6th Annual Illinois Symposium on Reproductive Sciences
University of Illinois at Chicago

Welcome! This rotating annual statewide symposium provides an opportunity to celebrate our strong research and educational heritage, to promote the exchange of scientific information in the reproductive sciences, to facilitate the career development of the next generation of Illinois reproductive scientists, and to establish a promising future of reproductive sciences research in the state of Illinois. We hope to leverage our collective institutional strengths to maintain Illinois in a preeminent nationwide position in this critical research field.

“Interact, Collaborate, and Succeed” is our theme this year. Accordingly, this meeting includes a “Meet the professor” lunch. We hope that this event will foster the sense of community embracing all the biologists in reproductive science in Illinois. We encourage all the trainees to interact with the leaders in the field and with other trainees to discuss their research and look for common ground to plant the seed of future collaborations.

Finally, a special recognition to the students involved in the organization of this year’s ISRS meeting. The student committee members have worked professionally and effectively together to plan an outstanding meeting. Well done!

Upcoming Meeting

7th Annual Illinois Symposium on Reproductive Sciences
University of Illinois at Urbana-Champaign

Cover: Mouse ovary immunostained against GATA4 in brown and counterstained with Gill’s hematoxylin. Jill Bennett (UIC).
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Program Overview
7:00 Registration/Continental Breakfast
8:00 Opening Remarks
8:15 Oral Session I
9:15 Coffee Break
9:30 Oral Session II
10:30 Poster Session I (Odd Numbers)
11:30 UIC Alumni Speaker – Dr. Gail Prins
12:30 Meet the Professor Lunch
1:45 Poster Session II (Even Numbers)
2:45 Oral Session III
3:45 Wine and Cheese Reception
4:15 Keynote Address – Dr. Bruce Murphy
5:30 Presentation of Awards
5:45 Closing Remarks
Alumni Speaker

“Developmental Programming of the Prostate by Estrogens: Implications for Later-life Disease”

Gail S. Prins, PhD, is the Michael Reese Endowed Professor in the Departments of Urology, Physiology & Biophysics and Pathology at the University of Illinois at Chicago (UIC) and is Director of the University Andrology Laboratories and Co-Leader of the Prostate Cancer Working Group in the UIC Cancer Center. She obtained her PhD in Physiology from the University of Illinois Medical Center, Chicago in 1979 under the mentorship of Laurens Zaneveld, DVM, PhD where she studied sperm enzymes and sperm transport mechanisms in the male reproductive tract. She went on to complete an NIH postdoctoral fellowship in the Department of Urology and Center for Reproductive Sciences at Northwestern University Medical School where she worked on prolactin regulation of prostate growth and function which began her life-long career focus in prostate biology and prostate cancer research. In 1983, Dr. Prins joined the faculty at Michael Reese Hospital & Medical Center as Assistant Professor of Obstetrics & Gynecology, University of Chicago. As the director of the In-Vitro Fertilization Laboratory, she worked with Dr. Antonio Scommegna to establish the state’s first IVF program and was successful in obtaining the first pregnancies and live births in Illinois using this new, cutting edge technology. She simultaneously built an active basic research program in prostate carcinogenesis, a clinical andrology laboratory, established the region’s first sperm banking program and directed the reproductive endocrinology laboratories at Michael Reese Hospital from 1983-1995. Dr. Prins joined the Department of Urology faculty at the University of Illinois at Chicago in 1996, moving her research team and clinical andrology laboratory to the UIC campus where she rose through the ranks to her current position.

Dr. Prins has maintained two active and highly successful research programs since the 1980s. Her translational research on human sperm cryopreservation led to the development of an optimal sperm freezing system widely used throughout the globe for both donor and surgically retrieved patient sperm samples. Her basic research program, continuously funded by the NIH for the past 25 years, is focused on prostate gland development, prostate stem cells, hormonal carcinogenesis, endocrine disrupting chemicals and the fetal basis of adult prostate disease. Her work has established that early life exposures to natural estrogens or chemicals such as bisphenol A permanently reprogram the prostate and increase its susceptibility to cancer with aging, using both rodent and human models. She has gone on to identify the molecular basis for altered prostate memory which includes epigenetic reprogramming of prostate stem cells. Her lab is currently utilizing novel models with human embryonic stem cells and human prostate stem/progenitor cells to dissect estrogen and EDC reprogramming in derived human tissues.

Dr. Prins is widely acclaimed for her research discoveries and is regularly invited as speaker at national and international scientific meetings on these topics. She has authored over 160 peer-reviewed manuscripts in addition to multiple book chapters and position papers and currently serves as principal investigator of three NIH grants as well as co-investigator of several other grants with fellow UIC faculty members. She is currently an Editor of Endocrinology, Associate Editor of Andrology and Editorial Board member on Hormones & Cancer, Steroids, Prostate, and Reproductive Toxicology. She has served on multiple scientific advisory panels including several NIH Study Sections, the DoD Integration Panel for Prostate Cancer Research Program, the NIEHS External Scientific Review Committee and is Past-Treasurer and Past-President of the American Society of Andrology (ASA). Dr. Prins is currently the Vice President and President-Elect of the Society for Basic Urologic Research. She is the recipient of multiple awards including the Distinguished Service Award from the American Society of Andrology (2001), the Excellence in Urologic Research Award from the Society for Basic Urologic Research (2009), the UIC Researcher of the Year Award in Basic Life Sciences (2011) and the Distinguished Andrologist Award from the ASA (2014).

It is a great pleasure to have Dr. Gail Prins as our Alumni Speaker at the 2014 ISRS.
Keynote Speaker

“The orphan nuclear receptor that rules reproduction”

Dr. Bruce D. Murphy, Ph.D. is currently the Director of the Center of Research in Animal Reproduction at the University of Montreal and holds a joint appointment in the Department of Obstetrics and Gynecology, at the same institution. He is also Adjunct professor at McGill University and Dalhousie University, and he has held visiting appointments at Cornell University and at the Institute of Genetics, Cellular, and Molecular Biology at Louis Pasteur University in France. Dr. Murphy received his B.Sc. degree in Biology in 1965, and his M.Sc. in Physiology in 1969, both from Colorado State University. He then obtained his Ph.D. in Reproductive Biology in 1973 at the University of Saskatchewan. Dr. Bruce D. Murphy is the Vice President (2014-2015) and President Elect (2015-2016) of the Society for the Study of Reproduction. Dr. Murphy was Editor-in-Chief of Biology of Reproduction in 2012/13 and serves or has served on editorial boards of several journals. He has been continuously serving on grant funding committees, including Canadian MRC Endocrinology, NSERC Strategic Research, NSERC Animal Biology, NIH REB, CIMR (ad hoc) Study Sections, and special NIH panels.

Dr. Murphy has been continuously funded for studies of embryo implantation, ovarian function and steroidogenesis. He has conducted research in two primary areas of reproductive biology: obligate delayed implantation and ovarian cell differentiation. His work has made a substantial impact in both fields. Dr. Murphy’s pioneering studies demonstrated the mechanisms of photoperiod influence on termination of embryonic diapause via inhibition of melatonin and consequent induction of prolactin for reactivating the corpus luteum. His group has developed culture systems allowing for the in vitro escape from embryonic diapause, and carnivore trophoblast stem cell lines to examine mechanisms of proliferation, differentiation, and invasion. His group described the role of pituitary hormones in lipoprotein utilization and has extensively studied the expression of cholesterol trafficking genes. Most recently, Dr. Murphy lab showed that the orphan nuclear receptor liver receptor homolog 1 (LRH-1) wires the postovulatory rise in progesterone production to progesterone-dependent preparation of the endometrium for pregnancy and that the lack of LRH-1 activity in either the ovary or uterus has catastrophic consequences for reproduction in mice. This study appeared in Nature Medicine. Dr. Murphy has more than 200 publications in refereed journals and has contributed to numerous book chapters, including chapters in recent editions of The Endometrium and The Ovary.

Dr. Murphy founded the Reproductive Biology Research Unit at the University of Saskatchewan. He is a founding member and was president of the Canada West Society of Reproductive Biology. Dr. Murphy also works extensively in international development of reproductive research especially in South and Central America and Africa, where he lectures, has established laboratories, and mentors research programs. He appears frequently on the programs of international congresses and his studies on carnivores led to invitations to speak at conferences and workshops on endangered species. Dr. Murphy cooperates and interacts with scientists professionally and compassionately. He supplies reagents and technical information to numerous colleagues. In recognition of his continued contributions, Dr. Murphy has been elected as a Fellow of the Canadian Academy of Health Sciences.

Dr. Murphy has received an extraordinary number of awards. Most recently he received the SSR Trainee Mentoring Award, an award that recognizes consistent support and guidance to trainees that far exceeds the basic responsibilities required of an academic advisor, and the Club de Recherches Cliniques du Québec, Prix du Mentor Scientifique, both in 2014. Dr. Murphy has supervised 21 Ph.D. and 23 M.Sc. students and directed 17 postdoctoral fellows. It is not surprising that many of his former trainees hold faculty positions throughout the world. He also holds awards for Excellence in Reproductive Medicine, Canadian Fertility and Andrology Society, and Career Achievement from the International Symposium on Animal Biology of Reproduction. He was also the recipient of the 2007 SSR Distinguished Service Award, which recognizes an active, regular member of the SSR who as a mentor has had a significant impact on trainee’s lives.

We are honored to have Dr. Bruce Murphy as the Keynote Speaker at the 2014 ISRS.
PROGRAM

7:00  REGISTRATION OPENS
      Continental Breakfast
      Poster Set-Up
      Check Presentation Slides

8:00  OPENING REMARKS:
      Dr. Carlos Stocco, Ph.D., University of Illinois at Chicago

8:15  ORAL SESSION I: Folliculogenesis: From the Pituitary to the Ovary
      Session Moderators: Bella Salamone and James Kashanian, MD, Northwestern University

8:15  T1. RBPJ-κ DEPENDENT NOTCH SIGNALING REGULATION OF ARCUATE
      KISSPEPTIN NEURON DEVELOPMENT. Matthew J Biehl¹, Lori T Raetzman¹
      ¹Department of Molecular & Integrative Physiology, University of Illinois at Urbana-Champaign

8:30  T2. MONO(2-ETHYLHEXYL) PHTHALATE, BUT NOT DI(2-ETHYLHEXYL)
      PHTHALATE, DIRECTLY ALTERS FACTORS WITHIN THE
      PHOSPHATIDYLINOSITOL 3-KINASE SIGNALING PATHWAY TO
      ACCELERATE EARLY FOLLICULGENESIS IN MICE. Patrick R. Hannon and
      Jodi A. Flaws. Department of Comparative Biosciences, University of Illinois, Urbana, IL.

8:45  T3. FOLLICLE STIMULATING HORMONE (FSH)-REGULATED IGF2
      EXPRESSION IS CRITICAL FOR HUMAN GRANULOSA CELL
      DIFFERENTIATION. Sarah C. Baumgarten¹, Scott M. Convissar¹, Michelle A. Fiero²,
      Nicola J. Winston², A. Musa Zamah², Bert Scoccia², and Carlos Stocco¹;¹Department of
      Physiology and Biophysics, ²Division of Reproductive Endocrinology and Infertility,
      Department of Obstetrics and Gynecology, University of Illinois College of Medicine

9:00  T4. IRX3 AND IRX5 PROMOTE SOMATIC CELL – GERM CELL
      COMMUNICATION TO ENSURE OOCYTE SURVIVAL AND FACILITATE
      FOLLICLE MATURATION. A. Fu¹, C. Holdreith¹, C. Kpegba¹, C.C. Hui² and J.S.
      Jorgensen¹. ¹Department of Comparative Biosciences, School of Veterinary Medicine,
      University of Wisconsin – Madison, Madison, WI. ²Program in Developmental & Stem Cell
      Biology, The Hospital for Sick Children, and Department of Molecular Genetics, University
      of Toronto, Toronto, ON, Canada.

9:15  COFFEE BREAK
9:30  ORAL SESSION II: Emerging Technologies for Reproductive Research
Session Moderators: Malavika Adur and Sarah Steinmann, University of Illinois at Urbana Champaign

9:30  T5. THE ROLE OF INTRACELLULAR ZINC FLUCTUATIONS DURING FERTILIZATION. Seth A. Garwin1,2, Emily L. Que1,2, Teresa K. Woodruff3,3, Thomas V. O’Halloran1,2. 1The Chemistry of Life Processes Institute, Northwestern University, Evanston, IL; 2The Department of Chemistry, Northwestern University, Evanston, IL; 3Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

9:45  T6. RE-LOCALIZATION OF SYNTAXIN IN MOUSE SPERM PRIOR TO THE ACROSOME REACTION. Sharif M1, Silva E1, Shah S1, Sivaguru M2, and Miller DJ1. Department of Animal Sciences1, Institute of genomic biology2, University of Illinois Urbana- Champaign, IL.

10:00  T7. MICROFLUIDIC PLATFORMS FACILITATE PRIMARY FETAL LEYDIG CELL CULTURE AND INVESTIGATIONS INTO THEIR RESPONSE TO ENDOCRINE DISRUPTORS. Jessica L. Muszynski1, Lindsay N. Strotman2, Dörte Döpfer3, David J. Beebe2, Ashleigh B. Theberge2, Joan S. Jorgensen1. Departments of 1Comparative Biosciences, 2Biomedical Engineering, and 3Medical Sciences, University of Wisconsin-Madison, Wisconsin, USA.

10:15  T8. ENGINEERED ENDOCRINE ORGAN TRANSPLANT UTILIZING A DECELLULARIZED OVARY SCAFFOLD. Monica M. Laronda1, Adam E. Jakus2,3, Kelly A. Whelan1, Ramille N. Shah2,3,4, Teresa K. Woodruff1. 1Division of Reproductive Biology, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; 2Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, IL; 3Department of Materials Science and Engineering, Northwestern University, Evanston, IL; 4Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL

10:30  POSTER SESSION I:
Odd Numbered Posters

11:30  ALUMNI SPEAKER:
Dr. Gail Prins, Ph.D.
"Developmental Programming of the Prostate by Estrogens: Implications for Later-life Disease"
Introduced by Dimple A. Modi, PhD Student, University of Illinois at Chicago

12:30  LUNCH BREAK: “Meet the Professor Lunch”
1:45 **POSTER SESSION II:**
Even Numbered Posters

2:45 **ORAL SESSION III: Understanding Reproductive Diseases**
*Session Moderators: Emily Isgur and Lan Hai, Southern Illinois University*

2:45 **T9. THE PRO-INFLAMMATORY CYTOKINE INTERLEUKIN-17 MAY PLAY A CRITICAL ROLE IN ESTABLISHMENT OF ENDOMETRIOTIC LESIONS.**
Malavika K. Adur, Jacqueline S. Juna, Romana A. Nowak. *University of Illinois at Urbana-Champaign, Illinois.*

3:00 **T10. INDUCTION OF THE DETOXIFICATION ENZYME NAD(P)H:QUINONE OXIDOREDUCTASE 1 (NQO1) BY LICORICE SPECIES USED IN BOTANICAL DIETARY SUPPLEMENTS FOR WOMEN'S HEALTH.**
Atieh Hajirahimkhan, Charlotte Simmler, Huali Dong, Dan Lantvit, Guannan Li, Shao-Nong Chen, Dejan Nikolic, Birgit M. Dietz, Guido F. Pauli, Richard B. van Breemen, Judy L. Bolton. *UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL.*

3:15 **T11. HIGH INCIDENCE OF TRIPLE NEGATIVE BREAST CANCERS FOLLOWING PREGNANCY AND THE ASSOCIATED GENE EXPRESSION SIGNATURE.**
Thao N.D. Pham¹, Szilard Asztalos¹, Peter H. Gann², Meghan K. Hayes¹, Ryan Deaton², Elizabeth L. Wiley², Rajyasree Emmadi², Andre Kajdacsi-Balla², Nilanjana Banerji³, William McDonald³, Seema A. Khan³, Debra A. Tonetti¹. ¹Department of Biopharmaceutical Sciences, ²Department of Pathology, ³Center for Clinical and Translational Science, University of Illinois at Chicago, Chicago, IL; ⁵Allina Hospitals & Clinics, Minneapolis, MN; ⁶Department of Surgery, Northwestern Feinberg School of Medicine, Chicago, IL.

3:30 **T12. THE ROLE OF PAX8 IN SEROUS OVARIAN CANCER.**
Ó hAinmhire, E.¹, Rodgers, L.H.¹, Eddie, S.L.¹, Quartuccio, S.M¹. Moyle-Heyrman, G.¹, Burdette, J.E¹. ¹Center for Pharmaceutical Biotechnology, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL

3:45 **WINE AND CHEESE RECEPTION**
❄ Please take down posters by 4:00 ❄

4:15 **KEYNOTE ADDRESS:**
Dr. Bruce Murphy
“The orphan nuclear receptor that rules reproduction”
Introduced by Sarah Baumgarten, MD/PhD Student, *University of Illinois at Chicago*

5:30 **AWARDS PRESENTATION**

5:45 **CLOSING REMARKS**
Scott Convissar, PhD Student, *University of Illinois at Chicago.*
Abstracts:

T1. RBPJ-κ DEPENDENT NOTCH SIGNALING REGULATION OF ARCULATE KISSPEPTIN NEURON DEVELOPMENT. Matthew J Biehl, Lori T Raetzman. Department of Molecular & Integrative Physiology, University of Illinois at Urbana-Champaign

The mammalian hypothalamic-pituitary-gonadal axis is a critical neuroendocrine feedback system involved in the onset of puberty and successful reproductive function later in an organism’s life. Kisspeptin neurons reside within the arcuate nucleus (Arc) of the hypothalamus and regulate the action of other the other hypothalamic neuronal population involved in reproduction, gonadotropin-releasing hormone (GnRH) neurons. Despite an understanding of the function of Kisspeptin neurons, the mechanism by which they are specified from a hypothalamic progenitor still remains unexplored.

We have previously shown that the Notch signaling pathway plays an important role in cell fate within the Arc of mice. Active Notch signaling prevented neural progenitors within the Arc from adopting the mature fate of feeding circuit neurons including Proopiomelanocortin (Pomc) and Neuropeptide Y (NPY), whereas conditional loss of Notch signaling lead to a premature differentiation of these neural subtypes within the Arc. In our current study, we hypothesized that Kisspeptin expressing neurons of the Arc would similarly be affected by Notch manipulation and would arise from a Pomc expressing lineage in a similar progression as NPY neurons. To address our hypotheses, we utilized mice with a conditional deletion of Rbpj-κ, an essential co-factor of active Notch signaling (Rbpj cKO) or mice persistently expressing the Notch1 intracellular domain (NICD tg) within Nkx2.1 expressing cells of the developing ventral hypothalamus. Interestingly, we have found that in both mouse models, a lack of Kisspeptin/Kiss1 expression is observed. These data would suggest that Notch signaling must be extinguished in order for neural differentiation to occur within the Arc, however Notch signaling is also necessary for the development of Kisspeptin neurons. To determine if Kisspeptin neurons of the Arc differentiate through a Pomc expressing intermediate, we utilized a genetic lineage tracing model expressing the tdTomato fluorescent protein in all cells which have expressed Pomc. We observed that some Kisspeptin expressing neurons labeled with our Pomc reporter, suggesting that at least a subset arose from a common, intermediate progenitor. Taken together, our findings suggest that active Notch signaling is an important molecular switch involved in instructing subpopulations of progenitor cells to differentiate into Kisspeptin neurons.

Kiss1 expression has been shown to be vital to sexual maturation and reproductive function. We then hypothesized that our Rbpj cKO mice lacking Kiss1 neurons in the Arc would show some degree of reproductive dysfunction that would persist into adulthood. Therefore, we assessed onset of puberty and reproductive success in both male and female mice. Our data has shown that male, but not female, mice show a delay in the onset of puberty. Both sexes show significantly reduced gonadal size at 8 weeks of age and have impaired fertility persisting into adulthood. Taken all together, these data suggest that the Notch signaling pathway plays an important role in the development of Arc Kiss1 neurons and appears necessary for successful reproductive function. Supported by R01 DK076647 and T32 ES007326.
Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer present in consumer, medical, and building products. DEHP is a ubiquitous toxicant to which humans are exposed daily via ingestion, inhalation, and dermal contact, representing a public health concern. Large doses of DEHP have been shown to disrupt normal ovarian function; however, the effects of DEHP at environmentally relevant levels and the mechanisms by which DEHP disrupts ovarian function are unclear. Our lab has shown that in mice, 10 and 30 day exposure to DEHP accelerates primordial follicle recruitment to the primary stage of development, and that this acceleration is a direct effect by mono(2-ethylhexyl) phthalate (MEHP), the bioactive metabolite of DEHP. Immature follicles rely on proper regulation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway for primordial follicle survival, quiescence, and activation of folliculogenesis so that overactivation of PI3K signaling leads to accelerated primordial follicle recruitment. Both pAKT, a stimulatory factor, and PTEN, an inhibitory factor, are two integral PI3K signaling proteins associated with early folliculogenesis. Thus, we tested the hypothesis that DEHP in vivo and MEHP in vitro increases protein levels of pAKT and decreases protein levels of PTEN to promote accelerated primordial follicle recruitment. To test this hypothesis, CD-1 mice (post-natal day 39) were orally dosed with corn oil (vehicle control) or DEHP (20µg/kg/day-750mg/kg/day) daily for 10 days. The ovaries were subjected to immunohistochemistry (IHC), and the percentage of positive staining in the whole ovary and the percentage of positively stained primordial and primary follicles for pAKT and PTEN were calculated and compared across treatment groups (n=3/group). In some experiments, ovaries from CD-1 pups (post-natal day 4) were cultured on filter paper suspended in media containing dimethylsulfoxide (DMSO; vehicle control), DEHP (0.2-20 µg/ml), or MEHP (0.2-20 µg/ml). After 6 days of culture, the ovaries were subjected to the same IHC methods described above. In the in vivo experiments, DEHP increased pAKT and decreased PTEN levels in the whole ovary compared to controls (pAKT: vehicle=1.7±0.2; 20µg/kg=5.3±0.9; 200µg/kg=6.7±2.0; 20mg/kg=7.8±3.4; 750mg/kg=7.6±1.6%; PTEN: vehicle=5.6±0.5; 20µg/kg=1.8±0.3; 200µg/kg=2.0±0.3; 20mg/kg=1.9±0.4; 750mg/kg=0.4±0.1%). Additionally, DEHP increased pAKT levels in primordial and primary follicles compared to controls (primordial: vehicle=2.8±0.5; 20µg/kg=10.4±2.6; 200µg/kg=8.8±2.3; 20mg/kg=7.2±0.7; 750mg/kg=8.3±1.4%; primary: vehicle=12.9±0.6; 20µg/kg=28.9±2.4; 200µg/kg=17.8±0.7; 20mg/kg=24.4±3.0%). In the in vitro experiments, MEHP decreased PTEN levels in the whole ovary compared to controls (DMSO=3.5±1.0; 2µg/ml=0.8±0.4; 20µg/ml=0.7±0.3%). Additionally, MEHP increased pAKT and decreased PTEN levels in primordial follicles compared to controls (pAKT: DMSO=23.7±2.5; 0.2µg/ml=50.7±2.3; 2µg/ml=52.1±2.7; 20µg/ml=49.7±2.1%; PTEN: DMSO=55.4±2.6; 0.2µg/ml=29.1±1.0; 2µg/ml=32.7±4.3; 20µg/ml=26.2±3.7%). Interestingly, DEHP did not alter the levels of pAKT and PTEN compared to controls in vitro. Collectively, these data suggest that DEHP, through MEHP, directly alters factors within the PI3K signaling pathway to promote an environment conducive for the acceleration of primordial follicle recruitment. Supported by R01ES019178 and an Environmental Toxicology Fellowship.
Infertility affects more than 7% of married couples in the United States, imposing significant physical, emotional, and financial burdens on these couples. Approximately 65% of these cases are attributed to female factors of infertility, including ovulatory failure, or the inability to produce and/or ovulate mature oocytes for fertilization. In women that seek Assisted Reproductive Technologies, such as *in vitro* fertilization (IVF), anovulation is often overcome by the administration of follicle stimulating hormone (FSH) to induce the development of the oocyte-containing ovarian follicles. FSH promotes follicle development from the pre-antral to the pre-ovulatory stage, inducing differentiation of granulosa cells into mural granulosa cells that line the wall of the follicle and cumulus cells that immediately surround the oocyte. Previous studies have indicated that other factors, such as insulin-like growth factors (IGFs), cooperate with FSH to promote the differentiation of the mural granulosa cells; however, we recently demonstrated that IGF action does not merely enhance FSH-induced differentiation, but is actually required for FSH-induced granulosa cell differentiation. The goal of the current study was to examine the relationship between FSH and IGFs in human granulosa cell differentiation. We collected cumulus granulosa cells from patients undergoing IVF at the University of Illinois Hospital and cultured them in serum-free, phenol red-free media. We found that cumulus granulosa cells express IGF2, as well as the IGF1 receptor (IGF1R) and IGF2 receptor; however, in contrast to rodents, human granulosa cells do not express IGF1. In human granulosa cells we observe that IGF2 administration, alone, upregulates aromatase expression and that IGF2 works synergistically with FSH to stimulate aromatase expression above that of either factor alone. Moreover, FSH stimulated IGF-2 expression (4-fold) through the P3 and P4 promoters in a dose- and time-dependent manner. We next sought to determine the mechanisms by which FSH regulates IGF2 expression in human granulosa cells. Cumulus granulosa cells were cultured and treated with FSH for 1h. Total protein was isolated from these cells and applied to a Human Phospho-kinase Array to simultaneously detect the phosphorylation state of 43 different kinases. We found that FSH stimulated the phosphorylation of ERK1/2 (5-fold), AKT (3-fold), and CREB (2-fold), and confirmed these findings by Western blot. To determine which of these cellular signaling pathways were necessary for the stimulation of IGF2 expression by FSH, cumulus cells were treated with U0126, MK2206, and H89 to inhibit the activity of ERK1/2, AKT, and PKA, respectively, before treatment with FSH. FSH-induced IGF2 expression was abolished in cells treated with MK2206 or H89, suggesting that AKT activity and PKA activity are critical for FSH to regulate IGF2 expression. U0126 had no effect on the ability of FSH to enhance IGF2 expression, indicating that ERK1/2 activation by FSH does not play a role in IGF2 expression. Taken together, our findings illustrate an interesting relationship between gonadotropins and IGFs in the ovary, where FSH stimulates the expression of IGF2, which, in turn, enhances FSH-induced differentiation in human granulosa cells. We propose that the interaction between the endocrine effects of FSH and the autocrine actions of IGF2 may play an essential role in the process of follicle selection and follicle dominance in humans. This work was supported by NIH grant number R01HD057110 (COS); SCB and SMC are supported by NIH training grant number T32HL07692.
T4. IRX3 AND IRX5 PROMOTE SOMATIC CELL – GERM CELL COMMUNICATION TO ENSURE OOCYTE SURVIVAL AND FACILITATE FOLLICLE MATURATION. A. Fu¹, C. Holdreith¹, C. Kpegba¹, C.C. Hui² and J.S. Jorgensen¹. ¹Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin – Madison, Madison, WI. ²Program in Developmental & Stem Cell Biology, The Hospital for Sick Children, and Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Follicle development and maturation within the ovary depend on intimate communications between the germ cell and its surrounding somatic cells. Our previous results using the Fused Toes (Ft) mutant mouse model, which contains a deletion of Fts, Fto, Ftm, Irx3, Irx5 and Irx6, showed disrupted oocyte – granulosa cell contacts leading to oocyte and follicle death. Among the genes in the Ft locus, however, only Irx3 and Irx5 exhibited ovary specific expression upon comparison of male versus female transcripts during gonad development. Thus, we hypothesized that Irx3 and Irx5 are critical for coordinating germ cell – somatic cell communications underlying oocyte and follicle survival. Real-time qPCR analysis showed that Irx3 and Irx5 had similar expression patterns during ovary development as their transcripts increased during germline cyst formation and peaked around birth when germline cysts broke down to form primordial follicles. Shortly thereafter, their expression diminished and remained at low levels throughout adulthood. Immunohistochemistry (IHC) analyses on ovaries at embryonic days E13.5, E15.5 and postnatal days P0 and P2 showed that IRX3 and IRX5 were co-localized to somatic cells during development, and then were detected in both germ cells and somatic cells around birth. Their expression decreased first in somatic cells of established primordial follicles, but was maintained for a few more days in germ cells. To evaluate the role of Irx3 and Irx5 during ovary development, we used a double knockout mouse model, Irx3/Irx5DKO (or Irx3/5 DKO). This mutation is embryonic lethal at E13.5; therefore, we used kidney capsule transplantation (KCT) of ovaries to facilitate further development equivalent to P0, P3, P7, and P14. Histological and transmission electron micrograph images of KCT ovary grafts showed that Irx3/5 DKO follicles developed abnormal granulosa cell morphology, gaps between germ and somatic cells, and oocyte death similar to that seen in the Ft mutant model at P7 and P14. We investigated whether Irx3 and Irx5 contribute to cell-cell communication pathways including gap junctions. Preliminary data showed that Irx3/5 DKO ovaries exhibited altered RNA transcript levels and IHC expression patterns of GJA1 (CX43) and GJA4 (CX37) when compared to wild type controls. Together, our results indicate that Irx3 and Irx5 work together in the ovary during follicle development to promote effective communication pathways between the oocyte and nascent granulosa cells to ensure oocyte survival and proper follicle maturation. Supported by NIH-R01HD075079 (JSJ).
T5. THE ROLE OF INTRACELLULAR ZINC FLUCTUATIONS DURING FERTILIZATION. Seth A. Garwin¹,², Emily L. Que¹,², Teresa K. Woodruff¹,³, Thomas V. O'Halloran¹,². ¹The Chemistry of Life Processes Institute, Northwestern University, Evanston, IL; ²The Department of Chemistry, Northwestern University, Evanston, IL; ³Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Initial work in the O'Halloran and Woodruff labs analyzing the zinc content of mammalian oocytes demonstrated that the mouse oocyte must acquire ca. 20 billion zinc atoms during meiotic maturation. Furthermore, upon fertilization it was observed that the egg undergoes periodic zinc exocytosis events termed “zinc sparks,” which involves the loss of 12-15 billion zinc atoms and coincide with intracellular calcium oscillations. Furthermore, utilizing a recently synthesized zinc probe, ZincBY-1, we found an inward flux of intracellular zinc termed the “zinc wave,” which happen concurrently with the zinc spark at the time of egg activation. In contrast to the “zinc spark,” which quickly diffuses away from the activated egg, the “zinc wave” persists and diffuses throughout the egg at a much slower rate.

By limiting the extracellular zinc availability during a narrow window time frame during activation, thereby preventing the “zinc waves,” we have shown that lower zinc availability negatively impacts blastocyst development. However, there are still many questions that remain as to the origin and mechanism behind the intracellular zinc fluctuations. Existing and newly invented cell-permeable, small molecules have been deployed to manipulate and monitor zinc in the female gamete; however, they act both the intracellular and extracellular space and are not targetable to particular regions inside a cell. Thus, we do not know if “zinc wave” results from extracellular zinc; nor do we know if it is contained within vesicles or freely 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 understanding intracellular zinc fluctuations and the development of a zinc nanosensor to further probe them. This work was supported by the W. M. Keck Foundation and National Institutes of Health Grants P01HD021921 and U54CA143869.
T6. RE-LOCALIZATION OF SYNTAXIN IN MOUSE SPERM PRIOR TO THE ACROSOME REACTION. Sharif M\textsuperscript{1}, Silva E\textsuperscript{1}, Shah S\textsuperscript{1}, Sivaguru M\textsuperscript{2}, and Miller DJ\textsuperscript{1}. Department of Animal Sciences\textsuperscript{1}, Institute of genomic biology\textsuperscript{2}, University of Illinois Urbana-Champaign, IL.

The principal role of SNARE proteins is to arbitrate vesicle fusion to a target membrane. Formation of tripartite SNARE protein complexes between SNARE proteins on opposing membranes is the minimal requirement for membrane fusion. The SNARE protein family is large, consisting of more than 60 members. One member of the SNARE family, syntaxin, is found on the sperm plasma membrane. During the sperm acrosome reaction, the outer acrosomal membrane fuses at hundreds of points with the overlying plasma membrane, resulting in release of the acrosomal contents. We hypothesize that syntaxin re-localizes within the sperm plasma membrane prior to the acrosome reaction to form SNARE complexes and promote membrane fusion. Immunofluorescence was used to localize syntaxin in mouse epididymal sperm before and after capacitation. Sperm were fixed using 4% paraformaldehyde, permeabilized with 0.05% triton X-100, blocked and incubated with a syntaxin antibody and Alexa 488 secondary antibody. Super resolution Structured Illumination Microscopy (SR-SIM) was used at time points 0, 10, 30, 60, and 120 min, to obtain 3D images of syntaxin localization. Quantification of the images was completed using IMARIS, which interpreted the total syntaxin-positive volume. Results show that syntaxin was found in a punctate pattern in the sperm head. The syntaxin-positive volume remained the same in capacitated and non-capacitated sperm but the location of syntaxin after capacitation was more restricted to the plasma membrane overlying the acrosome rather than over the entire head. The effect of bicarbonate and BSA, agents necessary in the medium for capacitation, was also investigated. Bicarbonate, which activates soluble adenylate cyclase, was not necessary for re-localization of syntaxin. On the other hand, BSA, which promotes cholesterol efflux, was required for syntaxin re-localization. Our results demonstrate that syntaxin shifts during the plasma membrane modifications that occur in sperm during capacitation and identify pathways that may regulate syntaxin localization and function, membrane fusion and the acrosome reaction. Supported by COMSATS Institute of Information and technology, Pakistan.
T7. MICROFLUIDIC PLATFORMS FACILITATE PRIMARY FETAL LEYDIG CELL CULTURE AND INVESTIGATIONS INTO THEIR RESPONSE TO ENDOCRINE DISRUPTORS. Jessica L. Muszynski\textsuperscript{1}, Lindsay N. Strotman\textsuperscript{2}, Dörte Döpfer\textsuperscript{3}, David J. Beebe\textsuperscript{2}, Ashleigh B. Theberge\textsuperscript{2}, Joan S. Jorgensen\textsuperscript{1}. Departments of \textsuperscript{1}Comparative Biosciences, \textsuperscript{2}Biomedical Engineering, and \textsuperscript{3}Medical Sciences, University of Wisconsin-Madison, Wisconsin, USA.

The development of the masculine fetus is a dynamic process that depends on the production of androgens by testicular fetal Leydig cells. Complete disruption of androgenic activity results in feminized external genitalia and brain, and nonfunctional internal genitalia. Although such extreme cases are rare, some of the most common birth defects are caused by subtle deficiencies in masculinization, including hypospadias and cryptorchidism. The incidence of these birth defects has been increasing in developed countries, suggesting a link to environmental toxins. The study of fetal Leydig cell biology is critical to understand the processes that surround fetal androgen production, but advances have been limited by factors including their small population size, the lack of appropriate cell lines, and their rapidly changing biology during development. We hypothesized that incorporation of microfluidic culture platforms would allow us to overcome these barriers to produce functional microcultures of primary fetal Leydig cells. Double reporter mT/mG mice were bred to Cyp11a1-Cre mice to achieve fluorescent green protein (mG+) labeled fetal Leydig cells. The mG+ fetal Leydig cell identity was validated with double-label immunohistochemistry and qPCR from cells sorted using fluorescent activated cell sorting (FACS). Microfluidic culture devices were employed to measure androstenedione and testosterone production of fetal Leydig cells that were cultured in 1) cell-cell contact within a mixed population 2) in isolation (mG+) but with media contact via compartmentalized co-culture with other testicular cells, or 3) in isolation (mG+). Results showed that fetal Leydig cells maintained their identity and steroidogenic activity for 3-5 days in each paradigm of primary culture. As expected, isolated fetal Leydig cells produced only androstenedione whereas mixed and co-cultures produced both androstenedione and testosterone. Next, we tested whether this culture system could be used to evaluate fetal Leydig cell function upon exposure to phthalates, a potent endocrine disruptor. Mixed culture microfluidic channels were treated with three increasing doses of mono-(2-ethylhexyl)phthalate (MEHP). Sox9 and Cytb5 transcripts were decreased at all doses showing the expected response to MEHP by Sertoli cells and redox status respectively. In contrast, fetal Leydig cell-specific genes, Sf1, Cyp17a1, and Insl3 exhibited a bimodal pattern of expression with increased levels at low and high doses and decreased transcripts at mid-range doses. These data were used to design a mathematical model that we intend to use to predict endocrine disruptor effects on fetal Leydig cell function in future exposures. Together our results show that primary fetal Leydig cells can be successfully cultured in mixed and co-culture paradigms and that this new technology can be used to evaluate fetal Leydig cell function after exposure to endocrine disrupting chemicals. Supported by NSF Graduate Research Fellowship Program DGE-0718123 (LNS), Wisconsin Multidisciplinary K12 Urologic Research Career Development Program K12DK100022 (ABT), University of Wisconsin-Madison Molecular and Environmental Toxicology Center NIH Grant T32ES007015 (ABT) and pilot project funding (JSJ), and University of Wisconsin-Madison Carbone Cancer Center Support Grant NIH P30 CA014520 (LNS, DJB,ABT, JSJ).
T8. ENGINEERED ENDOCRINE ORGAN TRANSPLANT UTILIZING A DECELLULARIZED OVARY SCAFFOLD. Monica M. Laronda¹, Adam E. Jakus²,³, Kelly A. Whelan¹, Ramille N. Shah²,³,⁴, Teresa K. Woodruff¹. ¹Division of Reproductive Biology, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; ²Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, IL; ³Department of Materials Science and Engineering, Northwestern University, Evanston, IL; ⁴Department of Surgery, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Certain cancer therapies can lead to impairment of fertility and endocrine function. Hormones produced by the ovary are important for reproduction, and maintain women’s overall health by protecting against brain, cardiovascular, and bone diseases. The field of oncofertility has sought to provide options for preserving and restoring fertility in patients at risk for iatrogenic ovarian failure, including freezing eggs and ovarian tissue for later use. Autotransplantation of cryopreserved ovarian cortical tissue has resulted in 17 reported live births, with short-term restoration of endocrine function, but also carries the risk of reintroducing cancer cells. After IRB approved consent, our lab obtained 20% of patient tissue for research, while 80% of the tissue was cryopreserved for patient use. Ovarian cortical tissues from 4 participants with acute lymphoblastic leukemia were selected for this study. These tissues stained positive for SALL4, confirming that they contained metastatic cancer cells within their ovaries and making them ineligible for current transplant techniques. We are investigating materials that will support long-term growth of ovarian cells, to reduce the risk of transferring diseased cells through ovarian tissue transplantation. We developed a technique by which all cells can be removed from ovary tissue pieces, leaving a decellularized scaffold of extracellular matrix on which patient ovarian cells can be seeded for transplantation. Because human ovarian tissue is rare, we used bovine ovaries as a proxy to develop the decellularization/recellularization procedures. We discovered, through high resolution SEM, the cortex is comprised of tightly organized collagen fibers, while the medulla contains additional flexible components such as laminin and elastin. Primary mouse granulosa and theca cells seeded onto the bovine medulla scaffold responded to FSH in vitro by producing estradiol. We tested the ability of seeded bovine ovarian scaffolds to function in vivo and produce hormones, by grafting these composites under the renal capsule of ovariectomized pre-pubertal mice. Mice with grafts produced estradiol at levels equivalent to controls. These data support further development of an artificial organ to provide an environment that sustains quiescent and growing ovarian follicles, restores endocrine function, and reduces the risk of re-introducing cancer cells to young women who survived treatment and wish endocrine or fertility restoration. This work is supported by the Watkins Chair of Obstetrics and Gynecology (TKW). This work made use of the EPIC facility (NUANCE Center – Northwestern University), which has received support from the MRSEC program (NSF DMR-0520513) at the Materials Research Center, The Nanoscale Science and Engineering Center (EEC-0118025)003), both programs of the National Science Foundation; the state of Illinois; and Northwestern University.

Endometriosis is an inflammatory, estrogen-dependent reproductive pathology affecting women and is estimated to account for up to 40% of otherwise unexplained infertility. Increased concentration of specific cytokines in the peritoneal fluid of patients with endometriosis promotes the establishment and proliferation of endometriotic lesions, and also may inhibit normal reproductive function. Cytokines can produce these effects by causing alterations in cellular proliferation, apoptosis, differentiation, motility, extracellular matrix remodeling and secretion of other cytokines. One such cytokine is Interleukin-17 (IL-17) which is mainly secreted by activated CD4+ Th17 cells and has been classified as a pro-inflammatory cytokine because of its ability to induce expression of a number of inflammatory mediators that promote peritoneal inflammation and adhesion. We have shown previously that women with endometriosis-related infertility have elevated expression of IL-17 due to increased numbers of Th17 cells in the secretory phase eutopic endometrium as compared to fertile women with or without endometriosis. We hypothesize that IL-17 may play a critical role in the alteration of endometrial and endometriotic cell proliferation and motility as well as the establishment of endometriotic lesions. To evaluate the effect of IL-17 on cell proliferation, endometrial epithelial (Ishikawa) cells and endometriotic epithelial (12Z) cells were treated with recombinant human IL-17 (recHuIL-17) at doses ranging from 2pg/ml to 100ng/ml. Cell proliferation was evaluated by tritiated thymidine incorporation and cell count assays after 48 hours of treatment. We observed that treatment with lower doses (2pg/ml-10ng/ml) of recHuIL17 had no effect on Ishikawa cell proliferation when compared to the vehicle control, but higher doses (50-100ng/ml) inhibited cell proliferation assessed by both thymidine incorporation and cell count assays. In contrast, treatment with these concentrations of recHuIL-17 had no effect on 12Z cell proliferation as compared to the vehicle control. We next evaluated the effect of recHuIL-17 on cell motility using the Cell Biolabs Inc. 24-Well Cell Migration Assay System in which cells are grown in 24 well plates containing a synthetic insert. Migration assays were set up with both Ishikawa and 12Z cells. Once the cells reached 80% confluency, the synthetic inserts were washed out and the cells were treated with 10pg/ml, 1ng/ml and 100ng/ml of recHuIL-17. Cell migration was assessed at 0, 6, 12, 24 and 48 hours of treatment. The 12Z cells showed an increase in migration over time in response to 100ng/ml of recHuIL-17 as compared to the lower concentrations and the vehicle control. In comparison, recHuIL-17 treatment had no effect on migration of Ishikawa cells when compared to the vehicle control. Pro-inflammatory factors such as IL-17 may promote the establishment of endometriotic lesions by inducing Epithelial to Mesenchymal Transition (EMT) through activation of the NFκB signal transduction pathway. We are now evaluating the effect of recHuIL-17 on the process of EMT and on activation of the pro-inflammatory NFκB pathway in Ishikawa and 12Z cells. Understanding the pathogenesis of endometriosis will generate critical knowledge about this debilitating disease and may have implications for therapeutic interventions both for endometriosis as well as the related infertility problems. Supported by NIH U54 HD40093 to RAN.
T10. INDUCTION OF THE DETOXIFICATION ENZYME NAD(P)H:QUINONE OXIDOREDUCTASE 1 (NQO1) BY LICORICE SPECIES USED IN BOTANICAL DIETARY SUPPLEMENTS FOR WOMEN’S HEALTH. Atieh Hajirahimkhan, Charlotte Simmler, Huali Dong, Dan Lantvit, Guannan Li, Shao-Nong Chen, Dejan Nikolic, Birgit M. Dietz, Guido F. Pauli, Richard B. van Breemen, Judy L. Bolton. UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL.

Induction of phase II detoxification enzymes plays a crucial role in eliminating the ultimate carcinogens and toxic entities in living cells. It is considered as one of the potential strategies for chemoprevention which might be addressed by changing people’s life style and diet. With Women’s health initiative demonstrating an increased cancer risk associated with hormone therapy, menopausal women turned to botanical dietary supplements such as hops (Humulus lupulus) and licorice for the alleviation of menopausal symptoms. Our previous studies have demonstrated chemopreventive potential for hops through activation of detoxification pathways in vitro and in vivo. Considering that prenylated chalcones and flavanones are present in hops and licorice, the major goal of the current study was to evaluate the chemopreventive potential of licorice species (Glycyrrhiza glabra, G. uralensis, G. inflata) used for women’s health. Our in vitro observations have shown that, similar to hops and its active constituent, the prenylated chalcone xanthohumol, licorice extracts containing the electrophilic chalcones, isoliquiritigenin and licochalconeA, induced NQO1 in normal breast epithelial (MCF-10A) and hepatoma (hepa1c1c7) cells. Induction of the antioxidant response element (ARE) in HepG2 cells implied the Keap1-Nrf2 pathway as the underlying mechanism. Significant induction of chemopreventive responses by isoliquiritigenin and licochalconeA over a narrow concentration range was comparable to other chemopreventive compounds such as curcumin and xanthohumol. The extracts showed dose-dependent NQO1 induction with no toxicity suggesting the possibility of generating designer formulations with optimal NQO1 activity. These observations were confirmed by in vivo analysis with fully characterized licorice extracts allowing the determination of the bioavailability of their active constituents and leading to a meaningful interpretation of NQO1 activity. Our data demonstrate the relevance of simultaneous biological and chemical standardization of botanical dietary supplements. Supported by P50 AT00155 provided by ODS and NCCAM.
T11. HIGH INCIDENCE OF TRIPLE NEGATIVE BREAST CANCERS FOLLOWING PREGNANCY AND THE ASSOCIATED GENE EXPRESSION SIGNATURE. Thao N.D. Pham¹, Szilard Asztalos¹, Peter H. Gann², Meghan K. Hayes¹, Ryan Deaton², Elizabeth L. Wiley², Rajyasree Emmadi², Andre Kajdacsi-Balla², Nilanjana Banerji³, William McDonald³, Seema A. Khan⁴, Debra A. Tonetti¹. ¹Department of Biopharmaceutical Sciences, ²Department of Pathology, ³Center for Clinical and Translational Science, University of Illinois at Chicago, Chicago, IL; ⁴Allina Hospitals & Clinics, Minneapolis, MN; ⁵Department of Surgery, Northwestern Feinberg School of Medicine, Chicago, IL.

Breast cancer is second leading cause of cancer death in US women. Various epidemiological studies have revealed that multiple factors including hormones, genetics, and reproductive history affect the incidence of breast cancer (Dupont et. al., 1985, N Engl J Med; Bernstein et. al., 1993, Epidemiol Rev; Henderson, 1993, Cancer). Among these, pregnancy is known to have a bidirectional effect on a woman’s risk for getting breast cancer. Compared to nulliparous women, parous women have a lower risk for breast cancer in a lifetime (Warner et. al., 2013, Breast Cancer Res Treat). However, several studies have confirmed that, in the period following pregnancy, breast cancer risk actually increases, and, if diagnosed, are termed pregnancy-associated breast cancers (PABC) (Albreksten et. al., 2005, Br J Cancer; Lambe et. al., 1994, N Engl J Med; Schedin, 2006, Nat Rev Cancer). These cancers are typically found at an advanced stage, have a higher incidence of lymph node metastases and are poorly differentiated (Bunker et. al., 1963, Am J Obstet Gynecol; Petrek et. al., 1991, Cancer; Petrek, 1994, Cancer;). By studying gene expression of human breast tissues following pregnancy, we have previously reported that they display higher expression of a number of inflammation related genes, suggesting a pro-tumorigenic environment as well as downregulation of ERα, and HER2 and upregulation of ERβ, suggesting a protective effect (Asztalos et. al., 2010, Cancer Prev Res). This finding helps us understand better the bidirectional nature of pregnancy on the risk of breast cancer. To examine the value of this gene set in predicting breast cancer, we evaluated expression of these genes in benign biopsy samples from group of women who were later on diagnosed with breast cancer (case), and compared it with that of age-matched women who were determined to be cancer-free (control). Even though this gene set failed to predict breast cancer cases, we found that some genes maintained differentially expressed between case and control. To examine the contribution of these genes in PABC, we investigated their expression in human breast tumors following pregnancy. Expression profiling of 64 pre-selected genes revealed that breast cancers detected within 10 years following pregnancy (parous group) display a different gene expression pattern than those detected in nulliparous breast cancer patients. The difference was found to be attributable to a triple negative (TNBC) subgroup under the parous group. Not only did our study find that TNBC subtype is more prevalent in the parous group, we were also able to derive a signature gene set that is able to discriminate PABC TNBCs from other breast cancer subtypes. Supported by AVON Foundation for Women
Ovarian cancer is the most lethal gynecological malignancy affecting US women. This is mainly due to lack of understanding of the early mechanisms of the disease, leading to poor diagnostic biomarkers and ineffective drug treatments. Recently, the cell of origin of ovarian cancer has been brought into question. This disease may arise from either the ovarian surface epithelium (OSE) or the fallopian tube epithelium (FTE), with much evidence supporting both progenitor cells. The OSE and FTE are derived from two separate cell types during development. Paired-box transcription factor 8 (PAX8) is a Müllerian expressed transcription factor involved in differentiation of thyroid, kidney and Müllerian derived cells such as the fallopian tube. The OSE does not express PAX8. PAX8 expression is seen in between 80-96% of high grade serous ovarian cancers (HGSOC), and often correlates with Wilm’s tumor protein 1 (WT-1) expression. Intriguingly, mouse models of HGSOC derived from the OSE show expression of PAX8, suggesting that it is not only a marker of Müllerian origin but an essential part of cancer progression potentially from both the OSE and TEC. Furthermore, previous studies suggest that PAX8 expression is essential for the survival of HGSOC because silencing resulted in apoptosis. While much work has been done on the role of PAX8 in carcinomas of the thyroid, very little is known about how PAX8 expression and function is regulated in HGSOC. Our hypothesis is that understanding the role of PAX8 in normal gynecological cells, and how it is regulated in HGSC will lead to a better understanding of the transformative mechanisms of the disease, while identifying potential therapeutic targets. PAX8 was knocked down in a non-cancerous mouse oviductal cell line (MOE cells). Additionally, PAX8 was expressed in a mouse ovarian surface epithelial cell line (MOSE). Down-regulation of PAX8 did not decrease proliferation of MOE Pax8 shRNA cells. The RNA expression of three Pax8 targets (WT-1, E2F1 and BRCA1) was reduced in the MOE Pax8 shRNA cells. Expression of PAX8 in MOSE cells leads to an increase in migration, along with migration-associated proteins. Interestingly, PAX8 target genes (WT1, E2F1 and BRCA1) were not altered in the same way between MOE and MOSE, suggesting that PAX8 has cell specific transcriptional targets. In order to understand the role of PAX8 in ovarian cancer, a panel of MOE cells containing alterations in pathways often associated with HGSOC was created. The altered pathways include knockdown of PTEN, oncogenic mutation in KRAS (G12V), mutation of p53 R273H, and constitutively activated myrIsolated-Akt (myrAkt). MOE-KRAS and MOE-PTEN shRNA increased PAX8 protein expression, while MOE-myrAKT and MOE-p53 R273H exhibited a decrease in PAX8. Understanding the role of PAX8 in normal epithelium of tubal origin, and how its expression and activity may be altered in HGSC will aid in the development of novel therapeutics and potential new biomarkers. This project was funded by the American cancer society RSG12-230-01-TBG
P1. HIGH LEVELS OF FOLLICLE STIMULATING HORMONE DURING AGING ARE ASSOCIATED WITH CHRONIC INFLAMMATION AND MALIGNANT TRANSFORMATION OF THE OVARY. A. Barua1,2,3, P. Bitterman2,3, Lauren M Rosen2, A. Guirguis3, S. Sharma3 and J.M. Bahr4. 1Department of Pharmacology, 2Pathology and 3Obstetrics and Gynecology, Rush University Medical Center, Chicago; 4Department of Animal Sciences, University of Illinois at Urbana-Champaign, IL.

Background: Ovarian cancer (OVCA) is an age-associated lethal malignancy of women and the median age of OVCA incidence is around 63 years (postmenopausal phase). Due to the lack of information on the molecular etiology, most cases of OVCA are diagnosed at late stages making it one of the fatal gynecological malignancies. Circulating levels of follicle stimulating hormone (FSH) have been reported to be persistently high in postmenopausal women although its pathophysiological role has not been well elucidated in the context of OVCA development. Objectives: the goal of this pilot study was to examine: (1) whether FSH receptor (FSHR) expression is associated with chronic inflammation or malignant transformation in the ovary of postmenopausal women or OVCA patients at early stage; and (2) to explore possible mechanism(s) of FSH associated OVCA development in patients. Methods and materials: Ovarian tissues (n=5) from postmenopausal healthy subjects (55-75 years old) and poorly differentiated serous ovarian tumors at early stage (n=5) and late stage (n=10) were examined for FSHR, hypoxia inducible factor (HIF-1), glucose regulated protein 78 (a marker of endoplasmic reticular stress and DNA damage) and IL-16 (a marker of chronic inflammation) expression as well as phosphorylation of AKT. In addition, the frequency of FSHR-expressing microvessels in postmenopausal ovaries and ovaries with tumors were also examined. Changes in expression intensity of each marker with respect to aging and OVCA stages were analyzed and $P<0.05$ was considered significant. Results: The intensity of FSHR expression increased significantly in association with ovarian aging in postmenopausal subjects and was highest in OVCA patients. Expression of HIF-1 and GRP78 was positively correlated with the increased FSHR expression. The frequency of ovarian IL-16 expressing cells was associated with the hypoxic condition and cellular stress in the postmenopausal ovary as well as in malignant ovaries. Moreover, increased FSHR expression together with persistent oxidative stress was associated with increased phosphorylation of AKT in normal aging ovaries and ovaries with tumor. In addition, FSHR was also expressed by the endothelium of ovarian tumor associated microvessels. Conclusion: The results of this pilot study suggest that increased expression of FSHR was associated with chronic hypoxic condition and cellular stress in menopausal ovaries. Together with longstanding unresolved inflammation these conditions may lead to malignant transformation of the ovary. Furthermore, high expression of FSHR by the tumor associated microvessels also suggests its potential role in the development of ovarian tumor associated neoangiogenesis. Support: Swim Across America grant (to AB).
Vast progress has been made in cancer treatment over the past few decades; however, a conventional cure without serious degenerative side effects has yet to be discovered. Cantharidin, commonly found in the excretion of blister beetles (family Meloidae), is a known toxin that causes cell apoptosis. A means of transporting this toxin to specific tumor cells without damaging healthy cells within the body is a potential approach to cancer treatment. Composites of liposomes and hollow gold nanoshells are potential selective carrier molecules for the toxin. The composites were imaged and sized using atomic force microscopy. Fluorimetry measurements showed the liposomes retained their cantharidin cargo during a 48-hour study. Cytotoxicity studies employing three breast cancer cell lines were implemented to measure cell apoptosis rates in the presence of the various constituents of the composites. Application of these composites presents a potentially less toxic, targeted defense against cancer. Supported by the Leighty Tabor Science Scholars Program (to LB), Millikin University Summer Undergraduate Research Fellowship Program (to LB), and the Howard L. Gravett Endowed Chair (to JRS).
In addition to being expressed developmentally and in the gastrointestinal tract, the gene Endothelin-2 (edn2) is highly and transiently expressed during ovulation in granulosa cells. We previously showed that global ablation of edn2 or antagonization of its receptor pathway inhibits ovulation. However, the molecular role that edn2 plays in the ovulatory process remains to be explicated. To accomplish this, we created novel granulosa cell-specific edn2 knockout mouse models. We first aimed to determine the consequences of losing edn2 expression selectively in the granulosa cells on ovulation, fertility, and litter size. Three conditional knockout mice were generated by crossing an Edn2Flox/Flox mouse with a PR-Cre, a Cyp19-iCre, or an Esr2-iCre mouse. Given that PR, Cyp19 and Esr2 expression start at the preovulatory follicular stage 4-6 hours prior to ovulation, at the secondary follicular stage, and at primary follicular stage, respectively, we hypothesized a gradient would exist in the severity of reproductive dysfunctional phenotypes in these different models owing to different temporal restrictions on Cre recombinase activity within follicles before ovulation. All three conditional knockout mice demonstrate normal rates of fertility in births per pairing (p=0.899), but Cyp19-iCre Edn2Flox/Flox mice have reduced litter sizes compared to control mice (4.67±0.71 vs 8.60±0.75, p=0.031). Similarly, Esr2-iCre Edn2Flox/flox mice trend towards a reduced litter size (4.00±1.00, p=0.111). Additionally, both Cyp19-iCre and Esr2-iCre Edn2Flox/Flox mice trend towards releasing fewer oocytes when treated to ovulate with gonadotropins (11.50±2.64 and 5.25±1.11 vs 18.72±1.97, p=0.141, 0.051, respectively). No significant differences in ovulation, fertility, or litter size were present in PRCre Edn2Flox/Flox mice (p>0.05), and histologically all conditional knockout ovaries form antral follicles and morphologically identifiable corpora lutea (n≥6). Taken together, this study confirms that granulosa cell-produced Endothelin is necessary to ovulate a normal number of oocytes and produce a litter of normal size, though conditional loss of edn2 does not eliminate pregnancy or corpus luteum formation. These animal models will be used to further describe the signaling cascade encompassing ovarian EDN2 during ovulation. Supported by NIH R01HD052694 to CK.
Silvestrol is a cyclopenta[b]benzofuran that was isolated from the fruits and twigs of Aglaia foveolata, which is indigenous to the island states of Southeast Asia. Previous testing of silvestrol revealed that it is a potent inhibitor of protein synthesis and has cytotoxic activity similar to or more potent than many FDA approved anticancer agents. Silvestrol is currently under preclinical development at the National Institutes of Health Experimental Therapeutic (NExT) program. The purpose of the current study was to determine if inhibition of protein synthesis caused by silvestrol could trigger autophagy and cell death. Silvestrol treatment of cultured human melanoma (MDA-MB-435) induces cell cycle arrest at G2. In addition, silvestrol treatment induces autophagosome accumulation. Silvestrol treatment also induces caspase-3 activation and apoptotic cell death in a time- and dose-dependent manner. Silvestrol-induced apoptosis was not blocked by the pan-caspase inhibitor Z-VAD-FMK, suggesting the involvement of an alternative cell death pathway. Silvestrol represents a natural product scaffold with the potential for the study of autophagy and cell death mechanisms in cancer cells. It may therefore be served as anticancer treatment in clinic with induction of autophagy. This project is supported by P01-CA125066 from National Institutes of Health.
Deletion of transcription factors GATA4 and GATA6 in ovarian granulosa cells results in anovulation and infertility. These defects are a result of a block in folliculogenesis where there is impairment of preovulatory follicle formation. Lack of preovulatory follicle formation precludes studies examining the role of GATA4 and GATA6 in luteal cells where GATA factors are highly expressed. Therefore, the role of GATAs in luteal cell function is still yet to be determined in vivo. The aim of this work is to determine the effect of GATA4 and GATA6 deletion at ovulation on corpus luteum function and fertility. To delete GATA4/6 in provulatory follicles, we crossed mice expressing Cre-recombinase driven by the P4 receptor (PR) with mice expressing single or combined floxed alleles for GATA4 and GATA6. Knockdown of GATA4 and GATA6 were confirmed by PCR. Since PR is highly expressed in the uterus, we investigated GATA expression here as well. We identified both GATA4 and GATA6 expression in uterine tissue however GATA mRNA levels were not affected in the presence of cre-recombinase. GATA4/6 double knockout mice were infertile while both single knockout mice were subfertile producing a reduced number of pups. No significant differences in the cycling between mutants and control animals or in the hormone induced ovulation rate could be detected. These results indicate that the infertility of GATA4/6-PRcre mice is not due to a lack of cyclicity, ovulation or normal sexual behavior. Ovarian histological sections confirmed the presence of corpora lutea 16h, and 96h after induction of ovulation. However, plasma P4 levels were significantly affected by GATA knockdown. At 16h post superovulation P4 levels were normal, however, at 96h there was a significant decline in plasma P4 levels. At 96h post superovulation, the GATA4/6-PRcre mice also showed significant inhibition of P450scc, and StAR mRNA expression; two important genes involved in P4 synthesis. Although, additional studies are needed to examine the effects of the deletion of GATA4 and GATA6 in other PR expressing tissues, these findings provide new insights into the roles these transcription factors have in female reproduction and demonstrate that they are not only crucial for the progression of folliculogenesis but also necessary for luteal progesterone production. Supported by NIH R01HD057110 and R21HD066233 (CS).
Obesity, metabolic syndrome and type 2 diabetes are some of the most serious health challenges facing the United States and other western countries. Recently there has been increased interest in the possible role of the gut microbiome and entero-endocrine system in the exacerbation of obesity and its sequelae. Our laboratory is studying the Ossabaw mini-pig as a novel animal model for obesity. The Ossabaw mini-pig has a mutation in the Val199→Ile region of the PRKAG3 gene (the γ isoform of AMPK) that causes increased intramuscular fat and contributes to their thrifty genotype. This pig breed, when fed an excess calorie high fat/cholesterol/fructose diet, naturally develops features of metabolic syndrome including visceral obesity, glucose intolerance, and dyslipidemia. Our objective was to determine whether feeding an excess calorie high fat diet and the consequent development of obesity would lead to alterations in the morphology of the small intestine and distribution of enteroendocrine cells. Nineteen Ossabaw gilts were fed either an excess calorie high fat diet/cholesterol/fructose diet (n=10) or control diet (n=9) for eight months after which the animals were euthanized and samples of the ileum collected for histological analysis. Ossabaw gilts on the high fat diet showed marked increases in overall body weight, heart girth and abdominal girth and also developed metabolic syndrome with elevated androgen levels. We used immunohistochemistry methods to measure intestinal villus height and assess expression of specific cell types in the ileum. An anti-chromagranin antibody was used to identify enteroendocrine cells, anti-glucagon-like peptide-1 (GLP-1) antibody to detect L cells, and Periodic Acid-Schiff stain to highlight goblet cells. Histological tissue sections were scanned using NanoZoomer Digital Pathology System (Hamamatsu Photonics) and the images obtained were analyzed using NDP.view2 software. Normal distribution of data was conducted using PROC UNIVARIATE and non-normal data were log transformed prior to analysis with an ANOVA using PROC MIXED (SAS, Inc., Cary, NC). The cut off level for statistical significance was set at \( p = 0.05 \).

The ileal intestinal epithelium of obese Ossabaw gilts had significantly greater length of the villi than that of lean Ossabaw gilts (obese: 488.9 \( \mu \)m ± 18.8 \( \mu \)m; lean: 389.4 \( \mu \)m ± 17.1 \( \mu \)m; \( p < 0.0001 \)). Obese Ossabaw gilts also had significantly more goblet cells per villus than lean Ossabaw gilts (obese: 31.3 ± 2.0 goblet cells/villus; lean: 26.0 ± 2.0 goblet cells/villus; \( p = 0.05 \)). We also identified numerous L cells that secrete GLP-1 and other enteroendocrine cells within the ileum. The L cells were located primarily in the intestinal submucosa with only a few in the villi. Lean Ossabaw gilts displayed a trend towards fewer L cells than the obese group. Our future studies will investigate potential differences in intestinal stem cells. We hypothesize that the differences in villi height and goblet cells may be due to increased numbers of proliferating stem cells in the crypts of the intestinal lining in obese animals. Funding: NIH R21HD060105 to RK and USDA ILLU-538-319 to RAN.
Identifying the cues governing progression of germ cells through meiosis is critical to our understanding of the mechanisms leading to the formation of healthy gametes. We have previously characterized the ENU-induced mouse mutation *repro42*, which causes both male and female infertility due to meiotic arrest. Genetic fine mapping combined to sequencing of candidate genes identified a nonsense mutation in *Spata22* (spermatogenesis associated 22). Analysis of mutant *repro42* surface-spread chromatin revealed defects in synaptonemal complex (SC) formation, synapsis and DNA double strand break repair. These data suggest that SPATA22 is required during prophase I in the mouse, but its precise function during gametogenesis remains elusive. To further define the role of SPATA22, we set forth to confirm its requirement by describing a novel allele of *Spata22* and by assessing its localization in meiotic male germ cells. First, we validated the need for SPATA22 during gametogenesis by examining *Spata22Gt* mice, a targeted gene trap allele of *Spata22*. Similarly to *repro42* mutant mice, adult homozygous mutant males and females are infertile but otherwise healthy. Furthermore, adult males present smaller testes devoid of spermatids and spermatozoa. Lack of SPATA22 protein was corroborated by immunoblotting analysis of mutant testis extracts, indicating that the *Spata22Gt* allele is indeed a null allele. We then performed a complementation analysis to genetically confirm the origin of the *repro42* mutation. Fertility testing of double heterozygous males and females revealed infertility, while all control mice consistently produced litters. Histological analysis of double heterozygous testes and ovaries confirmed interruption of gametogenesis at the same stage as homozygous mutant *repro42* or *Spata22Gt* mice. SPATA22 was previously localized to foci in the nucleus of spermatocytes, but precise spatiotemporal dynamics were not determined. Analysis of surface-spread chromatin revealed that SPATA22 foci are present in both oocyte and spermatocyte nuclei during prophase I. Although a few foci are initially visible in late leptonate oocytes and spermatocytes, 150-250 foci become clearly visible across the chromatin of early zygote meiocytes. In mid-to-late zygote oocytes and spermatocytes, foci become restricted to the synaptonemal complex (SC). The number of foci is considerably lower in early pachytene cells (> 10 foci per SC), and SPATA22 foci are completely absent in late pachytene oocytes and spermatocytes. Co-immunolabeling analysis confirmed that SPATA22 foci appear following DMC1 foci, and that these two factors are never present in the same foci. Furthermore, analysis of pachytene spermatocytes confirmed that SPATA22 foci are present on all chromosomal axes, including the XY axes, and that some persist until the mid-pachytene stage. Taken together, these data identify the *Spata22repro42* allele and further support a role for SPATA22 during meiotic prophase, most likely during meiotic recombination. Supported by funds from Midwestern University.
P8. FLAXSEED AND ITS COMPONENTS HAVE DIFFERENTIAL EFFECTS ON ENDPOINTS ASSOCIATED WITH OVARIAN CANCER. Anushka Dikshit, Chunqi Gao, Dale Buchanan Hales, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

We have previously established that flaxseed diet decreases the incidence and severity of ovarian cancer and a 15% flaxseed dose desirably alters the expression of certain cancer associated endpoints, in the laying hen. Anti-oncogenic properties of flaxseed are predominantly due to the omega-3-fatty acids and the lignan, Secoisolaricirescinol diglucoside (SDG). The omega-3-fatty acids cause a decrease in prostaglandins and as a result reduce inflammation. SDG is converted to weakly estrogenic compounds, (Enterodiol) ED and (Enterolactone) EL in the gut. Estradiol (E2) is metabolized by a series of CYP enzymes in the ovary and liver to form specific hydroxy-E2 metabolites. On oxidation, 4-hydroxyestradiol can form DNA adducts and ultimately become carcinogenic. Low 2-hydroxyestradiol/16-hydroxyestradiol ratio in urine or serum is correlated to increased risk of cancer. 2-methoxyestradiol (2-MeOHE2), a weak estrogen, is known to have anti-angiogenic and pro-apoptotic properties.

The objective of this study was to uncover the roles of the individual components of flaxseed and their effects on pathways implicated in cancer. Three year old chickens were fed either control/15%whole flax (WFX)/defatted flax meal (DFM), 5% flax oil, 5% corn oil diet for a period of 3 months. Blood was collected at specific time points throughout the study. Tissues were harvested at the end of the study and were used for analyzing mRNA and protein levels of target genes. Levels of 2-MeOHE2 and SDG metabolites were assessed in the serum using ELISA and LC MS/MS.

As expected, ED and EL were detected only in chickens that were fed the WFX and DFM diets. The expression of estrogen receptor alpha (ERα) decreased in the WFX group, demonstrated by Immunohistochemistry, qPCR and western blot analysis. Estrogen target, progesterone receptor mRNA expression was also decreased in the WFX. 2-methoxyestradiol levels increased in the DFM and WFX groups with a corresponding increase in CYP1A1 enzyme in the liver of DFM fed birds. Expression of CYP1B1 and CYP3A4 was not significantly altered among different diets. Different components of flaxseed might be targeting different pathways but their cumulative effect appears to be most promising in decreasing expression of ERα, a receptor associated with increasing tumor aggressiveness in ovarian cancer, without altering their normal ovarian function. The WFX diet also increased the levels of anti-oncogenic E2 metabolite, 2-MeOH2, further validating the anti-cancer effects of whole flaxseed. Supported by NIH AT00408
P9. FEW SEX DIFFERENCES EXIST IN THE EARLY POSTNATAL PITUITARY TRANSCRIPTOME. Kirsten Eckstrum, Karen Weis, Nick Baur, Lori Raetzman. Department of Molecular and Integrative Physiology, University of Illinois Urbana-Champaign

Sex differences in gene expression can arise from chromosomal influences or hormonal influences produced by the testosterone surge present in males. In fact, many sex differences in the brain can be induced by testosterone conversion to estradiol. The pituitary is a critical component of the hypothalamic-pituitary-gonadal (HPG) axis, utilizing signals from the hypothalamus of the brain to release luteinizing hormone (LH) and follicle stimulating hormone (FSH) from gonadotrope cells in the pituitary, which then influence circulating levels of sex steroids. It has been reported that serum levels of LH and FSH are different between males and females, even at an early age. However, it is unclear what pituitary sex differences exist at the level of the transcriptome at the onset of HPG axis function and how these differences occur. We hypothesize that, as in the brain, the testosterone surge is able to influence the genetic signature of the pituitary leading to important sex differences during the early postnatal period. We have shown that mRNA levels of Lhb and Fshb are higher in female mice as early as the day of birth (postnatal day (PND)0). However, these differences do not appear to be due to differences in gonadotrope number at this time point. Therefore, we did a microarray analysis to see if there were any other differences at the mRNA level, which may explain the striking differences in Lhb and Fshb mRNA levels. We discovered that at PND1, only a handful of genes are expressed at significantly different levels between males and females, six of which are chromosome dependent and the only three that are not are Lhb, Fshb, and Icam5. We analyzed the expression of these genes during the early postnatal period (PND0, 4, and 9) where Lhb and Fshb are higher in females and one time point just before puberty (PND20) where Lhb and Fshb are now lower in females than males. We analyzed mRNA expression of the X-linked gene Eif2s3x and found that it was higher in females at all time points. Icam5, though not significantly different at PND0, was higher in females at every other age tested as well. Importantly, the expression of this intercellular adhesion molecule gene has not been documented in the pituitary. Our next hypothesis was that we could influence the expression of hormonally regulated genes to make female gene expression patterns more male-like. To accomplish this, we administered 5μg/kg/day estradiol for one week to mice beginning on PND0 to mimic the male exposure to estradiol (E2) through aromatization of testosterone. E2 treatment decreased Icam5 mRNA levels in females to levels comparable to both treated and untreated males, indicating that Icam5 may be hormonally regulated. To determine the role of hypothalamic or direct hormonal influences on expression of these genes in the pituitary, PND0 pituitaries were cultured without any hormonal or hypothalamic stimulation. Sex differences between Lhb, Fshb, Eif2s3x, and Icam5 mRNA all persist. However, levels of Fshb and Icam5 are significantly lower in culture whereas levels of Lhb and Eif2s3x are comparable to an in vivo PND0 pituitary. This indicates that hypothalamic and hormonal signals may help to maintain expression of these genes. Overall, there are few differences in the transcriptome of male and female pituitaries during the early postnatal period despite the strong sexual dimorphism of Lhb and Fshb expression and the function of Icam5 warrants further exploration. Supported by grants R01 DK076647, T32 ES007326.
P10. NOTCH SIGNALING REGULATES EXPRESSION OF GRAINYHEAD LIKE 2: A POTENTIAL MECHANISM FOR CONTROLLING PROGENITOR CELL FATE IN THE DEVELOPING MOUSE PITUITARY. Whitney Edwards, Leah Goldberg, Lori Raetzman. Department of Molecular and Integrative Physiology. University of Illinois Urbana-Champaign

The pituitary gland controls crucial physiological functions such as growth, metabolism and reproduction. The vast functions of the pituitary are executed through the six distinct hormone-producing cell types, which are tightly regulated during development and adulthood. However, the signaling pathways that govern cell fate determination within the same region remain unclear. Previously we have demonstrated that the Notch signaling pathway is necessary to preserve the progenitor cell population and is important for terminal differentiation of the hormone producing cell lineages. However, the mechanism by which Notch controls the fate of these pituitary cell populations is unknown. To address this question, a microarray analysis of *Notch2* conditional knockout (cKO) mice revealed a significant decrease in expression of the transcription factor Grainyhead Like 2 (GRHL2) compared to littermate controls. In various tissues GRHL2 has been shown to be important in cellular morphogenesis, cell adhesion and the regulation of cell proliferation and differentiation. In addition, studies have revealed that GRHL2 directly regulates expression of proliferative genes such as proliferating cell nuclear antigen (PCNA) and antigen Ki-67 (Ki67). Based on its function in other tissues, the regulation of *Grhl2* mRNA may be a potential mechanism by which Notch maintains progenitor cells and promotes proliferation. In our studies we profiled the expression of *Grhl2* mRNA and examined GHRHL2 protein localization in the developing mouse pituitary. We show that *Grhl2* expression is most pronounced in the pituitary during the late embryonic and early postnatal time period and the protein appears to be present in progenitor cells. In addition, we have confirmed that Notch signaling is necessary for full expression of *Grhl2* mRNA and GHRHL2 protein using Notch 2 cKO mice and mice dosed with a chemical Notch inhibitor. Taken together, our studies define the spatial and temporal localization GRHL2 in the embryonic and postnatal mouse pituitary and show that Notch signaling is a potential regulator of *Grhl2*. Supported by R01 DK076647.
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 of the gonadal supporting cell lineages towards 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. However, 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 followed by next-generation sequencing (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.
Familial partial lipodystrophy (FPLD) syndromes may present with reproductive symptoms such as masculinization and menstrual irregularity as well as metabolic complications. We evaluated a 32-year-old woman with extensive hirsutism and irregular menses who was referred because of concern about an androgen secreting neoplasm. On physical examination, she had a FPLD2 (Dunnigan’s variety) phenotype. Laboratory evaluation was noteworthy for testosterone, triglycerides and glucose tolerance. By history, no other relatives had phenotypic features of FPLD. Her father has type 2 diabetes. We performed next generation exome sequencing on the proband and her parents. Sequencing was performed on the Illumina platform at BGI and results were aligned using BWA with build 37 as the reference genome. The standard workflow available through the Genome Analysis Toolkit (GATK) was utilized to prepare analysis-ready variants combining the trio family and annotated using ANNOVAR. In silico analyses yielded 19,329 quality variants with exonic gene locations and called in all individuals. Of these, 25 nonsynonymous variants were found to have a paternal autosomal dominant mode of inheritance (MAF<1%) and 9 nonsynonymous variants with an autosomal recessive mode of inheritance (MAF<5%) including a deleterious SNV within ACOXL, a gene involved in lipid metabolism. A candidate gene approach was used to evaluate variants within genes related to adipogenesis and previously known FPLD-associated genes (AKT1, AKT2, APOE, CD36, CEBPA, CEBPB, CIDEC, DGAT1, GSK3B, KLF5, KLF15, LMNA, LPIN1, LPIN2, LPL, LXR, PLIN1, PLIN2, PPARG, SIRT1 and SREBF1). The patient and her parents were heterozygous for a variant within LMNA, rs4641. This common SNV, located at the exon 10 splice site region, has been previously shown to lower transcript levels of lamin A and C and may contribute to the FPLD phenotype. Given that we did not find likely causal variants in previously identified FPLD genes, AKT2, CIDEC, LMNA, PLIN1 and PPARG, it is probable that the genetic variants contributing to the proband’s phenotype map to yet unidentified FPLD genes and/or noncoding regulatory elements. Supported by NIH grants P50 HD044405, R01 HD057223, T32 DK007196 and American Diabetes Association Career Development Award 7-09-CD-13.
P13. GENOMIC EVIDENCE OF STRESS INDUCED PHYSIOLOGICAL ADAPTATION IN THE PLACENTA. William E Gundling Jr¹,² Stacy Zamudio³, Nick Illsley³, Lourdes Echalar⁴, Derek Wildman¹,²,⁵. Department of Molecular and Integrative Physiology, University of Illinois Urbana-Champaign, Urbana, Illinois, USA. ²Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan, USA; ³ Department of Obstetrics and Gynecology Division of Maternal Fetal Medicine and Surgery, Hackensack University Medical Center, Hackensack, New Jersey, USA; ⁴ Instituto Boliviano de Biología de Altura, Universidad de San Andreas Mayor, La Paz, Bolivia; ⁵ Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA.

Placental hypoxia is associated with obstetrical syndromes including preeclampsia and intra-uterine growth restriction (IUGR). The incidence of these conditions is increased two-four fold at high altitude. We and others have shown that fetal growth is enhanced in populations with thousands of years of exposure to hypoxia due to altitude. We examined gene expression in term placentas from high and low altitude Native Americans versus recent European migrants to high altitude. Morphological results show that Europeans residing at high altitude exhibit significantly lower birth weights, while the native Andeans at the same location have birth weights similar to sea-level populations. Our global hypothesis is that evolution has favored genes that may permit greater fetal growth even in the presence of hypoxia. Using gene expression signatures of 45 villous placental samples we seek to identify genes involved in the Andean adaption to hypoxia occurring in the placenta. We identified 12 genes that were differentially expressed, with a Benjamini-Hochberg adjusted p-value < 0.1, between high altitude Andean and high altitude European populations. The most under-expressed gene in the high altitude Andeans is NAPRT1, a nicotinate phosphoribosyltransferase that plays a role in NAD synthesis and oxidative stress. The most over-expressed gene in the high altitude Andeans when compared to high altitude Europeans is arachidonate 5-lipoxygenase-activating protein (ALOX5AP), a gene that regulates pathways upstream of the hypoxia pathway. We found that there are genetic differences in high altitude Andeans when compared to their European counterpart in the same location. These differences can aid in explaining how the native Andeans are able to protect their offspring from hypoxia induced birth-weight decrease at high altitude. This work was supported by NIH grant R211HD068954-01.
Luteinizing hormone (LH) is a member of the glycoprotein hormone family and is secreted by the gonadotrope cells in the anterior pituitary. LH plays a very important role in reproductive function. In males, it binds to its specific G-protein coupled receptor (LHR) on the surface of Leydig cells to initiate testosterone production and is essential for spermatogenesis. Previous studies have identified several activating mutations in LHR in boys with sporadic or familial male-limited precocious puberty (SMPP or FMPP). The most common mutation in patients with FMPP is the amino acid change from aspartic acid 578 to glycine (D578G). Young boys with male-limited precocious puberty exhibit elevated testosterone levels, virilization, and Leydig cell hyperplasia, between the ages of 2 to 4. These patients are treated with inhibitors of testosterone or anti-androgen drugs and aromatase inhibitors until pubertal age to slow down the progress of virilization and epiphyseal maturation. However, there are few follow-up studies to determine if FMPP patients develop reproductive problems later in life. We have recently published a mouse model (KiLHR) with an activating mutation in LHR D582G that is equivalent to D578G in human. Male KiLHR mice exhibit precocious puberty, Leydig cell hyperplasia, and elevated testosterone levels, as seen in FMPP patients. In this study we examined the long-term consequences of premature LHR activation. A 6-month breeding study showed that the KiLHR become progressively infertile. Compared to 100% fertility of wild type (WT) mice (n=6), only 8% of KiLHR (n=13) were fertile at 6 months of age. The infertile KiLHR males were not able to form copulatory plugs in WT females although they exhibit normal sperm count and motility. During mating studies with superovulated WT females, sperm is not detected in the uterus and oviduct when there is no copulatory plug, suggesting that the KiLHR males are not capable of ejaculating. Behavioral testing reveals that the infertile KiLHR males try to mount the receptive WT females but are not able to achieve ejaculation suggesting a problem with erectile and/or ejaculatory function. H&E staining shows that the morphology of prostate and seminal vesicles are similar in WT and KiLHR males, while sperm is trapped in the vas deference before it joins to the urethra of mutant mice. Os and glans penis is normal but corpora cavernosa of the KiLHR penile body are filled with enlarged cells containing lipid-like droplets. These experiments indicate that activating mutations in LHR lead to age-related infertility most likely due to erectile and/or ejaculatory dysfunction. Our study suggests that patients with FMPP/SMPP are susceptible to infertility and should be monitored long-term.
P15. FLAXSEED DECREASES SEVERITY OF OVIDUCTAL POLYPS IN THE LAYING HEN MODEL OF LEIOMYOMAS. Emily Isgur¹, Malavika K. Adur², Romana A. Nowak², Dale Buchanan Hales¹.¹Department of Physiology, Southern Illinois University School of Medicine, Carbondale IL.²Department of Animal Science, University of Illinois, Urbana-Champaign IL.

Uterine fibroids or leiomyomas are benign smooth muscle tumors in the uterus that affect 70% of reproductive age women. Most women are asymptomatic but 40% of those affected are plagued with intense pelvic pain, menorrhagia, and infertility. These tumors are characterized by an overgrowth of extracellular matrix and fibrous tissue. What separates leiomyomas from other gynecological abnormalities is the need for both progesterone (P) and estrogen (E) for growth and maintenance. Laying hens are the only animals to spontaneously develop smooth muscle tumors (leiomyomas) in their reproductive tract, similar to women. Hen leiomyomas occur as polyps on the oviduct with increasing abundance as hens age and have the same histological and molecular markers as human uterine fibroids. Flaxseed has two major biologically active components, oil in the germ and phytoestrogen lignans in the hull. Flaxseed oil is very high in omega-3 fatty acids (alpha-linolenic acid) and these are known to act as inhibitors of prostaglandin pathways and as potent anti-inflammatories. Secoisolariciresinol diglucoside (SGD) in the hull is converted to the anti-estrogenic compounds, enterlactone and enterodiol. Our lab has previously shown that dietary intervention with flaxseed reduces incidence and severity of ovarian cancer. Similarly, flaxseed has also been shown to reduce the expression of steroidogenic enzymes in the ovary.

The objective of this study was to analyze samples from flax-fed and control-fed hens to investigate the potential for flax-based dietary intervention for the treatment of uterine fibroids. 1200 6-month-old White leghorn hens were fed a diet supplemented with 10% whole flaxseed over a period of 5 years. Every 6 months, 20 hens were sacrificed and oviductal polyps were collected to determine if the flax diet had any effect on polyp size, number and location. Normal oviductal, smooth muscle tissue and polyps were collected for histological analysis and for RNA and protein analysis from 42 month old hens, which displayed the peak incidence of leiomyomas. Hens fed flaxseed diet had decreased average polyp area per bird and decreased incidence when compared to control fed birds. qPCR showed polyps express steroidogenic enzymes, \textit{STAR}, \textit{HSD3B1}, \textit{CYP17}, and \textit{CYP19}. Flax fed hens had decreased mRNA expression of \textit{STAR} and \textit{HSD3B1}. qPCR also showed decreased levels of \textit{COX-2} and \textit{ER\textalpha} in flax fed hens. Immunofluorescence showed decreased nuclear staining for 3BHSD in oviductal polyps but no change in expression pattern of \textit{ER\textalpha} or COX-1.

Our study shows for the first time steroidogenic enzyme mRNA and protein levels in hen oviductal polyps. Flaxseed diet decreases average polyp area per bird and incidence at each time point. Flax diet decreased mRNA expression of \textit{STAR}, \textit{HSD3B1}, \textit{COX-1}, and \textit{ER\textalpha}. Immunofluorescence showed expression of 3BHSD protein as well as a shift from nuclear expression to cytoplasmic with flax diet. [Supported by NIH AT004085, AT005295, and P01 HD057877]
Basigin is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily. Basigin is known to regulate tissue remodeling and cellular differentiation and is critical for male and female reproductive processes including spermatogenesis and embryo implantation. Basigin is expressed by trophoblast cells beginning with the trophectoderm layer of the blastocyst. During the first trimester of pregnancy these trophoblast cells invade into the maternal endometrium and form the functional placenta at the implantation site. Pre-eclampsia, which is associated with shallow invasion of placental trophoblast cells, has been linked to reduced basigin expression in trophoblast. We hypothesized that knockdown of basigin expression in HTR-SVNeo trophoblast cells would decrease their migration ability. In order to knockdown basigin expression HTR-SVNeo cells were treated with 5nm of siRNA for basigin and compared to a negative control group treated with the same concentration of a scrambled siRNA. Cell lysates were collected using RIPA extraction buffer at 24, 48, 72 and 96 hours after treatment with siRNA to evaluate the knockdown efficacy. Immunoblotting revealed that siRNA treatment successfully knocked-down basigin protein expression in the HTR-SVNeo cells after 72 and 96 hours of treatment. Scratch migration assays were used to evaluate cell migration post knockdown. Migration was assessed at 24, 48, 72 and 96 hours after treatment with siRNA or scrambled siRNA control. Migration assays showed that basigin knockdown after 72-96 hours of siRNA treatment impaired cell migration compared to HTR cells treated with the scrambled siRNA negative control. We also carried out immunoblotting for basigin protein in cell lysates, conditioned medium and isolated microvesicles from HTR cells. We found that HTR cells secrete significant quantities of basigin protein into the medium. Isolation of microvesicles in the conditioned medium was carried out by ultracentrifugation and confirmed that >90% of BSG protein was in the microvesicle fraction. These results provide support for a local paracrine and/or autocrine mechanism by which basigin shed in microvesicles from trophoblast cells can act on neighboring cells to regulate invasive behavior. We are now evaluating the effects of basigin knockdown in HTR cells on MMP-2 and -9 expression as well as other possible factors that may be involved in regulating cell migration. Funding: NIH P01HD057877 to RAN
P17. COMPARISON OF CANTHARIDIN TOXICITY IN BREAST CANCER CELLS TO TWO COMMON CHEMOTHERAPEUTICS. K.M. Kern and J.R. Schroeder. Department of Biology, Millikin University, Decatur, IL.

As part of a larger study synthesizing a more directed form of chemotherapy, we have begun to assess the efficacy of different potential toxins that could be delivered locally rather than systematically. In doing so, we hope to reduce the systemic side effects commonly observed, while maintaining a high level of toxicity and eliminating the need for metabolic alterations. In a search for this more efficient method for killing cancerous cells, we have begun studying cantharidin, a toxin used in traditional Chinese medicine, as a potential chemotherapeutic. Using an MTT cell viability assay, the toxicity of cantharidin was compared to both cyclophosphamide and paclitaxel in three different breast cancer cell lines: MCF-7, MDA-MB-231, and SK-BR-3. Increasing the concentration of chemotherapy drugs did decrease cell viability in all cell lines when cantharidin and cyclophosphamide were applied; however differences for paclitaxel were cell-specific. Additionally, cantharidin exhibited the highest decrease in cell viability regardless of cell type, indicating it may be a much more potent and less specific chemotherapeutic. These results will help us move forward in developing a potentially more potent treatment for breast cancer that might eliminate the need for subtype-specific treatments. Supported by the Howard L. Gravett Endowed Chair (to JRS) and the Millikin University Department of Biology.
Previously, imatinib, an Abl kinase inhibitor, was shown to protect primordial follicles against cisplatin. Accordingly, the c-Abl has been implicated as the key regulator of chemotherapy-induced oocyte death. In proposed models, DNA damages caused by cisplatin activate c-Abl, and c-Abl in turn activates TAp63 by phosphorylation. We have demonstrated that oocytes specific conditional knockout (cKO) mice for Trp63 gene, encoding TAp63, were insensitive to cisplatin-induced follicle loss, supporting the essential role of TAp63 in cisplatin-induced oocyte apoptosis. On the other hand, involvement of c-Abl in oocyte apoptosis was indirectly supported by inhibitor studies: Two Abl kinase inhibitors with different modes of action, imatinib and GNF-2, protected primordial follicles from cisplatin.

In our previous study, induction of c-Abl by cisplatin was significantly attenuated in Trp63 null oocytes, suggesting that c-Abl might be a down-stream of TAp63. Moreover, cisplatin did not induce phosphorylation of TAp63, which occurred in g-irradiated oocytes. These observations prompted us the generation of c-Abl cKO mice to explore the function and requirement of c-Abl in the TAp63-regulated apoptosis of premature oocytes. Surprisingly, primordial follicles of oocyte specific c-Abl cKO mice underwent apoptosis in response to cisplatin, indicating that oocytic c-Abl is dispensable for the cisplatin-induced oocyte death. Moreover, phosphorylation of TAp63 was induced by radiation in the absence c-Abl, indicating that the previously proposed role of c-Abl in phosphorylation of TAp63 was incorrect. The presence and absence of TAp63 phosphorylation in the apoptosis of oocytes induced by radiation and cisplatin, respectively, strongly suggested that these two anti-cancer treatments activated TAp63 through different molecular mechanisms. Indeed, imatinib and GNF-2, which effectively protected oocytes from cisplatin, did not inhibit radiation-induced apoptosis and TAp63 phosphorylation in oocytes. In contrast, the radiation-induced oocyte death and TAp63 phosphorylation were effectively blocked by a CHK2 inhibitor, Chk2 Inhibitor II hydrate, suggesting that radiation activates TAp63 via phosphorylation by CHK2. Interestingly, although cisplatin did not induce phosphorylation of TAp63, the CHK2 inhibitor II hydrate still protected primordial follicles from cisplatin, suggesting that kinases activity that are inhibited by Chk2 Inhibitor II hydrate 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 II hydrate effectively protects oocyte against cisplatin as well as radiation, Chk2 Inhibitor II hydrate treatment might be a promising treatment for the prevention of ovarian reserve loss in women undergoing anti-cancer therapy. Funding National Institutes of Health Grants U54 HD076188, and P01HD021921 (to SYK, KE, MR, MHC, and TKW) and Grants U54 HD076188, R01CA154358 and R01HD064402 (to VAS and TK).
P19. 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. Supported by NIH Grant 5R00HD055330-05.
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. Additionally, endometrial cancers are also characterized in part by mutations occurring in the PTEN/PI3K pathway; and consequently, overactive Akt signaling persists in the majority of these cancers. Therefore, 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. Real-time PCR validation of angiogenesis-related target genes revealed an upregulation in the majority of target genes tested following siAkt and R5020 treatments. We also confirmed the upregulation in PRB target genes using the allosteric Akt inhibitor, MK-2206 (MK). Furthermore, Akt-dependent inhibition of PRB target genes was rescued using a constitutively active form of Akt (Myr-Akt), which is resistant to MK inhibition. 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 focused our analysis on the transcriptional coactivator, CBP, which is a known PRB cofactor and also subject to Akt regulation. We knocked down CBP in PRB-Ishikawa cells treated with MK and R5020 and subsequently performed real-time PCR analysis on PRB target genes. We found that in the majority of target genes tested, the upregulation observed in target gene expression following MK+R5020 treatment was significantly attenuated by knockdown of CBP. These results may implicate a role for CBP in the Akt-dependent regulation of PRB transcriptional activity. Finally, to determine the biological ramifications of combinatorial Akt inhibitor and progestin 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 more so than any other treatment. We also performed an endothelial tube formation assay and once again found that the combinatorial MK+R5020 treatment significantly decreased 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.
P21. PROGESTERONE ALLEVIATES ESTROGEN-DEPENDENT GROWTH OF ECTOPIC LESIONS IN AN IMMUNOCOMPETENT MOUSE MODEL OF ENDOMETRIOSIS VIA INHIBITION OF UTERINE CELL PROLIFERATION, INFLAMMATION AND ANGIOGENESIS. Q. Li¹, M.K. Adur², R.A. Nowak², M.K. Bagchi³, I.C. Bagchi¹. Departemnt of Comparative Biosciences, Department of Animal Science, Department of Molecular & Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL

Endometriosis is a common gynecological disease that affects many women of reproductive age. It involves growth of the endometrial cells outside the uterus on peritoneal surfaces, primarily due to retrograde menstruation. Although the estrogen (E2)-dependence of endometriosis is well characterized, the role of progesterone (P4) in establishment and progression of this disease remains less understood. In the present study, we developed and validated an immunocompetent mouse model of endometriosis, in which minced uterine tissue fragments collected from donor females were introduced into the peritoneal cavities of the syngeneic recipient females, to mimic human retrograde menstruation. These recipient females were then subjected to ovariectomy and treated with physiological doses of E2 or a combination of E2 and P4. Interestingly, ectopic lesions identified in the recipients were located at similar places to those observed in endometriosis patients, primarily on the surfaces of peritoneal wall, intestinal mesentery and outer uterine horns, fat tissues, and liver. E2-treated recipients had 2-5 large, light yellow color lesions with abundant blood vessels and extensive adhesions. Histological analysis revealed that these lesions were comprised primarily of stromal cells with cysts filled with turbid fluid. In contrast, 1-3 small, white colored, nonvascular lesions, with either small cysts or compact content, were seen in the E2 plus P4-treated group. Immunohistochemical analysis revealed extensive fibrogenesis in these lesions as indicated by the increased levels of collagen deposition and αSMA immunostaining. Moreover, a significant reduction in the expression levels of Ki67, F4/80, CYR61, NF-κB p65, and p-ERK1/2 as well as in the diameters of supporting blood vessels at the interface between lesion and peritoneum were observed in the E2 plus P4-treated lesions, when compared to those in E2-treated group. Collectively, these results indicate that P4 alleviates E2-dependent establishment and growth of ectopic lesions, presumably by inhibition of endometrial cell proliferation, inflammatory responses and angiogenesis. Interestingly, alteration in the numbers of pro-inflammatory T helper 17 (RORγt) cells and anti-inflammatory T-regulatory (FoxP3) cells as well as attenuation in the expression levels of estrogen receptor alpha and progesterone receptors were observed in the E-treated, but not in the E plus P-treated cystic lesions, when compared to the corresponding eutopic endometrium. Hence, this immunocompetent mouse model of endometriosis provides opportunities to study the impact of endometriosis on physiological functions of the uterus, as well as the progression and molecular pathogenesis of this disease that may be associated with aberrant steroid actions, genetic mutation, chronic inflammation, or exposure to environmental toxicants. Supported by U54 HD 055787 to MKB and ICB, and U54 HD40093 to RAN.
Preeclampsia affects 5 to 8 percent of pregnant women worldwide, and is one of the leading causes of maternal and neonatal morbidity and mortality. Vascular remodeling at the maternal-fetal interface is essential to maintain oxygen supply to the fetus during gestation. Pro-angiogenic growth factors like placenta growth factor (PGF) and its receptor FMS-like tyrosine kinase receptor (Flt1) are thought to be critical for maternal vascular adaptation. Alternative mRNA splicing regulates expression of membrane Flt1 (mFlt1) as well as soluble isoforms of Flt1 (sFlt1). During preeclampsia, generation of sFlt1 from trophoblast cells increases dramatically, while expression of PGF is decreased. This imbalance in pro-angiogenic growth factor expression contributes to the preeclampsia symptoms; hypertension and proteinuria. Mechanisms that regulate expression and alternative splicing of Flt1 in trophoblast are not known. Since hypoxia and a pro-inflammatory environment are associated with preeclampsia, we hypothesize that they could regulate expression patterns in trophoblast. Trophoblast cells were treated with hypoxia (1% oxygen) or activators of the NFkB pathway to determine effects on mFlt and sFlt mRNA expression. Hypoxia treatments (24 hr) caused at least a three-fold increase in sFlt-1 expression in JEG3 and Sw71 trophoblast cell lines, term placenta explants, and primary trophoblast. In term villous explants, both mFlt1 and sFlt1 expression increased about 2-4 fold after TNF-α treatment (20ng/ml), an activator of the NFkB pathway. To determine if increased transcriptional activity results in elevated sFlt-1, we cloned 1.7kb of the 5'UTR of human FLT1 into a luciferase reporter construct. Transfections with this construct produced limited expression in trophoblast cell lines and could not be increased by hypoxia. Stable trophoblast (JEG-3 and H8 cells) and nontrophoblast (hEK-293) cell lines with the 1.7kb flt1-luc construct produced limited expression also. These results suggest that key regulatory motif(s) may not be included in the cloned 1.7kb Flt-1 promoter region, or that increased expression of Flt1 mRNA by hypoxia/ TNF-α is not due to transcriptional activation. Alternatively, increased expression of sFlt1 may be due to post-transcriptional mechanisms. Recently, expression of Jumonji domain containing protein 6 (JMJD6) has been shown to regulate splicing of Flt1 in endothelial cells. Initial studies showed that JMJD6 mRNA and protein are expressed in Sw71, JEG3 (human trophoblast cell lines) and human term trophoblast. JEG3 and SW71 cells treated with hypoxia (1% oxygen) showed a two fold increase in JMJD6 RNA, but protein levels were not significantly altered. These preliminary results show that JMJD6 is expressed in human trophoblast; however, expression levels do not significantly change under hypoxia, a known inducer of sFlt1 splicing. Therefore, it could be that hypoxia induces post-translation modification(s) of JMJD6 that alter its functional activity in trophoblast. Further elucidation of mechanism regulating sFlt1 expression in trophoblast may provide new avenues for reversing the anti-angiogenic environment associated with preeclampsia. NICHD R15 HD073868
According to the American Cancer Society, over 200,000 new cases of invasive breast cancer are diagnosed and nearly 40,000 women will die from breast cancer annually. This leads to breast cancer being the second leading cause of cancer-based mortality in women, just behind lung cancer, with 23 deaths for every 100,000 women. Understanding how breast cancer cells function is crucial to finding effective treatments and possibly a cure. One element that is being considered is whether growth of these cells is being affected by chemicals that contaminate local water sources. Herbicides used in crop production and weed control contaminate water resources through runoff and ground seepage. Several of these compounds, especially chlorotriazines, are reported endocrine disruptors, thus mimicking sex hormones, binding to hormone receptors, and consequently leading to changes in gene expression. GPR30, a G protein-coupled receptor, has been recently been recognized as an estrogen receptor as it binds to estradiol. Since reports have indicated that atrazine may also act through GPR30, our goal was to identify whether atrazine also affected the expression of its own receptor, much like estrogen does. Two breast cancer cell lines, MDA-MB-231 and SK-BR-3, were treated with a 100-fold range of atrazine, cyanazine, or simazine, all chlorotriazine pesticides, with levels flanking the EPA safe level for each compound tested. Using real-time PCR, we assessed changes in GPR30 mRNA compared to a GAPDH control. Our results indicate that for most concentrations, treatment with chlorotriazines reduced GPR30 expression in MDA-MB-231 cells. In stark contrast, GPR30 expression doubled due to triazine exposure in the SK-BR-3 cell line. What we find then is that as GPR30 is expressed we may have some concerns as to how these herbicides may be contributing to the growth and spread of this disease. Supported by the Howard L. Gravett Endowed Chair (to JRS), the Brohard Cancer Research Fund, and the Millikin University Department of Biology.
P24. PROGRESSION OF EXPERIMENTAL DESIGN OF NANOSHELL TECHNOLOGY AS A POSSIBLE CHEMOTHERAPEUTIC DELIVERY SYSTEM. T.B. Mansur and J.R. Schroeder. 
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Of the advancements made in cancer research in the last few decades, one of the most promising is the use of nanotechnology as a mechanism for the detection and treatment of cancers. Our goal is to synthesize a liposome with other constituents, including our toxin, to allow the targeting of strictly cancerous tumors without affecting non-cancerous cells. There are several different assays that can test the toxicity of a drug or the viability of cells after a treatment, such as tetrazolium reduction, resazurin reduction assay, MTT assay, and an ATP assay. To verify the safety of our compounds, I assessed the toxicity of colloidal gold nanoparticles, cantharidin, liposomes, hollow gold nanoparticles, and cantharidin and fluorescein in liposomes, in both MDA-MB 231 and MCF-7 breast cancer cell lines using the MTT and crystal violet methods after a 72 hour exposure. Through these comparisons, I have been able to show which components are good candidates to be used in future projects, which will include in vivo trials, and which assay has a more reproducible result. Supported by the Millikin University Summer Undergraduate Research Fellowship Program (to TM), the Howard L. Gravett Endowed Chair (to JRS) and the Millikin University Department of Biology.
In the maturing mammalian oocyte, significant zinc fluxes in intracellular zinc availability have recently been shown to regulate the meiotic cell cycle. These findings warranted investigation into another organism named Caenorhabditis elegans, to understand if zinc fluxes are conserved events in developing oocytes across vertebrate and invertebrate phyla. While zinc fluxes have been shown to play a regulatory role in mammalian meiosis, the genetic and mechanistic underpinning are open question and it remains unknown how zinc plays a role in proper germline development. In C. elegans, Notch signaling governs germline development by orchestrating a switch from mitosis the gonad to forming sperm and oocytes. Notch utilizes zinc finger proteins to perform the switching events. Our objective is to define zinc how activity controls germline development. We hypothesize that a requirement for zinc fluxes in invertebrate oocyte maturation will follow the paradigm emerging in in vertebrate. Furthermore we will test the idea that zinc fluxes regulate switching from sperm to oocytes. We created a zinc deficient environment by sequestering zinc using a metal chelator named TPEN (N,N,N’,N’-tetrakis(2-pyridylmethyl)ethane-1,2-diamine) and examined young adult hermaphrodites for reproductive impairments. Zinc deficient hermaphrodites yielded multiple phenotypes, including reduced brood size, and misshapen gonads. Zinc deficient hermaphrodites produced fewer gametes and larger oocytes. Additionally we performed X-Ray Fluorescence Microscopy (XFM) to establish baseline metal quotas for total zinc in maturing oocytes. We examined the following stages: Prophase I arrested oocytes, cortically arranged -1 oocytes, 1-cell and 2-cell stage embryos. Results revealed large-scale zinc acquisition over embryonic development. Our next goal is to rigorously test whether this large-scale zinc increase may have an important role in defining a viable embryo in this invertebrate. We conclude that zinc fluxes developing embryos and gonads may be essential for their proper development. Perturbation of these types of zinc signaling pathways may lead to reproductive impairments. Further work will define the mechanism for which zinc regulates germline development and if zinc regulation is a conserved and important for viability. Future work will test new ideas for germline development and has the potential for a broad impact on numerous scientific fields. Supported by the The Chicago Biomedical Consortium & F31 GM112478-01
P26. PAX2 TRANSCRIPTION FACTOR REGULATION AND FUNCTION IN EARLY EVENTS OF FALLOPIAN TUBE EPITHELIAL-DERIVED SEROUS CANCERS. Dimple A. Modi and Joanna E. Burdette. Center for Pharmaceutical Biotechnology, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago IL 60607

Background and rationale: Fallopian tube epithelium (FTE) is one of the proposed progenitor populations for high-grade serous cancer (HGSC). One of the earliest reported changes identified in FTE is the loss of Pax2 expression in areas of secretory cell outgrowth (SCOUTs). SCOUTs are thought to progress to p53 signatures when p53 is mutated, and they may ultimately progress to serous tubal intraepithelial carcinomas and HGSC. Pax2 is highly expressed in normal FTE whereas in serous cancer, Pax2 is lost. Pax2 is a transcription factor and an epigenetic modifier that has been studied in thyroid and kidney development. Very little is known about what regulates Pax2 expression in the fallopian tube, the functional significance of its loss in the fallopian tube, and whether re-expression of Pax2 in HGSC may slow or halt tumor growth. Hypothesis: Deregulation of Pax2 expression contributes to tumor initiation and that understanding its regulation will allow for re-expression in cancer cells to induce cell death. Experimental methods and results: A) Defining the stepwise progression of oviduct-derived serous cancers – MOE cells stably expressing Pax2 knockdown or p53R273H or both have been generated and functionally validated. MOE cells with Pax2 knockdown mimic SCOUTs as described in human tissue. MOE cells with Pax2 knockdown and p53R273H mimic the protein profile observed in benign p53 signatures. These cells demonstrated increased proliferation (Sulforhodamine B assay), migration (scratch assay) and colony formation (soft agar assay) compared to MOE cells stably transfected with empty vector control. B) Functional significance of re-expressing Pax2 in HGSC cells – OVCAR3, OVCAR4 and Kuramochi cells were transiently transfected with pCMV-Myc-Pax2 to investigate whether re-expressing Pax2 was able to reverse or slow cancer progression characteristics. Re-expressing Pax2 in OVCAR4 cells reduced proliferation (statistically significant) of cancer cells by 22% ± 4% and migration by 57% ± 6% (± S.D.). Conclusion: MOE cells with p53R273H and Pax2 silencing demonstrated increased proliferation, migration and anchorage-independent growth. Pax2 re-expression in HGSC cells reduced proliferation and migration of HGSC cells. This project is designed to address the phenomena of developing an in-vitro model system to critically evaluate the evolvement and stepwise progression of serous cancer precursors in the FTE. Supported by ACS grant.
Preeclampsia is one of the most common complications of human pregnancy. Though its pathophysiology is not fully understood, it is thought that shallow migration of cytotrophoblast and the subsequent insufficient conversion of maternal spiral arteries into utero-placental arteries is the initiating defect. As fetal metabolic demands increase during gestation, this aberrant remodeling of the vasculature at the maternal-fetal interface results in a relatively hypoxic and nutrient-deficient placenta bed. Hypoxia decreases the expression the pro-angiogenic placenta growth factor (PGF), and increases expression of the anti-angiogenic soluble fms-like tyrosine kinase (sFlt-1), a non-membrane-bound variant of the PGF receptor. Inversion of the pro-angiogenic environment during preeclampsia may limit systemic vasodilation critical to maintain a healthy pregnancy by limiting serum levels of bioavailable PGF. Dysregulation of pro-inflammatory pathways, including NFκB, has also been implicated in preeclampsia development. The goal of our studies was to determine the mechanism governing NFκB regulation of PGF in trophoblast. Over-expression of NFκBp65, an active subunit of the NFκB complex, significantly decreases PGF mRNA expression in trophoblast – an effect which is ablated by co-expression of a dominant negative (dn) IκB which inhibits the NFκB complex by irreversibly binding and preventing nuclear translocation. Similarly, over-expression of NFκBp65 results in an ~90% reduction in the functional activity of glial cell missing 1 (GCM1), the primary transcription factor of PGF in trophoblast. The effect of NFκB activation on GCM1 mRNA expression, however, was less robust (~50% reduction), suggesting potential post-translational modifications of GCM1. Previous reports show that trophoblast GCM1 protein can be degraded during hypoxia. Because hypoxia and activation of NFκB have similar effects on both PGF and GCM1, we have investigated the possibility of cross-talk between these two pathways. Overexpression of NFκBp65 was able to significantly decrease GCM1 protein stability within 6 hours, similar to hypoxia. However, transient transfection with dnIκB was unable to prevent hypoxia-induced inhibition of PGF reporter activity. Collectively, these results indicate that hypoxia and NFκB activation utilize independent pathways to decrease PGF expression in trophoblast. To further investigate NFκB-mediated mechanisms regulating PGF and GCM1 expression, we established a novel stable human JEG-3 trophoblast cell line with a tet-inducible NFκBp65 expression cassette. With this construct, NFκBp65 expression is suppressed by tetracycline repressor (TetR). In the presence of doxycycline, TetR is displaced and transcription is allowed. Transduction with adenovirus expressing the viral protein 16 (VP16) transcription factor further increases NFκBp65 expression, as indicated by an ~35-fold increase in NFκB reporter activity compared to transient transfection of a CMV promoter-driven NFκBp65 vector. Induction of NFκBp65 suppresses PGF mRNA expression and significantly inhibits GCM1 mRNA expression by approximately two-fold. These results support that activation of NFκB in human trophoblast can limit PGF expression, independent of oxygen tension, and contribute to inversion of the pro-angiogenic status in preeclampsia. This work is supported by the National Institutes of Health grant NICHD R15 HD073868
P28. ASSOCIATION OF ADVERSE PREGNANCY OUTCOMES WITH FIRST-TRIMESTER SUBCHORIONIC HEMATOMA. Anna Palatnik, Timothy Janetos, Department of Maternal Fetal Medicine, Northwestern University Feinberg School of Medicine

**Objective:** It has been well established that subchorionic hematoma (SCH) is a risk factor for the development of a spontaneous abortion during pregnancy. The aim of this study was to look at the association between presence of SCH on first trimester ultrasound and adverse pregnancy outcomes.

**Methods:** In this cohort study, 512 women with a SCH on their first trimester ultrasound were compared with 1024 women without a first-trimester SCH. All women underwent cervical length measurement between 18 and 22 weeks. Women with multifetal gestation, cerclage or a uterine anomaly were excluded. Univariable comparisons of patients’ characteristics and pregnancy outcomes were conducted using chi-square for categorical data and student t-test for continuous measures. Nominal logistic regression was done to determine whether the presence of SCH was associated with adverse pregnancy outcomes, including placental abruption, preterm birth, preterm premature rupture of membrane, delivery method, apgar score, arteria pH, and birthweight

**Results:** 512 patients who were diagnosed with SCH on their first trimester ultrasound were matched with 1024 healthy controls. Pregnancy, in the presence of SCH, resulted in an increased rate of placental abruption (OR = 4.26; CI 1.09 – 20.64), an increase in preterm births (OR = 1.85; CI 1.28 – 2.67), and an increased rate of less than 10th percentile gestational size (OR = 1.36; CI 1.03 – 1.79).

**Conclusions:** SCH is a risk factor for the development of spontaneous abortion. We found that for successful deliveries, SCH was associated with adverse pregnancy outcomes including placental abruption, preterm birth, and a decrease in gestational size.
Glial cell line derived neurotrophic factor (GDNF) is a member of the TGF β protein super-family, and GDNF signals through RET receptor tyrosine kinase and a GPI-linked cell surface co-receptor, GFRα1. GDNF signaling plays crucial roles in urogenital processes ranging from cell fate decisions in germline progenitors to ureteric bud outgrowth and renal branching morphogenesis. Gene ablation studies in mice have revealed essential roles for GDNF and RET in urogenital development, although their roles in prostate development and growth are unclear.

Our objective was to examine the functional role of GDNF signaling in the developing mouse prostate. We observed expression of GDNF and GFRα1 in the urogenital sinus (UGS) prior to and during prostate development. GDNF shows time-specific and cell-specific expression during prostate development and growth in vivo. In embryos, GDNF and GFRα1 are expressed in the epithelium and mesenchyme of the UGS. GDNF expression declines in UGS mesenchyme during epithelial budding. During prostate branching morphogenesis and growth, GDNF expression is remarkably enriched in the mesenchyme/stroma of all prostate lobes. Using a well-established UGS organ culture system, we have obtained compelling evidence that exogenous GDNF increases proliferation of mesenchymal and epithelial cells in the UGS, altering prostate development. With regard to mechanism, the inhibition of RET kinase activity or ERK kinases (MEK1/2) suppressed GDNF-induced proliferation of the UGS mesenchyme. In addition, GDNF treatment dramatically increased Ret mRNA and protein expression in the UGS mesenchyme, and GDNF also increased phosphorylation of ERK1/2 (p44/42 MAPK) in immunohistochemical and immunoblot analyses of UGS mesenchyme. Taken together, these findings suggest that GDNF signaling influences cellular proliferation in the UGS and developing prostate. Furthermore, we propose that GDNF signaling in the UGS mesenchyme is mediated by RET receptor tyrosine kinase and involves activation of the MEK-ERK pathway, thus implicating GDNF-RET-MEK-ERK signaling in prostate development and growth.
Bisphenol A (BPA) is a synthetic estrogen found in commonly used polycarbonate plastics and epoxy resins that line food and beverage containers. BPA can leach out from products and be consumed by people. This is of concern because previous studies have shown that BPA inhibits follicle growth and induces atresia in the mouse ovary. Although the exact mechanism by which BPA inhibits follicle growth and induces atresia is not yet known, previous studies indicate that other endocrine disrupting chemicals inhibit follicle growth and induce atresia by causing oxidative stress. Oxidative stress occurs when more reactive oxygen species are present in tissues than the body can detoxify. Reactive oxygen species such as hydrogen peroxide and superoxide can cause damage to DNA, RNA, and proteins. To protect against oxidative stress, tissues are capable of expressing antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD1), glutathione peroxidase (GPX), and glutathione reductase (GSR). Thus, this study was designed to test the hypothesis that BPA exposure (1 μg/mL to 25 μg/mL) alters the expression of the genes Cat, Sod, Gpx, and Gsr. To test this hypothesis antral follicles were mechanically isolated from CD-1 mice at postnatal day 34, and cultured with vehicle control (dimethylsulfoxide; DMSO) or BPA (1, 10, 25 μg/mL) in supplemented α-minimum essential media for 96 hours. Throughout the culture, individual follicle diameters were measured every 24 hours to monitor follicle growth. At the end of the culture the follicles of each treatment group were collected and subjected to real-time PCR for analysis of the oxidative stress related genes Cat, Sod, Gpx, and Gsr. Our data indicate that exposure to BPA (10 and 25μg/ mL) decreases the expression of Gsr in ovarian follicles at 96h when compared to control (DMSO: 1.01 + 0.05GE; BPA 10μg/ mL: 0.80 + 0.02GE, BPA 25μg/ mL: 0.81 + 0.06GE; (n=3), p≤0.05). Exposure to BPA (25μg/ mL) increases expression of Cat in ovarian follicles at 96h when compared to control (DMSO: 1.24 + 0.17GE; BPA 25μg/ mL: 1.96 + 0.2GE; (n=3), p≤0.05). In conclusion, BPA alters antioxidant gene expression, potentially resulting in oxidative stress-related follicle growth inhibition. Support: NIH ES019178
Retinoic acid (RA) is an active derivative of vitamin A. RA is known to play a vital role in many systems through regulating the expression of target genes. However, its role in ovary development is not well understood. Aromatase, encoded by Cyp19a1, is an enzyme that converts androgens to estrogens and is prominently expressed in ovarian granulosa cells upon activation by gonadotropins. 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 or aromatase-inhibitor treated mice share similar ovarian pathologies/hormone profiles with a vitamin A deficient animal model that we have recently developed, suggesting converging mechanism(s). In order to better understand RA actions in the ovary, this study was designed to investigate the regulation of aromatase expression by RA. Primary ovarian granulosa cells were cultured and treated with FSH, RA, R115866 (a RA metabolism blocking agent), AGN193109 (a RA receptor inhibitor), RA plus FSH, 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. Real-time PCR results indicate that RA or R115866 increased Cyp19a1 mRNA levels at 24 hr. This increase in Cyp19a1 expression was confirmed at the protein level using western blot analysis. The stimulatory effect of RA or R115866 was specific as it was abolished by the RA receptor inhibitor. At 72 hr, no induction of Cyp19a1 mRNA levels by RA was detected, possibly due to the relatively short half-life of the mRNA. We are in the process of analyzing aromatase protein levels at 72 hr. To investigate the in vivo significance of our finding, we also examined aromatase expression in a transgenic mouse line, the MTα mice, which overexpress inhibin α. We have shown previously that MTα mice have increase CYP26B1 (the key enzyme that degrades RA) levels, suggesting decreased RA signaling in these mice. Both mRNA and protein analyses of whole ovary samples demonstrated that aromatase expression was significantly decreased in the MTα mice as compared to their normal littermates, supporting the idea of in vivo regulation by RA signaling. Overall, our study suggests that Cyp19a1 is a target of RA regulation and aromatase may mediate RA functions in the ovary. Supported by the DePaul-RFUMS Collaborative Research Grant and URC Grant.
Female reproduction is dependent on the ability to ovulate a healthy oocyte competent for fertilization. The oocyte develops in intimate association with the surrounding somatic support cells of the ovarian follicle, creating a critical niche that is established and maintained by a variety of endocrine or locally acting paracrine factors. Emerging evidence suggests that the Notch pathway, a juxtacrine signaling system dependent on cell-cell contact, also contributes to the formation and function of the ovarian follicle. Using pharmacological inhibition as well as conditional knockout approaches in mice, we have shown that Notch disruption results in aberrant follicle formation, changes in granulosa cell proliferation and survival, and reduced female fertility. Most of our work to date has focused on Notch signaling in neonatal mice. In this study, we examine Notch signaling in pre-pubertal mice undergoing standard superovulation protocol, so as to explore the changes that might ultimately explain the observed fertility defects and to gain insight into the interactions between Notch and gonadotropin signaling within the ovarian follicle. We first sought to determine if Notch signaling remained active in growing multilayered follicles, by examining Transgenic Notch Reporter (TNR) mice, which express eGFP in cells with active Notch signaling (mice from Dr. Nicholas Gaiano, Johns Hopkins University). Confocal microscopy was used to visualize the eGFP reporter in cultured ovaries, revealing robust expression in larger follicles at post-natal days (PND) 10 and 19. Fluorescence activated cell sorting of tdTomato marked granulosa cells (Foxl2-iCRE activating a CAG-LSL-tdTomato reporter) of PND19 TNR ovaries revealed expression of the TNR reporter in the majority of granulosa cells; however, Notch active cells are not limited to granulosa cells. To determine whether gonadotropins affect expression of Notch genes and activity, PND19 TNR mice were treated with pregnant mare’s serum gonadotropin (PMSG) for 48 hours followed by human chorionic gonadotropin (hCG), for various times. While there was little change following PMSG treatment, hCG treatment caused increases in eGFP reporter mRNA at 12 and 24 hours. This is consistent with our previous data of positive regulation of several Notch signaling molecules expression by hCG treatment. Examination of the TNR reporter mice following ovulation revealed continued eGFP expression in cumulus cell surrounding the ovulated oocyte as well as in the newly formed corpus luteum. The fertility defects in Notch pathway knockout mice were most robust in mice with a disruption of the gene encoding the ligand Jag1, and these females have litters about half the size of wild-type controls. We examined Jag1 knockout mice in the gonadotropin superovulation model, and found a trend toward reduced numbers of ovulated oocytes, likely because of a decrease in the number of antral follicles. In addition, we observed a significant decrease in the number of oocytes progressing to metaphase of meiosis II (MII) at 16 hours post-hCG, indicating a defect in oocyte maturation that likely contributes to the observed subfertility. Collectively, these data show that Notch signaling remains active in the peri-ovulatory period, is regulated by the gonadotropin LH, and contributes to oocyte maturation and female fertility. Supported by the Eunice Kennedy Shriver NICHD Program Project Grant (NIH P01 HD021921).
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P33. LOSS OF P38 SIGNALING IN EPITHELIAL OVARIAN CANCER IN THE LAYING HEN MODEL IS PARTIALLY RESTORED BY DIETARY FLAXSEED. Sheree Speckman1, Dale B. Hales1, Karen Hales2. 1Department of Physiology, 2Department of Obstetrics and Gynecology Southern Illinois University School of Medicine, Carbondale IL.

Epithelial ovarian cancer is the most lethal gynecologic malignancy, with a 5-year survival rate of less than 40%. This is due to a lack of specific early symptoms, a lack of early detection markers, and a high incidence of recurrent, chemoresistant disease. The laying hen is the only animal model which develops epithelial ovarian cancer spontaneously, with features that closely replicate the human disease. Dietary flaxseed, a rich plant source of omega-3 fatty acids and lignans, has been shown to exhibit chemopreventive properties; we have shown that a diet of 10% flaxseed reduces the incidence and severity of ovarian cancer when fed to laying hens over a period of 4 years. To investigate the specific molecular mechanisms by which flaxseed acts to suppress ovarian cancer, we have examined the effect of flaxseed upon expression and activation of a number of pathways known to be altered in ovarian cancer. The MAPK family of kinases is involved in the regulation of a wide variety of cellular processes, including cell fate, differentiation, proliferation, and apoptosis, as well as transducing signals to the nucleus. Activation of the p38 MAPK pathway has been shown to lead to cell cycle arrest, apoptosis, and differentiation, and is altered in many different cancer types. We have found a significant decrease in levels of phosphorylated p38 MAPK in ovarian cancer compared to normal ovaries. This loss in p38 phosphorylation is partially restored in ovarian tumors from flaxfed hens. Furthermore, we have found significant alterations in expression of kinases upstream of p38 MAPK in ovarian tumors from flaxfed hens compared to those of control-fed hens.
P34. PRENATAL DI-(2-ETHYLHEXYL) PHTHALATE EXPOSURE MAY AFFECT SEX RATIO IN MICE. Sarah Steinmann, Saniya Rattan, Jodi A. Flaws. Department of Comparative Biosciences, University of Illinois, Urbana, IL

Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer that is often found in polyvinyl chloride products such as shower curtains, swimming pool liners, rain coats, car upholstery, wire sheathing, baby toys, medical tubing, and blood transfusion bags. DEHP can readily leach from products into the environment. This is of concern because DEHP exhibits endocrine disrupting activity and is a known testicular toxicant. While effects of DEHP on the testes are well documented, little is known about the effects of DEHP on the ovary. Thus, we tested the hypothesis that prenatal DEHP exposure adversely affects ovarian development and reproductive outcomes in the offspring of mice. To test this hypothesis, timed pregnant female CD-1 mice were orally dosed with tocopherol-stripped corn oil (vehicle control) or 20μg/kg, 200μg/kg, 200mg/kg, 500mg/kg, or 750mg/kg DEHP daily from gestation day 10.5 until birth of the pups (n = 5-18 dams per treatment group). On postnatal day (PND) 0, pups were counted, weighed, and sexed. On PNDs 1, 8, 21, and 60, at least 1 female pup from each litter was euthanized and her ovaries were harvested and weighed. At PND8, one collected ovary from each pup was subjected to histological evaluation of primordial, primary, preantral, and antral follicle numbers. On PND21, at least one pup from each litter was weaned and subjected to daily vaginal smears to monitor estrous cyclicity. At 3 months, F1 females were mated with proven breeder males of the same age to evaluate fertility based on time to pregnancy, production of a live litter, and numbers of live versus dead pups. The results indicate that prenatal DEHP exposure does not significantly affect the numbers of pups born, birth weight, PND8 follicle numbers, estrous cyclicity, or overall fertility, but does alter the male-to-female ratio in each litter, with DEHP exposure (200 μg/kg) resulting in more males compared to females in each litter. Supported by: NIH P01 ES022848 and EPA RD-83459301.
Endometrial cancer affects over 52,000 women and leads to approximately 8,500 deaths in the United States each year. Two types of endometrial carcinomas have been distinguished, type I and type II. Type I endometrial carcinomas, which account for 80% of all endometrial carcinomas, are associated with hyperestrogenism, have a good prognosis and exhibit atypical hyperplasia. Type II endometrial carcinomas are estrogen independent, poorly differentiated tumors that behave in an aggressive manner that cause high recurrence and low survival rates. Thus, it is necessary to understand the molecular mechanisms regulating type II endometrial tumor growth and progression to formulate treatment strategies that lead to remission and increase long-term survival. The mutation of TP53 has been detected in approximately 90% of serous (type II) carcinomas, implicating a role for its inactivation in the development of this aggressive tumor type. Inactivation of E-cadherin (CDH1) has also occurred in 70-90% of type II carcinomas. Due to these two major molecular features of type II endometrial carcinomas, we generated a mouse model in which Cdh1 and Trp53 (Cdh1d/dTrp53d/d) were conditionally ablated in the uterus. The results from our study clearly showed that loss of Trp53 along with Cdh1 ablation induces endometrial carcinomas similar to human type II endometrial carcinomas, whereas mice with single gene ablation of Trp53 or Cdh1 in the uterus did not develop endometrial carcinomas. Thus, inactivation of Trp53 or Cdh1 alone is not likely to contribute to tumor initiation and progression. Cdh1d/dTrp53d/d uteri exhibited papillary proliferation of small sized cells of the surface epithelium that have very hyperchromatic nuclei similar to type II endometrial carcinomas pathogenesis. Further, tumors from Cdh1d/dTrp53d/d uteri were highly aggressive, and metastasized to nearby and distant organs within the peritoneal cavity. We also observed abundant cell proliferation, complex angiogenesis and abnormal steroid hormone receptor expression (ESR1 and PGR) in the uteri of Cdh1d/dTrp53d/d mice. Our transcriptional profiling found that most of the dysregulated genes in Cdh1d/dTrp53d/d uteri were linked to inflammatory responses. Specifically, we found evidence of activation of NFκB and STAT3 signaling and involvement of tumor-associated macrophages in Cdh1d/uteri. These results suggest that an inflammatory tumor microenvironment with dTrp53d/d immune cell recruitment is further supporting tumor development in Cdh1d/dTrp53d/d mice. When macrophage cells were cultured with conditioned media from type II endometrial carcinoma cells, abundant chemokines, cytokines and enzymes from our profiling were expressed in the macrophage cells. Interestingly, these results were clearly recapitulated in macrophage cells that were primed in media conditioned by AN3CA cells, which lack CDH1 and harbor a mutation in TP53, but not by KLE cells, which have abundant CDH1 and mutated TP53. In summary, our study highlights that chronic inflammation initiated in our mouse model with Cdh1 and Trp53 ablation modifies the tumor microenvironment and promotes aggressive tumor development. This work was supported by NIH/NCI CA179214.
Retinoic acid (RA) is an active metabolite of vitamin A (VA) and is involved in tissue organization, patterning, and growth. RA has been shown to regulate male reproduction, however information on its role in ovary development is limited. Using an in vivo dietary VA-deprivation animal model, we have shown that VA deficiency causes a variety of ovarian pathologies, including reduced numbers of total follicles and corpus lutea, formation of hemorrhagic and atretic follicles, and formation of bursa and follicular cysts. To further examine if the decrease in the number of corpus lutea represents a defect in ovulation, in this study, we first investigated the ovulation and oocyte maturation status of superovulated VA- mice. We fed CD-1 pregnant mice with either a VA-deprived diet (VA-) or a control diet, and at weaning, female pups were maintained on their respective diet. These female pups were then superovulated at D19 or Wk7 via subcutaneous injection of pregnant mare’s serum gonadotropin (PMSG) followed by human chorionic gonadotropin (hCG) 48 hours later. Oviducts from these animals were collected 14-16 hours later and ovulated oocytes in the oviducts were counted and classified. The results showed that although the number of ovulated oocytes was not different between the control and VA- groups at D19, the number of ovulated oocytes in VA- mice was significantly reduced at Wk7 as compared to the controls. In addition, a reduced percentage of GV-oocytes in D19 VA- mice and reduced percentages of GV-, GVBD-, and MII-oocytes in Wk7 VA- mice were observed as compared to the controls, suggesting abnormal oocyte maturation. Since VA functions through its conversion to RA, next, to investigate further a possible direct impact of RA on the ovulation event, we injected mice that were fed on a regular diet with the RA synthesis blocker WIN18446 for three days during the time of superovulation induction. It has been reported that a 3-day in vivo WIN18446 treatment can significantly suppress serum RA levels. Consistent with our observations in the VA- mice, oocyte count and classification demonstrated that a lower dose of WIN18446 suppressed ovulation and hindered oocyte maturation in Wk7 mice as the total number of ovulated oocytes and the percentages of GVBD- and MII-oocytes were all significantly reduced as compared to the controls. The effect of WIN18446 on D19 mice was not obvious. However, at a higher dose, WIN18446 also suppressed ovulation and reduced the percentages of GV- and MII-oocytes in D19 mice as compared to the controls. Overall, our studies suggest that in vivo RA signaling plays a critical role in ovulation and oocyte maturation and that older animals are more susceptible to the impact of VA/RA deficiency on reproduction. Supported by DePaul URAP, Summer Research Grant, and URC Grant.
P37. OXIDATIVE STRESS AND MnSOD ACETYLATION PROMOTE ACTIVATION OF THE PI3K/AKT PATHWAY IN UTERINE FIBROIDS. Vania Vidimar¹, David Gius², Jianjun Wei¹,³, J. Julie Kim¹. ¹Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology. ²Department of Radiation Oncology. ³Department of Pathology. Feinberg School of Medicine, Northwestern University, Chicago, IL

Approximately 70% of women in their reproductive age develop uterine fibroids or leiomyomas which are benign monoclonal tumors arising from the smooth muscular wall of the uterus (myometrium). The molecular pathogenesis of leiomyoma is poorly understood and insights are needed to identify valuable therapeutic targets to provide alternative non-surgical treatments. Previously, we have shown that the PI3K-AKT-mTOR pathway is upregulated in leiomyoma compared to normal myometrium. In leiomyoma cells, drugs targeting AKT promote senescence and inhibit growth suggesting that this pathway is crucial for their proliferation and survival. However, it is unclear what promotes and sustains AKT activation in leiomyoma. We hypothesize that oxidative stress caused by an intrinsic dysregulation of the redox system activates the AKT pathway and promotes survival in leiomyomas. In order to first demonstrate that oxidative stress can activate the AKT pathway, primary leiomyoma cells from fibroid tissues were treated with increasing concentrations of hydrogen peroxide (H2O2) and pAKT protein levels were assessed by Western Blot. H2O2 increased pAKT expression in a dose dependent manner in primary leiomyoma cells. Moreover, inhibiting AKT using either MK-2206 (a specific allosteric AKT inhibitor) or small interfering RNA in combination with H2O2 decreased cell viability further, compared to either MK-2206 or AKT silencing alone, indicating that AKT protects leiomyoma cells against oxidant-induced cell death. Alterations in oxidative stress can be estimated by changes in the activity/expression of antioxidant enzymes such as the Manganese superoxide dismutase (MnSOD). MnSOD is the primary mitochondrial ROS scavenging enzyme that detoxifies superoxide by converting it to hydrogen peroxide, which is subsequently converted to water by catalase and other peroxidases. Acetylation of MnSOD at lysine 122 (MnSODK122) leads to accumulation of inactive MnSOD. We observed that leiomyoma tissues from patients expressed significantly higher levels of MnSODK122 compared to the matched myometrium, suggesting that leiomyomas are characterized by a reduced detoxification capability that may sustain AKT activation. Moreover, the MnSOD mimetic GC4419 reduced pAKT protein levels suggesting that inactive MnSODK122 favors AKT activation in leiomyoma cells. In conclusion, leiomyomas are characterized by an impaired oxidative system due to the aberrant acetylation of MnSOD. This oxidative environment, also sustained by the hypoxic milieu of leiomyomas, upregulates the PI3K-AKT pathway in leiomyomas. Supported by P01.
Nanoparticle composites are a recent research hotspot, being examined as drug-delivery vehicles for more efficient treatment of malignant cancerous tumors. First, the chemotherapeutics are encapsulated in a liposome tethered to transition metal or hydroxide nanospheres. These composites are then injected at a tumor site, and can be opened with lasers or UV light to disperse the toxins in a localized manner. This method proposes to be relatively non-invasive, cost-effective, and more accurate for cancer treatment. Since the synthesis and fabrication of nanoparticle composites is a relatively new application, there must be further research to minimize toxicity and improve safety when using these composites. The focus of our study was to examine short (1 day and 1 hour) and long (1 week) term mutagenic effects of two gold nanoparticles synthesized at Millikin University, HPN1 and HGN2. HPN1 are 40 nm diameter colloidal gold nanoparticles, which reflect a purple hue, while HGN2 are 10 nm diameter colloidal gold nanoparticles that reflect a yellow-orange color. To measure short-term toxicity, β-galactosidase-based DNA damage assays were completed using four E. coli bacterial strains: KP266, KP263, BWλrec and BWλlex, which have either the LexA or RecA gene coupled to β-galactosidase. These strains indicate DNA damage through LexA or RecA transcription after the accumulation of single stranded DNA, which then gives a colorimetric indicator of DNA damage. The bacteria were grown in the presence of either HPN1 or HGN2 for up to twenty-four hours, followed by the β-galactosidase assay. We did not observe changes in β-galactosidase activity, thus indicating no change in the levels of DNA damage in any of the four strains upon exposure to either type of colloidal gold nanoparticle. In order to assess toxicity after a longer nanoparticle exposure, MTT cell viability assays were performed in two breast cancer cell lines (MDA-MB-231 and MCF-7) after exposure to nanoparticles for one week. Interestingly, we observed a cell type-specific effect where viability was unaffected in the MDA-MB-231 cell line, yet dramatically increased in MCF-7 cells. This occurred with both the 10 nm and 40 nm diameter nanoparticles. Taken together, these results indicate that we must be cautious as we move forward in the development of new chemotherapeutic techniques, since acute tests may not be indicative of the true toxicity of these compounds. Further studies will examine the mechanisms through which changes in viability occurred. Supported by the Leighty Tabor Science Scholars Program (to OGW), the Howard L. Gravett Endowed Chair (to JRS), and the Millikin University Department of Biology.
Ovarian follicle development is a complicated process critical for female reproduction and health. We have identified retinoic acid (RA), an active derivative of vitamin A, and CYP26B1, an enzyme that degrades RA, as novel ovarian factors that play a role in this process. We have shown that RA and an CYP26 inhibitor stimulate ovarian granulosa cell growth although the underlying mechanisms are not known. Intriguingly, tuning of the calcium signal is known to modulate cell proliferation and apoptosis, suggesting that the calcium signal may orchestrate multiple aspects of granulosa cell function. This study was designed to examine the effect of RA/CYP26B1 on intracellular Ca^{2+} signaling in granulosa cells and the role of their signaling in granulosa cell proliferation. To examine RA regulation of calcium signaling, we first assayed agonist-evoked Ca^{2+} in cells treated with RA or vehicle control. Intracellular Ca^{2+} was labeled with fluo-2 indicator and measured using live cell imaging. Purinergic agonist stimulation in granulosa cells causes release of Ca^{2+} store in the endoplasmic reticulum (ER) to produce an intracellular Ca^{2+} signal. We found that in response to ATP stimulation the amount of released Ca^{2+} was significantly larger in RA treated cells. We next assayed the Ca^{2+} content of the ER by blocking Ca^{2+} uptake into the store with the SERCA pump inhibitor CPA. We found that RA treatment increased ER store content. In addition, we measured Ca^{2+} influx after store depletion and found it to be increased after RA treatment. These results suggest that the RA/Cyp26b1 pathway exquisitely modulates calcium homeostasis in granulosa cells. To examine the roles of RA and calcium signaling in granulosa cell function, we transfected primarily cultured mouse granulosa cells with a Cyp26b1 overexpression construct or siRNA targeting Cyp26b1, or treated the cells with RA or the IP3 inhibitor 2-APB, a blocker of ER Ca^{2+} release and store-operated Ca^{2+} entry. BrdU incorporation assays were then carried out for cell proliferation analysis. Our results showed that Cyp26b1 overexpression suppressed while siRNA targeting Cyp26b1 increased BrdU incorporation, indicating a stimulatory effect of RA on cell proliferation. Importantly, BrdU incorporation is inhibited when the cells were treated with the IP3 inhibitor 2-APB, suggesting that granulosa cell proliferation requires calcium signaling. Overall, our data suggests a converging RA-Ca^{2+} signaling axis in the regulation of granulosa cell function. The results from this study promise a better understanding of the mechanisms of RA action in the ovary as well as new insights into the treatment and prevention of reproductive/endocrine diseases. Supported by the DePaul-RFUMS Collaborative Research Grant.
P40. STAGE SPECIFIC FOLLICLE SELECTION IMPROVES MOUSE OOCYTE MEIOTIC AND DEVELOPMENTAL OUTCOMES DURING IN VITRO FOLLICLE GROWTH (IVFG). Shuo Xiao¹,², Francesca E Duncan¹,², Lu Bai¹,³, Catherine T Nguyen¹,³, Lonnie D Shea⁴, and Teresa K Woodruff¹,².¹Department of Obstetrics and Gynecology, Feinberg School of Medicine, ²Center for Reproductive Science, ³Master of Biotechnology Program, ⁴McCormick School of Engineering, Northwestern University, Evanston, IL 60208, USA

Objective: In vitro follicle growth (IVFG) is an emerging technology, which not only introduces a critical model to study the basic biology of follicle development and oocyte maturation, but also provides fertility preservation options for young cancer patients without reintroducing cancer cells. Our three-dimensional alginate-based encapsulated IVFG system recapitulates the key events of mammalian folliculogenesis and oogenesis. To simplify IVFG experiments, follicles are typically cultured for a defined period and analyzed as a cohort before performing in vitro maturation (IVM). However, follicle growth is not synchronous and depends on the initial follicle size and quality. The aim of this study is to monitor mouse follicles individually with noninvasive markers to determine follicle maturity and oocyte quality in vitro. Methods: 150-180 μm multilayer secondary follicles were isolated from day 16 CD-1 mice and encapsulated in 0.25% alginate. Follicles were cultured individually for specific time periods or to different diameters followed by oocyte transcriptional activity analysis, IVM, spindle morphology investigation, and in vitro fertilization (IVF) and pre-implantation embryo development. Results: The follicle diameter increased from 158.88 ± 15.15 μm on day 0 to 390.42 ± 29.19 μm on day 10. The oocyte increased from 60.20 ± 3.06 μm on day 0 to 73.58 ± 3.76 μm on day 10. During IVFG, the transition of oocyte chromatin configuration from a transcriptionally active to quiescent state occurred when follicles developed to antrum stage with diameter around 300 μm, suggesting the oocyte was in transcriptional active state at the early stage of follicle development, and then transited to the transcriptional quiescent state after the follicle antrum formation. The metaphase II (MII) rate on day 6 was 85%, which was significantly higher than that on days 4, 8 and 10. However, when IVM was performed based on follicle size, follicles that were 300-350 μm had a 93% MII rate, significantly higher than the follicles ranging from < 250 μm, 250-300 μm and > 350 μm, and the MII rate on day 6. 90% of MII oocytes matured from follicles with diameters of 300-350 μm showed barrel-shaped bipolar spindles, which was significantly higher than the oocytes retrieved from other follicle diameter groups. Furthermore, oocytes with follicle diameters between 300-350 μm were fertilized through IVF, and developed to the 2-cell embryo and morula and blastocyst stages, at 84.62%, 80.77% and 46.15%, respectively, which were significantly higher than the rates of the follicles ranging from <250 μm, 250-300 μm and > 350 μm in diameter. These data suggests that the oocytes obtained both nuclear and cytoplasmic maturation when follicle diameter reached 300-350 μm. Conclusion: Our study demonstrates that the stage specific follicle selection, rather than absolute culture time, showed best oocyte meiotic and developmental competence during IVFG, and the immature or prolonged growth of follicles in vitro compromised the gamete quality. Through such individualized follicle monitoring, we will ultimately establish a culture system to grow human follicles in vitro for patients whose reproductive health may be compromised (funded by U54HD076188 and Sherman Fairchild Foundation).
The work from our lab has demonstrated that the egg releases concentrated boluses of zinc into the extracellular environment at the time of activation, which we have termed ‘zinc sparks’. Zinc sparks temporally coincide with intracellular calcium oscillations during fertilization. In previous work, the pattern of calcium oscillations has been correlated with subsequent embryonic developmental outcome. The purpose of this study is to test whether distinct zinc spark profiles are associated with general egg quality. We examined the patterns of extracellular zinc sparks and intracellular calcium transients in eggs that were matured from known optimal (in vivo ovulation, IVO) and suboptimal (in vitro maturation, IVM) conditions after strontium-induced parthenogenetic activation. In the IVO cohort, the number of zinc sparks positively correlated with the calcium transient frequency, with majority of the eggs exhibiting multiple transients and sparks during the imaging period. Eggs displaying fewer calcium oscillations had either a single spark or no spark. In contrast to IVO eggs, calcium oscillation frequency, number of zinc sparks and first spark amplitude were all lower in IVM eggs. These data indicate the existence of heterogeneity of calcium oscillations and zinc spark patterns during egg activation that may correspond to differences in egg quality. Supported by Ferring Pharmaceuticals Inc
P42. UNCERTAINTY IN CLINICAL DATA AND STOCHASTIC MODEL FOR IN-VITRO FERTILIZATION (IVF). K. M. Yenkie\textsuperscript{1, 2}, U. M. Diwekar\textsuperscript{1,2}. \textsuperscript{1}Department of Bioengineering, University of Illinois, Chicago, IL-60607, USA \textsuperscript{2}Center for Uncertain Systems: Tools for Optimization & Management (CUSTOM), Vishwamitra Research Institute, Clarendon Hills, IL-60514, USA

In-vitro Fertilization (IVF) is the most common technique in Assisted Reproductive Technology (ART). It has been divided into four stages; (i) superovulation, (ii) egg retrieval, (iii) insemination/fertilization and (iv) embryo transfer. The first stage of superovulation is a drug induced method to enable multiple ovulation, i.e., multiple follicle growth to oocytes or matured follicles in a single menstrual cycle. IVF being a medical procedure that aims at manipulating the biological functions in the human body is subjected to inherent sources of uncertainty and variability. Also, the interplay of the hormones with the natural functioning of the ovaries to stimulate multiple ovulation as against a single ovulation in a normal menstrual cycle makes the procedure dependent on several factors like the patient's condition in terms of cause of infertility, actual ovarian function, responsiveness to the medication. The treatment requires continuous monitoring and testing and this can give rise to errors in observations and reports. These uncertainties can be observed in the form of measurement noise in the available data. Thus, it becomes essential to look at the process noise and think of a way to account for it and build better representative models for follicle growth. The purpose of this work is to come up with a robust model which can project the superovulation cycle outcome based on the hormonal doses and patient response and hence provide a treatment guideline to enhance the success rate of the procedure. Supported by Vishwamitra Research Institute.
P43. DEVELOPMENT OF THREE DIMENSIONAL HUMAN ENDOCERVIX CULTURES THAT RESPOND TO MENSTRUAL CYCLE MIMIC OF ESTRADIOL AND PROGESTERONE. Sevim Yildiz Arslan1, Yanni Yu1, Joanna E. Burdette3, Mary Ellen Pavone1,4, Thomas J. Hope2 Teresa K. Woodruff1, J. Julie Kim1. 1Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology; 2Department of Cell and Molecular Biology, Northwestern University, Chicago, IL; 3Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL; 4Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL.

The endocervix has both anatomical and biological immune defense mechanisms that participate in the delicate balance between tolerance necessary for conception and protection from pathogens. Our goal was to develop a robust three-dimensional (3D) endocervix model that physiologically mimics the in vivo biology and achieves response to steroid hormones, during a 28-day menstrual cycle. Human endocervix cells were grown on polystyrene scaffolds and the morphologic and hormonal responses of cultured cells were assessed in response to fluctuating levels of estradiol (E2) or progesterone (P4). Endocervical cells survived for the entire 28-day culture and responded to appropriate hormonal treatment. Morphologically, the 3D cultures were composed of a mixed population of cells including epithelial and stromal cells. Treatment with E2 and P4 increased cell growth and proliferation as compared to no treatment control or E2 treatment only. Estrogen receptor and progesterone receptor were detected by immunohistochemical staining. Cells produced MUC16, as well as neutral and acidic mucins, as measured by Periodic Acid Schiff and Alcian Blue staining. In addition, secretory factors of the cultures were measured with the multiplex luminex assay. We identified Interleukin-1 beta (IL-1β) and Leukemia inhibitory factor (LIF) which significantly decreased in the presence of E2 and P4 as compared to the no hormone control at day-26. In order to verify the role of progesterone receptor in regulating IL-1β and LIF secretion, cells were treated with RU486, a progesterone receptor antagonist in combination with estradiol and progesterone. RU486 alleviated the progesterone-driven inhibition of IL-1β and LIF secretion. In summary, a robust 3D endocervical culture was developed and quantifiable endpoints were identified in response to the menstrual cycle mimic of estradiol and progesterone levels. Supported by UH2 grant (NIEHS/OWRH/UH2ES022920).
P44. ANTICANCER EFFECTS OF FURANODIENE IN DOXORUBICIN-RESISTANT HUMAN BREAST CANCER. Z. Zhong1, W. Qiang2*, Y.Wang1,* 1University of Macau, Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, Macau; 2Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine at Northwestern University, Chicago, Illinois

Drug resistance is the main reason for therapeutic failure in advanced cancer treatment. P-gp, a drug transporter protein, is overexpressed in most drug-resistant cancer cell lines, which represent one of the best characterized barriers to chemotherapy. Furanodiene is a sesquiterpene isolated from Rhizoma Curcumae. Accumulating evidence has indicated that in vitro and in vivo furanodiene displays potent anticancer activity in various cancer cell lines including human breast carcinoma MCF-7 cell line. However, whether furanodiene has anticancer effects on drug resistant cancer cells is unknown. We investigated the in vitro anticancer effects of furanodiene on doxorubicin-resistant human breast cancer cells MCF-7/ADR over expressing P-gp protein, originating from the breast cancer cell line MCF-7, using cell viability, cell proliferation, mitochondrial membrane potential, reactive oxygen species (ROS) levels, cell cycle, apoptosis, and P-gp function analysis. The results showed that furanodiene effectively decreased the cell viability and induced cytotoxicity and autophagy in MCF-7/ADR cells. The potential mechanism of furanodiene’s anticancer effect may result from induced oxidative stress and increased cellular calcium level and disrupted the mitochondrial membrane potential. Furthermore, furanodiene enhanced caspase 3/7 activity and apoptosis, which can be blocked by a caspase inhibitor. In addition, no effects of furanodiene on the uptake of Rho 123 and calcium AM and the expression level of P-gp protein in MCF-7/ADR cells indirectly suggested furanodiene is neither a P-gp substrate nor affect P-gp function. Taken altogether, these results suggested that furanodiene suppressed cell viability, blocked proliferation, enhanced apoptosis and autophagy in MCF-7/ADR cells, suggesting its potential as a promising anticancer agent against resistant human breast cancer. This study was supported by the Research Fund of University of Macau (UL016/09Y4/CMS/WYT01/ICMS, MYRG208 (Y3-L4)-ICMS11-WYT and CPG2014-00012-ICMS)) and the Macao Science and Technology Development Fund (077/2011/A3, 074/2012/A3).
Diethyl phthalate (DEP) is a synthetic plasticizer that is widely used in many common consumer products such as detergent bases and cosmetics. Though humans are frequently exposed to DEP, the health effects of this exposure are unclear. Previous studies show that high doses of DEP can damage the nervous system and reproductive organs in male rats. However, only limited information is available regarding the effects of environmentally relevant levels of DEP on the ovary. To investigate the effects of DEP on the neonatal ovary, we tested the hypothesis that DEP exposure alters germ cell and follicle numbers and induces oxidative stress in the ovary. To test this hypothesis, ovaries from neonatal CD-1 mice (postnatal day 4) were collected and cultured with dimethylsulfoxide (DMSO; vehicle control) or DEP (1, 10, 100 μg/ml; n=6-8/group) for 6 days. At the end of culture, the ovaries were processed for histological evaluation of follicle counts and percentages of each follicle type in each treatment group and were compared to control. Additionally, some neonatal ovaries were snap-frozen and further subjected to quantitative polymerase chain reactions to measure expression of the following key antioxidant enzymes Cu/Zn superoxide dismutase (Sod1), glutathione peroxidase (Gpx), and catalase (Cat). The results indicate that DEP does not alter the percentage of germ cells, primordial follicles, primary follicles, and pre-antral follicles compared to control (n=3; p>0.05). The results also indicate that DEP exposure significantly increases expression of Sod1 at the 100μg/ml dose (n=3; p<0.05), but does not affect Cat and Gpx expression at any dose. In conclusion, DEP exposure does not affect early folliculogenesis, but it partially affects the expression of a key antioxidant enzyme, which in turn may impair ovarian function in later life. Supported by NIHP01 ES022848 (JAF), EPA RD-83459301 (JAF), and an Environmental Toxicology Fellowship (PH).
Bisphenol A (BPA) is a monomer that is widely incorporated in polycarbonate plastics and epoxy resins. BPA is an endocrine disrupting chemical that can cause ovarian damage and infertility in animal models. For example, BPA (25 μg/ml) inhibits the growth of cultured mouse antral follicles compared to control. BPA (10 and 25 μg/ml) decreases estradiol levels synthesized by treated follicles compared to control. Additionally, exposure to BPA (25 μg/ml) significantly increases the expression of insulin receptor substrate 2 (Irs2) compared to control in antral follicles isolated from adult C57Bl6 mice (5.8 - fold change compared to control; p<0.05; n=3-5). Interestingly, IRSs are also part of the insulin-like growth factor 1 (IGF1) signaling pathway that is involved in ovarian follicular development and function. Other factors in the IGF1 pathway include IGF1, IGF1 receptor, and IGF binding proteins (IGFBP). In the current study, we hypothesized that exposure to BPA selectively alters expression levels of factors in the IGF1 signaling pathway in mouse cultured antral follicles. To test this hypothesis, we mechanically isolated antral follicles from CD-1 mouse ovaries (aged 30-34 days) and cultured them in vehicle control (dimethylsulfoxide) or BPA (1, 10, 25 μg/ml) for 96 hours. Following the cultures, follicles were subjected to measurements of expression of selected factors in the IGF1 signaling pathway by quantitative polymerase chain reactions. We specifically examined the following genes: Igf1, Igf2, Igfbp4, Igfr1, thymoma viral proto-oncogene 3 (Akt3), phosphatidylinositol 3-kinase catalytic delta polypeptide (Pik3cd), ribosomal protein S6 (Rps6), myelocytomatosis oncogene (Myc), and Irs2. Our results indicate that BPA exposure (25 μg/ml) significantly decreases expression of Igf2 (0.3 - fold change compared to control; p<0.05; n=3) and significantly increases expression of Irs2 and Igfbp4 (3.6 and 1.7 - fold change compared to control; p<0.05; n=3). Additionally, BPA exposure (10 μg/ml) significantly decreases expression of Igfr1 (0.5 - fold change compared to control; p<0.05; n=3) and significantly increases expression of Pik3cd (1.8 - fold change compared to control; p<0.05; n=3). In contrast, BPA exposure did not alter the expression of Igf1, Akt3, Myc, and Rps6 compared to control (p>0.05; n=3). These data suggest that BPA exposure may affect the health of mouse cultured antral follicles by altering expression of factors related to the IGF1 signaling pathway. Supported by: NIH ES019178 (JAF) and a Billie A. Field Fellowship (AZG).
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