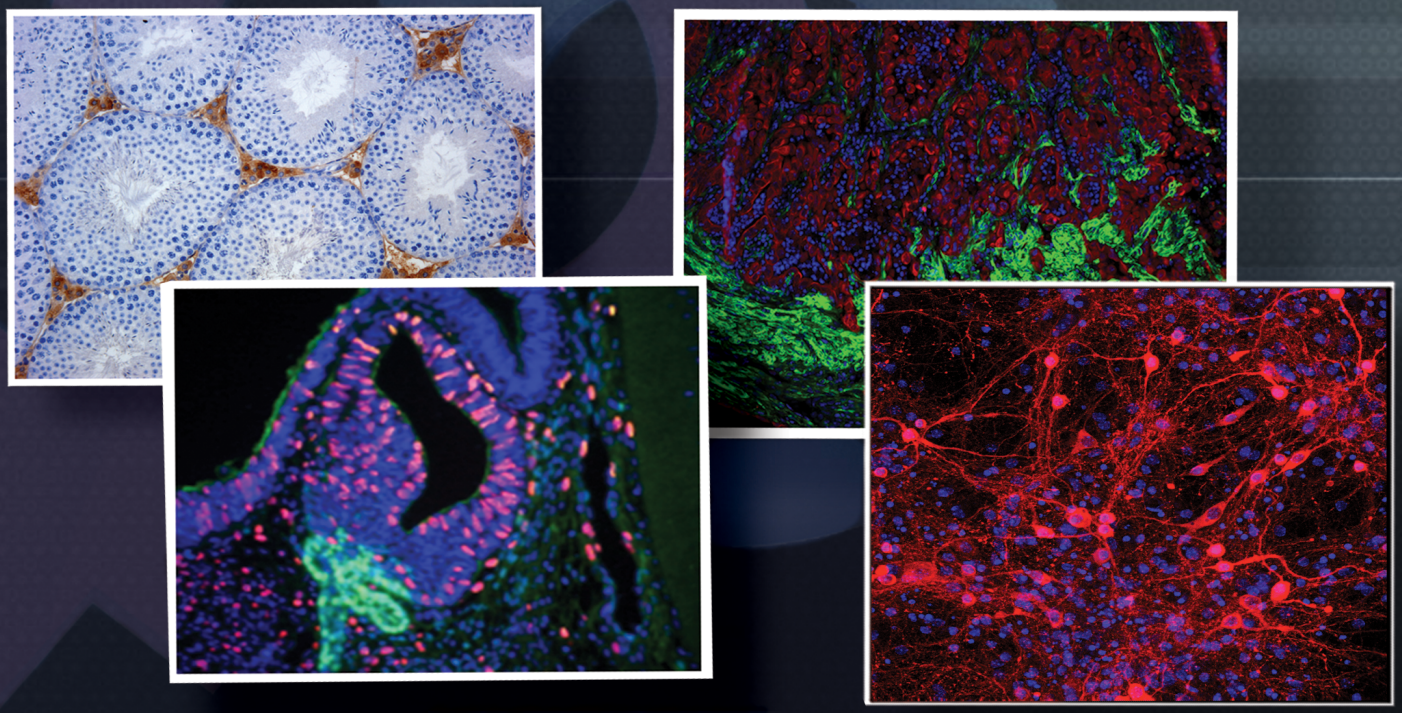
ISRS

5th Illinois Symposium on Reproductive Sciences



October 13-14 2013

Southern Illinois University Carbondale
Touch of Nature Environmental Center,
Makanda Illinois

SIU School of Medicine

SIU CARBONDALE Southern Illinois University

5th Illinois Symposium on Reproductive Sciences

Hosted by

Southern Illinois University

School of Medicine

October 13-14, 2013

Carbondale, Illinois

Welcome to ISRS 2013!!

Carbondale welcomes you to the 5th Illinois Symposium on Reproductive Sciences (ISRS), hosted by Southern Illinois University (SIU). This event blossomed out of the Mini-symposium in Reproductive Biology at Northwestern and the UIC-UIUC Biennial Reproductive Biology meeting. In 2008, the faculty at Northwestern, University of Illinois at Chicago (UIC) and University of Illinois at Urbana-Champaign (UIUC) came together to merge the two meetings and include SIU as one of the four host institutes. The first annual meeting was held in 2009, hosted by Northwestern. The ISRS meeting serves to showcase the quality of research in reproductive sciences, both in fertility and disease, within Illinois institutions as well as fostering collaborations and the exchange of scientific discoveries across the Midwest.

ISRS plays a key role in the development of careers for reproductive biologists during all phases of their training and beyond. The mission of the ISRS is to promote the productive exchange of ideas, initiate beneficial collaborations and perpetuate an environment in which trainees and young investigators, as well as senior scientists, can present their work in an academic setting in order to advance reproductive science and bring fresh ideas to research involving reproduction. To this end, the ISRS organizing committee is composed of trainees from Southern Illinois University, Northwestern University, the University of Illinois Chicago, and the University of Illinois at Urbana-Champaign. The trainee-centric mission of the ISRS meeting is also served by their integral role in shaping the meeting's program.

Reproductive Biology Research at Southern Illinois University

The SIU School of Medicine has over 100 laboratories working on both basic and clinical sciences. Outstanding scientists and pioneers in the field of reproduction including Andrzej Bartke and Lonnie Russell have been a part of the Department of Physiology at SIU School of Medicine (SIUSOM). Their remarkable contributions to this field, won them several accolades, internationally. We currently have reproductive biologists with research interests including development of the gonads and reproductive tract, neural and hormonal control of reproductive function, lactation, biological rhythms and temporal control of fertility, nutritional effects on reproduction, establishment and maintenance of pregnancy and gynecological cancers. The department is devoted to outstanding research and is committed to train budding scientists. We are honored to be hosting the 5th meeting of the ISRS and look forward to an exciting meeting! Welcome, everyone!

The cover page was designed by Jyoti Kapali (SIUSOM). The images were provided by several laboratories in the Department of Physiology at SIUSOM.

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Sunday, October 13

2:00 pm~	Registration Opens (Friends Room Lobby)
	Welcome Refreshment (Indian Room)
	Poster Mounting in Sledgefoot Lounge
4:00-4:15 pm	Opening Remarks (Friends Room)
4:15-5:30 pm	Oral Session 1: T1 - T5 (Friends Room)
5:30-5:45 pm	Refreshment Break (Indian Room)
5:45-6:45 pm	Keynote Lecture by Nancy Weigel, Ph.D. (Friends Room)
6:45-8:00 pm	Dinner (Freeberg Hall)
8:00-9:00 pm	Poster Session 1: Odd numbered posters manned (Sledgefoot Lounge)
9:00-11:00 pm	Social and Dance (Freeberg Hall and Patio)

Monday, October 14

7:00-8:00 am	Breakfast (Freeberg Hall)
7:30-8:30 am	Poster Session 2: Even numbered posters manned (Sledgefoot Lounge)
8:45-10:00 am	Oral Session 2: T6 – T10 (Friends Room)
10:00-10:15 am	Refreshment Break (Indian Room)
10:15-11:30 am	Oral Session 3: T11 – T15 (Friends Room)
11:30-11:45 am	Refreshment Break (Indian Room)
11:45 am-12:00 pm	Remarks by Chancellor, Rita Cheng, and Background on the Bialy Lecture Series (Friends Room)
12:00-1:00 pm	Bialy Lecture by Blanche Capel, Ph.D. (Friends Room)
1:00 pm~	Award Presentation (Friends Room)
	Closing Remarks (Friends Room)
	Lunch Boxes are available at Indian Room.
	All Posters must be taken down.

GABRIEL BIALY LECTURESHIP IN REPRODUCTIVE PHYSIOLOGY

The Gabriel Bialy Lectureship in Reproductive Physiology Endowment was established in the Department of Physiology through the SIU School of Medicine Foundation by Dr. Do Won Hahn (a distinguished research fellow at R.W. Johnson Pharmaceutical Research Institute in Raritan, N.J.) and Dr. Andrzej Bartke, who was then chairman of Department of Physiology. They were postdoctoral fellows in reproductive physiology at the Worcester Foundation in Shrewsbury, Massachusetts, in the late 1960's where they both formed a career long friendship with Dr. Gabriel Bialy.

Dr. Gabriel Bialy (1931-2009) was born in Poland and came to the United States after World War II. He received his undergraduate degree from Ohio State University and his doctorate from the University of Wisconsin. In 1959, he joined the laboratory of Gregory Pincus at the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, where he performed a wide variety of studies in reproductive physiology, with a particular emphasis on the pharmacological actions of steroid hormones. During that period, he was a member of the research team that developed the first oral contraceptive.

Dr. Bialy actively served NIH from 1971 in various administrative positions until his death making him one of the institute's longest-serving staff members.

During his career, he authored or co-authored numerous papers on reproductive biology, reproductive pharmacology and contraceptive development as well as stewarding a number of research collaborations, in particular between American and Indian researchers to further studies in order to improve reproductive health and maternal and child health in India.

Dr. Do Won Hahn established the Gabriel Bialy Lecture in Reproductive Biology in 2001 in recognition to Dr. Bialy's contributions to the field of reproductive biology and his service as a program official at NICHD. Dr. Bialy passed away on Feb. 17, 2009, but we continue to honor his contributions to reproductive biology research through this annual lectureship.

Keynote Lecture

"Phosphorylation, Cell Signaling, and Progesterone Receptor Action"

Nancy L. Weigel, Ph.D.
Professor
Scott Department of Urology
Department of Molecular and Cellular Biology
Baylor College of Medicine

Nancy Weigel received her B.S. from Cornell University and went on to earn her M.S and Ph.D. from John Hopkins University. Her thesis work was on the structure of *salmonella typhimurium* HPr in the laboratory of Dr. Saul Roseman. Next, she went to Houston, Texas to study steroid receptors during her post-doctoral training at Baylor College of Medicine with advisors Dr. Bert O'Malley and Dr. William Schrader. She was an NIH Postdoctoral Fellow from 1979-1981. Now, Dr. Weigel is a professor for the Scott Department of Urology and the Department of Molecular and Cellular Biology at Baylor College of Medicine.

Dr. Weigel has contributed greatly to the field of reproductive sciences. Her laboratory studies steroid receptor action and cell signaling in normal as well as in breast and prostate cancer tissue. Of major contribution to the field was the discovery of phosphorylation sites in the human progesterone receptor. Dr. Weigel's lab is also investigating the roles of splice variants of the androgen receptor, which gets reactivated in prostate cancer. Dr. Weigel's lab has also done much research on vitamin D receptors and has found that vitamin D is not beneficial in a sub-set of prostate cancer expressing a specific genomic rearrangement. These studies are advancing our understanding of prostate cancer and the role of steroid receptors.

Dr. Weigel has published over 100 peer reviewed journal articles and has written many book chapters. She has been the Associate Editor of Molecular Endocrinology since 2009. Also, she has been a board member of Steroids since 1994 and Hormones and Cancer since 2009. Dr. Weigel is an active member of the Endocrine Society. Currently, she is serving on the Awards Committee and has served on several committees including the Bridge grant review committee. She has also participated in many national and international review panels. Throughout the years at Baylor College of Medicine, Dr. Weigel has received several awards for her teaching and research including the Searle Scholar (1983-1986), the Marc Dresden teaching award (2006), the Roy O. Greep award (Endocrine Society) (2008), the Fulbright and Jaworski Faculty Excellence in Teaching Award (2009), the Prostate Cancer Foundation A. David Mazzone-PCF Challenge Award investigator (2012), and the SWIU/SBUR Award for Excellence in Urologic Research (2013).

Gabriel Bialy Lectureship in Reproductive Biology

"Macrophages: New Players in Vascular Patterning and Signaling"

Blanche Capel, Ph.D.
James B. Duke Professor
Department of Cell Biology
Duke University

Dr. Capel received her Bachelor of Arts as a double major in Literature/Art History and Molecular Biology from Hollins College in Roanoke, Virginia in 1968. From there, she studied Genetics at Haverford College in Haverford, Pennsylvania before continuing on and earning her Ph.D in Genetics from the University of Pennsylvania in Philadelphia, Pennsylvania in 1989. Following her Ph.D, she completed her postdoctoral training in mammalian development at the National Institute for Medical Research in Mill Hill, London in 1993. Dr. Capel then became an Assistant Professor at Duke University Medical Center in Durham, North Carolina in 1993 and has risen through the ranks of Associate and Full Professor to become a James B. Duke Distinguished Professor beginning in 2010.

Throughout her career, Dr. Capel has made tremendous contributions to the field of reproductive biology, publishing over 100 papers. Research during her post-doctoral training with Dr. Robin Lovell-Badge led to the discovery of the male sex determination gene, *Sry*. As an independent researcher, Dr. Capel pioneered the currently accepted model that primary sex determination relies on the antagonism between male and female signaling pathways and transcriptional regulation. Her current work focuses on the genetic pathways that control the reorganization of the bi-potential gonad into either testis or ovarian morphology and how internal and external factors, such as animal strain and temperature, respectively, can control this differentiation process. She was the first to develop organ culture techniques for studying the morphology of ovarian and testicular organogenesis, and to use live imaging to determine the role of the vasculature in gonadal morphology.

In addition to her research, Dr. Capel has served on the board of directors for the Society for Developmental Biology, the Society for the Study of Reproduction, and the Board of Scientific Advisors at the Jackson Laboratory. Over the course of her career, Dr. Capel has received numerous awards, including the Gorden G. Hammes Faculty Teaching Award at DUMC in 2006 and became a Fellow of the American Association for the Advancement of Science in 2011.

Program
5th Illinois Symposium on Reproductive Science (ISRS)

Sunday, October 13

2:00pm **Registration Opens**
Location: Friends Room Lobby

4:00pm **Opening Remarks**
***Anushka Dikshit**, Trainee Committee Chair, Department of Physiology, Southern Illinois University*
Location: Friends Room

Oral Session 1 - "Gene Expression and Reproductive Development"
Location: Friends Room
*Moderators: **Elizabeth Sefton**, Department of Obstetrics and Gynecology, Northwestern University; **Mary Laws, Ph.D.**, Department of Comparative Biosciences, University of Illinois Urbana Champaign*

4:15pm T1 **Gene Expression Analysis and Epigenetic Changes in Undifferentiated Male Germ Cells (uMGCs): Effects of Oxidative Stress and Aging**
Amber E. Kofman and Christopher J. Payne. Department of Pediatrics-Human Molecular Genetics, Department of Obstetrics and Gynecology, Center for Reproductive Science, Northwestern University

4:30pm T2 **Post-thaw Motility of Frozen Boar Sperm is not Related to In Vitro Fertilization Success**
Bradford W. Daigneault, Kelli A. McNamara, Phillip H. Purdy, Robert V. Knox, Rebecca L. Krisher, David J. Miller. Department of Animal Sciences, University of Illinois, Urbana-Champaign; USDA-ARS-NCGRP-NAGP, Fort Collins, Colorado

4:45pm T3 **Elucidation of a Genetic Cascade Regulating the Development of the External Genitalia**
Congxing Lin, Yan Yin, Sheila M. Bell, Michael G. Veith, Sung-Ho Huh, Hong Chen, David M. Ornitz, and Liang Ma. Department of Medicine, Department of Developmental Biology, Washington University School of Medicine, St. Louis; Division of Neonatology and Pulmonary Medicine, University of Cincinnati College of Medicine

5:00pm T4 **Visualization of follicle formation and development in the mouse ovary**
Dallas A. Vanorny and Kelly E. Mayo. Department of Molecular Biosciences and Center for Reproductive Science, Northwestern University

5:15pm T5 **Gene reporter profile of oocytes and spatial mapping of follicle activation using a VASA/GDF-9/ZP3 triple transgenic reporter line**
Marília H. Cordeiro, So-Youn Kim, Francesca E. Duncan, João Ramalho-Santos, Teresa K. Woodruff. Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University; Doctoral Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, Coimbra, Portugal

5:30pm **Refreshments Break**
Location: Indian Room

5:45pm **Keynote Lecture: "Phosphorylation, Cell Signaling, and Progesterone Receptor Action"**
Nancy Weigel, Ph.D., Professor Scott Department of Urology, Department of Molecular and Cellular Biology, Baylor College of Medicine
Introduction: ***Jyoti Kapali****, trainee committee member, Department of Physiology, Southern Illinois University*
Location: Friends Room

6:45pm **Dinner**
Location: Freeberg Hall

8:00pm **Poster Session 1: Odd numbered posters manned**
Location: SledgeFoot

9:00-11:00pm **Social and Dance**
Location: Freeberg Hall and Patio

Monday, October 14

7:00-8:00am **Breakfast**
Location: Freeberg Hall

7:30-8:30am **Poster Session 2: Even numbered posters manned**
Location: SledgeFoot

Oral Session 2 – "**Endocrine Signaling and Disruption"**
Location: Friends Room
Moderators: ***Jyoti Kapali****, trainee committee member, Department of Physiology, Southern Illinois University;* ***Scott Convissar****, trainee committee member, Department of Physiology and Biophysics, University of Illinois at Chicago*

8:45am T6 **FSH-induced expression of key differentiation genes is dependent on the activity of the insulin-like growth factor-1 receptor in human granulosa cells**
Sarah Baumgarten, Scott Convissar, Michelle Fierro, Nicola Winston, Jennifer Hirshfeld-Cytron, Humberto Scoccia, and Carlos Stocco. Univeristy of Illinois at Chicago, College of Medicine, Chicago

9:00am T7 **Bisphenol A down-regulates rate-limiting Cyp11a1 to acutely inhibit steroidogenesis in cultured mouse antral follicles**
Jackye Peretz and Jodi A. Flaws. Department of Comparative Biosciences, University of Illinois, Urbana-Champaign

9:15am T8 **Hyperandrogenemia and progesterone receptor expression and function in kisspeptin neurons**
Arnon Gal, Joe Cacioppo, Lin Po-Ching, CheMyong (Jay) Ko, Department of Pathobiology. Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, IL

9:30am T9 **Characterization of the ERβ-Regulated Genes, RERG and SGK1, in Endometriosis**
Monsivais D, Dyson M, Navarro A, Pavone ME, Yin P, Malpani S, Bulun SE. Division of Reproductive Biology Research, Northwestern University, Chicago, IL

9:45am T10 **Suppression of Endometriosis by Targeting Estrogenic and Inflammatory Pathways: Studies in a Mouse Model**
Yuechao Zhao, Ping Gong, Yiru Chen, Milan K. Bagchi, Robert N. Taylor, Kendall W. Nettles, John A. Katzenellenbogen and Benita S. Katzenellenbogen. Department of Molecular and Integrative Physiology, Department of Chemistry, University of Illinois at Urbana-Champaign, Department of Obstetrics and Gynecology, Wake Forest School of Medicine, Winston-Salem, NC, Department of Cancer Biology, Scripps Research Institute, Jupiter FL

10:00-10:15am **Refreshments Break**
Location: Indian Room

Oral Session 3 - "**Reproductive Cancer"**
Location: Friends Room
Session moderators: ***Kirsten Eckstrum****, trainee committee member, Department of Molecular and Integrative Physiology, University of Illinois Urbana Champaign;* ***Erfan Eilati****, Department of Physiology, Southern Illinois University*

10:15am T11 **Steroidogenic Factor 1 Drives Aggressive Prostate Cancer Cell Proliferation and Plays a Critical Role in Tumor Growth**
Samantha R Lewis, William A Ricke, Joan S Jorgensen. Departments of Comparative Biosciences and Urology, University of Wisconsin, Madison, WI. Endocrinology and Reproductive Physiology Program

10:30am T12 **Loss of *Cdh1* and *Trp53* in the uterus induces chronic inflammation with modification of the tumor microenvironment**
Genna R. Stodden, Mallory E. Lindberg, Mandy L. King, James A. MacLean II, Francesco J. DeMayo, John P. Lydon, Kanako Hayashi. Department of Physiology, Southern Illinois University School of Medicine, Carbondale, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston

10:45am T13 **Spontaneous Transformation of Oviductal Epithelial Cells: The Missing Link to Understanding Ovarian Carcinogenesis**
Michael P. Endsley, Georgette Heyrman, Suzanne M. Quartuccio, Daniel D. Lantvit, and Joanna E. Burdette. Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago,

11:00am T14 **Molecular signaling in the human fallopian tube- Insights into proliferation and p53 expression**
S.L. Eddie, S.M. Quartuccio, J.A. Shepherd, R. Kothari, and J.E. Burdette. Department of Medicinal Chemistry and Pharmacognosy and Department of Obstetrics and Gynecology, University of Illinois at Chicago

11:15am T15 **Dose dependent effects of flaxseed on Estradiol metabolism in the laying hen model of ovarian cancer**
Anushka Dikshit, Chunqi Gao, Dale Buchanan Hales. Department of Physiology, Southern Illinois University School of Medicine, Carbondale IL.

11:30am **Refreshments Break**
Location: Indian Room

11:45am Remarks by Chancellor, **Rita Cheng, Ph.D.**, Introduction: **Buck Hales, Ph.D.**
Background on the Bialy Lecture Series: **James MacLean, Ph.D.**
Location: Friends Room

12:00pm **Bialy Lecture: "Macrophages: New Players in Vascular Patterning and Signaling"**
Blanche Capel, Ph.D., James B. Duke Professor, Department of Cell Biology, Duke University
Introduction: ***Jackye Peretz Ph.D****. trainee committee member, Department of Comparative Biosciences, University of Illinois Urbana-Champaign*
Location: Friends Room

1:00pm **Award Presentation: Phil Jensik, Ph.D., Buffy Ellsworth, Ph.D., and Kanako Hayashi, Ph.D.**
Closing Remarks
Sarah Baumgarten, University of Illinois at Chicago
Location: Friends Room

ABSTRACTS

Oral Session 1- *Gene Expression & Reproductive Development*

T1. **Gene Expression Analysis and Epigenetic Changes in Undifferentiated Male Germ Cells (uMGCs): Effects of Oxidative Stress and Aging**

Amber E. Kofman and Christopher J. Payne

The genomic integrity of mammalian cells is subjected to numberous insults over the entirety of their lifespan. Resultant damage impacts both thegenetic fidelity of the DNA sequence and the epigenetic organization of the chromatin. These insults include aging and oxidative stress that, while detrimental to all cell types, are especially damaging to adult stem cell populations. Our lab studies undifferentiated male germ cells (uMGCs), a population that contains adult spermatogonial stem cells. Our research demonstrates that young mice (3-wk-old) treated with the longevity-promoting compound rapamycin exhibit increased transcription of oxidative stress response genes (*Sod1, Gsr,* and *Alad*) and stem cell self-renewal genes (*Gfra1, Nanos2,* and *Foxo1*) in their uMGCs. Conversely, aged (>1 yr) untreated uMGCs exhibit decreased transcription of oxidative stress response genes and increased transcription of histone deacetylase encoding genes characteristic of differentiating spermatogonia. These results suggest that aged uMGCs have diminished self-renewal potential when compared to younger cells or those treated with rapamycin and raise important, fundamental questions about gene regulation within an adult stem cell population. Our future directions will examine key genome-wide epigenetic modifications involving histone deacetylase family members and DNA methylation levels that may predispose uMGCs to these transcriptional changes.

T2. **Post-thaw Motility of Frozen Boar Sperm is not Related to In Vitro Fertilization Success**

Bradford W. Daigneault[1], Kelli A. McNamara[1], Phillip H. Purdy[2], Robert V. Knox[1], Rebecca L. Krisher[1], David J. Miller[1] [1]Department of Animal Sciences, University of Illinois, Urbana-Champaign, Illinois [2] USDA-ARS-NCGRP-NAGP, Fort Collins, Colorado

Using cryopreserved boar sperm rather than liquid semen for in vitro fertilization (IVF) improves IVF consistency by eliminating inter-ejaculate variability. However, the cryopreservation and thawing process results in reduced post-thaw motility and IVF success. Semen samples are often screened for motility prior to use for IVF. Sperm that fall below a designated motility threshold would be discarded. Our objective was to determine if post-thaw motility of boar sperm was predictive of successful IVF by identifying a threshold of acceptable motility. Semen from boars of different breeds was collected from commercial AI units and cooled to 15°C for overnight shipment prior to freezing. Sperm from 16 boars were cryopreserved, thawed at 50°C for 20 sec, diluted in Androhep Cryoguard extender to 35 x 10^6 total sperm/mL and held at 37°C for ~ 7 min. Post-thaw motility was evaluated using Computer Automated Sperm Analysis (Hamilton Thorne IVOS). Total motility ranged from 21 to 46%. IVF was performed using each sample and was repeated in two to three independent experiments. An average of 99 oocytes per boar was used for this experiment (n=1586). Regression analyses using Proc Mixed (SAS 9.0) were employed to determine if relationships existed between post-thaw motility and IVF outcomes. The mean percent (LSM) of fertilized oocytes using frozen-thawed sperm from all boars was 59%. Mean embryo cleavage and blastocyst development was 54% and 16%, respectively. Motility regression against fertilization measures included percentage of oocytes fertilized, monospermic fertilization and polyspermic fertilization. Motility was also regressed against embryo cleavage, blastocyst development, and blastocyst cell number. There was no relationship between post-thaw motility and any IVF measure except for a tendency for motility to be related to monospermic fertilization (P = 0.09). Our results indicate that post-thaw motility of frozen-thawed boar sperm is not a strong indicator of fertilization and developmental potential in IVF. Additional sperm traits such as sperm morphology, membrane integrity and other characteristics may prove useful for developing a model that is predictive of successful IVF. Individual cryopreserved boar sperm ejaculates may produce acceptable results with IVF even if post-thaw motility is low.

Supported by Agriculture and Food Research Initiative Competitive Grant no. 2010-85112-20620 from the USDA National Institute of Food and Agriculture.

T3. **Elucidation of a Genetic Cascade Regulating the Development of the External Genitalia**

Congxing Lin, Yan Yin, Sheila M. Bell, Michael G. Veith, Sung-Ho Huh, Hong Chen, David M. Ornitz, and Liang Ma. Department of Medicine, and Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO. Division of Neonatology and Pulmonary Medicine, University of Cincinnati College of Medicine, Cincinnati, OH.

The acquisition of the external genitalia allowed mammals to cope with terrestrial-specific reproductive needs for internal fertilization, and thus it represents one of the most fundamental steps in evolution towards a life on land. How genitalia evolved remains obscure, and the key to understanding this process may lie in the developmental genetics that underpins the early establishment of the genital primordium, the genital tubercle (GT).Development of the GT is similar to that of the limb, which requires precise regulation from a distal signaling epithelium. However, whether outgrowth of the GT and limbs is mediated by common instructive signals remains unknown. In this study, we used comprehensive genetic approaches to interrogate the signaling cascade involved in GT formation in comparison with limb formation. We demonstrate that the FGF ligand responsible for GT development is FGF8 expressed in the cloacal endoderm. We further showed that forced *Fgf8* expression can rescue limb and GT reduction in embryos deficient in WNT signaling activity. Our studies show that the regulation of *Fgf8* by the canonical WNT signaling pathway is mediated in part by the transcription factor SP8. *Sp8* mutants elicit appendage defects mirroring WNT and FGF mutants, and abolishing *Sp8* attenuates ectopic appendage development caused by a gain-of-function β-catenin mutation. These observations indicate that a conserved WNT-SP8-FGF8 genetic cassette is employed by both appendages for promoting outgrowth, and suggest a deep homology shared by the limb and external genitalia.

T4. **Visualization of follicle formation and development in the mouse ovary**

Dallas A. Vanorny and Kelly E. Mayo, Department of Molecular Biosciences and Center for Reproductive Science, Northwestern University, Evanston, IL 60208.

Morphogenesis of the mammalian ovary requires the correct spatial and temporal organization and function of multiple cell types, which is coordinated by juxtacrine, paracrine, and endocrine signaling mechanisms. Within the embryonic ovary, ovigerous cords containing partially fragmented syncytia of germ cells undergo follicle histogenesis, whereby somatic pregranulosa cells invade syncytia and encapsulate individual germ cells to form primordial follicles. As select cohorts of resting follicles are recruited, the oocyte grows, whereas pregranulosa cells become cuboidal and proliferate to form a multi-laminar structure enveloped by a basement membrane that supports a thecal layer. Following stimulation by the pituitary gonadotropins FSH and LH, follicles continue to grow, form antra through the differentiation of mural and cumulus granulosa cells, and ovulate a meiotically competent oocyte, while remaining somatic cells of the follicle luteinize to form corpora lutea. Using mouse lines that express fluorescent reporter proteins in specific ovarian cell types, as well as multiple imaging modalities, we have visualized cellular organization and interactions during the formation, growth and maturation, and ovulation and luteinization of ovarian follicles. Further, we have applied these techniques to investigate the role of Notch signaling, a highly conserved juxtacrine signaling system, during follicle histogenesis and development. At birth, Notch active somatic cells are arranged in an intricate cage-like pattern around germ cell syncytia and can be observed sending projections around individual germ cells. Notch active cells are seen in close association with collagen fibrils, detected by second harmonic generation, which could potentially facilitate pregranulosa cell migration and cellular reorganization during follicle histogenesis. Time-lapse imaging of *ex vivo* cultured ovaries reveals diverse cellular behaviors, including ovigerous cord fragmentation, cell-cell interactions, cell migration, and germ cell apoptosis. Additionally, Notch active cells can be visualized within growing follicles, cumulus oocyte complexes, and corpora lutea, suggesting important roles for Notch signaling between the various cell types of these structures. These approaches provide powerful techniques to follow the identity and behavior of multiple cell types in the ovary in a dynamic fashion, facilitating investigations into the cellular interactions, processes, and organization necessary for follicle formation and development.

Supported by NIH/NICDH P01 HD021921, NIH/NIGMS T32 GM08061, and NIH/NINDS P30 NS054850

T5. **Gene reporter profile of oocytes and spatial mapping of follicle activation using a VASA/GDF-9/ZP3 triple transgenic reporter line,**

Marília H. Cordeiro[1,2,3], So-Youn Kim[1], Francesca E. Duncan[1], João Ramalho-Santos[2], Teresa K. Woodruff[1]

[1]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2]Center for Neuroscience and Cell Biology, Coimbra, Portugal, [3]Doctoral Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, Coimbra, Portugal

Folliculogenesis is a complex process in which primordial follicles are selectively activated to grow and ultimately produce a female gamete capable of being ovulated and fertilized. To date, there is no robust method to track follicles/oocytes during the course of development *in vivo*. In this study, we generated a triple transgenic mouse in which oocyte-specific promoters (VASA, GDF-9 and ZP3), active at distinct stages of oocyte and follicle development, drive the expression of genes encoding exogenous fluorescent proteins (EGFP, mCherry and AmCyan, respectively). The mice were generated by pronuclear microinjection 1:1:1 ratio of the three independent DNA constructs (injections performed by NU Transgenic Core). The co-injection resulted in random insertion of exogenous DNA into the mouse genome and 9 independent transgenic lines were generated – 1 double and 8 triple transgenic founders. We then selected a line (#5570) in which the 3 transgenes segregated together after 4 generations and that had relatively robust expression for all three transgenes. We examined the expression pattern of the promoter-driven fluorescent proteins in ovaries from different age mice and found a follicle-class dependent pattern consistent with prior studies using VASA, GDF9, and ZP3 promoters. We examined the expression intensity of the oocytes and found that levels differed even within follicle classes, suggesting an underlying activity or intrinsic stratification of function not previously appreciated. Finally, we used the mouse line to examine the location of the first wave of follicle activation in the postnatal mouse. The first activated/growing follicles appear on the dorsal side of the ovary in a relatively posterior location (on the ovarian side nearest the rete ovarii). In conclusion, our triple transgenic mice represent the first oocyte specific reporter lineage in which the signal of fluorescent proteins can be use to follow oocyte gene activity and follicle activation. This transgenic mouse line will be an important tool for expanding our basic understanding of the mechanisms controlling follicle selection, oocyte quality and ovarian developmental dynamics. Similar utility may be found in the male offspring.

This work is supported by a Portuguese PhD Fellowship SFRH/BD/33886/2009 and endowed funds through the Watkins Chair in OB/Gyn, Northwestern University.

Oral Session 2- *Endocrine Signaling and Disruption*

T6. **FSH-induced expression of key differentiation genes is dependent on the activity of the insulin-like growth factor-1 receptor in human granulosa cells**

Sarah Baumgarten, Scott Convissar, Michelle Fierro, Nicola Winston, Jennifer Hirshfeld-Cytron, Humberto Scoccia, and Carlos Stocco. University of Illinois at Chicago, College of Medicine, Chicago, IL

During their progression from the preantral to the preovulatory stage, undifferentiated follicular granulosa cells proliferate and differentiate into two distinct populations: mural granulosa cells that line the wall of the follicle and cumulus granulosa cells that surround the oocyte. In rodents, it has been established that oocyte-secreted factors oppose the action of follicle stimulating hormone (FSH) on cumulus cells preventing their differentiation into mural cells; whereas, oocyte removal allows FSH stimulation of mural markers in cumulus cells. In humans, many aspects of the process of granulosa cell differentiation are not well understood because of the paucity of a source of preantral undifferentiated granulosa cells. We hypothesized that cumulus cells obtained from patients undergoing *in vitro* fertilization (IVF) treatment could serve as an experimental approach to study human granulosa cell differentiation, once the negative influence of the oocyte has been removed. Cumulus and mural granulosa cells were collected from follicular aspirates of IVF patients, cultured in serum free medium, and characterized according to gene and protein expression. Markers of mural granulosa cell differentiation, including the luteinizing hormone receptor (LHR) and steroidogenic genes such as aromatase, P450 side chain cleavage (P450scc), and steroidogenic acute regulatory protein (StAR) were expressed at significantly lower levels in cumulus cells when compared with mural cells. Additionally, cumulus cells responded to FSH with the up-regulation of several steroidogenic genes, while luteinized mural cells did not. These results mirror those found using mouse or rat undifferentiated granulosa cells. Next, we sought to investigate the actions of FSH and insulin-like growth factor 1 (IGF1) in human cumulus. These two factors are essential for folliculogenesis and synergize on the regulation of differentiation genes in laboratory animals. Strikingly, the FSH-induced stimulation of LHR, aromatase, P450scc, and StAR, which are markers of granulosa cell differentiation, was abolished by the inactivation of the IGF1 receptor (IGF1R) signaling. Additionally, inhibiting the activity of the IGF1R also abolished FSH-induced phosphorylation of Akt in the cumulus granulosa cells. Our findings provide strong evidence suggesting that isolated cumulus cells in culture behave as undifferentiated granulosa cells and could be used to study the mechanisms by which FSH induces granulosa cell differentiation in humans. Additionally, we demonstrate, for the first time, that in humans the stimulation of key differentiation genes by FSH depends on the activity of the IGF1R. This work was supported by NIH R01HD057110 (CS) and T32 HL07692-21 (SCB).

T7. **Bisphenol A down-regulates rate-limiting *Cyp11a1* to acutely inhibit steroidogenesis in cultured mouse antral follicles**

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Bisphenol A (BPA) is an endocrine disrupting chemical used in polycarbonate plastic products and the epoxy resin lining of food and beverage cans. Once released from these products, BPA readily enters the body and affects physiological processes such as steroidogenesis. Previous studies have shown that exposure to BPA decreases sex steroid hormone production in mouse antral follicles. The current study tests the hypothesis that BPA first decreases the expression levels of the steroidogenic enzyme cytochrome P450 side-chain cleavage (*Cyp11a1*) and steroidogenic acute regulatory protein (*Star*) in mouse antral follicles, leading to a decrease in sex steroid hormone production *in vitro*. Further, the current study tests the hypothesis that these effects are acute and reversible after removal of BPA. Exposure to BPA (10μg/mL and 100μg/mL) significantly decreased expression of *Cyp11a1* and *Star* beginning at 18h and 72h, respectively, compared to controls. Exposure to BPA (10μg/mL and 100μg/mL) significantly decreased progesterone levels beginning at 24h and decreased androstenedione, testosterone, and estradiol levels at 72h and 96h compared to controls. Further, after removing BPA from the culture media at 20h, expression of *Cyp11a1* and progesterone levels were restored to control levels by 48h and 72h, respectively. Additionally, expression of *Star* and levels of androstenedione, testosterone, and estradiol never decreased compared to controls. These data suggest that BPA acutely decreases expression of *Cyp11a1* as early as 18h and this reduction in *Cyp11a1* may lead to a decrease in progesterone production by 24h, followed by a decrease in androstenedione, testosterone, and estradiol production and expression of *Star* at 72h. Therefore, BPA exposure likely targets *Cyp11a1* and steroidogenesis, but these effects are reversible with removal of BPA exposure.

Supported by National Institute of Health R01 ES019178 and P20 ES 018163

T8. **Hyperandrogenemia and progesterone receptor expression and function in kisspeptin neurons**

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Kisspeptin is a key neuropeptide that is synthesized and secreted by hypothalamic Kisspeptin neurons (KPN) in the anteroventral paraventricular nucleus (AVPV) and arcuate nucleus (ARC). At the time of preovulatory rise of circulating estradiol, this neuropeptide is discharged from KPN and directly stimulates gonadotropin releasing hormone (GnRH) neurons in the hypothalamus to secrete GnRH, which in turn induces preovulatory luteinizing hormone surge. In this study, we aimed to determine the functional significance of progesterone receptor (PGR) expression in KPN under the hypotheses that 1) PGR plays a negative regulatory role in kisspeptin expression, and 2) that testosterone downregulates PGR expression. We first examined hypothalamic PGR expression pattern in cycling mice by immunohistochemistry. We found that both, hypothalamic AVPV and ARC KPN express PGR, and that the expression being highest in the AVPV during proestrus. We then knocked out *Pgr* gene selectively in the Kisspeptin neurons using Cre-LoxP approach (K-PGRKO, Kisspeptin-specific knockout) to determine if the loss of PGR expression alters Kisspeptin expression and/or secretion. The impact of *Pgr* gene deletion was first assessed by evaluating the onset of puberty and fertility, because altered kisspeptin expression/secretion are expected to impact such reproductive parameters. K-PGRKO mice had significantly earlier onset of puberty (29 days vs 32 days; p=0.007) and completely lost fertility by 5 months of age (p=0.03). At that age, induction of ovulation by PMSG/hCG was successful, but the ovulatory capacity of K-PGRKO mice was reduced (mean of 21 vs 14 oocytes; p=0.02). By immunohistochemical evaluation, we then examined the effect of elevated androgen levels using a mouse model of hyperandrogenemia - thERαKO mice (theca cell-specific ERα knockout) in which *ERα* gene is deleted in the ovarian theca cell (Lee S et. al., 2009). PGR expression was significantly lower by 30% in hypothalamic AVPV KPN of the thERαKO mice (n=3) compared to wild type littermates (n=3) or thERαKO (n=3) whose ovaries were surgically replaced with WT ovaries. We conclude that expression of PGR in hypothalamic KPN is biologically important for fertility and normal onset of puberty through modulation of the hypothalamic pituitary ovarian axis. We further conclude that high levels of androgens contribute to KPN PGR downregulation, and might be a possible cause for infertility in conditions in which hyperandrogenemia is present.

T9. **Characterization of the ERβ-Regulated Genes, RERG and SGK1, in Endometriosis**

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In endometriosis, stromal and epithelial cells from the endometrium form adhesions outside of the uterine cavity and persist in response to estrogen. Endometriosis affects 6-10% of women of reproductive age and is a major cause of chronic pelvic pain and infertility. In stromal cells derived from ovarian endometriosis, a hypomethylated ESR2 promoter region results in elevated ERβ mRNA and protein expression relative to the normal endometrium. Despite the important role of estradiol in mediating disease in endometriosis, the precise contribution of its receptor, ERβ, has not been fully characterized. Using gene expression profiles and ERβ ChIP-on-ChIP and ChIP-Seq datasets, we identify RERG and SGK1 as ERβ-target genes in primary human endometriotic stromal cells. Compared to the endometrium of healthy women, the mRNA and protein levels of RERG and SGK1 are significantly elevated in the stromal cells and endometriotic tissues from diseased patients. Using Real-Time RT-PCR, ERβ siRNA, and ERβ ChIP, we validate that RERG and SGK1 are ERβ targets in endometriotic stromal cells. To determine the contribution of ERα to the regulation of these genes, we used the ERα and ERβ specific agonists, PTT and DPN. We find that DPN-induced ERβ activity is a more potent activator of RERG and SGK1 than PPT-activated ERα. RERG is a member of the small GTPase family of proteins that is transcriptionally regulated by estrogen. Here we find that RERG contributes to endometriotic stromal cell proliferation and is post-transcriptionally modified by phosphorylation. The phosphorylation of RERG occurs in response to PGE2, and using the PKA inhibitor H89, we show that it is a PKA-dependent phosphorylation. Furthermore, we demonstrate that RERG phosphorylation increases its affinity for GTP. SGK1 is a serum and glucocorticoid-regulated kinase that contributes to the survival of endometriotic cells. In addition to being transcriptionally regulated by ERβ in response to estradiol, we observe that SGK1 expression is induced by pro-inflammatory and oxidative stress. This suggests that in endometriosis, SGK1 expression is induced in response to stress to confer apoptotic resistance. Our results show that estradiol, acting primarily via ERβ, regulates the transcription of RERG and SGK1 and that their expression is further potentiated by pro-inflammatory stress. Overall, these findings highlight the interplay between steroid hormone signaling via ERβ and PGE2-induced inflammation, two important mechanisms that contribute to disease in endometriosis. Research supported by NIH R37HD038691-12S1.

T10. **Suppression of Endometriosis by Targeting Estrogenic and Inflammatory Pathways: Studies in a Mouse Model**

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Endometriosis is a common estrogen-dependent gynecological disorder in which endometrial tissue forms inflammatory lesions in extra-uterine sites, leading to pelvic pain and reduced fertility. Current therapies focus on reducing systemic levels of estrogens; however, these treatments are not fully effective and are associated with side effects and frequent disease recurrence. Developed by us as novel estrogen receptor (ER) antagonists, chloroindazole (CLI) and oxabicycloheptene sulfonate (OBHS) showed strong ER-dependent anti-inflammatory activity, thus making them attractive candidates for possible treatment of endometriosis. In this study, surgical endometriosis was induced in ovariectomized mature immunocompetent mice supplemented with estradiol (E2). Several key events during endometriotic-like lesion establishment, including cell proliferation, cyst formation, vascularization and lesion growth, were strikingly interrupted by co-treatment of CLI or OBHS with E2, indicating their potential for preventing recurrence of the disease. To further evaluate their therapeutic effects, CLI or OBHS alone was administrated to intact animals beginning after lesion establishment, and this led to marked lesion regression and suppressed inflammation, based on: (1) greatly reduced production of cytokines and chemokines known to be highly stimulated in human endometriosis (i.e., IL6, CCL2, and RANTES); (2) suppression of NF-κB activation in endometriotic cells; and (3) marked inhibition of infiltrating immune cells into endometriotic lesions (i.e., T cells and macrophages). Notably, ligand treatment also greatly reduced neuron innervation, which is reported to be associated with inflammation and pain in patients. Importantly, low side effects are suggested by the fact that at effective doses neither ligand caused stimulation of the reproductive tract or alteration of estrous cycling. Hence, CLI and OBHS have very good ability to both restrain endometriosis progression and elicit regression of established lesions by suppressing estrogenic and inflammatory pathways. Our findings suggest that these compounds may have good potential as novel preventive and therapeutic agents for endometriosis treatment.

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Oral Session 3- *Reproductive Cancer*

T11. **Steroidogenic Factor 1 Drives Aggressive Prostate Cancer Cell Proliferation and Plays a Critical Role in Tumor Growth**

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The dependence of prostate cancer on androgens provides a targeted means of treating advanced disease. Unfortunately, androgen deprivation therapies that block gonadal steroid synthesis ultimately become ineffective, leading to the deadly form of prostate cancer. While there are likely many ways this transition to treatment refractive cancer can occur, one important factor is the ability of prostate adenocarcinoma cells to acquire machinery for *de novo* steroidogenesis and therefore, fuel their own growth. The mechanisms by which prostate cancer cells initiate and maintain steroidogenesis are unknown. We hypothesize that Steroidogenic Factor 1 (NR5A1, ADBP4, SF1), a key regulator of steroidogenesis in normal endocrine tissues, is expressed in castration resistant prostate cancer where it stimulates aberrant steroidogenesis and fuels malignant growth. Notably, SF1 is not expressed in normal prostate tissue. Our results indicated that SF1 was absent in benign prostate cell lines as expected, but present in aggressive prostate cancer cell lines. When ectopic SF1 expression was induced in benign prostate epithelial cells (BPH1), increased steroidogenic enzyme expression, steroid synthesis, and cell growth was observed. Converse experiments using shRNA-mediated knockdown of SF1 in an aggressive prostate cancer cell line (WR3) diminished steroidogenic activity and inhibited cell growth. SF1 depleted cells also exhibited signs of decreased cell cycle progression and defects in cell division. Xenograft studies were performed to evaluate the role of SF1 in tumor growth. Results comparing control versus SF1 deficient prostate cancer cells showed that knockdown of SF1 substantially impaired tumor growth under the kidney capsule in both castrated and intact nude mouse hosts. Based on these data, we conclude that aberrant SF1 expression in aggressive prostate cancers stimulates steroidogenesis and promotes aggressive tumor growth. These findings present a new potential mechanism and therapeutic target for deadly castration resistant prostate cancer. This work was supported by The University of Wisconsin-Madison Graduate School and NIH 5T32-HD041921-07.

T12. **Loss of *Cdh1* and *Trp53* in the uterus induces chronic inflammation with modification of the tumor microenvironment**

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Last year, we reported the effects of conditional ablation of *Cdh1* and *Trp53* in the uterus using *Pgr*-driven *Cre*. At 2 months of age, mice with conditional ablation of *Cdh1* and *Trp53* ($Cdh1^{d/d}$ $Trp53^{d/d}$) showed abnormal uterine development (metaplasia) including epithelial invasion into the myometrium, abnormal glandular development, as well as loss of PGR and ESR1. These results indicate that ablation of *Cdh1* and *Trp53* induces invasive phenotypes of endometrial carcinomas without steroid hormone signaling. However, $Cdh1^{d/d}$ $Trp53^{d/d}$ mice at 2 months of age did not exhibit any evidence of metastasis to the peritoneal cavity and distant organs. Our subsequent studies examining 6-month old $Cdh1^{d/d}$ $Trp53^{d/d}$ mice clearly demonstrate architectural features characteristic of type II endometrial carcinomas, including focal areas of papillary differentiation, protruding cytoplasm into the lumen (hobnailing) and severe nuclear atypia. Further, $Cdh1^{d/d}$ $Trp53^{d/d}$ tumors in 12-month old mice were highly aggressive, and metastasized to nearby and distant organs within the peritoneal cavity, such as mesentery and peri-intestinal adipose tissues, demonstrating that tumorigenesis in this model proceeds through the universally recognized morphologic intermediates associated with type II endometrial neoplasia. We also observed abundant cell proliferation and complex angiogenesis in the uteri of $Cdh1^{d/d}$ $Trp53^{d/d}$ mice. Our microarray analysis found that most of the genes regulated in the uteri of $Cdh1^{d/d}$ $Trp53^{d/d}$ mice were categorized by their involvement in inflammatory responses. The gene transcripts in $Cdh1^{d/d}$ $Trp53^{d/d}$ tumors were confirmed by quantitative RT-PCR. This finding clearly highlighted that many genes, especially those related to inflammation, were upregulated in $Cdh1^{d/d}$ $Trp53^{d/d}$ tumors, but not in single ablation of *Trp53* or *Cdh1* uteri. We also confirmed that key molecules related to inflammatory signaling, such as p-STAT3, cytokines, chemokines, MMPs and COX2 were abundant in the uteri of $Cdh1^{d/d}$ $Trp53^{d/d}$ mice. Thus, these results indicate that the tumor microenvironment under chronic inflammation represents a central regulator of tumor development leading to cell invasion, dissemination and metastasis. Further, CD68 and CD163, markers for tumor associated macrophages, were also detected in the uteri of $Cdh1^{d/d}$ $Trp53^{d/d}$ mice, suggesting that an inflammatory tumor microenvironment, with immune cell recruitment, is further augmenting tumor development in $Cdh1^{d/d}$ $Trp53^{d/d}$ uteri. In summary, ablation of *Cdh1* and *Trp53* in the mouse uterus initiates chronic inflammation with modification of the tumor microenvironment and promotes aggressive endometrial carcinomas. Supported by NIH/NCI R15CA179214.

T13. **Spontaneous Transformation of Oviductal Epithelial Cells: The Missing Link to Understanding Ovarian Carcinogenesis.**

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Epithelial ovarian cancer is the most lethal gynecological malignancy. The most common ovarian cancer subtype is high-grade serous carcinoma (HGSC). It has long been hypothesized that the origin of HGSC was the ovarian surface epithelium; however, emerging evidence implicates epithelial cells derived from the distal fallopian tube (TECs).

Since the tubal hypothesis emerged, most studies focused on engineering tumorigenic TEC through genetic manipulations. These studies are integral to determining the gene profiles that are altered in HGSC; however, they do not provide information regarding the stepwise progression leading to HGSC. One key element that contributes to spontaneous HGSC is aging. This study focused on developing a model of age-dependent TECs that are spontaneously transformed.

Two novel murine oviduct cell line (MTECs) variants were created. MTEC cells were isolated from mouse oviducts and were considered low (MTEC-L) when passaged less than 25 times. The second MTEC variant was serially passaged (to mimic aging) and examined from passages 85-120 (MTEC-H). MTEC-L and MTEC-H cells were confirmed to be oviductal based upon expression of markers including oviductal glycoprotein-1, acetylated tubulin, and Pax8.

Comparative analyses between MTEC-L and MTEC-H cells determined that MTEC-H cells have many altered phenotypic properties. First, MTEC-H are highly proliferative compared to MTEC-L due to disrupted regulation of the Rb pathway, depicted by reduced expression of $p16^{INK4A}$ (CDKN2A) and elevated expression of cyclin E1, phospho-RB1 (Ser780), c-myc, and PCNA. Second, MTEC-H cells have elevated p53 protein expression without a concurrent increase p21. This observation suggests that p53 is stabilized or potentially mutated, which is observed in 96% of HGSC tumors. Third, MTEC-H cells exhibit enhanced resistance to DNA damaging agents, such as cisplatin. Lastly, a fundamental phenotypic hallmark of cellular transformation is significant clonogenicity and anchorage-independent growth. MTEC-H cells displayed typical transformed in vitro functions, suggesting that prolonged MTEC passaging spontaneously induced transformation.

Transformed cells in culture are not always tumorigenic. Thus, MTEC-L and MTEC-H were xenografted subcutaneously (*s.c.*) into nude mice and monitored until palpable tumors formed. Within 57 days, all mice (5/5) injected with MTEC-H cells developed palpable tumors, which are continuing to grow. Collectively, this spontaneous tumorigenesis model offers the potential to uncover the premalignant changes that may yield novel therapies to improve the patients' quality of life. Furthermore, this model demonstrates that spontaneous transformation of TEC through aging modifies many of the pathways identified in HGSC such as p53, Rb, and resistance to cisplatin.

T14. **Molecular signaling in the human fallopian tube- Insights into proliferation and p53 expression**

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Ovarian cancer is the most lethal gynecological malignancy. This is due to patients presenting in late stage disease when the cancer has already metastasized. Contributing to delayed diagnosis is uncertainty regarding the origin of ovarian cancer. Ovarian cancer was thought to arise from the ovarian surface epithelium. Recently, it has been hypothesized that the distal fimbriae of the fallopian tube is the origin of 'ovarian' cancer, particularly the serous histotype. A potential precursor lesion, the 'p53 signature', has been identified in the fallopian tube. However, little is known about the normal p53 expression in the fallopian tube and how it might contribute to ovarian cancer.

To examine the role of the fallopian tube in serous cancer, a three-dimensional (3D) culture method was utilized. This 3D culture supports normal tissue architecture and retains epithelial-stromal contact using alginate hydrogels. This system was utilized for examination of normal human fimbriae collected from women undergoing salpingectomy. Donated tissues were treated with factors hypothesized to have a role in the onset of serous cancer for 2 and 7 days. Insulin was used to stimulate glycolysis, hydrogen peroxide as an oxidative stress mimetic, and the steroid hormones estrogen and progesterone were utilized to investigate hormone controlled cell growth.

Fimbriae cultures maintained normal morphology, with both epithelial sub-types (secretory and ciliated) retained in culture (PAX8 and acetylated-tubulin staining). Fimbriae cultures were further analyzed for changes in proliferation, DNA damage, and induction of p53 expression, which is thought to be linked to the onset of serous cancer. Initial findings reveal *ex vivo* p53 expression can be acquired. These hotspots of p53 expression are secretory cell enriched similarly to those seen *in vivo*, but are not always expressed concomitantly with γH2AX, a marker of DNA damage. Oxidative stress and insulin significantly increased the rate of proliferation. The effect of the steroid hormones on proliferation was less notable, with progesterone treatment consistent with possible promotion of fallopian epithelial cell death.

Characterization of biological function within potential preneoplastic lesions is integral to ovarian cancer research. Without identifying the origin, current research may be focusing on genetic or protein changes that are different only because they are compared to the wrong normal tissue. Targeted therapies might fail due to identification of targets in the wrong cell population. To evaluate the p53 signature as a potential precursor lesion, we must understand the factors that induce p53 expression and its functional significance.

T15. **Dose dependent effects of flaxseed on Estradiol metabolism in the laying hen model of 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 feeding 10% flaxseed diet decreases the severity and incidence of ovarian cancer in chickens. The two major components of flaxseed with anti-oncogenic properties are the omega-3-fattyacids and the lignan, Secoisolaricirescinol diglucoside (SDG). The omega-3-fattyacids, as we have shown before, decrease the synthesis of inflammatory prostaglandins. SDG is converted to Enterolactone (EL) and Enterodiol (ED) by the gut flora. ED and EL have weak anti-estrogenic/estrogenic properties, which allow them to antagonize 17β-estradiol (E2) by binding its receptors. The enzymes Cyp3A4, Cyp1B1 and Cyp1A1 act on 17β-estradiol (E2) to form 16-hydroxy E2 (16-OHE), 4-hydroxy E2 (4-OHE) and 2-hydroxy E2 (2-OHE), respectively. 16-OHE and 4-OHE are strongly estrogenic, on oxidation, 4-OHE is converted to quinones, forming depurating adducts that can ultimately become carcinogenic. Low 2OHE/16OHE ratio in urine or serum is correlated to increased risk of cancer in hormone sensitive organs.

The objective of this study was to determine the optimum dose of flaxseed affecting estrogen metabolism end points. Fifty, 2-year-old White leghorn chickens were fed 0%, 5%, 10% and 15% flaxseed in their diets for four months. Ovaries were collected and confirmed to be normal from gross pathology and histology. mRNA levels were measured using qPCR while protein levels were analyzed using IHC and western blotting. The levels of ED and EL were assayed using LC MS/MS analysis. Serum analysis for 2-OHE and 16-OHE was performed using an ELISA kit from Estramet (Immuna Care Corp.).

As expected, the levels of ED and EL increased with increase in percentage of flaxseed in the diet, with 15% having the highest concentrations. Estrogen receptor (ERα and ERβ) levels deceased in a dose dependent manner with flax diet. Cyp3A4 and Cyp1B1 mRNA levels were also down regulated in flax-fed hens. Cyp1A1 was undetectable in the ovary but its expression increased with flax diet in the liver. Serum analysis indicated that the 2OHE/16OHE ratio was significantly increased in the flax-fed birds, in turn showing the beneficial effects of flax.

Our dosage study indicates that 15% flaxseed diet is the most effective in decreasing the levels of carcinogenic catechol estradiols in the ovary of normal chickens without any toxic effects. This demonstrates the preventative effects of flaxseed in ovarian cancer.

[Supported by NIH AT00408]

Poster Sessions

Arranged Alphabetically by Last Name of Presenting Author
(Poster Session 1 – Odd Numbered Abstracts / Poster Session 2 – Even Numbered Abstracts)

P1. **Alteration in Endometrial Expression of Pro-inflammatory Th17 cells and their cytokine Interleukin-17 indicates a Pro-inflammatory Milieu in the Eutopic Endometrium of Women With Endometriosis-Related Infertility.**

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Endometrial receptivity is a complex phenomenon that requires a stage-specific and coordinated series of interactions between systemic and endometrial factors to allow attachment and controlled invasion of the fetal allograft. Endometriosis is an inflammatory, estrogen-stimulated disease affecting women and features clinical signs of chronic pelvic pain and infertility. However, only about 50% of women with endometriosis are infertile. To explore the phenomenon of infertility in patients with or without endometriosis, we performed a blinded study in which 100 human eutopic endometrial biopsies collected at the mid- to late-secretory stage of the menstrual cycle were evaluated for the expression of specific T cells. We focused on T-helper17 (Th-17) cells as these leukocytes are known to have pro-inflammatory effects in systemic immunity. We also evaluated the expression of Interleukin-17 (IL17), a specific cytokine secreted by Th17 cells, which when elevated is implicated in peritoneal inflammation and adhesions. Specific antibodies for Retinoic-acid-receptor-related Orphan Receptors-γt (RORγt- transcription factor for Th-17 cells) and Interleukin-17 were used to evaluate expression using the immunoperoxidase-ABC method. The numbers of RORγt+ cells per 10mm^2 of tissue were quantitated and IL17 expression was evaluated by H-score analysis. Moreover, endometrial cell lines were treated with recombinant human IL17 (recHuIL17) protein and observed for alteration in proliferation using thymidine incorporation assays as well as cell counts. The results showed that measuring the numbers and/or expression of these markers in the eutopic endometrium was a powerful assessment of endometrial receptivity when correlated with patient history. Infertile women with endometriosis had a significantly higher proportion of the pro-inflammatory Th-17 cells as compared to fertile women with or without endometriosis. Likewise, IL17 expression showed a higher H-score in infertile patients with endometriosis than in the fertile patient population. Our *in vitro* experiments with endometrial cell lines showed that treatment with low physiological doses (<10pg/ml) of recHuIL17 had no effect on cell proliferation when compared to the control, but higher pathological doses (>10pg/ml) inhibited cell proliferation as seen by both thymidine incorporation and cell counts. In summary, our results indicate that infertile women with endometriosis display a pro-inflammatory endometrial environment during the receptive phase which in turn alters the normal characteristics of endometrial cells and thus may make their uterus non-receptive to embryo implantation. Understanding this regulatory balance within the eutopic endometrial environment may lead to improved prediction and treatment of endometriosis related infertility and may have significant economic and health benefits. (Supported by NIH U54 HD40093 to RAN)

P2. THE POTENTIAL ROLE OF JMJD6 IN THE REGULATION SFLT-1 GENE EXPRESSION IN HUMAN TROPHOBLAST: IMPLICATIONS FOR PREECLAMPSIA

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Preeclampsia affects 5 to 8 percent of pregnant women worldwide. The placenta plays a major role in development of preeclampsia. Angiogenesis is essential to maintain oxygen supplies to the fetus through normal placental development. Factors that govern vascular remodeling during pregnancy and are highly expressed by the placenta include Placenta Growth Factor (PGF), and its receptor (Flt1). PGF high affinity binding to two Flt-1 splice variants receptors; a membrane bound (mFlt-1) and soluble Flt-1 (sFlt-1). Preeclampsia is associated with increased generation of this sFlt-1 isoform variant that sequesters available PGF causing an imbalance in angiogenesis and increased constriction of blood vessels leading to preeclampsia symptoms; hypertension and proteinurea after 20 weeks gestation. Over-expression of sFlt-1 as well as subjecting rodents to hypoxic conditions induces a preeclamptic-like state. Hypoxia up-regulates sFlt-1 expression in human trophoblast. Nitric oxide (NO) generation during hypoxia remarkably reverses the effect of hypoxia on sFlt-1 expression in human trophoblast. In addition, NFkB activation increases sFlt-1 expression. Recently, Jumonji domain containing protein 6 (Jmjd6) has been shown to regulate the splicing of Flt-1 in endothelial cells, although the role of Jmjd6 in trophoblast cells is unknown. We hypothesize that hypoxia and/or NFkB activation, decreases Jmjd6 expression which increases sFlt-1 in trophoblast. We found Jmjd6 is expressed in Sw71, JEG3 (trophoblast cell lines) and primary human trophoblast. Hypoxia (1% O_2) increases Jmjd6 RNA about two fold but does not affect Jmjd6 protein levels in JEG3 and SW71 cells. Over-expression of p65, an active subunit of the NFkB complex, has no significant effect on Jmjd6 RNA; however, it increases sFlt-1 RNA by two fold in JEG3 cells. Moreover, under hypoxia, Jmjd6 over-expression did not reverse the increases in sFlt-1 RNA expression in JEG3 and SW71 cells. Finally, treating trophoblast with SNP, a nitric oxide donor, decreases Jmjd6 and sFlt-1 expression under hypoxia conditions, but had no effect under normoxic conditions in SW71, JEG3, and the primary trophoblast. Future studies are to characterize Jmjd6 expression in preeclamptic versus normal placenta by western blot and immuno-histochemistry. We concluded that Jmjd6 is expressed in trophoblast, but expression levels do not significantly influence sFlt-1 expression in trophoblast. Therefore, it could be that hypoxia increases Jmjd6 expression, but it may induce post-translation modification(s) that alter its function in trophoblast cells. Understanding the relationship between Jmjd6 and the regulation of sFlt-1 may provide new therapeutic avenues for preeclamptic women.

P3. **Gene Profiling of Endocervical Tissues During Menstrual Cycle**

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Hormone fluctuation throughout the menstrual cycle contributes to changes in immune responses that render the upper female reproductive tract vulnerable to HIV infection. It is thought that HIV infection is more likely to occur during luteal phase because of dampening of protective immune responses. As it is becoming more evident that hormone production as well as function of immune cells are ideal candidates for HIV transmission, it is essential to understand their role on HIV infection in women. Dendritic cells (DCs) are potent antigen-presenting cells which can sample antigens at the mucosal surface, potentially making them one of the first cells that encounters HIV. DCs exert antiviral activity, which also can be regulated by sex hormones. The objective of this study is to identify genes in endocervical tissues of women that are differentially expressed in the follicular versus the luteal phases of the menstrual cycle using gene expression profiling. Cervical tissue samples of different hormonal status were obtained from routine hysterectomies to study the correlation of hormones to the subset of endocervical genes that may influence immune cell function. A microarray using the IIlumina platform was performed with 8 tissues from follicular and 8 tissues from luteal phases of the menstrual cycle. Data analysis revealed 450 genes significantly different between the two phases, with a p-value < 0.05 and a fold change cutoff of 1.5. Categorization of these genes, using Ingenuity Pathway Analysis, GeneGo as well as DAVID revealed genes associated with collagen fibril morphology, differentiation, proliferation, dendritic cell and leukocyte migration, among others. We focused on genes associated with dendritic cell maturation and migration as well as hormone regulation by assessing their differential expression using real time PCR as well as immunohistochemical staining to examine protein expression. Some of these genes include Leptin, STAT1, CREB1, PENK, IL-18, HLA-DRB4, FSCN1, among others. This is the first microarray analysis comparing gene expression in endocervical tissues in cycling women. This study identifies key genes and potentially novel mechanisms influenced by ovarian hormones and will significantly enhanced our understanding of HIV infection in women.

P4. **Effects of KNDy Neuron Peptides on Prolactin and Luteinizing Hormone in Pup-Deprived Lactating Rats**

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Hypothalamic arcuate nucleus KNDy neurons co-express the neuropeptides **K**isspeptin (Kp), **N**eurokinin B (NKB) and **Dy**norphin (Dyn) and these peptides may input to hypophysiotropic neurons, controlling luteinizing hormone (LH) and prolactin (PRL) secretion. Kp-10, a product of the *Kiss1* gene, increases LH secretion under a variety of endocrine conditions. Kp-10 can also increase prolactin (PRL), but the response is dependent on estrogen status in rodents. The role of NKB in regulating LH is less clear, however mice with mutations in the NKB gene or its receptor exhibit hypogonadotropic hypogonadism. Senktide is a commonly used NKB receptor agonist. Dyn acts on kappa opioid receptors (KOR) and KOR agonists decrease LH and increase PRL. Lactation is an endocrine state characterized by reduced fertility, high PRL levels and low LH levels. The roles and regulation of these peptides during lactation is sparsely understood. The objective of this study was to evaluate the effects of Kp-10, Senktide and DynA(1-13) on PRL and LH release in pup-deprived lactating rats. Lactating dams were implanted with a lateral intracerebroventricular (ICV) cannula on day 2 postpartum. On day 6, dams were implanted with a jugular cannula and pups were removed. Twenty-four hours later, dams were injected ICV with 3.0 nmol Kp-10, 3.0 nmol or 600 pmol Senktide, 0.5 nmol, 5.0 nmol or 50 nmol DynA, or 5 µl artificial cerebral spinal fluid vehicle. Blood samples were taken over a 2 hour period; plasma PRL and LH levels determined via radioimmunoassay. In Kp-10 treated rats, circulating PRL levels were low before treatment and showed a modest 2.8-fold increase 10 minutes after injection. LH levels initially were low before Kp-10 treatment, but were increased 10- to 15-fold at 60 and 120 minutes. Neither dose of Senktide had a significant response on either PRL or LH. Treatment with 50 nmol DynA increased PRL levels 43- to 45-fold at 10 and 20 minutes; treatment with 5.0 nmol DynA increased PRL 20-fold 10 minutes after injection. The 0.5 nmol dose of DynA did not have a significant effect on PRL. No significant change was measured in LH with any dose of DynA. PRL and LH levels were low and unchanged in vehicle-treated rats. These data indicate that LH is responsive to Kp-10 stimulation, in spite of reduced fertility. PRL is highly responsive to DynA stimulation and modestly responsive to Kp-10. Kp and Dyn may have roles in regulating PRL or LH during lactation.

P5. **Intrinsic and Extrinsic Factors Modulating Development of Hypothalamic Arcuate Nucleus Neurons**

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The hypothalamic arcuate nucleus (ARC) is a vital portion of the brain maintaining homeostasis and linking the brain to the endocrine system. Proopiomelanocortin (Pomc) and neuropeptide Y (Npy) neurons regulate food intake, growth-hormone-releasing hormone (Ghrh) neurons are crucial to body size, and kisspeptin neurons are important for fertility and the onset of puberty. Despite the central function of these neurons, intrinsic and extrinsic factors impacting their development remain unclear. It is important to understand how these neurons develop, as disruptions can lead to obesity, dwarfism, or impaired fertility.

We have previously demonstrated that Notch signaling prevents ARC progenitors from adopting a Pomc, Npy, or Ghrh fate. In our current study, we hypothesize that persistent activation of Notch will impede kisspeptin neuronal differentiation, whereas an absence of Notch will allow progenitors to prematurely differentiate into an overabundance of kisspeptin neurons, in parallel to what was previously observed for the other neuron types. To address this, we utilize mice with a conditional deletion of Rbpj-κ, an essential cofactor for Notch receptor signaling (LOF) or constitutive activation of Notch1 (GOF) in *Nkx2.1* expressing cells in the ventricular zone of the ventral hypothalamus. Interestingly, both LOF and GOF mice show a drastic reduction or complete ablation of kisspeptin neurons. This would suggest that Notch needs to be inactivated in order for ARC progenitors to differentiate, but is also necessary for kisspeptin neuronal differentiation.

In addition to intrinsic pathways, exposure to extrinsic factors may alter development or function of ARC neurons. Bisphenol A (BPA) is a common plasticizer which can act as an endocrine disrupting chemical (EDC). Studies have shown a correlation between levels of BPA in pregnant women and adverse outcomes in their offspring such as obesity and impaired fertility. BPA is detectable in the umbilical cord as well as fetal tissue, implying the prenatal environment exposes an embryo to EDCs. We hypothesize that prenatal exposure to BPA may change ARC neuron number or affect function. Preliminarily, environmentally relevant doses of BPA do not alter neuron number, but function has yet to be determined. Taken together, these studies suggest Notch signaling impacts ARC development, a critical brain center for controlling metabolism and reproduction. Supported by P01 ES022848, R01 DK076647, T32 ES007326.

P6. **Edn2-iCre transgenic mouse shows strong heterogeneous expression in the ovary, oviduct, and uterus.**

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Endothelin-2 (ET-2) is a potent vasoconstrictive peptide. Though similar to ET-1, recent studies suggest that ET-2 may act through distinct pathways, necessitating deeper study. ET-2 plays a role in inflammation, cancer metastasis, and macrophage invasion. In the ovary, it is transiently expressed and tightly regulated in the periovulatory ovary, where it aids in ovulation by inducing contraction of the thecal cell layer. Here, we present a transgenic mouse line that expresses iCre (codon-improved Cre recombinase) under the regulation of the promoter of the *endothelin-2* (*edn2*) gene, which was developed as a novel model for characterizing the expression of ET-2 and for conditional deletion of genes in cells where ET-2 is expressed. A vector was generated containing *iCre*, a polyadenylation signal sequence, and an frt-neomycin-resistance-frt cassette. Two homologous regions of the *edn2* gene flanking the ATG start codon were isolated from a BAC (bacterial artificial chromosome) clone and inserted upstream and downstream of the iCre-pA-FNF cassette. Homologous recombination was used to re-insert the cassette into the BAC plasmid. Following purification, the plasmid was injected into pronuclei of fertilized C57BL/6 mouse eggs, and resulting embryos were implanted into pseudopregnant mice. Twelve Edn2-icre transgene-containing lines of mice were established, and four lines were bred with ROSA26 reporter mice. Offspring were used to localize iCre-expressing cells through X-gal staining. Characterization of the staining pattern revealed that iCre was expressed most strongly in ovarian granulosa cells, the walls of the oviduct, and the myometrium of the uterus. Expression was also noted in the coronary vasculature, the lungs, the eyes, and the pituitary. This novel mouse model will be a useful tool for future studies on the expression and function of Endothelin-2 including its role in reproduction.

P7. **Directed differentiation of human embryonic stem cells into prostatic organoids *in vitro.***

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Prostate cancer is the most common type of cancer and the second leading cause of deaths in American men. However, the etiology of the disease remains poorly understood. Evidence in the rodent suggests a developmental basis for the adult disease. Data from our laboratory using a rat model shows that developmental exposures to natural or environmental estrogens (e.g. Bisphenol A or BPA) can predispose to prostate carcinogenesis with aging, a process referred to as estrogen reprogramming or developmental estrogenization. Epidemiologic evidence in humans suggests an increase in prostate cancer incidence with maternal estrogenic exposures. However, direct determination of estrogen reprogramming of the human prostate has been hard to accomplish due to the lack of a developmental human prostate model for testing.

Herein, we present a pioneer *in vitro* human prostate developmental model that permits direct testing of estrogenic exposures. In short, induction of human embryonic stem (hES) cells into prostatic organoids was undertaken using sequential exposure to a series of stage-specific growth factors, steroids and nutrients. hES cell cultures were driven into definitive endoderm (DE), the embryological origin of the prostate gland, by exposure to activin. Induction into prostatic fate determination was performed by exposure to growth factor cocktails post-DE differentiation and organoid development was carried out in a 3D milieu supplemented with prostatic media. To confirm that the organoids obtained are of prostatic nature, immunohistochemistry was performed utilizing specific prostatic markers. After establishing this human prostate model, it was utilized to assess whether developmental estrogenization occurs in the human as we previously observed in the rodent prostate. Cultures received 1 and 10nM BPA or vehicle during DE differentiation and throughout organoid development. Results show an increase in the stem-like/progenitor cell populations when organoids are exposed to BPA during development.

In summary, we have successfully established a novel *in vitro* human prostate developmental model from hES cells which has provided direct evidence that BPA can cause developmental estrogenization in human prostate. Supported by: ES015584

P8. **Notch Pathway Gene Expression in the Embryonic Mouse Ovary**

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The Notch signaling pathway influences cell proliferation, differentiation and death during development and in adult tissues. Notch signaling has been shown to have a role in follicle formation in the ovary, and genetic disruption of Notch signaling in the ovary results in abnormal follicle development and impaired female fertility. The goal of these studies is to investigate the expression of Notch signaling molecules in the ovary during embryonic development and into the early neonatal period, the time when ovarian follicles are being formed. In this study, RNA was isolated from pooled ovaries of CD1 mice for each time point from embryonic day 13.5 (E13.5), E14.5, E15.5, E16.5, E17.5, E18.5 and postnatal day 0 (PND0). The RNA was reverse transcribed to obtain complementary DNA. The expression of Notch pathway ligands and receptors such as *Jag1*, *Jag2*, *Dll1, 3, 4* and *Notch1-4* was determined using gene expression analysis via real time qRT-PCR. The data demonstrates that *Notch 2* is the most abundantly expressed receptor mRNA. *Jag1* and *Jag2* mRNAs were found to be up regulated at E18.5 and PND0. Also, *Hey2* mRNA, a Notch pathway target gene, was up regulated at E18.5 and PND0. These results indicate that Notch pathway genes are expressed during the interval when the initial events of meiosis occur. Primordial germ cells in the mouse ovary enter meiosis at E13.5 and are arrested in prophase 1 prior to birth. Oocytes will then complete meiosis at the time of ovulation. These same RNA samples were used to quantify several known markers of meiosis, Oct4 and Stra8 specifically, verifying a temporal relationship between meiosis and expression of the Notch signaling pathway. This relationship is being explored to establish whether Notch signaling has a direct role in regulating meiotic events. Overall, this work should provide an understanding of processes fundamental for fertility and point to mechanisms that may be disrupted leading to infertility.

P9. **Punicalagin attenuates hypoxia-induced apoptosis by down regulating p53 in the cultured human syncytiotrophoblasts**

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OBJECTIVE: Pomegranate juice (PJ) has potent antioxidant activity attributable to its rich polyphenols and has proven beneficial in functional studies of vascular cells and the responses of cancer cells. Our recent study shows that PJ and its major component punicalagin reduce oxidative stress and apoptosis in human placental trophoblasts (Chen et al. Am J Physiol 302:E1142, 2012). p53 is involved in the oxidative stress-induced apoptosis in trophoblasts. Here we test the hypothesis that punicalagin limits trophoblast injury *in vitro* by regulating the levels of p53.

STUDY DESIGN: Primary human cytotrophoblasts were cultured for 28 h in regular DMEM with 10% FBS and then for an additional 24 h in phenol-red free DMEM with 10% charcoal-stripped FBS, with medium supplemented with control (glucose, 7.5 mM), pomegranate juice (1% v/v), or punicalagin (33.8 mM) in 5%CO2-air. The cells were then cultured with glucose, pomegranate juice or punicalagin, with exposure to <1% oxygen with 5% CO_2, 10% H_2, and 84% N_2 for 24 h, with DMSO or p53 inhibitor Nutlin-3 (10 mM, Sigma, St Louis). The expression of p53, MDM2, p21, Hif 1a and selected members of Bcl 2 family proteins were analyzed by western blotting, qRT-PCR or immunofluorescence staining.

RESULTS: p53 activity was significantly decreased after exposure to punicalagin compared to vehicle control in the syncytiotrophoblasts under hypoxia, as measured by decreased levels of MDM2 and p21. The expression of Hif 1a, which regulates p53 via interaction with MDM2, was also decreased. There were no Bcl 2 family members examined (Bcl 2, Bcl X_L, Bak and Bax) showed significant changes after exposure to punicalagin compared to control.

CONCLUSION: Punicalagin attenuates hypoxia-induced trophoblast death through modulation of p53 activity in the cultured syncytiotrophoblasts. NIH RO1 HD 29190

P10. **Androgen sensitizes human prostate epithelial cells to apoptotic cell death**

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The androgen receptor (AR) is a ligand-activated transcriptional regulator that mediates the developmental, physiologic and pathologic effects of androgens such as 5-dihydrotestosterone (DHT). Although it plays a pivotal role in prostate development, function and neoplasia, the mechanisms through which AR regulates epithelial cell survival/death are not well understood.

Here, we report that androgen sensitizes HPr-1AR human prostate epithelial cells to apoptotic cell death. Treatment of HPr-1AR cells with DHT itself inhibits cell proliferation but does not trigger apoptosis or other types of cell death. However, co-treatment of HPr-1AR cells with DHT and an apoptosis inducer, such as staurosporine or AT101, synergistically increases cell death and decreases cell viability in comparison to treatment with apoptosis inducer by itself. Importantly, the sensitizing effect of androgen to apoptotic cell death is independent of the antiproliferative effect of androgen on cyclinD-CDK4/6 complexes that we have described previously.

Our current hypothesis is that DHT induces the expressions of UNC-51 like autophagy activating kinase 1 (ULK1) and autophagy-related gene 12 (ATG12). Transient overexpression of ULK1 indeed decreases HPr-1AR proliferation, possibly through inhibition of the mTOR signaling pathway. Up-regulation of ATG12 by DHT is correlated with increased levels of ATG12 free-form and ATG12-ATG3 conjugation form. Remarkably, both forms of ATG12 have been implicated in the initiation of mitochondrial apoptosis. As DHT does not increase the conversion of LC3-I to LC3-II, which is a hallmark of autophagy, the proapoptotic effect of DHT appears to be distinct from the well-documented “autophagy” functions of ULK1 and ATG12. Taken together, DHT sensitizes HPr-1AR cells to mitochondrial apoptosis, which might be mediated by “apoptotic” functions of ULK1 and ATG12. Notably, human prostate cancer cell lines, including LNCaP and PC3-AR, have altered regulation of cell survival/death in response to androgen. Our study provides insight into AR-mediated epithelial proliferation and survival as well as the roles of autophagy-related genes in apoptosis.

P11. **Follicle-stimulating hormone inhibits (FSH) phosphatase and tensin homolog (PTEN) activity in ovarian granulosa cells.**

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The Phosphatidylinositide 3-kinase/protein kinase B (PI3K/AKT) pathway plays a crucial role in the regulation of ovarian granulosa cell differentiation. This pathway is activated downstream of the FSH receptor since FSH stimulates AKT phosphorylation *in vivo* and *in vitro*. Although this relationship is known, the mechanisms involved in the regulation of AKT phosphorylation by FSH remain controversial. PI3K produces phosphatidylinositol 3,4,5-triphosphate (PIP3), which in turn anchors AKT to the plasma membrane, a step required for AKT activation. The activity of PI3K is counteracted by PTEN, which dephosphorylates PIP3. We hypothesize that FSH facilitates the activation of AKT through the inhibition of PTEN activity in granulosa cells. To test this hypothesis, we used undifferentiated granulosa cells cultured in serum free media and Western blot analysis to examine the effect of FSH on PTEN phosphorylation at the amino acid residues Ser380 and Thr 382 and Ser385. Phosphorylation of PTEN at Ser380/Thr382/Ser385 inhibits PTENs biological activity. The results demonstrated that in undifferentiated granulosa cells, treatment with FSH for 1 hour increased PTEN Ser380/Thr382/Ser385 phosphorylation. In addition, treatment with dbcAMP also increased PTEN phosphorylation. PTEN phosphorylation by FSH was not affected by co-treatment with insulin-like growth factor 1 (IGF-1), AEW-541, an inhibitor of IGF-1 receptor activity, U0126, an inhibitor of extracellular regulated kinase (ERK), or MK2206, an AKT inhibitor. In addition, we examined the effect of PTEN on AKT phosphorylation. The results demonstrated that treatment with the PTEN inhibitor, bpV(HOpic), led to an increase in AKT phosphorylation in the absence of FSH. Taken together these findings suggest that FSH inhibits PTEN activity, which allows PIP3 accumulation and AKT activation. This effect of FSH on PTEN seems to be mediated by cAMP but does not require the activity of ERK1/2 or AKT. Additionally, the results indicate that in the absence of FSH, endogenous IGF-1 activates PI3K since inhibition of PTEN is sufficient to increase AKT phosphorylation. In conclusion, these findings provide evidence for a novel mechanism by which FSH controls the activation of the PI3K/AKT pathway through inhibition of PTEN and suggests that the inhibition of PTEN activity may contribute to the differentiation of granulosa cells. This work was supported by NIH R01HD057110 and R21HD066233

P12. **RAC1 is critical for uterine angiogenesis during early pregnancy**

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During embryo implantation into the uterus, the uterine stromal cells differentiate into specialized decidual cells, which support the growth and development of the conceptus until placentation. During the process of stromal cell differentiation, new blood vessels form in the maternal decidua from pre-existing vasculature through the process of angiogenesis. Whereas it is clear that decidualization and angiogenesis play a crucial role during early pregnancy, the complex molecular pathways underlying these processes remain largely unknown. Our recent studies revealed that RAC1 (Ras-related C3 botulinum toxin substrate 1), a pleiotropic signaling factor, is induced in uterine stroma during decidualization. RAC1 is a member of the Rho family of GTPases that regulate a wide range of cellular processes, including cell proliferation, differentiation, and angiogenesis. Therefore, we hypothesized that RAC1 may play critical roles during early stages of pregnancy. To address the functional roles of RAC1 during pregnancy, we created a conditional knockout of the *Rac1* gene in the uterus by crossing mice carrying floxed *Rac1* allele with PR-Cre mice. A six-month breeding study indicated that *Rac1* conditional null female mice ($Rac1^{d/d}$) showed a severe fertility defect when these females were bred to *wild-type* males of known fertility. Furthermore, $Rac1^{d/d}$ female mice exhibited uterine hemorrhage, primarily in the decidual compartment, and embryo resorption around days 10-12 of gestation. Moreover, our analysis revealed that whereas the early phases of decidualization appear to be unaffected, the later stage of this process is markedly compromised in $Rac1^{d/d}$ females. The loss of *Rac1* expression in uterine stromal cells also resulted in a marked impairment in the development of maternal blood vessels as indicated by the reduced expression of PECAM-1, *Hif-2α/Epas-1*, and *Angpt2*. Collectively our studies indicate that RAC1 plays a role during the late stages of stromal differentiation and regulates production of critical local factors that impact on blood vessel formation at the maternal-fetal interface. We, therefore, propose that the uterine angiogenic defect is the main cause for pregnancy failure observed in $Rac1^{d/d}$ females at mid-gestation.

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P13. **Genome-wide DNA methylation differences in endometriosis identify an epigenetic switch in GATA expression**

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Endometriosis causes chronic pain and infertility in women due to the ectopic growth of endometrium-like tissue. These cells respond to estrogen but not progesterone signaling, and are characterized by increased inflammation and resistance to apoptosis. DNA methylation is thought to underlie the etiology of endometriosis, but only a handful of genes implicated in the pathology of the disease are differentially methylated. Using Illumina's high resolution 450K methylation array in concert with their HT12v4 gene expression array, we uncovered significant, focused differences in methylation between healthy human endometrial and endometriotic stromal cells that correlated with both baseline and steroid hormone-induced differences in gene expression. The majority of differential methylation was observed intragenically and at sites distal to CpG islands. Moreover, methylation was more likely to be negatively correlated with gene expression when CpGs distal to islands were observed near the transcriptional start site or the first exon of a gene. We developed an interaction analysis workflow to capitalize on the wide coverage of probes on the methylation array, allowing us identify genes that were both differentially methylated and differentially expressed in endometriotic cells. This workflow successfully confirmed the methylation profiles of previously characterized genes, such as *NR5A1* and *HOXA10*, and improved the details on how where they are differentially methylated. Additionally, we identified a novel set of epigenetically regulated transcription factors including several GATA family members. Functional analysis of the GATA family in primary cells revealed that GATA2 is necessary for the hormone-driven differentiation of healthy stromal cells, but this gene is hypermethylated and repressed in endometriotic cells. In contrast GATA6, which is hypomethylated and abundant in diseased cells, potently blocks hormone sensitivity, represses *GATA2*, and induces markers of endometriosis when expressed in healthy cells. This data uncovers a unique epigenetic fingerprint in endometriosis and identifies a novel role for the GATA family as key regulators of uterine physiology, suggesting that aberrant methylation in endometriotic cells underlies a shift in GATA isoform expression that facilitates progesterone resistance and disease progression.

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P14. **Dynamic expression of the *Rhox* homeobox cluster during the first wave of spermatogenesis depends on transcription, not translational control.**

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Homeobox genes encode transcription factors that have well-established roles in embryonic development. We recently discovered the *Rhox* genes, a new family of homeobox genes, which are selectively expressed in the developing embryo, postnatal and adult gonads, and accessory tissues associated with mammalian reproduction. The largest and best-characterized *Rhox* cluster is found in mouse. However, all mammals examined to date possess a set of RHOX factors that, while they may vary in number by species, appear relevant to reproduction and are located in the syntenic region of the X chromosome. There are 13 distinct *Rhox* genes expressed in the testes, but thus far only knockouts for the Sertoli-specific *Rhox5* and *Rhox8* have elucidated a function in supporting spermatogenesis. We previously reported the temporal mRNA expression pattern of the cluster during the first wave of spermatogenesis using qPCR and ribonuclease protection assays. *Rhox* genes exhibit unique and slightly overlapping expression patterns, indicating that they may play unique roles and in some cases have redundant functions in controlling germ cell differentiation. Interestingly, when *Rhox13* was characterized, it was discovered that while its mRNA was expressed in several germ cell types, and many phases of spermatogenesis, it was only translated in spermatogonia that lack NANOS2. To determine whether this phenomenon was true of the other germ cell-expressed *Rhox* genes, we examined RHOX expression from birth to post-natal day 23 in the testes of wild-type mice using novel antibodies provided by IMGENEX (San Diego, CA). RHOX5, RHOX8, and RHOX11 are expressed in somatic cells of the testes and were not included in our analysis. Using these previously uncharacterized antibodies, we could not detect RHOX6, RHOX9, and RHOX12 in the testes at any time point. However, RHOX1, RHOX2, RHOX3, RHOX4, and RHOX7 all displayed temporal expression in germ cells that correlated to their qPCR time courses. Our new RHOX10 antibody was of insufficient quality to assess its protein expression profile, but RHOX10 expression reported by another group, corresponded to its expected mRNA expression profile. Although *Rhox13* appears to be regulated via translational control mechanisms, we conclude that the remainder of the *Rhox* cluster is dependent upon transcriptional regulation. Supported by NIH/NICHD 55268 and 65584.

P15. **Postnatal Pituitary Proliferation and Differentiation: Sex Differences and Influence of BPA**

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During embryonic development of the pituitary gland, progenitor cells proliferate and differentiate into one of five cell types, including gonadotropes, which release luteinizing hormone (LH) and follicle stimulating hormone (FSH). The gonadotrope population expands postnatally as well, a time that is sensitive to hormonal input and potentially endocrine disrupting chemicals (EDCs). Bisphenol A (BPA) is a common EDC found in polycarbonate plastics that enters the body through oral or dermal routes. BPA interferes with hormone regulation at all levels of the hypothalamic-pituitary-gonadal (HPG) axis, negatively impacting fertility and accelerating the onset of puberty. Previous data from our lab demonstrated that low dose BPA exposure during embryonic development increased the proliferation of pituitary progenitors and increased their differentiation to gonadotropes in neonatal females, but not males. However, the lasting effects on the pituitary once BPA is removed remain unknown. Based on these data, we formed two hypotheses. First, we hypothesized that embryonic BPA exposure would have a persistent effect on pituitary development during the postnatal period. To address this, we looked at pups of female mice dosed from embryonic day 10.5 until birth (postnatal day (PND)0) with 0.5, 20, or 50 ug/kg/day of BPA. Pups were collected at PND4 and examined for Ki67, a marker of proliferation, by immunohistochemistry. The proliferation increases seen in PND0 females in response to BPA did not persist to PND4, indicating that BPA may need to be present to affect proliferation. Our second hypothesis is that there are inherent sex differences in gonadotrope differentiation that may explain the sexually dimorphic response to embryonic BPA exposure. To address this, we characterized proliferation and gonadotrope differentiation over time in untreated mice. We found that males and females had similar number of gonadotropes, but baseline differences in gene expression profiles of gonadotrope restricted genes were detected. QRT-PCR analysis of CD1 mice showed that females had significantly more *Lhb* and *Fshb* mRNA from PND0 to PND9 and significantly less *Lhb* and *Fshb* mRNA at PND20 and adult (diestrus) than males. Proliferation, as assessed by *mki67* qRT-PCR, was not different between sexes. This early sexual dimorphism in *Lhb* and *Fshb* expression may indicate an early role of intrinsic hormonal signaling in pituitary organogenesis or function. Identifying differences in pituitary development between sexes is important to our understanding of the different effects of BPA exposure and may elucidate the mechanism by which BPA and other endocrine disrupting chemicals act. Supported by P01 ES022848, R01 DK076647, T32 ES007326

P16. **Long Term Consumption of Flaxseed Enriched Diet Decreased Ovarian Cancer Incidence and Prostaglandin E_2 in Hens**

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Ovarian cancer is the most lethal gynecological malignancy. Prevention of ovarian cancer is the best approach for reducing the impact of this deadly disease. Progress in the treatment and prevention of ovarian cancer has been hampered due to the lack of a valid and appropriate animal model, and absence of effective chemo-prevention strategies. The domestic hens spontaneously develop ovarian adenocarcinomas that are similar in histological appearance to human ovarian carcinomas and share similar symptoms of the disease, such as perfuse ascites fluid and peritoneal metastatic dissemination. Flaxseed is the richest vegetable source of omega-3 fatty acids which may be effective in the prevention of ovarian cancer. There is a link between chronic inflammation and cancer. Prostaglandin E_2 (PGE_2) is the most pro-inflammatory ecoisanoid and one of the downstream products of two isoforms of cyclooxygenase (COX) enzymes: COX-1 and COX-2. Our objective was to determine if long-term consumption of a flaxseed enriched diet decreased ovarian cancer severity and incidence in the laying hen and to investigate its potential correlation with the expression of COX enzymes and PGE_2 concentration. 1200 white Leghorn hens were fed 10% flaxseed-enriched or standard diet for 4 years. The severity and incidence of ovarian cancer were determined by gross pathology and histology. COX-1 and COX-2 protein and mRNA expression and PGE_2 concentrations in ovaries were measured by Western blot, quantitative real-time qPCR and ELISA, respectively. Our results indicated that similar to humans, the incidence of ovarian cancer was increased in hens which further validates the chicken as a suitable model to study human ovarian cancer. In correlation with ovarian cancer incidence, the expression of both COX enzymes and concentrations of PGE_2 were elevated with age. The results demonstrated that there was a reduction in ovarian cancer severity and incidence in hens fed flaxseed diet. In correlation with decreased ovarian cancer severity and incidence, concentration of PGE_2 and expression of COX-2 were diminished in ovaries of hens fed flaxseed. Our findings suggest that the lower levels of COX-2 and PGE_2 are the main contributing factor in the chemo-suppressive role of long-term flaxseed consumption in ovarian cancer in laying hens. These findings may provide the basis for clinical trials of dietary intervention targeting prostaglandin biosynthesis for the prevention and treatment of ovarian cancer. [Supported by NIH (1R01 AT005295) National Center for Complementary and Alternative Medicine Grant AT004085 (DBH) and American Institute for Cancer Research Grant 06-A043 (DBH)]

P17. **Beclin-1 is necessary for adult Sertoli cell function and male fertility in the mouse.**

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It has been underappreciated that that autophagy occurs in the testes and could promote spermatogenesis and normal testicular function. Yet today, little is known about the function of autophagy in mammalian testes. A recent study using isolated rat leydig cells has positively correlated testosterone secretion to autophagic flux. Moreover, since autophagic flux is known to decline with age, it has been suggested that any decrease in androgen synthesis can be directly attributed to a decrease in autophagic flux by steroid-producing cells. To better understand this phenomenon, *in vivo*, we generated AMHR2-Cre; Beclin (fl/fl) mice to knockout an autophagy regulator in Sertoli and leydig cells.

cKOs are initially fertile and sire litters at 6 weeks of age but become infertile by 10 weeks. To explore the mechanism, we analyzed reproductive tracts from age-matched cohorts at 6, 8, 10, 12 and 15 weeks old. Testes weight is decreased in cKOs at 8 weeks and beyond with a maximal reduction of 50% at 15 weeks old. Seminal vesicle weight is equivilent, indicating that testosterone secretion is not affected. Histological analysis finds spermatocytes released into the lumen of the seminiferous tubules beginning at 6 weeks old. By 12 weeks old, a majority of the tubules show a Sertoli-cell only phenotype. Corroborating the loss of spermatocytes, cauda sperm numbers are reduced in 8-week and older cKOs. Using rt-PCR for cell-specific markers we estimated the ratio of cells. cKOs have increased leydig cells at all time-points. Sertoli, pre-meiotic spermatocytes, post-meiotic spermatocytes and spermatids are reduced in cKOs. Whereas, undifferentiated germ cells are not affected. Immunostaining resulted in finding the rare pocket of expanded germ cells in cKO tubules. Analysis of SQSTM1 indicates that autophagy is impaired in Sertoli cells but not leydig cells of cKOs. cKOs have increased TNF-alpha expression at all time-points analyzed suggesting spermatocyte death. Along with this result, cKOs have increased clusterin expression which is typically upregulated in response to tubule damage. Ultrastructural analysis at 6 weeks old demonstrates many unprocessed phagocytic vesicles and few lysosomes in the cKO.

All together, the results of this study show that Beclin-1 is necessary for the function of adult Sertoli cells. Beyond this the results suggest that Beclin-1 deficiency leads to an inability for the Sertoli cell to support spermatocytes beyond the first round of spermatogenesis and could cause a decline in the number of lysosomes leading to a metabolic imbalance in Sertoli cells.

P18. **Effective vaccine-induced protection against genital herpes is mediated by a polyclonal immune response towards many HSV-2 proteins.**

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A billion people worldwide are carriers of herpes simplex virus type-2 (HSV-2), and 2 to 5% of these individuals suffer from recurrent genital herpes. Neonates may be infected with HSV-2 during childbirth, and are at high risk for fatal CNS infections or severe cognitive disabilities. Thus, it is widely agreed that a HSV-2 genital herpes vaccine is a public health priority as it could eradicate diseases such as neonatal herpes. Previous attempts to develop an effective HSV-2 vaccine have focused on immunizing recipients with viral glycoprotein subunits in an attempt to induce neutralizing antibodies against HSV-2. Unfortunately, for the past 25 years, glycoprotein subunit vaccines have been unable to protect vaccine recipients from contracting HSV-2 genital herpes. As an alternative approach, our lab has developed a live-attenuated HSV-2 vaccine (0ΔNLS) which is 100 times more protective against HSV-2 challenge in animal models than the pharmaceutical industry's leading HSV-2 glycoprotein D (gD-2) subunit vaccine. The goal of the current study was to determine why the live HSV-2 0ΔNLS vaccine was more effective than the gD-2 vaccines which have been the focus of the HSV-2 vaccine campaign for decades. We present results that the IgG antibody titer against total HSV-2 antigen (i.e., a collection of 75 HSV-2 proteins) is ~50-fold greater in mice immunized with the HSV-2 0ΔNLS vaccine than mice immunized with a monovalent gD-2 subunit vaccine. Moreover, we present evidence that the antibody response to the HSV-2 0ΔNLS vaccine is highly polyclonal, as antibodies are directed against at least 10 HSV-2 proteins other than gD-2. Using a combination of Western blot analysis and immunoprecipitation-mass spectrometry, we have identified several of the dominant antigens of this novel, live-attenuated HSV-2 0ΔNLS vaccine. Intriguingly, the two most dominant antigens were intracellular HSV-2 proteins, UL29 and UL39, neither of which have ever been considered for use in a HSV-2 vaccine. These new data suggest that HSV-2 subunit vaccines might be more effective if they include multiple HSV-2 antigens which are more immunodominant than gD-2. However, one limitation of subunit vaccines is that they fail to recapitulate the repertoire of HSV-2 antigenic determinants presented during infection. Thus, we conclude that the next clinical trial of a HSV-2 vaccine would be far more likely to succeed if the immunogen were a safe, live-attenauted HSV-2 virus rather than the type of HSV-2 subunit vaccines that have been tested and re-tested in clinical trials for 25 years.

P19. **Ovarian Cancer and Diabetes: Can Metformin be used to Halt Tumor Growth and Proliferation?**

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Ovarian cancer and type II diabetes are diseases that are of importance to the scientific community because of their severity and widespread effects on the American population. Both diseases share a variety of risk factors, and some drugs used to treat diabetes are associated with a decreased cancer risk in individuals. In particular, Metformin (1-carbamimidamido-N,N-dimethylmethanimidamide, $C_4H_{11}N_5$), currently the number one worldwide anti-diabetes drug, shows promise in becoming a prescribed medication for cancer patients. Through activation of AMP-activated kinase in cancer cells, Metformin may inhibit cellular proliferation by causing cancer cells to decrease gluconeogenisis, starving the cells of their abundant glucose supply that previously allowed tumor growth.

This experiment was designed to study the effects of Metformin on cancer at a cellular level by measuring lactic acid production, a molecule that is present in excess when gluconeogenesis is inhibited by activation of AMP Kinase. In this experiment, cancerous (SKOV3) and noncancerous (IOSE) ovarian epithelial cells derived from humans were grown in culture in control media and media containing Metformin. Results showed that levels of lactic acid were significantly higher in Metformin-treated cancer cells compared to control cancer cells, indicating a decreased rate of gluconeogenisis, and therefore decreased glucose supply, which would point to the drug's ability to decrease development of cancer. Further testing of ATP content, cellular oxygen consumption, alterations in gene expression and effects on mitochondrial dynamics will provide further insight into the mechanisms through which Metformin acts in ovarian cancer cells. Supported by NIH AT00408 and AT005295

Keywords: Ovarian cancer, lactic acid production, gluconeogenesis, in vitro, SKOV3, IOSE

P20. **Reprogramming of NK-cell function by soluble immune mediators**

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Natural killer (NK) cells classically are associated with immune surveillance and destruction of nonself or tumor cells via cytotoxicity. This presents an enigma during normal pregnancy and early stages of tumor growth where large influxes of NK cells occur with no overt consequences. Recent studies indicate that NK cells with non-classical CD phenotypes (CD56brightCD16dim-negative) accumulate at the maternal-fetal interface during implantation, lose their cytotoxic capacity, release pro-angiogenic factors, and facilitate placentation. Mechanisms driving these changes are not established but soluble factors are thought to direct the unique phenotypic and functional differentiation of these CD56brightCD16- NK cells. We hypothesize that an analogous shift in NK cell phenotype/function could occur to support early stages of tumor growth. We used a combination of clinical specimens, primary cell culture, and a novel animal model to evaluate the effects of transforming growth factor beta (TGFβ) and prostaglandin E_2 (PGE_2) on NK cell phenotype and function in renal cell carcinoma (RCC). Plasma levels of PGE_2 for RCC patients (N=7) were 92 $\pm$ 39 pg/mL before surgery and 45 $\pm$ 11 pg/mL after nephrectomy (P=0.02, T-test). Post-operative levels of PGE_2 were approaching levels observed for healthy volunteers (27 $\pm$ 11 pg/mL), suggesting that PGE_2 is a RCC tumor-derived factor. Phenotype analysis revealed that peripheral blood NK (pNK) cells from healthy volunteers were characteristically CD56dimCD16bright (>95%); however, RCC tumor-derived NK cells exhibited the CD56brightCD16- phenotype and had elevated vascular endothelial growth factor (VEGF) transcripts (3-fold) compared to freshly isolated pNK cells. Trans-differentiation of CD56dimCD16bright pNK cells to the CD56brightCD16dim phenotype was achieved after culture with TGFβ; this conversion was coincident with impaired NK cytotoxicity (>50% reduction) and a modest augmentation of VEGF mRNA upon crosslinking of the activating receptor NKp46. A Balb/c mouse renal adenocarcinoma cell line (Renca) expressing moderate levels of TGFβ (300-350 pg/mL) was engineered for PGE_2 expression with murine Cox2 in antisense (Cox2-) or sense (Cox2+) orientations. ELISA demonstrated that Cox2- cells produced background levels of PGE_2 (35 pg/mL) while Cox2+ cells produced >5,000 pg/mL PGE_2. Exposure of freshly isolated human pNK cells to conditioned supernatants from Cox2- (TGFβ only) or Cox2+ (TGFβ and PGE_2) cells reduced cytotoxicity by 20% and 30%, respectively. In vivo studies demonstrated rapid tumor growth and metastasis over three weeks following kidney capsule injection in Balb/c mice. Lymphocytes isolated from these RCC-like tumors were infiltrated with NK-cells (CD3-p46+), nearly 60% of which demonstrated diminution or absence of CD16. Collectively, these studies support a role for TGFβ and PGE_2 in trans-differentiation of CD56dimCD16bright pNK cells to CD56brightCD16dim-negative phenotype characteristic of dNK cells. These cells have lost their normal cytotoxic capacities and produce pro-angiogenic factors. This trans-differentiation may be beneficial for supporting placentation of the fetal allograft, but may be exploited to support tumor growth and metastasis.

P21. **The *Rhox5* homeodomain protein directly regulates the expression of *Ins2* and cellular metabolism in the testis.**

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The Reproductive Homeobox X-linked, *Rhox*, genes encode transcription factors that are expressed exclusively in the testis, epididymis, placenta, and ovary. While there are 33 *Rhox* genes in mice, only *Rhox5* and *Rhox8* are expressed in Sertoli cells, suggesting that they alone regulate the expression of somatic-cell gene products crucial for testicular metabolism and germ-cell development. Targeted deletion of *Rhox5*, the founding member of the *Rhox* gene cluster, in mice, results in male subfertility via reduced sperm number, increased germ-cell apoptosis and a reduced proportion of sperm with normal motility. Microarray analysis was used to identify altered gene expression in *Rhox5*-null testis compared to control mice. 316 known genes were found to be altered more than 2-fold, the most highly downregulated gene being *Ins2*. Furthermore, *Ins2* exhibits a similar expression pattern to *Rhox5* during the first wave of spermatogenesis and a mouse model crippling insulin gene *Ins2* exhibits similar fertility defects to *Rhox5*-null mice. Together, these data support a model in which RHOX5 acts directly through insulin signaling to promote male germ cell survival. To study this hypothesis, 15P-1 and Tm4 Sertoli-cell lines were forced to exhibit *Rhox5* expression and responded by dramatically upregulating *Ins2* mRNA levels. To define the domains of RHOX5 required to induce this direct increase in expression, we generated and analyzed rodent RHOX5 mutants missing the homeodomain, the amino-terminal domain and/or the carboxy-terminal domain. Singular transfection of these mutants into immortalized Sertoli cell lines 15P-1 and MSC1 as well as co-transfection of these mutants with an *Ins2*-reporter vector allowed us to conclude the following: the RHOX5 homeodomain and amino-terminal domain are required but not sufficient for inducing *Ins2* transcription and the carboxy-terminal domain increases the magnitude of *Ins2* transcription but is not required. This evidence of the direct regulation and mechanism of action by which RHOX5 upregulates *Ins2* expression leads us to propose a model in which RHOX5 is a central transcription factor promoting the survival of male germ cells via effects on cellular metabolism thereby providing an appropriate metabolic state for the function of Sertoli and germ cells. In the future, it will be important to determine whether *Rhox5* regulation of *Ins2* is restricted to Sertoli cells or also occurs in other *Rhox5* expressing cell types such as the epididymis, placenta or ovary as well as to determine the identities of factors interacting with the domains of RHOX5 to promote the increased transcription of *Ins2*. Supported by NIH/NICHD 55268 and SIU-ORDA Faculty Seed Grants 2009.

P22. **Statin Therapy Is Associated With Improved Survival in Patients with Non-Serous-Papillary Epithelial Ovarian Cancer**

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Objective: To determine whether statin use is associated with improved epithelial ovarian cancer (EOC) survival.
Methods: This is a single-institution retrospective cohort review of patients treated for EOC between 1992 and 2013. Inclusion criteria were FIGO stage I–IV EOC. Exclusion criteria were noninvasive pathology or non-epithelial malignancies. The primary exposures analyzed were history of hyperlipidemia and statin use. Progression-free and disease-specific survivals were estimated using Kaplan-Meier methods. Cox proportional hazard models were utilized for multivariate analysis. To measure cell proliferation, EOC cell lines were treated with lovastatin (10 to 40 μM) for 24 hours and proliferation was measured using MTT assays.
Results: 442 patients were included in the study. 346 (78.3%) were non-hyperlipidemics, 68 (15.4%) were hyperlipidemics using statins, and 28 (6.3%) were hyperlipidemics not using statins. In a subgroup of EOC patients with non-serous-papillary histology, Cox proportional hazards models showed that there was a statistically significant decrease in hazards of both disease recurrence (HR:0.23, 95%CI 0.07-0.79, p=0.02) and disease-specific death (HR:0.23, 95%CI:0.05-0.96, p=0.04) among statin users when compared to non-hyperlipidemics. This was not true in those with serous-papillary (p=0.78 and p=0.98, respectively). This histology-specific effect of statins was further evaluated *in vitro* using proliferation assays. Here, statin treatment was more cytotoxic to non-serous (EG and ES2) cell lines compared to serous (SKOV3ip1 and OVCAR5) cell lines**.** In the dataset, when examining patients with all EOC subtypes, the protective effect of statins was lost. The median progression-free survival for non-hyperlipidemics was 14.7 months (95%CI: 13.2-18.6 months) compared to 21.7 months (95%CI:12.8-29.9 months) for hyperlipidemics using statins, and 13.6 months (95%CI:9.9-19.5) for hyperlipidemics not using statins (log-rank test comparing three groups p=0.69). Median disease-specific survival for non-hyperlipidemics was 41.5 months (95%CI:35.3-48.5 months) compared to 44.2 months (95%CI:27.8-104.9 months) for hyperlipidemics using statins, and 75.7 (95%CI:20.2-non-estimable) for hyperlipidemics not using statins (log-rank test comparing three groups p=0.43). Cox proportional hazards models showed no statistically significant decrease in disease recurrence (HR:0.84, 95%CI:0.56-1.27, p=0.41) or disease-specific death (HR:0.8, 95%CI:0.5-1.29, p=0.37) among statin users compared to non-hyperlipidemics.
Conclusion: Statin use among EOC patients with non-serous-papillary histologic subtypes was associated with a decreased risk of both disease recurrence and disease-specific death. This finding was further supported *in vitro*. However, due to the relative rarity of other histologies, larger studies are needed to identify these potentially statin-sensitive subtypes. That said, these findings add to an increasing body of literature indicating an anti-cancer effect of statins.

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P23. **Mono(2-ethylhexyl) phthalate, but not di(2-ethylhexyl) phthalate, directly accelerates early ovarian folliculogenesis in mice**

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Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in the manufacturing of products containing polyvinyl chloride. This ubiquitous toxicant represents a public health concern as humans are exposed daily via ingestion, inhalation, and dermal contact. Large doses of DEHP have been shown to disrupt normal ovarian function; however, the ovotoxic effects at environmentally relevant levels are unclear. We have shown that 30 day exposure to relatively low doses of DEHP accelerates primordial follicle recruitment; however, its effects at shorter exposure times are unknown. Furthermore, it is unknown if DEHP has direct effects on folliculogenesis. To further investigate the effects of DEHP on ovarian function, we tested the hypotheses that *in vivo* treatment with DEHP for a short period (10 days) accelerates primordial follicle recruitment, and the bioactive metabolite of DEHP, mono(2-ethylhexyl) phthalate (MEHP), is responsible for accelerating primordial follicle recruitment. To test these hypotheses, CD-1 mice (post-natal day 39) were orally dosed with corn oil (vehicle control) or DEHP (20 µg/kg/day-750 mg/kg/day; n=4/group) daily for 10 days. The ovaries were processed for histological evaluation of follicle counts and percentages of each follicle type were compared in each treatment group. Additionally, ovaries from CD-1 pups (post-natal day 4) were cultured for 6 days on filter paper suspended in supplemented media containing dimethylsulfoxide (vehicle control), DEHP (0.2-20 µg/ml), or MEHP (0.2-20 µg/ml; n=4-6/group). The ovaries were processed for histological evaluation of follicle counts and percentages of each follicle type were compared in each treatment group. After 10 day exposure to DEHP, the percentage of primordial follicles counted was significantly reduced at the 20 mg/kg/day and 200 mg/kg/day doses compared to controls (vehicle: 72.5±2.0; 20 mg/kg/day: 58.4±2.5; 200 mg/kg/day: 61.1±1.7). DEHP exposure significantly increased the percentage of primary follicles counted compared to controls (vehicle: 13.6±0.4; 20 µg/kg/day: 18.2±1.1; 200 µg/kg/day: 20.3±1.4; 20 mg/kg/day: 24.3±1.5; 200 mg/kg/day: 28.0±1.0; 750 mg/kg/day: 17.7±0.7). DEHP exposure *in vitro* did not significantly alter follicle numbers compared to controls. MEHP significantly reduced the percentage of germ cells (vehicle: 14.4±1.6; 0.2 µg/ml: 7.1±1.1; 2 µg/ml: 7.5±1.1; 20 µg/ml: 7.4±1.5, and significantly increased the percentage of primary follicles counted compared to controls (vehicle: 5.0±0.5; 0.2 µg/ml: 11.8±1.2; 2 µg/ml: 11.5±1.0; 20 µg/ml: 12.7±1.3). These data suggest that short term exposure to DEHP affects early folliculogenesis more than long term exposure, and the ovotoxic response of DEHP on folliculogenesis appears to be mediated by MEHP. Supported by R01ES019178 and an Environmental Toxicology Fellowship.

P24. **Micromechanical properties of mammalian meiotic chromosomes: a high frequency model of genomic instability**

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Prior to cell division, eukaryotic cells undergo a dramatic reorganization of chromatin whereby the long strands of DNA are bundled and condensed into the compact structures known as chromosomes. Proper chromosome condensation is essential for accurate segregation of homologous chromosomes or sister chromatids. The folding of the flexible chromatin fiber into a complex structure is due, in part, to the multi-subunit protein, condensin, and cells deficient in condensin fail to fold the chromatin properly, resulting in anaphase bridges and other defects. Meiosis is a specialized process with two sequential cell divisions that occur in the absence of an intervening interphase to produce haploid gametes. Compared to mitosis in healthy somatic cells, meiosis in the mammalian egg is remarkably error-prone, especially in females of advanced reproductive age. Thus, the mammalian egg represents a robust and unique model system to study molecular mechanisms of chromosome segregation errors and aneuploidy similar to what occurs in cancer cells. Measurement of the micromechanical properties of isolated individual chromosomes has provided a powerful tool for understanding the organization of chromosomes in mitotic cells of several species. However, the role of the micromechanical properties of mammalian chromosomes during meiosis is unknown. Our objective is to analyze the chromosome micromechanics during meiosis in oocytes from young mice and old mice, which have been shown previously to have high rates of aneuploidy. Chromosomes were isolated from *in vitro* matured eggs arrested at metaphase of meiosis II and, with micromanipulation, stretched in order to measure the force constant of a single chromosome. We show here individual chromosomes can be efficiently isolated from mouse eggs arrested at metaphase of meiosis II and these meiotic chromosomes have reversible elasticity and a force constant on the piconewton scale. Meiotic chromosomes have a force constant that is significantly higher than has been measured in human mitotic cells (~3000 pN vs. ~300 pN), indicating either a species-specific difference or an inherent difference in chromosome makeup between mitotic and meiotic chromosomes. We also demonstrate that chromosomes from young animals have a significantly smaller average force constant when compared to chromosomes from older animals. There is a significant difference in the micromechanical properties of chromosomes from oocytes with higher frequency of aneuploidy, suggesting a possible role for the architecture of chromosomes in contributing to chromosome segregation errors. Such analysis in a well-established model of increased genomic instability not only will reveal the relationship between changes in the micromechanical properties of meiotic chromosomes and aneuploidy, but will also have broad implications for reproductive health and associated interventions.

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P25. **Gene Expression in the Mouse Cerebral Cortex is Modulated by Estrogen and Hypoxia**

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17-beta estradiol (E2) is well known for playing vital roles in development, growth, and reproductive function. E2 also exerts a potent neuroprotective effect in the brains of rodents that have been subjected to middle cerebral artery occlusion. Mice that have been treated with E2 before artery occlusion have significantly less damage when compared to their vehicle-treated counterparts, particularly in the cerebral cortex. However, the mechanisms underlying the neuroprotective effect of E2 remain unclear.

To better understand how E2 protects the brain from ischemia, we examined the effects of E2 and hypoxia, a major component of ischemia. Female mice were ovariectomized, implanted with silastic tubing containing oil or E2, and then subjected to hypoxic or normoxic conditions. The cerebral cortices were isolated and RNA-Seq was used to identify transcripts that were altered by E2 or hypoxia. The transcript levels of 49 genes were altered by 1.2 fold or more in mice that had been treated with E2. Interestingly, the majority of E2-regulated genes were downregulated. Gene ontology revealed that the E2-regulated genes have been associated with heart development, chromosome organization, cell projection assembly, muscle organ development, and cell projection organization. These genes were also associated with pathways involved in gap junctions, MAPK signaling and cell adhesion. Recent work to examine the effects of hypoxia in the cerebral cortex revealed that 661 genes were altered by 1.5 fold or more. In contrast to the E2-regulated genes, a majority of the hypoxia-regulated genes were increased. These data provide novel insight to better understand how E2 and hypoxia regulate gene expression in the cerebral cortex and may help to identify mechanisms involved in E2-mediated neuroprotection. Supported by NIH Grant R01 DK 053884.

P26. **Conditional deletion of gene for forkhead transcription factor FOXO1 affects anterior pituitary function**

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Various mouse models have identified several transcription factors that are necessary for pituitary development. Lesions in the transcription factor genes result in pituitary hormone deficiency. Hormone deficiencies have been found to occur in approximately one in 4000 live births. Pituitary hormone deficiency may occur due to loss of a single hormone causing isolated hormone deficiency (IPHD) or several hormones that leads to combined pituitary hormone deficiency (CPHD). Defects in genes such as LHX3, LHX4, RPX, PROP1, and PIT1 are known to contribute to CPHD in humans. We are investigating FOXO1, which is a subclass of a large family of transcriptional regulators. FOXO1 is expressed in muscle, adipose tissue, liver, vascular epithelial cells, ovary, brain, heart. It is essential for metabolism, maintenance of hematopoietic stem cells, vascular development, regulating cell cycle progression and promoting apoptosis. Previous studies in mouse models have shown that FOXO1 is expressed in most of the somatotrope cells in mice at embryonic day 18.5 (e18.5) and in adulthood. In order to determine if FOXO1 is affecting pituitary development and/or function and determine the mechanism behind it, we are studying mouse embryos in which *Foxo1* has been deleted in the pituitary gland (*Foxo1*$^{\Delta pit}$ mice). We find that growth hormone (GH) production is reduced at e18.5 in the absence of FOXO1. Our hypothesis is that FOXO1 drives the expression of *Gh1* or upstream regulators that control somatotrope differentiation, proliferation and/or function presumably by binding to *Gh1* promoter region or the promoter region of upstream regulators. *Pitx2* and *Pit1* defects lead to loss of GH production. *Neurod4* is initially expressed at e13.5 shortly before differentiation of somatotropes. To determine if any of these transcription factors are altered in *Foxo1*$^{\Delta pit}$ mice, we performed qRT-PCR to measure their expression in *Foxo1*$^{\Delta pit}$ mice and wildtype (WT) littermates. We found a significant decrease ($p<0.05$) in the expression of *Neurod4* in *Foxo1*$^{\Delta pit}$ embryos as compared to the WT littermates. The data from these studies provide us with a greater understanding of pituitary organogenesis and expand the molecular diagnosis for congenital hypopituitarism.

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P27. **Constitutive activation of PI3K in oocytes stimulates the growth of mature follicles, but not activation of immature follicles**

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ABSTRACT

The majority of ovarian follicles remain in a dormant state constituting the ovarian reserve. In each cycle, a small number of primordial follicles in the ovarian reserve are stimulated to develop into mature follicles. It has been demonstrated that this process of cyclical progression is controlled by AKT-mediated phosphorylation of FOXO3. In the aforementioned studies, conditional deletion of PTEN increases PIP3 levels and activates AKT triggering spontaneous maturation of primordial follicles in neonatal mouse ovary. While the significance of PTEN-PI3K-AKT signaling in follicle maturation is established, the signaling that initiates activation of immature follicle is not fully understood. In order to dissect the signaling involved in the activation of immature follicles, we have generated transgenic mice expressing constitutively active PI3K (PIK3CA*) in oocytes of neonatal mouse ovary. Although PIK3CA* upregulated the phosphorylation of AKT in oocytes, immature follicles remained dormant. The immature follicles expressing PIK3CA* showed up-regulation and nuclear accumulation of PTEN, suggesting that PTEN antagonized PIK3CA* to attenuate nuclear PIP3 levels and maintained the dormant state of immature follicles. These data suggest that PTEN dominates the immature primordial follicles, while increased PI3K activity in the oocytes promoted the growth of activated follicles. Moreover, the PI3K activity within oocytes controls the function of granulosa cell.

Our results indicate the presence of intricate system that maintains the ovarian reserve for reproductive life by actively repressing the premature activation of primordial follicles. In this system, PTEN plays a central role in that maintenance of quiescence with PIK3 serving as the activator. These observations suggest that the activation of immature primordial follicles is triggered by an attenuation of PTEN activity rather than an activation of PI3K.

Keywords:

Ovarian reserve, PTEN, PI3K, follicle activation

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P28. **WNT7A/β-catenin/FGF1 signaling promotes ovarian cancer growth and is a novel therapeutic target**

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Aberrant canonical WNT/CTNNB1 signaling has been reported in epithelial ovarian cancer (EOC), and we have previously elucidated a role for the specific ligand WNT7A in EOC growth and progression. Further studies have revealed *FGF1* as a potential downstream target of activated WNT7A/*β*-catenin signaling, where high expression of *WNT7A* and *FGF1* leads to poor overall patient survival, *FGF1* expression is high in WNT7A overexpressing ovarian tumors, and overexpression of WNT7A or activation of CTNNB1 results in increased *FGF1* transcripts. In this study, we first determined if FGF1 is sufficient to drive the tumor promoting effects of WNT7A. Stable A2780 cells overexpressing WNT7A or FGF1 had reduced cell doubling time and increased cell adhesion compared to vector controls. Intraperitoneal injection into nude mice resulted in 100 and 67% tumor incidence in animals that received WNT7A or FGF1 overexpressing cells, respectively, while animals injected with vector control cells failed to develop any tumors during the experimental timecourse. Since FGF1 expression follows that of WNT7A, we used shRNA to knockdown FGF1 in WNT7A overexpressing cells and found that loss of FGF1 returned cell doubling times and adhesion to levels similar to control cells, despite sustained expression of WNT7A. Further, FGF1 knockdown in WNT7A expressing cells caused a significant reduction in tumor size. These results suggest that FGF1 is a critical downstream mediator of the oncogenic effects of WNT7A in ovarian tumorigenesis. We next sought to inhibit WNT7A/*β*-catenin/FGF1 signaling. We found that niclosamide efficiently abrogated WNT7A/*β*-catenin signaling and decreased *FGF1* in our model. In addition to inhibiting *β*-catenin transcriptional activity, cells treated with niclosamide had decreased WNT7A itself, decreased phospho-LRP6, and decreased active *β*-catenin. Niclosamide dose-dependently reduced cell viability and adherence, as well as increased cell death and cleavage of the apoptosis effector molecule caspase-3. Tumor growth and spread were reduced in nude mice treated with daily oral niclosamide following i.p. injection with A2780 or SKOV3.ip1 cells. Taken together, these results indicate that WNT7A/*β*-catenin/FGF1 signaling is a potential therapeutic target in ovarian cancer, and niclosamide is a promising inhibitor of this pathway that may have clinical relevance. Supported by NIH/NCI R15CA179214.

P29. **Characterization of SPATA22, a novel mammalian protein required for meiotic progression in mouse germ cells**

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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 *Spata22*$^{tm1a(KOMP)}$ 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 *Spata22*$^{tm1a(KOMP)}$ allele is indeed a null allele. Next, we assessed localization of SPATA22 in spermatocyte surface-spread chromatin. SPATA22 is diffusely detectable across the nucleus of leptotene spermatocytes, but numerous foci become visible in early zygotene spermatocytes. As spermatocytes progress through zygonema, foci become restricted to forming SC axes and decrease in number. By early pachynema, foci are exclusively located along the length of all SCs (5-8 foci per SC on average). By mid-to-late pachynema, SPATA22 foci are no longer visible. Immunoblotting analysis of proteins extracted from various subcellular fractions confirmed these findings, as SPATA22 is detected in both the soluble- and chromatin bound-nuclear fractions in addition to the cytoplasm. Since focal nuclear localization of SPATA22 is evocative of recombination nodules (RN), we assessed localization of additional DNA repair factors known to localize to RNs in wild type and mutant *repro42* spermatocytes. Although localization of RPA1 (replication protein A1) was not impaired in mutant *repro42* spermatocytes, MEIOB (meiosis-specific with oligonucleotide binding (OB) domains), a novel RPA paralog, was undetectable in these same cells. Taken together, these data further support a role for SPATA22 during meiotic prophase, most likely at recombination nodules. Supported by funds from Midwestern University and NIH HD042137.

P30. **A preclinical mouse model to study invasive endometrial carcinomas**

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Last year, we reported the impact of both *Cdh1* and *Pten* ablation ($Cdh1^{d/d}$ $Pten^{d/d}$) in the uterus using *Pgr-Cre* mice. $Cdh1^{d/d}$ $Pten^{d/d}$ mice exhibited curly horns with massive blood in the uterus. The uteri of $Cdh1^{d/d}$ $Pten^{d/d}$ mice resulted in extremely abnormal uterine development with active and complex angiogenesis, and widely disseminated epithelial invasion into myometrium, suggesting that ablation of *Cdh1* and *Pten* accelerates aggressive and invasive endometrial carcinomas leading to metastatic potential. However, $Cdh1^{d/d}$ $Pten^{d/d}$ mice died at day 15-19 of age due to excessive uterine bleeding (abnormal metrorrhagia). Thus, our current model does not allow sufficient time for the emergence of advanced, clinically aggressive endometrial tumorigenesis and metastasis. In this study, we developed a model of orthotopic transplantation to further examine tumor development including cell invasion, dissemination and metastasis. The uteri of control, $Cdh1^{d/d}$, $Pten^{d/d}$ and $Cdh1^{d/d}$ $Pten^{d/d}$ mice were collected and dissected to approximately 1 mm in diameter. Then, the tissue fragments were orthotopically implanted into the uterus (endometrium) of wild-type syngeneic host mice. The recipient mice were sacrificed 4 weeks post implantation, and uterine histology was examined whether donor tissues were successfully implanted and developed in the recipient uterus. Our preliminary results clearly demonstrated that the fragments of donor uterus from $Pten^{d/d}$ mice successfully incorporated into the recipient uterine tissue. The implanted donor tissues were histologically comparable to the $Pten^{d/d}$ uteri that normally present with complex atypical hyperplasia. Initially, donor tissues were directly implanted into the lumen of the recipient uterus. However, these tissues were not successfully retained, as the uteri were unable to adhere and incorporate into the recipient endometrium. Additionally, we have implanted uterine tissue fragments under the skin to determine whether they retain the original uterine histological and growth features. Both control and $Pten^{d/d}$ uterine tissues survived under the skin, and the tissue fragments from $Pten^{d/d}$ mice were larger than those from control mice at necropsy. However, the tissue fragments did not retain their uterine histoarchitecture, suggesting that subcutaneous implantation of uterine tissues is not the optimal model to observe the progression of endometrial tumors. Therefore, the orthotopic uterine explants will be a feasible way to characterize uterine tumorigenesis in $Cdh1^{d/d}$ $Pten^{d/d}$ mice while circumventing the complication of early death. Unlike injecting tumor cells directly, implanting tissue fragments into a syngeneic host mimics the natural tumor microenvironment and immune response of the donor. We are thereby capable of reconstructing the normal tumor progression of our transgenic mouse model. This technique would also permit us to examine tumor dissemination and metastasis beyond the lifespan of the mice. If the technique is well developed, this model will provide us insights for the discovery of potential therapeutic targets for the treatment of aggressive phenotypes of endometrial cancer.

Supported by NIH/NCI R15CA179214.

P31. **Pomegranate juice alters gene expression in human placental villous trophoblasts.**

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The epithelium of the human placenta is composed of an outer layer of syncytiotrophoblast and underlying cytotrophoblasts. The syncytiotrophoblast is a terminally differentiated, highly multinucleated cell type that is in direct contact with the maternal blood and that therefore mediates all fetal-maternal exchange. Underlying cytotrophoblasts are mononucleated cells that undergo division and differentiation, fusing with the syncytium allowing its growth and repair. During many complicated pregnancies (*e.g.* with intrauterine growth restriction and/or preeclampsia), evidence suggests increased oxidative stress leads to damage and death by apoptosis of villous trophoblasts, likely contributing to the observed placental dysfunction and poor pregnancy outcomes. We have previously shown that pomegranate juice (PJ) reduces oxidative stress in human trophoblasts *in vivo* and in cultured primary human trophoblasts *in vitro* (Chen B et al. Pomegranate juice and punicalagin attenuate oxidative stress and apoptosis in human placenta and in human placental trophoblasts. Am J Physiol Endocrinol Metab 2012;302:E1142-52), suggesting PJ is worth investigating as an antenatal therapy for complicated pregnancies. Here, we take a candidate gene approach to investigate the effects of PJ on gene expression in cultured primary human cytotrophoblasts and syncytiotrophoblasts, comparing expression of 22 genes in the presence or absence of PJ. genes showed altered expression. The gene with the most dramatically altered expression was NDRG1, which we previously found to protect trophoblasts from oxidative stress (Chen B, Nelson DM, Sadovsky Y. N-myc down-regulated gene 1 modulates the response of term human trophoblasts to hypoxic injury. J Biol Chem 2006;281:2764-72.). NDRG1 mRNA and protein levels were both increased several fold by PJ in trophoblasts cultured under normoxia or under oxidative stress. We propose this increase in NDRG1 contributes to the protective effects of PJ on trophoblasts exposed to oxidative stress.

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P32. **Sperm Release from the Functional Sperm Reservoir in Swine Involves Progesterone and and Sperm Cation Channels (CatSper)**

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Sperm storage in the female reproductive tract is a reproductive strategy used by a number of species. When insemination and ovulation are poorly synchronized the formation and maintenance of a functional sperm reservoir (SR) in the oviductal isthmus improves the odds of fertilization. Reproductive efficiency in species like swine relies on the formation of a SR because time of ovulation in the sow takes place over a variable period after the onset of estrus. The SR regulates sperm functions, including viability and capacitation. Once capacitation takes place, a fraction of the sperm attached to the oviductal epithelium is released to fertilize oocytes. Although detachment is a controlled process, the mechanisms and molecules that regulate sperm detachment are unclear. There is empirical association between hormonal milieu in the oviduct and sperm detachment. The goal of this work was to assess the influence of progesterone (P) on sperm release from porcine oviductal explants. We employed an in vitro assay to test the ability of P to induce sperm release from oviduct cell aggregates. Sperm were allowed to bind to the aggregates and then challenged with 80 and 800 nM P, which stimulated the release of up to 47.75 % of sperm from the cell aggregates. Calcium influx is associated with late stages of capacitation and development of hypermotility, essential to sperm fertility. We used Fluo-4 AM, a fluorescent Ca^{2+} indicator, to determine whether stimulation with P affected intracellular Ca^{2+} concentration. Incubation of sperm with 80nM P for 30 min resulted in a 12.88% increase in intracellular Ca^{2+} when compared to vehicle controls. P is known to activate CatSper, a sperm-specific Ca^{2+} channel. We blocked CatSper with 2 μM NNC 055-0396, a Ca_v channel inhibitor. After a 30 min-incubation, NNC 055-0396 reduced intracellular Ca^{2+} concentration by 9.63 % when compared to sperm treated with 80 nM P. Sperm treated with RU-486 did not differ from the control, ruling out the involvement of the classical nuclear receptors for P. We then tested whether functional CatSper channels were involved in sperm release from oviduct cells. Sperm bound to aggregates were treated with 2 μM NNC 055-0396 for 30 min. Blocking CatSper inhibited 93.72 % of sperm release when compared to groups treated with 80 nM P and vehicle. This is the first report indicating that release of sperm from the SR is induced by P action through CatSper channels. Supported by USDA NIFA 2011-67015-20099.

P33. **Effects of Hypoxia and Activation of the NFkB Pathway on FMS-Like Tyrosine Kinase 1 (flt-1) in Human Placenta Explants and Trophoblast Cells**

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FMS-Like Tyrosine Kinase 1 (Flt1) is a receptor for placenta growth factor (PGF). The two main forms of Flt1 receptors, that arise via alternative mRNA splicing, are membrane Flt1 (mFlt1) and soluble Flt1 (sFlt1). Successful pregnancy is thought to need PGF binding to mFlt1 on trophoblast and endothelial cells. During preeclampsia, generation of sFlt1 from trophoblast increases dramatically which acts as an antagonist to limit PGF from binding to the mFlt1 receptor. Mechanisms and controlling factors that regulate trophoblast production of sFlt1 are not well understood. Preeclampsia is thought to result from a relatively hypoxic and pro-inflammatory placental bed. The objective of this study is to determine the effects of hypoxia and activation of the NFkB pathway on Flt1 isoform expression. We used ex vivo human term placenta explants and trophoblast cell lines to determine the ability of hypoxia (1% O2) and cytokine-induced activation of NFkB pathway to influence mFlt1 and sFlt1 production. Following treatments, mRNA from these samples were analyzed via quantitative RT-PCR using mFlt and sFlt specific primer probe set. For several trophoblast cell lines and placenta explants sFlt1 expression increased at least three fold when treated with hypoxia for 24 hours. Also, TNF-α treatment (20ng/ml) increased both mFlt1 and sFlt1 expression 2-4 fold in term villi. One mechanism for these increases could be transcription activation. Thus, we cloned 1.7kb of the 5'UTR of Flt1 into a luciferase report construct to determine Flt1 transcription activity in the various trophoblast cell types after treatment with hypoxia and activation of the NFkB pathway. Initial studies showed that baseline Flt1 transcription activity was higher in hEK-293 cells than JEG3 trophoblast. For both cell types hypoxia did not produce a significant increase in Flt1 promoter activity. Transfections with the 1.7kbFlt-luc reporter and NFkBp65 were inconclusive. To facilitate analyses of the promoter activity we are creating a stable cell with 1.7kbFlt-luc reporter incorporated into the genome of trophoblast as well as non-trophoblast hEK-293 cell lines. Initial experiments seem to show some stable incorporation since the luciferase expression is higher in the stable clones than the parental population. Altogether, these results suggest that hypoxia and NFkB can increase sFlt production in trophoblast but this may not be the result of increased gene transcription.

P34. **Examination of gold nanoparticles and cantharidin as potential treatments for breast cancer**

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Nano-sized particles exhibit biocompatibility and have recently gained attention in the biomedical field. They have exhibited potential for use in a variety of areas such as clinical diagnosis (imaging), cell labeling, drug targeting, and enzyme immobilization. We have begun examining the potential for gold nanoparticles to be used as potential diagnostic or treatment tools for breast cancer, using a cytotoxicity assay. Several concentrations of colloidal gold nanoparticles were applied to non-cancerous and cancerous breast cancer cells and the level of cell survival was determined. Interestingly, variation in nanoparticle size for colloidal gold had a large effect upon cell viability in normal cells but not in cancerous cells. Another aspect of this study is examining what toxin to incorporate into these gold nanoparticles. Cantharidin has been indicated as a potential compound for the treatment of cancers as it is highly toxic. We assessed the toxicity of cantharidin in cancerous and non-cancerous breast cell lines to see the viability of the cells, both at several concentrations and time intervals. Taken together, this data will allow us to determine whether gold-encapsulated cantharidin may be a potential new method for cancer detection or treatment. Supported by the Millikin University Department of Biology and a Millikin University SURF grant.

P35. **Regulation of PAX2 expression in high-grade serous ovarian cancer**

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Ovarian cancer is the most lethal gynecological malignancy affecting American women. High-grade serous cancer (HGSC) is the most lethal form of the disease. Lack of precursor lesions to detect the disease at an early stage is attributed to the debatable source of origin; which could be the ovarian surface epithelium (OSE) or fallopian tube epithelium (TEC) or both. Elevated levels of mutant-p53 are noted in the distal end of fallopian tubes of women with genetic predisposition to ovarian cancer, suggesting common source of origin. PAX2, a transcription factor expressed by normal TEC, is lost in HGSC and putative precursor lesions of serous cancer in the tube. Its expression is downregulated in secretory cell outgrowths and p53 signatures, which are proposed to be benign precursors to serous tubal-intraepithelial carcinomas. In the present study, we screened mouse TEC (MTEC) and OSE (MOSE) cells for PAX2 expression. PAX2 was present in MTECs but absent in MOSEs when analyzed by western blot. Absence of PAX2 in OSE was confirmed *in vivo* by immunohistochemistry. To investigate the effect of loss of PAX2 in MTEC cells, series of PAX2 specific shRNA were transfected in MTEC cells. MTEC-PAX2^{KD} and MTEC^{SCR} (control) clones were validated for knockdown efficiency by western blot. Alteration in anchorage-independent growth and proliferation of cells with PAX2^{KD} was evaluated by soft agar and sulforhodamine-B assay respectively. Suspected serous cancer signaling pathways, including KRAS^{mut}, p53^{mut} and PTEN^{KD} were introduced in MTEC cells. Genetically modified MTEC cells demonstrated significant downregulation of PAX2, suggesting that PAX2 could be regulated downstream of common signal transduction pathways frequently implicated in serous tumors. Human serous cancer cell lines, including OVCA429, SKOV3, OVCAR5, OVCAR4, OVCAR3 and OVCA432 lacked PAX2 expression. p53 is a known transcriptional regulator of PAX2. PAX2 expression was detected in OVCA420 (p53^{WT}). OVCA420 cells transfected with p53^{mut}, downregulated PAX2, however SKOV3 (p53^{null}) transfected with p53^{WT} failed to re-express PAX2 protein indicating that mutations in p53 alone, can hinder but cannot induce PAX2 expression. Further, to investigate whether gain of PAX2 reduces cancerous potential of serous cell lines, OVCA432 and SKOV3 were transfected with pCMV-Myc-PAX2 and pCMV-Neo plasmids. Overexpression of PAX2 in these cell lines was confirmed by western blot. Preliminary results suggest that mutation in p53 in combination with KRAS^{mut} or PTEN^{KD} influence the loss of PAX2. Further studies are warranted to support PAX2 as an early sequential biomarker to detect precursors of serous cancer in the tube. Supported by ACS grant.

P36. **Activin and Notch signaling pathway cross-regulation in the mouse ovary.**

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The development and maintenance of ovarian follicles during a female's lifetime is coordinated by numerous endocrine, paracrine, and juxtacrine signaling pathways. Initiation of follicle assembly is characterized by the establishment of cell-cell contacts between the germ cell or oocyte and a single layer of somatic pre-granulosa cells. In response to intrinsic and extrinsic stimuli, small numbers of these follicles are selected to develop, promoting oocyte activation and somatic cell proliferation and differentiation. Both Activin and Notch signaling pathways are present in the developing ovary where they influence somatic cell proliferation and follicle assembly through local paracrine and juxtacrine mechanisms. Suppression of Activin or Notch signaling in the ovary results in the abnormal phenotype of multi-oocytic follicles, where multiple oocytes are contained within one follicle. This shared phenotype led us to hypothesize that Activin and Notch signaling might cooperatively regulate follicle development. To examine Activin and Notch signaling cross-regulation we tested whether activation or repression of Activin signaling alters the expression of Notch signaling receptors, ligands and target genes shown to be present during follicle development. Primary mouse granulosa cells cultured with recombinant Activin show an increase in mRNA expression of the Notch ligand, *Jag1* and the target genes, *Hey2* and *HeyL*. Furthermore, a model of Activin signaling suppression shows a decrease in mRNA levels for the receptor, *Notch2*, the ligand *Jag2*, and the target genes *Hey2* and *HeyL*. Conversely, attenuation of the Notch signaling pathway through conditional deletion of the *Notch2* receptor leads to a decrease in mRNA expression of the *Inh□A* and *Inh□B*, the genes that encode the protein subunits of Activin. Together, these results suggest a mechanism of cross-regulation between Activin and Notch signaling in the ovary. Supported by the Eunice Kennedy Shriver NICHD Program Project Grant (NIH P01 HD021921).

P37. **Response of tubal cells, a putative ovarian cancer precursor cell type, to estrogen receptor ligands**

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Ovarian cancer (OVCA) is the most lethal gynecological malignancy. One important factor contributing to the deadliness of OVCA is the unknown disease etiology, although current evidence indicates OVCA could arise from either the ovarian surface epithelium (OSE) or the fallopian tubal epithelium (TEC). A risk factor for OVCA is estrogen only hormone replacement therapy. Estrogen receptor (ER) and progesterone receptor (PR) are prognostic biomarkers in OVCA with the presence of both receptors correlating with improved survival. Despite the influence of estrogen in OVCA, selective estrogen receptor modulator (SERMs) therapy has been limited with only a 20% response rate. This low response could be due to many factors including the loss of estrogen signaling or the tissue of origin. The response of TEC to SERMs is not known, which if this cell type does give rise to OVCA would be useful when determining therapy options.

This study characterizes the response of ER ligands in mouse oviductal tubal epithelial cells (MTEC) and mouse ovarian surface epithelial cells (MOSE). Both cell types express ER and PR by Western blot analysis. The responsiveness to estrogen was investigated, with only MTECs demonstrating estrogen dependent PR upregulation.

MTEC cells were further probed for response to the SERMs tamoxifen (4OHT), raloxifene (RAL) and desmethylarzoxifen (DMA). PR was downregulated by treatment with SERMs by Western blot. A transcriptional luciferase reporter assay using a canonical ERE binding element revealed specific ER antagonist activity with all SERMs tested. A proliferation assay revealed no growth change in response to any ER ligands. Wound healing assays are in progress to determine if ER ligands affect cell migration.

Finally, the receptor status of a panel of OVCA cell lines was determined by Western blot revealing many OVCA cell lines do not express ER or PR, which may reflect the loss of receptors during tumorigenesis or these tumor cells arose from an estrogen unresponsive cell type. SKOV3 cells were further investigated by a reporter assay and qPCR. While SKOV3 cells were the most estrogen responsive cell line in the reporter assay, qPCR revealed no response to estrogen at endogenous loci consistent with previous reports that SKOV3 expresses a truncated form of ER that precludes full ER activity. If the TEC is the origin of OVCA, it is of great importance to understand the implications of SERM therapy, especially in women with a BRCA mutation taking prophylactic tamoxifen to reduce their risk of breast cancer.

P38. **NFκB regulates placenta growth factor and its primary transcription factor, glial cell missing 1 in human trophoblast**

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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 the maternal spiral arteries into utero-placental arteries play an important role. As fetal 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 is known to decrease placenta growth factor (PGF) expression in trophoblast and may play a key role in the suppression of PGF levels associated with preeclampsia. Systemic vasodilation, also critical to facilitate a healthy pregnancy, is significantly correlated to higher serum levels of PGF and lower levels of its soluble receptor, soluble fms-like tyrosine kinase-1 (sFLT-1); the inversion of this ratio is linked to an increased risk of preeclampsia. Dysregulation of certain pro-inflammatory pathways, including NFκB, has also been implicated in preeclampsia development. The goal of our studies was to determine the mechanism governing NFkB regulation of PGF in trophoblast. Over-expression of p65, 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 NFκB complex activity. Similarly, over-expression of p65 functionally inhibits glial cell missing 1 (GCM1), the primary transcription factor of PGF in trophoblast, but has little effect on GCM1 mRNA expression. Previous reports show that GCM1 protein is degraded during hypoxia. Because hypoxia and activation of NFκB have similar effects on both PGF and its transcription factor, GCM1, we have investigated the possibility of cross-talk between these two pathways. Unlike hypoxia, however, p65 did not significantly influence GCM1 protein stability, and 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 separate pathways to decrease PGF expression in trophoblast. Going forward, we are interested in elucidating the mechanism(s) behind the inhibition of GCM1 functional activity and suspect that a post-translational element is involved. Such a mechanism would account for the precipitous decrease in GCM1 functional activity without the concomitant decrease in expression levels of GCM1. Based on the current literature and our preliminary data, it is possible that p65 competes for critical post-translational modifications necessary for GCM1 function and PGF expression during pregnancy.

P39. **Murine Fetal Leydig Cells are Functional in Primary Culture**

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The development of the masculine fetus is a dynamic process that depends on the production of androgens by the testicular fetal Leydig cells. A deficiency of androgen production in the male fetus can lead to feminized genitalia and brain and birth defects such as hypospadias and cryptorchidism. Fetal Leydig cells within the developing testis are the sole source for androgen production in the male embryo. Unlike adult Leydig cells however, control of testosterone production by fetal Leydig cells is independent of gonadotropins and the mechanisms that promote steroidogenesis remain unclear. The study of fetal Leydig cells will help understand the processes that surround fetal steriodogenesis, but has been limited by factors including their small numbers, the lack of cell lines, and their rapidly changing biology during development. We aim to develop a method to effectively isolate and study fetal Leydig cells in primary culture. To achieve this we bred double reporter mT/mG mice to Cre recombinase expressing mice to achieve fluorescent green protein (mG+) labeled fetal Leydig cells. mG+ fetal Leydig cell identity was validated within sections of fetal testes using double-labeled immunohistochemistry against GFP and steroidogenic enzymes. RNA transcripts measured by qPCR verified unique mT+ and mG+ cell populations after fluorescent activated cell sorting (FACS). Cell culture was performed with mixed and sorted cell populations using microchannels that held a volume less than 20ul within a small surface area. The mixed population culture system allowed direct cell to cell contact of fetal Leydig cell with other testicular cell types. In contrast, the sorted cells were placed in a co-culture system that isolated fetal Leydig cells from remaining testicular cells, but allowed access to shared media. Media was harvested every 24 hours to measure testosterone concentration by enzyme-linked immmunosorbent assay (ELISA). Our results show that fetal Leydig cells can synthesize substantial quantities of testosterone for at least four days in culture whether they are in direct cell to cell contact or only shared media contact. This unique culture system will be useful in future studies of fetal Leydig cells to further describe androgen production and development of the masculine fetus. This work has been supported by the University of Wisconsin and the Merial Summer Scholar Program.

P40. **The role of Notch signaling in postnatal arcuate nucleus development**

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The hypothalamus is the neural regulator of reproduction. In particular, the arcuate nucleus (Arc) contains kisspeptin neurons, which interact with gonadotropin releasing hormone (GnRH) neurons in the median eminence (ME), to modulate pituitary luteinizing hormone release. Arc development first involves the prenatal generation of 3rd ventricle progenitor cells. Over time, these progenitor cells generate postmitotic neurons that migrate away and differentiate to contain distinct neuropeptides. Despite the fact that aberrant development of kisspeptin neurons can lead to Idiopathic Hypogonadotropic Hypogonadism and accompanying infertility, the mechanisms by which normal development of Arc neurons occurs is relatively unknown.

The Notch signaling pathway is a cell signaling pathway that is involved in the maintenance of progenitor cells during development. We have previously shown that Arc specific embryonic genetic manipulation of the Notch signaling pathway affects the development of neurons within the region. However, recent studies have shown neurogenesis postnatally within regions surrounding the Arc including the ME. The location of postnatal neurogenesis suggests that these cells could be generated in response to the environment to modulate neuron function. Components of the Notch signaling pathway have been shown to persist postnatally in the region. Based on this previous evidence, we hypothesize that Notch signaling regulates early postnatal neurogenesis.

To test this hypothesis, we first determined the postnatal peak age in newborn cell generation within the ME. We injected BrdU, a compound that incorporates into newborn cells, every two hours for twelve hours at three different ages. We observed that the peak number in BrdU-immunopositive cells, a marker of newborn cells, within the median eminence was postnatal day P7. After determining the peak age, we injected mice with the Notch signaling inhibitor DAPT, or the vehicle control DMSO during this identified time window. After chemical inhibition, we saw no significant difference in Hu-immunopositive cells, a marker of adult neurons, within the ME when compared to vehicle treated controls. Similarly, we observed no difference in GFAP expression, a marker of glia, within the ME. Interestingly, it appeared that DAPT treated mice may have reduced expression of Sox2, a progenitor cell marker, when compared to controls. Based on these results, Notch signaling during this time window does not appear to be involved in neurogenesis, but may play a role in postnatal progenitor cell maintenance.

P41. **The Role of Gdnf Signaling in Prostate Development**

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Glial cell line derived neurotrophic factor (Gdnf) is a member of the Tgfβ 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 form 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 proliferation and development are unclear. Our objective is to determine the functional role of Gdnf signaling in prostate development. 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 induction in vivo. In day 16 embryos, Gdnf and Gfrα1 are expressed in keratin-positive UGS epithelium and in keratin-negative, vimentin-positive UGS mesenchyme. As the prostate develops, Gdnf expression declines in UGS mesenchyme at postnatal day (P)0 and P7, restricting expression to UGS epithelium. We have also established a UGS organ culture system for prostate development. In UGS organ culture, we have obtained compelling evidence that exogenous Gdnf increases proliferation of mesenchymal and epithelial cells in the UGS, altering prostate morphogenesis. In addition, inhibition of Ret kinase activity inhibits prostate morphogenesis and inhibition of ERK kinases (MEK1/2) suppresses Gdnf-induced proliferation of the UGS mesenchyme. With regard to mechanism, 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.

P42. **STAT3 Regulates Uterine Epithelial Remodelling and Epithelial-Stromal Crosstalk During Implantation**

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Embryo implantation is regulated by a variety of endometrial factors, including cytokines, growth factors and transcription factors. Earlier studies identified the leukaemia inhibitory factor (LIF), a cytokine produced by uterine glands, as an essential regulator of implantation. LIF, acting via its cell surface receptor, activates the signal transducer and activator of transcription 3 (STAT3) in the uterine epithelial cells. However, the precise mechanism via which activated STAT3 promotes uterine function during implantation remains unknown. To identify the molecular pathways regulated by STAT3, we created $SW^{d/d}$ mice in which *Stat3* gene is conditionally inactivated in uterine epithelium. The $SW^{d/d}$ mice are infertile due to a lack of embryo attachment to the uterine luminal epithelium and consequent implantation failure. Gene expression profiling of uterine epithelial cells of $SW^{d/d}$ mice revealed dysregulated expression of specific components of junctional complexes, including E-cadherin, a- and β-catenin, and several claudins, which critically regulate epithelial junctional integrity and embryo attachment. Additionally, uteri of $SW^{d/d}$ mice exhibited markedly reduced stromal proliferation and differentiation, indicating that epithelial STAT3 controls stromal function via a paracrine mechanism. The stromal defect arose from a drastic reduction in the production of several members of the epidermal growth factor (EGF) family in luminal epithelium of $SW^{d/d}$ uteri and the resulting lack of activation of EGF receptor signaling and mitotic activity in the stromal cells. Collectively, our results uncovered an intricate molecular network operating downstream of STAT3 that regulates uterine epithelial junctional reorganization, and stromal proliferation and differentiation, which are critical determinants of successful implantation.

P43. **Gonadotropin Regulation of Notch Signaling Genes During Ovarian Follicle Growth and Development**

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Growth and ovulation of the ovarian follicle requires the coordinated actions of local paracrine factors and endocrine hormones. In mammals, the anterior pituitary secretes follicle stimulating hormone (FSH) and luteinizing hormone (LH) that serve as predominant endocrine cues for growth and ovulation of the follicle. Numerous paracrine regulatory pathways within the ovary participate in folliculogenesis, either throughout this process or at specific stages of follicular development. An emerging pathway that mediates juxtracrine signaling within the ovary is the Notch signaling pathway. Impaired fertility has been observed in knockout mouse models of attenuated ovarian Notch signaling developed by our group and others. We have also shown that chronic suppression of Notch signaling results in decreased granulosa cell proliferation and increased apoptosis in growing follicles of the prepubertal mouse ovary. Given the primal role of gonadotropins in regulating follicle growth and differentiation, we hypothesized that gonadotropin action might regulate or cooperate with Notch signaling during follicle development. To test this concept, we established expression profiles of genes belonging to the Notch signaling pathway following gonadotropin stimulation. We treated prepubertal mice with pregnant mare serum gonadotropin (PMSG) to activate FSH receptors followed by human chorionic gonadotropin (hCG) to activate LH receptors and measured mRNAs levels of select Notch receptors, ligands, and target genes using qRT-PCR. Treatment with PMSG resulted in a slight increase in expression of the Notch target gene *Hey2*, but other genes were unaffected. In contrast, hCG treatment resulted in significant increases in mRNAs encoding multiple Notch ligands (*Jagged1*, *Dll1*, *Dll3*, and *Dll4*) and receptors (*Notch1*, *Notch2*, *Notch3*, and *Notch4*). This was associated with increased expression of the Notch target gene *Hes1* and decreased expression of *Hey2*. The temporal patterns and extent of regulation of Notch pathway genes following hCG stimulation varied. These findings suggest a broad and complex regulation of local ovarian Notch signaling by circulating gonadotropins, especially in response to hCG initiation of the ovulatory cascade. Current efforts are focused on examining protein expression and localization of Notch signaling molecules in the ovary following gonadotropin stimulation and investigating the potential role of Notch signaling during ovulation and luteinization. Supported by the Eunice Kennedy Shriver NICHD Program Project Grant (NIH P01 HD021921) and NIGMS Cellular and Molecular Basis of Disease Training Grant (NIH T32 GM08061).

P44. **MED12 and HMGA2: Two independent genetic alterations in uterine leiomyoma**

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Uterine leiomyomata are the most common neoplasm in women of reproductive age with an incidence of over 70%. Recent identification of somatic *MED12* mutations in the majority of uterine leiomyomas brings a new venue for the study of the tumorigenesis of leiomyomas. We are particularly interested in the correlation of *MED12* and HMGA2 gene products in leiomyomas and leiomyosarcomas with and without *MED12* mutations. To address the issues, we examined *MED12* mutations in a large cohort of usual type leiomyomas (178 cases) and uterine leiomyosarcomas (32 cases). We found that 74.7% (133/178) of leiomyomas had *MED12* mutations, which was consistent with several studies. In contrast, only 9.7% (3/32) of leiomyosarcomas harbored *MED12* mutations. Expression analysis by Western blot and immunohistochemistry revealed that those leiomyomas with complex *MED12* mutations had significantly lower protein products than the controls. Most leiomyosarcomas without *MED12* mutations also had very low levels of MED12 expression in comparison to matched myometrium. These findings suggest a potential functional role of MED12 in both benign and malignant uterine smooth muscle tumors. Whether *MED12* mutations are the direct causal factor for the development of most leiomyomas is unclear, these clinically significant findings will accelerate medical research to characterize the functional role of *MED12* in the disease.

When we further examined HMGA2 expression in all leiomyomas and leiomyosarcomas, we found HMGA2 overexpression was exclusively present in leiomyomas with no *MED12* mutation, accounting for 10.1 % (18/178) of total leiomyomas and 40 % (18/45) of non-*MED12* mutant leiomyomas. ~25 % (8/32) of leiomyosarcomas had HMGA2 overexpression and no *MED12* mutations were found in HMGA2 positive leiomyosarcoma. These findings strongly suggest that MED12 mutations and HMGA2 overexpression are independent genetic events that occur in leiomyomas, and they may act different in the tumorigenesis of uterine leiomyomas. To summarize, our study of benign and malignant uterine smooth muscle tumors concludes that: 1) *MED12* mutations are common in usual type leiomyomas: 2) leiomyomas with HMGA2 overexpression do not share or harbor *MED12* mutations; 3) complex *MED12* mutations lead to loss or reduced MED12 protein product; and 4) leiomyosarcomas have a very low rate of *MED12* mutations, suggesting that most leiomyosarcomas have independent tumorigenic pathways from leiomyoma. Supported by Edna Leiomyosarcoma Foundation and R03 HD057380 (JJW), RO1 HD064402 and RO1 CA154358 (T.K). *These authors contributed equally, # indicates corresponding authors.

P45. **Downstream signaling from mutant p53 expression in normal oviductal tubal epithelium: Insights into serous cancer formation**

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Epithelial ovarian cancer is the fifth leading cause of cancer death among US women. Mounting evidence supports the hypothesis that "ovarian cancers" may originate in the distal end of the fallopian tube. Although a heterogeneous disease, 96% of serous ovarian tumors contain mutations in p53. In addition, the "p53 signature", or overexpression of p53 (usually associated with a mutation) is a potential precursor lesion to the disease suggesting an essential role for p53 mutation in early tumorigenesis. Most ovarian and tubal cell lines are immortalized with T (SV40T) antigen, precluding investigations into the role of mutant p53. To further our understanding of p53-mutation dependent effects on cells, normal mouse tubal epithelial cells (MTEC), which are able to passage in culture, were stably transfected with the R273H *p53* mutation, the most common mutation found in serous ovarian cancers, oncogenic *KRAS* (G12V) or SV40. The cells were analyzed for changes in morphology, proliferation, the ability to grow in soft agar, wound closure, gene profiling and protein expression. Mutation of p53 caused increased, stabilized expression of p53 as determined via western blot and immunofluorescent analyses. No significant change in morphology, proliferation or ability to grow in soft agar was observed in MTEC cells with mutant *p53* or *KRAS*, however SV40 MTEC showed a significant increase in proliferation by day 3 and formed large colonies when plated in soft agar. Expression of Aurora A and B kinase and Polo-like kinase 4 increased in mutant p53 MTEC and all three of these targets are overexpressed in ovarian tumors. These kinases ensure equal distribution of genetic material during cell division and their misregulation may lead to aneuploidy, a hallmark of serous ovarian tumors. *CMET* expression, which has been associated with poor patient prognosis, was increased in both mutant p53 and KRAS MTEC. Interestingly, expression of Wilm's Tumor protein, which is often upregulated in serous ovarian cancer, decreased in mutant *p53* MTEC. In the future, cells will be treated with commercially available inhibitors to determine potential candidates for targeted therapies that may act to prevent further accumulation of mutations in early lesions. Combining genetic manipulations, such as oncogenic KRAS activation or Rb silencing, with p53 mutation will be performed to determine their combined role in transformation. Understanding the mechanisms required to transform normal fallopian tube epithelium may help develop chemoprevention strategies and elucidate novel treatments to improve overall survival rates. Supported by the Department of Defense OC110133.

P46. **Imagining mouse ureterovesical junction (UVJ) formation**

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Abstract Solving control of tissue fusion malformations is an open problem that stands to benefit from collaborative investigations. Some variably penetrant CAKUT (Congenital Anomalies of the Kidney and Urinary Tract) phenotypes are influenced by correct coordination of nephric duct/cloacal fusion. While it is self-evident that clarifying observations cultivates scientific imagination, which impacts sustainability of productive collaborations, there are currently no clear cellular representations of this transient developmental process. We produced a map of pan-Laminin distributions during mouse ureterovesical junction (UVJ) formation from 8.5dpc to 12.5dpc using combinations of *Pax2:Cre;Smad4fl/fl;mTmG* alleles. We demonstrate that *Pax2:Cre* is activated in a field of cells that contributes to the leading edge during hindgut invagination, a region of which later interacts with the caudal nephric duct to form the UVJ. We classified different behavioral stages of nephric duct/cloacal fusion by taking advantage of the property of basement membranes to separate tissues. A partially penetrant, disconnected phenotype was scored at 11.5dpc in conditional nulls through comparison with the novel developmental series. We constructed an explicit agent-based computer model capable of producing variable solutions using open source Netlogo software. We contrasted our working model of nephric duct targeting with Mackie and Stephens-related models to situate shared histories and to highlight key issues that require attention. Our work demonstrates that Smad4 is required by *Pax2:Cre* labeled cells to promote robust coordination of mouse nephric duct/cloacal fusion and is intended to help sustain future collaboration by improving imagination of mouse UVJ formation. Supported by George M. Eisenberg Foundation for Charities and NIH/NCI R01 CA154358

P47. **Regulation of transcription factors by BMP4 and Activin A during the cell fate decision in vaginal epithelium.**

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The objective of this study is to identify the factors that determine the cell fate in MDE through the induction of *Δ*Np63.
In mammals the paramesonephric ducts of mesodermal origin, called Mülleran ducts (MDs), give rise to the female reproductive tract (FRT). The MD epithelium (MDE) provides the real contribution to the FRT epithelium development, differentiating into diverse epithelial cell types with unique morphology and functions in each organ. Tissue recombination studies have shown that epithelial–mesenchymal tissue interaction plays a critical role for the epithelium differentiation. In particular, factors secreted from uterine and vaginal mesenchyme are the responsible for the uterine and vaginal epithelium differentiation. Recently, we have demonstrated that BMP4 and Activin A produced by vaginal mesenchyme induce expression of *Δ*Np63 via the effector molecules SMAD4 and RUNX1. However, the pathways responsible of the activation of *Δ*Np63 in Müllerian duct epithelial cells are not fully understood.
We have identified that the expression of three transcription factors, SIX1, RUNX1 and PITX1 occurs in the neonatal vaginal epithelium preceding the expression of *Δ*Np63. Hence, the regulation of these transcription factors in MDE was studied utilizing the organ culture of neonatal FRT as well as the conditional knockout mice for SMADs and RUNX1.
Our results indicated that mesenchymal BMP4 and Activin A differentially regulate these three transcription factors in MDE via SMAD-dependent and –independent pathways. Their expression patterns strongly suggest that these three transcription factors in concert control the activation of *Δ*Np63 in MDE as the downstream of mesenchymal signals. Supported by the NIH/NCI R01 CA154358.

P48. **ESR1 and ESR2 activation differentially contribute to the expression of circadian rhythms in female mice**

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Estrogenic signaling shapes and modifies circadian rhythms, the disruption of which have been implicated in psychiatric, neurologic, cardiovascular, and metabolic disease, among others. To determine the activational impact of estrogen on daily behavior patterns and differentiate between the roles of ESR1 and ESR2, the primary CNS estrogen receptors, ovariectomized adult female mice were chronically administered estradiol (E), the ESR1 agonist propylpyrazole triol (PPT), the ESR2 agonist diarylpropionitrile (DPN), or cholesterol (CTL). Animals were singly housed with wheels in 12:12 LD or total darkness (DD), and wheel revolutions were recorded in 10 min bins.

E administration significantly increased average daily activity compared to CTL. This effect was recapitulated by chronic PPT, but not DPN administration, suggesting a role for ESR1, but not ESR2 in the regulation of total activity. Further, we found that E or PPT treatment consolidates activity to the dark phase, reducing the LD ratio compared to CTL. DPN treated animals did not differ from CTL animals with respect to LD ratio, suggesting that ESR1 alone is responsible for this effect. DPN, similar to E, resulted in a greater spread of activity throughout the active period. ESR2 activation also played a dominant role in the observed shift in acrophase to later in the subjective night, while ESR1 stimulation mimicked the elevated amplitude observed in E treated animals compared to CTL. DPN, E, or PPT at the highest dose reduced the phase angle of activity onset, suggesting that ESR1 and/or ESR2 activation is sufficient for advancing the onset of activity following the transition from L to D. Similarly, the length of the subjective day (tau) was shorter in animals administered PPT, DPN, or E. Finally, the phase delay associated with a pulse of light during the early subjective night in CTL animals was absent in animals treated with either E or DPN, demonstrating both that E opposes the plastic phase response and that the mechanism underlying this phenomena is ESR2-dependent. Importantly, we show here that estrogen has strong activational effects on the temporal patterning and expression of circadian behavior, and that these effects are due to distinct mechanisms elicited by ESR1 and ESR2 activation. Supported by Campus Research Board, UIUC (MMM), Billie Fields Foundation (SER), and a Neuroscience Program Fellowship (SER).

P49. **PI3K and AKT inhibition regulate distinct biological outcomes in uterine leiomyoma cells.**

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Uterine leiomyomas (UL), benign tumors of the myometrium, are the number one indication for hysterectomies in the U.S. due to a lack of effective therapy. The pro-survival PI3K and AKT pathway is hyperactive in UL compared to normal myometrium. We previously demonstrated that AKT inhibition using MK-2206 induced caspase independent UL cell death *in-vitro* and reduced UL xenograft volume *in-vivo*. PI3K activates AKT, but if PI3K inhibition would also elicit caspase independent UL cell death is unknown. In addition, data suggests that mTORC1, a downstream component of the PI3K and AKT pathway is hyperactive in UL cells but the cellular function of mTORC1 in UL is unknown. Therefore, the effects of PI3K and dual PI3K/mTOR inhibitors were tested on UL and myometrial cells using proliferation, viability, and cell death assays. PI3K inhibitor LY294002, and PI3K/mTOR inhibitors BEZ235 and GDC0980 reduced primary UL cell viability and proliferation over 48 and 72 hours. Interestingly, dual PI3K/mTOR inhibition was not significantly more effective at reducing UL cell viability compared to PI3K inhibition alone indicating that PI3K inhibition may be sufficient to inhibit mTOR signaling in UL. However, UL cells remained AnnexinV negative with intact cell membranes after 48 hours indicating a lack of cell death in response to PI3K and PI3K/mTOR inhibition. UL cells also lacked cleaved caspase 3 in response to BEZ235 and GDC-0980 supporting the Annexin V results. In conclusion, although PI3K is a major upstream activator of AKT, PI3K inhibition and AKT inhibition caused distinct cellular outcomes in UL cells. PI3K and PI3K/mTOR inhibition reduced cell proliferation in the absence of UL cell death, while our previous data showed that AKT inhibition promoted caspase independent UL cell death. These results provide molecular evidence for the involvement of PI3K and AKT in UL cell survival via modulation of cell proliferation and protection from cell death. The distinct responses of UL cells to PI3K and AKT inhibition suggest AKT independent proteins are involved in UL cell proliferation downstream of PI3K. The results support PI3K and PI3K/mTOR inhibition may be a viable option for the treatment of UL. This work was funded by NIH P01HD057877.

P50. **Investigating the Effect of Dietary Flaxseed on Oxidative Stress in Ovarian Cancer**

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy, with a 5-year survival rate of less that 50%. Lack of early detection markers and a high rate of recurrent, chemoresistant disease emphasizes the importance of chemopreventive approaches, including dietary intervention. While murine models are limited in their ability to recapitulate the etiology of human EOC, the laying hen develops EOC spontaneously in a manner very similar to the human disease. Reactive oxygen species (ROS) are a normal byproduct of cellular metabolism and are neutralized by a variety of cellular antioxidant defense mechanisms; however, an alteration in this balance in favor of ROS production results in oxidative stress. Chronic oxidative stress damages many different biomolecules and results in DNA mutations, and has been implicated as a causal factor in cancer and many other diseases. Flaxseed lignans possess antioxidant activity, and have been shown to reduce oxidative damage to biomolecules. Nrf2 is a transcription factor that drives expression of over 200 antioxidant and phase 2 genes, and has been shown to be dysregulated in ovarian and other cancers. We have shown previously that whole flaxseed reduces incidence and severity of ovarian cancer, indicating that flax influences both tumor development and metastasis. Microarray analysis shows that flaxseed modulates a number of genes involved in oxidative stress signaling pathways in normal ovaries and ovarian tumors, including targets of the mitogen-activatived protein kinase (MAPK) pathways. In the present study we show that Nrf2 protein is expressed in the tumor epithelia of early and late stage tumors. Phosphorylated p38 MAPK is expressed in both the tumor epithelia and stromal cells of early and late stage tumors. Profiling change in stress-responsive pathways during the course of tumor development in the hen spontaneous model of ovarian cancer may yield insight into the etiology of the disease, and the mechanism by which dietary flaxseed ameliorates the incidence and severity of the disease.

P51. **The somatic-specific *Rhox* genes exhibit unique regulation and putative independent functions in the testes.**

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The Reproductive Homeobox X-linked (*Rhox*) genes encode transcription factors that are expressed exclusively in the testis, epididymis, placenta, and ovary and are therefore good candidates to regulate fertility. The founding member of this 33 gene cluster *Rhox5*, previously known as *Pem*, is important for both spermatogenesis and sperm maturation. This is supported by the findings that *Rhox5*-null mice are subfertile due to increased apoptosis of meiotic germ cells in the testis and motility defects in epididymal sperm. In this report, we characterize the dynamic expression and subcellular localization of the 3 members of the *Rhox* cluster that are restricted to somatic cells in the testes, RHOX5, RHOX8, and RHOX11. During our investigation, we characterized two new anti-RHOX5 antibodies that are commercially available, but have not yet been vetted. RHOX5 exhibited androgen-dependent and stage-specific expression in Sertoli cells. Interestingly, while *Rhox8* exhibited high expression in all Sertoli cells from E11.5 to the adult, its expression was not androgen-dependent as it exhibited no change in the testes of androgen receptor mutants (SCARKO and ARKO). Our prior studies demonstrated that *Rhox8*-knockdown male mice exhibit similar subfertility to *Rhox5*-null mice, suggesting that these genes may be partially redundant. However, given their differential expression and regulation, we predict these genes may synergize to regulate distinct downstream genes from either the androgen-dependent or androgen-independent pathways for maximal spermatogenic output. *Rhox11* was previously thought to be Sertoli-specific, based on qPCR analysis of fractionated testes generated by differential enzymatic digestion and centrifugal elutriation. However, our novel anti-RHOX11 antibody demonstrated expression specifically in peritubular myoid cells around the basement membrane of all testis tubules during the window which qPCR analysis indicated it should be found. Currently, the putative functions in RHOX11 in myoid cells remain to be investigated, but it is likely to regulate gene products that cooperate with Sertoli cells to promote spermatogenesis, as well as promoting the integrity and contractility of the extracellular matrix defining the tubule interstitium boundary. Supported by NIH/NICHD 55268 and 65584.

P52. **Establishment of renal capsule xenografts of human primary endometrial cancer tissues to study invasion and metastasis**

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Endometrial cancer is the seventh most frequent cancer of women in the world with 142,000 new cases every year. Approximately 42,000 women die of this malignancy every year. Endometrial cancer can be aggressive exhibiting invasion into the myometrium and metastasis to distal sites. Currently, treatment for aggressive disease is suboptimal and there are no model systems to study the aggressive nature of these tumors from patients. The objective of this study was to establish a mouse xenograft model of primary endometrial tumor tissues in order to study invasion and metastasis. Approximately 1.5mmX1.5mm primary endometrial tumor fragments from patients were transplanted under the renal capsule of NSG mice. After 6-8 weeks, tumors were excised and serially transplanted into other mice for propagation. Among 11 cases tested thus far, (Uterine Papillary Serous Carcinoma (UPSC), cases 1-5 ; Uterine Clear Cell Carcinoma (UCCC), case 1; Endometrioid Carcinoma (EEC), case 1-5), UPSC1, UPSC3, ECC2 and ECC4 grew under the kidney capsule. UPSC1 grew outward and also into the kidney with a clear demarcation between tumor and kidney. In contrast, UPSC3, ECC2 and ECC4 preferentially invaded kidneys and grew tumors at distal site. Expression of plasminogen activator urokinase, PLAU/uPA, estrogen receptor (ER) and progesterone receptor (PR) was measured using immunohistochemical staining. Positive PLAU staining was observed with UPSC3, ECC2 and ECC4 xenografted tissues, but not in tissues from UPSC1. Additionally, UPSC1, UPSC3, ECC2 and ECC4 xenografted tissues were all ERα negative, while UPSC1 and ECC2 tissues were partially positive for PR. In conclusion, endometrial cancer tissues transplanted under renal capsule can grow, invade, and metastasize, providing a novel model to study the aggressive subtypes of endometrial cancer.

P53. **Leiomyoma cells undergo necrosis-like cell death upon inhibition of AKT signaling.**

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Uterine leiomyomas (ULs) are common benign tumors of the myometrium and the leading cause of hysterectomies in the United States. Since little is know about the etiology or pathogenesis of ULs, new insights are needed to identify valuable therapeutic targets and contribute to alternative non-surgical treatments for managing this disease.

Activation of the AKT pathway has been found to be prevalent in leiomyomas compared to normal myometrium. We have previously shown that AKT inhibition caused mitochondria disruption and caspase-independent cell death in leiomyoma cells. We therefore addressed the question of which molecular mechanisms underlie leiomyoma cell death irrespective of caspase activation. Primary leiomyoma cells from uterine fibroids were treated with the AKT inhibitor, MK-2206, at different times and concentrations. Concomitant with cell death, cyclophilin A (a marker of necrotic cell death) was released into the media following MK-2206 treatment, suggestive of a cell death mechanism other than caspase-dependent apoptosis, such as necrosis. Since mitochondrial were disrupted by MK-2206 treatment, levels of the BCL2 family proteins, which are mitochondrial-associated proteins that regulate cell death, were measured. We observed that cell death-promoting concentrations of MK-2206 reduced the protein levels of the anti-death BCL2 and increased those of the pro-death BAX, shifting the BAX/BCL2 balance towards a pro-death condition. Furthermore, BCL2 down-regulation by MK-2206 was associated with increased miR-182 levels, a redox sensitive microRNA which targets BCL2 and has recently been found to play a key role in cancer tumorigenesis. In this study, we demonstrate that AKT inhibition in leiomyoma cells induces expression of miR-182, downregulates BCL2, upregulates BAX and promotes a necrosis-like cell death. In conclusion, AKT is a major survival pathway in leiomyomas that could be a viable therapeutic target.

P54. ***Rhox8* ablation using a novel siRNA transgenic mouse model yields down regulation of sex-determining factor, *Sox9***

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The Reproductive Homeobox X-linked, *Rhox*, genes encode transcription factors that are expressed exclusively in the testis, epididymis, placenta, and ovary. While there are 33 *Rhox* genes in mice, only *Rhox5* and *Rhox8* are expressed in Sertoli cells, suggesting that they alone regulate the expression of somatic-cell gene products crucial for germ-cell development. Indeed, *Rhox5*-null mice are subfertile, exhibiting excessive germ-cell apoptosis and poor sperm motility. The continued expression of RHOX8 in *Rhox5*-null testes is most likely to explain why they are subfertile and not infertile. Thus, we sought to ablate *Rhox8* and potentially derive *Rhox5/Rhox8* double knockouts to answer this question. However, *Rhox5* and *Rhox8* are too close on the X chromosome to examine redundancy by combination of individual knockouts. To circumvent this issue, we used a novel tissue-specific RNAi approach to knockdown RHOX8 *in vivo*, in which the *Rhox5* promoter was used to drive *Rhox8*-siRNA transgene expression in Sertoli cells. Western and immunohistochemical analysis confirmed Sertoli-specific knockdown of RHOX8. However, other Sertoli markers, *Gata1*, *Ar*, and *Rhox5*; maintained normal expression patterns, suggesting the knockdown affect was specific. Male *Rhox8*-knockdown animals showed reduced fecundity in 5-day and 6-week timed breeding experiments, ~50% decline in spermatogenic output, and 30% decrease in sperm motility, in four independent *Rhox8*-knockdown lines. While we have not yet characterized the mechanism of RHOX8 action, it is significant to note that both *Sox8* and *Sox9* were downregulated in *Rhox8*-knockdown testes. Ablation of either of these genes causes male infertility at 5 months of age that is accelerated in double knockouts. Thus, the Sox E genes may be essential mediators of RHOX8 action. We are currently investigating this hypothesis, as well as other genetic interactions; through the development of *Rhox8* overexpressing cell lines established using the Invitrogen Flp-In system. Furthermore, we are currently developing Floxed *Rhox8* mice which will allow tissue-specific ablation of the gene to examine function earlier than our current model allows, as well as developing a second *Rhox8* knockdown model utilizing expression of an shRNA via a Cre-LoxP system that can be activated in the embryo allowing for elucidation of *Rhox8*'s role in embryonic development. This latter strategy has the advantage of allowing us to examine the fertility of *Rhox5/Rhox8* double knockout animals. Supported by NIH/NICHD 55268 and SIU-ORDA Faculty Seed Grants 2009.

P55. **Analysis of the human follicular and luteal endocrinology using encapsulated *in vitro* follicle growth (eIVFG) systems**

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The alginate-based encapsulated *in vitro* follicle growth (eIVFG) system supports coordinated folliculogenesis and oogenesis in follicles from all mammalian species examined to date. One essential marker of follicle health is the ability to produce peptide and steroid hormones in a characteristic profile. In this study, we performed a comprehensive analysis of the hormones produced by human preantral follicles during eIVFG. Secondary stage human follicles (120um to 260um diameter) were isolated from ovarian tissue donated for research under IRB approval and following informed consent. Follicles were encapsulated in 0.3% alginate, and levels of estradiol, progesterone, inhibin A, inhibin B, AMH, and activin A were measured in the spent culture media throughout culture. The follicular phase of development was tracked for each individual follicle by measuring estradiol, and once these levels plateaued, luteal conversion was triggered by hCG administration for 36 hours. The luteal phase of development was monitored for an additional 15 days post-hCG, resulting in total cultures ranging from 40-65 days. Although the absolute levels of steroid and peptide hormones produced by individual follicles varied, the synthesis patterns were similar. Inhibin B was the predominant inhibin in the small antral follicle, rising in the early to mid-follicular phase but dropping in the later follicular phase. Inhibin A and estradiol levels increased and reached a peak as the follicles reached maturity during the follicular phase. Post-hCG, progesterone levels increased and there was a trend towards higher activin A. Inhibin A levels dropped transiently with hCG treatment, but then rose and remained elevated throughout the luteal phase. In contrast, Inhibin B levels remained low during the luteal phase. AMH is a hormone made by small follicles and it reached its peak in the early follicular phase and dropped before mid-follicular phase. These results suggest that the endocrinology of the human follicle is faithfully recapitulated in the eIVFG system. Although this culture technique provides a robust method to study follicular and luteal endocrinology, access to human follicles is limited. Thus, we attempted to phenocopy the hormones of the human menstrual cycle using mouse follicles. Late primary stage follicles were isolated from 12-day old mouse ovaries and encapsulated in 0.5% alginate in cohorts of 5 or 10 follicles. Follicles were cultured for 14 days (follicular phase) upon which hCG was administered to induce ovulation followed by an additional 15 days of culture (luteal phase). The patterns of estradiol, inhibin A, inhibin B, and AMH during this 29-day culture mimicked a complete human menstrual cycle. Taken together, these results demonstrate that eIVFG is a powerful technique that allows the controlled study of human follicular and luteal endocrinology.

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P56. **Neonatal diethylstilbestrol exposure alters the metabolic profile of uterine epithelial cells.**

Yan Yin, Congxing Lin, G. Michael Veith, Hong Chen, Maulik Dhandha, Liang Ma

Developmental exposure to diethylstilbestrol (DES) causes reproductive tract malformations, affects fertility and increases the risk of clear cell carcinoma of the vagina and cervix in humans. Previous studies on a well-established mouse DES model demonstrated that it recapitulates many features of the human syndrome, yet the underlying molecular mechanism is far from clear. Using the neonatal DES mouse model, the present study uses global transcript profiling to systematically explore early gene expression changes in individual epithelial and mesenchymal compartments of the neonatal uterus. Over 900 genes show differential expression upon DES treatment in either one or both tissue layers. Interestingly, multiple components of peroxisome proliferator-activated receptor-γ (PPARγ)-mediated adipogenesis and lipid metabolism, including PPARγ itself, are targets of DES in the neonatal uterus. Transmission electron microscopy and Oil-Red O staining further demonstrate a dramatic increase in lipid deposition in uterine epithelial cells upon DES exposure. Neonatal DES exposure also perturbs glucose homeostasis in the uterine epithelium. Some of these neonatal DES-induced metabolic changes appear to last into adulthood, suggesting a permanent effect of DES on energy metabolism in uterine epithelial cells. This study extends the list of biological processes that can be regulated by estrogen or DES, and provides a novel perspective for endocrine disruptor-induced reproductive abnormalities.

P57. **E3 Ubiquitin Ligase Cullin 4B in Mouse Spermatogenesis**

Yan Yin, Liren Liu, Congxing Lin, G. Michael Veith, Pengbo Zhou and Liang Ma

CUL4B is a member of the Cullin ubiquitin-ligase family, the largest E3 ligase family which accounts for one fifth of total protein degradation through the ubiquitin-proteasome pathway. CUL4B shares extensive sequence identity and structural similarity with the other CUL4 member, CUL4A. We have previously reported that the two CUL4 genes exhibited complimentary expression pattern in the mouse testis, and revealed that genetic ablation of Cul4a in the mouse led to male infertility, due to aberrant meiotic progression. In the current study, we generated germ cell–specific Cul4b conditional knockout to investigate its role in spermatogenesis. Complete removal of CUL4B in all male germ cells is achieved by VasaCre in the cKO testis, and the resulted animals are infertile without any apparent morphological abnormalities of the testis. The Cul4b cKO males produce comparable number of spermatozoa, however, the mobility of the sperms is significantly impaired. Reduced or absence of mitochondria activity is observed in the mitochondria sheath of ~60% cKO sperms. Extremely low, if any, ATP level can be detected in isolated epididymal spermatozoa. Furthermore, the cKO spermatozoa exhibit defective arrangement of microtubules and outer dense fibers, and abnormal connecting piece structure. These results indicate the indispensable role for CUL4B in spermatogenesis, particularly during spermiogenesis.

P58. **Estrogen-Induced Expression of CCN1 Promotes Vascular Network Formation during Establishment of Endometriosis-like Lesions in a Mouse Model**

Yuechao Zhao[1], Quanxi Li[2], Benita S. Katzenellenbogen[1], Lester F. Lau[3], Robert N. Taylor[4], Indrani C. Bagchi[2], and Milan K. Bagchi[1]*

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Dysregulated estradiol (E) signaling has been implicated in endometriosis, a prevalent gynecological disorder in which endometrial tissue proliferates in extra-uterine sites, such as the peritoneal cavity, giving rise to painful, invasive lesions. To investigate the mechanisms by which E controls this condition, we investigated the role of CCN1, an E-regulated cysteine-rich matricellular protein, which exhibit highly elevated expression in human endometriosis. We used a mouse model of endometriosis in which endometrial tissue from donor mice was surgically implanted on the peritoneal wall of immunocompetent syngeneic recipient mice, leading to establishment of cystic endometriosis-like lesions. Our studies revealed that E markedly increased the size of these lesions and stimulated the expression of CCN1. When mice lacking uterine *Ccn1* were used as donors, the resulting ectopic lesions were strikingly smaller in comparison to wild-type lesions due to reduced cell proliferation and cyst formation. Notably, loss of CCN1 disrupted development of vascular networks and reduced the expression of several angiogenic factors. Thus, CCN1, acting downstream of E, critically controls cell proliferation and neovascularization, which promote the growth and survival of endometriotic tissue at ectopic sites. Blockade of CCN1 signaling during early stages of lesion establishment may provide a therapeutic avenue to control endometriosis.

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P59. **Plasma Membrane Proteomics Identifies Potential Targets for Breast Cancer Diagnosis and Treatment**

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The use of broad spectrum chemotherapeutic agents to treat cancer results in substantial and debilitating side effects, necessitating the development of targeted therapies to limit tumor proliferation and prevent metastasis. In recent years, the list of approved targeted therapies has expanded, and it includes both monoclonal antibodies and small molecule inhibitors that interfere with key proteins involved in the uncontrolled growth of cancer cells. The targeting of plasma membrane proteins has been most successful to date, and this is reflected in the large representation of these proteins as targets of newer therapies. In view of this fact, experiments were designed to investigate the plasma membrane proteome of a variety of breast cancer cell lines representing hormone responsive, HER2 over expressing, and triple negative cell types, as well as a benign control. Plasma membranes were isolated using an aqueous two phase system, and the resulting proteins were subjected to mass spectrometry. The resulting data set was biologically validated by selecting candidate proteins for Western blot, RT-PCR, and immunofluorescent analysis. After validation, the data was further examined for patterns of expression and to search for current as well as novel targets. Interestingly, a number of tyrosine kinases were up-regulated, some of which are targeted by therapies already in use to treat very different types of cancer. Surprisingly, several major histocompatibility complex proteins were also found to be highly up-regulated in some of the cell lines. Overall, the data set provides a very rich and complex picture of plasma membrane proteins on breast cancer cells, and the sorting and categorizing of this data is providing interesting insights into the biology, classification, and potential treatment of this prevalent yet debilitating disease.

P60. **Bisphenol A treatment of cultured mouse ovarian antral follicles may affect the aryl hydrocarbon receptor signaling pathway**

Ziv-Gal A, Craig ZR, Wang W, and Flaws JA. Comparative Biosciences, University of Illinois, Urbana, IL

Bisphenol A (BPA) is a commonly used plasticizer in the manufacture of polycarbonate plastics. Previous studies indicate that BPA exposure has toxic effects on the female reproductive system. For example, BPA (50-100 μg/ml) inhibits growth and steroidogenesis in cultured adult mouse antral follicles. Nevertheless, not much is known about the underlying mechanism. The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that regulates cellular processes in the ovary including transcriptional activation of cytochrome P450, family 1, subfamily B, polypeptide 1 (*Cyp1b1*). Interestingly, previous studies have shown that in-utero BPA treatment alters expression levels of AHR signaling pathway related genes in mouse embryonic ovaries. Hence, our current study was designed to examine whether the toxic effects of BPA observed in adult mouse antral follicles are mediated by the AHR signaling pathway. We hypothesized that BPA treatment of cultured adult mouse antral follicles alters expression levels of *Ahr*, aryl hydrocarbon receptor nuclear translocator (*Arnt*), aryl hydrocarbon receptor repressor (*Ahrr*), and *Cyp1b1*. To test this hypothesis, we mechanically isolated antral follicles from mouse ovaries (C57BL/6) and cultured them in vehicle control or BPA (0.001-50 μg/mL) for 24 and 96 hours. At the end of the cultures, follicles were further processed for gene expression analyses. Our results indicate that at 24 hours, BPA 50 μg/mL treatment significantly decreased *Ahrr* and *Cyp1b1* expression levels compared to the control group ($p \leq 0.05$). At 96 hours, BPA treatment (0.001-10 μg/mL) did not alter expression levels of any of the examined genes compared to the control group. These data suggest that relatively low doses of BPA do not affect the expression levels of selected AHR related genes. A high dose of BPA (50 μg/mL) may affect the expression of selected genes in the AHR signaling pathway as early as 24 hours. Supported by: NIH ES019178 (JAF), NIH K99ES021467 (ZRC), the Environmental Toxicology Scholar Program (WW, AZG).

Abstract Judges

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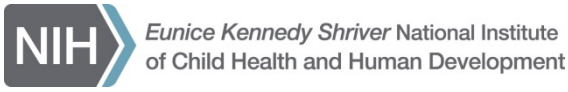

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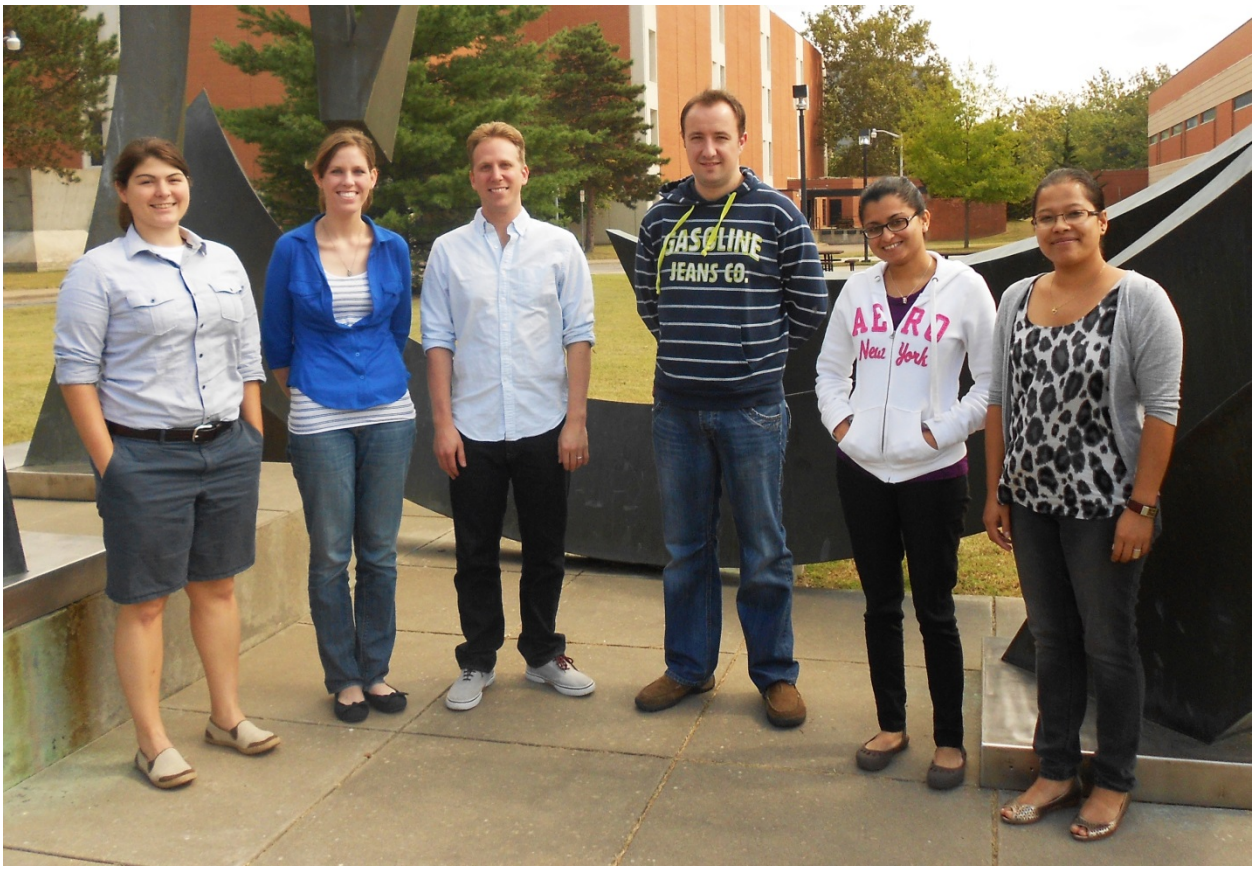

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