SIGNATURES WITH REGRESSING CORPORA LUTEA

Madeline J. Perry [1], Shweta S. Dipali [1], Michele T. Pritchard [2], Francesca E. Duncan [1], Aubrey Converse [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago IL 60611, USA; [2] Department of Pharmacology, Toxicology, & Therapeutics, Institute for Reproductive and Developmental Sciences, University of Kansas Medical Center, Kansas City, KS 66160, USA

The ovary is one of the first organ systems to show signs of aging, with the ovarian microenvironment characterized by increased inflammation, fibrosis, and stiffness, as well as the presence of macrophage-derived multi-nucleated giant cells (MNGCs). MNGCs are highly penetrant in reproductively old, chronically inflamed ovaries but their etiology is not known. One potential driver could be the accumulation of ovulation-induced debris with age. Ovulation is a classic example of wound repair as the ovarian surface epithelium and follicular wall rupture to enable the release of an egg and then the ruptured region is repaired. The residual follicular cells form the corpus luteum (CL), a transient endocrine structure which produces progesterone. In the absence of pregnancy, murine CL functional lifespan is approximately 2 days and subsequent non-functional CL undergoes regression. With advanced reproductive age, we have observed an enrichment of CL-like structures with a vacuolar appearance, termed foamy corpora lutea (FCL). These FCLs are nearly 4 times more prevalent in ovaries from reproductively old (12m) mice compared to young (6-12 wk) mice and may represent CLs that have failed to properly regress due to age-associated abrogation of the ovarian debris clearance system. These FCLs share similarities with MNGCs as they both contain lipofuscin, an age-associated waste product. We hypothesize that abrogated CL regression with advanced age results in FCL accumulation and contributes to macrophage fusion into MNGCs to aid in FCL clearance. To test this hypothesis, we isolated CLs, FCLs, and MNGCs from mouse ovaries using laser capture microdissection (LCM) technology and performed transcriptomic analyses. Bioinformatic analyses indicated FCLs and CLs are related structures with high expression of *Cyp11a1*, a steroidogenic marker indicative of CL functional capability. However, pathway analyses show that in FCLs, inflammatory GO pathways are upregulated, including leukocyte activation and inflammatory response, indicating a unique inflammatory profile of FCLs compared to CLs. Furthermore, FCLs and MNGCs share this unique immune transcriptomic profile. *Apoe, Fth1*, and *Gpnmb*, which are genes associated with debris clearance and inflammation, are highly expressed in both FCLs and MNGCs, but not in CLs. Both the steroidogenic capacity of CLs and FCLs and the unique inflammatory profile of FCLs and MNGCs offer compelling evidence that FCLs may serve as an intermediate between attenuated CL regression and MNGC formation with advanced reproductive age. Ongoing research aims to define the effect of aging on CL regression and establish the relationship between FCLs and MNGC formation and their contribution to age-associated ovarian inflammation. This work was supported by the Master of Science in Reproductive Science and Medicine Program, the Global Consortium for Reproductive Longevity and Equality (to AC & MTP) and the Duncan Startup.

P66: INCREASED AGE-RELATED OVARIAN STIFFNESS IMPAIRS FOLLICLE DEVELOPMENT AND OOCYTE QUALITY BY MODULATING FOLLICLES' TRANSCRIPTOME

Sara Pietroforte [1], Farners Amargant i Riera [1]

[1] Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO, USA.

Oocyte quantity and quality are severely compromised with female reproductive aging. We and others reported that with aging mouse and human ovary becomes stiffer. However, the role of stiffness on ovarian function and oocyte quality is unknown. This study aims to use an alginate-encapsulated in vitro follicle culture that mimics age-associated changes in ovarian microenvironment to identify whether stiffness is a novel mechanism mediating the progressive decline in oocyte quality observed with aging. To evaluate how stiffness impacts follicle development and oocyte quality, we synthesized hydrogel recapitulating the soft (young: 0.5%, 1.79±0.08kPa) and stiff (old: 2%, 4.56±2.03kPa) environments. Secondary follicles from CD1 (D12-D13, N=3 replicates) were cultured in 0.5% or 2% alginate for up to 12 days. Follicles cultured in stiff environment showed significant reduction in follicle size compared to follicles in soft environment (0.5% 226.9±17.4um, 2% 160.8±9.9um, p< 0.0001). These differences were triggered by granulosa layers since no changes were detected in oocyte size (oocytes: 0.5% 66.19±5.7um, 2% 60.6±4.5um, p=0.401; granulosa cells: 0.5% 160.04±13.4um, 2% 103.37±16.5um, p<0.0001) suggesting that granulosa cells are not proliferating in a stiff environment. To explore this, we assessed estradiol synthesis and found that estradiol levels were reduced in 2% (0.5% 11.3±15.9ng/ml, 2% 0.3±0.5ng/ml, p=0.296), confirming that stiff environment impact granulosa cells viability. We then evaluated whether stiff environment impact gamete quality. At D12, oocytes were isolated from follicles and inspected morphologically. Oocyte quality significantly declined in 2%, with 68.9±16.8% of oocytes degenerated, compared to 23.6±9.2% in 0.5%. We repeated all the analyses using the CB6F1 strain yielding comparable results. Since the effects of stiffness on follicle growth were already evident at D4, we investigated early-changes in follicles' transcriptome that might mediate the observed phenotypes. 120 secondary follicles were cultured in 0.5% or 2%, and analyzed by RNAseq at 3h, 6h, 12h and 24h. We analyzed follicles' gene expression (*DESeq2, R/Bioconductor*) at each timepoint comparing 0.5% and 2% (significance padj<0.05; log2FoldChange±2). We found few differentially expressed genes (DEG) at 3h, a peak of DEG at 6h, followed by a decrease which reached a plateau at 12h and 24h. DEGs at 6h were involved in response to external stimuli, inflammation and apoptosis, suggesting that at 6h follicles' transcriptome is more sensitive to changes in ovarian biomechanics. To further evaluate how stiffness impacts ovarian transcriptome, we analyzed DEGs in a time-dependent manner. We identified 1029 DEGs in follicles cultured in 2% at 24h compared to 3h. Most of the upregulated genes were associated with extracellular matrix (ECM) remodeling, collagen, and connective tissue development. In the 0.5% condition, 1282 DEGs were identified, with upregulation of genes related to metabolism and cell cycle at 24h. Overall, we demonstrated that the age-associated increase in ovarian stiffness impacts follicle development and oocyte quality by impairing granulosa cell viability and estradiol production. In addition, our transcriptome analysis reveals that follicles are highly mechanosensitive and quickly respond to stiffness triggering the expression of inflammation- and ECM-related genes. These results show that stiffness might be a novel regulator of folliculogenesis and oocyte quality.

P67: THE MECHANOTRANSDUCER YAP1 IS HIGHLY EXPRESSED IN GROWING FOLLICLES AND ITS ACTIVITY IS REGULATED BY THE AGE-ASSOCIATED INCREASE IN OVARIAN STIFFNESS

Makenzie L Plough [1], Sara Pietroforte [1], Courtney Murphy [1], Farners Amargant i Riera [1]

[1] OB & GYN Department, Washington University in St. Louis, MO

YAP1 (Yes-Associated Protein 1) is a transcriptional co-activator and the main mechanotransducer in cells. In soft environments, YAP1 is sequestered in the cytoplasm while in stiff environments, YAP1 is dephosphorylated and translocates to the nucleus, where it associates with TEA domain family member (TEAD) transcription factors to regulate gene expression. In the ovary, YAP1 nuclear localization promotes primordial follicle activation and granulosa cell proliferation. YAP1 inhibition results in impaired follicle and embryo development. These observations highlight the importance of mechanotransducers like YAP1 in ovarian function. Importantly, we previously showed that with advanced maternal age the mouse ovary becomes stiffer (6-12 Weeks: 1.98±0.42 kPa, 14-17 Months: 4.36±1.24 kPa). However, the role of YAP1 in the growing follicle and its dependence on the ovary's mechanical properties remains unclear. Therefore, this study aims to investigate YAP1 activity during folliculogenesis and to examine whether YAP1 function is affected by the age-associated increase in ovarian stiffness. To determine YAP1 expression, we used RNAscope with a Yap1 probe on ovary sections from reproductively young mice (6-12 weeks, CB6F1, N=4). We found that Yap1 expression increased progressively during folliculogenesis (Primordial: 2.19±1.16 tpc (transcripts per cell), Primary: 3.95±1.97 tpc, Secondary: 5.48±1.23 tpc), with the highest expression in oocytes from secondary follicles (>250 time higher than in oocytes from primordial/primary follicles). We then investigated YA1 protein expression levels. YAP1 is expressed in all follicle stages, but it was particularly enriched in the theca cell layer, which is in direct contact with the ovarian microenvironment. Interestingly, YAP1 localized primarily in the oocyte cytoplasm, while in granulosa and theca cells, it showed both nuclear and cytoplasmic localization, suggesting active YAP1 in granulosa and theca cells but less so in oocytes. To investigate whether stiffness can modulate YAP1 function in ovarian follicles, we used a hydrogel-based in vitro follicle growth assay. Secondary stages follicles (N=120) were isolated from 12-day-old CB6F1 mice, encapsulated in alginate hydrogels of different stiffness (0.5% and 2%) mimicking the stiffness of young and old ovaries, respectively. Follicles were collected at 3, 6, 12, and 24 hours for RNAseq analysis. YAP1 expression remained unchanged under mechanical stimulation, but YAP1 downstream genes were downregulated (Ctgf 6h: p=0.033, 12h: p=0.017; Ankrd1 12h: p=0.047, Cyr61 3h: p=0.019), suggesting that physiologically relevant mechanical inputs can regulate YAP1 activity. Finally, we evaluated if these patterns are also observed in ovaries from mid-age (7 months, N=5) and reproductively old (14-17 months, N=5) mice. We found no differences in Yap1 expression with aging. Protein-level and downstream gene analysis are currently underway. These findings demonstrate that YAP1 is highly enriched in the growing follicles and actively transported to the nucleus in granulosa and theca cells. Our results suggest that age-associated increase in ovarian stiffness may impact YAP1 activity without altering its expression levels. Ongoing studies are examining whether YAP1 signaling pathway is altered in ovaries from reproductively old mice, and the role of YAP1 in theca cells. This work was supported by the National Institute of Child Health and Human Development (K99HD108424) to F.A.R.

P68: NEUROANATOMICAL AND TRANSCRIPTIONAL ANALYSIS OF HYPOTHALAMIC RESPONSE TO LACTATION

I. C. Possa-Paranhos [1], S. Congdon [1], P. Sweeney [1,2]

[1] Department of Molecular and Integrative Physiology, University of Illinois Urbana-Champaign, Urbana, Illinois, USA.
[2] Neuroscience Program, University of Illinois Urbana-Champaign, Urbana, Illinois, USA.

During the lactational period, mammals increase their energy demands due to milk and heat production for their offspring. In rodents, there is an increase in food intake 3 to 4 times the levels observed in non-lactating animals, while humans are recommended to increase their energy intake by 400 kcal per day. Lactation also drastically alters the neuroendocrine and metabolic profile of animals in an effort to accommodate the energy demands associated with caring for young. However, the neuroanatomical regions and transcriptional changes important for the metabolic and neuroendocrine response to lactation are not fully understood. Given the established role of hypothalamus in feeding, metabolism, and neuroendocrine function, here we used RNA fluorescent in situ hybridization (RNAscope) to compare neuronal activity in discrete hypothalamic cell types involved in feeding, energy homeostasis, neuroendocrine control. To characterize the dynamics of neuronal activity changes we compared the neuronal activity and transcriptional changes in non-lactating and lactating female mice after ad libitum access to food, 90-minute, or 10-hours of food deprivation.

Firstly, looking at the genes and regions important in the leptin-melanocortin pathway, with colocalization for the early expressing mRNA cfos as a marker for neuronal activity, we observed that neurons expressing agouti-related protein (AgRP) are significantly more active in lactating mice after 90 minutes of fasting and the overall the activity of proopiomelanocortin (POMC) expressing neurons is lower during lactation. Additionally, during lactation there is overall lower expression of melanocortin-4-receptor (MC4R) in paraventricular hypothalamus nucleus (PVN), and these cells are also less active compared to non-lactating mice. Furthermore, we analyzed other cell types in PVN that have shown importance for energy intake regulation and neuroendocrine function, and we observed that pituitary adenylate cyclase-activating polypeptide (PACAP) is significantly less expressed during lactation, and these cells are also less active in lactating animals. Similar changes were observed in corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) expressing cells in PVN, indicating broad changes in multiple hypothalamic neuroendocrine populations during lactation. Finally, within the lateral hypothalamus, GABAergic and glutamatergic cells are also known to be important for food intake regulation in opposing directions. Preliminary data presented here indicate that these neurons exhibit opposing dynamics during lactation, providing a potential neuroanatomical mechanism for altered food motivation and hedonic feeding during lactation.

Together, these results highlight that lactation results in significant transcriptional and functional changes in multiple hypothalamic populations important for feeding behavior, metabolism, and neuroendocrine function. Further work is required to establish the mechanism(s) leading to these transcriptional changes, and the importance of these changes to the lactational state.

P69: MINFLUX NANOSCOPY REVEALS ULTRA-FINE STRUCTURE OF AXIAL AND CENTRAL ELEMENTS IN SYNAPTONEMAL COMPLEX

Reza Rajabi-Toustani [1], Sasha Kakkassery [2], Angela Ho [2], Kingsley Boateng [1], and Huanyu Qiao [2]

[1] Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign
[2] Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign

The synaptonemal complex (SC) is a vital structure in meiosis, playing a crucial role in ensuring proper chromosome segregation and genetic diversity through crossing over. It is essential for stabilizing synapsis and facilitating homologous chromosome pairing. While previous studies have utilized various microscopy techniques to study the SC's structure and protein localization, they have been limited in visualizing structures at the nanometer level.

A novel technology called Minimal Photon Flux (MINFLUX) nanoscopy has emerged as a powerful tool for studying the SC. MINFLUX offers unparalleled resolution, allowing researchers to visualize structures at the nanometer scale, which was previously impossible. By employing MINFLUX, we investigated the intricate architecture of the SC in both wild-type and Rec8-/- mutant mice.

Our study revealed significant differences in the SYCP3 width between wild-type and Rec8-/- mutants. In wild-type mice, the SC forms between two homologous chromosomes or four sister chromatids, while Rec8-/- mutants experience pairing between only two sister chromatids. Additionally, we observed a decrease in the width and depth of SIX6OS1 in Rec8-/- mutants compared to wild type.

Overall, our findings highlight the critical role of REC8 in maintaining the stability and structure of the SC. MINFLUX nanoscopy has provided unprecedented insights into the SC's architecture, offering new avenues for studying complex biological structures with exceptional detail.

P70: A PHENOTYPIC SCREENING ASSAY IDENTIFIES NOVEL INHIBITORS TARGETING WEE2 KINASE ACTIVITY TO BLOCK EGG ACTIVATION FOR NON-HORMONAL CONTRACEPTION

Alisha F. Ramos [1], Hoi Chang Lee [1], Jeffrey Pea [1], Prianka H. Hashim [1], Hidenori Takahashi [2], Christian Andersen [2], Felicia Gray [2], Shelby Ellery [2], Francesca E. Duncan [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL
[2] Schrödinger LLC, New York, NY, Portland, OR and San Diego, CA

WEE2 is an oocyte-specific kinase crucial for meiotic resumption from metaphase II (MII) arrest and egg-to-embryo transition during fertilization. Selective inhibition of WEE2 kinase activity without impacting other WEE family kinases, such as MYT1 and WEE1, offers a targeted strategy for non-hormonal contraception. We established a phenotypic assay to monitor mouse egg activation, characterized by second polar body (PB) extrusion, pronuclei formation (PN), and two-cell cleavage. Strontium chloride (SrCl2), an inducer of calcium transients, was used to synchronize egg activation. In the absence of selective commercially available WEE2 inhibitors, we used Adavosertib as a reference compound to validate the platform. Treatment with Adavosertib (500 nM) significantly inhibited egg activation and developmental progression compared to control. We then tested a novel series of highly potent and selective Wee2 inhibitors developed by Schrodinger (B-1 and B-2, Ki <30 nM) along with a less active enantiomer of analogue B-2 (B-3, Ki >1,000nM). We found that treatment with these compounds still allowed for second PB extrusion but reduced PN-formation (Control: 100%, B-1: 0%, B-2: 0%, B-3: 81%) and two-cell cleavage (Control: 100%, B-1: 0%, B-2: 0%, B-3: 59%). These phenotypes were dose-dependent and exhibited similar but less potent effects in egg activation via intracellular sperm injection (ICSI). As WEE2 phosphorylates CDC2 to drive egg activation, we assessed target engagement by evaluating pCDC2 expression in activated oocytes treated with 10μM B-1 and B-2 using Western blotting. While pCDC2 expression increased following egg activation, it was significantly inhibited by B-1 and B-2 exposure. We evaluated the safety profile of these compounds by examining their impact on follicular growth, survival, and ovulation over an 8-day period. Follicles were treated with the minimum effective concentrations identified from the egg activation assay (B-1: 100 nM; B-2: 1 μM). There was no statistically significant difference in survival rates between the control group and follicles treated with B-1 and B-2, with all surviving follicles demonstrating comparable growth trajectories and successfully ovulating mature MII oocytes under both treatment conditions. These findings highlight the potential of WEE2 inhibitors as non-hormonal contraceptives, with ongoing studies to further improve their safety and efficacy in vivo.

P71: CHARACTERIZING THE EXPRESSION AND FUNCTION OF THE ACTIN-BINDING PROTEIN NEXILIN IN MAMMALIAN OOCYTES

Shams M. Saad [1], Lauren McGinnis [2], Janice P. Evans [1]

[1] Department of Biological Sciences, Purdue University [2] Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health

The progression of female gametes through meiosis is crucial for producing competent eggs capable of supporting fertilization and normal embryonic development. The actin cytoskeleton and actin-binding proteins (ABPs) within oocytes play a vital role in facilitating the highly organized asymmetric cell division necessary for this process. Nexilin is a novel F-actin-binding protein, and our lab is the first to demonstrate that nexilin has a role in oocyte meiotic maturation. Nexilin is known for its critical role in maintaining the structural integrity and normal function of the heart by stabilizing Z-disks within sarcomeres, the functional units of striated muscles. Additionally, nexilin is essential for the initiation and formation of transverse (T)-tubules in junctional membrane complexes (JMCs) in cardiomyocytes. Furthermore, nexilin is involved in cell adhesion, migration, differentiation, and cell-matrix adherens junctions (AJs). Mutations in NEXN gene are associated with various cardiovascular disorders (CVDs), particularly dilated cardiomyopathy (DCM), a condition in which the heart becomes enlarged and weakened, impairing its ability to pump blood effectively. In various species, Nexn transcripts are highly abundant in cardiac, skeletal, and smooth muscles. Interestingly, a mouse transcriptome database also revealed that Nexn transcripts are highly abundant in oocytes and fertilized eggs. In this study, we investigate the role of nexilin in mammalian oocyte meiotic maturation, identifying the splice variants present in oocytes, and exploring their functional differences. We assessed nexilin expression at different meiotic stages, finding consistent expression throughout meiosis. We also examined the localization of nexilin at different meiotic stages, showing that nexilin is localized in the cytoplasm and cortex from prophase I to metaphase II. Additionally, nexilin is found in the actin cap of metaphase II eggs, with some of these eggs also displaying meiotic spindle localization. Using reverse transcription (RT)-PCR and immunoblots, we examined the two splice variants, B-nexilin and S-nexilin, and demonstrated that S-nexilin is the predominant splice variant in oocytes, while B-nexilin is barely detectable. Using siRNA-mediated nexilin knockdown, we showed that nexilin-deficient oocytes become arrested at meiosis I with abnormal actin organization and spindle localization. To further investigate the effects of nexilin depletion in oocytes over a shorter time frame, we will utilize a posttranslational reverse genetics protein depletion method called Trim-Away . We are validating antibody specificity for Trim-Away, using immunoblotting of proteins separated by native gel electrophoresis and immunofluorescence, and analyzing the functions of the two structurally different nexilin splice variants. This study is the first to investigate the role of nexilin, a novel actin-binding protein, in the progression of oocytes through meiosis, which could have implications for infertility. With growing evidence suggesting a link between infertility and CVDs, nexilin may act as a molecular mediator of female fertility and cardiovascular health.

P72: LONG-TERM DIETARY EXPOSURE TO AN ENVIRONMENTALLY RELEVANT PHTHALATE MIXTURE AFFECTS OVARIAN AGING MARKERS.

Adira M. Safar [1], Ramsés Santacruz-Márquez [1], Mary J. Laws [1], Daryl D. Meling [1], Jodi A. Flaws [1]

[1] Department of Comparative Biosciences, University of Illinois Urbana-Champaign.

Phthalates are a class of chemicals used in the production of polyvinyl chloride products such as food packaging, medical tubing, and children's toys. They are also used as solvents in personal care products and paints. Humans are chronically exposed to phthalates, primarily through ingestion, due to their presence in food packaging and their tendency to leach out of consumer products and packaging at high temperatures. Exposure to phthalates can accelerate the onset of reproductive aging, leading to premature ovarian failure. Reproductive aging is characterized by changes in ovarian follicle populations, altered expression of telomere associated genes (TAGs), increased fibrosis, decreased serum estradiol levels, and increased follicle-stimulating hormone (FSH) levels. Previous research in our lab demonstrated that long-term dietary exposure to di(2-ethylhexyl) phthalate or diisononyl phthalate accelerates ovarian aging in mice. However, humans are exposed daily to a mixture of phthalates. Thus, it is important to investigate the effects of phthalate mixtures on ovarian aging markers. In this work, we focused on the effects of long-term dietary exposure to an environmentally relevant phthalate mixture (Mix) on ovarian aging markers. We exposed mice to Mix (0.15 ppm, 1.5 ppm, 1500 ppm) through the chow for 6 months. One ovary per mouse was fixed and used for histological evaluation of follicle populations and fibrosis using Masson's trichrome (MT) and picrosirius red staining (PSR). The other ovary was snap-frozen and used for qPCR analyses to evaluate the expression of TAGs (*Pot1a1, Trf1, Trf2, Terc*, and *Tert*). Sera were processed for analysis of FSH and luteinizing hormone (LH) levels. Long-term exposure to Mix significantly increased the expression of Pot1a1 (1.5 ppm) and borderline increased expression of Pot1a1 and Trf2 (1500 ppm), with no changes in expression of *Trf1, Tert*, or *Terc*. Interestingly, exposure to Mix significantly decreased the percentage of collagen 1 (positive signal of MT) in the ovary (1500 ppm), and borderline increased the combined percentage of collagens 1 and 3 (positive signal of PSR) in the ovary (1.5 ppm) compared to control. Exposure to Mix borderline increased serum FSH levels (0.15 ppm) and significantly decreased serum LH levels at each dose compared to control. Interestingly, exposure to Mix borderline decreased the percentage of antral follicles (1.5 ppm), borderline decreased the percentage of preantral follicles (1500 ppm), significantly decreased the percentage of antral follicles (1500 ppm), and significantly increased the number of primordial follicles (1500 ppm). Together, these data indicate that long-term dietary exposure to Mix affects fibrosis, follicle dynamics, expression of TAGs, and serum gonadotropin levels, suggesting that Mix can accelerate the onset of ovarian aging. Supported by R01 ES028661, R01 ES034112, T32 ES007326.

P73: THE FEASIBILITY OF ADENO-ASSOCIATED VIRUS-MEDIATED TRANSDUCTION IN HUMAN PRIMARY GRANULOSA CELLS

Natasha Salpeter [1], Meredith Lohman [1], Dilan Gokyer [1], Anna Kleinhans [2], Joan K. Riley [2], Elnur Babayev [1,2]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL
[2] Department of Obstetrics and Gynecology, Northwestern Medicine Center for Fertility and Reproductive Medicine, Chicago, IL

Background: Infertility affects approximately 10% of women with an estimated half of these cases linked to genetic variants. Some of the infertility cases are due to single gene mutations. However, there is currently no targeted gene therapy available for these conditions. A promising advancement in genetic therapies is the use of Adeno-associated viruses (AAVs) that can infect human cells without causing disease or integrating into the host genome. Over 200 clinical trials are currently underway to evaluate the efficacy of AAV-based gene therapies in humans, with five products already FDA-approved. However, the feasibility of using AAVs in human reproductive tissues has not been previously assessed. Our aim is to test whether AAVs can be used to transduce human primary granulosa cells and to determine the optimal transduction protocols.

Study Design: Human primary granulosa cells were obtained from infertility patients undergoing ovarian stimulation and egg retrieval, cryopreserved, thawed, and cultured. These cells were transduced with 17 native and capsid-engineered AAV serotypes expressing Green Fluorescent Protein (GFP) across two multiplicities of infection (MOIs: 30,000 and 100,000), on two different days after cell plating (Day 1 and Day 2), and two incubation periods (24 and 48 hours). The efficiency and intensity of transduction were analyzed. Seven of the 17 initial serotypes were selected for further study to determine the lowest possible MOI that can transduce human primary granulosa cells.

Key Findings: Of the 17 AAV serotypes tested, seven (AAV2, AAV3, AAV6, AAV9, AAVDJ, AAVX1.1, and AAVPV38) exhibited at least 10% transduction efficiency at MOI of 30,000. Additionally, transduction on Day 1 of the culture and 48-hour incubation time demonstrated the highest transduction efficiency and fluorescent signal intensity per cell (e.g., AAVDJ 100k 63.38%, 26.07 au) compared to the Day 1-24 Hour (e.g., 59.63%, 24.56 au), Day 2-24 hour (e.g., 47.17%, 15.96 au), and the Day 2-48 hour (e.g., 46.78%, 20.60 au). We then tested the transduction efficiency of these 7 AAVs from the initial screen at MOIs of 5000, 10,000 and 20,000 and used the optimized (Day1-48hr) protocol. Only 2 AAVs (2, DJ) were able to transduce human primary granulosa cells at the lowest MOI of 5000.

Conclusions: Our findings provide evidence of the feasibility of AAV transduction in human granulosa cells. Studies are ongoing to determine the optimal MOIs for transduction as well as characterization of possible AAV toxicity on human granulosa cells. This research lays the groundwork for the development of gene therapies for women with single gene mutations disruptive to their ovarian function, and has broad implications for the modulation of gene expression in human ovaries.

P74: CHARACTERIZING EXTRACELLULAR VESICLES FROM THE BOVINE UTERINE EPITHELIUM

Kassandra Sandoval [1], Malia Berg [2], Madison Hall [2], Matthew Dean [1,2]

[1] Division of Nutritional Sciences, [2] Department of Animal Sciences, University of Illinois at Urbana Champaign, Urbana, IL

Lactate and glucose are important sources of nutrition for early embryonic development. Too much or too little of these nutrients can be lethal to the embryo. It is known that extracellular vesicles (EVs) can transfer material from the uterine epithelium to the embryo. Many factors regulate EV secretion, uptake, and cargo profiles. The role of estradiol and progesterone on EVs has not yet been fully elucidated. Here we aimed to explore the localization of proteins related to EV secretion in the bovine uterine epithelium, confirm secretion of EVs by immortalized bovine uterine epithelial (BUTE) cells, and explore the metabolic contents of uterine EVs.

Transmission electron microscopy (TEM) analysis of uterine biopsies from abattoir collected bovine samples showed EVs in the glandular lumen, confirming their presence in vivo. Immunohistochemical localization of proteins involved in vesicular trafficking/recycling, endocytosis and exocytosis, RAB11, FLOT1 and RAB27A, respectively, was observed in uterine sections on day 1 and day 11 of the reproductive cycle. On day 1 of the cycle, RAB11 was localized in the glandular epithelium but also expressed diffusely throughout the stroma. On day 11, RAB11 was mainly localized to the glandular epithelium. On day 1, FLOT1 staining was diffusely expressed and appeared puncta-like in the stromal tissue with some staining observed in uterine glands. On day 11, FLOT1 exhibited similar puncta-like staining in stromal tissue along the basal membrane but was mainly localized to the uterine glands. RAB27A was similarly localized to the glandular and stromal epithelia; however, RAB27A was more intensely expressed on day 11 sections. These results indicate there are cyclic differences in vesicular trafficking and exocytosis at different stages of the reproductive cycle.

Ultra-centrifugation was used to isolate EVs from the conditioned media from BUTE cells. TEM showed nanoparticles that matched the morphology of EVs observed in vivo. Similarly, NanoSight NS300 data showed an abundance of particles in the 50-200 nm size range. Confirming our ability to isolate EVs, western blot confirmed the expression of known extracellular vesicle markers, CD9, CD63, HSP70 and β-tubulin. This confirms our ability to isolate EVs from BUTE cells for further study.

Untargeted gas chromatography-mass spectrometry (GC-MS) was performed the identify the metabolites contained within EVs. Metabolomics performed on untreated EV samples revealed an abundance of fatty acids, DNA, RNA, and amino acids. Most importantly, lactic acid and glucose were identified within the extracellular vesicles. This supports our working theory that the uterus secretes EVs with nutrients to nourish the early embryo. Future work will study the effects of estradiol and progesterone treatment on the metabolic and proteomic contents of uterine EVs.

P75: LONG-TERM DIETARY EXPOSURE TO DI(2-ETHYLHEXYL) PHTHALATE INDUCES INFLAMMATION AND FIBROSIS IN THE OVARY

Ramsés Santacruz-Márquez [1], Adira M. Safar [1], Mary J. Laws, Daryl D. Meling [1], Jodi A. Flaws [1]

[1] Department of Comparative Biosciences, University of Illinois Urbana-Champaign

Phthalates such as di-(2-ethylhexyl) phthalate (DEHP) are used as plasticizers in polyvinyl chloride plastics, flooring, food packaging, personal care products, medical tubing, plastic toys, and paints. Humans are continuously exposed to phthalates. Phthalates are ubiquitously found in consumer products and the environment and humans can be exposed to them through ingestion. Previous studies in our lab have shown that short-term exposure (between 10 and 30 days) to phthalates accelerates the onset of reproductive aging in mice. However, limited data exist regarding the effects of long-term exposure to phthalates on markers of ovarian aging and the onset of reproductive senescence. Reproductive aging is characterized by increased inflammation, induction of fibrosis, decreased follicle numbers, decreased estradiol levels, and shortening of telomeres in ovarian cells. The goal of this study was to determine whether long-term dietary exposure to DEHP affects markers of reproductive aging, including systemic and ovarian inflammatory profiles, fibrosis levels in the ovary, and telomere-associated gene expression in the ovary. Adult female mice were exposed to DEHP at 0.15 ppm, 1.5 ppm, or 1500 ppm via the chow for 6 months. After dosing, the mice were euthanized, and some ovaries were collected and processed for picrosirius (PSR), and Masson's trichrome staining (MTS) to determine fibrosis levels in the ovary. Other ovaries were used for qPCR analysis to determine changes in inflammatory-, fibrosis-, and telomere-related genes. Sera were collected and processed using a cytokine array for the analysis of the systemic inflammatory profile. The data show that long-term DEHP exposure affects the systemic inflammatory profile by increasing systemic inflammatory molecules compared to control. Long-term DEHP exposure also affected the ovarian inflammatory profile by dysregulating several inflammatory molecules compared to the control. In addition, DEHP increased fibrosis levels as evidenced by increased PSR and MTS positive signals compared to control. Finally, long-term DEHP exposure increased the expression of the telomere associated gene *Trf2* compared to control. Collectively, these data indicate that long-term exposure to DEHP alters inflammation at both the systemic and ovarian levels, induces ovarian fibrosis, and induces the expression of *Trf2*, suggesting that long-term exposure to DEHP may accelerate ovarian aging. Supported by R01 ES028661, R01 ES034112, T32 ES007326.

P76: THROUGH THE TROUGH: ENGINEERING 3D TESTICULAR TUBULES TO SUPPORT SPERMATOGENESIS *IN VITRO*

Anika Schipma [1], Anjali Patel [2], Olivia Printy [1], Monica M Laronda [3-5]

[1] Center for Reproductive Science, Feinberg School of Medicine, Northwestern University, Chicago, IL
[2] Feinberg School of Medicine, Northwestern University, Chicago, IL
[3] Stanley Manne Children's Research Institute, Ann & Robert Lurie Children's Hospital of Chicago, Chicago, IL
[4] Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL
[5] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL

1 in 285 children in the US is diagnosed with cancer. As a result of modern therapeutics such as platinum analogs, radiation therapy, and alkylating agents, up to 82% of these children will likely survive to adulthood. Unfortunately, many of these treatments confer a high risk of permanent infertility in pediatric patients. Thus, the increasing number of childhood cancer survivors introduces a need for novel technologies to support fertility preservation and restoration for pediatric cancer patients. Spermatogenesis—the process in which spermatogonial stem cells (SSC) develop into sperm--requires a specific hormonal and structural niche. Our murine in vitro testicular aggregate system aims to recapitulate the in vivo structural support necessary for spermatogenesis. Within our 3D-printed agarose trough culture, preliminary data has shown that donor testicular organoids will develop continuous tubule-like aggregates containing native-similar localization of Sertoli cells, Leydig Cells, and Peritubular Myoid Cells. We predict that such organoids will have improved capacity to support the survival and migration of spermatogonial stem cell (SSC) populations. Testicular organoids were created from 5dpp CD_1 murine testes and enriched with TdTomato+ murine SSCs at time of cell seeding, time of trough culture, or via aggregate injection. Trough cultures consisted of widths of <700 um or >700 um. After three days of culture in microwells, spherical organoids were transferred to the trough culture for 9 days, where they aggregated prior to fixation and analysis. Aggregates were sectioned and analyzed via immunofluorescence with staining for testicular somatic cells and germ cells. Testicular organoids cultured in trough widths of <700um developed aggregates containing tubule-like structures, with peritubular myoid cells and Sertoli cells basally localized, and Leydig cells interstitially located between tubules. Preliminary data has indicated that germ cells expressing DDX4 localize to Sertoli Cells—their native location *in vivo*--most often when organoids are enriched with THY1+ cells at the timepoint of trough aggregation. Improved localization of germ cells to Sertoli cells would indicate progress in the generation of an *in vitro* niche that could support Spermatogenesis. Further studies aim to enhance survival and localization of spermatogonia within continuous, tubule-like testicular aggregates. Through the generation of an *in vitro* platform for spermatogenesis, the above work has the potential to expand fertility restoration options for pediatric cancer patients at risk for fertility loss, patients with differences in sex development, and patients planning to undergo gender-affirming hormone care. Supported by R01 grant.

P77: LONG-TERM DIETARY EXPOSURE TO PHTHALATES ALTERS EXPRESSION OF OXIDATIVE STRESS MARKERS IN THE OVARY

Taylor A. Seaton [1], Ramsés Santacruz-Márquez [1], Zane C. Inman [1], Adira M. Safar [1], Mary J. Laws [1], Jodi A. Flaws [1]

[1] Department of Comparative Biosciences, University of Illinois Urbana Champaign

Phthalates such as diethyl (2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP) are used as plasticizers and solvents in many products including cosmetics, medical tubing, and food packaging. Phthalates are non-covalently bound to these products, which leads to them leaching out and entering the human body, resulting in continuous and long-term exposure of phthalates in humans. In our previous studies, short-term exposure to DEHP and DiNP (between 10 and 30 days) was shown to increase the levels of oxidative stress markers in the mouse ovary; however, few studies have examined the impacts of long-term phthalate exposure. Oxidative stress is indicated by an imbalance in the production of reactive oxygen species and antioxidant defense mechanisms. Prolonged oxidative stress in ovarian cells leads to cellular damage and impaired follicular development, which may impact fertility. This study tested the hypothesis that long-term dietary exposure to phthalates affects markers of oxidative stress in the mouse ovary. Adult female CD-1 mice were fed a diet formulated with vehicle control or various concentrations (0.15ppm, 1.5ppm, or 1500ppm) of phthalates (DEHP, DiNP, or an environmentally relevant phthalate mixture (Mix) containing diethyl phthalate (DEP), dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), DEHP, benzyl butyl phthalate (BzBP) and DiNP) for 6 or 12 months. The doses of 0.15ppm and 1.5ppm were chosen because they fall within the typical range of daily human exposure. The highest dose of 1500ppm was chosen to represent occupational exposure. After dosing, the mice were euthanized, and the ovaries were collected to perform qPCR for gene expression analyses of genes that regulate oxidative stress (superoxide dismutase 1 (*Sod1*), glutathione S-transferase pi 1 (*Gstp1*), glutamate-cysteine ligase catalytic subunit (*Gclc*), and glutamate-cysteine ligase modifier subunit (*Gclm*)). The results indicate that at 6 months, DEHP at 1.5ppm increased *Gclm* expression and DEHP at 1500ppm increased *Sod1* expression compared to control. Additionally, DiNP at 1.5ppm and 1500ppm decreased *Gstp1* expression compared to control. At 12 months, DEHP at 0.15ppm decreased the expression of Gclc and DEHP at 1.5ppm decreased *Gstp1* and *Gclc* expression compared to control. DiNP and Mix at 0.15ppm, 1.5ppm, and 1500ppm increased *Sod1* expression compared to control. Collectively, these data suggest that long-term exposure to DEHP, DiNP, and Mix may interfere with oxidative defense mechanisms promoting oxidative stress in ovarian tissue. Supported by NIH R01 ES034112.

P78: HYPERPLASTIC OVARIAN STROMAL CELLS EXPRESS GENES ASSOCIATED TO TUMOR PROGRESSION: A CASE STUDY

A. Sharma [1,2], F. Becker [2], X. Tao [2], V.S. Baddela [2], D. Koczan [3], C. Ludwig [2] and J. Vanselow [2]

[1] Department of Animal Sciences, University of Illinois at Urbana Champaign, Urbana, IL
[2] Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany
[3] Institut für Immunologie, Universität Rostock, Rostock, Germany

The current study presents the analysis of ovarian stromal cells obtained from an hyperplastic left-ovary of a Holstein cow. Cultured hyperplastic stromal cells displayed a fibroblast-like morphology and ceased proliferation after the 8th passage. The non-cancerous nature of stromal cells was confirmed by in vitro cell proliferation and migration assays. Negligible amounts of 17β-Estradiol (E2) were detected in the spent media of cultured stromal cells, which suggests that stromal cells were non-estradiol synthesizing cells. As revealed in immunofluorescence and gene expression analysis, the hyperplastic stromal cells explicitly expressed vimentin in their cytoskeleton. Upon hematoxylin staining, a highly dense population of stromal cells was observed in the stromal tissue of the hyperplastic ovary. Further, to explore genome-wide alterations, mRNA microarray analysis was performed using Affymetrix Bovine Gene 1.0ST Arrays compared to normal ovarian derived stromal cells. The microarray identified 1396 differentially expressed genes, of which 733 were up- and 663 down-regulated in hyperplastic stromal cells. Importantly, asporin (*ASPN*) and vascular cell adhesion molecule 1 (*VCAM1*) were among the highly up-regulated genes. Higher expression of *ASPN* was also confirmed by immunohistochemistry and RT-qPCR analysis. Ingenuity pathway analysis (IPA) identified about 98 significantly enriched (-log (p value ≥1.3) canonical pathways, importantly of which the "Sirutin Signaling Pathway" and "Mitochondrial Dysfunction" were highly activated while "Oxidative phosphorylation" was inhibited. Additionally, higher proportion of hyperplastic stromal cells in the S-phase of cell cycle, could be attributed to higher expression levels of cell proliferation genes such as *CCND2* and *CDK6*.

P79: LONG-TERM DIETARY EXPOSURE OF MICE TO A MIXTURE OF PHTHALATES CAUSES DYSREGULATION OF ESTROGEN RECEPTOR SIGNALING AND LEADS TO ENDOMETRIAL HYPERPLASIA

Ritwik Shukla [1], Athilakshmi Kannan [1], Mary J. Laws [1], Jodi A. Flaws [1], Milan K. Bagchi [2], and Indrani C. Bagchi [1]

[1] Departments of Comparative Biosciences, [2] Molecular & Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL

Phthalates, synthetic chemicals widely utilized as plasticizers and stabilizers in various consumer products, are of concern due to their persistent presence in daily human life. While past research predominantly focused on the effects of individual phthalates, real-life human exposure typically encompasses complex mixtures of these compounds. The cumulative effects of prolonged exposure to phthalate mixtures on uterine health remain poorly understood. Thus, we conducted studies in which adult female mice were exposed for 6 and 12 months to two doses, 0.15 ppm and 1.5 ppm of a mixture of phthalates via chow ad libitum. Our studies revealed that consumption of phthalate mixture at 0.15 ppm and 1.5 ppm for 6 months led to excessive accumulation of extracellular matrix components, such as collagen fibers in the uterus. Further investigation employing RNA-sequencing revealed a significant upregulation of a subset of genes encoding proinflammatory cytokines and several genes known to be regulated by estrogen receptor in the uterus. Since excessive action of the estrogen receptor in the uterus and chronic exposure to proinflammatory cytokines are common factors leading to hyperplasia, we investigated changes in endometrial histology after 12 months of phthalate exposure. In alignment with these findings, we observed a significant increase in the gland/stroma ratio with enhanced proliferation of glandular epithelial cells, indicating development of hyperplasia in phthalate-exposed uteri. Future studies will address the mechanisms by which chronic exposure to phthalates leads to dysregulation of estrogen receptor-regulated pathways in the uterus contributing to endometrial hyperplasia.

P80: INNOVATION FOR INSUFFICIENCY: PRECURSOR TO AN IN VITRO ASSAY TO PREDICT ADRENAL CORTEX FUNCTION USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Janavi Thyagraj [1], Monica M. Laronda [2-4]

[1] Center for Reproductive Science, Feinberg School of Medicine, Northwestern University
[2] Stanley Manne Children's Research Institute, Ann & Robert H Lurie Children's Hospital of Chicago
[3] Department of Pediatrics, Feinberg School of Medicine, Northwestern University
[4] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University

The adrenal cortex secretes steroid hormones which maintain homeostasis of the body. Like the gonads, the cortex also produces sex steroids. Hence, mutations in the steroidogenic genes will disrupt the synthesis of steroids in the adrenal cortex leading to adrenal insufficiency. Adrenal insufficiency (AI) is a chronic condition characterized by the adrenal cortex's inability to adequate amounts of mineralocorticoids, glucocorticoids, and sex steroids. Owing to mutations in certain gene variants, the condition of AI can co-exist with differences in sex development (DSD), infertility etc. Unfortunately, predicting the prognosis of sex steroid deficiency is challenging before puberty. Therefore, we seek to develop an in vitro model of the adrenal cortex function that can provide early predictive insights on potential steroid insufficiency, adrenal gland co-morbidities or unknown adrenal pathologies.

IISH2i-BM9 stem cells were differentiated into human adrenal cortex-like cells (hACLCs) using a previously published and established differentiation protocol. hACLCs generated using this protocol were characterized to define baseline ranges of steroid hormones. The stem cells were aggregated and treated with a series of ligands for 28-days to steer their differentiation towards adrenal cortex-like lineage. hiPSCs were aggregated in V bottom plates. They were cultured in U bottom plates (floating culture) from day 1 to 21 followed by an air-liquid interface (ALI) culture in trans-wells from day 22 through day 28. On day 28, the hACLCs were validated for expression of a specific adrenal cortex cell surface marker. DHEA and cortisol ELISAs were performed on media collected on day 22, 25, 28 and 32 of culture.

Using 12 thawed hACLC aggregates, we found that 65.4% of live cells expressed the adrenal cortex cell surface protein DLK-1, indicating both the efficiency of differentiation and feasibility of utilizing cryopreserved cells. On average, the hACLC aggregates produced the most DHEA (14.97 ng/ml) on day 22 of floating culture and significantly less DHEA (4.32-6.49 ng/ml; p=0.0123, p=0.0071, p=0.0096) on days 25, 28 and 32. However, in ALI culture hACLC aggregate DHEA production was relatively consistent from day 22 to 32 (4.73-9.90 ng/ml). The differentiated hACLCs also produced cortisol in the range of 21.64-26.62 ng/ml and 20.57-21.77 ng/ml in the floating and ALI culture, respectively. Overall, the levels of cortisol remained steady in the two culture systems.

Our preliminary results indicate that the aggregates differentiated into adrenal cortex-like cells. The hACLC aggregates comprises cells of adrenal cortex lineage which can secrete DHEA and cortisol. The decreasing levels of DHEA from day 22 to 32 can be attributed to its utilization in sex steroid biosynthesis. Contrary to previous literature, our results display a notable decrease in DHEA which needs to be validated with expression of sex steroid secretion. Additional assays such as qPCR will be conducted on cryopreserved aggregates to identify the expression of steroidogenic genes of these aggregates. Later, we will model adrenal insufficiency by culturing the hACLC aggregates in media devoid of sonic hedgehog ligand. Then, we will conduct experiments to compare the structure and function of the hACLC aggregates at baseline and in hiPSC lines generated from patients with known adrenal insufficiency.

P81: EFFECT OF LONG-TERM PROPYLPARABEN EXPOSURE ON COLLAGEN DEPOSITION AND GENE EXPRESSION IN MURINE OVIDUCT

Swarna Pal Tisha [1], Jie Li [1], and Ayelet Ziv-Gal [1]

[1] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois

Parabens, extensively used as preservatives in cosmetics, food products, and pharmaceuticals, are classified as endocrine-disrupting chemicals capable of interfering with hormone signaling, including estrogen. Women are particularly at risk due to the prevalent use of paraben containing products, yet little is known on paraben effects on female reproductive health. The oviduct is crucial for female fertility, serving as the site for fertilization and facilitating the transport of pre-implantation embryos to the uterus. Thus, changes in oviduct's structure and function may lead to infertility. We investigated the effects of long-term propylparaben exposure on the murine oviduct. Propylparaben is one of the most used parabens and is consistently detected in women biological samples. We hypothesized that chronic daily exposure of propylparaben will alter the expression of estrogen and fibrosis-related genes and enhance collagen deposition in the oviduct. To that end, adult female CD-1 mice were exposed to human relevant doses of propylparaben (2, 20, and 200 µg/kg/day, n=15/group) over a six-month period, daily. Oviducts were examined using Masson's trichrome histochemical staining for collagen deposition (n=4 per group) and RT-qPCR for gene expression analysis (n=5-6 per group) of estrogen receptor alpha (*Esr1*), collagen types 1, 3, 4, and 5 (*Col1a1, Col3a1, Col4a1, Col5a1*), transforming growth factor beta 1 (*Tgfβ1*), and progesterone receptor (*Pgr*). Data were statistically analyzed by one-way ANOVA or Kruskal-Wallis tests followed by post-hoc tests. A p-value of ≤0.05 was considered statistically significant compared to controls. Results indicate a marginal statistical difference in collagen deposition across treatment groups (p-value = 0.08). Post-hoc analysis showed a statistically significant increase in the percent area stained for collagen in the 200 µg/kg/day propylparaben group (median = 3.74, IQR = 3.87) compared to the control group (median = 1.09, IQR = 1.63), with an adjusted p-value of 0.05. No significant differences were observed between the control group and the propylparaben groups at 2 µg/kg/day (median = 3.74, IQR = 3.87) and 20 µg/kg/day (median = 3.74, IQR = 3.87), with adjusted p-values >0.05. Further, analysis of *Esr1* expression levels indicate an overall marginal statistical difference (p-value = 0.06) between the propylparaben and control groups, with fold change in expression levels of 1.02 ± 0.23 (mean ± SD) for controls, 1.09 ± 0.10 for 2 µg/kg/day, 0.94 ± 0.22 for 20 µg/kg/day, and 0.78 ± 0.22 for 200 µg/kg/day. Fold change in expression levels of the other genes (*Col1a1, Col3a1, Col4a1, Col5a1, Tgfb1*, and *Pgr*) were similar across treatment groups (p-values > 0.05). Overall, these results suggest that long-term exposure to low levels of propylparaben may have a limited impact on fibrosis-related processes in adult female mice.

P82: DETERMINING BIOCOMPATIBILITY OF 3D PRINTING RESINS AND EFFECT OF PARYLENE-C COATING FOR APPLICATIONS IN COMPLEX REPRODUCTIVE TISSUE CULTURES

Uyen Tran [1], Hannes Campo [1], Francesca E. Duncan [1,2]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611, [2] Buck Institute for Research on Aging, Novato, CA, 94945.

Resin 3D printing has become a popular method to prototype and manufacture intricate tissue-engineered in vitro cultures, including microfluidic devices. This is because it is an approachable technique that can print complex models with high fidelity and enables design progress through rapid iteration. However, it has been well-established that most photopolymerizable resins are highly cytotoxic to cell cultures. Furthermore, research from our lab has shown that supposedly biocompatible dental resins have severe negative effects on oocyte culture, making them incompatible with research that uses gamete-containing cultures.

Several approaches can be applied to resolve this issue. A common method to improve biocompatibility of materials is to use parylene-C. This polymer can be deposited chemically to create micron-thin barriers that are impermeable to many molecules, including water and possible leachates from 3D prints. Additionally, an expanding line of biocompatible (BioMed) resins has been made commercially available from Formlabs. All these resins passed FDA-approved ISO standards (biological evaluation of medical devices, ISO 10993), some of which even passed ISO 10993-3 which accounts for reproductive toxicity. It is unclear which tests were used to establish these standards and if these resins can be used in vitro ovarian tissue cultures for extended periods of time.

Here we present a proposed workflow to determine the biocompatibility of five resins, and if parylene-C coating can reduce possible deleterious effects. The potential reproductive toxicity will be tested using an in vitro maturation (IVM) assay of denuded mouse oocytes, a highly sensitive and well-established mammalian cell model.

We designed an insert for 24-well plates using computer-aided design software, printed it in Clear V4, Biomed Elastic 50A, BioMed Flex 80A, BioMed Clear and BioMed Durable resin using a Form 3B+ printer and post-processed them according to the manufacturer's specifications. Half of the inserts received a 5µm thick parylene-C coating. The inserts were then sterilized using 100% ethanol, or with other manufacturer-approved methods. Next steps in this work are to isolate denuded oocytes from mice that were hyperstimulated with 5 IU pregnant mare serum gonadotropin and to culture them directly with the inserts. Prior to this, 500 ul of IVM media will be placed in a standard 24 well plate with or without 3D printed inserts for 24 hours to allow possible leachates to be released into the culture media. After 14 to 16 hours of culture the meiotic stage of each oocyte will be scored and MII stage eggs will be fixed and analyzed for spindle morphology.

Determining which resins or treatments yield non-ovotoxic and biocompatible materials gives more options to create microfluidic devices and physiomimetic constructs that include these sensitive cells. Hence, this work will lay the foundation to advance the in vitro models for testing of drugs, environmental contaminants and improve basic research in reproductive studies.

P83: PTBP1 MEDIATES SERTOLI CELL ACTIN CYTOSKELETON ORGANIZATION BY REGULATING ALTERNATIVE SPLICING OF ACTIN REGULATORS.

Yuexi Wang [1], Ullas Valiya Chembazhi [2,7], Danielle Yee [1,8], Sijie Chen [1], Jie Ji [1,9], Yujie Wang [1,10], Ka Lam Nguyen [1], PoChing Lin [1], Antonia Ratti [3], Rex Hess [1], Huanyu Qiao [1], CheMyong Ko [1], Jing Yang [1], Auinash Kalsotra [2,4,5,6], and Wenyan Mei [1,4,5,6]

[1] Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA
[2] Department of Biochemistry, The School of Molecular and Cellular Biology, College of Liberal Arts & Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA
[3] Department of Medical Biotechnology and Translational Medicine, Universita degli studi di Milano, Via Fratelli Cervi 93, 20090, Segrate, Milan, Italy
[4] Cancer Center at Illinois, University of Illinois Urbana-Champaign, Urbana, IL, USA
[5] Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA
[6] Division of Nutrition Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA
[7] Current address: Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
[8] Current address: University of Maryland School of Medicine, Baltimore, MD, USA
[9] Current address: Department of Integrative Biology & Physiology, University of California, Los Angeles, Los Angeles, CA, USA
[10] Current address: College of Arts and Science, Vanderbilt University, Nashville, TN, USA

Spermatogenesis is a biological process within the testis that produces haploid spermatozoa for the continuity of species. Sertoli cells are somatic cells in the seminiferous epithelium that orchestrate spermatogenesis. Cyclic reorganization of Sertoli cell actin cytoskeleton is vital for spermatogenesis, but the underlying mechanism remains largely unclear. Here, we report that RNA-binding protein PTBP1 controls Sertoli cell actin cytoskeleton reorganization by programming alternative splicing of actin cytoskeleton regulators. This splicing control enables ectoplasmic specializations, the actin-based adhesion junctions, to maintain the blood-testis barrier and support spermatid transport and transformation. Particularly, we show that PTBP1 promotes actin bundle formation by repressing the inclusion of exon 14 of *Tnik*, a kinase present at the ectoplasmic specialization. Our results thus reveal a novel mechanism wherein Sertoli cell actin cytoskeleton dynamics is controlled post-transcriptionally by utilizing functionally distinct isoforms of actin regulatory proteins, and PTBP1 is a critical regulatory factor in generating such isoforms. Supported by National Institute of Health [R03AI138138, R03AI146900, and R01GM140306 to W.M].

P84: CHEMICAL MIXTURE DYSREGULATES PLACENTAL CELL SIGNALING PATHWAYS, HORMONE SECRETION, AND ANTIANGIOGENIC PROPERTIES IN TERM HUMAN PLACENTAS

A. A. Waye [1], E. Ticiani1, Z. Sharmin1, V. Perez Silos1, C. A. Murga-Zamalloa1, and A. Veiga-Lopez [1]

[1] Department of Pathology, University of Illinois at Chicago, Chicago, IL

Complex environmental chemical mixtures can reach the feto-placental unit and directly target the placenta. Certain chemicals perturb activation of epidermal growth factor receptor (EGFR) which is essential for placental trophoblast growth, differentiation, and invasion. We hypothesize that EGFR-disrupting chemicals also impair placental hormone synthesis and disrupt additional pathways that control trophoblast cell function. To test these hypotheses, human placental explants and human primary cytotrophoblast cells (hCTBs) were utilized. Placental villus explants were obtained from three different healthy term placentas and exposed them for 96 h to: 1) vehicle (0.1% DMSO), 2) EGF (30 ng/ml), 3) a chemical mixture (Chem-Mix: PCB-126, PCB-153, atrazine, niclosamide, trans-nonachlor, and bisphenol S; 100 ng/ml) demonstrated by us to blunt EGFR activation in hCTBs, or 4) Chem-Mix+EGF. Media was collected after 24, 48, and 96 h of cultures to measure the following by ELISA: human chorionic gonadotropin (hCG) to evaluate hormone synthesis and sFlt-1, a tyrosine kinase protein with antiangiogenic properties implicated in the pathogenesis of preeclampsia. At 96 h, explants were fixed and processed for TUNEL to evaluate apoptosis. hCTBs (6 primary cell cultures) from healthy pregnancies at term were isolated and exposed for 24 h to the same exposure groups as the placental explants. hCTBs were subjected to RNA sequencing. Exposure to the Chem-Mix for 96 h did not induce cell death compared to the control group. EGF exposure tended to increase hCG secretion, while the Chem-Mix with or without EGF did not affect hCG. The effects of the Chem-Mix, EGF, and Chem-Mix+EGF on sFLT-1 secretion were patient sample dependent. The RNA sequencing approach revealed that the Chem-Mix upregulated 128 genes with dysregulated pathways including Ras, PI3K-Akt, JAK-STAT, and T cell receptor and chemokine signaling. In addition, 740 genes were downregulated by the Chem-Mix with affected pathways related to cytokine-cytokine receptor interaction, cell cycle, tight junction, protein processing in the endoplasmic reticulum, and chemokine signaling. A more pronounced effect was observed in the Chem-Mix+EGF compared to EGF, with 1,090 downregulated genes affecting the peroxisome, phosphatidylinositol signaling system, VEGF signaling, and regulation of actin cytoskeleton. In contrast, the upregulation of 155 genes influenced pathways such as, cell adhesion molecules, PI3K-Akt, calcium signaling, MAPK, cAMP, and cell cycle. We have demonstrated that exposure to the chemical mixture dysregulates signaling pathways associated with cell proliferation, differentiation, and cell cycle in primary hCTBs. In the placental villi explants, the chemical mixture exposure resulted in patient dependent hCG and angiogenic factor sFLT-1 secretion. Since abrogation of trophoblast cell functions and reduction of secretory capacity have been associated with pregnancy complications such as preeclampsia, our findings highlight the need to understand whether complex mixtures contribute to the placental abnormalities. Funded by NIEHS/NIHR01ES027863 to A.V-L

P85: MEIOTIC SILENCING VIA ASYNAPSIS AND GEL-LIKE PHASE SEPARATION

Yiheng Peng [1,1], Shuangqi Wang [1,1], Royal Shrestha [1], Xing Tian [1], So Maezawa [2], Marcus Smolka [3], Paula Cohen [4], Satoshi Namekawa [5], Huanyu Qiao [1], *

[1] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA
[2] Department of Applied Biological Science, Tokyo University of Science, Noda-shi, Chiba-ken, Japan
[3] Department of Molecular Biology and Genetics, Cornell University, Ithaca, USA
[4] Department of Biomedical Sciences, Cornell University, Ithaca, USA
[5] Department of Microbiology and Molecular Genetics, University of California – Davis, Davis, California, USA

In mammals, meiotic silencing of unsynapsed chromatin (MSUC) occurs in both males and females and is driven by the DNA damage response (DDR) pathway, as indicated by the DDR marker γH2AX. During normal male meiosis, MSUC is confined to the unsynapsed X and Y chromosomes, a process known as meiotic sex chromosome inactivation (MSCI), which is required for the process of spermatogenesis. The broader MSUC can occur in autosomes when asynapsis happens. However, the variability in MSUC regions across cells makes their precise silencing location and levels hard to define. To address this, we developed an approach called "digital chromosome banding" to accurately assess the meiotic silencing for both MSCI and MSUC at single cell level. We observed two severe silencing steps in MSCI during the zygotene-to-early-pachytene transition and early-to-mid pachytene transition. The mechanism of second-step silencing associates with the formation of mature sex bodies, which achieve silencing through a gel-like phase separation that creates a barrier to the diffusion of small molecules. Our analysis of three synapsis-defective mouse mutants (*Spo11*-/-, *Tdp43*-/-, and *Nelfb*-/-) revealed unique MSUC patterns among them. Overall, the X chromosome more frequently involved in silencing than autosomes. Although MSUC is proposed to form a pseudo-sex body, its cytological characteristics resemble the first-step silencing of MSCI. Comparative analysis between sexes also reveals sexual dimorphisms in meiotic silencing.

P86: EXPOSURE TO AN ENVIRONMENTALLY RELEVANT PHTHALATE MIXTURE ALTERS LEVELS OF OVULATORY ANGIOGENIC FACTORS AND RECEPTORS IN MOUSE GRANULOSA AND ENDOTHELIAL CELLS *IN VITRO*

Brittney A. Williams [1], Caroline V. Harper [2]; Gretchen L. Ruschman [2]; Madison M. Wilson [2]; Patrick R. Hannon [1]

[1] Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, United States
[2] Department of Obstetrics and Gynecology, University of Kentucky, Lexington, KY, United States

After the initiation of the ovulatory cascade and during subsequent physiologic changes to the follicle, blood vessels develop from existing vasculature to penetrate the previously avascular granulosa cell layer. This process of ovulatory angiogenesis has been shown to be critical for ovulation and relies on granulosa cell production of pro-angiogenic factors that bind to receptors on endothelial cells. Specifically, the ovulatory cascade induces granulosa cells to produce prostaglandins (PGE2, PGF2α), vascular endothelial growth factors (VEGFA, VEGFC, VEGFD), and angiopoietins (ANGPT1, ANGPT2). Endothelial cells express prostaglandin E receptors (PTGER1-4), prostaglandin F receptor (PTGFR), VEGF receptors (FLT1, KDR, FLT4), and angiopoietin receptor (TEK). This signaling is enhanced by endothelial cofactors, such as neuropilins (NRP1, NRP2). The communication between these cells could be disrupted by endocrine-disrupting chemicals, such as phthalates. People are ubiquitously exposed to phthalates, and these chemicals have been found to reach the ovary and exert toxic effects. We hypothesized that phthalates would alter the production of angiogenic factors by granulosa cells and the expression of angiogenic receptors on endothelial cells. Mouse granulosa cells were isolated from immature mice 48 hours after pregnant mare serum gonadotropin (PMSG) injection. The cells were treated with dimethylsulfoxide (DMSO) or a mixture of 6 phthalates (MPTmix, 1-500µg/mL) derived using urinary phthalate levels in pregnant women. Following one hour of treatment, the cells received human chorionic gonadotropin (hCG) to induce the ovulatory cascade. The cells and media were collected after 11 hours and subjected to gene expression analyses and enzyme-linked immunosorbent assays (n=7, p≤0.05). In complementary experiments, mouse ovarian endothelial cells were treated with DMSO or MPTmix (1-500µg/mL)±VEGFA before collection at 24 and 48 hours for gene expression analyses (n=7, p≤0.05). Exposure to hCG+MPTmix decreased granulosa cell levels of PGE2 at 1, 10, 100, and 500µg/mL and PGF2α at 10, 100, and 500µg/mL compared to hCG. Of the prostaglandin receptors, treatment with MPTmix increased *Ptger4* at 500µg/mL (24hr) relative to DMSO. For the VEGFs in granulosa cells, exposure to hCG+MPTmix decreased the levels of *Vegfd* at 100 and 500µg/mL and increased protein levels of VEGFA at 500µg/mL. Of the VEGF receptors, MPTmix decreased endothelial cell expression of Kdr at 1, 10 (24hr), and 500µg/mL (48hr); and decreased *Flt4* expression at 10, 100, and 500µg/mL (24hr). MPTmix decreased granulosa cell expression of *Angpt1* and *Angpt2* at 100 and 500µg/mL. On endothelial cells, MPTmix decreased *Tek* expression at 500µg/mL (24,48hr); and *Nrp1* at 500µg/mL (48hr). Depending on the dose and timepoint, supplementation with VEGFA to endothelial cells fully restored (*Ptger4, Nrp1, Kdr*), partially restored (*Kdr, Flt4, Tek*), or did not restore (*Kdr, Flt4*) the MPTmix-induced changes in receptor expression to control levels. These data demonstrate that exposure to an environmentally relevant phthalate mixture altered the levels of granulosa cell-derived angiogenic factors, altered the expression of key angiogenic receptors on endothelial cells, and some of these toxic effects could not be overcome with a potent angiogenic stimulus. Thus, phthalate exposure may induce dysregulation of the communication between granulosa and endothelial cells necessary for ovulatory angiogenesis. Supported by R01ES033767.

P87: EXPRESSION OF SEX HORMONE RECEPTORS IN LEVATOR ANI MUSCLE TISSUE FROM PATIENTS WITH PELVIC ORGAN PROLAPSE

Tianming You [1], Ariel Halle [1], William Xu [1], Tanvi Potluri [1], Hoang-Hoa Nguyen [1], Julia Geynisman-Tan [1], Serdar E. Bulun [1], Hong Zhao [1]

[1] Department of Obstetrics and Gynecology (Division of Reproductive Science in Medicine), Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA.

Introduction: Women with pelvic floor disorders usually have weakened pelvic floor muscles, which may cause urinary incontinence, fecal incontinence, or pelvic organ prolapse (POP). Histologically, the pelvic floor musculature in POP patients displays increased fibrosis and muscle atrophy. However, the underlying molecular mechanism for POP has not been fully investigated. We and others previously found that estrogen causes pelvic skeletal muscle fibrosis and inguinal hernias in male animal models. Additionally, clinical trials from the Women's Health Initiative showed that women receiving estrogen-only or estrogen plus progestin treatment had statistically significant increases in the risk for urinary incontinence. However, the role of estrogen, progesterone, androgen, and their receptor expression in pelvic floor muscle is not well studied.

Methods: TruCut biopsy needles were used to obtain the major pelvic floor muscle levator ani (LA, the major pelvic floor muscle) biopsies from four groups of patients undergoing surgery: (i) 5 premenopausal women with stage II/III pelvic organ prolapse (age 38-52 years) and (ii) 5 postmenopausal women with stage III pelvic organ prolapse (59-75 years) as well as (iii) 5 age and parity matched premenopausal controls and (iv) 5 age and parity matched postmenopausal controls without any pelvic floor prolapse (stage 0) or with sole stress urinary incontinence. H&E staining, Masson's trichrome staining, and immunohistochemistry (IHC) staining for estrogen receptor α (ERα), progesterone receptor (PGR), and androgen receptor (AR) were performed in LA muscle tissue. Standard patient demographics (age, parity, and diagnosis) were collected.

Results: We used H&E staining to morphologically assess stromal and myofibers and found myofibers and/or stroma obtained from LA muscle biopsies. Masson's trichrome staining showed ECM deposition in the LA muscle from women with or without POP. In addition, we found ERα was present in the stromal cells from nearly every LA biopsy using IHC staining. ERα expression (H-Score) was similar in four groups of patients. ERα IHC staining was absent in LA myofibers. AR was highly expressed in stromal cells in all LA muscles and weakly expressed in myofiber in ~70% of LA tissues. AR expression (H-Score) was significantly decreased in postmenopausal women with stage III pelvic organ prolapse. PGR was only expressed in a small portion of the LA sample (30%), with PGR exclusively expressed in the stroma of the LA muscle.

Conclusion: Our findings indicate that sex hormone receptors (ERα, AR, and PGR) are present in LA muscle stroma, providing the foundation to further define the roles of sex steroid hormones in pelvic organ prolapse.

P88: DECODING PSEUDOURIDINE, THE EMERGING ROLE OF DYSKERIN PSEUDOURIDINE SYNTHASE 1 IN UTERINE FIBROIDS

Qiwei Yang[1] and Ayman Al-Hendy[1]

[1] Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL 60637. USA

Introduction: Uterine fibroids (UFS) are benign monoclonal neoplasms of myometrium, representing the most common tumors in women worldwide. The UFs cause morbidity because of abnormal uterine bleeding and pelvic pressure. The development of UFs involves a complex and heterogeneous constellation of hormones, growth factors, stem cells, genetic, and epigenetic abnormalities. The emerging evidence demonstrates that RNA modifications play a central role in regulating cell functions. Pseudouridine (Ψ) is the most abundant RNA modification. Ψ is catalyzed by Ψ synthases. The dyskerin Ψ synthase 1 (DKC1) gene is a member of the Ψ synthase family, which encodes a protein catalyzing the isomerization of uridine (U) nucleosides to (Ψ) nucleosides in its target RNAs, which include ribosomal RNAs, small nuclear RNAs, mRNAs, snoRNAs, and long noncoding RNAs. In addition, recent reports show that dysregulated expression of DKC1 triggers cancer cell growth and metastasis and is associated with patient prognosis and outcome in various types of human cancer. However, the role of DKC1 in the pathogenesis of UFs is unknown.

Methods: We performed immunoblot analysis using patient-matched tissue sample sets, each comprising one myometrium (MM) and at least 2 different UF tumors from the same patient's uterus (MM: N=7, UFs: N=23). Cell viability was performed using a trypan blue exclusion assay. Western blot was performed to determine the levels of proteins. We employed RNA-seq approaches to map the transcriptome-wide profile in vehicle- and pyrazofurin (PF, DKC1 inhibitor)-treated UF cells. Library quality and quantity were assessed using the Agilent bio-analyzer, and libraries were sequenced using an Illumina NovaSEQ6000. Differentially expressed genes (DEGs) were called by DESeq2 with Log2FC≥0.58 and padj <0.1. The enriched pathways were called by DAVID tools. Real-time qPCR was performed to validate the RNA-seq data. A comparison of 2 groups was carried out using a student t-test for parametric distribution and the Mann Whitney test for nonparametric distribution. The significant difference was defined as $p<0.05$.

Results: Here, we showed that DKC1 is aberrantly overexpressed in UFs compared to adjacent myometrium. Targeted inhibition of DKC1 with its inhibitor PF, suppressed UF cell proliferation identified by cell viability assay and Western blot analysis using cell proliferation marker PCNA. To further characterize the mechanistic basis for PF inhibition of UF cell growth, we performed a comparative RNA-seq analysis of vehicle-treated (n=4) and PF-treated UF cells (n=3). Bioinformatic analysis revealed that PF treatment distinctly altered the UF cell transcriptome. The gene ontology analysis demonstrated that inhibition of DKC1 targeted ribosomal RNAs in UF cells. Notably, the RNA expression of genes encoding many ribosomal proteins and components of ribosomal subunits was significantly downregulated in PF-treated UF cells. This study is consistent with previous findings that DKC1 can stabilize the mRNA of ribosomal proteins via Ψ RNA modification.

Conclusion: In this study, we demonstrated for the first time that the protein levels of DKC1 are higher in UFs, and targeted inhibition of DKC1 suppressed UF proliferation via inhibiting ribosomal function. Our novel findings uncover new mechanisms, potentially leading to innovative strategies to prevent and treat UFs by targeting the Ψ-reprogrammed pathways.

P89: ROLE OF PROGESTERONE RECEPTOR AS A DOWNSTREAM MEDIATOR OF ESTROGEN-INDUCED MUSCLE FIBROSIS IN MOUSE MODELS OF INGUINAL HERNIA

Tianming You[1], Mehrdad Zandigohar[2], Tanvi Potluri[1], Elizabeth Baker[1], Maya Kafali[1], Yang Dai[2], Richard Lieber[3], Jonah Stulberg[4], Hong Zhao[1] and Serdar Bulun[1]

[1] Department of Obstetrics & Gynecology, Northwestern University Feinberg School of Medicine
[2] Department of Biomedical Engineering, University of Illinois at Chicago
[3] Department of Physical Medicine and Rehabilitation, Northwestern University Feinberg School of Medicine
[4] Department of Surgery, McGovern Medical School, University of Texas Health Science Center of Houston

Introduction: Inguinal hernias affect up to 27% of men in their lifetime, with 1,000,000 hernia repair surgeries performed annually in the US alone. Despite this high prevalence, non-surgical approaches are not available to treat this disease, as the molecular mechanisms underlying hernia formation are poorly understood. Using a transgenic mouse model expressing the human aromatase gene ($Arom^{hum}$), we previously showed that high levels of estradiol (E2) production in lower abdominal muscle (LAM) would activate LAM fibroblasts via estrogen receptor alpha (ERα) signaling, leading to extensive LAM fibrosis and hernia formation. These ERα-positive fibroblasts in $Arom^{hum}$ LAM also expressed high levels of progesterone receptor (PGR) while wild-type (WT) LAM fibroblasts did not. PGR is a well-established E_2/ERα-responsive gene in the female uterus and breast. However, the role of progesterone (P_4)/PGR signaling in abdominal muscle hernia formation in men is unknown.

Methods: Two unique hernia mouse models (E_2/P_4-induced and $Arom^{hum}$), primary LAM fibroblasts from $Arom^{hum}$ mice, and LAM and serum samples from human hernia patients were used for this study. Mice were treated with E_2, P_4, and/or RU486 (P_4/PGR antagonist) for 12 weeks while tracking hernia development. After treatment, LAM was harvested for histological analysis as well as single-nuclei multiomic analysis (snRNAseq and snATACseq).

Results: In cultured $Arom^{hum}$ LAM fibroblasts, PGR expression was induced by E_2 and blocked by fulvestrant (ERα-antagonist). LAM fibroblasts exhibited increased proliferation and expression of fibrosis-related genes upon treatment with E_2+R_{5020} (P_4 analog), but not E_2 or R_{5020} alone. These effects were blocked by concurrent RU486 administration. In $Arom^{hum}$ male mice, 12-week RU486 treatment starting at 4 weeks of age strikingly reduced hernia size or even prevented hernia development entirely. In WT male mice, E_2 treatment induced the development of small hernias (125-175mm^2) after 10-12 weeks, while P4 treatment did not. Intriguingly, all mice treated with E_2+P_4 concurrently developed significantly larger hernias (>225mm^2) within just 2-4 weeks. The large hernias in E_2+P_4 treated mice were completely prevented with concurrent RU486 treatment. IHC analysis showed increased expression of PGR in LAM fibroblasts of $Arom^{hum}$ mice as well as WT mice treated with E_2. In both models, LAM fibrosis was significantly reduced with RU486 treatment. Single-nuclei multiomic analysis of LAM tissue from the E_2+P_4-treated WT mice showed an increased proportion of fibroblast-like cells and a unique population of ERα$^+$/Pgr$^+$ fibroblasts. Consistent with our mouse findings, human fibroblasts from fibrotic LAM of hernia patients exhibited strikingly higher proliferation and PGR expression compared to fibroblasts from adjacent healthy muscle.

Conclusion: Our results suggest that P_4/PGR signaling induced by E_2/ERα in highly P_4-sensitive LAM tissues is critical for LAM fibrosis and hernia development. Our findings are one of the first to show the importance of PGR signaling in men and provide evidence that PGR antagonists may be useful as a therapeutic alternative to hernia repair surgery.

P90: A NOVEL 3D-PRINTED AGAROSE MICROMOLD SUPPORTS SCAFFOLD-FREE MOUSE EX VIVO FOLLICLE GROWTH, OVULATION, AND LUTEINIZATION

Emily J. Zaniker [1], Prianka H. Hashim [1], Samuel Gauthier [1], James A. Ankrum [2], Hannes Campo [1], Francesca E. Duncan [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611, USA
[2] Roy J. Carver Department of Biomedical Engineering, Pappajohn Biomedical Institute, University of Iowa, Iowa City, IA, USA.

Ex vivo follicle growth is an essential tool enabling interrogation of folliculogenesis, ovulation, and luteinization. Novel techniques to culture ovarian follicles in a biomimetic scaffold could have clinical implications for fertility preservation, studies of reproductive toxicology, and contraceptive drug discovery. Currently, most *ex vivo* follicle culture techniques uses alginate hydrogel encapsulation. This method is technically challenging and is not compatible with automated imaging and analysis. In this study, we developed a custom agarose micromold, which enables scaffold-free follicle culture. We established an accessible and economical manufacturing method using stereolithographic 3D printing and silicone molding that generates biocompatible hydrogel molds without the risk of cytotoxicity from leachates. Each mold supports simultaneous culture of ten multilayer secondary follicles in a single focal plane, allowing for constant time-lapse monitoring and automated analyses of the morphology and growth kinetics of each individual follicle. Mouse follicles cultured in this system reach larger terminal diameters (406.1 ± 4.1 μm vs. 374.0 ± 4.1 μm, P-adj <0.0001) and have greater ovulatory capacity (97% + 2% compared to 82% + 4%, P-adj 0.031) relative to established encapsulated in vitro follicle growth (eIVFG) systems. Follicle survival, hormone production, and oocyte maturation were comparable. Additionally, follicles recapitulated aspects of in vivo ovulation physiology with respect to their architecture and spatial polarization, which has not been observed in eIVFG systems. This system offers simplicity, scalability, integration with morphokinetic analyses of follicle growth and ovulation, and compatibility with existing microphysiological platforms. Future studies will optimize the agarose mold for culture of multiple stages of follicles and integration of specialized biomaterials using modified poly(ethylene glycol) hydrogels.

P91: EFFECTS OF POSTPARTUM ACETYLSALICYLIC ACID ADMINISTRATION AND CALCIUM SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE AND CULLING IN HOLSTEIN DAIRY COWS

P. Zarei [1,2], E. Jimenez [1,3], J. Spring [1], M. Dailey [1], M. Martinez [1], j. Lection [1,4], R. Sorto [1], E. Hovingh [1], and A.A Barragan [1]

[1] Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA, 16802, [2] Department of Animal Sciences, University of Illinois Urbana-Champaign, Urbana, IL, 61801, [3] College of Veterinary Medicine, Iowa State University, Ames, IA, 50011, [4] College of Veterinary Medicine, North Carolina State University, Raleigh, NC, 27607.

In dairy cows, the transition period (TP) encompasses three weeks before and after calving and is referred to as the most critical period in dairy cattle reproduction as they pass through different physiological challenges. Among these challenges, systemic inflammation stands out as one of the most prominent, since prolonged and exacerbated inflammatory response has been associated with decreased fertility. The application of anti-inflammatory management, including non-steroidal anti-inflammatory drugs (NSAIDs), has been proposed as a potential approach to modulate post-calving inflammation. In this regard, acetylsalicylic acid (ASA) commonly known as Aspirin has been widely suggested by literature. In addition to systemic inflammation, hypocalcemia (HC) is one of the most common postpartum metabolic disorders in dairy cattle characterized by a sudden and drastic drop in the serum concentration of calcium (Ca). Calcium supplementation has also been showing notable improvements in cow metabolism, reproductive performance, and overall health throughout the postpartum period. Therefore, combining oral ASA administration along with Ca supplementation may exhibit synergistic positive effects on modulating postpartum systemic inflammation and reproductive health. This study aimed to assess the effects of postpartum acetylsalicylic acid administration and Ca supplementation on reproductive performance and culling in multiparous Holstein dairy cows. Within 12 h after calving, cows were randomly allocated to one of four groups: 1) ASA (n=155) = Cows received two oral administrations with ASA 24 h apart (125 g/cow/d; 4 480-grain aspirin boluses); 2) ASACAL (n=164) = Cows received two oral administrations with ASA (125 g/cow/d; 4 480-grain aspirin boluses) and Ca (43 g/cow/d; 2 Ca boluses) 24 h apart, 3) CAL (n=171) = cows received two oral administrations with Ca (43 g/cow/d; 2 Ca boluses) 24 h apart, and 4) UNT (n=156) = cows remained untreated. Vaginal discharge was scored weekly until 21±3 days in milk (DIM). On-farm records were collected to assess reproductive performance and culling for 300 DIM. The data were analyzed using MIXED, GLIMMIX, and LIFETEST procedures of SAS. Cows treated with ASA tended (p=0.1) to have a lower prevalence of clinical metritis compared to CAL cows (ASA=15.92±3.56%; CAL=29.92±4.40%). ASACAL cows tended to require fewer days (p=0.10; ASACAL=101.77±4.18 d; UNT=111.45±4.13 d) and fewer services (p=0.05; ASACAL= 1.91±0.13 svc.; UNT=2.27±0.13 svc.) to become pregnant compared to UNT cows. However, when assessed using LIFETEST procedure there was no significant difference in days in milk to conception (DIMC) by 150 DIM between ASACAL and UNT cows (p=0.17). There was no difference in culling rate between treatment groups (ASA=29.06±3.71%; ASACAL=28.52±3.60%; CAL=24.52±3.33%; UNT=24.67±3.49 %; p=0.68). These results suggest that postpartum acetylsalicylic acid combined with Ca might positively affect fertility in multiparous cows.

Keywords: Postpartum period, Calcium and acetylsalicylic acid, reproductive performance

P92: ANOMALOUS HIGH TEMPERATURE EXPOSURE EFFECTS ON MOUSE WEIGHT AND OVARIAN DEVELOPMENT

Luhan Tracy Zhou [1], Elnur Babayev [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA

Greenhouse gas emissions trap excess heat in the atmosphere leading to rising global temperatures. Increased frequency, duration, and intensity of extreme heat have the most immediate and direct impact on human physical and mental health. The impact of exposure to high temperatures on fertility and reproduction is an area of increasing concern. Although the negative impact of heat on spermatogenesis is well recognized, little is known about the impact of heat on female reproduction and offspring outcomes. We hypothesized that increased frequency of exposure to anomalous high temperatures during pregnancy and after birth impacts offspring development, their ovarian reserve and reproductive aging. To test our hypothesis, we used a climate change model to apply predicted environmental changes in a physiologically aging mouse model. CD-1 mice were mated in temperature adjustable chambers at mouse thermoneutral temperature of 28°C. The experimental group was exposed to 35°C once every 7 days during pregnancy and after birth, compared to the control group exposed to 35°C once every 40 days. Mouse body weight, crown-rump length, ovary weights and lengths were measured on postnatal day 6 (PND) and 6 weeks. On PND6, significant decrease in body weight of experimental animals were noted (4.95±0.35g vs. 3.79±0.26g; p<0.01); however, this was no longer significant when normalized to litter size. Crown-rump length did not significantly differ between control and experimental groups (36.50±1.21mm vs. 34.30±1.33mm). Ovarian length was significantly decreased in the experimental animals (1056±44.80μm vs. 790.80±27.13μm; p<0.01); however, this was also nonsignificant when normalized to body weight. At 6 weeks, body weight was significantly decreased in the experimental group, (26.93±0.61g vs. 23.39±0.57g; p<0.01). However, similar to the PND6, litter size normalization rendered the result insignificant. The same trend was observed for crown rump length (82.67±0.49mm vs. 79.17±1.17mm). Ovary weight was significantly decreased in the experimental group (5.16±0.36mg vs. 3.87±0.20mg; p<0.05), but the effect was no longer statistically significant after normalization to litter size. The same trend was observed for ovary length (2630±80.39μm vs. 2296±86.04μm). Studies are ongoing to assess the ovarian reserve at these two ages, as well as examine the above measurements at 3-4 months of age. Overall, our research indicates that increased frequency of exposure to an anomalous high temperature of 35°C does not affect offspring body weight, ovarian weight and size at PND6 and 6 weeks of age. This study will help uncover the impact of high temperature exposure on ovarian reserve and reproductive aging, which has implications for female fertility and overall health.

P93: A COMPLEX PHENOTYPIC SCREENING ASSAY OF OOCYTE MEIOTIC MATURATION IDENTIFIES NOVEL TARGETS FOR NON-HORMONAL CONTRACEPTIVE DISCOVERY

Yiru Zhu [1], Hoi Chang Lee [1], Jeffrey Pea [1], Lauren R. Haky [1], John Proudfoot [2], Richard Nelson [2], Francesca E. Duncan [1]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
[2] Ovarian Contraceptive Discovery Initiative (Independent Consultant)

Oocyte meiotic competence is the ability of the oocyte to exit prophase I arrest and resume meiosis. During meiotic maturation, the oocyte undergoes breakdown of its intact nucleus, or germinal vesicle (GV), completion of meiosis I with extrusion of the first polar body (PBE), and arrest at metaphase II. Notably, this process can be recapitulated ex vivo with hallmarks of GVBD and PBE being visible via transmitted light microscopy. Given that meiotic maturation is critical for generating a fertilization-competent gamete, this biological process provides a promising target for non-hormonal contraceptive drug discovery. Therefore, we screened 818 compounds based on target and structural diversity in the Selleckchem Bioactive Compound library using a complex phenotypic assay to identify potential inhibitors of meiotic maturation. Oocytes at the GV stage were in vitro matured in the presence of 10μM compound, DMSO (vehicle control), or milrinone, a Pde3a inhibitor known to inhibit meiotic maturation, as a positive control. Brightfield images were taken before and after IVM for analysis of maturation status. A positive hit was defined as a compound with a ≤40% maturation rate in 10 oocytes across two replicates. We identified 31 positive hits and proceeded to verify them using independently sourced compounds, with 25 showing consistent maturation results. Subsequent concentration-response experiments showed that 18 compounds exhibited good response curves, with five compounds maintaining meiotic arrest at the GV stage. Of these, several have known inhibitory effects on meiosis, including Cacna1h, Pde3, and Kif11, thus validating our platform. We also identified novel targets that had no previous known function in meiotic maturation, such as Galr3 and Vipr1. Chemical and biological validations, including testing structural analogs and gene modulations, are ongoing. These results indicate that this screening pipeline can identify inhibitors of oocyte meiotic maturation and candidate targets for non-hormonal contraceptive drug development.

P94: THERAPEUTIC TARGETING OF THE TRYPTOPHAN-KYNURENINE-ARYL HYDROCARBON RECEPTOR PATHWAY WITH APIGENIN IN MED12-MUTANT LEIOMYOMA CELLS

Takashi Iizuka [1,2], Azna Zuberi [1], Helen Wei [1], John S. Coon V [1], Melania Lidia Anton [1], Kadir Buyukcelebi [1], Mazhar Adli [1], Serdar E. Bulun [1], and Ping Yin [1]

[1] Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine at Northwestern University, Chicago, Illinois, USA
[2] Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan

Approximately 77.4% of uterine leiomyomas carry MED12 gene mutations (mut-MED12), which are specifically associated with strikingly upregulated expression and activity of the tryptophan 2,3-dioxygenase (TDO2) enzyme, leading to increased conversion of tryptophan to kynureine. Kynurenine increases leiomyoma cell survival by activating the aryl hydrocarbon receptor (AHR). We used a leiomyoma-relevant model, in which a MED12 Gly44 mutation was knocked in by CRISPR in a human uterine myometrial smooth muscle (UtSM) cell line, in addition to primary leiomyoma cells from 14 patients to ascertain the mechanisms responsible for therapeutic effects of apigenin, a natural compound. Apigenin treatment significantly decreased cell viability, inhibited cell cycle progression, and induced apoptosis preferentially in mut-MED12 versus wild-type primary leiomyoma and UtSM cells. Apigenin not only blocked AHR action but also decreased TDO2 expression and kynurenine production, preferentially in mut-MED12 cells. Apigenin did not alter TDO2 enzyme activity. TNF and IL-1β, cytokines upregulated in leiomyoma, strikingly induced TDO2 expression levels via activating the NF-κB and JNK pathways, which were abolished by apigenin. Apigenin or a TDO2 inhibitor decreased UtSM cell viability induced by TNF/IL-1β. We provide proof-of-principle evidence that apigenin is a potential therapeutic agent not only for mut-MED12 leiomyomas but also for TDO2-overexpressing malignant tumors.

MEET THE UMSRS 2024 ORGANIZERS

Milan K. Bagchi, Ph.D
UIUC

Matthew J Dean, Ph.D
UIUC

Lori Raetzman, Ph.D
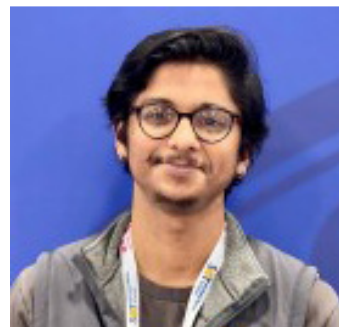UIUC

Ayelet Ziv-Gal, Ph.D
UIUC

TRAINEES

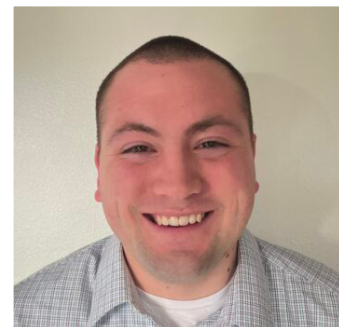**Arpita Bhurke**, Ph.D
UIUC

Sameeksha Bhaye
UIUC

Kavyashree Basavaraju
UIUC

Ritwik (Rick) Shukla
UIUC

Jacob Reed Beal
UIUC

Shams Mowafak Saad
Purdue University

Hannah McDowell
Northwestern University

Janavi Thyagraj
Northwestern University

Cary Brandolino
DePaul University

ORGANIZERS

Faculty, University of Illinois at Urbana-Champaign

Milan K. Bagchi, Ph.D. — Department of Molecular and Integrative Physiology
Matthew J. Dean, Ph.D. — Department of Animal Sciences
Lori Raetzman, Ph.D. — Department of Molecular and Integrative Physiology
Ayelet Ziv-Gal, Ph.D. — Department of Comparative Biosciences

TRAINEES

Arpita Bhurke, Ph.D., Trainee Committee Chair — University of Illinois at Urbana-Champaign
Sameeksha Bhaye — University of Illinois at Urbana-Champaign
Kavyashree Basavaraju — University of Illinois at Urbana-Champaign
Ritwik (Rick) Shukla — University of Illinois at Urbana-Champaign
Jacob Reed Beal — University of Illinois at Urbana-Champaign
Shams Mowafak Saad — Purdue University
Hannah McDowell — Northwestern University
Janavi Thyagraj — Northwestern University
Cary Brandolino — DePaul University

** A special thanks to Pranav Volety for his excellent work in designing the conference booklet cover page.

SPONSORSHIP

Olga G. Nalbandov Lecture Fund
Department of Animal Sciences, College of ACES
Department of Comparative Biosciences, College of Veterinary Medicine
Department of Molecular and Integrative Physiology, College of Liberal Arts and Sciences
University of Illinois Office of the Vice Chancellor for Research & Innovation
Society for the Study of Reproduction
R13 conference grant from the NIH/NICHD (R13 HD113376)