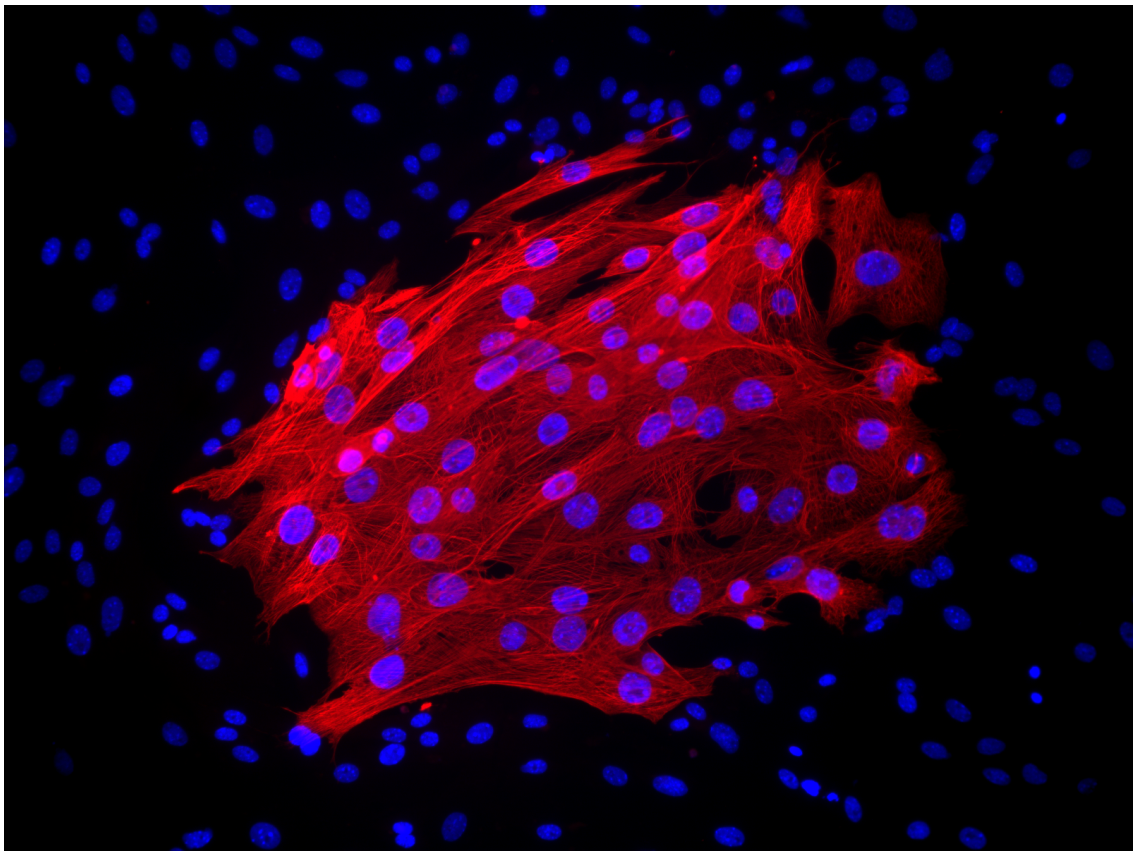
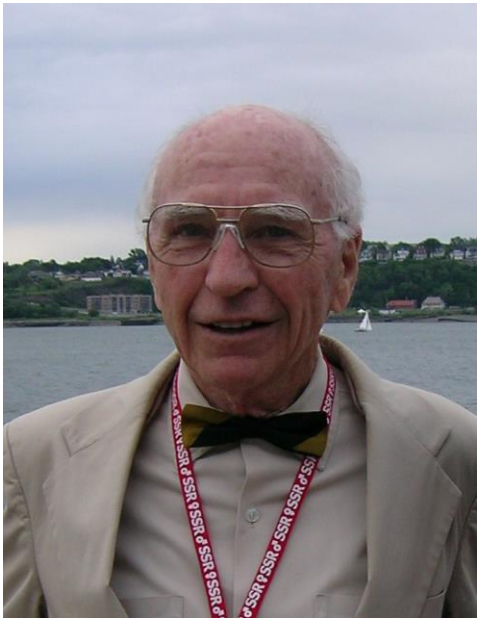
7th Annual Illinois Symposium on Reproductive Sciences



UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
iHOTEL and CONFERENCE CENTER
OCTOBER 12, 2015

In Memoriam



Dr. Philip J. Dziuk

Pioneer in the Field of Reproductive Sciences
Founding Member of Society for the Study of Reproduction

Died July 8, 2015

TABLE OF CONTENT

SYMPOSIUM OVERVIEW

7:00 - 8:00 am	Registration and Continental Breakfast
8:00 - 8:15 am	Welcome and Opening Remarks
8:15 - 9:15 am	Oral Session I (4 talks)
9:15 - 9:40 am	Alumnus Speaker: Dr. Jennifer Marcinkiewicz
9:40 - 10:00 am	Break
10:00 - 10:25 am	Alumnus Speaker: Dr. Peter J. Thomford
10:25 - 10:50 am	Alumnus Speaker: Dr. Thaddeus G. Golos
10:50 - 11:50 am	Oral Session II (4 talks)
11:50 - 12:40 pm	Lunch
12:40 - 2:10 pm	Poster Presentations
2:10 - 3:10 pm	Oral Session III (4 talks)
3:10 - 3:35 pm	Alumnus Speaker: Dr. Suzanne Moenter
3:35 - 4:05 pm	Coffee Reception
4:05 - 5:10 pm	A.V. Nalbandov Memorial Lecture: Dr. Richard L. Stouffer
5:10 - 5:25 pm	Student Awards
5:25 - 5:30 pm	Closing Remarks

7TH ANNUAL ILLINOIS SYMPOSIUM ON REPRODUCTIVE SCIENCES

Alma welcomes you…

…to the 7th Illinois Symposium on Reproductive Sciences (ISRS) at the University of Illinois at Urbana-Champaign. What once was known as the Minisymposium, which was conceived by Northwestern University, has evolved into a rotating, statewide annual symposium that continues to provide an opportunity to celebrate the strong heritage in the reproductive sciences research of Illinois (and neighboring states.) The main goals of the ISRS are to foster the exchange of scientific information in the reproductive sciences, to facilitate the career development of the next generation of reproductive scientists in Illinois (and surrounding states), and to establish a promising future for reproductive sciences research in the state of Illinois (and the Midwest.)

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

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

PROGRAM IN ENDOCRINOLOGY, EMBRYOLOGY & REPRODUCTION

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

Our program has a number of invited outside speakers, named lectures, including the A.V. Nalbandov Memorial Lectures and the Thanis "Billie" Alexander Field Lectures, as well as presentations from faculty, graduate students, and postdoctoral fellows in our weekly reproductive biology seminar series, a tradition started by Drs. Nalbandov and Gorski more than 50 years ago.

Dr. Juanmahel Dávila, a postdoctoral research associate in the laboratory of Indrani Bagchi, UIUC, provided the cover image. The image shows a 96 h co-culture of a mouse embryo (in red) on a bed of differentiated uterine endometrial stromal cells. Immunocytochemistry: KRT8 (red) and DAPI (blue).

A.V. NALBANDOV MEMORIAL LECTURESHIP

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

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

To honor Andy's legacy, friends and colleagues established the "A.V. Nalbandov Memorial Lectureship", which provides funding for an annual lecture by a distinguish scientist in the field of reproductive sciences. This year, Richard L. Stouffer, Ph.D., Senior Scientist and Division Head, Division of Reproductive Sciences, Oregon National Primate Research Center, will deliver the keynote address and receive the 2015 lectureship award.

A.V. NALBANDOV LECTURESHIP RECIPIENT IN REPRODUCTIVE SCIENCE

"Endocrine and Local Control of Folliculogenesis in Primates"

Richard L. Stouffer, Ph.D.
Senior Scientist and Division Head, Division of Reproductive Sciences, Oregon National Primate Research Center, with joint appointments in the School of Medicine and Oregon Graduate Institute, Oregon Health & Science University.

Dr. Richard Stouffer's primary focus, since his dissertation research in the laboratory of Dr. David Schomberg, Duke University (1971-1975), has been on understanding the structure, function, and regulation of the ovary. Moreover, as a staff fellow with Dr. Gary Hodgen from 1975-1977, in the intramural NICHD program at NIH, he began studies using the nonhuman primate (NHP) as a "translational model" for direct application to ovarian function and its disorders in women.

Dr. Stouffer was a member of the Department of Physiology, University of Arizona College of Medicine, where he rose to the rank of tenured Associate Professor. In 1985, he move to the ONPRC where his research program utilizes whole animal, cellular and molecular approaches to elucidate the mechanisms controlling follicle development, ovulation and the functional lifespan of the corpus luteum during the menstrual cycle and early pregnancy in rhesus macaques. A major focus of his research is to unravel the factors and mechanisms regulating the growth and maturation of follicles, ovulation, and luteinization of the single mature follicle at midcycle and the functional lifespan of the corpus luteum during the menstrual cycle and early pregnancy, with emphasis on the local actions of steroid hormones, especially progesterone and androgens.

Dr. Stouffer has received numerous NIH grants including a NIH R01 grant that has been active for over 30 years at University of Arizona and the Oregon National Primate Research Center that focuses on the endocrine and local control of the primate ovulatory follicle and corpus luteum. This project resulted in a landmark discovery (Hibbert et al., PNAS 93:1897-1901, 1996) that progesterone produced by the dominant follicle in response to midcycle gonadotropin surge is required for ovulation and development of the primate corpus luteum. The latter, which differs from the mouse model, is supported by novel studies detailing the expression of genomic progesterone receptor in macaque (and human) luteal cells. This led to Dr. Stouffer's important studies elucidating the gonadotropin- and

progesterone- responsive genes in the ovulatory follicle, as well as the corpus luteum as it ages during the menstrual cycle and early pregnancy.

Currently, Dr. Stouffer, in collaboration with Dr. Jon Hennebold, is using attenuated adenoviral vectors to deliver siRNAs to PR and PGRMC1 into the macaque periovulatory follicle to elucidate the role(s) of genomic and non-genomic progesterone receptor signaling pathways in ovulation and corpus luteum development in primates. Furthermore, Dr. Stouffer's collaborations with members of the Department of Obstetrics & Gynecology, OHSU, and industry (e.g., Bayer Health Care, Berlin), catalyzed formation of a Contraceptive Development & Research Center using the nonhuman primate to discover the next generation of non-hormonal contraceptives for women that selectively block oocyte maturation, ovulation or gamete transport. Further, a collaboration with Drs. Mary Zelinski and Teresa Woodruff resulted in a NIH Director-sponsored Roadmap Initiative, the Oncofertility Consortium. Their projects utilized macaques to develop and optimize techniques for growing primate follicles (and the enclosed oocytes) for assisted reproductive technology procedures and for transplanting cryopreserved/thawed ovarian cortex, to expand fertility options for female cancer patients after successful irradiation or chemotherapy.

In his career, Dr. Stouffer has trained 14 graduate students, 20 postdoctoral students, and numerous summer undergraduate students. He has received numerous awards including the SSR Research Award from the Society for the Study of Reproduction and the Distinguished Researcher Award from the American Society for Reproductive Medicine.

Dr. Stouffer has extensive leadership experience and mentorship as he has served on the Board of Directors and President of SSR, member then chair of the NIH REN Study Section, director/PI and Co-PI of the Oregon Infertility SCCPIR/NCTRI centers, and the Oregon Contraceptive CDRC center. He is currently the chair of the Oregon Stem Cell Research Oversight Committee, chair of the ONPRC Faculty Appointment & Promotions Committee, and Ambassador of the OHSU Center for Women's Health.

Because of his accomplishments and contributions to the field of Reproductive Sciences, Dr. Richard Stouffer will deliver the Keynote lecture at the 7th Annual Illinois Symposium on Reproductive Sciences and will receive the A.V. Nalbandov Memorial Lectureship Award.

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN ALUMNI SPEAKERS

Dr. Jennifer L. Marcinkiewicz
Associate Professor, Kent State University
Ph.D. Advisor: Dr. Janice M. Bahr

Dissertation Title: ***Identification and Characterization of Luteotrophic Activity in the Rabbit Placenta***

"Blooming Where You're Planted: Cultivating a Meaningful Career in Science Education and Outreach"

Dr. Peter J. Thomford
Study Director, Covance Laboratories Inc.
Ph.D. Advisor: Dr. Philip Dziuk

Dissertation Title: ***The Possible Role of Extra-Gonadal Metabolism of Steroids in the Regulation of Plasma Steroid Levels***

"Some Career Ruminations – 40 Years On"

Dr. Thaddeus G. Golos
Professor, University of Wisconsin at Madison
Ph.D. Advisor: Dr. O. David Sherwood

Dissertation Title: ***Regulation of Serum and Ovarian Relaxin Levels and Corpus Luteum Function during the Second Half of Pregnancy in the Rat***

"There and Back Again: a Physiologist's Journey to UIUC and Beyond"

Dr. Suzanne Moenter
Professor, University of Michigan at Ann Arbor
M.S. Advisor: Philip Dziuk

"No matter where you go, there you are"

PROGRAM FOR THE 7TH ILLINOIS SYMPOSIUM ON REPRODUCTIVE SCIENCES

7:00 am **Registration and Continental Breakfast.**

8:00 am **Welcoming remarks** – *Milan K. Bagchi, Ph.D., Head of the Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign*

Oral Session 1: Uterine Physiology and Pathophysiology

Session Moderators:

***Juanmahel Dávila, Ph.D.**, University of Illinois at Champaign-Urbana*
***Genna Prather**, Southern Illinois University at Carbondale*

8:15 am T1 **Niclosamide as a potential therapeutic drug for endometriosis**

Genna R. Prather, Daniel Boadu, James A. MacLean II, and Kanako Hayashi
Southern Illinois University at Carbondale

8:30 am T2 **Loss of basigin expression in the uterus reduces fertility in female mice**

Kailiang Li and Romana A. Nowak
University of Illinois at Urbana-Champaign

8:45 am T3 **Identifying extracellular vesicles originating from the placenta and uterus using RNA expression signatures**

Don L. Armstrong, Monica Uddin, and Derek Wildman
University of Illinois at Urbana-Champaign

9:00 am T4 **Activation of NFκB decreases glial cell missing 1 DNA binding: Implications for Preeclampsia**

Mark Schroeder, Timothy Murphy, Kathy Groesch, Trenae Smith, Andrew Wilber, Ronald Torry, Donald Torry
Southern Illinois University at Springfield and Drake University

University of Illinois Alumni Speaker

9:15 am **Alumni Speaker: Dr. Jennifer Marcinkiewicz**

Introduced by: *Deepak Hiremath, Southern Illinois University at Carbondale*

9:40 am **Refreshment break**

University of Illinois Alumni Speakers

10:00 am **Alumni Speaker: Dr. Peter J. Thomford**
Introduced by: *William E. Gundling, University of Illinois at Urbana-Champaign*

10:25 am **Alumni Speaker: Dr. Thaddeus G. Golos**
Introduced by: *Kailiang Li, University of Illinois at Urbana-Champaign*

Oral Session 2: Ovarian Development and Pathogenesis
Session Moderators:
***Shreya Patel**, University of Illinois at Urbana-Champaign*
***S. Alexandra García-Moreno**, Northwestern University*

10:50 am T5 ***Irx3*** **and** ***Irx5*** **are regulated by canonical WNT signaling in the somatic cells of the developing ovary**
Megan Hornung, Barbara Nicol, Humphrey Yao, and Joan Jorgensen
University of Wisconsin at Madison, and the National Institute of Environmental Health Sciences, NIH

11:05 am T6 **Notch functions in regulating steroidogenesis during the periovulatory period in the mouse ovary**
Rexxi D. Prasasya and Kelly E. Mayo
Northwestern University

11:20 am T7 **In vitro follicle growth supports human oocyte meiotic maturation**
Shuo Xiao, Jiyang Zhang, Megan M. Romero, Kristin N. Smith, Lonnie D. Shea, and Teresa K. Woodruff
Northwestern University and University of Michigan

11:35 am T8 **Decrease in DNA repair enzyme 8-oxo-guanine glycosylase is associated with the development and progression of ovarian cancer**
Animesh Barua, Janice M. Bahr, Lauren M Rosen, Lindsey Franklin, Sameer Sharma, Paolo Gattuso, and Pincas Bitterman
Rush University Medical Center and University of Illinois at Urbana-Champaign

11:50 am **Lunch Break**

12:40 pm **Poster Session A** (Odd Numbered Abstracts)

1:25 pm **Poster Session B** (Even Numbered Abstracts)

Oral Session 3: Mechanisms of Disease

Session Moderators:

***Scott Convissar**, University of Illinois at Chicago*
***Subbulakshmi Karthikeyan**, University of Illinois at Chicago*

2:10 pm T9 **Regulation of PAX2 in fallopian tube epithelium and high-grade serous ovarian carcinoma**

Dimple A. Modi, Rosemarie D. Tagare, Joanna.E. Burdette
University of Illinois at Chicago

2:25 pm T10 **Defining critical developmental windows of exposure to Bisphenol A in pituitary development and elucidating sex differences in response to chemical exposures**

Kirsten Eckstrum, Karen Weis, Lori Raetzman
University of Illinois Urbana-Champaign

2:40 pm T11 **Androgen-sensitized apoptosis in human prostate epithelial cells**

Congcong Chen, Jason Dienhart, and Eric C. Bolton
University of Illinois at Urbana-Champaign

2:55 pm T12 **Effects of the cholesterol metabolite, 27-hydroxychoesterol on t cells and metastasis**

Amy E. Baek and Erik R. Nelson
University of Illinois at Urbana-Champaign

University of Illinois Alumni Speaker

3:10 pm **Alumni Speaker: Dr. Suzanne Moenter**

Introduced by: *Changqing Zhou, University of Illinois at Urbana-Champaign*

3:35 pm **Refreshment break**

4:05 pm **Remarks on the A.V. Nalbandov Lectureship in Reproductive Science –** *Janice M. Bahr, Ph.D., Professor Emerita in the Department of Animal Science, University of Illinois at Urbana-Champaign and past trainee of Professor Andrew Nalbandov*

4:10 pm **A.V. Nalbandov Lectureship in Reproductive Science and ISRS Keynote Lecture – Richard L. Stouffer, Ph.D.**

Introduced by: *Sudipta Dutta, Ph.D., University of Illinois at Urbana-Champaign*

5:10 pm **Research Awards for Posters and Oral presentations**

5:25 pm **Closing Remarks** – *Romana A. Nowak, Ph.D., Professor in the Department of Animal Science, University of Illinois at Urbana-Champaign and Chair of the 2015 ISRS*

ABSTRACTS FOR THE ORAL SESSIONS

T1 NICLOSAMIDE AS A POTENTIAL THERAPUTIC DRUG FOR ENDOMETRIOSIS

Genna R. Prather, Daniel Boadu, James A. MacLean II, and Kanako Hayashi, Department of Physiology, Southern Illinois University School of Medicine, Carbondale IL 62901

Endometriosis affects 6-10% of women of reproductive age. Although endometriosis is a benign disorder, approximately 50% of affected women experience severe chronic pelvic pain and infertility. Because endometriosis is estrogen-dependent, the most widely used medical drugs are oral contraceptives, GnRH agonists and progestins, which suppress ovarian function and subsequently reduce pelvic disease and associated pain. However, these hormonal treatments are often of limited efficacy, elicit side-effects, temporarily inhibit fertility, and ultimately result in high recurrence rates of symptoms. Therefore, it is necessary to identify therapeutic targets and efficient drug(s) that improve current treatment. The progression of endometriosis is marked by an increase in proinflammatory signaling eliciting remarkably increased macrophage, cytokine and chemokine content in the peritoneal fluid. Although nonsteroidal anti-inflammatory drugs, such as ibuprofen, have also been used for the treatment of endometriosis, these drugs primarily relieve dysmenorrhea. We have recently identified a small molecule, niclosamide, which modulates the NFκB and STAT3 signaling pathways. Therefore, we hypothesize that niclosamide inhibits the endometriotic microenvironment suppressing proinflammatory mechanisms via NFκB and STAT3 signaling. To determine the inhibitory mechanisms of niclosamide in endometriosis, endometriotic lesions were induced in mice. During diestrus, the uteri of GFP expressing donor mice were excised and disks of endometrial tissue were implanted into the peritoneal cavity of cycle-matched wild-type recipient females. After three days recovery from the surgery, mice were given niclosamide (100 or 200 mg/kg b.w./day) or vehicle orally for 3 weeks. We observed a significant difference in the pattern of progression of endometriotic lesions after 3 weeks. Oral administration of niclosamide (100 or 200 mg/kg b.w.) reduced the total lesion volume 23.15 ± 5.03 mg (n=5), 13.73 ± 3.05 mg (n=11), respectively, compared with controls (43.32 ± 5.52 mg, n=7). Niclosamide treated animals had fewer proliferating Ki67 positive cells in their lesions. Immunohistochemical analysis revealed a reduction in phospho-IKK and iNOS following treatment of niclosamide. Current treatments for endometriosis temporarily inhibit fertility. However, niclosamide treatment did not disturb normal reproductive function in a six month breeding study. Additionally, niclosamide treatment following surgical induction of endometriosis did not alter time to achieve pregnancy, gestational length, or neonatal pup weight. Thus, these results suggest that niclosamide could be a potential therapeutic drug for the treatment of endometriosis by targeting inflammatory mechanisms while preserving normal fertility.

Supported by Southern Illinois University School of Medicine CDA.

NOTES

T2 LOSS OF BASIGIN EXPRESSION IN THE UTERUS REDUCES FERTILITY IN FEMALE MICE

Kailiang (Adam) Li and Romana A. Nowak; Department of Animal Sciences, University of Illinois, Urbana, IL.

Basigin (BSG) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. BSG is involved in many physiological and pathological processes including cell proliferation, induction of MMPs, stimulation of angiogenesis, and tissue remodeling. BSG has been shown to play an important role in male and female reproduction since global knockout of Bsg leads to severe infertility. Studies of the functional role of BSG in the uterus have been complicated by the fact that most Bsg null mutants are embryonic lethal. We hypothesize that BSG is necessary for normal proliferation and differentiation of the uterine endometrium at the time of implantation and early pregnancy. In order to investigate our hypothesis, we generated a conditional BSG knockout mouse to ablate BSG expression specifically in the uterus. Mice carrying floxed basigin1 alleles were crossed with mice expressing CRE recombinase driven by the progesterone receptor. Knockout of BSG was confirmed by PCR and histology. A six-month breeding study was carried out and our results showed $Bsg^{d/d}$ females had significantly reduced fertility as compared to $Bsg^{f/f}$ females when bred to wild type males. We found that $Bsg^{d/d}$ females produced only 4.3 pups per litter over six months compared to 7.9 pups per litter produced by $Bsg^{f/f}$. $Bsg^{d/d}$ females averaged 3.3 litters whereas $Bsg^{f/f}$ females averaged 5 litters over the 6 month study. Both the number of pups per litter and the average litter number per female for the conditional knockout group were significantly lower than the $Bsg^{f/f}$ ($p<0.05$). The average weight of pups and the sex ratio were not different. In the next experiment, eight-week old females were mated with fertile wild type males and sacrificed at different days of gestation. Uteri and ovaries were collected for histology and the weights of the tissues were measured. Our results showed that on day 4 of pregnancy, the uterine weights of the $Bsg^{d/d}$ female mice were 30% less than those of the litter mate wild-type controls. The uterine horns also were shorter in the conditional knockouts. Immunohistochemical staining for the cell proliferation marker KI67 showed no significant difference in the proliferation of stromal cells in the conditional knockout on day 1 and day 4 of pregnancy. However, there was noticeably reduced proliferation in the uterine epithelium on day 1. Immunostaining for epithelial cell markers such as claudin and cytokeratin revealed no difference in the number of glands or defects in tight junctions in the glands between the two groups. Data from pregnancy day 6, 9, and 12 showed that there were many fewer implantation sites in the uteri of the $Bsg^{d/d}$ females. Uterine horns were flushed on day 4 to determine the number of embryos in the two genotypes and were found not to be different. This suggests that the reduced fertility in the Bsg conditional knockouts is not due to differences in ovulation or fertilization rates. In addition to impaired implantation and fewer numbers of pups born, we also observed an increased incidence of dystocia and neonatal death suggesting that loss of BSG expression in the uterus and/or cervix leads to problems in parturition. Future studies will focus on delineating the role of BSG in endometrial differentiation and placental angiogenesis during gestation.

Supported by Arnold Beckman award to RAN.

NOTES

T3 IDENTIFYING EXTRACELLULAR VESICLES ORIGINATING FROM THE PLACENTA AND UTERUS USING RNA EXPRESSION SIGNATURES

Don L. Armstrong[1], Monica Uddin[1,2], Derek Wildman[1,3]. [1]Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign. [2]Department of Psychology, University of Illinois, Urbana-Champaign. [3]Department of Molecular and integrative Physiology, University of Illinois, Urbana-Champaign.

Extracellular vesicles (EVs) are small (40-1000nm) compartments that can be found in blood, saliva, and urine. EVs originate from the plasma membrane or multivesicular endosome of many cell types and contain proteins, lipids, mRNA, miRNA, and many other non-coding RNAs. Because EVs contain mRNA from the cell of origin and can be obtained without invasive procedures, EVs are remote sensors of the transcriptomes of otherwise inaccessible tissues. One requirement for remotely sensing the transcriptome is identifying the source tissue of a specific EV. To identify the source tissue of a specific EV, we have identified tissue-characterizing ncRNA and mRNA markers for multiple organs and tissues from existing publicly-available RNAseq transcriptomes by calculating a tissue specificity index. Our approach identified PAEP and MYH11 as well as non-coding RNAs FTX_1 (71bp), ZNFX1-AS1_3 (87bp), PVT1_3 (95bp) as characteristic of uterus. We identified CGA, members of the CSH family, members of the PSG family, as well as ncRNAs AC026803.1 (82bp), MIR126 (85bp), MIR662 (95bp), AC108933.1 (93bp) and NEAT1_3 (159bp) as highly-expressed (FPKM $\geq$ 1024) transcripts characteristic of placenta. Using machine learning techniques we also trained a Support Vector Machine to classify the tissue of origin. The accuracy of the classifier was verified using RNAseq transcriptome data not in the training set. We show the applicability of the classifier to simulated EVs by down sampling sequencing reads and accurately identify placenta with only 10 reads, whereas uterus requires 5×103 reads. When coupled with single-EV sequencing (or microchimerism-aware variant calling in whole blood samples for placental tissue of fetal origin), our classifier enables clinicians to monitor disease-specific markers over the course of treatment without biopsies, increasing the ability of clinicians to adapt treatment modalities to the individual patient.

NOTES

T4 ACTIVATION OF NFκB DECREASES GLIAL CELL MISSING 1 DNA BINDING: IMPLICATIONS FOR PREECLAMPSIA

Mark Schroeder[1], Timothy Murphy[1], Kathy Groesch[1], Trenae Smith[1], Andrew Wilber[1], Ronald Torry[2], Donald Torry[1].[1]Department of Medical Microbiology, Immunology, and Cell Biology, Department of Obstetrics and Gynecology, Southern Illinois University School of Medicine, Springfield, IL. [2]College of Pharmacy and Health Sciences, Drake University, Des Moines, Iowa.

Preeclampsia (PE) is a gestational disease that occurs in 5-8% of pregnancies. PE is described by a two stage process. The first stage (8-16 weeks gestation) is characterized by aberrant trophoblast invasion, and insufficient remodeling of maternal spiral arteries leading to a relatively hypoxic placenta. This hypoxia decreases expression of pro-angiogenic placental growth factor (PlGF) and increases anti-angiogenic soluble fms-like tyrosine kinase-1 receptor (sFlt-1). The second stage of PE is characterized by the onset of hypertension and proteinuria occurring at >20 gestation. Hypertension and proteinuria are attributed to the angiogenic imbalance and poor relaxation of the maternal systemic vasculature.

An inflammatory state during PE is also implicated in contributing to the angiogenic imbalance. Because hypoxia and activation of NFκB pathways have shown to have similar effects on PlGF expression in trophoblast, we hypothesized that the pathways may work through a similar mechanism. PlGF mRNA was decreased ~60% under hypoxia; however, overexpression of the dominant negative form of the IκB complex (dnIκB) which inhibits NFκB activation, was unable to rescue expression of PlGF, therefore we believe that NFκB and hypoxia can function independently of each other to decrease PlGF expression. The focus of this project is to determine the mechanism through which NFκB regulates PlGF expression. Over expression of NFκB-p65, the active subunit of NFκB, decreases PlGF transcription by ~60%. This effect can be reversed by a dnIκB. Glial cell missing 1 (GCM1) is a primary transcription factor regulating PlGF expression in trophoblast. Over expression of NFκB decreased GCM1 protein stability and also inhibited transcriptional function by ~95%.

GCM1 is subject to many posttranslational modifications that effect its function. Sumoylation of the DNA binding domain of GCM1 decreases its DNA binding capacity. Therefore, we hypothesized that activation of NFκB leads to sumoylation of the DNA binding domain of GCM1 and decreased functional activity. Over expression of p65 significantly decreased (~50%) GCM1 binding to a synthetic oligo that contains GCM1 consensus binding sequences. Over expression of SUMO1 showed no significant decrease in GCM1 DNA binding. However, co-expression of p65 and SUMO1 produced a ~90% reduction in GCM1 ability to bind to its consensus sequence. These data suggest that NFκB activation leads to an increase in sumoylation in the DNA binding domain of GCM1, resulting in decreased binding to its consensus sequence. Further studies will need to confirm that NFκB activation increases sumoylation of GCM1 DNA binding domain and whether this modification is responsible for decreased endogenous expression of PlGF in trophoblast and in PE.

Supported by NICHD R15 HD073868.

NOTES

T5 *IRX3 AND IRX5* ARE REGULATED BY CANONICAL WNT SIGNALING IN THE SOMATIC CELLS OF THE DEVELOPING OVARY

Megan Hornung[1], Barbara Nicol[2], Humphrey Yao[2], Joan Jorgensen[1]. University of Wisconsin Madison, Madison, WI[1]; National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC[2]

Canonical Wnt/β-catenin signaling is critical for proper ovarian development. Oocyte death is a prominent feature of *Wnt4* null ovaries caused by disrupted interactions between oocytes and supporting somatic cells. Previously, our laboratory discovered that members of the Iroquois Homeobox transcription factor family, specifically *Irx3* and *Irx5 (Irx3/5)*, were required to maintain oocyte-somatic cell interactions in the developing follicle. Significant gaps separate oocytes and supporting somatic cells in *Irx3/5* double knockout ovaries that ultimately lead to oocyte death, suggesting a link to the Wnt/β-catenin pathway. *Irx3* has been used as a marker for active Wnt signaling in several tissues including the developing ovary; however, the mechanism by which Wnt regulates this factor is unclear. We hypothesize that *Irx3* and *Irx5* are directly regulated by canonical Wnt/β-catenin signaling during ovarian development. To test this hypothesis, we manipulated gonadal β-catenin activity *in vitro* and *in vivo* and investigated changes in *Irx3* and *Irx5* expression. Embryonic day (E) E11.5 ovary explants were cultured with iCRT14, a potent inhibitor of β-catenin stimulated transcription. As expected, positive controls *Axin2* and *Fst* were significantly decreased (0.23x, $p<0.001$ and 0.13x, $p<0.001$, respectively) and negative control *Rps29* was unaffected. Expression of both *Irx3* and *Irx5* were significantly decreased by 0.27 and 0.24 fold, respectively ($n=4$, $p<0.003$ for both). In a converse experiment, E11.5 testis explants were cultured with LiCl, a GSK3β inhibitor that results in stabilized and activated β catenin. Results showed little change in *Rps29* and significantly increased expression of *Axin2* (4x, $p<0.03$), *Fst* (10x, $p<0.01$), *Irx3* (8x) and *Irx5* (5x) ($n=5$, $p<0.01$ for both). Next, β-catenin activity was manipulated *in vivo* by breeding SF1-Cre (somatic cell specific) to loss-of-function *Ctnnb1*$^{f/f}$ or gain-of function *Ctnnb*$^{\Delta ex3/\Delta ex3}$ mice. Gonads were harvested from E14.5 embryos and subjected to qPCR analysis to evaluate β-catenin function. Control ovaries (*SF1-Cre*$^{+/Tg}$*; Ctnnb1*$^{f/+}$) were compared to somatic cell specific knockout ovaries (*SF1-Cre*$^{+/Tg}$*; Ctnnb1*$^{f/f}$). *Rps29* was unaffected while *Axin2* (0.24x, $p<0.01$), *Fst* (0.04x, $p<0.0001$), *Irx3* (0.35x, $p<0.001$) and *Irx5* (0.40x, $p<0.01$) expression was significantly decreased in mutant verses control ovaries. In the converse study, control testes (No Cre; *Ctnnb1*$^{\Delta ex3/+}$*)* were compared to stabilized β-catenin testes (*SF1-Cre*$^{+/-}$*; Ctnnb1*$^{\Delta ex3/+}$). No significant change was seen in the negative control, *Rps29*. Positive controls *Axin2* and *Fst* had increased expression (11x, $p<0.005$ and 7x, $p<0.01$, respectively). *Bmp2*, a pro-ovarian gene that is not regulated by Wnt, was not affected. However, *Irx3* expression increased 16 fold compared to the control testes, while *Irx5* expression increased almost 20 fold ($p<0.001$ and $p<0.01$, respectively). In conclusion, our results from complementary *in vitro* and *in vivo* experiments suggest canonical Wnt/β-catenin signaling is responsible for *Irx3* and *Irx5* regulation. Taken together, these data suggest *Irx3/5* respond to canonical Wnt/β-catenin signaling in ovarian somatic cells to set up the proper foundation for essential interactions between somatic cells and oocytes. This relationship begins within germline nests during development and translates to their intimate connections within primordial follicles that ensure oocyte survival.

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NOTES

T6 NOTCH FUNCTIONS IN REGULATING STEROIDOGENESIS DURING THE PERIOVULATORY PERIOD IN THE MOUSE OVARY

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Coordinated endocrine, paracrine, and juxtacrine signaling is critical to the formation, growth and function of the ovarian follicle. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior pituitary serve as predominant endocrine cues for growth and ovulation of the follicle. In addition, numerous local ovarian regulatory pathways work in cooperation with these hormones to ensure a robust, albeit complex, control of female fertility. We, and others, have shown that the highly conserved Notch signaling pathway mediates juxtacrine signaling within the ovary. Models of ovarian Notch deficiency, developed by our group and others, exhibit abnormal follicle formation, decreased cell proliferation and increased apoptosis in growing follicles, and female subfertility. The Transgenic Notch Reporter (TNR) mice, which express eGFP in cells with active Notch signaling (from Dr. Nicholas Gaiano, Johns Hopkins University) show Notch activity within the granulosa cells at all stages of follicle development, as well as in luteal cells. We found that activation of LH receptors by human chorionic gonadotropin (hCG) treatment of pregnant mare serum gonadotropin (PMSG)-primed mice leads to increased Notch activity (*eGFP* expression) during the early luteinization period (24h post-hCG). This is consistent with findings that expression of multiple Notch ligands and receptors, including the highly abundant *Jag1* and *Notch2*, are positively regulated by hCG during the periovulatory period (4-12h post-hCG). NOTCH2 localization is limited to granulosa cells of follicles, while JAG1 is localized to the oocyte prior to ovulation. Surprisingly, we found that JAG1 localization shifted to highly steroidogenic cells (thecal-interstitial cells and luteal cells) post ovulation, potentially serving as a ligand for Notch activation in these cells. Knockdown of *Jag1* in preovulatory granulosa cells using siRNAs resulted in a striking downregulation of genes involved in steroidogenesis including, *Cyp19b*, *Cyp11a1*, and *StAR*. These data suggest that in ovarian somatic cells, JAG1 has the potential to promote steroidogenesis, a function likely distinct from its early expression in the oocyte of developing follicles.

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NOTES

T7 *IN VITRO* FOLLICLE GROWTH SUPPORTS HUMAN OOCYTE MEIOTIC MATURATION

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In vitro follicle growth has great potential to provide an alternative fertility preservation option for young cancer patients who are facing a risk of premature ovarian failure (POF) caused by radiation or chemotherapy. Several in vitro follicle culture systems have successfully supported the growth and maturation of ovarian follicles in mice and several large mammalian species. However, translation of this work to humans has been challenging. The objective of this study was to develop a two-step follicle culture strategy to recapitulate the dynamic human follicle growth environment and support follicle and oocyte maturation in vitro. Human ovarian tissues were obtained from 44 patients and 65 multilayer secondary follicles were mechanically isolated. Human follicles were encapsulated and cultured in 0.5% alginate hydrogels with initial diameter at 165.8 ± 32.3 μm. By 10-15 days of culture, 32 follicles started to display an antrum formation and had a diameter of 400-500 μm. To mimic the dynamic follicle growth environment with a shift from a rigid cortex to permissive perimedullary region observed in vivo, a portion of follicles were released from the alginate hydrogels at the antral stage, and continued the cultures in low-attachment plates for up to 40 days. Compared to follicles cultured in the alginate hydrogels, follicles cultured using this two-step strategy had significantly greater terminal diameters starting on day 20, higher hormone levels of estradiol, progesterone and anti-Müllerian hormone (AMH). In vitro maturation (IVM) was performed to determine whether the in vitro follicle growth promotes oocyte maturation. Follicles cultured only with alginate encapsulation produced oocytes that either remained in the germinal vesicle (GV) stage (8 out of 12) or degenerated (4 out of 12). In contrast, 20% (4 out of 20) of follicles cultured using the two-step strategy produced meiotic competent MII oocytes that extruded the first polar body, and with barrel-shaped bipolar spindles and with tightly aligned chromosomes on the metaphase plate. Human follicles that reached MII stage had cumulus cells that responded to hormone stimulation with cumulus expansion, and with significantly higher expression levels of cumulus expansion markers of pentraxin-related gene (*Ptx3*), hyaluronan synthase 2 (*Has2*), and prostaglandin-endoperoxide synthase 2 (*Ptgs2*). Our study demonstrates the two-step in vitro follicle growth supports human follicle growth and maturation, and, for the first time, produced meiotically competent MII oocytes, which will one day provide an additional fertility preservation option for young women and girls facing diseases or treatments that threaten their reproductive health.

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NOTES

T8 DECREASE IN DNA REPAIR ENZYME 8-OXO-GUANINE GLYCOSYLASE IS ASSOCIATED WITH THE DEVELOPMENT AND PROGRESSION OF OVARIAN CANCER

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Background: Surface epithelium of the ovary (OSE) and Fimbria (FSE) are constantly exposed to endogenous (ovulatory) or exogenous (pathogens/carcinogens) insults. These insults result in chronic inflammatory condition leading to the generation of reactive oxygen species (ROS). Chronic inflammation and oxidative stress (OS) are hallmarks of malignant transformation. 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxo-dG) is an oxidized derivative of deoxyguanosine and a major DNA damage adduct involved in tumor associated mutation. OGG1 (8-oxoG DNA glycosylase) is one of the enzymes which excises 8-oxo-dG from DNA to repair damaged DNA to prevent mutation. Thus, the level of OGG1 expression indicates the extent of oxidative damage in the DNA. The goal of this study was to examine the association of OGG1 expression with ovarian tumor development and progression.

Materials and Methods: Experiment1: Expression levels of OGG1 was examined in normal ovaries from healthy postmenopausal subjects (n=10), BRCA1+ subjects (n=5), ovaries with benign serous tumors (n=5), ovarian cancer (OVCA) at early (n=5 serous, 5 mucinous, 5 clear cell) and late stages (serous, n=10). Experiment 2: Hen ovarian tissues with postovulatory follicles (n=5) as well as early and late stage OVCA, n= 5, each) were examined. OGG1 was detected by immunohistochemistry and Western blotting.

Results: Only epithelial cells of ovarian surface invagination in normal subjects and subjects with BRCA1 mutation expressed OGG1. No or very weak expression of OGG1 was observed for benign tumors. Malignant cells expressed OGG1 strongly in early serous OVCA while the expression decreased in serous OVCA at late stages. Expression of OGG1 was detected in hen ovarian postovulatory follicular tissues. As observed for OVCA in women, OGG1 expression increased significantly in early stage OVCA in hens and decreased in late stages.

Conclusion: The results of this study show that OGG1 expression is associated with oxidative stress including ovulation and increases during OVCA development to prevent DNA damage associated with 8-OXO-dG.OVCA progresses to late stages with the decrease in OGG1 expression. Thus, 8-OXO-dG represents a marker of OVCA associated oxidative stress and offers a target for OVCA prevention.

Support: Swim Across America grant (to AB).

NOTES

T9 REGULATION OF PAX2 IN FALLOPIAN TUBE EPITHELIUM AND HIGH-GRADE SEROUS OVARIAN CARCINOMA

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Background: Fallopian tube epithelium (FTE) is one of the proposed progenitor populations for high-grade serous cancer (HGSC). One of the earliest reported changes identified in FTE is loss of PAX2 expression in areas of secretory cell outgrowth (SCOUTs). SCOUTs are clusters of secretory cells that are thought to progress to p53 signatures when p53 is mutated, that may stepwise progress to HGSC. PAX2 is expressed in normal FTE whereas in serous cancer, PAX2 is lost. PAX2 is a transcription factor and very little is known about what regulates PAX2 in FTE, functional significance of its loss and whether PAX2 re-expression in HGSC may halt tumor growth.

Hypothesis: Deregulation of PAX2 expression combined with mutant p53, contributes to tumor initiation and that understanding its regulation will allow for re-expression in cancer cells to induce cell death.

Rationale and results: Pathologists have reported lack of PAX2 expression in fixed tumor tissue, which limits their ability to understand molecular basis of lesion development. Mouse FTE (mFTE) cells with PAX2 knockdown ($PAX2^{shRNA}$), p53 mutation ($p53^{R273H}$) and combination ($PAX2^{shRNA}/p53^{R273H}$) were generated. $PAX2^{shRNA}/p53^{R273H}$ cells retained migratory phenotype of $p53^{R273H}$. These cells were more proliferative and formed soft agar colonies, but not tumors. Serially passaged $PAX2^{shRNA}/p53^{R273H}$ cells ($PAX2^{shRNA}/p53^{R273H\text{-}tumorigenic}$) formed peritoneal tumors in mice. $PAX2^{shRNA}/p53^{R273H\text{-}tumorigenic}$ showed higher proliferation, migration and anchorage independent growth compared to $PAX2^{shRNA}/p53^{R273H}$. A recent study compared human SCOUTs to HGSC and validated that subset of SCOUTs have similar expression profiles to tumors. Comparison of gene targets in mFTE cells with $PAX2^{shRNA}$, $p53^{R273H}$, $PAX2^{shRNA}/p53^{R273H}$ and $PAX2^{shRNA}/p53^{R273H\text{-}tumorigenic}$, revealed that likely PAX2 targets are Stathmin, Fut8 and MME. Stathmin expression is lost from PAX2 silencing, upregulated by $p53^{R273H}$, and highly upregulated by the combination and in tumorigenic line. MME is upregulated by PAX2 loss and combination. Fut8 is upregulated by PAX2 loss, and is lost as cells become tumorigenic. MME and Fut8 are novel genes with little information on their role in HGSC. Based on TCGA, PAX2 is neither mutated nor heavily methylated, while gene locus has loss of heterozygosity. HGSC cells do not express PAX2. Stable PAX2 expression in HGSC cells failed due to cell death. Transient PAX2 expression in HGSC cells resulted in reduced proliferation and migration compared to control transfection. While HGSC cells are of unknown origin, mFTE cells harboring PTEN silencing ($PTEN^{shRNA}$) that produced peritoneal tumor explants were derived from known source; murine oviducts. Reduced levels of PTEN negatively regulated PAX2 expression in mFTE and stable re-expression of PAX2 reduced pro-proliferative and pro-migratory effects from PTEN silencing.

Conclusion: $PAX2^{shRNA}/p53^{R273H\text{-}tumorigenic}$ cells accrued significant changes over time that lead to tumor formation, providing a useful model to compare with changes identified in HGSC. Stathmin, Fut8 and MME are possible novel targets downstream of PAX2 in mFTE. Stable overexpression of PAX2 in $PTEN^{shRNA}$ but not HGSC cells; suggests that additional mutations occur in human tumors that contribute to toxic effects when PAX2 is re-expressed. These novel *in vitro* and *in vivo* models enable us to compare PAX2 targets that are regulated during stepwise progression of HGSC from FTE.

Supported by ACS grant.

NOTES

T10 DEFINING CRITICAL DEVELOPMENTAL WINDOWS OF EXPOSURE TO BISPHENOL A IN PITUITARY DEVELOPMENT AND ELUCIDATING SEX DIFFERENCES IN RESPONSE TO CHEMICAL EXPOSURES

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Exposures to endocrine disrupting chemicals (EDCs) are linked to a number of reproductive deficits including altered onset of puberty, infertility, and miscarriage. EDC exposures are often more detrimental during critical periods of development and can have persistent phenotypes into adulthood. One EDC that is ubiquitous in the environment is Bisphenol A (BPA), a compound found in polycarbonate plastics, epoxy resins, and thermal paper. Exposure to BPA during mouse embryonic development has previously been shown to increase pituitary proliferation and influence progenitor cells to become luteinizing hormone (LH) and follicle stimulating hormone (FSH) producing gonadotrope cells, potentially leading to disruptions in reproductive function. Interestingly, this effect was seen only in females. However, the neonatal period, a time at which the hypothalamic-pituitary-gonadal axis has begun to function and sex differences have been initiated through the testosterone surge in males, has not been examined. Our goal was to determine if this second period was sensitive to BPA exposure. To address this question, we dosed neonatal mice from postnatal day (PND)0 (day of birth) to PND7 with three doses of BPA, 50μg/kg/day, which is the oral reference dose for BPA, and two doses that would be within the range of human exposure: 0.5 and 0.05μg/kg/day BPA. BPA is considered to have effects similar to 17β-estradiol (E2) in many scenarios, therefore, to see how E2 affects neonatal pituitary development and to compare its effects to that of BPA, we used one dose of E2 and examined the effects on proliferation and cell fate choices within the pituitary. E2 decreased proliferation, measured by *mki67* mRNA levels, in males only. BPA had no effect on proliferation. To determine if these compounds influenced cell fate choices, we looked at lineage specific genes. *Nr5a1* is specific to the gonadotrope lineage and necessary for its differentiation. E2 increased *Nr5a1* in females only and there was no effect with BPA exposure. We found that BPA exposure at 0.5μg/kg/day and 0.05μg/kg/day was able to decrease the transcript levels of *Pit1*, which is specifically expressed in the somatotrope, thyrotrope, and lactotrope lineages, in males, but had no effect at the higher BPA dose of 50μg/kg/day or E2. However, 50μg/kg/day BPA was able to increase levels of *Tpit* mRNA, which is a marker of the corticotrope and melanotrope lineages, in females only and no other BPA dose or E2 had any effect on this gene. Overall, these data demonstrate that the neonatal period of pituitary development is sensitive to exposure to both E2 and BPA and that both these compounds can have sex-specific effects; however, E2 and BPA do not appear to be producing similar responses, indicating that BPA may be acting through a different mechanism at the concentrations chosen. The hypothesis that these changes in transcript levels may be paralleled with differences in cell number persisting into adulthood is currently being explored. It is important to determine the critical windows of exposure as these would be the most sensitive to EDCs found in the environment and we have uncovered that different stages of pituitary development have unique responses to EDC exposure.

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T11 ANDROGEN-SENSITIZED APOPTOSIS IN HUMAN PROSTATE EPITHELIAL CELLS

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Androgen receptor (AR) signaling is crucial to the development and homeostasis of the prostate gland, and its dysregulation mediates common prostate pathologies. The mechanisms whereby AR regulates growth suppression and differentiation of luminal epithelial cells in the prostate gland and proliferation of malignant versions of these cells have been investigated in human and rodent adult prostate. However, the apoptotic stress response of human prostate epithelial cells is not well understood, though it is central to prostate homeostasis and pathology. Here, we report that androgen sensitizes HPr-1AR human prostate epithelial cells to apoptotic cell death. Although 5α-dihydrotestosterone (DHT) treatment alone did not induce cell death, co-treatment of HPr-1AR cells with DHT and an apoptosis inducer, such as staurosporine (STS) or TNFα, synergistically increased apoptotic cell death in comparison to treatment with apoptosis inducer by itself. We found that the synergy between DHT and STS or TNFα led to activation of the intrinsic/mitochondrial apoptotic pathway, which is supported by robust cleavage activation of caspase-9 and caspase-3. Further, the dramatic depolarization of the mitochondrial membrane potential that we observed upon cotreatment with DHT and STS is consistent with increased mitochondrial outer membrane permeabilization (MOMP) in the pro-apoptotic mechanism. Interestingly, the synergy between DHT and apoptosis inducer was abolished by AR antagonist (enzalutamide), transcription inhibitor (DRB), and protein synthesis inhibitor (cycloheximide), suggesting that AR mediates pro-apoptotic synergy through transcriptional regulation of MOMP genes. Expression analysis revealed that pro-apoptotic genes (BCL2L11/BIM and AIFM2) were DHT-induced, whereas pro-survival genes (BCL2L1/BCL-XL, MCL1, and BCL2A1) were DHT-repressed. Hence, we propose that the net effect of these AR-mediated expression changes shift the balance of BCL2-family proteins, such that androgen signaling sensitizes mitochondria to apoptotic signaling, thus rendering HPr-1AR more vulnerable to cell death signals. Our study offers insight into AR-mediated regulation of prostate epithelial cell death/survival.

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NOTES

T12 EFFECTS OF THE CHOLESTEROL METABOLITE, 27-HYDROXYCHOESTEROL ON T CELLS AND METASTASIS

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Background: Obesity rates have steadily climbed in recent decades, and in the US alone, 68.5% of adults are either overweight or obese. This has negative implications for breast cancer outcomes, as obesity is associated with metastatic relapse. Elevated cholesterol, a comorbidity of obesity, has also been shown to be a risk factor for relapse. Conversely, pharmacological inhibition of cholesterol biosynthesis by means of HMG Co-A reductase inhibitors (statins), increases relapse-free survival in breast cancer patients. Intriguingly, our group has found that the metastatic actions of cholesterol require its conversion to 27-hydroxycholesterol (27HC). Further, 27HC was recently shown to mediate the tumor growth-promoting effects of cholesterol by activating the estrogen receptors (ERs) and liver X receptors (LXRs). Interestingly, we found that the pro-metastatic effects of 27HC required the presence of myeloid cells. *Therefore, we hypothesized that elevated 27HC changes the host environment to promote the recruitment of specific myeloid cells, which primes distal sites for colonization by cancer cells.*

Method: Mature and immature bone marrow derived cells were purified by density gradient. Cells were treated with vehicle or 27HC and then analyzed by qRT-PCR. Bone marrow derived cells were also co-cultured with splenocytes with or without 27HC treatment, and then analyzed by flow cytometry. Female C57Bl/6 mice were grafted with E0771 cancer cells in an axillary fat pad, followed by administration of 27HC or vehicle for two weeks before tissue analysis by flow cytometry.

Results: mRNA analysis of bone marrow derived cells showed that cytokines involved in myeloid cell differentiation (*Csf1, Csf3),* and LXR target genes such as *Abca1* were elevated in 27HC treated samples relative to vehicle. Further cellular analysis showed that after co-culture with splenocytes, the percentage of $CD3^+/\gamma\delta$ T cell receptor $(TCR)^+$ cells was elevated in 27HC treated samples. γδ T cells have been reported by others to have a suppressive effect on cytotoxic $CD8^+$ T cells, and the absence of these γδ T cells has been shown to reduce metastases. These *in vitro* findings were confirmed *in vivo* where we observed that $CD25^+$ and γδ TCR^+ are elevated in mice treated with 27HC compared to placebo. Therefore, it is likely that one mechanism by which 27HC facilitates metastasis is by suppressing the immune system's anti-cancer ability via increasing the presence of γδ T cells.

Conclusion: Hypercholesterolemia is a highly associated co-morbidity of obesity and elevated plasma cholesterol is in itself a prominent risk factor for breast cancer. 27HC has been shown to mediate the effects of cholesterol on breast cancer metastasis, but the precise mechanism has not been described. Our observations highlight 27HC as a novel immune suppressive agent, permitting metastatic cancer cells to escape immune-attack. Therefore, the synthesis or downstream actions of 27HC likely represent a viable therapeutic target in the prevention and treatment of metastatic breast cancer; a stage of disease with a current paucity of targeted treatment options.

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NOTES

ABSTRACTS FOR POSTER SESSION A (ODD NUMBERED POSTERS)

P13 CROSSTALK BETWEEN THE RETINOIC ACID AND CALCIUM SIGNALING PATHWAYS IN MOUSE OVARIAN GRANULOSA CELL FUNCTION

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Normal development of ovarian follicles is critical for female reproduction and endocrine function. We have identified retinoic acid (RA) and the RA-degrading enzyme CYP26B1 as regulators of ovarian follicle development and shown that RA and a CYP26 inhibitor stimulate ovarian granulosa cell proliferation. The mechanism underpinning RA-dependent proliferation, however, is not known. The current study was designed to examine the role of intracellular calcium (Ca^{2+}) signaling in mediating the effects of RA on primary mouse granulosa cell proliferation. In single-cell Ca^{2+} imaging experiments, treatment of cultured granulosa cells with RA was found to increase the steady-state Ca^{2+} content of the endoplasmic reticulum (ER) stores. This correlated with increased store-operated Ca^{2+} entry (SOCE) and enhanced IP_3-dependent Ca^{2+} release in response to purinergic stimulation. In proliferation assays, RA treatment or *Cyp26b1* knockdown stimulated proliferation while *Cyp26b1* overexpression inhibited proliferation. When cells were treated with 2-Aminoethoxydiphenylborane (2-APB), a blocker of IP_3-dependent ER Ca^{2+} release and SOCE, or with Xestospongin C, a selective IP_3 receptor antagonist, cell growth was inhibited. When RA was given together with 2-APB or Xestospongin C, the stimulatory effect of RA on cell proliferation was abolished. Further investigation showed that treatment with 2-APB or the specific SOCE blocker, 3,5-bis(trifluoromethyl)pyrazole (BTP-2), inhibited RA induction of RA response element (RARE) activation, confirming an important role for Ca^{2+} signaling in mediating RA actions. Overall, these data support a model in which RA regulates ovarian follicle development by stimulating granulosa cell proliferation and this stimulatory effect is at least in part driven by the modulation of Ca^{2+} signals mediated by increased ER Ca^{2+} store filling and IP_3-dependent Ca^{2+} release from the ER.

P15 INTRAOVARIAN ENDOTHELIN-2 EXPRESSION IS FUNDAMENTAL FOR NORMAL OVULATION AND SUBSEQUENT FECUNDITY.

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Rupture of mature follicle is the culmination of ovulatory process that is inherently dependent upon a myriad of intrinsic ovarian and extrinsic factors that signal for follicular growth, vascularization, leukocyte invasion, and ultimately the release of an oocyte prior to fertilization. Prior to follicle rupture, granulosa cells of periovulatory follicles transiently produce the potent vasoconstrictor EDN2, a gene product of endothelin-2 (edn2). We previously showed that antagonization of the Endothelin receptor significantly reduces ovulation, indicating a potential role of contraction in ovulation. Here, we show that follicle rupture and therefore fertility is significantly impaired in the absence of ovarian EDN2 expression. Using a novel transgenic mouse that expresses improved Cre recombinase (iCre) in the granulosa cells, end2 gene was selectively ablated in the granulosa cells. This specific deletion of edn2 significantly reduced the numbers of ovulated oocytes compared to wild type littermates when treated with gonadotropins for ovulation induction (3.75±0.88 vs. 16.36±1.85 oocytes/ovary, p=0.001). Furthermore, edn2 ablation resulted in smaller litters than controls (4.29±l.02 vs. 8.50±0.60 litters/mouse, p=0.008). Though fecundity was decreased, the number of pregnancies to term per pairing was not different between groups (p=1.000), implying that other than the ovulatory machinery, the reproductive axis of the mutant mice is intact. Histological examination showed that the ovaries of the conditional edn2 knockout mice had a significantly higher percentage of antral follicles and fewer corpora lutea than controls (p=0.019 and p=0.013, respectively), suggesting that follicles progress to the antral stage but some are unable to rupture at the time of ovulation. RT-PCR profiler array data suggest that EDN2 does not cause transcriptional modification to induce follicle rupture, but instead acts through its cognate receptors to induce follicular contraction and oocyte expulsion. Taken together, our data demonstrate that EDN2 is a critical and potentially the last trigger of follicle rupture.

This work was supported by NIH R01 HD052694 and P01 HD071875 (CK).

P17 THE ROLE OF GERM CELLS IN ACTIVATION OF NOTCH SIGNALING IN SOMATIC CELLS OF THE NEONATAL MOUSE OVARY

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Bidirectional signaling between the oocyte and somatic granulosa cells is critical for the establishment and growth of ovarian follicles. While endocrine and paracrine signaling mechanisms have been extensively investigated, less is known about the potential roles of juxtacrine, or contact-dependent, signaling in this context. The Notch pathway is one type of juxtacrine signal implicated in these processes, and previous studies from our lab and others using pharmacological inhibition or gene knockout approaches have demonstrated that Notch signaling is important during early ovarian development for primordial follicle formation. Additional studies have revealed ongoing functions for Notch signaling in the growth and survival of ovarian follicles. However, the spatial and temporal mechanisms of Notch activation in the ovary are poorly understood. Our prior data show the expression of the Notch ligand, Jagged1, in germ cells of the neonatal ovary and the receptor, Notch2, in somatic cells, suggesting that the oocyte may serve as the source of Notch ligand required to activate the Notch pathway in somatic cells as follicles begin to form. However, not all Notch signaling is likely to be dependent on the oocyte, as the pathway remains active in granulosa cells of multi-layered follicles in the absence of any direct contact with the oocyte. This project therefore seeks to determine the contribution of germ cells to the activation or maintenance of Notch signaling within granulosa cells of the follicle. To address this question, Transgenic Notch Responsive (TNR) reporter mice (from Dr. Nicholas Gaiano, John Hopkins University), in which GFP expression labels Notch active cells, were crossed with Sohlh1-mCherry transgenic mice (from Dr. Alex Rajkovic, University of Pittsburgh), in which mCherry is expressed in early germ cells, in order to visualize Notch active cells and germ cells simultaneously. Pregnant females were injected at day 11.5 of gestation with busulfan at 75 mg/kg intraperitoneally, which disrupts primordial germ cells colonizing the bipotential gonad. The ovaries from 3 busulfan treated offspringcarrying both the TNR and Sohlh1-mCherry transgenes were examined at E18.5 by confocal microscopy. The absence of mCherry reporter activity confirmed the loss of germ cells compared to vehicle-injected controls. Additionally, activation of the TNR reporter was clearly reduced in 2 of the 3 ovaries, while it appeared unchanged in the third ovary. These preliminary data suggest that Notch activation in somatic cells is altered when germ cells are not present, consistent with a requirement of Jagged1 expression from the oocyte as a stimulus for Notch activation in the neonatal ovary. Ongoing studies include the use of genetic approaches to ablate germ cells within the ovary to complement the chemical targeting strategy. Understanding how Notch is activated in the developing ovary is vital to appreciating its overall importance in follicle function and female fertility.

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P19 DEVELOPMENT OF A TUNABLE PHYSICAL ENVIRONMENT USING MMP-DEGRADABLE HYDROGELS FOR GROWTH OF OVARIAN FOLLICLES

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Hydrogel-encapsulating platforms can provide a physical environment conducive to ovarian follicle growth. Such platforms may provide a viable fertility preservation option for female cancer patients. Natural hydrogel matrices, composed of fibrin, have been combined with *in vitro* culture to support follicle development. Fibrin hydrogels at a concentration of 20 mg/mL resulted in 40% follicle survival (at all stages) after a 2 day *in vitro* culture, whereas a higher concentration (40 mg/mL) resulted in decreased survival (~20%). However, fibrin at a concentration of 20 mg/mL rapidly degrades *in vitro* and may not provide a long-term physical environment to grow follicles *in vivo.* Consequently, we explored combining fibrin with natural materials, such as alginate and collagen, to control degradation and thus the rigidity of the follicle's microenvironment. Rigidity is a critical factor to regulate preantral follicle development as a very rigid environment change can result in their altered gene expression, altered hormone production, inhibited growth, and ultimately reduced gamete quality. We found that follicle density was highest, approximately double, in fibrin compared to fibrin-alginate and fibrin-collagen hydrogels tested *in vivo* at day 9 in the bursa. Thus, fibrin was used in conjunction with vascular endothelial growth factor (VEGF) to aid in two live births to date. Our objective is to now replicate these results in a synthetic hydrogel platform that allows follicles to control degradation of their physical environment according to their developmental needs. Synthetic hydrogels are particularly attractive as they can be designed to be semi- or fully degradable via protease-specific sequences. Such hydrogels allow follicles to control the rigidity of their physical environment. Polyethylene glycol (PEG) vinyl sulfone (VS) hydrogels with plasmin-sensitive degradable sequences have been demonstrated to result in a 17-fold volumetric expansion of encapsulated mouse secondary follicles and enhanced *in vitro* follicle growth (IVFG) outcomes compared to natural-derived hydrogels. This indicates the encapsulated follicle can remodel its physical environment, which can result in improved follicle growth and oocyte quality. We aim to re-create the dynamic physical ovarian environment using hydrogels that containing specific peptide sequences that are fully degradable via matrix metalloproteinases (MMPs). MMPs dynamically change the microenvironment of the follicle by regulating extracellular matrix (ECM) composition. Remodeling of the ECM by MMPs can have dramatic effects on cellular proliferation, differentiation, and survival, all of which may be critical in preserving microenvironments conducive to follicular growth and function. We hypothesize that PEG-VS hydrogels degradable by MMPs will allow encapsulated follicles to alter the rigidity of their environment and support high meiotic competence *in vitro* and *in vivo*. As specific proteases become active at distinct stages of folliculogenesis, the follicle will be able to appropriately break down the PEG network, which will result in loss of rigidity. Such changes in rigidity are critical for primary follicles, located in the rigid ovarian cortex, to transition to larger growing follicles in the less rigid medulla. Our ultimate goal is to apply this approach to grow bovine and human follicles.

P21 CDH6 - TISSUE SPECIFIC MARKER FOR HIGH GRADE SEROUS OVARIAN CANCER

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Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer related death in American women. High-grade serous ovarian cancer (HGSOC) is the most lethal and malignant histotype of EOC. The high mortality rate of the disease is primarily due to lack of understanding on the source of origin and targeted therapy. Evidences supports the hypothesis that fallopian tube epithelium (FTE) may be the potential precursors for HGSOC with elevated levels of mutant p53. A mutation in tumor suppressor protein p53 is one of the known early events and reported in 96% of HGSOC. Mutant p53 exerts dominant negative (DN) effects by inhibiting wild type p53 function and is additionally reported to exhibit gain-of-function (GOF) activity, resulting in increased tumor metastases in animal models. The goal is to identify mutant p53 regulated cell specific markers of disease progression to classify FTE vs OSE derived HGSOC. Previous findings using murine oviductal cells (MOE, a human equivalent of fallopian tube) showed that $p53^{R273H}$, a high frequent DNA binding domain mutation in HGSOC, increases cell migration. Microarray data confirmed expression changes of pro-migratory genes in $p53^{R273H}$ transfected MOE cells compared to parental cells. Similar p53 mutation did not change migration in murine ovarian surface epithelial cells (MOSE), a human equivalent of ovarian surface epithelium (OSE). OSE is another proposed progenitors for HGSOC. A comparative pro-migratory gene expression analysis in MOE and MOSE cells revealed that $p53^{R273H}$ decreases CDH6 (cadherin – 6 type 2, K- cadherin) expression in MOE not in MOSE cells. CDH6 is the member of cadherin glycoproteins family that mediates homophilic cell-to-cell adhesion. A decrease in Cdh6 expression may be associated with metastasis. Examining CDH6 expression in a transgenic mouse model with tissue specific expression of mutant p53 in oviducts but not in ovaries supported the FTE specific regulation of CDH6. To further clarify if all p53 DNA binding domain mutants regulate CDH6, MOE cells were stably transfected with a construct encoding for $p53^{R248W}$, the second common DNA binding domain mutation. A decreased CDH6 expression was observed in $p53^{R248W}$ MOE cells compared to the control cells. Chromatin immunoprecipitation analysis (ChIP) showed high confidence of mutant p53 occupancy on CDH6. To identify the signal transduction pathways of mutant p53 on CDH6 regulation in HGSOC, MOE cells with Snai2 knockdown was tested for CDH6 expression. Snai2, encodes for SLUG, a mutant p53 target that binds to E-box motifs to repress cadherin expression. Decreased CDH6 expression by $p53^{R273H}$ is restored by SLUG knockdown suggesting that CDH6 is downstream of SLUG and mutant p53. OVCAR3, a human HGSOC cell line, with $p53^{R248W}$ mutation had decreased CDH6 expression. We are currently investigating the mutant p53 knockdown effects on CDH6 expression and whether its regulation is mutant p53 GOF or DN activity. Expression and characterization of mutant p53 tissue specific marker provides new target for identifying FTE derived HGSOC and blocking the peritoneal spread of the disease, which is what results in poor survival.

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P23 INVESTIGATING THE ROLE OF SMALL HETERODIMER PARTNER (SHP) IN BREAST CANCER PATHOLOGY

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Although there have been impactful advancements in the treatment of breast cancer, relapse with endocrine-therapy resistant disease, and the lack of targeted therapy for triple negative disease remain significant clinical problems. Therefore, with approximately 1 in 8 women being diagnosed with invasive breast cancer over their lifetime, breast cancer still remains the second leading cause of cancer related death in women. Thus, there is strong rationale for the development of novel strategies for treating breast cancer. In this regard, members of the nuclear receptor superfamily all contain a 'ligand binding domain' making them highly amenable to drug development. While screening nuclear receptors with potential involvement in breast cancer, we found that the tumoral expression of Small Heterodimer Partner (SHP) was associated with a significantly increased relapse free survival time among breast cancer patients. Importantly, this beneficial association was apparent in all subtypes of breast cancer with the exception of HER2+ cases. Based on these data, we initiated a series of experiments to evaluate the specific roles of SHP in breast cancer. SHP is a unique nuclear receptor in that it lacks a DNA binding domain. Furthermore, an endogenous ligand for SHP has not yet been described. It functions by binding and inhibiting other nuclear receptors such as the Liver X Receptors (LXRs). Here, we report that SHP is differentially expressed across different, commonly used breast cancer cell lines. Furthermore, treatment with a specific small molecule antagonist (GSK1268) to SHP led to expected changes in the expression of target genes indicating the functionality of SHP in breast cancer cells. Somewhat unexpectedly however, treatment with GSK1268 was without significant effect on the proliferation of various breast cancer cell lines. Moreover, the SHP antagonist significantly decreased the migratory capacity of MDA-MB-453 cells, a triple negative breast cancer cell line. We are currently extending these findings to other cell lines and are manipulating the expression levels of SHP using RNAi and CRISPR/Cas9. However, given the paradoxical observations between the protective role of SHP in the clinic compared to the decreased migration observed when SHP is antagonized, we are now considering cancer-cell-extrinsic roles for SHP within the tumor microenvironment. In conclusion, clinical data strongly support a role for SHP in breast cancer pathophysiology. Since ligands targeting SHP are already available, our mechanistic studies will set the framework for rapid drug development and novel therapies for the treatment of breast cancer.

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P25 DETERMINING THE ROLE SPHINGOSINE-1-PHOSPHATE (S1P) PLAYS IN PROGRESSION AND METASTASIS OF OVARIAN CANCER

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Purpose:

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that has been implicated in numerous biological processes including cell migration, survival, proliferation, angiogenesis and immune functionality. S1P signals through five sphingolipid receptors (S1P1-S1P5). Of particular importance is how the sphingolipid pathway controls cytokine or chemokine secretion and metastasis. We reported the expression of S1P1 in ovarian tumors in both women and hens; however, its role in ovarian cancer pathogenesis is uncertain. Ongoing *in vitro* studies will determine if S1P alters cell invasion, cell proliferation, and apoptosis of ovarian cancer cells.

Methods:

S1P1 and S1P2 expression were confirmed by immunohistochemistry and Western blot in several ovarian cancer cell lines. Biological activity of S1P agonists and antagonists were assayed to determine the function of these receptors with respect to cell proliferation. Ovarian cancer cells (ES2, SKOV, EG, and HeyA8) were grown to confluency and treated with different dosages of S1P, CYM5442, SEW2871, FTY720, W123, and Fumonosin. MTT (5mg/mL) assay was used to measure cell proliferation at 24 and 48 hours. Invasion was measured as the average number of cancer cells/field that invaded collagen (I)-coated wells. Flow cytometry was also used to determine if apoptosis of ovarian cell lines was induced when treated with S1P agonists and antagonists via propidium iodide (PI)/FITC- annexin V staining.

Results:

Our preliminary data suggests that S1P and agonists reduces invasion of ovarian cancer cells as measured by the ability of cancer cells to invade a Boyden chamber coated with collagen. Also, the different ovarian cancer cell lines (which represent the different histological tumor types in humans) have varying sensitivities to S1P agonists in terms of proliferation. Ongoing studies at the time of abstract submission will address S1P role in cell proliferation and ability to induce apoptosis. Current findings indicate a dichotomy between proliferation and invasion with respect to S1P.

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P27 PAX8 LEADS TO EARLY PROGRESSION OF HIGH-GRADE SEROUS OVARIAN CARCINOMA THROUGH UPREGULATION OF THE FOXM1 PATHWAY

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Ovarian cancer is the 5th leading cause of cancer mortality in women in the United States. High-grade serous ovarian carcinoma (HGSC) is the most common form of ovarian cancer and the most lethal. Part of the reason for this high mortality rate is lack of early detection and the heterogeneity of disease. It is crucial, therefore, for research to elucidate the early mechanistic changes that facilitate the development of ovarian cancer. Paired-box transcription factor 8 (Pax8) is a transcription factor expressed in cells of Müllerian origin, including the fallopian tube, but it is not found in the ovarian surface epithelium. Amongst HGSC, Pax8 is expressed in ~8096% of cells. While this might suggest a fallopian tube cell of origin for HGSC, recent studies have shown Pax8 expression in models of HGSC derived from the mouse ovarian surface epithelium (MOSE). In this study, we show that Pax8 increases proliferation and migration in MOSE cells through increased expression of several EMT factors such as N-cadherin, Fibronectin, and Slug. Pax8 is also known to regulate p53, which is mutated in 96-100% of HGSC cases and governs expression of FOXM1. The FOXM1 pathway is altered in ~70% of tumors and can be targeted with cell penetrating peptides. In this study, we show Pax8 expression enhanced FOXM1 levels in MOSE cells. Targets downstream of FOXM1, such as PLK1, AURKB, CCNB1 and BIRC5a were also upregulated. In contrast, PAX8 knockdown in the mouse oviductal epithelium (MOE) cells decreased proliferation by increasing apoptosis and downregulation of the FOXM1 pathway. Finally, despite an unknown cellular origin, most ovarian tumors and ovarian cancer cell lines express Pax8. Silencing Pax8 in these cancer cell lines resulted in apoptosis with a decrease in the anti-apoptotic protein Bcl2. There was also a decrease in FOXM1 and its downstream proteins. The results presented here suggest that Pax8 has a role in ovarian cancer development and adds to our current understanding of the early mechanisms facilitating the progression of ovarian cancer.

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P29 ACTIVATING MUTATION IN THE LUTEINIZING HORMONE RECEPTOR CAUSES ERECTILE/EJACULATORY DYSFUNCTION RESULTING IN AGE-RELATED INFERTILITY IN MALE MICE

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The luteinizing hormone receptor (LHCGR) is crucial for fertility, and genetic mutations in LHCGR cause adverse effects in reproductive development. Among the activating mutations identified in LHCGR, replacement of aspartic acid 578 by glycine (D578G) is the most common inherited mutation. Boys with this mutation undergo puberty by 2-4 years, caused by high testosterone in the context of prepubertal luteinizing hormone (LH) levels and present with Leydig cell hyperplasia. Clinically, these symptoms are associated with familial male-limited precocious puberty (FMPP). Our lab has published a mouse model for FMPP (KiLHR) with D582G mutation equivalent to D578G in human LHCGR. We demonstrated that KiLHR mice exhibited identical phenotypes and was a good model for FMPP. We observed that the KiLHR mice became progressive infertile. A 6-month breeding study showed that compared to 100% fertility of wild type (WT) mice, only 8% of KiLHR were fertile at 6 months of age. The infertile KiLHR males were unable to form copulatory plugs in WT females although they exhibited normal sperm count and motility. In this study, we sought to determine if the reason for the infertility was due to a mating problem or accessory gland dysfunction resulting in lack of plug formation. To test this, we mated WT and KiLHR males at two ages with superovulated WT females. At 10-12 weeks, 3/5 mutant males produced plugs compared to 5/6 WT males. When the animals were retested at 20-22 weeks, only 1/7 mutant males produced a plug compared to 4/5 WT males. Sperm was detected in the uterus and oviduct only when a plug was formed. Sperm from both WT and KiLHR males were able to fertilize oocytes indicating that the sperm from KiLHR mice was functional. These results suggest that the KiLHR males are not capable of mating/ejaculating. Sexual behavioral testing revealed that the infertile KiLHR males were capable of mounting the receptive WT females but were unable to achieve ejaculation indicating the erectile/ejaculatory dysfunction. To address the reason for this, we performed histopathological analysis of the accessory glands and penis. Hematoxylin and eosin staining showed that the morphology of prostate and seminal vesicles were similar between KiLHR and WT mice. However, sperm accumulation was visible in the ampulla of ductus deferens of mutant mice as early as 7-8 weeks of age. The epithelium of the ampulla was hyperplastic multifocally primarily reflected by a stratification of epithelial cells. This finding suggested a possible luminal obstruction of the sperm at this location. The urethral lumen was normal and open in the entire length. Additionally, corpora cavernosa of the KiLHR penile body showed several aggregates of chondrocyte metaplasia as early as 12 weeks of age. Immunohistochemistry indicated that the chondrocytes stained positive for the androgen receptor in the nucleus and for LHCGR in the cytoplasm. Anti-alpha smooth muscle actin and Masson's trichrome staining for collagen showed no apparent difference in sections of the body penis between the genotypes. Positive staining in the areas around chondrocytes indicated type II collagen secretion by the cells. These experiments suggest that failure of the KiLHR mice to ejaculate is likely due to a functional defect in the penis. Our studies indicate that FMPP patients may be susceptible to age-related infertility, likely due to erectile/ejaculatory dysfunction.

P31 CHARACTERIZATION OF EXTRACELLULAR MATRIX COMPONENTS IN THE DEVELOPING FETAL TESTIS AND THEIR POTENTIAL INFLUENCE ON STEROIDOGENESIS

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Previous work performed in fetal adrenal glands found that specific spatial patterns of extracellular matrix (ECM) components were identified in discrete zones of the cortex, and this appeared to influence steroid synthesis. Just prior to the onset of steroidogenesis, the fetal testis undergoes massive reorganization that separates the organ into two distinct regions: the cords, which contain germ and Sertoli cells, and the interstitium, which includes Leydig and other mesenchymal cells. While the ECM is present during this reorganization, especially in the interstitium, the components and their actions are unknown. We hypothesized that specific ECM components are expressed in the interstitium where they contribute to stimulating androgen synthesis in fetal Leydig cells. To test our hypothesis, embryonic testes from wild type mice were collected and processed for immunohistochemistry for laminin and fibronectin, and histological stains for collagen. The onset of steroidogenesis occurs just after cord formation at approximately embryonic day E13.5. Thus, we focused our analysis on three time points that encompassed organizational events of the interstitium and ECM during testis maturation: E11.5, pre-steroidogenesis, cords actively being formed; E13.5, onset of steroidogenesis, cords formed; and E16.5, peak of steroidogenesis, cords elongating. Results showed collagen expression was limited to specific locations depending on developmental age. At E13.5, collagen was present at the gonad-mesonephros border. At E16.5, collagen was also present at the gonad-mesonephros border, but confined to a discrete region that corresponds to the developing rete testes. In addition, preliminary data indicates that fibronectin expression is dispersed throughout the interstitium and surrounding the testis cords, while laminin is localized to the basement membrane of testis cords. These data suggest that ECM components are dynamic and are found in distinct locations in the developing fetal testes. Current studies are underway to evaluate ECM components within 2D and 3D cell culture matrices and their effects on steroid output.

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P33 SYNAPTOBREVIN PUNCTA ARE PRESENT IN THE APICAL RIDGE BUT DO NOT CO-LOCALIZE WITH SYNTAXIN PUNCTA TO FORM TRANS-SNARE COMPLEXES DURING MOUSE SPERM CAPACITATION

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The principal role of SNARE proteins is to arbitrate vesicle fusion to a target membrane. Formation of tripartite protein complexes between SNARE proteins on apposing membranes is the minimal requirement for membrane fusion. A member of the SNARE family, syntaxin, is found on the sperm plasma membrane while synaptobrevin, is found on the outer acrosomal membrane. During the sperm acrosome reaction, the outer acrosomal membrane fuses at hundreds of points with the overlying plasma membrane, resulting in release of the acrosomal contents. We hypothesize that, during capacitation, syntaxin and synaptobrevin shift within their respective membranes to form trans-SNARE complexes that promote membrane fusion at hundreds of specific points during the acrosome reaction. Immunofluorescence was used to localize both syntaxin and synaptobrevin in mouse epididymal sperm before and after capacitation. Sperm were fixed, permeabilized, and incubated with antibodies to syntaxin, synaptobrevin and then fluorescent secondary antibodies. Super resolution Structured Illumination Microscopy (SR-SIM) was used to examine samples collected at 0-120 min of capacitation time, to obtain 3D images of SNARE localization. At 60 min of capacitation, syntaxin was localized in puncta that were mostly restricted to the apical ridge of the plasma membrane overlying the acrosome in over 90% of sperm. Syntaxin was localized in this restricted pattern in less than 20% of the sperm that were not capacitated or were incubated in medium lacking albumin that is required for capacitation. In contrast, synaptobrevin was already found in the puncta at the apical ridge of the sperm head in 83% of sperm prior to capacitation, where it remained during capacitation. When co-localization was assessed, the puncta containing syntaxin and synaptobrevin did not precisely co-localize at the beginning or end of capacitation. Our results demonstrate that, in contrast to syntaxin, the acrosomal SNARE synaptobrevin is already localized to the apical edge of capacitated sperm. But the puncta that contain synaptobrevin do not co-localize with those containing syntaxin, even after 60 min of capacitation time. Therefore, the formation of trans-SNARE complexes does not occur until after capacitation, during acrosomal exocytosis.

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P35 BLIMP1 CONTROLS PENIS DEVELOPMENT THROUGH ESTROGEN RECEPTOR SIGNALING

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B lymphocyte–induced maturation protein-1 (Blimp-1), also know as PR Domain Containing 1 with ZNF Domain (PRDM1), was discovered 17 years ago as a transcriptional repressor of the IFNβ promoter, plays fundamentally important roles in many cell lineages, early development and a master regulator of plasma cell differentiation, also controls patterns of gene expression in T lymphocytes and, macrophages, the sebaceous gland and skin epidermis. In the mouse embryo, Blimp-1 controls germ cell specification and regulates development of the posterior forelimb, caudal pharyngeal arches, secondary heart field and sensory vibrissae. In postnatal stages, unfortunately, only one study shows Blimp1 regulates the transition of neonatal to adult intestinal epithelium. In this study, we discovered the function of Blimp1 on penis development. Blimp1 strongly expressed in mesenchyme, urethra and penis epithelium of developing penis. Knockout Blimp1 in mesenchyme led to micropenis formation; Deletion of Blimp1 from ectoderm-derived epithelium using Msx2Cre causes reduced number of penis spines; when blimp1 was knocked out in urethra epithelium using ShhCre, No obvious phenotype could be observed. Additional study shows that Blimp1 governs penis development through repressing estrogen receptor (ERα and ERβ) expression, then regulate key morphogen genes like Ihh, affect cell proliferation in penis development. Our study discovered a possible micropenis causal gene and linked immune system to steroid hormone signaling and reproductive organ development.

P37 UNCOVERING THE ROLE OF NOTCH SIGNALING IN EARLY HYPOTHALAMIC FATE CHOICES USING PRIMARY NEUROSPHERES AND MICROENVIRONMENT ARRAYS

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The hypothalamus is a key regulator of homeostatic function within the body. A multitude of cell types including neurons containing distinct neuropeptides and glial cells each arise from a common early progenitor to carry out these complex processes. During embryogenesis, both intrinsic and extrinsic signals instruct early progenitor cells to adopt a certain fate and receive additional cues to further differentiate into their respective mature cell types. Dysregulation of any of the aforementioned cell types can result in physiological consequences persisting into adulthood including obesity and reproductive deficits.

Previous *in vivo* work from our lab has shown that the Notch signaling pathway acts as a critical molecular switch during early development of the hypothalamus. Interestingly, active Notch signaling not only maintains early progenitors as SOX2-positive progenitors of the hypothalamic ventricular zone, but also appears to promote expression of Glial Fibrillary Acidic Protein, a common glial marker, and may play an important role in differentiation of Kisspeptin neurons within the hypothalamus. Additional *in vitro* studies have suggested that extrinsic signals such as leptin or insulin may also regulate Notch signaling, further suggesting a role for its importance. However, the interaction between Notch signaling and extrinsic signals including growth factors and the extracellular matrix is unknown.

In our current study, we developed a hypothalamic progenitor cell culture in which we can activate or inhibit Notch signaling in a controlled extracellular environment. We hypothesized that inhibition of the Notch signaling pathway as well as removal of growth factors may promote primary hypothalamic progenitor cells to adopt specific differentiated fates. To address our hypotheses, primary neurospheres were treated with the γ-secratase inhibitor of Notch, DAPT, in the presence or absence of fibroblast growth factor (FGF) and epidermal growth factor (EGF) to assess their lineage bias. Preliminarily, we have determined that acute treatment with DAPT is sufficient to significantly reduce the downstream Notch target genes *Hes1* and *Hey1* and induce the proneural gene *Mash1*. Additionally, removing the growth factors in the presence of DAPT induces expression of the immature neuronal marker TUJ1, suggesting a more concrete bias towards a neuronal lineage. Taken together, these data provide *in vitro* evidence for the direct role of Notch signaling in cell fate choices in the developing hypothalamus. Next, to activate Notch signaling, we utilized a Notch ligand array coupled with multiple extra-cellular matrix (ECM) proteins to determine which Notch ligands may direct the fate of cultured neurospheres. Previous work using this technology has shown that Notch signaling can indeed bias cell fate decisions in bipotential mouse embryonic liver cells. Preliminarily, we find that hypothalamic progenitors prefer to adhere to laminin and fibronectin preferentially compared to collagen subtypes as their ECMs and that these progenitors robustly express SOX2 when presented with the Notch ligands Jagged1, Delta-like ligand 1 or Delta-like ligand 4. These ongoing studies will help further elucidate the role of Notch signaling in early progenitor fates.

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P39 ELUCIDATING A ROLE FOR NOTCH SIGNALING IN THE REGULATION OF GLUCOCORTICOID SIGNALING DURING POSTNATAL PITUITARY DEVELOPMENT

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The Notch signaling pathway is a developmental pathway that has been shown to regulate progenitor cell maintenance and cell fate choice in numerous tissues. Our lab has demonstrated that *Notch2* conditional knockout mice have a decrease in progenitor cell number during postnatal pituitary expansion. This is coincident with an increase in corticotrope cell number and a decrease in somatotropes, thyrotropes and lactotropes. These studies indicate that the Notch signaling pathway is essential for development of the correct number of each cell type during postnatal pituitary development. However, the mechanism by which Notch influences these cell fate choices remains unknown. To identify novel Notch targets that may regulate lineage specification, a microarray analysis was conducted to compare *Notch2* cKO and control pituitaries. Interestingly, one candidate gene uncovered that may be involved in corticotrope differentiation is 11-beta Hydroxysteriod Dehydrogenase (*Hsd11b1)*. It has been demonstrated in other glucocorticoid responsive tissues that the enzyme HSD11b1 increases intracellular levels of active glucocorticoids, however its expression and function in the pituitary is unknown. There is compelling evidence to indicate that glucocorticoid signaling is essential for pituitary development because studies have demonstrated that removal of Glucocorticoid Receptor (GR) in conditional knockout mice results in increased corticotrope cell number during postnatal development. To better elucidate the role of the glucocorticoid signaling pathway in pituitary gland development we characterized *Hsd11b1* and *GR* mRNA expression throughout gland development. We show that *Hsd11b1* and *GR* expression is highest during the postnatal period coincident with a hyperproliferative period of the pituitary. In addition, *in situ* hybridization studies show that at postnatal day 1 (p1) *Hsd11b1* is highly expressed in the cleft cells that surround the lumen of the pituitary as well as in the intermediate lobe and cells scattered throughout the anterior lobe. This localization suggests that *Hsd11b1* is expressed in both progenitor and differentiated cell populations. In contrast, *in situ* analysis of *Notch2* cKO mice show a substantial decrease in *Hsd11b1* expression throughout the pituitary. This finding is further confirmed by quantitative reverse transcriptase PCR data that shows a significant reduction in *Hsd11b1* levels in *Notch2* cKO mice compared to wildtype littermates at p1. Taken together these data indicate Notch as a novel regulator of *Hsd11b1* expression in the developing pituitary. Furthermore, our data suggests Notch regulation of the glucocorticoid signaling pathway as a mechanism for regulating corticotrope number during postnatal pituitary expansion.

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P41 LOSS OF CANONICAL NOTCH SIGNALING RESULTS IN ALTERED DEVELOPMENT OF HYPOTHALAMIC REGIONS CONTROLLING REPRODUCTION

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The mammalian reproductive system relies on a well-regulated feedback loop between the hypothalamus, pituitary, and gonads to properly develop. The hypothalamus is responsible for signaling the pituitary to release the sex hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Within the hypothalamus lies the anteroventral periventricular nucleus (AVPV), a region important for neural control of reproductive maturation. Past findings show that Notch signaling regulates progenitor maintenance and formation of Kisspeptin neurons during development of the Arcuate nucleus and that Kisspeptin neurons are also lost in the AVPV, however Notch's role in AVPV development is not well understood.

To determine the mechanism by which Notch signaling regulates neurogenesis in the AVPV, we first characterized the Notch components of the AVPV. At embryonic day 13.5, *Notch1* and *Notch2* were robustly expressed in the presumptive AVPV suggesting a role for Notch signaling within this region. To address the necessity for Notch signaling in the AVPV, we utilized an *Nkx2.1* Cre mouse to create a conditional knockout (cKO) of *Rbpj-κ*, a transcription factor necessary for canonical Notch signaling. Our results suggest a loss of the downstream target gene *Hes5* within a specific region of the AVPV in cKO mice. Additionally, we observed a reduction of the proneural gene *Mash1* in the same region of cKO mice. Given the role of Notch signaling on cellular self-renewal, we next characterized Ki67 expression, a marker of proliferation. Similarly, we observed a noticeable decrease in Ki67 immunopositive cells within the region devoid of *Hes5* and *Mash1* in cKOs. Interestingly, expression of SOX2, a cellular progenitor marker, was still observed throughout the entire AVPV in cKO mice.

Since cKO mice show severely disrupted ventricular morphology within the arcuate nucleus (ARC), we next sought to determine if a similar phenotype was observed within the AVPV as well as to determine any other cellular alterations. Day of birth (P0) and adult (P35) mice were collected to characterize morphology and cellular subtypes of the AVPV. Preliminarily, we observed a striking reduction of ventricular size, as well as aberrant dispersion of tyrosine hydroxylase (TH) neurons. Interestingly, we also observed a clear reduction in SOX2-positive cells within the AVPV at p0 as well as a reduction and misplacement of ERα cells at both p0 and p35.

Given the observed disruption in both ARC and AVPV development as well as the near absence of fertility in cKO mice, we next analyzed gonadotrope transcript levels in the pituitary gland since hypothalamic signaling to the gonads occurs through this gland. In P0 male and female cKO mice, a reduction in *Lhb*, *Fshb*, and *Gnrhr* (Gonadotropin releasing hormone receptor) was observed in the cKO mice compared to controls. However, levels of *Nr5a1*, a gonadotrope lineage marker, remained the same, indicating that gonadotropes are likely differentiating, but are not receiving input from the brain to express gonadotropin subunits. These data all together suggest an important role for Notch signaling in hypothalamic development and control of reproduction.

P43 REGULATION OF FMS-LIKE TYROSINE KINASE 1 (FLT-1) ESPRESSION IN HUMAN TROPHOBLAST

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Although preeclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality that affects 5-8 percent of women worldwide, currently there are no predictive tests or curative treatments. Clinical diagnosis of preeclampsia is determined by hypertension and proteinurea after 20 weeks gestation and both are mediated by aberrant expression of angiogenic and antiangiogenic factors. An increase in sFlt1, a soluble antagonist receptor for placenta growth factor (PGF), and decreased PGF expression are evident in preeclamptic patients' sera. Regulation of Flt1 expression in trophoblast is not understood. Thus, the goal of our project is to characterize mechanisms that regulate Flt1 expression in human trophoblast. Endogenous expression of Flt1 gene products is high in primary term trophoblast, but are significantly lower in trophoblast cell lines. Hypoxia increases Flt1 expression in JEG3 and Sw71 trophoblast cell lines and primary trophoblast. To determine if this increase in Flt1 expression is due to transcriptional activity, we constructed a luciferase reporter containing 1.7kb of the 5'UTR of human *FLT1* gene. Transient transfections with this reporter plasmid in JEG3, H8, and hEK-293 cells showed limited expression. In addition, hypoxia did not significantly increase transcriptional activity of the reporter in trophoblast cell lines. Finally, stable JEG3 and Sw71 trophoblast containing the reporter construct had limited luciferase expression. These data suggest that key regulatory motif(s) may not be included in this 1.7kb Flt1 promoter region or that trophoblast cell lines have specifically down regulated transcription of Flt1. One possibility is that the Flt1 promoter is hypermethylated, which blocks expression of Flt1 in trophoblast cell lines. We treated cells with varied concentrations of the demethylating agent 5-Aza2'deoxycytidine (AZA) and cultured them under normoxia and hypoxia. Aza treatment increased total Flt1 and sFlt1 mRNA under both normoxic and hypoxic conditions in H8 cells. Treatments of JEG3 cells with AZA increased total Flt1 and sFlt1 expression in normoxic culture conditions. Importantly, hypoxia increased both total Flt1 and sFlt1 expression in JEG3 cells and this was greatly augmented with AZA treatment. Collectively, these results suggest that trophoblast cell lines have specifically down regulated expression of Flt1 gene.

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P45 ADAPTATIONS TO HYPOXIA DURING PREGNANCY: THE BLIND MOLE RAT, SPALAX, PLACENTA TRANSCRIPTOME

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Introduction: Exposure to atmospheric hypoxia *in utero* has been associated with adverse birth outcomes such as reduced birth weight as well as metabolic disorders later in life. Species that reside in high altitude environments as well as those that live underground are exposed to hypoxic conditions and have evolved mechanisms to adapt to these conditions. To study the adaptations of subterranean mammals we sequenced the placenta transcriptomes of two species of the blind mole rat.

Methods: We performed RNA-seq on villous placenta tissue from two species of blind mole rat (*Spalax galili*, and *Spalax carmeli*) using the Illumina HiSeq2000 platform. 100bp paired-end reads were generated and only the high quality reads (i.e. >Q20) were assembled *de novo* using Trinity, aligned to the assembled transcripts using STAR, called using Cufflinks, and annotated using Diamond. We used phylogenetic methods to infer the evolutionary history of several placenta-specific genes including capthepsins and prolactins. We examined genes in the GO category "response to hypoxia" (GO:0001666) as well as those genes known to be imprinted in the mouse placenta to determine if they are expressed in the *Spalax* placenta with a FPKM > 1. To identify orthologous genes that are not imprinted in the *Spalax* placenta but are imprinted in the mouse placenta, we called variants using samtools among genes known to be imprinted in the mouse placenta. We reasoned that transcripts that have heterozygous variants are not imprinted in the *Spalax* placenta.

Results: We assembled 47,270 and 54,035 transcripts with FPKM > 1 for the *Spalax galili* and *Spalax carmeli* transcriptome, respectively. Through maximum likelihood analysis we confirmed that expression of several placenta specific gene families have been features of rodent pregnancy for at least 45 million years. Of the 255 genes in the GO category "response to hypoxia" 74% are expressed in the *Spalax* placenta transcriptome. The results of our variant calling analysis showed that 106 of the 167 genes imprinted in the mouse placenta and expressed in the *Spalax* placenta exhibited bi-allelic expression in the *Spalax* transcriptome and thus are unlikely to be imprinted. This means in genes that are not imprinted there is the potential for maternal paternal conflict because both the maternal and paternal alleles are being expressed. Genes that are maternally imprinted in mice but not imprinted in the *Spalax* placenta, such as *Igfr2*, could result in increased fetal growth through paternal influence. Genes that are paternally imprinted in mice but not in *Spalax*, including *Peg3*, could result in a conservation of maternal resources.

Conclusions: Species that are chronically exposed to hypoxic conditions can be used as natural models to examine adaptations during human pregnancy. The results of our phylogenetic and variant calling analysis point to a complex evolutionary history of gene expression in the placenta. We show changes in the number of members of several placenta specific gene families as well as increased potential for conflict between the maternal and paternal genomes in *Spalax* over *Mus musculus*. There is an increased potential for conflict in the *Spalax* transcriptome because many of those genes seen imprinted in the mouse placenta express both the paternal and maternal allele in the *Spalax* placenta transcriptome.

P47 PLACENTAS OF HIGH ALTITUDE ANDEANS SHOW DIFERENTIAL MIRNA EXPRESSION AS A POSSIBLE ADAPTIVE EVOLUTIONARY RESPONSE TO HYPOXIA

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Background: Hypoxic environments of high altitude are associated with 2-4 fold increases in obstetrical syndromes including preeclampsia and intrauterine growth restriction (IUGR). Prior research has shown that fetal growth is enhanced in populations with thousands of years of residence at high altitude. Morphological studies show that native Andeans at high altitude have birth weights comparable to nearby sea-level populations, whereas migrant Europeans at high altitude have significantly lower birth weights than their sea-level counterparts. We hypothesize that evolution has favored specific microRNA expression as a means of translational repression in placental tissue to permit normal placental development and fetal growth. We seek to identify microRNAs involved in placental adaptation to hypoxia in high altitude Andeans.

Methods: We performed gene expression analysis on 45 term villous placental samples delivered through caesarian section from low and high altitude Andeans and Europeans using an Illumina HT12v4 gene expression microarray. We performed small RNA sequencing on the same tissues. We aligned the raw reads from small RNA sequencing to miRBase (v. 21) with Bowtie 2.0 to obtain read counts for hairpin and mature miRNA. We then used a general linearized model implemented in EdgeR (v. 3.10.2) to normalize counts and determine which genes and miRNAs were differentially expressed (DE) according to altitude and ancestry. A gene or miRNA was considered DE if it had a fold change >1.25 and q <0.10. We used multiMiR to search databases to determine if DE genes were either validated targets or in the top 10% of predicted targets.

Results: We identified 1 DE miRNA in its mature and hairpin forms between Andeans and Europeans at high altitude. We identified 39 DE microRNAs (4 mature and 35 hairpin) between high and low altitude Andeans. We identified 72 genes expressed according to altitude or ancestry. We predicted 7 interactions between the DE microRNAs and mRNAs of genes of high and low altitude Andeans. Two of these predictions negatively correlated with our differential expression data. We were unable to predict any microRNA-mRNA interactions of DE miRNA and genes between Andean and European ancestries.

Conclusion: We found that there are significant differences at the level of microRNA expression between Andeans and Europeans at high altitude. Results suggest that two miRNAs could be responsible for some amount of differential gene expression, but the mechanisms of the other predicted miRNA interactions remain unclear. Previous studies show many of the uncorrelated miRNAs to be associated with cell division, proliferation, and gene expression. We believe there are other miRNA-targeted pathways that also play a significant role in placental adaptation to high altitude.

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P49 CHARACTERIZATION OF THE FECAL MICROBIOME OF CHINESE HOLSTEIN DURING THE TRANSITION PERIOD

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The objective was to characterize the fecal microbiome during the transition period in 3 Chinese Holstein cows. Fecal samples were collected weekly starting at day 21 before parturition until day 21 after calving. Total DNA of each sample was extracted and the V4 region of 16S rRNA gene was sequenced using the MiSeq Illumina platform. As a result, a total of 1,800,487 filtered sequences reads with an average 54,523 reads per sample classified into 945 species based on 97% similarity. Small variation on diversity of microbiome was across the seven collected time-points when evaluated by Shannon, Chao1 and mean relative abundance of OTUs. Overall the fecal flora community of these 3 Chinese Holstein cows had a little of transformation in the different stages but in general with phylum *Firmicutes* and *Proteobacteria* being dominant. Interestingly, the proportion of these two largest phylum presented opposite changes. A similar situation appeared in the genus level, that cellobiose utilizing bacteria *Bacteroidia* fluctuated with cellulose digesting organism *Clostridium* but have a contrast tendency to the protein decomposing one, *Gamma-Proteobacteria*. These fecal bacteria may be not as same as rumen microbiota that notably related to the ratio of grain in the diet but are still highly responsive to the feeding changes. In this study, we present a case to understand the characterization and transformed tendency of dairy cows fecal microbiome in the transition period.

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P51 GENSITEIN EXPOSURE INHIBITS GROWTH AND ALTERS HORMONE LEVELS THROUGH DYSREGULATION OF STEROIDOGENIC ENZYMES IN CULTURD MOUSE ANTRAL FOLLICLES

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Genistein is a naturally occurring isoflavone phytoestrogen commonly found in many plant products, such as soybeans, lentils, and chickpeas. Genistein, like other phytoestrogens, has the potential to mimic, or alter the estradiol biosynthesis pathway, thereby potentially altering ovarian follicle growth. Previous studies have inconsistently indicated that genistein exposure affects granulosa cell proliferation and hormone production, but no studies have examined the effects of genistein on intact antral follicles. Thus, this study was designed to test the hypothesis that genistein exposure inhibits follicle growth and steroidogenesis in mouse antral follicles. To test this hypothesis, antral follicles were mechanically isolated from adult CD-1 mice and cultured in supplemented α-minimum essential media treated with either a vehicle control (dimethylsulfoxide; DMSO) or genistein (6.0 and 36μM) for 96 hours (h). Individual follicle diameters were measured every 24 h as a measurement of growth. At the end of the culture period, media were collected and subjected to enzyme-linked immunosorbent assays for measurements of progesterone, androstenedione, testosterone, and estradiol levels. Additionally, the follicles were collected and subjected to quantitative real time polymerase chain reaction (qPCR) assays to measure the gene expression of key steroidogenic enzymes. The results indicate that genistein (36μM) significantly inhibits antral follicle growth beginning at 24h when compared to control (n=3, $p \leq 0.05$). Exposure to genistein (6.0 and 36μM) significantly decreases estradiol levels at 72h and 96h when compared to control (n=3, $p \leq 0.05$). In contrast, genistein (6.0 and 36μM) increases progesterone levels at 48h, 72h, and 96h (36μM only) when compared to control (n=3, $p \leq 0.05$). Genistein exposure (6.0 and 36μM) also increases testosterone levels at 72h when compared to control (n=3, $p \leq 0.05$). Androstenedione levels remained unaffected at every time point. The results also indicate that genistein exposure increases the expression of steroidogenic acute regulatory protein (*Star*) at 72h (DMSO: 1.01 $\pm$ 0.10, genistein 36μM: 3.57 $\pm$ 0.96 relative fold change) and 96h (DMSO: 1.23 $\pm$ 0.49, genistein 36μM: 8.46 $\pm$ 3.25 relative fold change) and increases expression of cytochrome P450 cholesterol side-chain cleavage (*Cyp11a1*) at 96h (DMSO: 1.06 $\pm$ 0.24, genistein 36μM: 2.74 $\pm$ 0.56 relative fold change) when compared to control (n=3, $p \leq 0.05$). Additionally, genistein decreases the expression of 3β-hydroxysteroid dehydrogenase (*Hsd3β1*) at 24h (DMSO: 1.02 $\pm$ 0.10, genistein 36μM: 0.42 $\pm$ 0.01 relative fold change) and the expression of cytochrome P450 17-α-hydroxylase 1 (*Cyp17a1*) at 24h (DMSO: 1.76 $\pm$ 0.65, genistein 36μM: 0.42 $\pm$ 0.11 relative fold change) and 96h (DMSO: 1.05 $\pm$ 0.20, genistein 36μM: 0.16 $\pm$ 0.04 relative fold change) when compared to control (n=3-5, $p \leq 0.05$). Genistein exposure did not significantly affect the expression of 17β-hydroxysteroid dehydrogenase 1 (*Hsd17β1*) or cytochrome P450 aromatase (aromatase (*Cyp19a1*). Collectively, these data indicate that genistein exposure inhibits antral follicle growth through dysregulation of the estradiol biosynthesis pathway.

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P53 EFFECTS OF PRENATAL DI-(2-ETHYLHEXYL) PHTHALATE EXPOSURE ON OVARIAN FOLLICLE NUMBERS IN MICE

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Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in medical tubing, building materials, car seats, and children's toys. Humans are exposed to DEHP on a daily basis by ingestion, inhalation, and dermal contact. It is known that prenatal exposure to DEHP can adversely affect the reproductive organs and functions of the male reproductive system, but limited information exists about the effects of prenatal DEHP exposure on female reproductive organs, especially the ovary. Thus, we tested the hypothesis that prenatal DEHP exposure affects the numbers and histologic appearance of ovarian follicles in the F1 generation of mice. To test this hypothesis, pregnant CD-1 mice (7-14 dams per treatment group) were orally dosed every day with tocopherol-stripped corn oil (vehicle control) or DEHP (20 µg/kg/day, 200 µg/kg/day, 200 mg/kg/day, 500 mg/kg/day, or 750 mg/kg/day) from gestation day 10.5 until the birth of pups. On postnatal days (PNDs) 8, 21 and 60, at least one female pup from each litter was euthanized, the ovaries were collected, and fixed in Dietrich's solution. The fixed ovaries were embedded in paraffin, sectioned at 8 µm, and stained with Weigert's hematoxylin and methyl blue. Every tenth ovarian section then was used to count the numbers of primordial, primary, pre-antral, and antral follicles. The results show that prenatal exposure to DEHP does not affect primordial, primary, pre-antral, or antral follicles in F1 ovaries collected at PND 8 or 60 (n=3-11 dams/treatment group, except n=2 for the 20 µg/kg/day group; $p \geq 0.05$). In contrast, DEHP (200 µg/kg/day and 500 mg/kg/day) exposure significantly increased the number of pre-antral follicles compared to controls on PND 21. Specifically, numbers of pre-antral follicles in control ovaries were 38.3±3.4, but were 63.4±11.0 in ovaries treated with 200 µg/kg/day DEHP and were 78.5±18.6 in ovaries treated with 500 mg/kg/day (n=3-11 dams/treatment group; $p \leq 0.05$). These data suggest that prenatal DEHP exposure may not affect follicle numbers in F1 ovaries at PND 8 or 60, but it may increase pre-antral follicle numbers at PND 21.

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P55 BISPHENOL A EXPOSURE DISRUPTS GERM CELL NEST BREAKDOWN IN CULTURED NEONATAL MOUSE OVARIES

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Bisphenol A (BPA) is a known endocrine disrupting chemical and reproductive toxicant in animal models. In females, studies suggest that BPA exposure during development has the potential to affect germ cell nest breakdown and follicle formation in the ovary. Previous studies indicate that low-dose *in utero* BPA exposure increases the number of germ cells in nests and decreases the number of primordial follicles compared to controls. However, the mechanism by which BPA affects germ cell nest breakdown is unknown. Thus, we tested the hypothesis that BPA inhibits germ cell nest breakdown by interfering with oxidative stress and apoptosis pathways. To measure oxidative stress, we focused on the expression of major antioxidant genes [superoxide dismutase 1 (*Sod1*), catalase (*Cat*), glutathione peroxidase (*Gpx*), and glutathione reductase (*Gsr*)], as well as the level of reactive oxygen species (ROS). To measure apoptosis, we focused on the expression of several key anti-apoptotic and pro-apoptotic genes [B cell leukemia/lymphoma 2 (*Bcl2*), Bcl2-like 1 (*Bclxl*), Bcl2-associated X protein (*Bax*), Bcl2-related ovarian killer protein (*Bok*), Bcl2-associated agonist of cell death (Bad), first apoptosis signal (*Fas*), and caspase 8 (*Casp8*)]. To test our hypothesis, ovaries from newborn mice [postnatal day (PND) 0] were collected and cultured with vehicle (dimethylsulfoxide, DMSO) or low doses of BPA (0.1, 1, 5, and 10 µg/mL) for 1, 2, 4, and 8 days. After culture, the ovaries were collected for histological evaluation of germ cell nest breakdown and for biochemical analyses of oxidative stress and apoptosis pathways. Our results indicate that on PND 4 (all doses) and PND 8 (1, 5, and 10 µg/mL), BPA significantly increased the percentage of germ cells remaining in nests and decreased the percentage of primordial follicles compared to control ($n=3\text{-}6$, $p\leq0.05$). On PND 8, BPA (1, 5, and 10 µg/mL) significantly decreased the percentage of primary follicles compared to control ($n=3$, $p\leq0.05$). Further, our results indicate that BPA treatment did not alter *Sod1* expression at any doses, but it did significantly increase *Cat*, *Gpx*, and *Gsr* expression levels on PND 1, 2, and 4 compared to control (5 and 10 µg/mL, $n=3\text{-}4$, $p\leq0.05$). Additionally, BPA treatment did not affect ROS levels compared to control on PND 2 and 4 ($n=3$, $p>0.05$), but BPA (5 µg/mL) significantly increased ROS levels at PND 8 ($n=3$, $p<0.05$). Our results from analyses of the apoptosis pathway indicate that BPA significantly increased expression of anti-apoptotic genes (*Bcl2, Bclxl*), and reduced expression of some pro-apoptotic genes (*Fas, Casp8*) ($n=3\text{-}4$, $p\leq0.05$). Collectively, these data suggest that low doses of BPA exposure significantly inhibit germ cell nest breakdown by inhibiting the expression of key ovarian apoptotic genes, but not by interfering with the oxidative stress pathway.

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P57 IN VITRO EXPOSURE OF DOXORUBICIN INHIBITS MOUSE MULTILAYER SECONDARY FOLLICLEGROWTH, SURVIVAL, HORMONE SECRETION, AND INDUCES FOLLICLE APOPTOSIS

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Advances in chemotherapy significantly improve the cancer survival rate, particularly in young cancer patients. However, the ovarian toxicity caused by chemotherapy is the main concern for female patients. It increases the risk of premature ovarian failure (POF) and leads to early menopause and infertility. Doxorubicin, one of the most efficacious anticancer drugs for many malignant tumors, has been shown to induce apoptosis in mouse germinal vesicle (GV) and metaphase II (MII) stages of oocyte. However, most of the studies were conducted by treating doxorubicin in the denuded oocytes instead of intact ovarian follicles, which is less representative to the physiological condition when female cancer patients receive chemotherapy. The objective of the present study was to investigate the adverse effect of *in vitro* exposure of doxorubicin to mouse multilayer secondary follicles. 150-180μm multilayer secondary follicles were isolated from day 16 CD-1 mice and encapsulated in 0.5% alginate. Follicles were exposed to doxorubicin at different concentrations (0, 2, 20, 40, 100 and 200 nM) or time durations (0, 0.5, 1, 4 and 24h), and cultured for up to 8 days followed by monitoring the follicle growth, survival, steroid hormone secretion, mRNA expression of follicle pro-apoptosis genes, and follicle apoptosis. In the control group, follicles developed from secondary to antral stage with follicle diameter increased from 152 ± 7 μm on day 0 to 300 ± 41μm on day 8, and the follicle survival rate was more than 80%. However, in the 100 and 200 nM doxorubicin treated follicles, the follicle size did not change over time, the estradiol secretion was significantly inhibited, and the follicle survival rates were significantly decreased with the oocytes extruded and dissociated from granulosa cells, indicating the toxicity of doxorubicin on mouse multilayer secondary follicles. When follicles were treated by doxorubicin at 200 nM for different time durations, the follicle survival rates were significantly decreased beginning at 1 h, and with 58%, 83% and 100% of follicles died on day 8 after 1h, 4h, and 24h doxorubicin treatment on day 0, respectively.At molecular level, the doxorubicin treatment for 4h significantly increasedthe mRNA expression of *Casp3*, *Bcl2l11*, *Foxo1* and *Foxo3*, and the number ofTUNEL-positive granulosa cells, suggesting the induced follicle death by doxorubicin is through the mechanism of granulosacell apoptosis. Our study demonstrates that the doxorubicin treatment at human exposure level inhibits mouse multilayer secondary follicle growth and hormone secretion, and induces follicle death,which is through the follicle granulosa cell apoptosis.

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P59 OXIDATIVE STRESS RESPONSE OF MCF-7 CANCER CELLS EXPOSED TO TRIAZINE CLASS HERBICIDES

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Triazine class herbicides are widely used in the United States for crop production. However, these same herbicides are commonly detected in drinking water and groundwater at high enough concentrations that they were banned from use by the European Union. Studies have indicated these compounds to be potential endocrine and reproductive disruptors, but have not addressed the oxidative state of treated cells. For our study, we examined the oxidative effects of two triazine herbicides, atrazine and simazine, on estrogen-dependent MCF-7 mammary epithelial carcinoma cells. Cells were treated with environmentally relevant concentrations of the two herbicides for 48 hours and the oxidation levels were assessed using four different bioluminescent assay techniques. At all concentrations of atrazine and simazine, no statistical differences were found in the levels of oxidized glutathione or total oxidized and reduced nicotinamide adenine dinucleotides phosphates. In stark contrast, the levels of hydrogen peroxide were found to be statistically different from the control at all concentrations of atrazine and simazine tested. Thus, exposure to triazines alters the amount of hydrogen peroxide produced, which in turn can greatly affect the stability of the cell milieu. Further analyses will be performed to elucidate the mechanisms by which this reactive oxygen species is increased.

P61 27-HYDROXYCHOELSTEROL INCREASES THE EXPRESSION OF INTERLEUKIN-6 IN MAMMARY CANCER ASSOCIATED MACROPHAGES IN A NOTCH DEPENDENT MANNER

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Breast cancer is currently the second leading cause of cancer death in women. The vast majority of breast cancer related mortality is due to the metastatic spread of the disease. Therefore, there continues to be a need for new therapies and/or lifestyle strategies aimed at preventing and treating metastatic disease. Importantly, there is a positive correlation between obesity and metastatic relapse, although the precise mechanisms behind this observation remain unclear. One potential contributor is elevated cholesterol, which is often a comorbidity to obesity. In this regard, elevated cholesterol levels are a risk factor for relapse, while patients taking cholesterol-lowering drugs (HMG-CoA reductase inhibitors, statins) experience a prolonged relapse free survival time. Previous work from our lab using murine models has shown that high cholesterol diets increase mammary cancer metastasis in a CYP27A1 dependent manner. That is, the metastatic effects of cholesterol are mediated by its metabolite, 27-hydroxycholesterol (27HC). Furthermore, the effects of 27HC were found to require the presence of the host myeloid cells. Metastasis and tumor growth are mediated by uniquely differentiated macrophages. Monocytes are capable of differentiating into mammary tissue macrophages (MTMs), which exist in breast tissue as part of the innate immune system, or into tumor associated macrophages (TAMs) which exhibit a M2-like polarization state. Recent studies have shown that TAMs require activated Notch signaling for their differentiation. Therefore, we hypothesize that the pro-metastatic effects of 27HC may be in part due to its influence on Notch signaling in TAMs. Using an *in vitro* model system, we differentiated the murine monocytic RAW 264.7 cells into unpolarized (M0), M1 and M2 polarized macrophages. When screening cytokines that have been previously implicated in metastasis, we found that 27HC significantly increases the expression of Interleukin-6 (IL6) in undifferentiated, M0 and M2 macrophages. Furthermore, the 27HC induction of IL6 was attenuated when cells were cotreated with the γ-secretase inhibitor RO4929097. Therefore, 27HC increases IL6 expression in a Notch dependent manner. Interestingly, when probing mechanisms by which this may occur, we made the observation that 27HC also increases the expression of the Notch ligand, DLL1. Current work is aimed at testing the hypothesis that 27HC induces DLL1 which then activates Notch signaling, leading to a subsequent increase in IL6 expression. We are also confirming our results in *in vivo* mammary tumor models. Since elevated IL6 and Notch signaling are both associated with poor prognosis among breast cancer patients, the synthesis of or downstream targets of 27HC may represent novel therapeutic options in the treatment of metastatic disease.

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P63 ASSESSMENT OF NANOPARTICLE SAFETY FOR USE IN BREAST CANCER TREATMENTS

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From detection to treatment of diseases, synthesized nanoparticle composites are a recent research hotspot. This study focused on nanoparticle-based anti-cancer composites, where a chemotherapeutic is encapsulated either in a transition metal nanoshell or within a liposome tethered to metal nanospheres, and is injected at a tumor site. The composite can then be opened with UV light to disperse the toxins in a localized manner, minimizing the undesirable side effects of drugs in other tissues. One main issue surrounding the application and introduction of this technique into the field is if the composites are safe to use. The focus of our study was to examine mutagenic effects of two synthesized gold nanoparticles, HPN1 and HGN2. HPN1 are 40 nm diameter colloidal gold nanoparticles, which reflect a purple hue, while HGN2 are 10 nm diameter and reflect a yellow-orange color.

In order to assess any toxicity with these nanoparticles, MTT cell viability assays were performed in two breast cancer cell lines (MDA-MB-231 and MCF-7). Cells were exposed to varying amounts of relatively fresh nanoparticles for one week instead of the traditional forty-eight hours, thus ensuring that any changes in cell viability were not missed due to shorter treatment times. We observed a cell type-specific effect where viability was unaffected in the MDA-MB-231 cell line, yet dramatically increased in MCF-7 cells.

Several months after synthesis, similar assays were run and the nanoparticles begin to alter cell viability in MCF-7 breast cancer cells. This indicates that changes in the nanoparticles may have occurred during the lag period between experimentation. This result highlights the need for multiple model systems, as well as the effect of time upon potential results. Further, our study indicates that we must be cautious moving forward in the development of new chemotherapeutic techniques, as initial results may not tell the full story.

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P65 THE EFFECTS OF OBESITY IN FEMALE OSSABAW MINI PIGS ON THE MICROBIOME OF THE UROGENITAL TRACT

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Two thirds of Americans are overweight or obese, resulting in a significant financial burden on the healthcare system in the United States. Obesity negatively impacts both the immune and reproductive systems, resulting in increased systemic inflammation and abnormal ovarian function. Utilizing next generation sequencing technology, research has shown that the dynamic of the microbial community in mucosal tissues correlates with different states of physiological "health". The goal of our research program is to examine how triggers of inflammation – obesity as an example – alter reproductive function as assessed by investigations of the urogenital microbiome. Specifically, we hypothesize that obesity negatively impacts the immune protection of the microbial community profile within the urogenital tract and alters serum progesterone and estradiol concentrations throughout the estrous cycle. To test this hypothesis we utilized the Ossabaw pig as an animal model of a "thrifty" metabolic phenotype. Ossabaw pigs have a loss of function mutation in the Val199→IIe region of the PRKAG3 gene (the γ3 isoform of AMP-activated protein kinase) that is associated with accumulation of increased intramuscular fat. Five nulliparous, sexually mature Ossabaw pigs were fed an excess calorie, high fat/cholesterol/fructose diet (n=3) or a control diet (n=2) for seven months. After a six cycle (~ three-month) diet induction period, pigs remained on their respective diets for an additional four months and had collection of vaginal swabs, cervical flushings, ovarian ultrasound, and paired serum samples on cycle days 1 (estrus), 4, 8, 12, 16, 18, 20 and 22 for two estrous cycles. Vaginal swabs and cervical flushings were paired with serum samples to measure microbial changes within the urogenital tract in relationship to ovarian hormone profiles. Ovarian ultrasound was used to help assess ovarian function. Sample days 4, 8, and 12 corresponded with the luteal phase and sampling days 1, 16, 18, 20, and 22 corresponded with the follicular phase of the estrous cycle. Bacterial phylotype profiles were defined from the urogenital tract (vaginal swabs and cervical flushings) from control and obese animals. Briefly, microbial community structures were generated using deep rDNA sequencing of the hyper-variable V3-V5 region of the 16S ribosomal RNA (rRNA) gene and then sequenced using the MiSeq platform. We were able to isolate bacterial DNA in all samples collected longitudinally from all animals. Bacterial DNA has been sent for sequencing, and clustering of sequence data into specific operational taxonomic units (OTUs) will determine ecological dynamics through the inference of taxonomy utilizing Qiime, Mothur and LefSeq software programs. With these data, we will identify urogenital microbiome profiles induced by obesity as well as the influence of cycling ovarian hormones on urogenital microbial dynamics. The data from this pilot study may indicate important pieces of information about previously unrecognized bacterial species, genera, families, or phyla that may cause or exacerbate reproductive and immune disorders in obesity or may, in fact, maintain "health" in an obese environment.

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P67 AN ANIMAL MODEL OF SHIFT WORK: THE INTERACTION OF SEX, SCHEDULED FOOD< AND SUSTAINED ATTENTION ON DAILY ACTIVITY RHYTHMS

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Circadian rhythms are critical for homeostasis and health as they regulate numerous biological processes. These endogenously generated rhythms are regulated by daily signals; the most salient are the light:dark cycle and timed meals. Disruptions to biological rhythms as occurs with shift work results in significant health consequences including an increased risk for cardiovascular, metabolic and reproductive dysfunction. Relatively few studies however have determined the impact of "shift-work" on attention processes. Furthermore, almost no research has examined the impact of disrupted rhythms on males compared to females. Here, we tested male and female rats on a well-established behavioral measure of attention, the 5-choice serial reaction time task (5CSRTT, n= 6-8/group). Task difficulty was modified by altering cue light duration (0.1, 0.5 and 1 sec) and the delay between cues (0, 2, 4 sec). Animals were food restricted so they were motivated to complete the task to receive sucrose pellets. Experimental rats (EXP) were tested during the light or dark (4 hrs after lights-on or off, respectively). Controls (CON) were moved to the testing room and only received sucrose rewards. We analyzed activity patterns and task performance. Within all "day" rats, we observed two phenotypes; a predominantly nocturnal rat that was also active at the time of the task/food pellets, and 2) a predominantly diurnal rat that began daily activity at lights-on and remained active until lights-off. As expected, in CON rats, scheduled food rewards resulted in animals becoming diurnal (42% of females and 71% of males). When animals had food rewards coupled with the attention task (EXP), there was an increase in the number of diurnal rats (66% of females and 83% of males) thus suggesting attention tasks can also alter daily activity rhythms. Furthermore, entraining cues may alter activity rhythmicity in a sex specific manner. Within day-tested EXP rats, we found diurnal rats made significantly less incorrect choices than those that remained nocturnal, indicating that cognitive function is improved if the animals align their rhythms to the time of the attention task. This performance difference, however, was only detected at the easiest task condition of 1 sec cue duration and 0 sec cue delay. Finally, regardless of activity phenotype, day-tested females made fewer incorrect responses than day-tested males indicating there a sex difference in the impact of time of day on attention. These data are among the first to use a 5CSRTT as a model for shift work and will enable us to identify the mechanisms by which vigilance can modulate biological rhythms.

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P69 SEXUAL DIMORPHIC DEVELOPMENTAL PATTERNS OF THE EXTERNAL GENITALIA IN GUINEA PIG

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Sexual dimorphism of external genitalia is a major phenotypic difference in human. It has been reported that abnormal external genital features, such as ambiguous and micropenis, may occur during the process of sex differentiation. Unlike mice and rats, the morphology of adult external genitalia in guinea pigs is similar to that in human. To understand developmental differentiation of external genitalia in guinea pigs, we examined male and female external genitalia in Harley guinea pigs on embryonic days (E) 20, E26, E28, E30, E35, E40, E45. Scanning electronic microscopy indicated that the early sign of sex differentiation was starting at E28. We observed that the regions of male glans protruded distally more than female glans, whereas the lateral sides of the female tubercle extended horizontally. Between E28 and E30, the male tubercle showed significantly changed morphology. The distal region of male glans grew forward to the ventral side, and the two original lateral swellings form the lateral walls surrounding the medial urethral seam. The whole male genital tubercle adopted a "head-lower" position at E30. By E35, the lateral walls of E30 male tubercle fused together gradually from the proximal to distal region, which resulted in the formation of a distal urethra. The preputial walls of male tubercle finally fused together, and form a completed urethra during late stages of development (E40-E45). The female tubercle, on the other hand, did not change between E28 and E35. It showed a further extension to widen the entire genital tubercle, and formed a platelet structure by E35. This special female plate then folded forward and fusion to form clitoris and urethra (E40-E45). On E45, morphology of both male and female external genitalia was similar to that of neonate. Compared to mice, which showed no difference between male and female during embryonic stage, the sex differentiation of guinea pig initiated during prenatal development like human. Immunohistochemical analysis showed that abundant androgen receptor was observed around the urethra and preputial epithelium in male, and reduced estrogen receptor alpha was detected in the female tubercle. Our findings suggest guinea pig could be a good model to study human disorders of sex differentiation. Future research will attempt to identify sexual dimorphism of neural development in the brain of guinea pig.

ABSTRACTS FOR POSTER SESSION B (EVEN NUMBERED POSTERS)

P14 CROSS REGULATION BETWEEN THE ACTIVIN AND NOTCH SIGNALING PATHWAYS IN MOUSE GRANULOSA CELLS

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Multiple signals and signaling mechanisms influence the structure and function of the ovarian follicle, thus impacting female fertility and reproductive health. The process of follicle assembly establishes important cell-cell interactions that allow for juxtacrine and paracrine signaling that potentiates folliculogenesis and hormone secretion during the reproductive lifespan. Activin and Notch are evolutionarily conserved signaling pathways that are important for regulating developmental cellular processes in many tissues. In the ovary, Activin and Notch are necessary for regulating follicle formation and somatic cell proliferation, and disruption of either pathway leads to ovarian pathologies. Recent micro-array and quantitative PCR (qPCR) analysis of cultured mouse granulosa cells have shown that select Notch signaling genes are Activin responsive. These observations lead us to postulate that Activin and Notch signaling have coordinated actions in promoting ovarian follicle formation and growth that may, in part, be the result of cross-regulatory interactions between these pathways at the transcriptional level. Suppression of Activin signaling in cultured granulosa cells by the antagonist Follistatin or in the ovary by overexpression of the antagonist Inhibin results in a suppression of several Notch receptors, ligands and target genes. By contrast, Activin treatment of cultured granulosa cells results in a rapid increase in Notch gene expression (within 4 hours), suggesting that Activin's actions may be exerted at the level of Notch gene transcription. Interestingly, the most robust increases in gene expression were observed with the Notch target and effector genes, *Hey2* and *Heyl*. When granulosa cells were co-cultured with Activin and the transcriptional inhibitor Actinomycin D, Activin's stimulatory effect on *Hey2* and *Heyl* was abolished. Complementary experiments using RNA isolated from ovaries with attenuated Notch signaling (*Notch2* and *Jag1* knockout mice) have suppressed expression of the *Inhβ*A and *Inhβ*B genes that form the isoforms of Activin, as well as *Acvr1b* the gene for the Activin type 1 receptor. These data further support a reciprocal cross-regulatory relationship between these two pathways. Functionally, Activin and Notch have important roles in promoting granulosa cell proliferation. In cultured granulosa cells, treatment with the Notch inhibitor, DAPT, results in a decrease in granulosa cell proliferation as measured by EdU incorporation and FACs analysis. Importantly, this decrease in proliferation is rescued when DAPT-treated cells are treated with Activin, indicating that Activin's stimulatory effect can compensate for Notch suppression. Ongoing studies are exploring whether this effect is a consequence of the ability of Activin to enhance the transcription of Notch effector genes.

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P16 OOCYTE SURVIVAL AND FOLLICLE MATURATION REQUIRE *IRX3* AND *IRX5* TO PROMOTE COMMUNICATION BETWEEN SOMATIC AND GERM CELLS IN THE MOUSE OVARY

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Follicle development and maturation within the ovary depend on intimate communications between the germ cell and its surrounding somatic cells. Our previous results using the Fused Toes (*Ft*) mutant mouse model showed disrupted oocyte – granulosa cell contacts leading to oocyte and follicle death. Among the genes deleted in the *Ft* locus, only *Irx3* and *Irx5* (*Irx3/5*) exhibited ovary specific expression upon comparison of male versus female transcripts during gonad development. Therefore, we hypothesized that *Irx3/5* were critical for coordinating germ cell – somatic cell communications underlying oocyte and follicle survival. The current study characterizes the location of *Irx3/5* transcripts and proteins over time and determines their functions within the developing ovary. Real-time qPCR results showed that *Irx3/5* had similar expression patterns during ovary development as their transcripts increased during germline nest formation and peaked around birth when nests broke down to form primordial follicles. Shortly thereafter, their expression diminished. Immunohistochemistry analyses on ovaries at embryonic days (E) 13.5, 15.5 and postnatal days (P) 0 and 2 showed that IRX3 and IRX5 were co-localized to somatic cells during development and then were detected in both germ and somatic cells around birth. Their expression decreased first in somatic cells of established primordial follicles, but was maintained for a few more days in germ cells. Next, we characterized Irx3/5 double knockout, $Irx3^{\Delta}Irx5^{G}/Irx3^{\Delta}Irx5^{G}$ (*Irx3/5* DKO), ovaries. This mutation is embryonic lethal at E13.5; therefore, we used kidney capsule transplantation (KCT) of ovaries to analyze time points equivalent to P0, 3, 7 and 14. Histology and transmission electron micrograph of KCT ovary grafts showed that *Irx3/5* DKO follicles developed abnormal granulosa cell morphology, gaps between germ and somatic cells, and oocyte death similar to that seen in the *Ft* mutant model at P7 and 14. Next, we generated a somatic cell specific double knockout mouse model using *Sf1*Cre, $Sf1\text{Cre}^{+/\Delta}$; $Irx3^{f}Irx5^{G}/Irx3^{\Delta}Irx5^{G}$ (*Irx3/5* sFΔ), to evaluate the role of *Irx3/5* in somatic cells in the developing ovary. Histological analysis of adult *Irx3/5* sFΔ mutant ovaries displayed an overall smaller size with more zona pellucida remnants and rare corpora lutea. Because the *Irx3/5* sFΔ mice were small and too weak to perform breeding studies, we examined fertility using superovulation followed by *in vitro* fertilization. Our current results indicates that *Irx3/5* sFΔ mutant females ovulates fewer oocytes, have a higher incidence of egg fragmentation, and fewer 2-cell embryos (3; 66.67%; 33.33%; n = 1) compared to no Cre (12; 14.68%; 41%; n = 4) and wild-type controls (21; 6.09%; 68.86%; n = 4). To investigate female fertility through natural breeding, we generated another somatic cell specific double knockout model, $Sf1\text{Cre}^{+/\Delta}$; $Irx3^{f}Irx5^{G}/Irx3^{f}Irx5^{G}$ (*Irx3/5* sFF). *Irx3/5* sFF mutant mice are robust, and preliminary breeding study results indicate that mutant females reproduce, but are subfertile. Together, our results indicate that *Irx3/5* work together during follicle development in the ovary to promote effective communication between the oocyte and nascent granulosa cells to ensure oocyte survival and proper follicle maturation. These functions may depend on *Irx3/5* expression specific to ovarian somatic cells.

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P18 OVARY-MIMETIC 3D PRINTED FOLLICLE NICHES (3DP-FNS) SUPPORT SURVIVAL, HORMONE PRODUCTION AND OOCYTE MATURATION

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Iatrogenic cancer treatments, such as radiation and chemotherapies, can impair fertility and endocrine function. The field of oncofertility creates options for preserving and restoring fertility in patients at risk of iatrogenic ovarian failure. Autotransplantation of cryopreserved ovarian cortical tissue has resulted in human live births, with short-term restoration of endocrine function. However, this technique carries the risk of reintroducing cancer cells since the tissue is removed prior to treatment. To minimize cancer transplant risk we bioengineered an artificial ovary consisting of follicles seeded within a 3D printed hydrogel scaffold modeled from the extracellular matrix (ECM) composition and architecture of decellularized bovine and human ovaries. The scaffolds were printed with gelatin in an intricate microporous pattern, engineered to accommodate multi-layered secondary ovarian follicles. Scaffolds having 'tortuous' architectures and porosities were investigated, including those printed with 30, 60 and 90 degree advancing angles. Ovarian follicles were supported in the artificial scaffold setting, as measured by survival, and were functional, based on measurement of secreted estradiol over 8 days and their ability to respond to luteinizing hormone (LH). The follicles settled within 3D printed follicle niche (3DP-FN) created by the overlaid struts and somatic cells along the follicle periphery made contacts with the struts through cell adhesion molecules, such as vinculin. An essential feature of an ideal 3DP-FN was the number of engineered contacts; scaffolds that allowed for 2 or more contacts of the follicle within the niche out performed configurations that supported only 1 contact (81±1.7% versus 31±15.6% survival, n=113 follicles). Follicles within a tortuous 3DP-FN also released a meiotically competent egg in response to LH. Characterizing the ideal features of an engineered ovarian follicle niche is the first step in creating a safer artificial ovary that will provide both endocrine support and fertility to young women who have survived cancer.

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P20 THE ROLE OF THE OVARY IN HIGH GRADE SEROIUS CANCER ORIGINATING IN THE FALLOPIAN TUBE EPITHELIUM

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High-grade serous ovarian cancer (HGSOC; the most common and lethal histotype) can originate from the Fallopian tube epithelium (FTE, oviductal epithelium in other species). Mice expressing *Brca*^{Mut}/*Tp53*^{Mut}/*Pten*$^{-/-}$ in the oviductal epithelium develop tumors that migrate to the ovary and then throughout the peritoneum, and ovariectomy reduces peritoneal metastasis of these tumors. In this study, we sought to identify ovarian factors that stimulate migration of murine oviductal epithelial (MOE) cells. Ovary conditioned media stimulated migration of MOE cells 600% over control, but media conditioned with oviducts only increased migration 60%. HGSOC is characterized by mutations in the tumor suppressor protein p53 and loss of PTEN expression. Introduction of mutant p53 (R273H or R248W) reduced the pro-migratory effect of ovarian conditioned media, while PTEN^{shRNA} did not alter the effect. Size exclusion chromatography indicated the active component(s) of ovarian conditioned media were > 3 kDa, and the pro-migratory effect was heat-liable, indicative of a protein. We analyzed 8 proteins produced by the ovary and likely to stimulate migration (MCP1, IP10, SDF1, TIMP1, KC, MCSF, activin A, and TGFβ). The six cytokines did not have any effect. In contrast, activin A, but not TGFβ, increased migration in a dose dependent manner. Next, the role of small G-proteins (RhoA, Rac1, and Cdc4) in mediating activin's migratory effect was tested. Neither rhosin nor MLS573151 (RhoA and Cdc42 inhibitor, respectively) altered activin's migratory effect. In contrast, the Rac1 inhibitor, NSC23766, completely abrogated activin's actions. Confirming activin's actions in HGSOC, activin also simulated migration in OVCAR3 cells and the effect was blocked by MLS573151. In OVCAR4 cells activin also stimulated migration, but only with the highest concentration tested (40 ng/ml). Interestingly, OVCAR4 cells expressed INHBA mRNA, indicating OVCAR4 cells may produce activin. In support of this, SB431542, an ALK4/5/7 inhibitor, inhibited migration. Further size exclusion chromatography of conditioned media indicated an active component in the 50-100 kDa range (i.e. larger than the previously analyzed proteins). Interestingly, sub-fractionation of the 50-100 kDa fraction with fast protein liquid chromatography produced 7 fractions, none of which had a significant effect on migration. However, when the sub-fractions were recombined, the migratory effect was still present, suggesting that the migratory effect of the 50-100 fraction was due to multiple proteins. Future work is focused on elucidation the pathway by which activin stimulates migration of MOE cells and identifying the proteins responsible for the migratory effect in the 50-100 kDa fraction. Identification of the proteins that stimulate migration of the Fallopian tube cells to the ovary represent potential targets for blocking ovarian colonization and metastasis of ovarian cancers.

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P22 OOCYTE-DRIVEN GRANULOSA CELL TUMORIGENESIS IN MOUSE OVARY

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Cell-cell interactions play crucial roles in the maintenance of tissue homeostasis, a loss of which often leads to a number of diseases, including cancer. Here, we report that uncontrolled phosphoinositide 3-kinase (PI3K) activity within oocytes irreversibly transforms granulosa cells (GCs) through perturbed local cell communication and causes GC tumors (GCTs). Previously, we reported reproductive phenotypes of transgenic mice in which expression of constitutively active mutant PI3K (PIK3CA*) was induced in primordial oocytes by Gdf9-iCre. The transgenic mice (Cre+) demonstrated severe ovarian phenotypes, including an excess number of follicles and follicle overgrowth. Surprisingly, the Cre+ female mice developed progressive cancer-related cachexia wasting syndrome by the age of PD80 due to bilateral ovarian tumors. Pathological analysis identified the ovarian malignancy as GCTs. While local interaction with PK3CA*-positive oocytes during folliculogenesis was essential for the transformation of GCs, the growth of transformed GCs was independent of oocytes. Thus, tumor fragments free of oocytes grew rapidly and invaded the host kidney in subrenal transplants. Based on prior studies showing that ACTIVIN drives symptoms associated with cachexia, we suspected that these tumors produce high levels of this cytokine. Indeed, the tumors secreted activin A and the downstream effector SMAD3 was phosphorylated and located in the nucleus of the tumor cells. However, the growth rate of GCT transplants was attenuated in the presence of normal ovaries. We suspect that gonadal derived INHIBINs and follistatin blocked and/or bioneutralized the effect of ACTIVIN. It has long been known that active communication between the oocyte and surrounding somatic cells is necessary to normal development; for the first time, this study shows that oocyte derived factors can also impose a somatic cell transformation signal. Therefore, GCs can be irreversibly programmed by local extrinsic factors to grow independently of oocytic and endocrine factors. These studies raise important new opportunities to understand a potential mechanism of tumor initiation and ways to intervene in the disease.

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P24 A METABOLITE OF CHOLESTEROL IS IMPLICATED IN THE PATHOPHYSIOLOGY OF OVARIAN CANCER

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Ovarian cancer remains one of the leading causes of cancer deaths among women and causes more than 140,000 deaths annually worldwide. The current standard-of-care for ovarian cancer treatment includes de-bulking surgery, followed by chemotherapy. Unfortunately, 50%-75% patients relapse within 18 month of treatment. Therefore, there is an urgent need for new therapeutic or lifestyle strategies to prolong progression free survival. It is significant therefore, that recent epidemiological studies have shown that elevated circulating LDL-cholesterol is associated with poor prognosis while statin therapy correlates with increased progression free survival, strongly implicating cholesterol in the pathophysiology of ovarian cancer. However, the precise mechanisms by which cholesterol influences ovarian cancer remain unknown. Adopting a bioinformatics approach to screen genes involved in cholesterol metabolism, we found that increased expression of CYP27A1, the enzyme that synthesizes 27-hydroxycholesterol (27HC) from cholesterol, is associated with poor prognosis, while CYP7B1, the enzyme responsible for the catabolism of 27HC, is associated with increased progression-free survival. Based on this clinical evidence, coupled with previous reports demonstrating that 27HC can serve as a ligand for the estrogen receptors (ERs) and liver X receptors (LXRs), we hypothesized that 27HC mediates the impact of cholesterol on ovarian cancer. Surprisingly, we have found that *in vitro* 27HC treatment actually decreases the proliferation of ER-negative ovarian cancer cell lines and had very little effect on ER-positive lines. Subsequent studies have revealed that this antiproliferative effect is due to the activation of the LXRs by 27HC resulting in cholesterol efflux and thus decreased proliferation. We are currently testing the hypothesis that 27HC may alter the tumor microenvironment in such a way as to decrease progression free survival. Strikingly, our preliminary data indicate that 27HC significantly enhances the infiltration of CD11b+MHCIIly6c+ monocytic myeloid derived suppressor-like cells (Mo-MDSCs), which are known to be protumorigenic by suppression of cytotoxic CD8+ T cells. Future studies will characterize how 27HC influences tumor associated immune cells as well as determine whether the synthesis of 27HC might be a novel therapeutic target for the treatment of ovarian cancer. These translational studies are very important given the clear associations between cholesterol and patient outcome, coupled with the knowledge that 60%-80% of women between the ages of 55 and 74 are hypercholesterolemic.

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P26 IDENTIFICATION OF THE SIGNALING PATHWAYS DOWNSTREAM OF PTEN LOSS IN FALLOPIAN TUBES REQUIRED FOR THE GENESIS OF HIGH-GRADE OVARIAN CANCER.

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High-grade serous ovarian carcinoma (HGSOC) is the most common histotype of ovarian cancer with the highest mortality rate. The lethality of these tumors is due mainly to the difficulty in detecting the disease at early stages. Investigations on hereditary ovarian cancers characterized by alteration of BRCA 1/2 genes (also known as breast cancer type 1 susceptibility genes), failed to detect early lesions in the ovary; conversely, occult lesions were found in the fallopian tube suggesting that HGSOC may originate from the fallopian tube epithelium (FTE). Early lesions have mutations in the tumor suppressor p53, considered to be one of the earliest genetic alterations present in 100% of HGSOC. However, mutation in the p53 gene alone is not sufficient to generate malignant transformation in cell models. Recent studies revealed that loss of PTEN (phosphatase and tensin homolog) is also a common alteration in STIC lesions.

Transgenic animal models of FTE-derived HGSOC have pointed out the critical role of loss of PTEN in generating HGSOC when in combination with p53 mutation and alteration of BRCA1/2, but not by itself. Our lab has shown for the first time that deletion of PTEN alone in FTE is sufficient to lead to tumor formation and peritoneal dissemination suggesting that the importance of PTEN in the early events of the tumorigenic cascade may have been underestimated.

The understanding of the sequence of the molecular alterations behind the genesis of HGSOC from FTE remains poorly defined and pivotal to early detection of HGSOC. Herein, we generated a novel transgenic model of PTEN loss in fallopian tube that presents tumors with p53 signature and ovarian carcinoma. This model may help uncover the genetic and molecular alterations behind the genesis of HGSOC. Our data reveal that loss of PTEN in FTE leads to increased mRNA levels of signaling components of the Notch and Wnt pathway. Blocking this pathway may provide new ways to treat HGSOC and loss of PTEN may help stratify patient populations for these new therapies.

P28 DIAGNOSIS AND EFFECTS OF URINE CONTAMINATION IN COOLED EXTENDED STALLION SEMEN

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Urospermia is known to affect semen quality in many mammals, including stallions. Determinations of semen pH and creatinine and urea concentrations have been used to diagnose urine contamination in raw stallion semen. Unfortunately, practitioners suspecting urine contamination in cooled-shipped samples have no proven means to confirm the presence of urine. Therefore, the objectives of this study were: (i) to assess the effects of urine contamination on sperm motility of extended fresh and cooled-stored stallion semen, (ii) to evaluate the usefulness of semen color, odor, pH, creatinine and urea concentrations for urospermia diagnosis, and (iii) to evaluate the accuracy of a commercial blood urea nitrogen test strip in diagnosing urine contamination in extended-cooled stallion semen. Thirty-seven ejaculates were obtained from eleven stallions with no history of urospermia. Initial total and progressive motility was assessed using Computer-Assisted Sperm Analysis, and concentration measured with a spectrophotometer. The ejaculates were divided into 5 ml aliquots, and 0, 0.25, 1, 1.5, or 5 mLs of stallion urine was added. Each resulting sample was assessed for color, odor, pH, creatinine, and urea nitrogen concentration using both a semi-quantitative test strip (Azostix®), and a quantitative automated analyzer. The samples were extended with INRA96 to 25 million sperm/mL, packaged, and stored in an Equitainer I. At 24 hours motility, color, odor, pH, urea, creatinine, and Azostix® were assessed for all samples. Sperm motility parameters, pH, creatinine and urea concentrations were analyzed using mixed models. Urine contamination decreased motility in all samples pre and post cooling ($p<0.05$). Odor assessment presented moderate sensitivity (65%) and high specificity (100%), while color assessment presented low sensitivity (47%) and moderate specificity (79%) for urine in extended semen. Urine contamination was associated with increased pH in fresh samples, but this difference was not noted upon cooling storage. Azostix® strips were highly sensitive (95%) and specific (97%). Assessment of color, odor, and pH are not reliable methods to diagnose urine in experimentally contaminated in cooled-stored stallion semen. The results of the present study confirmed that urea and creatinine can be used to diagnosis urospermia, and that Azostix® can be used as a point care method for diagnosing urine contamination in extended cooled stallion semen.

P30 A COMPARISON OF DIFFERENT EXTENDERS FOR CRYOPRESERVATION OF SEMEN IN WHITE-TAILED DEER.

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Deer farming is an economically important and continuously growing industry in the United States, with its sustainability relying on the use of artificial insemination with frozen semen to effectively disseminate valuable genetics. While anecdotally, egg yolk-based semen extenders have been used with some success in white-tailed deer, there is currently no data comparing its use to soybean-based (AM, AndroMed®) or phospholipid-based (OP, OptiXcellTM) extenders. The objective of the current study was to compare the use of AM and OP extenders to different egg yolk-based extenders (OR, Ovine Red®; TR, Triladyl®; BI4, Biladyl® 4%; BI6, Biladyl® 6%; BI8, Biladyl® 8%). Our hypothesis was that semen extended in egg yolk-based extenders would exhibit a greater decline in sperm motility than semen extended in AM or OP extenders. In each experiment (EXP1, EXP2), white-tailed deer (n = 6 and 8, respectively) were anesthetized with tiletamine-zolazepam (0.4 mg/lb) and xylazine (1 mg/lb) IM via dart gun. Semen was collected using electroejaculation. The ejaculate from each buck was divided amongst six extenders for EXP1: AM, OR, TR, BI4, BI6, BI8; and three extenders for EXP2: AM, OR, OP. Semen was diluted to a concentration of 120 million sperm/mL, cooled to 5 °C, and incubated for 2 to 4 h. Semen was loaded into 0.5 mL straws and frozen manually by placing straws on a rack in liquid nitrogen vapor at a distance of 4 cm horizontally above the liquid nitrogen level for 10 m before submerging them into the liquid nitrogen for final freezing and storage. Each sample was thawed in a 37 °C water bath for 30 s for post-thaw analysis. Overall and progressive sperm motilities were assessed using computer-automated semen analysis before and after freezing, and percent motility decline was calculated for each parameter. Data were analyzed using a General Linear Models procedure for all analyses of variance in SAS with a Tukey-Kramer test for post-hoc analysis. Significance was declared at $P < 0.05$, with tendencies described at $P = 0.05 - 0.09$. All data is presented as mean ± SEM. In EXP1, percent decline in overall sperm motility in AM (49 ± 7.9%) and OR (50 ± 3.8%) extenders was less than BI4 (71 ± 3.6%; $P < 0.05$) and tended to be less than BI6 (69 ± 3.2%; $P \leq 0.09$). Percent decline in overall sperm motility did not differ between AM, OR, TR (51 ± 9.2%), or BI8 (62 ± 3.7%; $P \geq 0.42$), nor did it differ between TR, BI8, BI6, or BI4 ($P \geq 0.51$). Percent decline in progressive sperm motility for AM (51 ± 8.3%) was less than BI4 (90 ± 1.8%), BI6 (85 ± 2.6%), BI8 (82 ± 3.6%), and TR (69 ± 9.9%; $P < 0.05$). Percent decline in progressive sperm motility for OR (67 ± 2.4%) also differed from that of BI4 ($P = 0.02$), but did not differ from AM ($P = 0.14$) or any of the other extenders ($P \geq 0.10$). In EXP2, percent decline in both overall and progressive sperm motility did not differ between AM, OR, or OP extenders ($P \geq 0.2$). The use of non-egg yolk-based semen extenders (AM and OP) exhibited improved or comparable protection against decline in sperm motility after cryopreservation when compared to egg yolk-based extenders. These extenders are acceptable substitutes to the traditional egg yolk-based extenders used for semen cryopreservation in white-tailed deer.

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P32 EFFECT OF SORTING BOAR SPERMATOZOA BY SEX CHROMOSOMES ON OVIDUCT CELL BINDING

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The following study examined how flow cytometrically sorted sperm bind to oviduct cells and purified oviduct glycans. Previous data has shown that there are two oviduct glycan motifs, bi-sialylated lactosamine (bi-SiaLN) and Lewis X trisaccharide (Le^X) that bind noncapacitated boar spermatozoa with high affinity and specificity. The sperm rich fraction from boars (n=5) was collected, sperm were stained with Hoechst 33342 and sorted in Wisconsin. Sperm were separated into either X or Y chromosome-bearing cells and placed into the following treatments: 1) sperm sorted for the X chromosome, 2) sorted for the Y, 3) an equal mixture of sorted X and Y, and 4) a control of non-sorted sperm from the same collection. Samples were then transported to Illinois and tested for oviduct cell binding within 12 h of sorting. Additionally we observed motility characteristics, acrosome status and glycan binding to three soluble fluoresceinated glycans bi-SiaLN, sulfated Le^X ($suLe^X$) and the control lactosamine disaccharide (LacNAc). The data showed that the number of sperm binding to oviduct cells was reduced by more than half in the three sorted samples compared to the control. When binding of fluoresceinated soluble glycans was investigated, the proportion of sperm that bound bi-SiaLN or $suLe^X$ averaged 81% whereas 42% of sperm bound LacNAc. The glycans bound to sperm in three patterns (Pattern A: glycan binding to the apical ridge and post-acrosomal area, Pattern B: post-acrosomal binding only and Pattern C: apical ridge binding only). For $suLe^X$ and bi-SiaLN glycans, pattern A was present on 38% of the sperm, pattern B on 29%, pattern C on 20% and no fluorescence was observed on 12% of sperm from each of the four samples. The percentage of sperm that were motile in the sorted samples was reduced on average by 15% from the unsorted control. However, Computer Assisted Semen Analysis did not detect other differences in motility parameters between the sorted and control samples. All samples maintained >97% acrosome integrity after the sorting process. In conclusion, sperm binding to the complex matrix around oviductal cell aggregates was reduced after sorting but binding to purified soluble fluoresceinated glycans was not different among sperm preparations, probably due to a requirement for higher affinity binding and motility to contact and bind intact oviduct cells. The reduction in sperm fertility observed following sorting may be due to reduced ability to bind the oviduct epithelium.

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P34 GRAINYHEAD LIKE 2, A NOVEL DOWNSTREAM TARGET OF THE NOTCH SIGNALING PATHWAY IN THE DEVELOPING MOUSE PITUITARY

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The pituitary gland controls crucial physiological functions such as growth, metabolism and reproduction. The vast functions of the pituitary are executed through the six distinct hormone- producing cell types, which are tightly regulated during development and adulthood. However, the signaling pathways that direct their cell number remain unclear. Previously we have demonstrated that the Notch signaling pathway is necessary to preserve the progenitor cell population and is important for terminal differentiation of the hormone producing cell linages. However, the mechanism by which Notch controls the fate of these pituitary cell populations is unknown. To address this question, a microarray analysis of *Notch2* conditional knockout (cKO) mice revealed a significant decrease in expression of the transcription factor Grainyhead Like 2 (GRHL2) compared to littermate controls. GRHL2 has been shown to be a regulator of cellular morphogenesis, cell adhesion and cellular proliferation and differentiation. Recently, studies have revealed that GRHL2 directly regulates expression of proliferative genes such as proliferating cell nuclear antigen (PCNA) and antigen KI-67 (Ki67). In addition, GRHL2 has been identified as a direct transcriptional regulator of cell-to-cell contact proteins including E-cadherin (CDH1).In our studies we profiled the expression of *Grhl2* mRNA and examined GRHL2 protein localization in the developing mouse pituitary. We show that *Grhl2* expression is most pronounced in the pituitary during the late embryonic and early postnatal time period and the protein appears to be present in progenitor cells. Notch signaling is necessary for full expression of *Grhl2* mRNA and GRHL2 protein, demonstrated in both *Notch2* cKO mice and mice dosed with a chemical Notch inhibitor. Our data also suggests that that loss of *Notch2* and subsequent reduction of GRHL2 in the postnatal pituitary results in decreased proliferation and mis-expression of cellular adhesion molecules. Additionally, we demonstrate that GRHL2 expression is increased in Ames Dwarf mice. These mice harbor a point mutation in the *Prop1* gene, resulting in an increase *Notch2* mRNA levels coincident with an increase in progenitor cell number at the expense of differentiated cells. These data further suggest Notch as a regulator of *Grhl2* expression. Taken together, our studies define *Grhl2* as a novel, Notch regulated gene in pituitary progenitors. In addition, our finding also indicate that GRHL2 may be a regulator of progenitor cell maintenance and proliferation in the postnatal pituitary.

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P36 PITUITARY PROGENITOR CULTURE AS A TOOL TO DEFINE THE MECHANISM OF NOTCH MEDIATED CELL-FATE CHOICE

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The pituitary gland controls many body systems including reproduction and growth using six distinct hormone-producing cell lineages. These lineages arise from non-hormone producing pituitary progenitor cells characterized by self-renewal and robust expression of SOX2 and SOX9, common markers of progenitor cells. Previous studies have shown the necessity of these progenitor cells in gland development and mobilization in response to hormonal challenges experienced through adulthood. However, the processes and signals involved in progenitor cell maintenance and differentiation remains unclear. Past findings indicate that Notch signaling influences progenitor cell-fate choice. *Notch2* conditional knockout mice showed a progressive decrease in progenitor cell number during postnatal pituitary development. The mice also showed a decrease in thyrotropes, somatotropes and lactotropes, however, there was an increase in corticotrope cell number. While these studies indicate Notch signaling is important in developing the correct number of progenitor cells and differentiated cells, the underlying mechanism by which Notch controls these cell-fate choices remains unclear. To further elucidate the role of Notch signaling in lineage specification in pituitary progenitor cells, we employed a colony forming unit assay. The assay isolated progenitor cells by allowing dissociated pituitary cells to grow and expand in culture under conditions that strictly selected for progenitor cells. The colony forming units were treated for 48 hours in culture with a chemical Notch inhibitor, DAPT. The treatment showed a decrease in mRNA levels of canonical Notch downstream targets and an increase in corticotrope cell number, reflecting the *in vivo* observations. Our study characterizes a novel tool that will be useful in gaining insight into the Notch-specific mechanisms involved in progenitor cell-fate choice. Furthermore, understanding these mechanisms will provide a framework for manipulating cellular processes to create new pituitary cells from progenitors in order to combat hormone deficiencies due to pituitary dysfunction.

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P38 GENE EXPRESSION CHANGES IN THE ARCUATE NUCLEUS OF LACTATING RATS

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Many important physiological changes occur during lactation to allow for nourishment of the offspring. Specific neuronal groups within the hypothalamus respond to both sensory inputs via suckling stimulus and changing levels of various hormones associated with lactation. The arcuate nucleus is a chief regulator of lactation influencing serum hormone levels and metabolic and reproductive changes. The goals are to evaluate lactation-associated changes in the transcriptome of the arcuate nucleus and examine the expression of selected genes influenced by suckling stimulus. On day 1 postpartum, all rats were overiectomized and half were deprived of pups. On day 7 of lactation, brains of suckled and pup-deprived dams were collected. RNA was isolated from micro punches containing the arcuate nucleus and subjected to RNA-seq analysis. Thirty-eight differentially expressed genes including neuropeptides, signaling molecules, receptors, ion channels and enzymes were identified between the suckled and pup-deprived groups. Selected genes were then evaluated in ovariectomized virgin, 24 hour pup-deprived lactating and suckled lactating rats by qRT-PCR. Relative to the ovariectomized virgin group, expression of tyrosine hydroxylase, kisspeptin, neurokinin B and growth hormone releasing hormone showed a significant decrease in suckled lactating rats. The expression of enkephalin, parathyroid hormone 2 receptor, insulin-like growth factor binding protein 3, membrane progesterone receptor β, suppressor of cytokine signaling 2 and cytokine-inducible SH2 domain-containing protein showed a significant increase in suckled lactating rats. When dams were deprived of pups for 24 hours, the expression of parathyroid hormone 2 receptor, insulin-like growth factor binding protein 3, membrane progesterone receptor β, suppressor of cytokine signaling 2 and cytokine-inducible SH2 domain-containing protein was significantly decreased as compared to suckled lactating rats. The expression of tyrosine hydroxylase was significantly increased as compared to suckled lactating rats. There was no difference in the expression of kisspeptin, neurokinin B, growth hormone releasing hormone and enkephalin between pup-deprived and suckled lactating rats. However, when dams were deprived of pups for 48 hours, the expression of enkephalin showed a significant decrease in pup-deprived compared to suckled lactating rats. Many genes identified in RNA-seq as differentially expressed in an ovariectomized lactating model also showed changes between ovariectomized virgin and intact lactating rats. Of the genes examined, it is interesting that the expression of neuropeptides were not altered after 24-hour pup deprivation, but the other genes which included an enzyme, cell signaling molecules and receptors were altered with pup removal. These data support the critical role of the arcuate nucleus in regulating hormone secretion, metabolism and fertility during lactation.

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P40 *EPH-EPHRINA*1 EXPRESSION, METHYLATION AND CLONING DURING PORCINE EMBRYO ATTACHMENT

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Introduction

In the establishment of pregnancy, embryo attachment is an important step because most of the early embryonic (pregnancy day 10-30) deaths take place during this period, and the embryonic death is the main cause of piglets loss. Therefore, it is urgent to study the molecular mechanism of porcine embryo attachment. *Eph-EphrinA*1 is the important pair of receptor-ligand of Eph-Ephrin (Erythropoietin-Producing Hepatocellular receptor and ligand) system, which have been evidenced to play an important role in cell migration and adhesion during embryonic development in human and murine. The objective of the study is to detect *Eph A1* methylation effect on its expression, clone *EphrinA*1 CDS sequence followed by transfected to porcine endometrial epithelial cells.

Experimental Details

In this study, the mRNA expression, protein expression and CpG island methylation of *EphA*1 was investigated in Suzhong, Meishan, Huai and Erhualian pigs using real-time quantitative PCR, western blot and bisulfate sequencing technology. Among these pigs, nine Meishan sows were killed on pregnancy day 13, 18 and 24 (n = 3/day). Reproductive tissues were collected, including ovary, oviduct, endometrial attachment site of uterine horn (attachment site), endometrial inter-attachment site of uterine horn (inter-attachment site), uterine body, cervix and embryo.

Results and Discussion

The results showed that *Eph-EphrinA*1 mRNA expression increased from 13 d to 18 d and decreased from 18 d to 24 d at endometrium attachment sites ($P < 0.05$), while *EphA*1 protein expression decreased from 13 d to 24 d, and expression of both genes in pregnant sows was significantly higher than that of non-pregnant sows ($P < 0.05$). There is a highest CpG island methylation level in *EphA*1 promoter region on 13 d, followed by 18 d and 24 d. *EphrinA*1 coding sequence contained five exons and encoded 205 amino acids, *EphrinA*1 protein was hydrophilic and instable, has a signal peptide and a conservative region and no transmembrane region. *EphrinA*1 was successfully transfected to porcine endometrial epithelial cells. These findings suggest that CpG island methylation in promoter region of *EphA*1 might affect its expression and further affect the regulation of swine embryo attachment. *Eph-EphrinA*1 might play an important role in porcine embryo implantation.

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P42 EFFECT OF STATINS ON ANGIOGENIC GENE EXPREESION IN PLACENTA AND ENDOTHELIUM

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Preeclampsia (PE) can be a serious pregnancy disorder characterized by new onset maternal hypertension and proteinuria after 20 weeks of gestation. PE complicates ~ 2-5% of pregnancies in the USA and is one of the major causes of maternal and fetal morbidity. Delivery is the only cure. The etiology of PE is not certain but insufficient trophoblast invasion early in gestation is thought to lead to hypoxic placenta and aberrant expression of angiogenic and anti-angiogenic molecules that contribute to the clinical manifestations of this disorder. Elevated expression of the antiangiogenic factor, soluble fms-like tyrosine kinase-1 receptor (sFlt-1), becomes evident weeks before clinical onset of preeclampsia. Two sFlt-1 variants (sFlt-1i13 and sFlt-1e15a) are highly expressed in trophoblast and endothelial cells. Truncated Flt-1 receptors antagonize functional activity of pro-angiogenic molecules, placenta growth factor (PGF) and vascular endothelial growth factor (VEGF), which leads to PE. Cardiovascular disease and PE share similar pathophysiology and risk factors. This has led to potential use of statins (3-hydroxy-3-methyl-glutaryl-coenzyme) as therapy for PE. Statins are classified based on their hydrophilic (pravastatin) or lipophilic (simvastatin) nature. Recent studies show that statins can reverse the angiogenic imbalance in preeclamptic animal models. The objective of this study is to determine the ability of statins to regulate sFlt-1, PGF, and VEGF expression in primary trophoblast and endothelial cells. We hypothesize that statins will decrease sFlt-1 and increase PGF and VEGF expression.

Primary human umbilical vein endothelial cells (HUVECs) and trophoblast were isolated from normal term deliveries, cultured, and treated with serial concentrations of statins under hypoxia (1% O_2) for 24 hours. Total RNA was extracted and expression of sFlt-1i13, sFlt-1e15a, PGF, and VEGF were analyzed by real time PCR. Cell viability was measured using MTS assays.

The statins had no significant effect on cell viability under hypoxia or normoxia. Different concentrations of pravastatin tended to decrease PGF expression (~0.2-0.6 fold) in HUVECs and primary trophoblast. Simvastatin had little effect on PGF expression in HUVECs. Both pravastatin and simvastatin tended to down-regulate sFlt-1i13 and sFlt-1e15a expression (~0.3-0.6 fold) in HUVECs. Hypoxia significantly increased sFlt-1i13 and sFlt-1e15a expression in primary trophoblast and pravastatin had little effect on reversing this expression, except at high concentration (1000ug/ml). The statins had no effect in augmenting VEGF expression in either cell type. Future studies will need to verify whether statins may influence angiogenic factor protein production or release from trophoblast or HUVECs. Although more research is required, these results suggest that statins may exert more of an effect in regulating angiogenic factor expression in endothelial cells than trophoblast.

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P44 PHENOTYPIC AND FUNCTIONAL SIMILARITIES BETWEEN TUMOR-INFILTRATING AND DECIDUA NATURAL KILLER CELLS

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Natural killer (NK) cells are classically associated with immune surveillance and destruction of tumor cells via cytotoxicity. However, large influxes of NK cells are found in advanced tumors, a finding inconsistent with normal NK function. Similarly, large numbers of NK cells accumulate in the decidua during implantation. These decidua NK (dNK) cells possess non-classical CD phenotypes ($CD56^{+}CD16^{dim/negative}$), are poorly cytotoxic, elaborate pro-angiogenic factors and are thought to facilitate placentation. Transforming growth factor beta (TGFβ) mediates some of these alterations, but mechanistic details are largely lacking. As similarities between embryo implantation and tumor growth have long been noted, we investigated whether an analogous shift in NK cell phenotype/function occurs in advanced renal cell (RCC) tumors; an effect potentiated by tumor-derived TGFβ and hypoxia. NK cells from peripheral blood (pNK) and resected tumor tissue (TiNK) of RCC patients (n=6) were compared to pNK from healthy, tumor-free donors (n=5). pNK cells were cultured in the absence or presence of TGFβ (2 ng/mL) and exposed to 21% or 1% O_2 to assess conversion by monitoring expression of surface markers and angiogenic genes as well as the ability to directly kill target cells. pNK cells of healthy donors were uniformly $CD56^{+}CD16^{+}$ (94±1%) and cytotoxic, but acquired characteristics of dNK cells (reduced cytotoxicity and augmented vascular endothelial growth factor (VEGF) expression) when exposed to TGFβ or 1% O_2. Addition of TGFβ to NK cells maintained in 1% O_2 further suppressed cytotoxic function and induced expression of urokinase plasminogen activator (uPA) and its inhibitor PAI-1, two factors with known roles in tumor progression and tissue remodeling. pNK cells of RCC patients were phenotypical similar to healthy donors (89±2%, $CD56^{+}CD16^{+}$), but lacked full cytotoxic ability which we attributed to 3-fold higher levels of TGFβ in patient serum. Alternatively, RCC TiNK cells were significantly enriched for dNK-like $CD56^{+}CD16^{dim/negative}$ cells (47±12%) had limited cytotoxic capacity and increased VEGF and uPA expression. Analogous alterations in TiNK cells were observed using an orthotopic mouse model of RCC. These collective studies support a role for TGFβ and hypoxia in conversion of pNK cells to a dNK-like phenotype in RCC tumors that can promote neovascularization (VEGF) and tissue remodeling (uPA). These characteristics are conceivably beneficial for placentation, but exploited to support tumor growth and metastasis.

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P46 PRENATAL EXPOSURE TO DI-(2-ETHYLHEXYL) PHTHALATE ADVERSELY AFFECTS SELECTED FEMALE REPRODUCTIVE OUTCOMES IN THE F2 AND F3 GENERATIONS OF MICE

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Di-(2-ethylhexyl) phthalate (DEHP) is a plasticizer commonly used in materials such as vinyl flooring, medical devices, baby toys, automotive parts, and food containers. DEHP can leach from products and expose the general population through ingestion, inhalation, and dermal contact. Prenatal exposure to DEHP has been shown to adversely affect the development and function of the male reproductive system in multiple generations, but little is known about the multi-generational effect of DEHP on the female reproductive system. Thus, the objective of this study was to test the hypothesis that prenatal DEHP exposure affects female reproductive outcomes in the F2 generation of mice. To test this hypothesis, pregnant CD-1 mice (7-10 dams per treatment group) were orally dosed with tocopherol-stripped corn oil (vehicle control) or DEHP (20µg/kg/day, 200µg/kg/day, 500mg/kg/day, or 750 mg/kg/day) daily from gestation day 10.5 until birth of the pups. The subsequent generation (F1) offspring were mated with proven male breeders to produce the F2 generation, without further DEHP treatment. On postnatal days (PNDs) 8, 21, and 60 of the F2 offspring, at least one female pup from each litter was euthanized, and the ovaries and uteri were collected and weighed. At PNDs 21-60, the pups were weaned and subjected to measurements of puberty onset and estrous cyclicity. At PND 90, the mice were mated with untreated, proven male breeders to produce a set of offspring. The results show that prenatal exposure to DEHP did not affect the litter size or the male to female ratio of the F2 mice compared to controls (n=3-10 dams per treatment; $p \leq 0.05$). However, prenatal DEHP exposure (750mg/kg/day) significantly altered estrous cyclicity so that prenatally DEHP exposed mice spent less time in proestrus compared to controls (n=3-10 dams per treatment; $p \leq 0.05$). Further, DEHP exposure (500mg/kg/day) significantly influenced the weight at the time of weaning so that prenatally DEHP exposed mice weighed more when compared to controls (n=3-10 dams per treatment; $p \leq 0.05$). In contrast, DEHP exposure did not affect the mating ability of the dams (n=3-10 dams per treatment; $p \leq 0.05$). Further, DEHP exposure did not affect the ability of the dams to become pregnant, litter size, or the male to female ratio of the pups produced by the F2 generation (n=3-10 dams per treatment; $p \leq 0.05$). These data suggest that prenatal DEHP exposure may have two-generational effects on some female reproductive outcomes such as amount of time spent in proestrus and weight at the time of weaning.

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P48 PHTHALATE METABOLITES ARE ASSOCIATED WITH HOT FLASHES IN MIDLIFE WOMEN

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Hot flashes are the most common symptom that perimenopausal women report to their health care providers and the primary reason that perimenopausal women seek medical care. Despite the high prevalence of hot flashes and their negative impact on quality of life, little is known about the risk factors for hot flashes. Thus, the objective of this study was to test the hypothesis that an environmental chemical exposure (phthalates) is associated with the occurrence, frequency, and severity of hot flashes in midlife women. Phthalates are synthetic chemicals commonly used as plasticizers in consumer goods such as children's toys, deodorants, lotions, and cosmetics. Phthalates are also used in adhesives, pesticides, solvents, wood finishes, lubricants, and in medical devices including surgical gloves, tubing, blood bags, and dialysis equipment. With 18 billion pounds of phthalates used worldwide each year and exposure occurring through inhalation, ingestion, and dermal absorption, human exposure to phthalates is ubiquitous. To test whether phthalate exposure is associated with hot flashes, data were obtained from 195 women aged 45 to 54 years enrolled in the Midlife Women's Health Study. Each woman completed a self-administered questionnaire that provided detailed information on hot flash history and potential confounders such as race, body mass index, and reproductive history. Additionally, each woman provided urine samples that were subjected to measurements of phthalate metabolites by isotope dilution high-performance liquid chromatography negative-ion electrospray ionization-tandem mass spectrometry. Specifically, the following phthalate metabolites were measured: monomethyl phthalate (MMP), mono-(5-carboxy-2-ethylpentyl) phthalate (MCEPP), mono(3-carboxypropyl) phthalate (MCPP), monobutyl phthalate (MBP), monoethyl phthalate (MEP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHPP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and monoethylhexyl phthalate (MEHP). Further, serum samples were subjected to measurements of estradiol using enzyme-linked immunosorbant assays. The data indicate that higher mean MCEPP levels were positively associated with ever having hot flashes ($p < 0.04$), severe hot flashes ($p<0.02$), and trended towards an association with daily hot flashes ($p<0.09$). Additionally, higher MEHHP levels were positively associated with ever having hot flashes ($p<0.04$), severe hot flashes ($p<0.03$), and trended towards a positive association with daily hot flashes ($p<0.06$). MEP levels were also positively associated with ever having hot flashes ($p<0.005$), having hot flashes in the past 30 days ($p<0.01$), daily hot flashes ($p<0.008$), and trended towards a positive association with severe hot flashes ($p<0.06$). There were no statistically significant associations between the other metabolites and hot flashes outcomes. The data also indicate that MEP levels are negatively correlated with estradiol levels ($p<0.05$) and that MBP and MBzP levels trended towards a negative correlation with estradiol levels ($p<0.06$). Collectively, these data suggest that some, but not all, phthalate metabolites are associated with an increased risk of hot flashes in perimenopausal women. These data also suggest that some phthalates may be associated with hot flashes through a mechanism that includes low estradiol levels.

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P50 EXPOSURE TO DEHP DURING PRENATAL GONADAL DEVELOPMENT NEGATIVELY IMPACTS FERTILITY IN MALE MICE

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This study tested the hypothesis that prenatal Di-(2-ethylhexyl) phthalate (DEHP) exposure affects gonadal development in the male offspring. Prenatal exposure was achieved by orally dosing pregnant female CD-1 mice with tocopherol-stripped corn oil (vehicle control) or DEHP (20 μg/kg/day - 750 mg/kg/day) from gestation day 11 to birth. The body and gonadal weights of male pups were measured during the early postnatal and peripubertal periods. Gonadal histology was performed at the ages of postnatal days (PND) 1 and 60 to assess the impact of prenatal DEHP exposure to gonadal development. When the mice reached adulthood, their fertility was assessed at 3-7 months of ages and anogenital distance and serum testosterone levels measured at the ages of PND 21 and 16 month. The mice that were exposed to environmentally relevant doses (20-200 μg/kg/day) did not exhibit an obvious anatomical malformation in their gonadal development or fertility defect. However, those exposed to a high dose (750 mg/kg/day) displayed a significantly reduced fertility ($p=0.028$), a lower serum testosterone concentration ($p=0.07$), and a shorter anogenital distance ($p=0.0031$) compared to control group. Importantly, the mice that were exposed to environmentally relevant doses (20 and 200 μg/kg/day) also had significantly lower serum testosterone concentrations ($p=0.04$ and $p=0.03$, respectively), indicating the adverse effect of DEHP on gonadal steroidogenesis. Interestingly, these low dose groups of mice also performed poorly at a spatial memory function test, indicating a harmful effect of prenatal exposure of DEHP on the brain development as well. Taken together, this study finds that a prenatal exposure to a high dose of DEHP may give a lifelong impact on the male fertility and memory function.

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P52 POTENTIAL OF CANTHARIDIN-CONTAINING LIPOSOME AND GOLD NANOSHELL COMPLEXES TO INDUCE APOPTOSIS

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One of the primary concerns with traditional chemotherapy is that there are few targeted delivery methods, allowing drugs access to all cells within the body. One solution to this dilemma is to encapsulate an apoptotic agent within a liposome to minimize exposure to non-targeted cells, and tether it to a hollow gold nanoshell in order to guide the liposome to tumor sites. These complexes will then be heated to change liposomal membrane conformation to release cantharidin, a strong apoptotic agent. However, it is uncertain whether the nanoshells pose any threat to cells. In order to examine any potential toxicity, MCF-7 and MDA-MB-231 breast cancer cells were exposed to various samples of hollow gold nanoshells of various sizes, as well as several nanoshell-liposome complexes, both heated and unheated. A standard MTT cell viability assay was performed after 48 hours of treatment, with a wavelength reading of 570 nm. Overall, though there were differences in viability between the various samples, no cells exposed to nanoparticles had a statistically significant difference from the control viability. However, when solutions of nanoparticles and cell media were stored in refrigeration for periods of one week or more, there were significant differences in viability that differed from the control. When samples were not stored, these differences in viability were not observed. Due to this issue, there were large differences in viability, resulting in large error ranges and no statistically significant changes in viability. Interestingly, the liposome complexes have displayed a variety of effects on cell viability, with some inducing apoptosis and others no effect. This may indicate loss of cantharidin from some complexes without an induced release. Further studies of both nanoparticles and complexes are needed.

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P54 EQUOL INHIBITS GROWTH AND ESTRADIOL PRODUCTION IN MOUSE ANTRAL FOLLICLES IN VITRO

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Equol is a non-steroidal estrogen metabolite exclusively produced by microbial conversion of the ingested soy isoflavone daidzein in the gut of some humans and many animal species. Daidzein is one of the major isoflavone phytoestrogens found in soybeans. A few studies show that phytoestrogens can affect the endogenous estradiol biosynthesis pathway by mimicking, potentiating, or impairing different components of this pathway, and this in turn, could alter ovarian follicle growth. However, no studies have examined the effects of equol exposure on intact antral follicles. Thus, we tested the hypothesis that equol (6, 36, and 100 µM) inhibits follicle growth and estradiol production in mouse antral follicles. To test this hypothesis, antral follicles were manually isolated with forceps from ovaries of cycling CD-1 mice at postnatal days 33-35, and cultured with vehicle control (dimethylsulfoxide; DMSO) or equol (6, 36, and 100 µM) in supplemented alpha-minimum essential medium for 96 hours (h). Individual follicle diameters were measured every 24 h, and at completion of culture, media were collected and subjected to enzyme-linked immunosorbent assays for measurement of estradiol levels. The results indicate that equol at the highest concentration tested (100 µM) significantly inhibited follicle growth at 72 h (DMSO: 127.3 $\pm$4.03 percent change; Equol 100 µM: 109.23 $\pm$1.08 percent change, n=4, $p \leq 0.05$) and 96 h (DMSO: 146.85 $\pm$5.35 percent change; Equol 100 µM: 120.1 $\pm$2.72 percent change, n=4, $p \leq 0.05$) compared to control. Additionally, equol at 100 µM significantly inhibited estradiol levels measured at 96 h compared to control (DMSO: 4135.08 $\pm$592.32 pg/mL; Equol 100 µM: 218.4 $\pm$75.8 pg/mL; n=3-4, $p \leq 0.05$). Exposure to the lower doses of equol (6 and 36 µM) did not significantly affect follicle growth or estradiol levels when compared to control. Collectively, these data suggest that equol inhibits growth and estradiol production in cultured mouse antral follicles.

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P56 SILVESTROL INDUCES AUTOPHAGY AND APOPTOSIS IN HUMAN MELANOMA CELLS

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Silvestrol is a cyclopenta[b]benzofuran that was isolated from the fruits and twigs of Aglaia silvestris, which is indigenous to the island states of Southeast Asia. Previous testing of silvestrol revealed that it is a potent inhibitor of protein synthesis and has cytotoxic activity similar to or more potent than many FDA approved anticancer agents. Silvestrol is currently under preclinical development at the National Institutes of Health Experimental Therapeutic (NExT) program. The purpose of the current study was to determine if inhibition of protein synthesis caused by silvestrol triggers autophagy and apoptosis in solid tumors. By 24h a clear decrease in cyclin B and cyclin D expression was observed in silvestrol-treated cells relative to control. In addition, silvestrol blocks progression through the cell cycle at the G_2-phase. Silvestrol treatment also induced caspase-3 activation and apoptotic cell death in a time- and dose-dependent manner. Next, DAPI staining of nuclear chromatin showed nucleosomal fragments. Annexin V staining also showed an increase in apoptotic cells after silvestrol treatment. Furthermore, both silvestrol and SAHA enhanced autophagosome formation in MDA-MB-435 cells. Quantitation of the acidic vacuoles measured by flow cytometry further confirmed these results. MDA-MB-435 cells responded to silvestrol treatment with accumulation of LC3-II and dose-dependent p62 degradation. However, bafilomycin A, an autophagy inhibitor, resulted in the accumulation of LC3 in cells treated with silvestrol. Silvestrol represents a natural product scaffold with the potential for the study of autophagy and apoptosis mechanisms in cancer cells. It also highlights the direction of future drug development.

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P58 DIRECT COMPARISON OF COLORIMETRIC ASSAYS FOR VIABILITY USING A BREAST CANCER CELL MODEL

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Cancer is a dangerous disease that affects many people nationwide. Breast cancer is one of the most common types that exist in today's society. Different cancer cell lines originate with different types of cancer. The main type of cancer that my cell lines represented were carcinomas. The purpose of my experiment was to identify an optimal assay for determining compound toxicity. Cancer cells are a good model system, as they are available in several cell types and exhibit robust growth. In this experiment, I used two different breast cancer cell lines, MCF-7 and MDA-MB-231, and compared three different colorimetric assays: MTT, resazurin, and crystal violet to counting viable cells directly. I hypothesized that the MTT assay, which we use regularly in our studies, would be more reproducible than the other assays and would show strong toxicity. The toxin I used was cantharidin, which we have previously identified as highly toxic to these cell types. Our results indicate that the crystal violet assay was not as reliable, and therefore we concluded that it is not an ideal assay to use. In contrast, the MTT and resazurin assays are highly reproducible and show the strong toxicity of cantharidin. These assays gave similar results to counting viable cells directly and thus we conclude that the crystal violet assay is not an effective method for testing cancer cell lines, as opposed to the MTT and resazurin, are very effective.

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P60 THE PRO-APOPTOTIC EFFECT OF FURANODIENE IN THE DOXORUBICIN-RESISTANT MCF-7 HUMAN BREAST CANCER CELLS IS AMPK-DEPENDENT

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Furanodiene is a bioactive compound isolated from Curcuma wenyujin Y.H.Chen et C.Ling (C. wenyujin) that is a commonly prescribed Chinese medicine for cardiovascular and tumor therapy. We previously showed that furanodiene can inhibit breast cancer cell growth and suppresses metastatic breast cancer cell migration and invasion, but little is known about the underlying mechanisms of anti-cancer activities of furanodiene. The role of AMP-activated protein kinase (AMPK), as an energy sensor in eukaryotic cells, in controlling energy homeostasis in cancer development is well-established. However, AMPK's role in furanodiene induced apoptosis has yet to be examined. In the present study, we aimed to investigate the effects of furanodiene on mitochondria function, AMPK activation, tumor cell growth, and apoptosis in doxorubicin-resistant MCF-7 cells. Intracellular ATP levels and the AMPK-related energy metabolic signaling were also inspected. Our results demonstrated that furanodiene decreased cellular ATP levels and mitochondria function, but induced DNA damage and apoptosis in doxorubicin-resistant MCF-7 human breast cancer cells. AMPK inhibitor compound C (CC) abolished the tumor cell growth inhibition and AMPK activation effects of furanodiene, while AMPK activator AICAR augmented those effects. In addition, knockdown of AMPK in doxorubicin-resistant MCF-7 human breast cancer cells presented with furanodiene showed increased mitochondrial function, decreased AMPK activation, and decreased apoptosis. Furanodiene also inhibited the phosphorylation of ATP-citrate lyase and GSK-3β, signaling intermediates in AMPK-related energy metabolic regulation. These findings extend our understanding of furanodiene and the molecular mechanisms of its pro-apoptotic effect. This study further suggests that AMPK may be a potential therapeutic target for treating chemo-resistant breast cancer.

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P62 MASS SPECTROMETRY ANALYSIS OF CONDITIONED MEDIUM FROM 3D BREAST CANCER CELL CULTURES IDENTIFIES CANDIDATE PROTEINS INVOLVED WITH PROLIFERATION AND METASTASIS

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The tumor microenvironment plays a significant role in mammary gland oncogenesis. Deciphering the exchange of information among the cells within the diseased microenvironment could add significantly to our comprehension of oncogenesis and disease progression. To this end, estrogen and progesterone receptor positive MCF-7 cells, triple negative breast cancer MDA-MB-231, DT22, and DT28 cells, and MCF-10A non-transformed mammary epithelial cells were grown in 3D cultures in the absence and in the presence of human mammary fibroblasts. When MCF-10A cells were exposed to conditioned medium (CM) from each of the cancer cell lines, the growth of these benign and relatively quiescent cells was significantly stimulated. However, the degree of stimulation varied and was dependent on the cell line utilized, indicating that soluble secreted growth factors were present in the CM of the cancer cell cultures. The CM from each cell line was subjected to mass spectrometry analysis. A variety of secreted proteins were identified, including chemokines, proteases, glycolytic enzymes, insulin-like growth factor binding proteins, and exosomal components. An investigation of each cell line's secretome yielded clues about strategies used for breast cancer proliferation and metastasis. A serum-based test for breast cancer could conceivably utilize some of these protein signatures for detection, diagnosis, and the monitoring of disease progression.

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P64 GENERATION OF ESTROGEN RECEPTOR ALPHA- AND BETA-ICRE KNOCK-IN MICE

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Two novel knock-in mouse lines that express codon-improved Cre recombinase (iCre) under the regulation of the estrogen receptor alpha (*Esr1*) or beta (*Esr2*) promoter are developed for conditional deletion of genes and for the spatial and/or temporal localization of Esr1 and Esr2 expression, respectively. ESR1 (ERα) is one of two classical estrogen receptors and displays a spatio-temporal expression pattern and functions that are different from the other estrogen receptor, ESR2 (ERβ). A cassette was constructed that contained iCre, a polyadenylation sequence, and a neomycin selection marker. This construct was used to insert iCre in front of the endogenous start codon of the Esr1 and Esr2 gene, respectively, of a C57BL/6J embryonic stem cell line via homologous recombination. Resulting Esr1-iCre and Esr2-iCre mice were bred with ROSA26-lacZ and Ai9-RFP reporter mice to visualize areas of functional iCre expression. A faithful expression of iCre was observed in the reproductive tissues where ERS1 and ESR2 expression are well characterized. In addition, these transgenic mouse models revealed novel sites of ESR1 and ESR2 expression in the tissues of digestive, nervous and immune systems. These novel transgenic mice were successfully used for conditional ablation of progesterone receptor (Esr1-iCre/Pgr-flox/flox and Esr2-iCre/Pgr-flox/flox), which resulted in complete loss of fertility in both lines of conditional knockout mice. Overall, Esr1-iCre and Esr2-iCre mice will serve as novel mouse lines for conditional gene deletion in *Esr1*- or *Esr2*-expressing tissues.

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P66 NUCLEAR ORGANIZATION DURING DIFFERENTIATION OF THE BIPOTENTIAL GONAD

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During mammalian sex determination, a population of bipotential progenitor cells directs the fetal gonad to become either a testis or an ovary. XX and XY gonads are initially indistinguishable and express components of both developmental pathways, suggesting that progenitor cells are poised to initiate either fate. In XY gonads, the expression of *Sry* triggers progenitor differentiation into preSertoli cells; lacking *Sry*, XX progenitor cells differentiate into pregranulosa cells. Highlighting the truly bipotential nature of these cells, mutation of sex-determining (SD) genes such as *Sry* leads to male-to-female sex reversal at the SD stage. However, mutation of female SD genes does not disrupt initial progenitor cell differentiation and, instead, female-to-male sex reversal occurs postnatally. Thus, there may be distinct stages where gonadal cells are permissive to sex reversal that differ between males and females. Furthermore, the ability of the adult gonad to transdifferentiate suggests that, post-differentiation, XX and XY gonadal cells retain a memory of their progenitor state. In many cell types, chromatin is spatially organized into distinct nuclear domains based on whether genes are activated or silenced. To probe the role of nuclear remodeling during cellular differentiation and plasticity, my project will test whether differentiating gonad progenitor cells undergo changes in nuclear patterning. We predict that sex determination requires changes in nuclear patterning for differentiation to proceed normally. However, the balance between retention of differentiated cell fates and maintenance of plasticity may require nuclear organization to remain relatively similar to the bipotential gonad despite changes in sex-specific gene expression. To test this, we will use DNA FISH to probe for the localization of the male and female sex-determining genes in the nucleus of XX and XY supporting cells. Additionally, we will identify global differences in the distribution of histone modifications between embryonic and adult gonadal cells in order to examine the establishment and maintenance of repressive chromatin during permissive and resistant periods of sex reversal. Using the sex determination model, we aim to better understand the mechanisms whereby cells make and maintain cell fate decisions while still retaining some measure of plasticity.

P68 HUMAN FALLOPIAN TUBE EPITHELIUM CO-CULTURE WITH MURINE OVARIAN FOLLICLES REVEALS CROSSTALK IMPACTING ESTROUS CYCLE HORMONES.

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The human fallopian tube is well known as a critical organ in the female reproductive tract that facilitates the transport of the cumulus-oocyte complex (COC), sperm, and embryos. Until now, research has focused on developing in-vitro fallopian culture methods to study the impact of hormonal signals on the fallopian epithelium and transcriptional changes during the estrous cycle. Currently, there is no direct evidence showing that fallopian tissue affects ovarian function during a reproductive cycle; however, luteal phase deficiency and irregular menstrual cycles have been reported in women after tubal sterilization. In this study, an in-vitro co-culture model was developed to study the human fallopian tube epithelium and murine follicles and the interaction between them during a reproductive cycle. Our results indicate that human fallopian tissue acquired from routine hysterectomy can be cultured on a transwell insert membrane for at least 14 days and retain viable morphology, as well as estrogen receptor (ER), progesterone receptor (PR), and oviduct-specific glycoprotein (OVGP1) expression. The cultured fallopian tube epithelium was composed of both ciliated and secretory cells. Estradiol enhanced the cilia beating rate and OVGP1 expression, while progesterone suppressed both functions. Human fallopian tube epithelium was co-cultured with murine multilayered secondary follicles in 3D, secreting steroid hormones as the follicle matured and luteinized, effectively mimicking a reproductive cycle. The fallopian tube epithelium experienced cyclic changes, both in the morphology and in the production of secreted factors, such as OVGP1, IGF1, VEGFA, and IL8. However, the epithelial tissue did not exhibit similar cyclic changes with regards to VEGFA and IL8 secretion in the exogenous stepwise steroid hormone treatment. Using this co-culture system, the fallopian epithelial tissue positively impacted corpus luteal function to produce significantly higher levels of progesterone and maintain corpus luteal function longer than follicles cultured alone. The in-vitro co-culture model between human fallopian epithelial tissue and murine follicles serves as a unique tool to explore the interactions between the fallopian tube and the ovarian follicle across the menstrual cycle and provides direct evidence to better understand how normal tubal function could benefit the luteal phase in the reproductive cycle.

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