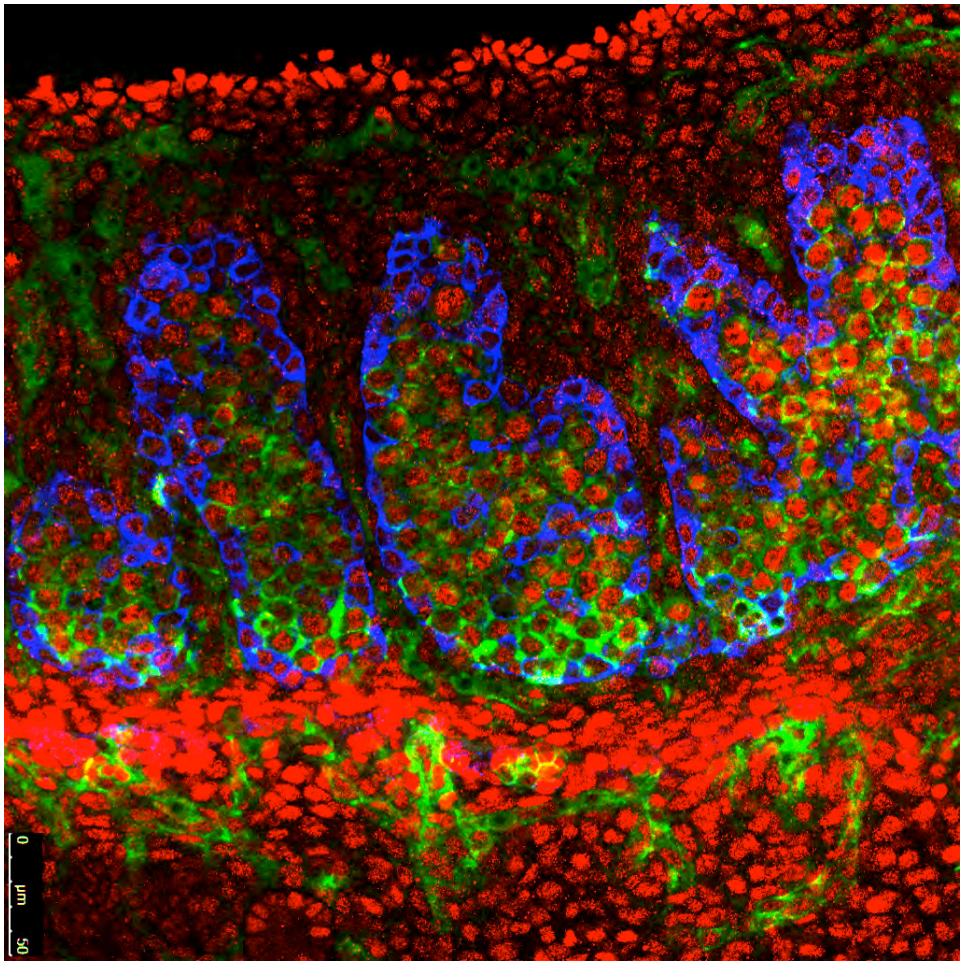
34th Annual
Minisymposium on Reproductive Biology

January 26th, 2015

Lurie Medical Research Center
303 E. Superior St, Northwestern University, Chicago, IL



Sponsored by

Center for Reproductive Science
Northwestern University
2205 Tech Drive, Hogan 2-171
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Front cover photograph courtesy of Bella Salamone (Maatouk Lab)
Immunofluorescent staining of E12.5 male mouse gonad for the repressive histone modification H3K27me3 (red), Sertoli cell marker AMH (blue), and germ cell and vasculature marker PECAM1 (green).

About the Center for Reproductive Science

The Northwestern University Center for Reproductive Science (CRS) includes faculty whose research is pushing the boundaries of reproductive science and medicine. CRS includes 43 faculty members, from across the University, who share common interests in research and training in the reproductive sciences. CRS has members from 14 departments in the Weinberg College of Arts and Sciences, the McCormick School of Engineering, Stanley Manne Children's Research Institute, and the Feinberg School of Medicine. Research areas of current faculty interest include: germ cell development, clinical fertility and infertility, gonadal development and function, diseases of the female reproductive tract, contraception, relationship of circadian rhythms and metabolism to reproduction, neuroendocrinology, ovarian function, and reproduction and society.

The Center's mission is to enhance and coordinate research in the reproductive sciences at Northwestern; to promote the application of this research toward human welfare; and to optimize the training of future researchers, educators, and clinicians in the reproductive sciences.

CRS administers a multidisciplinary NIH P01 Program Project, *"Hormonal Signals that Regulate Ovarian Differentiation"* (Kelly Mayo, Director), while also facilitating a number of other reproductive science related grants and training programs. For further information about the center, please visit the CRS website at http://www.crs.northwestern.edu**.** The CRS Newsletter *"Reproduction Matters"* is also available on the CRS website.

CRS Sponsored Awards

Constance Campbell Research Awards
The Constance Campbell Memorial Fund provides awards that are presented to trainees with the best oral and poster presentations at symposia sponsored by the Center for Reproductive Science including the *Minisymposium on Reproductive Biology* and the *Illinois Symposium on Reproductive Sciences*.

Constance Campbell Trainee Travel Awards
Funded through the Constance Campbell Memorial Fund**,** this travel award is for graduate students, postdoctoral fellows, and clinical residents or fellows conducting research in reproductive science and medicine to help cover travel expenses as they present their work at a national meeting.

Marcia L. Storch Scholarship for Undergraduate Women
Marcia L. Storch, MD was an outstanding gynecologist and dedicated feminist who died from ovarian cancer on November 9, 1998. She was always interested in the education of young women, and stipulated that donations in her name be made to the Center for Reproductive Science for the purpose of introducing undergraduate women to research. This scholarship is awarded to undergraduate women working in some aspect of ovarian research and provides a sum for supplies in the designated laboratory.

Minisymposium Overview

Robert H. Lurie Medical Research Center of Northwestern University
303 E. Superior, Chicago, IL

9:30 AM	Registration (Lurie Atrium)
10:15 AM	Welcome/Announcements (Baldwin Auditorium)
10:30 AM	Alumni Speaker – Dr. David Keefe (Baldwin Auditorium)
11:30 AM	Lunch Break (Atrium- Lunch will be provided)
12:30 PM	Oral Session (Baldwin Auditorium)
1:30 PM	Poster Session A (Lurie Atrium)
2:10 PM	Poster Session B (Lurie Atrium)
3:00 PM	Neena B. Schwartz Lectureship – Dr. Richard Stouffer (Baldwin Auditorium)
4:00 PM	Constance Campbell Awards Presentation and Closing Comments (Baldwin Auditorium)
	Reception to follow

Neena B. Schwartz Lectureship in Reproductive Science

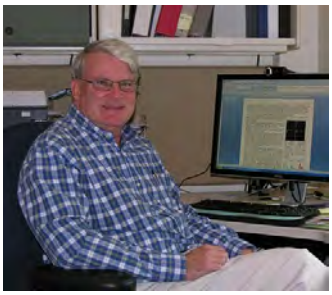
The idea for the Minisymposium, a day-long event focused on providing trainees with an early opportunity to present their research, came primarily from Dr. Neena B. Schwartz, William Deering Professor Emerita of Biological Sciences and founder of the Center for Reproductive Science.

Dr. Schwartz is a native of Baltimore who has spent most of her research and teaching career in the greater Chicago area. After obtaining her undergraduate degree from Goucher College, Dr. Schwartz completed her MS and PhD degrees in physiology at Northwestern University. Her first faculty position was at the University of Illinois College of Medicine, where she rose to the rank of Professor before moving to Northwestern University in 1974 as Chair of the Department of Biological Sciences. Dr. Schwartz founded the Program in Reproductive Research, now the Center for Reproductive Science, in 1974, and developed the program into a premier training site for students and young investigators interested in reproductive endocrinology.

Dr. Schwartz has had a distinguished and productive research career and has made many seminal contributions toward understanding the hypothalamic-pituitary-gonadal axis and its control. Her early studies on the rat estrous cycle established many of the basic tenets of cyclical changes in gonadotropin secretion upon which our current views of the HPG axis are based. Much of Dr. Schwartz's research has focused on the issue of differential regulation of pituitary FSH and LH secretion. It was this interest that led her and the late Cornelia Channing to describe a nonsteroidal feedback factor from the ovary involved in controlling the secondary FSH surge, and Dr. Schwartz's laboratory went on to make many important contributions to the study of ovarian inhibin.

To honor Dr. Schwartz, on the occasion of the 25th Minisymposium, the Center for Reproductive Science named the keynote address the *Neena B. Schwartz Lectureship in Reproductive Science*. This year, ***Richard Stouffer PhD***, a senior scientist in the Division of Reproductive & Developmental Sciences at the Oregon National Primate Research Center, with active joint appointments as Professor in the Departments of Obstetrics & Gynecology and Physiology & Pharmacology at the Oregon Health & Sciences University, will give the keynote address and receive the 2015 lectureship award.

34th Minisymposium on Reproductive Biology
Neena B. Schwartz Lectureship in Reproductive Science



Richard L. Stouffer, PhD

Dr. Richard Stouffer, a native of Hagerstown, Maryland, earned his B.Sc. from Virginia Polytechnic Institute and State University before receiving his doctoral degree in physiology at Duke University in 1975. Since his dissertation research in the laboratory of Dr. David Schomberg, Dr. Stouffer's primary focus has been on understanding the structure, function and regulation of the ovary. As a staff fellow with Dr. Gary Hodgen, in the intramural NICHD program at NIH, he began studies using the nonhuman primate (NHP) as a "translational model" for direct application to ovarian function and its disorders in women. For 8 years, Dr. Stouffer was a member of the Department of Physiology, University of Arizona College of Medicine, where he rose to the rank of tenured Associate Professor. In 1985, he moved to the Oregon National Primate Research Center (ONPRC). He is now a senior scientist in the Division of Reproductive & Developmental Sciences at ONPRC with active joint appointments as Professor, Departments of Obstetrics & Gynecology and Physiology & Pharmacology, at ONPRC's home institution, the Oregon Health & Sciences University (OHSU).

Dr. Stouffer's research program utilizes whole animal, cellular and molecular approaches to unravel the mechanisms controlling follicle development, ovulation and the functional lifespan of the corpus luteum during the menstrual cycle and early pregnancy in rhesus macaques. His NIH R01 grant (which has been active for over 30 years at Arizona and Oregon) focuses on the endocrine and local control of the primate ovulatory follicle and corpus luteum. This project resulted in the landmark discovery that progesterone produced by the dominant follicle in response to midcycle gonadotropin surge is required for ovulation and development of the primate corpus luteum. The later feature, which differs from the mouse model, is supported by novel studies detailing the expression of genomic progesterone receptor in macaque (and human) luteal cells, and spurred Dr. Stouffer's important studies to elucidate the gonadotropin (LH or CG)- and progesterone– responsive genes in the ovulatory follicle, as well as the corpus luteum as it ages during the menstrual cycle and early pregnancy. These gene (GED) databases are publicly available, and being used for comparative studies of ovarian function by other investigators. Currently, Dr. Stouffer, in collaboration with Dr. Jon Hennebold, is using attenuated adenoviral vectors to deliver siRNAs to PR and PGRMC1 into the macaque periovulatory follicle to elucidate the role(s) of genomic and nongenomic progesterone receptor signaling pathways in ovulation and corpus luteum development in primates.

In addition to world-renown research program pertaining to women's reproductive health, as recognized by the Society for the Study of Reproduction (SSR Research Award, 2007) and the American Society for Reproductive Medicine, (ASRM Distinguished Researcher Award, 2010), Dr. Stouffer has broad experience in leadership and mentorship both locally and nationally. He served on the Board of Directors and President of SSR, and member then chair of the NIH REN Study Section. He is director/PI and Co-PI of the Oregon Infertility SCCPIR/NCTRI centers and the Oregon Contraceptive CDRC center. Dr. Stouffer served as chief of the Division of Reproductive & Developmental Sciences from 1996-2014, until he recently stepped down to return to full-time research. He remains active in center/institutional oversight as chair of the Oregon Stem Cell Research Oversight Committee, chair of the ONPRC Faculty Appointment & Promotions Committee, and Ambassador of the OHSU Center for Women's Health. Dr. Stouffer has mentored over 40 graduate students and postdoctoral fellows, including MD resident researchers and research fellows. He was actively involved in mentoring beginning faculty in his division and in the Obstetrics & Gynecology department.

The Legacy of Dr. Constance Campbell

Dr. Constance Campbell received her PhD in Psychology from University of Illinois at Chicago where she also trained as a postdoctoral fellow under the mentorship of Dr. Neena Schwartz. In 1974, Dr. Campbell joined the faculty at Northwestern University Department of Biological Sciences as an assistant professor. Dr. Campbell's research was focused on the relationship of sexual and other behaviors to environmental cues. During her time at Northwestern University, Dr. Campbell administered the undergraduate honors program and served as a preceptor in the Program for Reproductive Biology, now the Center for Reproductive Science (CRS), which was spearheaded by Dr. Schwartz. Dr. Campbell was promoted to associate professor with tenure in the department of Neurobiology and Physiology before her untimely death in 1981.

Dr. Campbell was fiercely dedicated to her students. Fellow colleagues noted her presence in the laboratory to mentor her graduate students even as she began to fall ill. To commemorate Dr. Campbell's legacy and her dedication toward student research, her family, friends, former students, and colleagues established the Constance Campbell Memorial Fund. Since 1989, this fund has supported the **Constance Campbell Research Awards**, presented to trainees with the best oral and poster presentations at symposia sponsored by the CRS, including the Northwestern University *Minisymposium on Reproductive Biology* and the *Illinois Symposium on Reproductive Sciences*. To date, over 100 trainees have benefited from these awards. Many recipients of the award have gone on to become world-renowned scientists and notable leaders in the field of Reproductive Science. This year's Minisymposium will mark the 26th presentation of these awards. We are pleased to welcome **Dr. David Keefe**, as our alumni speaker, to present this year's awards.

Additionally, recognizing the importance of attendance and participation at national meetings as part of trainees' education, the CRS has utilized the growth of the Constance Campbell Memorial Fund to establish the **Constance Campbell Trainee Travel Awards (C^2T^2)** to support trainees in presenting their research at national meetings of their choosing.

34th Minisymposium on Reproductive Biology
Northwestern Alumni Speaker

David L. Keefe, MD

Dr. Keefe earned his bachelor's degree from Harvard College and his medical degree from Georgetown University School of Medicine. He completed a residency in Psychiatry at the University of Chicago and an NIH funded fellowship in Neuroendocrinology at Northwestern University before completing his residency in Obstetrics and Gynecology at Yale-New Haven Hospital and fellowships in Reproductive Endocrinology and Clinical Reproductive Endocrinology/Infertility at Yale University School of Medicine. At Yale, he received the John Meehan-Clifford Miller and Irving Friedman awards, as well as the Kennedy-Dannreuther Research Fellowship from the American Gynecological & Obstetrical Society. He went on to direct the Division of Reproductive Medicine and Infertility at Women and Infants Hospital, Brown University and serve as the James M. Ingram Professor and Chair of Obstetrics and Gynecology at the University of South Florida, before joining the NYU School of Medicine as the Stanley Kaplan Professor and Chair of the Department of Obstetrics and Gynecology.

Dr. Keefe is a physician-scientist whose research focuses on egg infertility, reproductive aging and stem cells. He has received funding from the National Institutes of Health, the American Society for Reproductive Medicine, and the King Foundation. His research has also been awarded several U.S. patents and his work has been presented to peers both nationally and internationally. As an award-winning teacher and researcher, Dr. Keefe has published extensively with more than 150 papers and abstracts. He has won the ASRM General Program, Society for Assisted Reproductive Technology and New England Fertility Society Prize Paper Awards. Dr. Keefe is also a long-time participant in a number of key professional associations and served as the Scientific Chair of the Annual Meeting of the American Society for Reproductive Medicine, on the Board of Resolve, the Editorial Boards of the journals Human Reproduction, the Journal of Assisted Reproductive Technology and Genetics, and as a reviewer for such publications as the American Journal of Obstetrics & Gynecology and the New England Journal of Medicine. His clinical practice focuses on the diagnosis and treatment of infertility, including *in vitro* fertilization (IVF).

From 1982 to 1985 Dr. Keefe conducted neuroendocrinology research in the laboratory of Center for Reproductive Science member Professor Fred Turek at Northwestern University and we are pleased to welcome him as this year's alumni speaker.

PROGRAM FOR THE
34th MINISYMPOSIUM ON REPRODUCTIVE BIOLOGY

January 26th, 2015
Robert H. Lurie Medical Research Center of Northwestern University
303 E. Superior, Chicago, IL

9:30 – 10:15 AM	Registration	Ryan Family Atrium
	Run-through talks	Baldwin Auditorium
	Setup Posters	Ryan Family Atrium

10:15 AM **Welcome** (Baldwin Auditorium)
Sevim Yildiz Arslan, Nan Zhang, and Lia Bernardi
(Trainee Committee Members)

Opening Remarks and Introduction of Alumni Speaker
Teresa K. Woodruff, **PhD**
Thomas J. Watkins Professor of Obstetrics & Gynecology
Northwestern University

10:30 AM **Northwestern Alumni Address**
David L. Keefe, MD
The Stanley H. Kaplan Professor and Chair
Department of Obstetrics and Gynecology
New York University School of Medicine

11:30 AM ***Lunch Break*** (Ryan Family Atrium)

12:30 – 1:30 PM **ORAL SESSION** (Baldwin Auditorium)
Session Moderators: **Lia Bernardi**, **MD** Reproductive Endocrinology and Infertility Fellow, Obstetrics and Gynecology Department, Northwestern University; **Sevim Yildiz Arslan**, PhD, Postdoctoral Fellow, Obstetrics and Gynecology Department, Northwestern University; **Nan Zhang**, PhD, Postdoctoral Fellow, Obstetrics and Gynecology Department, Northwestern University

Abstract # Presenting author underlined

12:30 PM O1 **Inhibition of Akt is Sufficient to Upregulate Progesterone Receptor B Dependent Transcription and Decrease Angiogenesis in Endometrial Cancer Cells.** Irene Lee, J Julie Kim, Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Northwestern University

12:45 PM O2 **Molecular Signatures of Fibroid Stem Cells (SCs) Implicate IGF2 as a Potential New Target.** Molly B Moravek, Ping Yin, John S Coon V, J Julie Kim, Serdar E Bulun, Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Northwestern University

1:00 PM O3 **Polycomb-mediated Chromatin Remodeling During Sex Determination.** Sara García-Moreno, Isabella Marie Salamone, Danielle Marie Maatouk, Department of Obstetrics and Gynecology, Northwestern University

1:15 PM O4 **Personalized Follicle Monitoring Improves Oocyte Reproductive Outcomes During Encapsulated *In Vitro* Follicle Growth (eIVFG) in Mouse And Human.** Shuo Xiao[1,2], Francesca E. Duncan[3], Lu Bai[1,4], Catherine T. Nguyen[1,4], Lonnie D. Shea[5], Teresa K. Woodruff[1,2] [1]Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL; [2]Center for Reproductive Science, Northwestern University, Evanston, IL; [3]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [4]Master of Biotechnology Program, Northwestern University, Evanston, IL; [5]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI.

1:30 - 2:50 PM **POSTER SESSIONS** (Ryan Family Atrium)
See Poster Session Abstracts in this program.

1:30 - 2:10 PM **POSTER SESSION A** (odd numbered posters presented)

2:10 – 2:50 PM **POSTER SESSION B** (even numbered posters presented)

3:00 – 4:00 PM **Introduction of Keynote Speaker and Presentation of the *Neena B. Schwartz Lectureship in Reproductive Science*** (Baldwin Auditorium)
Serdar E. Bulun, MD
John J. Sciarra Professor and Chair of Obstetrics and Gynecology
Northwestern University

Keynote Address – *"Endocrine and Metabolic Control of Folliculogenesis in Rhesus Monkeys: Relevance to Polycystic Ovarian Syndrome?"*
Richard L. Stouffer, PhD
Senior Scientist and Head,
Oregon National Primate Research Center
Professor, Departments of Obstetrics & Gynecology and Physiology & Pharmacology, Oregon Health and Science University

4:00 – 4:30 PM **Presentation of Constance Campbell Awards**
Kelly E. Mayo, PhD, Director, Northwestern University Center for Reproductive Science and David L. Keefe, MD, Alumni Speaker

Closing Comments –Minisymposium Trainee Committee members

4:30 - 5:30 PM **Reception** (Ryan Family Atrium)

**** ***Please take down posters after reception at 5:30 pm*** ****

Abstract # O1

Inhibition of Akt is sufficient to upregulate Progesterone Receptor B dependent transcription and decrease angiogenesis in endometrial cancer cells, Irene Lee and J. Julie Kim; Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL.

Progestins have long been used clinically for the treatment of endometrial cancers, however, the response rates to progestin therapy vary considerably and the molecular mechanisms behind progestin insensitivity are not well understood. We hypothesized that in *PTEN* mutated endometrial cancers, hyperactive Akt signaling downregulates Progesterone Receptor B (PRB) transcriptional activity, leading to impaired progestin responses in endometrial cancer. In order to identify the specific PRB gene targets that are modulated by Akt signaling, we performed microarray gene expression analysis using PRB-Ishikawa cells transfected with either siCtrl or siAkt and then treated with either vehicle or the progestin, R5020. Upon microarray analysis, we identified 126 differentially expressed genes in the siAkt+R5020 dataset. We then performed gene ontology analysis of these differentially expressed genes, and have identified angiogenesis as the principle process enriched in the siAkt+R5020 dataset. To further interrogate the mechanism by which inhibition of Akt modulates PRB transcriptional activity, we sought to determine whether inhibition of Akt affects PRB interaction with its cofactors. We performed ChIP-Mass Spectrometry to identify those cofactors that differentially interact with PRB in the presence of R5020 and MK+R5020. We identified 294 differentially expressed proteins present in the MK+R5020 treatment group compared to the R5020 only group. Upon gene ontology analysis of these differentially expressed proteins, we found that the 14-3-3 pathway is enriched, implicating a role for 14-3-3 in the Akt-dependent regulation of PRB transcriptional activity. Finally, to determine the biological ramifications of MK+R5020 treatments, we performed in vitro angiogenesis assays examining the effects of treated conditioned media from PRB-Ishikawa cells on uterine microvascular endothelial cells. We found that the combinatorial MK+R5020 treatment significantly decreased endothelial cell invasion and endothelial tube formation more so than any other treatment. Taken together, these data may suggest a combinatorial therapeutic approach utilizing Akt inhibitors with progestins to improve the efficacy of progestin therapy for the treatment of endometrial cancer.

This work is supported by NIH/NCI grant R01CA155513, NIH/NCI training grant T32CA09560, and by the Malkin Scholars Program from the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

Abstract # O2

Molecular signatures of fibroid stem cells (SCs) implicate IGF2 as a potential new target, Molly B Moravek, Ping Yin, John S Coon V, J Julie Kim, Serdar E Bulun; Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL

We previously identified 3 populations of fibroid cells—CD34+CD49b+ (+/+, SCs), CD34+CD49b- (+/-), and CD34-CD49b- (-/-). We sought to determine differential gene expression among the pathways and explore critical pathways for SC function and fibroid pathogenesis. Fibroid cells were isolated from surgical specimens and either cultured or sorted by flow cytometry based on CD34 and CD49b expression. Microarray was performed with differential expression defined as fold change >1.5 and FDR<5%. Cells were treated with IGF2 (100ng/mL) or vehicle and analyzed by real-time PCR, Western blot, or flow cytometry. All experiments were performed in triplicate. Microarray revealed that the 3 populations are distinct with >1500 differentially expressed genes, and suggested a transition from +/+ SCs through +/- cells to -/- cells. Pathway analysis indicated a significant role for IGF signaling. IGF1 and IGF2 were overexpressed in +/- cells (5-fold and 150-fold, respectively, $p<0.05$), whereas insulin receptor-A (IR-A) and IGFBP3 were significantly overexpressed in +/+ SCs (26-fold and 19-fold, respectively, $p<0.05$). Total cell number was increased 1.5-fold in IGF2-treated cells. IGF2 treatment of unsorted cells activated both Akt and ERK signaling, and resulted in increased levels of proliferating cell nuclear antigen compared to vehicle. In sorted cells, treatment with IGF2 preferentially activated the ERK pathway in +/+ SCs and the Akt pathway in -/- cells, with no effect seen in +/- cells. IGF2 also increased expression of IR-A 3-fold in +/+ SCs, without change in apoptosis, measured by bcl2 and Annexin V. Additionally, proliferation increased in all three populations with IGF2 treatment, with highest EdU incorporation in +/+ SCs and lowest in -/- cells. These data suggest IGF2 signaling is important for fibroid SC function and tumor proliferation and growth, likely through IR-A and the ERK pathway. IGF2 appears to act through different pathways in SCs vs differentiated cells, making it possible to specifically target SCs. The IGF2/IR-A pathway in SCs has recently been suggested as a therapeutic target in several cancers, and should be explored in fibroids as well. Treatments targeting pathways necessary for fibroid SC function may both address existing fibroids and prevent the development and growth of new tumors.

Supported by the ASRM In-Training Grant in Heavy Menstrual Bleeding (MBM), NIH/NICHD P01-HD057877

Abstract # O3

Polycomb-mediated chromatin remodeling during sex determination, Sara García-Moreno, Isabella Marie Salamone, and Danielle Marie Maatouk; Department of Obstetrics and Gynecology, Northwestern University, Chicago IL.

Prior to sex determination, gonads are identical in XX and XY individuals and the somatic cells that compose them are bipotential, with the ability to differentiate into either Sertoli cells leading to testis formation, or into pregranulosa cells leading to ovary formation. Over 35 sex-determining genes have been identified involved in the differentiation toward the preSertoli or pregranulosa cell fate. Mutation of these genes lead to disorders of sexual development (DSD), a group of congenital conditions in which there is inconsistency between chromosomal, gonadal and phenotypic sex. Disruption of known sex-determining genes accounts for only 20% of DSD cases. Interestingly, mutations of several chromatin remodeling enzymes, including the Polycomb-group (PcG) subunit *Cbx2* have been linked to sexual disorders, suggesting a critical role for chromatin remodeling in sex determination. CBX2 binds the repressive histone modification mark H3K27me3. We hypothesize that the PcG-complex directs bipotential gonadal cells toward the testis pathway by maintaining sexually dimorphic patterns of H3K27me3 on sex-determining genes. To understand the involvement of PcG during mammalian sex determination, we performed carrier chromatin immunoprecipitation (cChIP) for H3K27me3 on <50,000 purified preSertoli and pregranulosa progenitor cells at E10.5 (pre-sex determination) and at E13.5 (post-sex determination). In accordance with our hypothesis, cChIP revealed differential H3K27me3 enrichment patterns in XY and XX supporting cells at E13.5, and similar patterns at E10.5. At E13.5, repressed ovary-determining genes were highly enriched for H3K27me3 in preSertoli cells but not in pregranulosa cells, suggesting an important role for chromatin remodeling in the repression of the female pathway during testis development in XY gonads. Our results are the first to uncover sex-determining gene targets of PcG and demonstrate dynamic histone modifications during sex-determination. Future studies using ChIP-seq will allow us to analyze genome-wide Polycomb-group targets in XY and XX supporting cells and their potential role in sex determination.

Abstract # O4

Personalized follicle monitoring improves oocyte reproductive outcomes during encapsulated *in vitro* follicle growth (eIVFG) in mouse and human, Shuo Xiao[1, 2], Francesca E. Duncan[3], Lu Bai[1, 4], Catherine T. Nguyen[1, 4], Lonnie D. Shea[5], Teresa K. Woodruff [1, 2*]
[1]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2]Center for Reproductive Science, Northwestern University, Evanston, IL; [3]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [4]Master of Biotechnology Program, Northwestern University, Evanston, IL; [5]Department of Biomedical Engineering, College of Engineering and Medical School, University of Michigan, Ann Arbor, MI.

One fertility preservation technology under active investigation for females facing a fertility threatening condition is encapsulated *in vitro* follicle growth (eIVFG). The alginate hydrogel-based eIVFG recapitulates key events of folliculogenesis and oogenesis. However, there is significant room to improve as the efficiency of *in vitro* fertilization (IVF) remains low in mouse and there are unique challenges in translating this work from mouse to human. We hypothesized that the personalized follicle monitoring based on follicle size, rather than absolute culture time, could identify high quality oocytes. Multilayer secondary follicles were isolated from day 16 CD-1 mice and cultured individually either for defined time periods or up to specific follicle diameter ranges, at which point multiple reproductive endpoints were analyzed. The metaphase II (MII) percentage for follicles cultured to a terminal diameter of 300-350 μm was the highest at 93%, which was significantly higher than that of follicles cultured for specific days or in other follicle size groups. More than 90% of MII oocytes matured from follicles with diameters of 300-350 μm showed normal spindle morphology and chromosome alignment, 85% of oocytes showed 2 pronuclei after IVF, 81% developed into the 2-cell embryo stage, and 46% developed into the morula or blastocyst stage, all significantly higher than the percentages for follicles in the other follicle size groups. When this personalized follicle monitoring based on size was applied to human follicle, the two-step culture strategy with alginate removal after follicle diameter reached 500 μm significantly improved human follicle growth and stage-specific follicle development *in vitro*. After *In vitro* maturation (IVM), 20% (4 out of 20) of human follicles produced oocytes in MII stage and with expression of developmental markers. Our results demonstrate that the personalized follicle monitoring based on sized improves oocyte meiotic and developmental outcomes in both mouse and human.

(Funded by U54HD076188 and Sherman Fairchild Foundation).

Abstract # P1

The legacy of Constance Campbell, Northwestern University Center for Reproductive Science.

Abstract # P2

Development of PDX tumor models of gynecologic cancers for the discovery of novel therapeutics, Wenan Qiang[1,2,3], Ruifen Dong[2], Stacy Druschitz[1], Kenji Unno[1], Abigail Winder[1], Zhenxiao Lu[1], Jian-Jun Wei[2,3], and J. Julie Kim[1,3], [1]Department of Obstetrics and Gynecology - Division of Reproductive Science in Medicine, Women's Cancer PDX Core, [2]Department of Pathology, [3]Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL.

Patient-derived xenografts (PDX) established by the transplantation of surgically resected tumor specimens into immunodeficient mice are reported to be more predictive of the clinical situation while maintaining the histopathology and molecular diversity of patient tumor compared to conventional cell lines. 13 ovarian cancer (9 serous, 3 endometrioid, and 1 mucinous) and 11 endometrial cancer tumors were obtained from Northwestern University Prentice Hospital and xenografted through subcutaneous, subrenal, intraperitoneal, or intrabursal/orthotopical in immunodeficient NSG female mice to establish the ovarian and endometrial cancer PDX model.
All of 9 serous ovarian cancers have been propagated from one to four passages. Serous ovarian cancer was subsequently re-transplanted into the intrabursa of the mouse ovary, which resulted in ascites and metastasizes to many organs in the abdominal cavity including the omentum, cecum, mesentery, pancreas, liver, kidney, diaphragm, and spleen. Cryopreserved PDX tumors also re-grew in the mice. The morphological features between all methods of transplantation were similar, and the histology of the xenografted tumor resembled the original cancer. Cisplatin resistant tumors as well as tumors carrying fluorescent and luciferase reporters were also established as models to study efficacy of targeted agents. Four aggressive endometrial cancer PDX tumor lines, successfully generated through subrenal xenografting, are available in the core for testing of targeted compounds to study their effects on tumor growth and metastasis. In conclusion, the PDX tumor model was successfully established in NSG female mice, which recaptures patterns of human ovarian and endometrial cancer growth and metastasis. Currently, the PDX tumors are used in the preclinical development of new therapeutics.

The ovarian cancer PDX was supported by Baskes Foundation and by NIH/ NCI grant 1R21CA167038 and award to J.J. Wei and the endometrial PDX was funded by NIH/NCI grant R01CA155513 and awarded to JJK.

Abstract # P3

Intrinsic redox dysregulation promotes AKT activation in uterine fibroids, V. Vidimar[1], D. Gius[2], J.J. Wei[1,3], J.J. Kim[1]. [1]Obstetrics and Gynecology, Northwestern University, Chicago, IL, United States, 60611; [2]Radiation Oncology, Northwestern University, Chicago, IL, United States, 60611 and [3]Pathology, Northwestern University, Chicago, IL, United States, 60611.

Uterine fibroids (leiomyomas) are noncancerous tumors of the myometrium that appear in approximately 70% of reproductive-age women. We previously reported that the AKT pathway is upregulated in leiomyoma (ULM) compared to normal myometrium (MM). However, it is unclear what promotes and sustains AKT activation in ULMs. We hypothesize that oxidative stress caused by an intrinsic dysregulation of the redox system activates the AKT pathway promoting ULMs survival. Oxidative stress often results from alterations in MnSOD levels. Acetylation of MnSOD leads to accumulation of inactive MnSOD. Immunostaining of a tissue microarray containing over 100 matched ULM/MM tissues showed that acetylated MnSOD levels were higher in ULM than MM. Moreover, the MnSOD mimetic GC4419 reduced pAKT protein levels in primary ULM cells supporting our hypothesis. The different expression status of MnSOD between ULM and MM cells reflects a differential response to reactive oxygen species (ROS) of these two cell types. Exposure to H_2O_2 or paraquat increased pAKT levels in ULM cells but not in MM cells. Interestingly, while high doses of ROS reduced ULM cell viability, viability of MM cells was minimally affected. Inhibition of AKT with MK-2206 led to cell death in ULM cells without activation of any apoptosis-related event. Here, we showed that doses of MK-2206 that trigger cell death in ULM cells resulted in increased superoxide and decreased glutathione (GSH) levels, suggesting that MK-2206 triggers oxidant-induced cell death of ULM cells by impairing the GSH/ROS balance. We found that leiomyomas are characterized by an impaired oxidative system due to the aberrant acetylation of MnSOD that promotes AKT activation. In contrast, myometrium displays a functional redox detoxification system that explains both low acetylated MnSOD and pAKT levels. This study allowed us to better understand leiomyoma biology highlighting the differential response to oxidative stress between leiomyoma and myometrium that may be considered for future therapeutic use.

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Abstract # P4

Novel progesterone receptor target gene perilipin 2 regulates proliferation and collagen formation in breast cancer and uterine leiomyoma, Ping Yin, Molly B Moravek, John S Coon V, Hye Ok Kim, Matthew T Dyson, and Serdar E Bulun. Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, 303 E Superior St, Chicago, IL 60611.

Progesterone exerts an overall tumorigenic effect on both uterine leiomyoma (fibroid) and breast cancer tissues, whereas the antiprogestin RU486 inhibits growth of these tissues through an unknown mechanism. Previously, using an unbiased ChIP-sequencing technique, we uncovered perilipin 2 (ADRP/ADPH/PLIN2) as a novel PR target gene in T47D breast cancer cells and primary human uterine leiomyoma cells. RU486 treatment induced PLIN2 gene expression via the same DNA regulatory region in both cell types. PLIN2, an adipose differentiation-related protein, is ubiquitous in non-adipose lipid droplet-containing cells and plays important roles in lipid droplet formation and stabilization, but its loss is linked to the expression of fibrogenic genes in hepatic fibrosis. Moreover, in clear cell renal carcinoma, higher PLIN2 expression is associated with better cancer-specific survival and cancer-free survival. Until now, little is known about the roles of PLIN2 in the regulation of the growth of human uterine leiomyomas or breast cancers. In this study, we examined the impact of PLIN2 on the function of uterine fibroid cells and T47D cells by depleting its expression via small inference RNA (siRNA). We found that knockdown of PLIN2 with siRNA dramatically increased protein levels of proliferating cell nuclear antigen (a marker for cell proliferation) in both cell types. In T47D cells, PLIN2 knockdown also significantly stimulated the expression of fibrogenic genes including TIMP1 (2-fold), collagen 1a1 (1.5-fold), and collagen 1a2 (3-fold). Furthermore, we discovered that 75% of subjects' leiomyoma tissues expressed lower mRNA levels of PLIN2 compared to the adjacent myometrial tissues. Our conclusions suggest that PLIN2 regulates cell proliferation and extracellular matrix formation in human T47D breast cancer cells and primary uterine leiomyoma cells, and we predict that PLIN2 plays an important role in mediating the therapeutic effects of RU486 in uterine leiomyomas and breast cancers.

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Abstract # P5

Retinoic acid induces *Cyp19a1* expression in the mouse ovary, S. Persaud and J. Kipp. Department of Biological Sciences, DePaul University.

Retinoic acid (RA) is an active derivative of vitamin A and plays a vital role in many biological systems through regulating gene expression. We have recently shown that RA is an important regulator of ovary development although the underlying mechanisms are not known. Aromatase, encoded by *Cyp19a1*, is an enzyme that converts androgens to estrogens and is prominently expressed in ovarian granulosa cells upon gonadotropin activation. Aromatase has been suggested to be a target of RA regulation in breast cancer cells and placental cells in a cell-type specific manner. In the ovary, aromatase regulates follicle growth and maturation and prevents follicle atresia. Aromatase knockout mice or aromatase-inhibitor treated mice share similar ovarian pathologies and/or hormone profiles with a vitamin A deficient mouse model that we developed, suggesting converging mechanism(s). To better understand RA actions in the ovary, this study investigated the regulation of aromatase expression by RA. Primary ovarian granulosa cells were cultured and treated with RA, R115866 (a RA metabolism blocking agent), AGN193109 (a pan RA receptor inhibitor), RA plus AGN193109, R115866 plus AGN193109, or vehicle (control). After treatments for 24- or 72-hrs, mRNA and proteins were collected for real-time PCR and western blot analysis. The results showed that RA or R115866 treatment increased *Cyp19a1* mRNA levels at 24 hr. The stimulatory effects were specific as they were abolished by AGN193109. Western blot results confirmed R115866 induction of aromatase protein expression. At 72 hr, R115866 increased while AGN193109 decreased *Cyp19a1* mRNA levels, and the stimulatory effect of R115866 was abolished by AGN193109. Although the effect of RA on *Cyp19a1* mRNA levels was not significant at 72 hr, it significantly increased aromatase protein levels, possibly due to the relatively short half-life of *Cyp19a1* mRNA. Overall, our study suggests that *Cyp19a1* is a target of RA regulation and aromatase may partially mediate RA functions in the ovary.

Supported by a URC Grant to Kipp.

Abstract # P6

In vivo **impact of vitamin A deficiency or retinoic acid synthesis inhibition on ovulation and oocyte maturation in mice,** Rebecca L. Ursin, J.L. Kipp. Department of Biological Sciences, DePaul University, Chicago, IL.

Our previous studies have indicated that retinoic acid (RA), an active metabolite of vitamin A (VA), plays a role in regulating ovary development. Using an *in vivo* dietary VA-deprivation animal model, we have demonstrated that VA deficiency (VA-) causes a variety of ovarian pathologies, including reduced numbers of total follicles and corpus luteum, formation of hemorrhagic and atretic follicles, and formation of bursa and follicular cysts. The decreased number of corpus luteum in the VA- mice may result from ovulation defects. To test this hypothesis, current study examined both longterm and acute impact of VA- or RA-deficiency on ovulation and oocyte maturation in mice. To examine the longterm impact of VA-, VA- or control female mice were superovulated at D19 or Wk7 via a subcutaneous injection of pregnant mare's serum gonadotropin (PMSG) followed by human chorionic gonadotropin (hCG). Ovulated oocytes were collected, counted and classified. Oocyte count showed that the number of ovulated oocytes was significantly reduced in the VA- mice as compared to the controls at Wk7 but not at D19. Oocyte classification showed a reduced percentage of GV-oocytes at D19 and reduced percentages of GV-, GVBD-, and MII-oocytes at Wk7 in the VA- mice, suggesting abnormal oocyte maturation. To investigate whether acute RA-deficiency may affect ovulation event, mice on a regular diet were injected with the RA synthesis blocker WIN18446 at D19 or Wk7 for three days during superovulation induction. The results showed that a lower dose of WIN18446 suppressed ovulation and hindered oocyte maturation in Wk7 mice, as the number of ovulated oocytes and the percentages of GVBD- and MII-oocytes were reduced. At a higher dose, WIN18446 also suppressed ovulation and reduced the percentages of GV- and MII-oocytes in D19 mice. Overall, our results suggest that RA signaling plays a critical role *in vivo* in regulating ovulation and oocyte maturation and older animals are more susceptible to the impact of VA- and RA-deficiency.

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Abstract # P7

Investigating the spatio-temporal properties of intracellular zinc waves during fertilization, Seth A. Garwin[1,2], Emily L. Que[1,2], Teresa K. Woodruff[1,3], Thomas V. O'Halloran[1,2]. [1]The Chemistry of Life Processes Institute, Northwestern University, Evanston, IL; [2]The Department of Chemistry, Northwestern University, Evanston, IL; [3]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL

Previous work in the O'Halloran and Woodruff labs illustrated that the mouse oocyte must acquire ca. 20 billion zinc atoms during meiotic maturation, and upon fertilization it undergoes periodic zinc exocytosis events termed "zinc sparks," which involves the loss of 12-15 billion zinc atoms. By manipulating zinc availability in the oocyte using a zinc chelator (TPEN) and a zinc ionophore (Zn-pyrithione), we demonstrated that these large zinc fluxes were required for proper progression from oocyte to egg to embryo. Furthermore, utilizing a recently synthesized zinc probe, ZincBY-1 (Que, et. al., Nat. Chem. 2014), we discovered an intracellular "zinc wave" occurring with the zinc spark at the time of egg activation. Existing and newly invented cell-permeable, small molecules have been deployed to manipulate and monitor zinc in the female gamete; however, they act both intracellularly and extracellularly and are not targetable to particular regions inside a cell. Thus, we do not know the origin of the observed "zinc wave" nor do we know if it is contained within vesicles or diffusing through the cytosol. In order to address the technical gap of this family of agents, we have designed a new generation of fluorescent zinc probes to measure the zinc status of the oocyte in a targeted manner. Injectable zinc nanosensors are being synthesized through modifications of the previously developed ZincBY-1 that will allow us to localize probes in different regions of the cell and precisely track and quantify labile zinc movement within the cytosol. Herein we will present our preliminary results in both understanding the properties of the zinc wave and the development of a zinc nanosensors. We will also present our plans for probing zinc in the mammalian egg during activation.

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Abstract # P8

Engineering a three-dimensional human ectocervical tissue model to study hormonal regulation and immune response of the female reproductive tract, Kelly McKinnon[1], Paul Hoover[2], Teresa Woodruff[1], Spiro Getsios[2], Department of Obstetrics and Gynecology[1], Department of Dermatology[2], Northwestern University Feinberg School of Medicine, Chicago, IL.

The ectocervix plays a major role in childbirth, infectious disease transmission, and contraception. It has a stratified squamous epithelium that is composed of multiple differentiated cell layers. The basal layer contains progenitor cells that produce the parabasal, intermediate and superficial layers. This epithelium undergoes constant regeneration throughout life and is modified during the menstrual cycle in response to ovarian hormones. In addition to the morphological changes that occur throughout the menstrual cycle, the innate immune system of the female reproductive tract (FRT), including the ectocervix, is directly and indirectly regulated by ovarian hormones. Though much is known about hormonal regulation of other tissues in the FRT, the ectocervix remains vastly understudied. Mouse models and 2D cultures have traditionally been used to study reproductive biology; however, mouse models do not accurately represent human FRT anatomy or physiology, and more importantly are not natural hosts to pathogens that can infect humans, whilst 2D human cell cultures do not accurately reflect the stratified architecture of a differentiated ectocervix. Therefore, using primary epithelial cell cultures initiated from human ectocervix, we have engineered a 3D model of this stratified epithelium that recapitulates aspects of the *in vivo* human physiology. Primary ectocervix epithelial cells were grown on a collagen-based stromal scaffold at an air-liquid interface, and formed multiple differentiated layers, as shown by histology and cytokeratin profiles. Cytokeratin-14 was found in the basal layer, and cytokeratin-13 in the more intermediate and superficial layers. Additional differentiation markers typical of native ectocervix, such as p63 and MUC4 were also present in these 3D tissue cultures. This model will be a critical tool for understanding fundamental mechanisms of the ectocervix involved in homeostasis as well as diseased states of the ectocervix, such as host-pathogen interactions and oncogenic transformation.

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Abstract # P9

The Reproductive Science and Medicine Training Cluster, Northwestern University Center for Reproductive Science.

Abstract # P10

The relationship between obesity and ovarian reserve in a population of young African American women (AAW), Deborah E Ikhena, MD[1], Lia A Bernardi[1], Peter de Chavez, MS[2], Randall B Barnes, MD[3], Mercedes Carnethon, PhD[2] and Erica Elizabeth Marsh, MD MSCI[4], [1]Division of REI, Dept of OBGYN, Northwestern University, Chicago, IL, [2]Department of Preventative Medicine, Northwestern University, Chicago, IL, [3]Division of REI - Department of OBGYN, Northwestern University, Chicago, IL, [4]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL.

Objective: The objective of this study was to determine the relationship between obesity and ovarian reserve.
Methods: This is a cross sectional study of 1696 AAW recruited to the Study of Environment, Lifestyle and Fibroids (SELF). Inclusion criteria comprised of AA race, age 23-34 at enrollment, and no prior radiation/chemotherapy or current medication for cancer or autoimmune disease. Obesity is defined as BMI $\geq$ 30 kg/m^2. BMI and serum adipokines; leptin and adiponectin were used as markers for obesity and AMH as a marker of ovarian reserve. Serum AMH, leptin and adiponectin levels were obtained using ELISA assays (CV <10%). Log-transformed AMH was used as the outcome in simple and multiple linear regression models.
Results: The mean subject age was 28.7 ± 3.5 years (mean ±SD) and the mean AMH was 4.00 ± 3.49 ng/ml (range <0.002 -39.4). The mean BMI was 33.6 ± 9.4 (range 15.9 -79.4), with 59.5% of the subjects being obese. There was a significant inverse relationship between log-AMH and BMI (β=-0.012, SE =0.003, P<.0001). The mean leptin level was 16.11 ± 9.68 ng/ml and the mean adiponectin level was 5.287 ± 2.69 μg/m. There was no association between log-AMH and adiponectin levels (β= <0.001, SE = <0.001, p=0.963). There was a significant association between leptin and log-AMH levels (β= -0.017, SE = 0.004, p=<0.001). In multivariate analysis adjusting for age, BMI and current contraception use, this relationship is no longer significant, but approaches significance (p=0.088).
Conclusions: In conclusion, the trend towards significance in the relationship between serum leptin and AMH suggests that the negative effects of obesity on ovarian reserve are at least in part mediated by leptin. Our findings indicate that for this population of young AAW, higher BMI is associated with a lower AMH level and contributes to disparities seen in ovarian reserve.

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Abstract # P11

Pharmacological inhibition of Notch signaling *in vivo* to study roles of Notch on follicle formation and growth in the mouse ovary, Nisan Hubbard and Kelly Mayo, Department of Molecular Biosciences and Center for Reproductive Science, Northwestern University, Evanston, IL.

The formation and development of ovarian follicles is an essential determinant of female fertility in mammals. Studies from our laboratory using pharmacological inhibition of Notch signaling in ovary culture and conditional Notch knockout mouse models showed that Notch is important in follicle formation and growth during ovarian development. While evidence suggests a vital role for Notch signaling during follicle development, it remains unclear when and how this pathway is activated and regulated. This project seeks to determine the temporal importance of Notch's role in follicle formation and subsequent follicle growth using a pan-Notch inhibitor, DAPT (which blocks proteolytic cleavage of the Notch receptors by the γ-secretase complex), during specific windows of follicle development. While DAPT has been used to examine follicle formation *in vitro*, we investigated its application *in vivo* in mice. This provides the advantage of using a pharmacological, transient drug that can be used during a precise temporal window of study. Initial studies with DAPT focused on optimizing the injection dose needed to suppress the Notch signaling pathway, while limiting toxicity, using CD-1 PND17-20 prepubertal mice injected with differing doses (648ng; 865ng per mouse). PND21 ovarian mRNA was then extracted for gene expression studies of Notch target genes. Injected mice showed no effects of toxicity with the selected DAPT doses, exhibiting high survival during treatment. Ovarian mRNA from injected mice showed a significant decrease in the expression of *Hes1* with both doses, signifying a potential knockdown in Notch activity during this window. This suggests that this approach is valid for studying if transient Notch inhibition results in aberrant follicle formation. We are now in the process of testing Notch inhibition during two temporal windows surrounding follicle formation (E17.5-PND1; PND0-3), and are measuring the effects of Notch inhibition on follicle numbers and aberrant follicles. This investigation will provide an approach to decipher the specific mechanisms of Notch actions in the ovary to understand the importance of Notch signaling in follicle development.

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Abstract # P12

Investigating the role of zinc fluxes during sperm activation and fertilization, Amanda R. Bayer[1, 2], Emily L. Que[3], Francesca E. Duncan[4], Teresa K. Woodruff[1, 5], Thomas V. O'Halloran[1,2]. [1]The Chemistry of Life Processes Institute, Northwestern University, Evanston, IL; [2]The Department of Chemistry, Northwestern University, Evanston, IL; [3]The Department of Chemistry, University of Texas at Austin, Austin, TX; [4]The Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [5]The Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Bioinorganic transition metals such as zinc are abundant in all cell types and play important structural, enzymatic, and signaling roles. Since male and female gametes are held in a transcriptionally silent state during meiosis, inorganic signaling through metal fluxes can lead to changes in metalloproteins and pathway activation, which can induce cellular changes. In the male reproductive tract, there is a high concentration of zinc in seminal fluid (0.2 M), which is thought to hold the sperm in a quiescent state until post-ejaculation. Although extracellular zinc effects on sperm have been studied, intracellular zinc concentration and localization of sperm undergoing activation (capacitation and the acrosome reaction) has remained relatively convoluted. Recently, it was discovered by x-ray fluorescence microscopy that spermatozoa acquire 50 million zinc atoms during capacitation that are localized to the sperm head and midpiece. Using fluorescent probes, it was found that labile zinc is localized to the acrosome during uncapacitated and capacitated stages but is exocytosed during the acrosome reaction as sperm prepare for fertilization. In this Project, we test the hypothesis that a spermatozoon must undergo a series of spatio-temporal zinc fluxes that are key to its ability to successfully fertilize the egg. We hypothesize that the decrease in extracellular zinc concentration during the transition from male to female reproductive tract leads to an increase in intracellular zinc. This intracellular zinc flux is essential for sperm to reach a fully capacitated state, successfully undergo the acrosome reaction, and ultimately penetrate and fertilize a mature egg to lead to a viable zygote.

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Abstract # P13

Cisplatin and radiation induce loss of ovarian reserve by activation of TAp63 through different mechanism, So-Youn Kim[1], Katy Ebbert[1], Megan Romero[1], Marilia H. Cordeiro[1], Teresa K. Woodruff[1], and Takeshi Kurita[2,1]. [1]Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago IL, [2]Department of Molecular & Cellular Biochemistry, The Comprehensive Cancer Center, Ohio State University, Columbus OH.

Imatinib was shown to protect primordial follicles against cisplatin and the phosphorylation of TAp63 by c-Abl/ABL1 has been implicated as the key pathway of oocyte death. While the essential role of TAp63 in cisplatin-induced oocyte apoptosis was demonstrated using oocytes specific conditional knockout (cKO) mice for *Trp63* gene, involvement of c-Abl in oocyte apoptosis was indirectly supported by inhibitor studies: Two Abl kinase inhibitors, imatinib and GNF-2, protected primordial follicles from cisplatin. To explore the function of c-Abl, *Abl1* cKO mice were generated. Surprisingly, primordial follicles of oocyte specific *Abl1* cKO mice underwent apoptosis in response to cisplatin and radiation, indicating that oocytic c-Abl is dispensable for the cisplatin and radiation-induced oocyte death, showing p-TAp63 was induced by radiation in the absence c-Abl. The presence and absence of p-TAp63 in the apoptosis of oocytes induced by radiation and cisplatin, respectively, suggested that these two anti-cancer treatments activated TAp63 through different molecular mechanisms. Imatinib and GNF-2, which protected oocytes from cisplatin, did not inhibit radiation-induced apoptosis and p-TAp63 in oocytes. Contrastly, the radiation-induced oocyte death and p-TAp63 were effectively blocked by a CHK2 inhibitor, suggesting that radiation phosphorylates TAp63 by CHK2. Interestingly, although cisplatin did not induce p-TAp63, the CHK2 inhibitor protected primordial follicles from cisplatin, suggesting that kinases activity that are inhibited by Chk2 Inhibitor is essential for cisplatin-induced apoptosis in primordial oocytes. Our study elucidated that anti-cancer therapies with different mode of action induce apoptosis of immature oocytes by activating TAp63 through different mechanisms. Since CHK2 Inhibitor effectively protects oocyte against cisplatin as well as radiation, CHK2 Inhibitor treatment might be a promising treatment for the prevention of ovarian reserve loss in women undergoing anti-cancer therapy.

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Abstract # P14

Impacts of advanced age and high-fat diet on the epigenetic regulator sirtuin 6 and its target histone h3k9 during spermatogenesis, A.E. Kofman and C.J. Payne, Departments of Pediatrics and Obstetrics & Gynecology, Northwestern University Feinberg School of Medicine, Stanley Mann Children's Research Center, Chicago, IL.

Although its effects are more widely documented in females, advanced age has a negative impact on germ cell development in males. Moreover, decreased fertility in both sexes correlates with a diet high in fat. The nuclear epigenetic regulator Sirtuin 6 (SIRT6) mediates aging and metabolic processes as a deacetylase and mono-ADP-ribosyltransferase, and influences spermatid elongation through a mechanism that remains unclear. We hypothesize that both advanced age and high-fat diet alter the chromatin occupancies of SIRT6 and the acetylation levels of its target H3K9, inducing transcriptional changes that impact spermiogenesis. SIRT6 and acetylated H3K9 (H3K9ac) exhibit stage-specific distributions in the seminiferous tubules of 8-week-old mice on a standard lab diet. To examine how aging and dietary intake affect SIRT6 and H3K9 in elongating spermatids, we are comparing 1-year-old mice to 8-week-old mice on standard diets, and comparing 6-month-old mice fed for 16 weeks on a high-fat diet (60% kcal fat) to mice on a control low-fat diet (10% kcal fat). Fluorescence-activated cell sorting of testicular cells labeled with the live cell DNA stain Hoechst 33342 is being used to enrich for 1N spermatids. The chromatin occupancies of SIRT6 and H3K9ac in these recovered cells will be examined using chromatin immunoprecipitation coupled with next-generation DNA sequencing (ChIP-Seq), and gene expression levels will be characterized using RNA-Seq. We expect target genes such as *Pfk1* and *Hif1α*, which respond to metabolic and oxidative stress, to be differentially regulated in elongating spermatids under conditions of advanced age and high-fat diet.

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Abstract # P15

Ex-vivo female reproductive tract integration in a 3D microphysiologic system, Jie Zhu[1], Shuo Xiao[1], Sevim Yildiz Arslan[1], Jackie Shepard[2], Jonathan Coppeta[3]*, Elizabeth C. Sefton[1], Brett Isenberg[3], Linda Griffith[2], Mary Ellen Pavone[1], Joanna Burdette[4]*, J. Julie Kim[1]*, Jeff Borenstein[3], Teresa K. Woodruff[1]*. [1]Department of Obstetrics and Gynecology, Northwestern University, Chicago IL, 60611; [2]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA; [3]The Charles Stark Draper Laboratory, Cambridge, MA; [4]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607; * sub-project leader; All authors contribute equally to this initiative.

Our objective is to develop an ex-vivo female reproductive tract that can be used for reproductive toxicology and therapeutic discovery. The ex-vivo female reproductive tract will consist of 3D cultures of ovarian follicles, fallopian tube epithelium, endometrial and myometrial co-cultures, and endocervix connected by unidirectional flow of cell culture media via a microfluidic culture system. An important goal of our system is to model ovarian hormone production and their effects on the downstream gynecologic tissues. Static 3D culture protocols of primary human fallopian tube, uterus (endometrial and myometrial co-culture), and endocervix have been defined. In addition, murine ovarian follicles can phenocopy the human menstrual cycle reducing dependence on healthy human ovarian tissue. Recent efforts have focused on compound testing and optimizing ovarian follicular microfluidic culture. Testosterone (T4) and RU486 were used as test compounds in static 3D cultures of human fallopian tube and endocervix, respectively. T4 treatment in the presence of E2 modified secreted factor production and reduced human fallopian cilia beating frequency. RU486, a selective progesterone receptor modulator, alleviated inhibition of IL-1beta secretion in the presence of E2/P4 demonstrating that IL-1beta secretion is mediated via P4 in the human endocervix. In addition, RU486 reduces endocervical mucin staining. We plan to include all tissues of the reproductive tract in future compound testing. To facilitate static to microfluidic tissue culture, microfluidic culture modules for follicles were designed and quality assurance (QA) testing was performed. Testing demonstrated that the microfluidic platform consistently pumps cell culture media through MPS modules for 28 days. Overall, we have delivered 3D static cultures of gynecologic tissues with quantifiable endpoints as markers for physiologic responses and proof of concept for microfluidic culture over 28 days. Future plans include culturing follicles in the microfluidic system to phenocopy hormone secretion across 28 days.

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Abstract # P16

Efficacy of metarrestin against ovarian cancer, Margaux J. Kanis, MD, Wenan Qiang, MD, PhD, Sui Huang, PhD, J. Julie Kim, PhD, Department of Reproductive Biology, Northwestern University, Chicago, IL.

Metarrestin was identified through a screen of compounds that disrupted the perinucleolar compartment (PNC) which is a nuclear structure associated with the metastatic potential of cancer cells. In this study, the effect of metarrestin on ovarian cancer cells was tested. The PNC prevalence (the percentage of cells containing at least one PNC) in SKOV3 and OVCAR3 ovarian cancer cell lines treated with two doses of metarrestin was determined by immunofluorescence with SH54, a monoclonal antibody that specifically recognizes PTB, an RNA binding protein that is highly concentrated in the PNC. Cell viability using a WST assay and a Matrigel invasion assay using transwell system were performed. Both SKOV3 and OVCAR3 cells are high in PNC prevalence. When treated with metarrestin, PNC prevalence and the number of cells that contain multiple PNCs were significantly reduced in SKOV3 and OVCAR3 cells. Cell viability assays showed minimal cell toxicity, in which treatment up to 20 uM metarrestin did not induce growth inhibition. Significant decreased cell viability was only observed when treated with metarrestin at 50 uM. Furthermore, metarrestin significantly inhibited invasion of SKOV3 cells. Metarrestin effectively reduces PNC prevalence in ovarian cancer cell lines, and significantly attenuates invasion of ovarian cancer cells without significant growth inhibition. We are currently testing metarrestin on metastasis of ovarian tumors in mouse xenograft models.

Abstract # P17

Scrotal ultrasound for mass: Findings and correlations, James Kashanian, Christopher Morrison, Marah Heheman, Valery Raup, Andrew Choi, Brian Trinh, Mohammed Said, Daniel Mazur, Daniel Oberlin, Robert Brannigan, Northwestern Memorial Hospital, Department of Urology.

Introduction: Scrotal ultrasound (US) is the most common adjunctive test performed in the assessment of patients with palpable scrotal abnormalities. We hypothesize that the majority of scrotal US performed for the evaluation of a palpable scrotal abnormality exhibit findings that are consistent with benign processes. On the other hand, we also hypothesize that the majority of testicular neoplasms detected on US coincide with a palpable mass on physical exam. Additionally, we predict that a small fraction of all testicular neoplasms are found incidentally.
Methods: After receiving IRB approval, we performed a retrospective review of all scrotal US performed from 2002 to 2014 at our tertiary care institution. We separately examined A) scrotal US performed for a clinical history of a palpable scrotal mass, nodule or swelling and, B) scrotal US with findings of intra-testicular mass suspicious for neoplasm. Individual US results were reviewed and analyzed.
Results: A total of 18,593 scrotal US were performed from 2002-2014 at our institution. There were 3,487/18,593 (18.7%) US performed for palpable abnormality (Group A). Of this group only, 198/3487 (5.68%) US identified discrete intratesticular masses concerning for malignancy. A total of 259/18,593 (1.4%) discrete testicular masses (group B) were identified on US for any indication. Of this group, 198/259 (76.4%) were performed because of a palpable abnormality and the remaining 61/259 (23.5%) were found incidentally on US. The 61 incidentally found intra-testicular masses accounted for just 0.33% of all US performed.
Conclusion: To our knowledge, this is the largest study addressing findings of scrotal ultrasound. Based on our 12 year retrospective analysis, we found that only a small minority (5.68%) of US performed for scrotal masses on physical exam confirmed a discrete intratesticular mass concerning for malignancy. Based on these results, we believe that patients presenting with a palpable testicular mass should undergo initial scrotal US to rule out malignancy. Interestingly, though, the vast majority (94.3%) of palpable abnormalities correspond to non-malignant US findings. Finally, incidentally found intra-testicular masses are extremely rare (0.33%).

Abstract # P18

Crosstalk between the retinoic acid and calcium signaling pathways in mouse ovarian granulosa cell function, C. White[1], M. Demczuk[2], H. Huang[1], J. Kipp[2]. [1]Department of Physiology and Biophysics, Rosalind Franklin University of Medicine and Science, North Chicago, IL and [2]Department of Biological Sciences, DePaul University, Chicago, IL.

Normal development of ovarian follicles is critical for female reproduction and endocrine function. We have identified retinoic acid (RA) and the RA-degrading enzyme CYP26B1 as regulators of ovarian follicle development. We have shown that RA and a CYP26 inhibitor stimulate ovarian granulosa cell growth. Tuning of the calcium signal is also known to modulate cell proliferation and apoptosis, suggesting that the calcium signal may orchestrate multiple aspects of granulosa cell function. The current study was designed to examine the role of intracellular Ca^{2+} signaling in mediating the effects of RA/CYP26B1 on primary mouse granulosa cell proliferation. In Ca^{2+} imaging experiments, treatment of cultured granulosa cells with RA was found to increase the steady-state Ca^{2+} content of the endoplasmic reticulum (ER) stores. This correlated with increased store-operated Ca^{2+} entry and enhanced IP_3-dependent Ca^{2+} release in response to purinergic stimulation. In proliferation assays, RA treatment or *Cyp26b1* knockdown stimulated proliferation while *Cyp26b1* overexpression inhibited proliferation. When cells were treated with 2-APB, a blocker of ER Ca^{2+} release and store-operated Ca^{2+} entry, or treated with Xestospongin C, a selective IP3 receptor antagonist that specifically blocks ER Ca^{2+} release, cell growth was inhibited. When RA was given together with 2-APB or Xestospongin C, the stimulatory effect of RA on cell proliferation was abolished. These data support a model in which the RA regulates ovarian follicle development by stimulating granulosa cell proliferation that is at least partly driven by the modulation of Ca^{2+} signals mediated by increased ER Ca^{2+} store filling.

Support for this work was provided by a DePaul-RFUMS Collaborative Research Grant.

Abstract # P19

Effect of usage and type of hormonal contraception (HC) on anti-Mullerian hormone (AMH) levels in young African American women, Marissa L Steinberg, MD[1], Peter de Chavez, MS[2], Donna D Baird, PhD[3], Mercedes Carnethon, PhD[2] and Erica E Marsh, MD, MSCI[1]. [1]Obstetrics & Gynecology - REI Division, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, 60611; [2]Preventative Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, 60611 and [3]Epidemiology, NIEHS, Research Triangle Park, NC, United States, 27709.

A number of studies have suggested HC use results in lower AMH levels, while others have found no effect. Few studies have looked at the effect of specific types of HC on AMH, beyond oral contraceptive pills (OCPs). Our objective is to determine if there is an impact on AMH level among young African American women currently using HC and, further, to determine if specific types of HC have a differential impact. 1654 of the 1696 women participating in the Study of Environment, Lifestyle & Fibroids had blood samples and covariate data for inclusion in this cross-sectional analysis. Samples were run on an ultrasensitive AMH ELISA assay. Log AMH was used for the linear regression model, as AMH was not normally distributed. The mean age of the subjects was 28.7±3.5 years (mean±SD). The mean AMH level (ng/mL) was 3.99±3.48. 27.4% of the participants were currently using some form of HC. Mean AMH of current HC users (3.31±2.56) was significantly lower than prior HC users (4.25±3.90, p<.001), and never HC users (4.26±3.02, p<.001). Among current HC users, the most commonly used types were OCPs (44%), hormonal shot (23%), and levonorgestrel IUD (22%). Mean AMH among those currently using OCPs, hormonal implant, vaginal ring, hormonal shot, and levonorgestrel IUD were significantly lower than those not currently using HC. After controlling for age and BMI, OCPs (ß=-.25; SE=.080; P=.002), vaginal ring (ß=-1.15; SE=.21; P<.0001), and hormonal shot (ß=-.37; SE=.011; P=.001) remained associated with lower log AMH values in the multivariate analysis. This study supports an association between current HC use and lower AMH levels, and suggests a differential impact of various types of HC. The mean AMH of prior HC users was not significantly different from those who had never used HC, suggesting the effect of HC on AMH is temporary.

Abstract # P20

Cross-regulation of Activin and Notch signaling pathways in the ovary, Pamela Monahan, Kelly E. Mayo. Department of Molecular Biosciences, Center for Reproductive Sciences, Northwestern University, Evanston, IL.

Ovarian follicle assembly and maturation are crucial developmental events that are integral to female fertility and reproductive health. As the mouse ovary develops, somatic cells proliferate and encapsulate individual oocytes, initiating follicle assembly and establishing cell-cell interactions that allow for juxtacrine and paracrine signaling. Both the Activin and Notch signaling pathways are known to play important roles in ovarian development and follicle growth. Activin, a TGF-β superfamily member, promotes granulosa cell proliferation during development and in the adult. Suppression of Activin signaling, results in the presence of multi-oocytic follicles (MOFs). Recent studies show that suppression of Notch signaling in the ovary also results in MOFs and impaired follicle formation. These common phenotypes shared between Activin and Notch deficient mice, suggests that cooperative regulation between these pathways may play a role in ovary development. To examine whether cross-regulation between the Activin and Notch signaling occurs in the ovary, we tested whether activation or repression of the Activin pathway could impact Notch signaling gene expression. Primary granulosa cells cultured for 24 hours with Activin show an increase in *Jagged1* ligand and downstream Notch effector gene expression, while treatment with the Activin inhibitor, Follistatin, suppresses gene expression. Activin regulation of Notch effector mRNA is still apparent in cells treated for 4 hours with Activin and Follistatin, suggesting a direct transcriptional affect. Therefore, cells were treated with the transcriptional inhibitor Actinomycin D and mRNA analysis shows abolishment of Activin's stimulatory affect. We have conversely tested whether attenuation of Notch signaling through conditional deletion of the receptor *Notch2* or the ligand *Jagged1* could affect Activin signaling gene expression in isolated ovaries. Conditional deletion of either of these Notch components results in a reduction in mRNA expression of *Inhβ*A and *Inhβ*B, subunits that form isoforms of Activin. Together, these results strongly suggest a cross-regulatory mechanism between Activin and Notch signaling in the ovary.

Supported by the Eunice Kennedy Shriver NICHD Program Project Grant (NIH P01 HD021921).

Abstract # P21

Antiproliferative activity and induction of cell cycle arrest by evodiamine on Taxol-sensitive and -resistant human ovarian cancer cells, Zhangfeng Zhong[1], Wenan Qiang[2], Yitao Wang[1*], [1]University of Macau, Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, Macau; [2]Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine at Northwestern University, Chicago, Illinois.

Ovarian cancer is the fifth most common cancer among women, and it causes more fatalities than any other type of cancer involving the female reproductive system. Unfortunately, ovarian cancer cells also eventually become resistant to the widely used chemotherapeutic agent (such as Taxol) so it is imperative to find an alternative chemotherapy. Accumulating evidence has indicated that evodiamine displays broad-spectrum and potent anticancer activity *in vitro* and *in vivo*; however it is unclear whether evodiamine has any effect on ovarian cancer cells. In this study, we investigated the anticancer activities of evodiamine in human ovarian cancer cell lines including A2780-WT and Tax-resistant cells, A2780/Tax cells. Both human ovarian cancer cell lines were treated with different concentrations of evodiamine, and cell viability, cell proliferation, cell cycle, cell apoptosis, P-gp function, and the relevant proteins were analyzed. Overall, evodiamine showed strong anti-proliferative effects, but no cytotoxic effects in A2780/WT and A2780/TAX as verified by LDH and a CFSE assay. Cell cycle analysis suggested that the anti-proliferative effects of evodiamine on ovarian cancer cells may be attributed to its ability to induce G2/M phase cell cycle arrest through p27 regulation. Furthermore, evodiamine increased oxidative stress and regulated the AMPK signaling pathway in ovarian cancer cells, as revealed by an increase in the calcium level and mitochondrial function in evodiamine-treated cells. Evodiamine also enhanced caspase3/7 activity in ovarian cancer cells, and treatment of the cells with a caspase inhibitor reversed the effect of evodiamine. In addition, evodiamine is not a specific substrate of P-gp in resistant ovarian cancer cells. These results indicate that evodiamine suppresses cell proliferation and enhances apoptosis in ovarian cancer cells suggesting that it has the potential to be developed as an effective anticancer agent against Taxol-sensitive and -resistant human ovarian cancer.

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Abstract # P22

Investigation into the dynamic Zn proteome during oocyte maturation, Andrew B. Nowakowski[1], Teresa K. Woodruff[1,2], Thomas V. O'Halloran[1,3], [1]Chemistry of Life Processes Institute, Northwestern University, Evanston, IL 60208, [2]Department of Obstetrics and Gynecology, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, [3]Department of Chemistry, Northwestern University, Evanston, IL.

Zinc is an essential cofactor in hundreds of metalloproteins. Initial work from the O'Halloran and Woodruff labs have shown that mouse oocytes at the germinal vesicle (GV) stage must accrue approximately 20 billion zinc ions in order to successfully mature to a metaphase II (MII) arrested egg. Further, once activated, the MII egg begins ejecting zinc, resulting in the loss of 10 billion zinc ions by the time the embryo reaches the two-cell stage. To determine the utility of the zinc in flux during this developmental progression, a bioinformatic approach was undertaken to determine which Zn-proteins are present at specific stages during the maturation process. In parallel with total intracellular zinc content, there was a rise and fall in the number of Zn-proteins present from a GV oocyte to an MII egg to two-cell embryo. This mouse oocyte Zn-proteome data was compared to the Zn-proteome in oocytes from three other species (*C.elegans*, Sea anemone, and Zebrafish) to determine how universal the Zn-proteome is within oocytes. Lastly, a radioactive zinc tracer was used to monitor zinc flux during the maturation process, which will be used to determine when specific metalloproteins obtain their metal cofactor in the developmental process.

Funding: Chemistry of Life Processes Institute Innovation Award

Abstract # P23

Notch signaling during gonadotropin-dependent follicle growth in the mouse ovary, R.D. Prasasya, K.E. Mayo, Department of Molecular Biosciences, Center for Reproductive Science, Northwestern University, Evanston, IL.

In mammals, growth and ovulation of the ovarian follicle requires integration of endocrine and paracrine signaling. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior pituitary serve as predominant endocrine cues, while within the ovary, numerous paracrine signals participate in folliculogenesis. Among these local factors, the juxtacrine signaling system Notch is emerging as an integral means of intra-follicular cell communication. Disruption of Notch signaling using pharmacological and genetic approaches in mice during the period of germ cell nest breakdown and follicle formation has been shown to result in aberrant follicle formation and development, as well as female subfertility. In this study we show that Notch signaling remains active in granulosa cells of multilayered, growing follicles. Using a superovulation protocol, we tested the effects of gonadotropins on ovarian Notch gene expression and activity. Using the Transgenic Notch Reporter (TNR) mice, which express eGFP in cells with active Notch signaling (from Dr. Nicholas Gaiano, Johns Hopkins University), activation of LH receptors by human chorionic gonadotropin (hCG) treatment caused increased expression of the eGFP reporter mRNA during the periovulatory period. This is consistent with our previous data showing positive regulation of several Notch signaling mRNAs by hCG treatment, suggesting the importance of Notch signaling during the gonadotropin dependent stages of folliculogenesis. Since the abundantly expressed Notch2 receptor is expressed in granulosa cells of larger follicles, a *Notch2*$^{flox/flox}$ mouse was crossed with a mouse expressing the *Cre recombinase* under the control of the *Cyp19a1* or *Aromatase* promoter (Cyp19-Cre) to obtain *Notch2* disruption in the granulosa cells of multilayer and gonadotropin responsive follicles (N2KO^{cyp}). These mice are being used to study the role of Notch signaling during this later stages of follicle development. Early characterization of this line showed significant down regulation of *Notch2* mRNA in both whole ovaries and isolated granulosa cells. Current efforts are focused on characterizing potential follicle and fertility phenotypes of this new N2KO^{cyp} line.

This work was supported by the Eunice Kennedy Shriver NICHD Program Project Grant (NIH P01 HD021921) and NIGMS Cellular and Molecular Basis of Disease Training Grant (NIH T32 GM08061).

Abstract # P24

Ovarian follicle interaction and maturation in a bioengineered 3D printed artificial ovary scaffold, Monica M. Laronda[1], Alexandra L. Rutz[2,3], Adam E. Jakus[2,4], Shuo Xiao[1], Kelly A. Whelan[1], Ramille N. Shah[2,4,5], Teresa K. Woodruff[1]**.** [1] Division of Reproductive Biology, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2] Simpson Querrey Institute for BioNanotechnology Northwestern University, Chicago, IL; [3] Department of Biomedical Engineering, Northwestern University, Evanston, IL; [4] Department of Materials Science and Engineering, Northwestern University, Evanston, IL; [5] Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL;

Iatrogenic cancer treatments, such as radiation and chemotherapies, can impair fertility and endocrine function. The field of oncofertility pursues options for preserving and restoring fertility in patients at risk of iatrogenic ovarian failure. Autotransplantation of cryopreserved ovarian cortical tissue has resulted in human live births, with short-term restoration of endocrine function. However, this technique carries the risk of reintroducing cancer cells since the tissue is removed prior to treatment. To minimize cancer transplant risk we bioengineered an artificial ovary from follicles and a 3D printed hydrogel scaffold modeled from the extracellular matrix (ECM) composition and architecture of decellularized bovine and human ovaries. The scaffolds were printed with gelatin in an intricate microporous pattern engineered to accommodate multi-layered secondary ovarian follicles. Scaffolds of different tortuous architectures and porosities were investigated. Ovarian follicles produced estradiol when cultured within the 3D printed scaffolds. Confocal microscopy revealed ovarian follicle contacts with the 3D printed struts. Follicles remained intact and survived at an increased rate in scaffolds that allowed for more contacts with struts. Contacts between somatic cells and oocytes and somatic cells and 3D printed struts were investigated. Secondary follicles matured and ovulated in response to human chorionic gonadotropin (hCG) within these scaffolds. These are the first steps in creating a safer artificial ovary that will provide both endocrine and gametic support to young women who have survived cancer.

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Abstract # P25

Evidence for zinc involvement in mouse ovarian follicle activation and oocyte growth, Ru Ya[1], Seth A Garwin[3], Stefan Vogt[2], Thomas V. O'Halloran[3], and Teresa K Woodruff[1,3], [1]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611 [2]X-ray Science Division, Argonne National Laboratory, Argonne, Illinois 60439 [3]The Chemistry of Life Processes Institute, Northwestern University, Evanston, Illinois.

Recent studies have shown that zinc regulates meiotic progression in the mammalian oocyte, however little work has been done on the accrual or functional role of zinc in follicle-enclosed oocytes. Here we quantified zinc levels in these earlier times of oocyte activation and growth and manipulated zinc activity through supplementation or chelation. Elemental analysis of mouse follicles was done by synchrotron-based X-ray fluorescence microscopy (XFM). Zinc is the most abundant transition metal in a cohort of 90-100μm primary follicles, with an average of ~70 billion atoms per follicle, which is more abundant than iron (~3-fold) and copper (~10-fold). We next examined whether zinc levels changed during oocyte growth. Oocytes were isolated from individual follicles and categorized by diameter then stained with a zinc sensor, ZincBY-1. Primordial oocytes (~15μm in diameter) showed strong zinc punctate staining surrounding the pronuclei. This staining pattern disappeared and became more homogenous in activated oocytes (~30μm in diameter). It is known that zinc exhibits relatively uniform cortical distribution in mature germinal vesicle (GV) stage oocytes (72μm); this distribution pattern was readily observed in ~60μm growing oocytes. Genes encoding twenty-four zinc transporters and four zinc-binding proteins known as metallothioneins (MTs) are present in the mouse genome and several have been assigned organ-specific roles in zinc homeostasis. Here we measured expression levels of all transporters and two MTs in isolated primordial follicles. Interestingly, the transcript levels of several zinc importers are higher than exporters in this follicle class; and, MT1 and 2 are both present. We next tested whether the zinc transporters are metal responsive by supplementing the media with zinc sulfate or by depleting zinc levels with a metal chelator, N, N, N′, N′-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN). Preliminary data show some regulation of these transcripts and additional studies are ongoing. To further test the effect of zinc on follicle activation, ovaries from 4-day old CD-1 mice were isolated and cultured *ex-vivo* with increasing doses of zinc sulfate or TPEN. Supplementation of zinc sulfate at 10 and 50μm concentrations significantly increased the number of activated primary follicles after four days of culture. In contrast, limiting zinc levels by TPEN addition did not show any stimulatory effect. To test whether addition of zinc released primordial follicles from dormancy, we measured phospho-AKT levels by immunohistochemistry. Consistent with the follicle activation data, there was an increase in pAKT levels in the zinc-treated group. Collectively, our preliminary data suggest that dynamic zinc uptake and redistribution occurs during oocyte growth, and that zinc stimulates follicle activation likely through the canonical AKT signaling pathway.

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Abstract # P26

Promotion of scrotal hernias by elevated aromatase: Involvement of Kisspeptin, Hong Zhao[1], Robert T Chatterton[1], Jeremy Zhang[1], Enze Jiang[1], Matthew Dyson[1], David Christopher Brooks[1], Francesco J. DeMayo[2] and Serdar E. Bulun[1], [1]Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2]Department of Molecular and Cellular Biology, Baylor College.

In the US, more than 1 in 4 men develop symptomatic inguinal hernias, and more than 600,000 inguinal hernia repair surgeries are performed annually. While, the underlying molecular mechanisms of inguinal hernias are currently unknown, exogenous estrogen induces scrotal hernia development in male mice, which we propose to serve as the counterpart of inguinal hernia in humans. To develop a more physiologically relevant source for estrogen, we have generated a humanized aromatase ($Arom^{hum}$) mouse model. Aromatase is a key enzyme for estrogen biosynthesis. We demonstrated that locally increased estrogen levels in the lower abdominal muscle tissue in $Arom^{hum}$ mice, but not circulating estrogen levels, lead to the development of scrotal hernias with nearly complete penetrance. The aromatase inhibitor letrozole completely reversed this phenotype. In both wild type (WT) and $Arom^{hum}$ mice, the expression of estrogen receptor α (ERα) mRNA and protein in skeletal muscle varied in different muscle groups, and was highest in the lower abdomen, modest in the upper abdomen, and lowest in thigh. Skeletal muscle tissue from the lower abdomen had significantly greater proportion of fibroblasts than the upper abdomen in all mice, and similarly all mice had a greater proportion of ERα positive fibroblasts in the lower abdomen than in upper abdomen. However, human aromatase expression resulted in a striking increase the total number of fibroblasts in the lower abdominal tissue relative to wild type. The percentage of Ki67-positive fibroblasts indicated proliferation was approximately 3-fold higher in the lower abdomen of $Arom^{hum}$ mice than that of WT mice and the upper abdominal muscle tissue of $Arom^{hum}$ mice. E2 stimulated incorporation of BrdU in primary cultured fibroblasts from WT mice with a maximum at 0.1 nM in lower abdominal muscle tissue and at 1.0 nM in upper abdominal muscle tissue, indicating higher estrogen sensitivity in fibroblasts of the lower abdomen. Fulvestrant (ICI) inhibited these responses. In the hypothalamus, kisspeptin is a functional mediator of ERα. Interestingly, a microarray analysis of mRNA revealed a high concentration of kisspeptin only in the lower abdominal muscle tissue of $Arom^{hum}$ mice, which was confirmed by real-time RT-PCR analysis. Kisspeptin staining was observed in fibroblasts, but not in muscle cells of both lower and upper abdomen. C2C12 myoblast differentiation was increased with E2 treatment as demonstrated by increased mRNA levels of *Myh2, Myog, Myomaker* and *CCKN1*. In conclusion, aromatase expression in the lower abdominal skeletal muscle tissue can cause scrotal hernias by increasing local estrogen production, which triggers the abnormal expansion of ERα positive fibroblasts and the improper differentiation of surrounding muscle possibly due to elevated kisspeptin expression.

34th Minisymposium on Reproductive Biology
List of Presenters

Amanda Bayer
O'Halloran lab
amandabayer2012@u.northwestern.edu

Michael Demczuk
Kipp lab
mmdemczuk@gmail.com

Sara Garcia-Moreno
Maatouk lab
sagm@u.northwestern.edu

Seth Garwin
O'Halloran lab
sethg@u.northwestern.edu

Nisan Hubbard
Mayo lab
nisanhubbard2012@u.northwestern.edu

Deborah Ikhena
Reproductive Endocrinology &
Infertility Fellow
d.e.ikhena@gmail.com

Margaux Kanis
Kim lab
margaux.kanis@northwestern.edu

James Kashanian
Urology Fellow
james.kashanian@northwestern.edu

So-Youn Kim
Woodruff lab
so-youn-kim@northwestern.edu

Amber Kofman
Payne lab
amberkofman2015@u.northwestern.edu

Monica Laronda
Woodruff lab
m-laronda@northwestern.edu

Irene Lee
Kim lab
irenelee2014@u.northwestern.edu

Kelly McKinnon
Woodruff & Getsios labs
kmckinnon@u.northwestern.edu

Andrew Nowakowski
O'Halloran lab
andrew.nowakowski@northwestern.edu

Pamela Monahan
Mayo lab
pamela-monahan@northwestern.edu

Molly Moravek
Reproductive Endocrinology &
Infertility Fellow
mollymoravek@gmail.com

Wenan Qiang
Women Cancer Xenograft Core
w-qiang@northwestern.edu

Sarah Persaud
Kipp lab
sap74@case.edu

Rexxi Prasasya
Mayo lab
rexxiprasasya2016@u.northwestern.edu

Rebecca Ursin
Kipp lab
rebecca.ursin@yahoo.com

Elizabeth Sefton
Woodruff lab
e-sefton@northwestern.edu

Marissa Steinberg
Obstetrics & Gynecology Resident
marissa.steinberg@northwestern.edu

Ru Ya
Woodruff lab
ru.ya@northwestern.edu

Vania Vidimar
Kim lab
vania.vidimar@northwestern.edu

Shuo Xiao
Woodruff lab
shuoxiao@northwestern.edu

Hong Zhao
Bulun lab
h-zhao@northwestern.edu

Ping Yin
Bulun lab
p-yin@northwestern.edu

Trainee Organizing Committee for the 34th Minisymposium on Reproductive Biology

Sevim Yildiz-Arslan
Postdoctoral Fellow
Julie Kim Laboratory
Division of Reproductive Science in Medicine
Department of Obstetrics and Gynecology
Feinberg School of Medicine

Lia Bernardi
Reproductive Endocrinology and Infertility Fellow
Serdar Bulun Laboratory
Department of Obstetrics & Gynecology
Feinberg School of Medicine

Nan Zhang
Postdoctoral Fellow
Teresa K. Woodruff Laboratory
Department of Obstetrics and Gynecology
Feinberg School of Medicine

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Northwestern University
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2205 Tech Drive, Hogan 2-171
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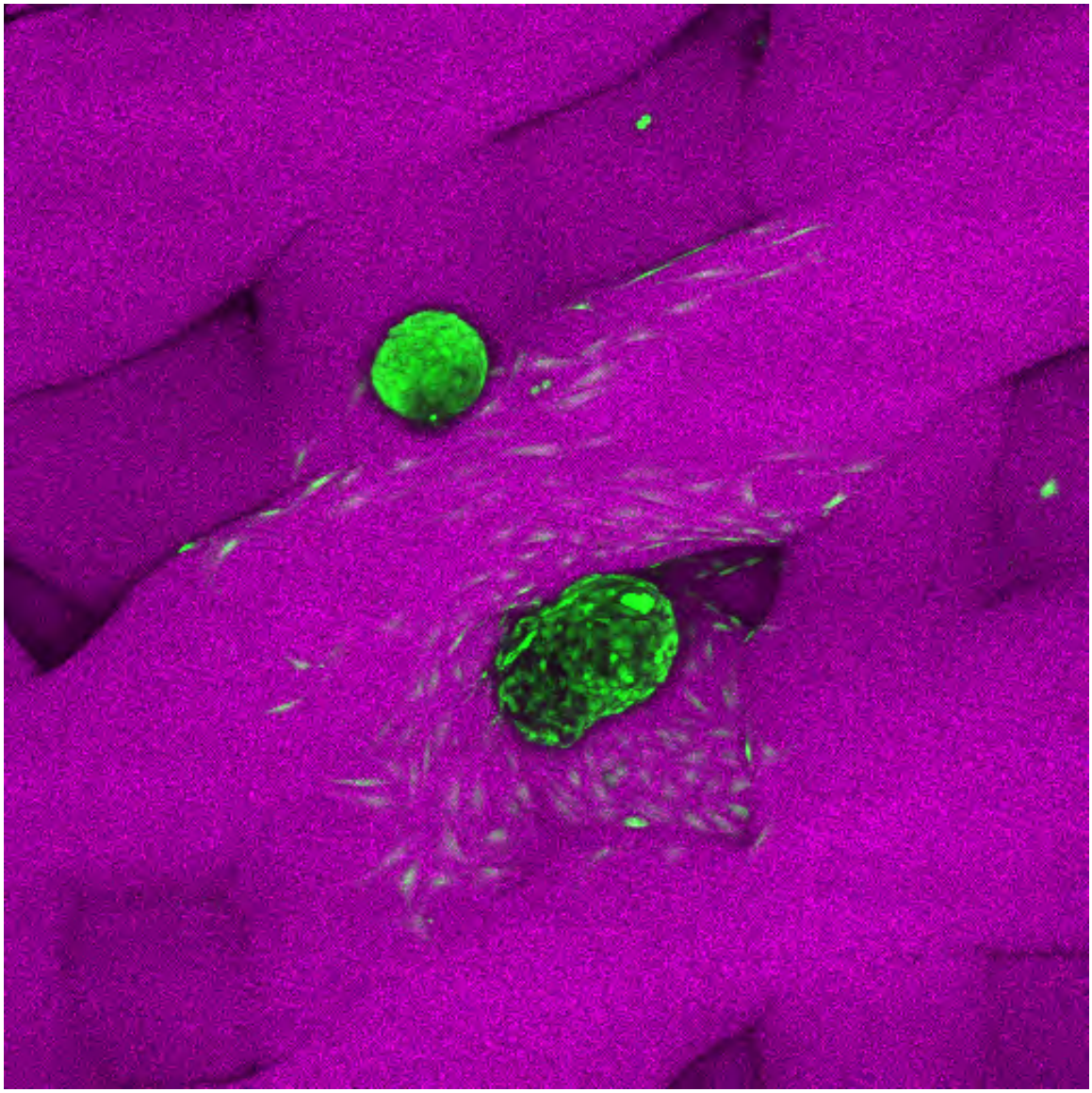

Photograph courtesy of Monica M. Laronda (Woodruff Lab) and Alexandra Rutz (Shah Lab).
Mouse follicles (green, calcein) and shed stromal cells nesting in a 3D printed gelatin scaffold (red, rhodamine-> false colored purple).