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Program Overview

8:00 - 8:30 AM Registration/Continental Breakfast
8:35 - 8:45 AM Opening Remarks
8:45 - 9:00 AM UIC Alumni Speaker – Dr. Selva Nataraja
9:00 - 10:15 AM Oral Session I
10:15-10:30 AM Coffee Break
10:30-10:45 AM UIC Alumni Speaker – Dr. Rachel Duan
10:45-12:00 PM Oral Session II
12:00-1:00 PM Lunch
1:00 - 3:00 PM Poster Session
3:00 - 3:15 PM UIC Alumni Speaker – Dr. Constance Albarracin
3:15 - 4:30 PM Oral Session III
4:30 - 5:15 PM Wine and Cheese Reception
5:15 - 5:20 PM Intro to Keynote Address
5:20 - 6:20 PM Keynote Address – Dr. Joanne Richards
6:20 – 6:30 PM Presentation of Awards
6:30 – 6:35 PM Closing Remarks
Introduction to the Symposium on Reproductive Sciences in Health and Disease

We welcome you to the Symposium, and hope to see you all in future years at similar events that will take place in different universities all over the state of Illinois. This rotating annual statewide symposium provides an opportunity to celebrate our strong research and educational heritage, to foster the exchange of scientific information in the reproductive sciences, to facilitate the career development of the next generation of Illinois reproductive scientists and to establish a promising future of reproductive sciences research in the state of Illinois. We hope to leverage our collective institutional strengths to maintain Illinois in a preeminent nationwide position in this critical research field.

The great importance of this annual meeting is the opportunity it affords for interaction between faculty and trainees at several institutions. Pioneers in reproductive sciences including Andy Nalbandov, Neena Schwartz, and Jack Gorski did groundbreaking discoveries and made Illinois a forefront center in reproductive sciences. A new generation of scientists has taken up this responsibility, succeeding at obtaining a significant fraction of research funded by the NICHD Reproductive Sciences Branch and at generating highly respected research programs. Many became impressive 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.

The student organizing committee members are from several state institutions, and have worked professionally and effectively together to plan an outstanding meeting. We are very pleased to have a record number of abstracts submitted not only from throughout Illinois but also from several neighboring states.

The next Illinois Symposium on Reproductive Sciences will be hosted by UIUC in the fall of 2011.
Keynote Address

“Gatekeepers of Ovulation and Luteinization”

One of the major issues facing the world today is the explosion in world population. New ways to approach fertility control are needed and depend on our understanding the biology of the mammalian ovary. The biological challenge to be met by the mammalian ovary is to maintain the continuous development of small follicles and, at the same time, to allow other follicles to ovulate and release a fertilizable egg. The dynamics of this are orchestrated by many interwoven biochemical and hormonal processes that ultimately allow less than 1 percent of the follicles contained within the ovary to ovulate. There are many key regulators of ovarian cell differentiation including the gonadotropins (follicle stimulating hormone, FSH and luteinizing hormone, LH) that act via G-protein coupled receptors, steroid hormones such as estradiol and progesterone that act via nuclear hormone receptors (ESR1/2 and PGR) and various growth factors that activate tyrosine kinases.

Our efforts are focused on the molecular mechanisms by which some of these regulatory molecules (especially, FSH, LH and progesterone) control ovarian cell gene expression and alter cell function during follicular growth, ovulation and luteinization. Our most recent studies have focused on the role of specific factors in ovarian cancer such as β-catenin, Pten and Foxo1a. In addition, our studies have revealed that during the process of ovulation, cumulus cells within the cumulus oocyte complex are induced to express genes characteristic of immune cells. These include the Toll-like receptors and CD14, activated cell adhesion molecule (ALCAM), programmed cell death one (PDCD1) associated with autoimmune diseases and CD36, a scavenger receptor. Moreover, we have shown that cumulus cells and granulosa cells can respond to external cues in a manner similar to immune cells, including the uptake of bacterial particles into lysosomes. That ovarian cells can acquire specific immune-related functions opens many new avenues of investigation into the ovulation process. Lastly, factors that control early stages of ovarian follicular formation are among the projects being studied.

To identify specific genes at specific stages of follicular growth and ovulation we utilize microarray technologies combined with Q-PCR, in situ hybridization and immunocytochemical approaches. To analyze the function of specific genes, we use transient transfection analyses, adenoviral delivery of specific genes into granulosa cells as well as various transgenic mouse models. The next decade promises new insights into how the ovary is formed, where ovarian stem cells are found, what endocrine signals and genes regulate follicular growth and follicular cell function, how ovulation occurs and what role do the immune cell-related factors have and what genes mediate the transformation of a follicle into a corpus luteum.
### UIC Alumni Speakers

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Affiliation</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selva Nataraja</strong></td>
<td>Group Leader</td>
<td>EMD Serono Research Institute</td>
<td>&quot;Drug Discovery Research for treating Infertility: The other side of the grass&quot;</td>
</tr>
<tr>
<td><strong>Rachel Duan</strong></td>
<td>Medical Director</td>
<td>Global Pharmaceutical R&amp;D</td>
<td>&quot;Clinical Research in Drug Development: From Bench to Bedside&quot;</td>
</tr>
<tr>
<td><strong>Constance Albarracin</strong></td>
<td>Associate Professor</td>
<td>Dept. of Pathology</td>
<td>&quot;Multidisciplinary Team Approach to Translational Research: Opportunities in a Cancer Center &quot;</td>
</tr>
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PROGRAM

8:00 - 8:30 AM  Registration  
Continental Breakfast  
Run-through talks  
Setup Posters

8:35 AM  **Opening Remarks**  – Dr. Geula Gibori, Ph.D. Department of Physiology and Biophysics, University of Illinois at Chicago. Chicago IL

8:45 AM  **UIC Alumni Speaker**  – "Drug Discovery Research for treating Infertility: The other side of the grass"  Dr. Selva Nataraja, Ph.D., Senior Principal Investigator, EMD Serono Inc., Rockland, MA

**ORAL SESSION I: Gonadal Development and Function**

*Session Moderators*: Eugene Galdones, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL and Leah Goldberg, Department of Molecular and Integrative Physiology, University of Illinois, Urbana, IL.

Abstract #  * Presenting author underlined

9:00 AM  **T1**  The conserved RNA-binding protein Boule regulates RNA stability during male germ cell differentiation, M.J.W. VanGompel and E.Y. Xu, Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL.

9:15 AM  **T2**  Zinc is required for proper dynamics of maturation promoting factor during mouse oocyte maturation, M.L. Bernhardt¹, A.M. Kim¹, T.V. O’Halloran²,³, and T.K. Woodruff¹,³, ¹Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; ²Department of Chemistry, Northwestern University, Evanston, IL; ³Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.

9:30 AM  **T3**  Infertility in Basigin null mutant male mice may be due to impaired interactions between gametes and Sertoli cells, J. Bi¹, Y. Li¹, F. Sun², M.A. Handel², and R.A. Nowak¹, ¹Department of Animal Sciences, University of Illinois, Urbana, IL; ²The Jackson Laboratory, Bar Harbor, ME.

9:45 AM  **T4**  Cadmium induced oxidative stress and altered testicular steroidogenesis and spermatogenesis: the protective role of melatonin, N. Joshi¹,², S. Banerjee¹, R. Kukherjee³, and A.V. Ramachandran¹, ¹Division of Reproductive Toxicology, Department of Zoology, Faculty of Science, The M.S. University of Baroda, Sayajigunj Vadodara, India; ²Department of Obstetrics, Gynecology, and Reproductive Biology, College of Human Medicine, Michigan State University, Grand Rapids, MI.
10:00 AM T5 Di-\textit{n}-butyl phthalate suppresses mouse antral follicle growth in vitro, Z.R. Craig, P.R. Hannon, M.S. Basavarajappa, B.N. Karman, T. Paulose, and J.A. Flaws, Department of Veterinary Biosciences, University of Illinois, Urbana, IL.

10:15 - 10:30 AM Coffee Break

10:30 AM UIC Alumni Speaker – "Clinical Research in Drug Development: From Bench to Bedside" Dr. Rachel Duan, M.D., Ph.D., Medical Director, Global Pharmaceutical Research and Development, Abbott Pain Care, Abbott Park, IL

ORAL SESSION II: Signaling Mechanisms and Gene Regulation

Session Moderators: Mary Laws, Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL and Doug Luccio-Camelo, Department of Urology, University of Illinois at Chicago, Chicago, IL

10:45 AM T6 SF-1 driven steroidogenic differentiation of murine embryonic stem cells, U. Jadhav and J.L. Jameson, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.

11:00 AM T7 The Notch inhibitor Numb is present in gonadotropes and may be necessary for regulating LH expression and function, L.B. Goldberg, T.B. Moran, K.E. Brannick, and L.T. Raetzman, Department of Molecular and Integrative Physiology, University of Illinois, Urbana-Champaign, IL.

11:15 AM T8 Germ-cell specific deletion of Jagged1, a Notch ligand, leads to the formation of multi-oocytic follicles in the ovary, D.A. Vanorny, S.M. Kilen, and K.E. Mayo, Department of Molecular Biosciences and Center for Reproductive Science, Northwestern University, Evanston, IL.

11:30 AM T9 Novel induction of EMMPRIN release via the cell surface G protein coupled estrogen receptor GPER, L.A. Burnett$^{1,2}$, R.A. Nowak$^1$, $^1$Department of Animal Sciences; $^2$College of Medicine, University of Illinois, Urbana, IL.

11:45 AM T10 Genetic polymorphism in the aryl hydrocarbon receptor gene is associated with an increased risk of hot flashes in generally healthy midlife women, A. Ziv-Gal and J.A. Flaws, Department of Comparative Biosciences, University of Illinois, Urbana, IL.

12:00 - 1:00 PM Lunch Break

1:00 - 3:00 PM POSTER SESSION

P2 Provision of emergency contraception after sexual assault: a national survey, 2004 and 2009, A Patel, MD, MPH, S Tilmont, MPH, A Sheth, MD, L Nguyen, MD, S Chaparala, MD, L Keith, MD, PhD, 1Division of Family Planning, Department of Obstetrics and Gynecology, John H. Stroger; Jr. Hospital of Cook County, Chicago, IL; 2Feinberg School of Medicine, Northwestern University, Chicago, IL.

P3 Comprehensive medical care management to victims of sexual assault: a national survey of hospital emergency departments in United States, A Patel, MD, MPH, Sushma Chaparala, MD, Sandra Tilmont, MPH, Luan Nguyen, MD, Amish Sheth, MD, Mrunal Ghanshyam Parab, MD, L Keith, MD, PhD, 1Division of Family Planning, Department of Obstetrics and Gynecology, John H. Stroger; Jr. Hospital of Cook County, Chicago, IL; 2Feinberg School of Medicine, Northwestern University, Chicago, IL.

P4 Methoxychlor induces atresia and decreases estradiol levels in antral follicles simultaneously, M. S. Basavarajappa, I. Hernández-Ochoa, J. Singh, and J. A. Flaws, Veterinary Biosciences, University of Illinois, Urbana, IL.


P6 The Notch effector gene Hes1 regulates migration of hypothalamic neurons, neuropeptide content and axon targeting to the pituitary, Paven K. Aujla, Adriana Bora, Pamela Monahan, Jonathan V. Sweedler, and Lori T. Raetzman, Department of Mol. Integrative Physiol, University of Illinois at Urbana-Champaign, Urbana, IL.

P7 Increased activation of the PI3K/Akt pathway compromises decidualization of stromal cells of endometriosis, Xunqin Yin, Mary Ellen Pavone, Zhenxiao Lu, J. Julie Kim Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL.

P8 Molecular mechanism of vaginal epithelial differentiation, Kenji Unno, Vanida A. Serna, Kazutomo Ishi, *Alea A. Mills and Takeshi Kurita, Division of Reproductive Biology Research, Ob/Gyn Department and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

P9 Secretion of Basigin By human trophoblast-like cells through microvesicle shedding is regulated by interleukin-1β and hypoxia, Victoria Ermilova, Ying Chen, Richard Leach, Stephen Charnock-Jones, and Romana A. Nowak; Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL., Department of OB/GYN, Michigan State University, Grand Rapids, MI and Department of OB/GYN, Cambridge University, Cambridge, UK.

P10 Alterations in gene expression levels in estrogen receptor alpha overexpressing antral follicles treated with methoxychlor, Paulose T., Hernández-Ochoa I., Flaws JA, Comparative Biosciences, University of Illinois, Urbana, IL.
P11 Ontogeny of the Relaxin receptor (Rxfp1) in the female mouse reproductive tract, Juanmahel Davila†, O. David Sherwood§, Alexander I. Agoulnik‡, and Paul S. Cooke† ¶ †, Department of Comparative Biosciences, University of Illinois, Urbana, IL; §Department of Molecular and Integrative Physiology, University of Illinois, Urbana, IL; ‡Department of Human Molecular Genetics, Florida International University, Miami, FL; ¶Division of Nutritional Sciences, University of Illinois, Urbana, IL.

P12 The cytokine IL-1β includes epithelial to mesenchymal transition (EMT) in peritoneal mesothelial cells through direct and indirect effects involving Basigin, Farzaneh Masoud, Pavni Mehrotra, and Romana A. Nowak, Department of Cellular and Developmental Biology, University of Illinois, Urbana, IL, Department of Animal Sciences, University of Illinois, Urbana, IL.

P13 Estrogen-dependent ERα activation is required for normal branching morphogenesis of the mouse prostate gland, Esther L. Calderon¹, Kerstin Sinkevicius², Muriel Laine², Geoffrey L. Greene², Gail S. Prins¹, Departments of Urology and Physiology & Biophysics, University of Illinois at Chicago, Chicago, IL; ²The Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

P14 Retinoic Acid action is deficient in endometrium in a baboon endometriosis model, Mary Ellen Pavone¹, Elizabeth Pearson¹, You-Hong Cheng¹, Asgi Fazleabas², Serdar Bulun¹, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; ²Obstetrics, Gynecology, & Reproductive Biology, Michigan State University, Grand Rapids, MI.

P15 Spontaneous and programmed transformation of ovarian surface epithelium using a three-dimensional organ culture system. S.M. King, D.A. Davis, Joanna E. Burdette, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL.

P16 Regeneration of normal prostate gland and prostate cancer tissue using normal and tumorigenic prostate progenitor cells, Wen-Yang Hu, Guang-Bin Shi, Dan-Ping Hu, Ikenna Madueke, Gail Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

P17 Follicle-stimulating hormone and luteinizing hormone induce expression of oncogenes in a 3D model of normal ovarian surface epithelium. T. S. Hilliard, J. E. Burdette, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL.

P18 DNA methylation analysis of human uterine leiomyomas, Antonia Navarro, Ping Ying, Jianjun Wei, Pan Du, Simon Lin, Serdar E. Bulun. Department of Reproductive Biology Research, Feinberg School of Medicine, Northwestern University, Chicago, IL.

P19 MEK1 and 2 are essential for the survival of the adult population of Leydig cells in the mouse, Soichi Yamashita¹, Jean Charron², CheMyong Ko³, and Mario Ascoli¹, Department of Pharmacology, The University of Iowa, Iowa City, IA, ²Centre de Recherche en Cancérologie de l’Université Laval, Québec, Canada and ³Division of Clinical and Reproductive Sciences, Department of Clinical Sciences, University of Kentucky, KY.
P20 **Extracellular matrix-mediated regulation of proliferation in leiomyoma smooth muscle cells**, Faezeh Koohestani and Romana Nowak, University of Illinois at Urbana-Champaign, Urbana, IL.

P21 **Identification and characterization of AP3 binding proteins in luteal Cells**, Deepika Talla and Carlos Stocco, Department of Physiology and Biophysics, College of Medicine, University of Illinois at Chicago, Chicago, IL.

P22 **The granulosa cell inositol phosphate cascade is essential for fertility**, S.M. Breen1, S. Offermans2, J.A. Gossen3, and M. Ascoli1, 1Department of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA, 2University of Heidelberg, Heidelberg, Germany, 3 Target Discovery Oss, Schering-Plough Corporation, Molenstraat, Oss, The Netherlands.

P23 **The Forkhead transcription factor, FOXP3, is essential for normal reproductive function**, Deborah O. Jung, David B. Schwartz, Corrie L. Farris, Elaina Rathbun, and Buffy S. Ellsworth, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL.

P24 **The role of the Forkhead transcription factor, FOXO1, in pituitary gland development**, Sreeparna Majumdar, Corrie L. Farris, Deborah O. Jung, Buffy S. Ellsworth, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL.

P25 **Identification of developmental competence related genes in mature porcine oocytes**, Y. Yuan, R. Krisher, Department of Animal Sciences, University of Illinois, Urbana, IL.

P26 **17β-estradiol increases the expression of the oxidative stress response protein apurinic/apyrimidinic endonuclease or redox factor-1 (Ape1) in the cerebral cortex**, Alicia K. Dietrich and Ann M. Nardulli, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL.

P27 **Testosterone, not 5α-dihydrotestosterone stimulates LRH-1 leading to FSH independent expression of Cyp19 and P450sc in granulosa cells**, Yan-Guang Wu, Jill Bennett, Deepika Talla, and Carlos Stocco. Department of Physiology and Biophysics, College of Medicine, University of Illinois at Chicago, Chicago, IL.

P28 **Sertoli cell specific Rhox8 knockdown via siRNA**, Matt Davis, Raquel Brown, Kanako Hayashi, and James A. Maclean II, Department of Physiology, Southern Illinois University, Carbondale, IL.

P29 **Rhox homeobox gene cross-regulation in granulosa cells**, Raquel Brown, Matthew Davis, Kanako Hayashi, and James A. MacLean II, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

P30 **Functional mechanism of WNT7A in ovarian carcinomas**, Shin Yoshioka1, Sophia Ran2, Hiroshi Okuda2, James A. MacLean II1, Mary E. McAsey3, Kounosuke Watabe 2 and Kanako Hayashi1, Department of Physiology1, Medical Microbiology, Immunology and Cell Biology2, Obstetrics and Gynecology3, Southern Illinois University School of Medicine, Carbondale, IL.
Targeting PTB (polypyrimidine tract-binding protein): promising therapeutic tool for ovarian cancer, A. D. Arslan¹, X. He¹², W. T. Beck¹², ¹Department of Biopharmaceutical Sciences, College of Pharmacy and Cancer Center, University of Illinois at Chicago, Chicago, IL; ²Gynecologic Oncology Group (GOG) Core Laboratory for Molecular Pharmacology, Chicago, IL.

Evolution and patterns of reproduction in Philippine mammals, Vince FitzPatrick, Department of Mammals, Field Museum of Natural History, Chicago, IL.

Mice lacking two sperm serine proteases, ACR and PRSS21, are subfertile, but the mutant sperm are infertile in vitro, Woojin Kang¹, Natsuko Kawano¹³, Misuzu Yamashita¹, Yoshitaka Koga¹, Taiga Yamazaki¹, Tamako Hata², Kenji Miyado³, Tadashi Baba¹, ¹Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki, Japan; ²National Institute of Agrobiological Sciences, Tsukuba Science City, Ibaraki, Japan ³Division of Game and Reproductive Biology, National Research Institute for Child Health and Development, Setagaya, Tokyo, Japan.

Possible involvement of SDK2 in sperm/oviductal epithelium interaction, Yusuke Suzuki, Masayo Ogawa, Ekyune Kim, Keiko Tsumura, Misuzu Yamashita, Woojin Kang, Fumi Tanaka, Tadashi Baba, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki, Japan.

Endogenous EMMPRIN expression by human uterine fibroblast cells regulates metalloproteinase production, proliferation and decidualization, Andrea Braundmeier, Jiajia Bi, Pavni Mehrotra, and Romana A. Nowak, Department of Animal Sciences, University of Illinois, Urbana, IL.

Effects of levonorgestrel and mifepristone on endometrial receptivity and embryo implantation in a 3-dimensional in vitro model system, C.X. Meng¹², P.G.L. Lalitkumar¹, F. Hambiliki¹, K. Gemzell-Danielsson¹, ¹Department of Women’s and Children’s Health, Karolinska Institute, Stockholm, Sweden, ²Department of Obstetrics, Gynecology & Reproductive Biology, Michigan State University, MI.

FOXM1 is expressed in actively proliferating cells during pituitary gland development, Adam Ploegman, Bufféy S. Ellsworth, Department of Physiology, School of Medicine, Southern Illinois University at Carbondale, Carbondale, IL.

Regulation of the inhibin alpha subunit gene by the NR4A orphan nuclear receptors in ovarian granulosa cells, K.M. Meldi, A.D. Burkart, PhD, S.M. Thomas, W.B. Pearse, and K.E. Mayo, PhD, Department of Molecular Biosciences, Center for Reproductive Science, Northwestern University, Evanston, IL.

Differential and interactive effects of ligand-bound progesterone receptor A and B isoforms on tyrosine hydroxylase promoter activity, Philip J. Jensik and Lydia A. Arbogast, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

FOXO1 expression is reduced in human pituitary adenomas, Corrie Farris, Deborah Jung,
Buffy S. Ellsworth, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

P41 Analysis of protein expression differences in MCF-7 breast cancer cells due to exposure with varied concentrations of acetochlor and chlorpyrifos, Jessica D. Rich and Jennifer R. Schultz-Norton, Department of Biology, Millikin University, Decatur, IL.


P43 GATA-4 role in ovarian granulosa cell function and female fertility, Jill Bennett, Carlos Stocco, Dep. of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL.

P44 Smad Crosstalk in Ovarian Cancer, Roshan Ahmed, Kari Inoue, Joanna E. Burdette, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL.

P45 Effects of negative energy balance on kisspeptin and gonadotropin-releasing hormone in the rat hypothalamus. A.E. Flowers¹, J.E. Levine², T.H. Horton², B.G. Mann², C.M. Muller², ¹Master of Biotechnology Program and ²Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL.

P46 Germ cell loss, development delay and globozoospermia in mice lacking Pumilio, Y.H. Shih¹, W. Qiang², E. Y. Xu², ¹Master of Biotechnology Program, Northwestern University Robert R. McCormick School of Engineering and Applied Science, Evanston, IL, ²Division of Reproductive Biology research, Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL.

P47 Sonic Hedgehog is dispensable for mouse gonad development, Jeff Huang, Paul Cooke and Humphrey Yao, Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, IL.

P48 Impact of estrogen receptor alpha phosphorylation site mutations on hormone responsiveness and endocrine resistance in breast cancer, Kyuri Kim and Benita S. Katzenellenbogen, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana IL.

P49 Capacitation regulates oviduct glycan receptors on boar sperm, Kadirvel Govindasamy, S. Machado and David J. Miller, Department of Animal Science, University of Illinois, Urbana-Champaign, IL.

P50 Antioxidant enzyme activity in reproductive tract fluids of tropically-adapted rams, J.P.A. Rêgo¹, C.E. Souza¹, D.M.F. Gondim², J.T.A. Oliveira², A.A.A. Moura¹, ¹Department of Animal Science, ²Department of Biochemistry, Federal University of Ceará, Fortaleza, Brazil.

P51 Murine homologs of highly conserved germline stem cell factors-Pum1 and 2 work redundantly to regulate growth and cell proliferation in dosage-sensitive manner, W.
Qiang, Y. Chen, Y. Shih, T. Kurita, and E. Y. Xu, Division of Reproductive Biology Research, Department of OB & GYN, and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.

P52 Growth hormone potentiates estrogen action on proliferation and gene expression in spontaneous dwarf rat mammary gland and in T47D human breast cancer cells, Dana L. Felice1, Qi Shen2, Dan Lantvit2, Shuangping Zhao1, Steven M. Swanson2, Terry G. Unterman3, Jonna Frasor1. 1Department of Physiology and Biophysics; 2Department of Medicinal Chemistry and Pharmacognosy; 3Department of Medicine, University of Illinois at Chicago, Chicago, IL.

P53 Pro-inflammatory cytokines influence estrogen activity at the promoter of BIRC3, a member of the inhibitor of apoptosis family, by facilitating recruitment of the estrogen receptor (ER) to a latent ERE, Madhumita Pradhan, Leslie Bembinter, Jonna Frasor, Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL.

P54 Gene dosage of the coregulator REA is critical of uterine function and fertility, Sunghee Park1, Sangyeon Yoon1, Seongeun Park1, Yuechao Zhao1, Jianming Xu2, John P. Lydon3, Francesco J. DeMayo2, Bert O’Malley2, Milan K. Bagchi1, and Benita S. Katzenellenbogen1, 1Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana,IL; and 2Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.

P55 Health of primate ovarian tissue after long distance transport, J.E. Hornick, F.E. Duncan, M. Xu, and T.K. Woodruff, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

P56 Progesterone and prostaglandins in periovulatory leukocyte infiltration in the rat ovary, Oliver R. Oakley, HeyYoung Kim, Ismail El-Amouri, Po-Ching Patrick Lin, Jongki Cho, Mohammad Bani-Ahmad, Thomas Muse, and CheMyong Ko, Center of Excellence in Reproductive Sciences, Department of Clinical Sciences, College of Health Sciences, University of Kentucky, Lexington, KY.

P57 Role of Wnt4 in the prostate development, Wen-Yang Hu, Ikenna Madueke, Lynn Birch, Dan-Ping Hu, Guang-Bin Shi, Gail S. Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

P58 Characterization and estrogen modulation of human adult prostate progenitor cells, Guang-Bin Shi, Wen-Yang Hu, Ikenna Madueke, Dan-Ping Hu, Jason L. Nelles, Hung-Ming Lam, Gail S. Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

P59 Regulation of Granulosa Cell Proliferation by Retinoic Acid and Cyp26b1 in the Mouse Ovary. Guadalupe Rodriguez1, Ann Golebiowski1, Michael Demczuk1, Eemahn Haralelli1, Kelly Mayo2,3, and Jingjing Kipp1. 1Department of Biological Sciences, DePaul University, Chicago, IL 60614; 2Department of Molecular Biosciences; 3Center for Reproductive Science, Northwestern University, Evanston, IL 60208.

P60 A novel role for prolactin signaling through the short form of its cognate receptor in the ovary. Y. Sangeeta Devi, Anita Seibold, Aurora Shehu, Julia Helparin, Jamie Le, Lei Bao,
P61 **ZPBP2 is one of SBTI-binding proteins during the acrosome reaction of mouse sperm.**
Yuichi Izumi, Misuzu Yamashita, Woojin Kang, and Tadashi Baba, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki

Please take down posters by 4:45 PM

**3:00 PM UIC Alumni Speaker - "Multidisciplinary Team Approach to Translational Research: Opportunities in a Cancer Center "**
Dr. Constance Albarracin, M.D., Associate Professor, Department of Pathology, University of Texas M.D. Anderson Cancer Center, Houston, TX

**ORAL SESSION III: Hormones and Gene Perturbations in Reproductive Diseases**

*Session Moderators: Tyvette Hilliard, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago and Kristen Meldi, Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.*

**3:15 PM T11** A novel human prostate cancer model using adult prostate progenitor cells, W-Y. Hu, G-B. Shi, I. Madueke, D-P. Hu, and G.S. Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

**3:30 PM T12** Generation of human-in-mouse breast cancer models and identification of microRNAs that regulate breast cancer stem cells and metastasis, H. Liu¹², Y. Shimono², J. Bockhorn¹, R. Dalton¹, F. Olopade³, M.F. Clarke², G. Greene¹; ¹Ben May Department for Cancer Research, University of Chicago, Chicago, IL; ²Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Palo Alto, CA; ³Department of Medicine, University of Chicago, Chicago, IL.

**3:45 PM T13** Cofilin and Slingshot localization in the epithelium of uterine endometrium changes during the menstrual cycle and in endometriosis, K.J. Morris, I. Ihnatovych, E. Ionetz, J. Reed, and Z. Strakova, Departments of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL.

**4:00 PM T14** Ovulation induces oxidative stress associated with inflammation in fallopian tube epithelial cells *in vivo*, S.M. King¹, T.S. Hilliard¹, L.Y. Wu¹, A.T. Fazleabas², and J.E. Burdette¹, ¹Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL; ²Department of Obstetrics, Gynecology, and Reproductive Biology, College of Human Medicine, Michigan State University, Grand Rapids, MI.

**4:15 PM T15** Perturbation of estrogen receptor signaling in the hypothalmo-pituitary-ovarian (HPO) axis leads to ovarian tumorigenesis in mice, M.A. Laws¹, A.
Kannan¹, M.K. Bagchi², and I.C. Bagchi¹, ¹Department of Veterinary Biosciences and ²Department of Molecular and Integrative Physiology, University of Illinois, Urbana, IL.

4:30 - 5:15 PM  Wine and Cheese Reception

5:15 - 5:20 PM  Introduction of Keynote Speaker – Shelby M. King, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL.

5:20 - 6:20 PM  Keynote Address – “Gatekeepers of Ovulation and Luteinization” Dr. JoAnne S. Richards, Ph.D., Professor, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.

6:20 – 6:30 PM  Presentation of Awards

6:30 - 6:35 PM  Closing Comments: Dr. Carlos Stocco, Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL.
Abstracts

Oral Sessions

T1:  The conserved RNA-binding protein Boule regulates RNA stability during male germ cell differentiation, M.J.W. VanGompel and E.Y. Xu, Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL.

Post-transcriptional regulation is a common mechanism used throughout germ cell development. In males, for example, during the differentiation of post-meiotic round spermatids into highly specialized motile sperm, transcripts are often stored for several days before they are activated for translation. Though many RNA-binding proteins have been identified in this process, validated mRNA targets of these proteins remain scarce, and how such protein-RNA interactions mediate RNA storage and translation activation remains unclear. Boule is one such RNA-binding protein that is thought to activate the translation of associated mRNAs. Boule is the ancestral member of the DAZ (Deleted in Azoospermia) family, with orthologs in nearly all metazoans, and gave rise to homologs Dazl (DAZ-Like) and DAZ in vertebrates and higher primates, respectively. DAZ genes are important fertility factors in many species, including humans. Boule mutations lead to a pachytene arrest in Drosophila males and C. elegans females, and human BOULE can rescue meiosis in the fly testis, suggesting a conservation of Boule meiotic function. We have shown that Boule is not required for meiosis in mice, but instead is only required for differentiation beyond the round spermatid stage. To further determine Boule function, we sought to identify RNA targets of Boule and any associated functions. Boule was immunoprecipitated from whole testes, and coprecipitating RNA was purified and analyzed. We detected interactions between Boule and mRNAs important for spermatid differentiation. To further determine how Boule regulates these RNA targets, we performed microarrays on control and knockout 24-day old testes, when round spermatids are the most advanced cells present and defects first appear in mutants, and also on control and Boule null purified adult round spermatids. We found that several mRNA targets are absent specifically in mutant round spermatids, suggesting that Boule is involved in RNA stability. This is a novel function for Boule, and the first indication of a role for any DAZ family protein beyond translational activation. Further studies are needed to determine if DAZ and Dazl function similarly, but our results have widened the possibilities of how Boule and the DAZ family control fertility.

T2:  Zinc is required for proper dynamics of maturation promoting factor during mouse oocyte maturation, M.L. Bernhardt1, A.M. Kim1, T.V. O’Halloran2,3, and T.K. Woodruff1,3, 1Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; 2Department of Chemistry, Northwestern University, Evanston, IL; 3Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.

Zinc is essential for many biological processes. Our laboratory recently demonstrated that zinc levels undergo dramatic fluxes during oocyte maturation and that bioavailability of zinc is required for proper meiotic progression. Limiting intracellular zinc availability during in vitro maturation of mouse oocytes using the membrane permeable heavy metal chelator N,N,N',N'-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) causes formation of large polar bodies and arrest at telophase of meiosis I (MI). The goal of this project was to investigate the mechanism by which this “zinc insufficiency” interferes with meiotic maturation, focusing on regulation of maturation promoting factor (MPF), a complex of Cyclin B1 (CCNB1) and CDK1. Histone H1 kinase assays demonstrate that zinc-insufficient oocytes fail to increase MPF activity following the first meiotic division. In zinc-insufficient oocytes, CCNB1 protein levels are greatly reduced following MI,
indicating that failure to re-accumulate CCNB1 is likely the cause of reduced MPF activity. Rescue experiments were performed to test whether this reduction in CCNB1 is responsible for the observed telophase-I arrest. Oocytes in vitro matured in the presence of TPEN were treated with the proteasome inhibitor MG132 following the first meiotic division in order to limit CCNB1 degradation. Proteasome inhibition resulted in a partial recovery of CCNB1 protein levels, as well as a partial rescue of the telophase-I arrest phenotype, with 69% of oocytes showing some degree of MII spindle formation. In addition, expression of a nondegradable form of CCNB1 (Δ90) after MI in zinc-insufficient oocytes also resulted in a partial rescue of meiotic progression; of oocytes with visible first polar bodies, 89% had MII spindles, although many of these spindles were disorganized. MPF activity was also elevated in CCNB1(Δ90) expressing cells, relative to uninjected controls. These results indicate that zinc insufficiency likely impacts pathways controlling CCNB1 levels, causing failure to re-accumulate CCNB1 necessary for cell cycle progression. Current experiments are centered on possible roles for zinc in the control of the anaphase promoting complex/cyclosome (APC/C) ubiquitin ligase responsible for targeting CCNB1 for degradation. Particular focus is being concentrated on potential modulation of the zinc-binding APC/C regulator Emi2. Supported by NIH/NICHHD P01 HD021921 and T32 HD07068 and by the W. M. Keck Foundation.

T3: Infertility in Basigin null mutant male mice may be due to impaired interactions between gametes and Sertoli cells, J. Bi1, Y. Li1, F. Sun2, M.A. Handel2, and R.A. Nowak1, 1Department of Animal Sciences, University of Illinois, Urbana, IL; 2The Jackson Laboratory, Bar Harbor, ME.

Basigin (Bsg) is a multifunctional transmembrane glycoprotein that plays an important role in male reproduction since male knockout (KO) mice are infertile. In the wild type testis, Bsg protein is detectable in all types of germ cells as well as Sertoli cells. The phenotype of the Bsg KO testis is similar to that of the recently described alpha-mannosidase IIx (MX) KO with the testis lacking spermatids and mature spermatozoa. This enzyme regulates formation of intermediate asparagine-linked carbohydrates (N-glycans). N-acetylglucosamine (GlcNAc) terminated N-glycan structures were shown to play a key role in germ cell-Sertoli cell adhesion. The goals of the present study were to determine 1) whether spermatocytes of Bsg KO mice were capable of completing the first or second meiotic divisions; 2) whether there is an increase in apoptosis of spermatocytes in these KOs; and 3) whether GlcNAc terminated N-glycans and MX are altered in the testes of Bsg KO mice. Our results indicated that Bsg KO spermatogonia displayed identical spermatogenic progression to wild type spermatocytes. Bsg KO spermatocytes showed normal homologous chromosome synapse formation and progression to the midpachytene stage as indicated by labeling of the X-Y bivalent with gamma H2AX. In addition, both MI and MII spermatocytes were visible in the KO preps. Next, we found that there was a large increase in the number of germ cells undergoing apoptosis in Bsg KO testes. These cells were determined to be spermatocytes by their position in the seminiferous epithelium as well as their morphology. Next, using lectin blotting, we found that GlcNAc terminated N-glycans are one of the carbohydrates linked to Bsg. In the Bsg KO testis, both GlcNAc terminated Nglycan and MX were significantly reduced. These results indicate that Bsg may regulate the synthesis of MX and also act as a germ-Sertoli cell attachment molecule during spermatogenesis. In conclusion, although Bsg KO spermatogonia can undergo normal progression to the spermatocyte stage, Bsg-mediated germ-Sertoli cell adhesion via GlcNAc terminated N-glycans may be necessary for further spermatocyte progression to mature spermatozoa. Funded by NIH U54 HD40093 to RAN.
T4: Cadmium induced oxidative stress and altered testicular steroidogenesis and spermatogenesis: the protective role of melatonin, N. Joshi\textsuperscript{1,2}, S. Banerjee\textsuperscript{1}, R. Kukherjee\textsuperscript{1}, and A.V. Ramachandran\textsuperscript{1}, \textsuperscript{1}Division of Reproductive Toxicology, Department of Zoology, Faculty of Science, The M.S. University of Baroda, Sayajigunj Vadodara, India; \textsuperscript{2}Department of Obstetrics, Gynecology, and Reproductive Biology, College of Human Medicine, Michigan State University, Grand Rapids, MI.

Cadmium (Cd) induced testicular toxicity was assessed in the context of its role as an environmental toxicant. The study undertaken in the rats was designed to reflect the conditions to which humans are exposed to Cd in their diet in industrialized areas such as Vadodara (India). Adult Wistar rats were exposed to Cd (9mg/Kg BW) through drinking water with or without simultaneous administration (i.p.) of melatonin (10mg/Kg BW) at 1800hrs for 15 days. All animals were maintained as per the guidelines of CPCSEA, India. On completion of the treatment schedule, animals were sacrificed and testes were used for estimation of metal load by inductive coupled plasma atomic emission spectroscopy and enzymatic (SOD, CAT, GPx) and non enzymatic (GSH, Vit. C) antioxidants along with lipid peroxidation and the activity levels of 3βHSD and 17βHSD using appropriate assay procedures. Histopathology of testes, epididymal sperm analysis and assay of serum titres T, E2 & Melatonin were also carried out. Cadmium treatment resulted in significant increase in lipid peroxidation and decrease in antioxidant levels together with decreased activity of steroidogenic enzymes and lowered serum T and E2 levels. Significant increase in testicular cadmium load and reduced serum melatonin level were also observed. Disruption in spermatogenesis and severe sperm abnormality were also a result of Cd exposure. Co-administration of melatonin showed significant protection against the Cd induced toxicity. Overall the present study clearly shows toxic effects of Cd on the male reproductive system in rats and, a potent action of melatonin in protecting against the Cd induced toxic effects.

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<tr>
<th>Treatment</th>
<th>C</th>
<th>C + M</th>
<th>Cd</th>
<th>Cd + M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml of serum)</td>
<td>3.59 ± 0.05</td>
<td>3.07 ± 0.05\textsuperscript{a}</td>
<td>1.83 ± 0.01\textsuperscript{b}</td>
<td>2.61 ± 0.02\textsuperscript{c}</td>
</tr>
<tr>
<td>Estradiol (ng/ml of serum)</td>
<td>34.5 ± 0.22</td>
<td>33.01 ± 0.12\textsuperscript{a}</td>
<td>26.8 ± 0.19\textsuperscript{b}</td>
<td>27.45 ± 0.34</td>
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Di-n-butyl phthalate (DBP) is an industrial solvent present in many consumer products such as cosmetics, insecticides, nail polish, some printing inks and the coating of certain oral medications. In animal studies, fetal exposure to DBP has been shown to cause decreased levels of testosterone, testicular atrophy and Sertoli cell abnormalities in male rats. In female rats, exposure to DBP from weaning through puberty, mating and gestation has been shown to cause midpregnancy abortions and decreased ex vivo hormone production. Further, di-2-ethylhexyl phthalate (DEHP), another widely used phthalate, has recently been shown to inhibit antral follicle growth and reduce estradiol production in vitro. However, our knowledge about the effects of DBP exposure on ovarian function is to date very limited. This study was designed to determine if DBP directly targets mouse ovarian follicles and alters follicle growth in vitro. We hypothesized that DBP, like DEHP, targets ovarian follicles and suppresses follicular growth in vitro. To test this hypothesis, antral follicles isolated from female CD-1 mice (ages 34-35 days old; 6-10 follicles/group) were exposed to media containing dimethylsulfoxide (DMSO, vehicle control) or DBP (1-100 μg/ml equivalent to 3.59 - 359.2 μM) in culture for 96 hours. Antral follicle diameter was measured every 24-hour period to assess follicular growth. Follicle diameter data were
expressed as percent change and averaged from 3 separate experiments. DMSO-treated follicles remained viable and grew throughout the entire 96-hour culture period (100 to 130.8 ± 3.2 % change over time) while follicles treated with DBP showed decreased growth (DMSO: 130.8 ± 3.2 %; 1 μg/ml: 127.4 ± 5.6 %; 10 μg/ml: 117.8 ± 5.1%; 100 μg/ml:108.2 ± 6.8 %; n=3; P<0.05). These results show that DBP treatment suppresses mouse antral follicle growth in vitro and support the hypothesis that the adult mouse ovary is a target for DBP toxicity. Supported by NIH R01 ES012893 (JAF), R01 ES019178 (JAF) and a Billie A. Field Fellowship in Reproductive Biology (ZRC).

T6: SF-1 driven steroidogenic differentiation of murine embryonic stem cells. Unmesh Jadhav, J. Larry Jameson, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611.

Steroidogenic Factor-1 (SF-1) is a nuclear receptor expressed in multiple cell types within the Hypothalamic-Pituitary-Gonadal-Adrenal axis. SF-1 is known to control multiple processes like development, differentiation and maintenance of cells in each of these organs. It also plays an important role in steroid production in the gonads (testis, ovary) and the adrenal gland. SF-1 acts as a master transcriptional regulator to control multiple genes involved in these processes. As SF-1 knockout mice show complete agenesis of the adreno-gonadal primordium, it can't be used as in vivo model to study the molecular actions of SF-1 in early development of steroidogenic cells and tissues. Embryonic stem cells can be induced to differentiate into multiple cell lineages in culture. These in vitro systems provide useful models for identification of key regulatory genes involved in differentiation and developmental processes and their mechanisms of action. We created ES cell lines that stably express SF-1 (SF-1-ESCs) and subjected these cells to multiple conditions that elicit steroidogenic differentiation. Under these conditions, we saw marked differences in cell morphology and cell proliferation/survival between native ES cells and SF-1-ESCs. Differentiating SF-1-ESCs showed induction of genes involved in cholesterol processing, steroidogenesis and adreno-gonadal development. Upon differentiation through embryoid bodies, the SF-1-ESCs also showed increased induction of early lineage markers of the mesendodermal lineage as compared to the native ES cells. We developed a 2D culture protocol for effective differentiation of SF-1-ESCs that produces homogeneous population of steroidogenic cells in vitro. The SF-1-ESCs thus provide a valuable model to study the role of SF-1 in early differentiation events towards the steroidogenic lineage. To study the molecular targets of SF-1, we created cell lines expressing biotin tagged SF-1 (Bio-SF-1). Biotin-ChIP assays using these lines showed binding of SF-1 to the promoter regions of known target genes. We are currently using Biotin-ChIP assays and high throughput expression analysis to study transcriptional control by SF-1 in these cells. Grant Support: NIH RO1 HD044801.

T7: The Notch inhibitor Numb is present in gonadotropes and may be necessary for regulating LH expression and function, L.B. Goldberg, T.B. Moran, K.E. Brannick, and L.T. Raetzman, Department of Molecular and Integrative Physiology, University of Illinois, Urbana-Champaign, IL.

The synthesis and release of the gonadotropins LH and FSH from the anterior pituitary are essential for fertility in males and females. Many cases of infertility that present with altered gonadotropin levels are idiopathic, making the identification of genes controlling gonadotrope development and function critically important. Notch signaling may be necessary for early gonadotrope lineage determination, however, it must be suppressed for timely hormone production in future gonadotropes. A candidate for suppressing Notch activity is Numb, which is known to cause degradation of the Notch receptor. Although Numb is known to suppress Notch during
development, it can also play a role in adult homeostasis. In fact, loss of Numb is correlated with ER, PR-negative breast cancer and Numb has been shown to play a critical role in regulating p53 stability. In the present study, we show by immunostaining that Numb is detectable in αGSU expressing cells in the anterior lobe beginning at e15.5 and is preferentially localized in LH and FSH containing cells in the anterior lobe of the adult mouse pituitary. To determine the function of Numb in the gonadotropes, Cre recombinase driven by the αGSU promoter was used to delete Numb and its homolog Numblike in gonadotropes and thyrotropes. During the period of gonadotrope specification, from e15.5 to e16.5, loss of Numb results in higher levels of LH in gonadotropes. The increase in LH levels appears to be ameliorated by birth, as confirmed by the observation that LH mRNA levels are similar between 21 day old male or female wildtype and conditional knockouts(cKOs). Although LH mRNA levels are not changed in adult mice, we find that cKO females display precocious puberty, with vaginal opening four days earlier than wildtype litters. All cKO females used for breeding are fertile, however, the litter size is reduced to an average of 3.8 pups per litter as compared to 7.7 pups per litter in wildtypes. While female cKOs have decreased reproductive fitness, all cKO males used for breeding have produced litters of the same size as their wildtype litters. This study suggests Numb may have a previously unidentified critical role in gonadotropin synthesis and/or secretion, impacting timing of puberty and reproductive fitness in female mice. Future studies will be critical in order to elucidate the mechanism by which Numb affects gonadotrope function. Supported by NIH Grant R01 DK076647A to L.T.R.

T8: Germ-cell specific deletion of Jagged1, a Notch ligand, leads to the formation of multi-oocytic follicles in the ovary, Dallas A. Vanorny, Signe M. Kilen, and Kelly E. Mayo, Department of Molecular Biosciences and Center for Reproductive Science, Northwestern University, Evanston, IL.

Previous work from our laboratory has suggested an important role for Notch signaling in the breakdown of germ cell nests and the formation of primordial follicles within the mouse ovary. In the murine ovary, Notch2 is the most highly expressed Notch receptor and is restricted to granulosa cells, while Jagged1, the most highly expressed Notch ligand, localizes to germ cells. The demarcation of Notch2 and Jagged1 expression suggests the importance of Notch signaling for somatic and germ cell communication. To investigate the potential roles of Notch signaling within the ovary, Jagged1 was conditionally deleted using a VasaCre line that expresses Cre-recombinase specifically within germ cells beginning 12.5 dpc. Ovaries from newborn mice conditionally deleted for Jagged1 in germ cells (J1-/-) and ovaries from heterozygous (J1+/-) controls both have germ cells contained within nests. This suggests that Notch signaling within the ovary may not be important for the formation of nests. However, by Day 5, J1-/- ovaries possess structures resembling seminiferous tubules, devoid of germ cells, but containing Sertoli-like cells found along the basal lamina of the tubules, a phenotype also seen in models where germ cells are lost. In addition, Day 5 J1-/- ovaries contained multi-oocytic follicles (MOFs), defined as follicles containing more than one oocyte. Examination of Day 21 J1-/- ovaries revealed the extensive presence of MOFs, some containing as many as six oocytes within a single follicle. MOFs were only rarely seen in J1+/ controls. The presence of MOFs may suggest that nest breakdown is being delayed or inhibited by disruption of Notch signaling, consistent with earlier results from our group in which γ-secretase inhibitors were used to inhibit Notch signaling. Interestingly, the tubular structures were absent from Day 21 J1-/- ovaries suggesting that they may be transient. Current studies are aimed at better understanding the mechanisms by which loss of Jagged1 in the oocyte leads to abnormal follicle development. Supported by NIH/NICHD P01 HD021921 and CMBD Training Grant T32 GM12453.
**T9: Novel induction of EMMPRIN release via the cell surface G protein coupled estrogen receptor GPER, L.A. Burnett¹², R.A. Nowak¹, ¹Department of Animal Sciences; ²College of Medicine, University of Illinois, Urbana, IL.**

Metastatic cancer cell invasion and normal tissue remodeling in the uterus are both highly dependent on matrix metalloproteinases (MMPs). Secretion of MMPs can be induced by the transmembrane, glycosylated protein known as Extracellular Matrix Metalloproteinase Inducer (EMMPRIN). EMMPRIN expression is highest in luminal and glandular epithelial cells during the late proliferative phase of the menstrual cycle suggesting that expression is E dependent. Previous studies in our laboratory have shown that estrogen (E) increases EMMPRIN protein expression and release by uterine epithelial cells in vitro and that EMMPRIN induces MMP production by uterine stromal cells. Estrogen can elicit cellular response by interacting with conventional nuclear estrogen receptors or alternatively by acting on the cell surface estrogen receptor GPER. Clinically GPER overexpression is correlated with highly invasive endometrial adenocarcinomas and poor survival as endometrial carcinomas with overexpression of GPER more frequently exhibited deep myometrial invasion. These studies suggest that GPER is an E responsive receptor that is over-expressed and functionally relevant in uterine endometrial cells. Here we examined the role of GPER signaling on EMMPRIN production and secretion in uterine epithelial cells (EECs) to provide insight into E-mediated cell invasion and proliferation. We found that GPER mRNA is expressed in EECs indicating that GPER may play a role in E responsiveness in these cells. We also found that treatment with the GPER-selective agonist G-1 results in induction of EMMPRIN release comparable to that seen with estrogen. In contrast EMMPRIN mRNA levels remained unchanged with G-1 or estrogen stimulation. These findings confirm that GPER is present in EECs and show that stimulation of signaling through GPER promotes EMMPRIN protein release but does not alter EMMPRIN mRNA transcription. These data support a novel mechanism of estrogen-mediated EMMPRIN release via stimulation of the G protein coupled estrogen receptor GPER. Supported by NIH U54HD40093.

**T10: Genetic polymorphism in the aryl hydrocarbon receptor gene is associated with an increased risk of hot flashes in generally healthy midlife women, Ayelet Ziv-Gal, Jodi A. Flaws, Department of Comparative Biosciences, University of Illinois, Urbana, IL.**

The aryl hydrocarbon receptor (AHR) is a ligand–activated nuclear transcription factor that is present in numerous tissues including reproductive ones. Absence of the AHR in mice results in impaired reproductive function. Specifically, the AHR plays a role in ovarian function by modulating estradiol production and regulating the onset of reproductive senescence. Interestingly, the most reported symptom by women who undergo reproductive senescence is hot flashes (HFs). HFs are a worldwide debilitating condition, but yet the definitive etiology and risk factors for them are unclear. HFs are mainly associated with low estradiol levels, smoking, obesity, race/ethnicity, molecular variation in genes encoding enzymes of the estrogen biosynthesis and metabolic pathway, and the later menopausal transition stages. The involvement of the AHR in the female reproductive system along with its effects on enzyme activity (e.g. CYP1B1, CYP1A1) and steroidogenesis have led us to examine whether a nonsynonymous genetic polymorphism (SNP) in the AHR gene (R554K) is associated with HFs. Specifically, we examined whether a SNP in the AHR gene was associated with ever experiencing HFs, or the severity, frequency and duration of HFs. To test this hypothesis, we genotyped 633 DNA samples (isolated from peripheral blood lymphocytes) that were collected in a cross-sectional study of generally healthy midlife women using allele specific polymerase chain reactions (AS-PCR) and agarose gel electrophoresis. Associations between the AHR (R554K) polymorphism, and HFs then were statistically analyzed using regression analysis.
The results indicate that carrying the selected SNP in the AHR (R554K) is significantly associated with daily HFs (odds ratio: 1.97; 95% confidence interval: 1.04-3.72, after adjusting for race, BMI, and age). However, the selected SNP was not significantly associated with ever experiencing HFs or duration and severity of HFs. Collectively, these data suggest that the AHR may play a role in the experience of daily menopausal HFs. Supported by: NIH R01 AG18400.

**T11:** A novel human prostate cancer model using adult prostate progenitor cells, Wen-Yang Hu, Guang-Bin Shi, Ikenna Madueke, Dan-Ping Hu, Gail S. Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

Prostate cancer is the leading diagnosed non-cutaneous malignancy for men in the US. Recent identification of prostate stem cells and prostate cancer stem cells strongly supports the stem cell hypothesis for this malignancy, that a small number of stem cells are responsible for prostate cancer initiation and progression, and subsequently recapitulate the disease. It has been long understood that sex hormones are involved in regulating the development of prostate cancer. Progress toward understanding the hormonal carcinogenesis in prostate cancer has been hindered by the lack of a suitable research model. We have developed a method to isolate and characterize human prostate progenitor cells that can regenerate normal prostate tissue in vivo. Prostate stem cells were isolated and enriched from primary normal prostate epithelial cells (PrEC) using a 3-D culture prostasphere assay. Approximately 0.2% of the PrEC can self-renew and form prostaspheres in this culture system. The differentiation ability of prostaspheres was confirmed by the formation of lumen-like and ductal branching structures in vitro. Regenerative capacity of these prostasphere cells was demonstrated by formation of humanized prostate gland tissue in vivo following tissue recombination and grafting under the renal capsule of nude mice. Differentiation of human prostasphere cells in grafts was confirmed by immunohistochemical analyses using prostate basal (p63) and luminal (CK8/18, AR) epithelial cell markers. The human origin and functionality of the regenerated grafts were confirmed by expression of human antinuclear antigen and secretion of prostate specific antigen, respectively. Furthermore, we successfully induced prostate cancer in these tissues by exposing to elevated testosterone and 17-β-estradiol for 2 months. By using open biopsy, we were able to longitudinally monitor the process of cancer initiation in stem cell-generated prostate tissue progressing from normal through epithelial hyperplasia, intraepithelial neoplasia and malignant tumor. We have generated a true model of human prostate cancer initiation and progression from normal prostate stem cells to full malignancy. Unlike using human cancer cell lines or tissue from histologically normal regions of prostatectomy specimens which may be affected by a pre-malignant field effect, the unique and novel idea of the human prostate cancer model that we have established and applied, originated from normal human prostate stem cells. This study was supported by NIEHS grant RC2 ES 018758.

**T12:** Generation of human-in-mouse breast cancer models and identification of microRNAs that regulate breast cancer stem cells and metastasis, H. Liu1,2, Y. Shimono2, J. Bockhorn1, R. Dalton1, F. Olopade3, M.F. Clarke2, G. Greene1; 1Ben May Department for Cancer Research, University of Chicago, Chicago, IL; 2Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Palo Alto, CA; 3Department of Medicine, University of Chicago, Chicago, IL.

Cancer stem cells (CSCs) have been identified from various tumor types including breast cancer. However, it is not clear whether CSCs mediate metastasis. To examine the role of breast cancer stem cells (BCSCs) in metastasis, we generated human-in-mouse breast cancer orthotopic models using patient tumor specimens, labeled with optical reporter fusion genes. These models are maintained in NOD/SCID mice but recapitulate human cancer features not captured with previous
cell line models, including spontaneous metastasis in particular, and provide a useful platform for studies of breast tumor initiation and progression. With non-invasive imaging approaches, as few as 10 cells of stably labeled BCSCs could be tracked in vivo, enabling studies of early tumor growth and spontaneous metastasis. These advances in BCSC imaging revealed that CD44+ cells from both primary tumors and lung metastases are highly enriched for tumor initiating cells. With the established human-in-mice breast cancer models, we are investigating the role of miRNAs in regulating BCSCs and metastasis. We demonstrated clusters of miR-200 family members regulate both human BCSCs and normal mammary stem cells, by targeting Bmi1. Identification of additional miRNAs regulating both BCSCs and metastasis is in progress. In combination with human breast cancer gene profiles, miRNA-gene network will give us the comprehensive signals contributing to personalized medicine in the future. Funded by DOD BCRP Postdoctoral Fellowship, University of Chicago Fellows Program, University of Chicago Cancer Research Center Pilot Project Award, and University of Chicago BSD Imaging Research Institute Pilot Project Award.

T13: Cofilin and Slingshot localization in the epithelium of uterine endometrium changes during the menstrual cycle and in endometriosis, K.J. Morris, I. Ihnatovych, E. Ionetz, Jennifer Reed, and Z. Strakova, Departments of Obstetrics and Gynecology University of Illinois at Chicago, Chicago, IL, 60612.

Regulation of the actin cytoskeleton plays a role in establishment of epithelial cell polarity and protein trafficking within human uterine epithelium. Actin dynamics are regulated by actin binding proteins such as cofilin. Inactive cofilin binds to monomeric G-actin; active cofilin promotes depolymerization of F-actin and cytoskeleton reorganization. We have previously shown that cofilin participates in actin dynamics regulation during decidualization of human stromal fibroblasts (Ihnatovych et al., 2009). To investigate cofilin in human endometrium during the menstrual cycle, we localized cofilin through immunohistochemical staining. During the proliferative phase of the menstrual cycle, dual staining of cofilin and G-actin (represented by staining for DNAse I) indicated cofilin-G-actin colocalization in both the apical and basolateral sides of luminal epithelial cells. Interestingly, during the secretory phase of the menstrual cycle this colocalization was only present on the basolateral side of luminal epithelium cells; cofilin was not detected on the apical part. To investigate whether the disease endometriosis will cause a different pattern of actin remodeling in endometrium, we investigated the localization of cofilin and cofilin regulators (LIM kinase 1/2, pLIMK1/2, slingshot, and 14-3-3) in a baboon (Papio anubis) model of induced endometriosis. The staining pattern of cofilin in healthy baboons confirmed our previous observations in human tissue. However, the cofilin pattern in secretory phase endometriosis baboon epithelium was very similar to the proliferative phase in normal animals; cofilin was observed in the apical parts of the luminal and glandular epithelium. The phosphatase regulating activity of cofilin, slingshot, revealed a similar staining pattern within the tissues. These patterns were confirmed through quantitative image analysis. Real-time PCR quantification revealed that the mRNA expression levels of slingshot and LIMK2 were upregulated in endometriosis. Our data indicate that there is a severe dyssynchrony in menstrual cycle phases in animals with endometriosis which is connected with improper cytoskeleton rearrangements. In summary, we suggest that cofilin-mediated actin reorganization of uterine epithelial cells during the secretory phase might be important in preparation for implantation; disregulation of this reorganization may lead to decreased fertility in women suffering from endometriosis. ARRA NIH HD044713 grant to ZS.

T14: Ovulation induces oxidative stress associated with inflammation in fallopian tube epithelial cells in vivo, S.M. King1, T.S. Hilliard1, L.Y. Wu1, A.T. Fazleabas2, and J.E. Burdette1,
Ovarian cancer is the most lethal gynecological malignancy affecting American women. Advanced serous carcinoma shares several biomarkers with fallopian tube epithelial cells, indicating that some forms of ovarian carcinoma may originate in the fallopian tube. Current hypotheses concerning the etiology of ovarian cancer propose that a reduction in the lifetime number of ovulations reduces ovarian cancer risk. However, the impact of ovulation on the tubal epithelium is unknown. In CD1 mice, ovulation did not increase tubal epithelial cell proliferation as measured by bromodeoxyuridine incorporation and proliferating cell nuclear antigen staining as compared to unstimulated animals. However, ovulation induced increased levels of phospho-γH2A.X in tubal epithelial cells, indicating that these cells were susceptible to double-strand DNA breakage in response to ovulation. To determine which components of ovulation contributed to DNA damage in the fallopian tube, an immortalized baboon tubal epithelial cell line and a three-dimensional organ culture system for mouse oviduct and baboon fallopian tubes were developed. Tubal epithelial cells did not proliferate or display increased DNA damage in response to the gonadotropins or estradiol alone. *In vivo*, ovulation induced macrophage infiltration into the oviduct, providing a local source of inflammation. Oxidative stress generated by treatment with hydrogen peroxide increased DNA damage in tubal epithelial cells in culture. Ovulation may impact fallopian tube epithelium by upregulating inflammatory pathways and generating oxidative stress. This work was supported by BIRCWH grant K12HD055892, NIH grant R03CA139492, NIH grant C06RR15482, UIC Cancer Center grant, and UIC Center for Clinical and Translation Science grant to J.E.B. This research was supported by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement [(U54HD40093 to A.T.F.) as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research.

**T15:** Perturbation of estrogen receptor signaling in the hypothalamo-pituitary-ovarian (HPO) axis leads to ovarian tumorigenesis in mice, M.A. Laws\(^1\), A. Kannan\(^1\), M.K. Bagchi\(^2\), and I.C. Bagchi\(^1\), \(^1\)Department of Veterinary Biosciences and \(^2\)Department of Molecular and Integrative Physiology, University of Illinois, Urbana, IL.

Ovarian cancer is the most lethal malignancy of the female reproductive system. Due to the absence of specific symptoms and the lack of strategies for early detection of ovarian malignancies, the majority of women with ovarian cancer are diagnosed at a late stage. The etiology of ovarian cancer is poorly understood, mainly due to the lack of an appropriate experimental model for studying the onset of this disease. We have recently developed a mouse model in which aberrant estrogen receptor alpha (ER\(\alpha\)) signaling in the HPO axis leads to ovarian tumorigenesis. In this mouse model, termed ER\(\alpha\)d/d, a conditional deletion of ER\(\alpha\) gene occurred in the anterior pituitary, but ER\(\alpha\) expression remained intact in the hypothalamus and the ovary. Selective ablation of ER\(\alpha\) in the pituitary created a systemic hormonal imbalance in these mice. Loss of the negative-feedback regulation of estrogen (E) in the pituitary led to elevated levels of luteinizing hormone (LH). LH hyperstimulation of ovarian cells resulted in elevated steroidogenic activities, leading to high circulating levels of steroid hormones. The ER\(\alpha\)d/d mice exhibited formation of ovarian tumors with 100% penetrance. These tumors grew up to 11 mm in size by 8 months of age and most mice died 12 months. The ER\(\alpha\)d/d mice displayed an elevated ER\(\alpha\) signaling in the surface epithelial cells of the ovary, which is apparently linked to ovarian tumorigenesis. We observed a marked elevation in the expression of the transcriptionally active, phosphorylated ER\(\alpha\) (at Ser-118) in the epithelial cells of ER\(\alpha\)d/d compared to its wild-type counterparts. The phosphoinositide-3 kinase/AKT pathway was also activated, as indicated by increased expression of phospho-AKT in
the ovaries of mutant mice. Immunohistochemical analyses revealed that the cells within the ovarian tumors of ERα/d/d mice are characterized by intense expression of cytokeratin 8 and 19 as well as nuclear staining of Wilms tumor 1, a well known marker of ovarian tumorigenesis. In summary, we have developed an animal model, which will serve as a powerful tool for exploring the involvement of ERα-dependent signaling pathways in the etiology of ovarian cancer.

**Poster Section**


Bisphenol A (BPA) is used as the backbone for plastics and epoxy resins, including various food and beverage containers. BPA has also been detected in 95% of random urine samples and ovarian follicular fluid of adult women. Few studies have investigated the effects of BPA on antral follicles, the main producers of sex steroid hormones and the only follicles capable of ovulation. Thus, this study tested the hypothesis that postnatal BPA exposure inhibits antral follicle growth and steroidogenesis. To test this hypothesis, antral follicles isolated from 32-day-old FVB mice were cultured with vehicle control (dimethylsulfoxide; DMSO), BPA (4.4-440µM), pregnenolone (10µg/mL), pregnenolone + BPA 44µM, and pregnenolone + BPA 440µM. During the culture, follicles were measured for growth daily. After the culture, media was subjected to enzyme-linked immunosorbent assays for hormones in the estradiol biosynthesis pathway, and follicles were processed for quantitative real-time polymerase chain reaction of steroidogenic enzymes. The results indicate that BPA (440µM) inhibits follicle growth and that pregnenolone co-treatment was unable to restore/maintain growth. Further, BPA 44µM and 440µM inhibit progesterone, dehydroepiandrosterone, androstenedione, estrone, testosterone, and estradiol production. Pregnenolone co-treatment was able to increase production of pregnenolone, progesterone, and dehydroepiandrosterone and maintain androstenedione and estrone levels in BPA treated follicles compared to DMSO controls, but was unable to protect testosterone or estradiol levels. Further, pregnenolone was unable to protect follicles from BPA (44-440µm) induced inhibition of steroidogenic enzymes compared to the DMSO control. Collectively, these data show that BPA targets the estradiol biosynthesis pathway in the ovary. This work was supported by the National Institute of Health [R01 ES019178].

**P2: Provision of emergency contraception after sexual assault: a national survey, 2004 and 2009, A Patel, MD, MPH1, S Tilmon, MPH1, A Sheth, MD1, L Nguyen, MD1, S Chaparala, MD1, L Keith, MD, PhD2, 1Division of Family Planning, Department of Obstetrics and Gynecology, John H. Stroger Jr. Hospital of Cook County, Chicago, IL; 2Feinberg School of Medicine, Northwestern University, Chicago, IL.**

OBJECTIVE: A random sample of U.S. and territories’ emergency departments were surveyed in 2004 and again in 2009 to obtain information on sexual assault victims and their ability to obtain emergency contraception provision at point of visit. DESIGN: A representative sample was assembled; 20% of hospitals stratified by state/territory were selected from the American Hospital Association list in order to conduct a 13-question telephone survey. Questions included: “Is there a written protocol for counseling about emergency contraception to sexual assault patients?”; “Are sexual assault patients at risk of pregnancy counseled about emergency contraception?”; and “Are sexual assault patients at risk of pregnancy provided emergency contraception?”. A cross-sectional prevalence survey was administered in 2004 and again in 2009.
SETTING: Emergency departments, the main point of care for recent sexual assault victims.

PARTICIPANTS: Emergency department personnel (e.g., charge nurses).

OUTCOME MEASUREMENTS: Reported provision of emergency contraception to sexual assault victims.

RESULTS: Provision of emergency contraception has changed very little from 63% in 2004 to 66% in 2009. Provision varies by number of victims seen, region of the country and state legislation status.

CONCLUSIONS: Provision of emergency contraception for sexual assault victims in emergency departments has not greatly increased over time and does not reflect regulatory changes, such as over-the-counter administration. Prophylaxis against possible pregnancy is an important and necessary part of treatment and its provision should be improved. Prophylaxes against STIs and pregnancies are handled differently for SA survivors, perhaps reflecting a separation of sexual health and reproductive health in clinical practice. This research was not funded.

P3: Comprehensive medical care management to victims of sexual assault: a national survey of hospital emergency departments in United States, A Patel, MD, MPH1, Sushma Chaparala, MD1, Sandra Tilmont, MPH1, Luan Nguyen, MD1, Amish Sheth, MD1, Mrunal Ghanshyam Parab, MD1, L Keith, MD, PhD2, 1Division of Family Planning, Department of Obstetrics and Gynecology, John H. Stroger; Jr. Hospital of Cook County, Chicago, IL; 2 Feinberg School of Medicine, Northwestern University, Chicago, IL.

BACKGROUND: Sexual assault, a major public health problem affecting 17.7 million women in the United States, results in a variety of physical, emotional and psychological complications. Hospital emergency departments often are the primary points of care for these women. OBJECTIVES: (1) To describe the medical care services provided to sexual assault victims in hospital emergency departments; and (2) to identify the percentage of hospitals which always provide all 10 elements of comprehensive medical care management. METHODS: A representative sample of the 50 states and territories was assembled; 20% of hospitals in each state/territory were selected from the American Hospital Association (AHA) list in order to conduct a 13-question telephone survey. Information on the following items was collected: (1 and 2) acute medical care, including history and physical examination; (3 and 4) acute and long-term rape crisis counseling; (4 and 5) testing and prophylactic therapies for sexually transmitted infections; (6 and 7) counseling and provision of emergency contraception (EC); and (9 and 10) testing and prophylactic therapy for HIV. RESULTS: An 84% response rate of eligible hospital emergency departments provided the following results: 100% provided acute medical care upon presentation; 69% provided rape crisis counseling; (4 and 5) testing and prophylactic therapies for sexually transmitted infections; (6 and 7) counseling and provision of emergency contraception (EC); and (9 and 10) testing and prophylactic therapy for HIV. Only 17% of hospitals provide all 10 elements of comprehensive medical care management. CONCLUSION: Although published recommendations from various medical organizations support provision of all ten elements of comprehensive medical care management to victims of sexual assault, less than one-fifth of American hospitals provide comprehensive medical care management. Improvements must be made nationwide to provide quality comprehensive medical care to victims of sexual assault. This research was not funded.

P4: Methoxychlor induces atresia and decreases estradiol levels in antral follicles simultaneously, M. S. Basavarajappa, I. Hernández-Ochoa, J. Singh, and J. A. Flaws, Veterinary Biosciences, University of Illinois, Urbana, IL.

The endocrine disruptor methoxychlor (M XC) is organochlorine pesticide widely used in many countries against insects that attack field crops, vegetables, fruits, gardens, livestock, and domestic pets. M XC exposure causes adverse effects on reproductive functions in adult females and induces ovarian atrophy. Further, M XC inhibits growth and induces atresia of mouse antral follicles.
in vitro. Previous studies have shown that MXC induces atresia and decreases estradiol levels after 96 hrs of follicle culture. The goal of the present study was to determine whether MXC induces atresia and decreases estradiol levels earlier than 96 hrs of culture. Further, the present study was designed to determine whether MXC-induced changes in atresia precede MXC-induced changes in estradiol levels or vice versa. To complete these goals, antral follicles were mechanically isolated from ovaries of CD1 female mice aged 35-40 days. The isolated antral follicles (10-15 per treatment) were cultured in supplemented α- minimum essential media in the presence of dimethylsulfoxide (control; DMSO) or MXC (1, 10, 100 μg/ml) for 24 and 48 hrs at 37ºC and 5%CO2. After culture, the media was collected and follicles were fixed in Dietrick’s solution. Fixed follicles were embedded in plastic blocks, cut into 2 μm sections, stained with Lee’s methylene blue-basic fuchsin, and graded for atresia on a scale of 1 to 4 based on the percentage of apoptotic bodies (1=0%, 2=<10%, 3=10-30%, and 4=>30% apoptotic bodies). The media was subjected to measurements of estradiol levels by enzyme-linked immunosorbent assays. The results show that MXC does not induce atresia at 24 hrs, but it does induce atresia by 48 hrs (24 hrs: DMSO = 1.73 ± 0.11, MXC1 μg/ml = 1.59 ± 0.16, MXC10 μg/ml = 1.78 ± 0.20, MXC100 μg/ml = 1.90 ± 0.16; 48 hrs: DMSO = 1.25 ± 0.14, MXC1 μg/ml = 1.79 ± 0.09, MXC10 μg/ml = 2.22 ± 0.15, MXC100 μg/ml = 4 ± 0; n=3 separate experiments; p ≤ 0.05 for DMSO versus MXC at 48 hrs). The results also indicate that MXC does not alter estradiol levels at 24 hrs, but it does decrease estradiol levels by 48 hrs (24 hrs: DMSO = 17.10 ± 4.98, MXC1 μg/ml = 18.73 ± 2.50, MXC10 μg/ml = 26.46 ± 7.95, MXC100 μg/ml = 18.63 ± 4.27 pg/ml; 48 hrs: DMSO = 599.38 ± 68.97, MXC1 μg/ml = 225.65 ± 18.79, MXC10 μg/ml = 120.48 ± 35.31, MXC100 μg/ml = 32.85 ± 2.97 pg/ml; n=3 separate experiments; p ≤ 0.05 for DMSO versus MXC at 48 hrs). Collectively, these data suggest that MXC decreases estradiol levels and induces atresia as early as 48 hrs and that these effects of MXC on antral follicles occur simultaneously. Support: NIH R01 ES012893 and the Environmental Toxicology Program at UIUC.


Leiomyomas (uterine fibroids) cause significant morbidity of 25% of women in their 30’s. Leiomyomas are the top reason for hysterectomy in pre-menopausal women in the U.S. largely because therapies are limited and unsafe for long term use. Progesterone (P), PI3K, and AKT are thought to be important for tumor growth although detailed signaling leading to proliferation are unknown. We hypothesize that progesterone promotes PI3K activation in leiomyoma cells involving an interaction between PR and PI3K. We examined activation of AKT following treatment with P. AKT phosphorylation (p-AKT) and GSK3b, a direct target of AKT, phosphorylation (p-GSK3b) increased in a time dependent manner in myo-hTERT (immortalized myometrial) and DD-HLM (immortalized leiomyoma) cells following treatment with 100nM P. Overexpression of wild type p85a, the regulatory subunit of PI3K, enhanced p-AKT and p-GSK3b in response to P and at the basal level. Expression of PRA and PRA/B promoted p-AKT while only PRA promoted p-FOXO1, another AKT substrate, and p-GSK3b in response to P. Importantly PR and p85a interacted in T47D cells with and without P. We next examined DD-HLM and myo-hTERT cell proliferation in the presence of R5020 (R5) and PI3K inhibitors. Treatment with R5 promoted cell proliferation of DD-HLM cells while myo-hTERT cells experienced a decrease in cell proliferation. PI3K inhibition decreased myo-hTERT and DD-HLM cell proliferation. In summary, differences between tumor and normal cells were seen at the level of cell proliferation in response to R5 and short term signaling to AKT substrates depended upon PR isoform. Overall, these results suggest that P can activate PI3K and that AKT, GSK3b, and FOXO1 are regulated
downstream. We have also shown for the first time an interaction between PR and p85a in mammalian cells. We are continuing experiments to support that PR activates PI3K in leiomyoma cells leading to cell proliferation. Supported by NIH P01HD057877.

**P6:** The Notch effector gene *Hes1* regulates migration of hypothalamic neurons, neuropeptide content and axon targeting to the pituitary, Paven K. Aujla¹, Adriana Bora², Pamela Monahan¹, Jonathan V. Sweedler² and Lori T. Raetzman¹, Department of Mol. Integrative Physiol.¹, Department of Chemistry², University of Illinois at Urbana-Champaign, Urbana, IL.

The failure of the hypothalamus and pituitary to form coordinately during embryonic development can lead to hypopituitarism, with infertility as a possible consequence. The hypothalamic magnocellular neurons that synthesize arginine vasopressin (AVP), found in the paraventricular (PVN) and supraoptic nucleus (SON), send their terminal axons to the posterior pituitary to regulate homeostasis. Parvocellular hypothalamic neurons that synthesize somatostatin (SS) originate in the anterior periventricular nucleus (aPV) and send their axons to the median eminence (ME) to regulate growth. We hypothesized that the Notch signaling pathway is necessary to specify AVP and SS neurons and to project their axons to the pituitary. The Notch signaling effector gene *Hes1* is present in the developing pituitary and hypothalamus. *Hes1* null mice survive until embryonic day 18.5 (e18.5) and show reduction in the size of the posterior pituitary, which contains the terminal axons of AVP neurons. We found that *Hes1* null animals show no significant difference in cell proliferation or cell death in the developing diencephalon at e10.5 or e11.5. By e16.5, AVP neurons are formed and specified in the SON and PVN, but are abnormally placed in and around these nuclei, suggesting that Notch signaling within the hypothalamus may be necessary for migration of AVP neurons. There is continued misplacement of AVP-positive cell body location within the PVN and SON at e18.5 in *Hes1* null embryos, and a reduction of SS neurons in the aPV. Interestingly, *Hes1* null mice also have abnormal AVP and SS axonal projections to the pituitary at e18.5, as well as decreased AVP-positive axon terminals and aberrant expression of SS-positive cells in the posterior pituitary. Using a novel mass spectrometry-based approach, we found that *Hes1* null pituitaries have altered AVP and SS peptide content compared to littermate controls at e18.5. The alternations in cell body location, peptide content, axon pathfinding to and termination in the pituitaries of *Hes1* null mice indicate that Notch signaling facilitates migration of hypothalamic neurons, guidance of hypothalamic axons to the pituitary as well as neuropeptide processing. Supported by NIH Grant R01 DK076647, NIH Grant T32 HD007333, and NIDA Grant P30DA018310.

**P7:** Increased activation of the PI3K/Akt pathway compromises decidualization of stromal cells of endometriosis, Xunqin Yin, Mary Ellen Pavone, Zhenxiao Lu, J. Julie Kim Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL.

The effects of progesterone on the human endometrium is profound, opposing estrogen driven growth and promoting remodeling and differentiation of the endometrium. In endometriotic lesions, however, the response to progesterone is inadequate. The objective of this study was to investigate a potential mechanism for the suboptimal in vitro decidual response. Stromal cells from endometrium from disease free women (HSC) or from ovarian endometriomas (Osis) were grown in culture and treated with 1uM MPA and 0.5mM dbcAMP (M+A) for 48h. Cells were also treated with 10uM LY294 or infected with adenoviral construct containing mutant FOXO1 in the presence or absence of M+A for 48h. Real time PCR was used to measure expression of IGFBP1 and PRL mRNA, Western blot and immunohistochemical staining were used to measure levels of PR, FOXO1, p-AKT, AKT, p-ERK and ERK proteins. Expression of the decidual genes, IGFBP1 and
PRL were significantly lower in Osis cells compared to normal HSCs in response to M+A. Levels of PRA, PRB and FOXO1 proteins were also dramatically lower in Osis cells. Interestingly, AKT, the upstream kinase of FOXO1, was more highly phosphorylated in Osis cells compared to HSC, with a concomitant decrease in FOXO1 protein levels. Upon treatment of Osis cells with the PI3K inhibitor, LY294, the levels of p-AKT decreased as predicted, with a concomitant increase in FOXO1 protein, and IGFBP1 and PRL mRNAs. Furthermore, addition of the MEK inhibitor, U0126 with M+A treatment did not affect levels of p-AKT or FOXO1 protein, however, it did increase IGFBP1 mRNA levels, compared to M+A treatment alone. In addition, overexpression of the DN-AKT in the presence of M+A treatment, increased FOXO1 protein levels as well as expression of IGFBP1 and PRL in Osis cells suggesting that inhibition of AKT activity partially rescues FOXO1 from degradation and increases expression of decidual genes in endometriotic stromal cells. In endometriotic stromal cells, the down-regulation of FOXO1 mediated by the hyperactivation of Akt pathway contributes to the decreased expression of decidual genes.

P8: Molecular mechanism of vaginal epithelial differentiation, Kenji Unno, Vanida A. Serna, Kazutomo Ishi, *Alea A. Mills and Takeshi Kurita, Division of Reproductive Biology Research, Ob/Gyn Department and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

The developmental origin of vaginal epithelium has been controversial for nearly a century, with speculation that vaginal epithelium originates from the Müllerian duct, Wolffian duct, and/or urogenital sinus. None of these possibilities has been definitively proven or disproven by direct scientific data. To define precisely the origin of vaginal epithelium, epithelial cells of the Müllerian duct, Wolffian duct, or urogenital sinus were fluorescently labeled in mouse embryos by crossing tdTomato-EGFP dual-reporter transgenic mice with transgenic mouse lines that express Cre recombinase in each type of epithelium. The cell fate tracing experiment determined that the entire epithelium of adult mouse vagina is derived solely from Müllerian duct epithelium (MDE). Accordingly, the mechanism underlying the differentiation of MDE into vaginal epithelium was studied. The conditional deletion of Trp63 gene in MDE transformed vaginal epithelium into uterine-like glandular epithelium. Thus, p63 transcription factor is essential for MDE to differentiate into vaginal epithelium. The vaginal epithelial promoter of p63 contained multiple smad-binding elements (SBEs), and the binding of smad4 on the SBEs was detected only in the vaginal but not uterine epithelium. The expression levels of bone morphogenetic proteins (BMPs) and Activin-A were significantly higher in the mesenchyme of developing vagina than uterus, and the expression of p63 in MDE was inhibited by neonatal treatment with inhibitors for BMPs or transforming growth factor (TGF) β / Activin. Moreover, Bmp4 plus Activin A treatment induced squamous differentiation of uterine epithelium in organ culture. In conclusion, differentiation of MDE into vaginal epithelium is regulated by BMPs and Activin-A secreted from vaginal mesenchyme via induction of p63. Supported by NIH/NCI grant.

P9: Secretion of Basigin By human trophoblast-like cells through microvesicle shedding is regulated by interleukin-1β and hypoxia, 1Victoriya Ermilova, 2Ying Chen, 3Richard Leach, 3Stephen Charnock-Jones, and 1Romana A. Nowak; 1Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL., 2Department of OB/GYN, Michigan State University, Grand Rapids, MI and 3Department of OB/GYN, Cambridge University, Cambridge, UK.

Basigin (Bsg), also known as CD147, extracellular matrix metalloproteinase inducer (EMMPRIN), HT7 antigen, OX-47, and neurothelin, is a glycosylated transmembrane protein expressed on the surface of normal and tumor cells and is a member of the immunoglobulin superfamily. Basigin expression has been confirmed previously in human placenta. The goals of our
study were to determine, 1) whether human trophoblast-like cell lines secrete BSG and whether this occurs through microvesicle shedding; 2) whether microvesicle release can be regulated by external stimuli such as the inflammatory cytokine IL-1β or activators of PKC; and 3) whether expression of BSG by trophoblast cells and microvesicle release are regulated by hypoxia. We determined that the trophoblast-derived cell lines HTR8-SVneo, Jeg-3, JAR, and BeWo all secreted BSG into culture medium. Isolation of microvesicles by ultracentrifugation confirmed that >90% of BSG protein in the conditioned medium was in the microvesicle fraction. Treatment of trophoblast-like cells with an activator of protein kinase C, PMA, stimulated release of microvesicles in a time- and dose-dependent manner. Thus, microvesicle shedding can be regulated through cytoplasmic signaling mechanisms. Treatment of HTR8-SVneo cells with 1, 2 or 4 ng/ml interleukin-1β for 4 or 8 hours increased microvesicle shedding as measured by the BCA protein assay. However, while BSG release in microvesicles was increased at 4 hours it was downregulated at 8 hours as analyzed by immunoblotting. Culture of HTR cells under hypoxic conditions (2% oxygen) showed that BSG mRNA levels were increased between 2-2.6 fold at 2, 4 and 22 hr as measured by microarray and quantitative RT-PCR. Microvesicle release was also increased under hypoxic conditions (2% oxygen) at 4 and 22 hr as shown by BCA protein assay and immunoblotting analysis for BSG and monocarboxylate transporter 1. Our results show that human trophoblast-like cells secrete BSG in microvesicles and this is upregulated by hypoxia. BSG is secreted by these cells through microvesicle shedding which can be regulated through the PKC pathway. Microvesicle shedding is increased under hypoxic conditions and under the influence of cytokines such as IL-1β. Our findings suggest that BSG may be important for paracrine interactions between trophoblast cells and other uterine cells at the implantation site. Supported by the Centre for Trophoblast Research at Cambridge University and NIH U54HD40093.


Antral follicles in the ovary are capable of synthesizing and secreting sex steroid hormones including estrogen (E). E binds to estrogen receptors (ESRs) to bring about follicle development. Overexpression of ESRs due to either an exposure to estrogenic chemicals or genetic polymorphisms may increase the susceptibility of the ovary to estrogenic compounds such as the organochlorine pesticide methoxychlor (MXC). To test this hypothesis, we developed and validated a mouse model in which ESR alpha (ESR1) is overexpressed in several tissues, including the ovary. When the antral follicles of control and ESR1 overexpressing mice (ESR1 OE) were treated in vitro with MXC (1-100 µg/ml) or vehicle dimethylsulfoxide (DMSO) for 96 h, MXC10 and MXC100 inhibited growth in both genotypes compared to DMSO treatment. Interestingly, MXC1 inhibited growth of ESR1 OE antral follicles compared to controls. Gene expression of Esr1, androgen receptor (Ar) and an oxidative stress- response gene catalase (Cat) was also analyzed at the end of the culture. Esr1 and Ar were chosen because MXC is known to bind to ESR and AR while Cat was analyzed MXC is known to cause oxidative stress in antral follicles. Esr1 is significantly upregulated in ESR1 OE antral follicles compared to controls. However, in controls, MXC treatment caused a dose dependent increase in Esr1 expression (controls: DMSO = 0.19 ± 0.09, MXC1 = 0.12 ± 0.00, MXC10 = 0.05 ± 0.03, MXC100 = 0.03 ± 0.03; ESR1 OE: DMSO = 5.02 ± 0.98, MXC1 = 4.43 ± 0.42, MXC10 = 10.09 ± 1.01, MXC100 = 29.06 ± 7.08 genomic equivalents (ge); n = 3, p ≤ 0.05). Ar was significantly up-regulated in controls and ESR1 OE follicles in the highest treatment group (MXC100) compared to DMSO. Further, Ar levels in ESR1 OE follicles at M100 were significantly higher compared to control follicles (controls: DMSO = 26.89 ± 4.31, MXC100 = 49.63 ± 7.14; ESR1 OE: DMSO = 25.53 ± 2.59, MXC100 = 87.54 ± 8.85 ge; n = 3, p ≤
Cat was not different in the control follicles treated with DMSO or MXC, whereas in the ESR1 OE follicles, MXC100 significantly increased Cat expression compared to DMSO (DMSO = 18.86 ± 3.01, MXC100 = 79.12 ± 16.27 ge; n = 3, p ≤ 0.05). Collectively, these data suggest that ESR1 overexpression may increase the sensitivity of antral follicles to MXC-induced slow-growth by altering gene expression levels. Support: NIH R21ES13061, R01ES012893 and an Eli Lilly Fellowship in Toxicology.

**P11: Ontogeny of the Relaxin receptor (Rxfp1) in the female mouse reproductive tract.**
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Relaxin, acting through its G-protein receptor, Rxfp1, has important functions in the female reproductive tract. Relaxin produced and secreted by the corpus luteum leads to cell proliferation, growth and increased extensibility of the lower reproductive tract, thereby facilitating parturition. Although Rxfp1 was identified in 2002, to date the key regulatory events that govern the ontogeny and adult expression of this receptor remain poorly understood. The goal of the present study was to use a mouse model with an IRES-LacZ reporter cassette incorporated into the Rxfp1 gene (Rxfp1KO) to understand the ontogeny and the factors controlling the expression of Rxfp1 in the reproductive tract of female mice during the neonatal and juvenile periods. Uteri, cervix, vagina, and oviducts from Rxfp1KO and wild-type controls female mice were collected, prepared and processed for β-galactosidase (LacZ) activity using X-gal as the substrate. LacZ staining, indicative of Rxfp1 expression, was not observed at day 0, 10, or 20 postpartum (PP) in the oviduct, uterus, cervix and vagina. However, LacZ staining was present in all organs by the age of 35 days at levels similar to those seen in adults (> 60 PP). Rxfp1 expression was localized in the subepithelial stroma and myometrial compartments of the cervix, uterus, vagina and oviduct, while the epithelium was negative. To determine whether normal Rxfp1 ontogeny is influenced by the ovary, mice with the reporter gene were ovariectomized at 21 PP, and LacZ staining was examined at 35 PP. Rxfp1 expression is not regulated by the ovary, since LacZ expression was present at 35 PP in ovariectomized females, indicating that removal of the ovary does not preclude Rxfp1 ontogeny. In summary, Rxfp1 appears pubertally in female reproductive organs and has similar distribution in the various organs examined. The ontogeny of Rxfp1 does not appear to be regulated by ovarian hormones. These data provide new insights into the expression, ontogeny, and potential sites of action of Rxfp1. Supported by Billie A. Fields Endowment

**P12: The cytokine IL-1β includes epithelial to mesenchymal transition (EMT) in peritoneal mesothelial cells through direct and indirect effects involving Basigin.**
Farzaneh Masoud, Pavni Mehrotra, and Romana A. Nowak, Department of Cellular and Developmental Biology, Department of Animal Sciences, University of Illinois, Urbana, IL.

Endometriosis is a common disease among reproductive aged women and is a leading cause of infertility. Establishment of endometriotic lesions involves contact between endometrial fragments consisting of uterine epithelial and stromal cells with the mesothelial cells lining the peritoneum. Endometrial cells ultimately invade beyond the basement membrane of the mesothelium and develop into vascularized lesions. This process may involve transformation of epithelial-like mesothelial cells to a more fibroblastic cell type capable of migration and leads to exposure of the underlying basement membrane. The peritoneal fluid of women with endometriosis shows elevated levels of several cytokines including interleukin 1Beta (IL1B). The goals of this
study were 1) to compare the ability of IL1B to induce EMT of mesothelial cells with epidermal growth factor (EGF), a known potent stimulator of EMT in mesothelial cells and 2) to determine whether IL1B can also regulate EMT by stimulating secretion of the pro-inflammatory protein basigin by endometrial epithelial cells (EEC). Basigin is a glycosylated transmembrane protein released by EECs in microvesicles. It has been implicated in EMT in some cancer cells. We utilized an in vitro cell culture system consisting of normal human mesothelial cells (LP-9) and an immortalized human endometrial epithelial cell line (EEC). Mesothelial cells were cultured for 2-3 weeks in medium lacking EGF to induce formation of an epithelial monolayer. These LP-9 cells were then treated with either EGF (10 ng/ml) or IL1B (2, 10 ng/ml) for 3 days. Changes in morphology of LP-9 cells were assessed along with changes in specific EMT markers (cytokeratin, N-cadherin, vimentin, Twist and Snail) every 24 hrs. We also assessed the effects of these factors on production of metalloproteinases (MMPs) -1 and -2 by LP-9 cells. Our results showed that both EGF and IL1B were potent stimulators of EMT in LP-9 cells. While both caused increases in expression of Twist, Snail and vimentin, EGF caused a more marked increase in Snail than IL1B. Both EGF and IL1B increased expression of basigin in the LP-9 cells but the effect of IL1B was greater. Interestingly, while IL1B caused a 0.5 fold increase in MMP-1 expression in LP-9 cells, EGF stimulated MMP-1 expression 12-14 fold. Treatment of LP-9 cells with basigin (1.0 or 10 ug/ml) also induced EMT in LP-9 cells. We next tested the effects of IL1B on secretion of basigin by EEC cells. Cells were treated with IL-1β (2 and 10 ng/ml) for 48 hrs. Treatment with IL1B increased basigin mRNA expression in EEC cells by approximately 0.4 fold and increased basigin protein release into culture medium 2-3 fold. In contrast treatment of EEC cells with transforming growth factor beta (TGFβ) inhibited basigin expression. In summary our results show that IL1B is as potent an inducer of EMT in mesothelial cells as EGF. However the two proteins do not act in the very same way as EGF had a much more potent effect on MMP-1 expression than IL1B. IL1B acts directly on mesothelial cells to induce EMT but can also act on uterine epithelial cells to stimulate release of proinflammatory factors such as basigin that can induce EMT. IL1B in the peritoneal fluid may play a significant role in promoting EMT and thereby facilitating movement of endometrial cells through the mesothelium into the underlying peritoneum and establishment of endometriotic lesions. (NIH U54 HD40093 to RAN).

P13: Estrogen-dependent ERα activation is required for normal branching morphogenesis of the mouse prostate gland. Esther L. Calderon1, Kerstin Sinkevicius2, Muriel Laine2, Geoffrey L. Greene2, Gail S. Prins1, 1Departments of Urology and Physiology & Biophysics, University of Illinois at Chicago, Chicago, IL; 2 The Ben May Department for Cancer Research, University of Chicago, Chicago, IL.

Increasing evidence indicates that estrogens acting through estrogen receptors (ER) play an important role during prostate gland development and abnormal growth associated with prostatic diseases such as BPH and cancer. Studies with ER knock-out mice have shown that developmental effects of estrogens on the prostate gland are mediated through ERα and not through ERß (Prins et al, Can Res 2001). Recent studies using ACTB-Cre/ERα/- mice demonstrated reduced branching morphogenesis of the prostate glands suggesting a critical role for ERα in this process (Chen et al, Endocrinology 2001). ERα actions can be mediated through both estrogen-dependent and estrogen-independent activation, the later effects being driven through growth factor induced phosphorylation of ERα at serine 118. Since multiple growth factor pathways are involved in regulating prostate morphogenesis and differentiation, the present study sought to determine whether estrogen-dependent and/or ligand-independent actions of ERα are involved in prostate gland development. To do so, we examined the prostate glands of male estrogen nonresponsive ERα knock-in (ENERKI) mice. These animals have a point mutation (G525L) in the ligand-binding
domain of ERα that interrupts interaction with endogenous estrogens but does not affect the activation of ligand-independent ERα pathways (Sinkevicius, *Endocrinology* 2008). Prostate lobes from peri-pubertal (day 30) male ENERKI and wt littermates mice were analyzed morphologically and histologically for branching and differentiation endpoints. Branch tip number in microdissected ventral prostates was significantly reduced from 77.9 ± 4 in wt mice to 65.6 ± 1.5 in ENERKI males (p<0.05). While histologic inspection revealed normal epithelial cell differentiation, the lumens were markedly enlarged in ENERKI prostates as compared to wt males. Together these findings suggest that ERα activated by endogenous estrogens is involved in branching morphogenesis and planar cell division controlling circumferential growth of the prostatic ducts. (Supported by DK40890 and CA89089)

**P14:** Retinoic Acid action is deficient in endometrium in a baboon endometriosis model. Mary Ellen Pavone¹, Elizabeth Pearson¹, You-Hong Cheng¹, Asgi Fazleabas², Serdar Bulun¹, ¹Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL; ²Obstetrics, Gynecology, & Reproductive Biology, Michigan State University, Grand Rapids, MI.

**Introduction:** Retinoic acid controls many biologic processes including cell survival. We have shown that expression of STRA6, the gene responsible for uptake of the RA precursor retinol, CRABP2, a gene necessary for the apoptotic and anti-estrogenic actions of RA, and HSD17B2, the anti-estrogenic RA target gene, are severely deficient in human endometriosis compared to eutopic endometrium. However, FABP5 which mediates the anti-apoptotic actions of RA remains unchanged. Here, a baboon model is used to study in vivo roles of these genes in eutopic endometrium of animals with and without endometriosis. **Design:** Eutopic endometrial tissues from baboons with and without experimentally induced endometriosis were used. We characterized tissues levels of STRA6, CRABP2, FABP5 and HSD17B2 mRNA or protein throughout the menstrual cycle and investigated eutopic endometrium of animals with induced endometriosis as a function of disease duration. **Methods:** Endometriosis was induced in female baboons with intraperitoneal inoculation of menstrual endometrium(n=2-4). Endometrial tissue was harvested by endometriectomy days 9 to 11 post-ovulation 3, 6 and 12 months after induced endometriosis. Control endometrium was harvested in a similar fashion from disease-free animals(n=2-4). Real-time PCR was used to quantify STRA6, CRABP2, FABP5 and HSD17B2 mRNA levels. Immunohistochemistry was used to evaluate protein expression. **Results:** Highest levels of HSD17B2 mRNA were found in late secretory phase. STRA6 and CRABP2 were differentially expressed throughout the menstrual cycle, with highest levels in early follicular phase. Expression of endometrial STRA6 or CRABP2 was significantly lower in animals with disease vs disease free controls. As disease progressed, expression of STRA6 or CRABP2 decreased significantly. FABP5 expression remained stable. Protein expression as determined by IHC showed differences comparable to mRNA levels. **Conclusions:** Expression of STRA6 and CRABP2, responsible for the pro-apoptotic actions of RA, are significantly lower in eutopic endometrium of baboons with endometriosis. Expression of these genes is further suppressed as disease progresses, suggesting that resistance to apoptosis is directly related to disease duration. Thus, early rather than later treatment of endometriosis may reduce the rate of disease progression.

**P15:** Spontaneous and programmed transformation of ovarian surface epithelium using a three-dimensional organ culture system. S.M. King, D.A. Davis, Joanna E. Burdette, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL.

Ovarian cancer is the fifth leading cause of cancer death among women in the United States, and is the most lethal of the gynecologic cancers due in part to a lack of early detection methods.
Over 95% of ovarian cancers are believed to arise from the ovarian surface epithelium (OSE), a single layer of flat-to-cuboidal epithelial cells covering the surface of the ovary. The “tear-and-repair” hypothesis proposes that repeated ovulations during a woman’s lifetime, during which the OSE must rupture to allow release of an oocyte, encourages spontaneous DNA damage in OSE cells as they proliferate to cover the wound caused by ovulation. These spontaneous changes accumulate, leading to hyperproliferation of the OSE and potentially malignant changes in the ovarian surface.

To study changes in the OSE induced by aspects of ovulation, our lab has developed a three-dimensional organ culture system in which mouse ovaries are wounded with a scalpel and embedded in an alginate hydrogel matrix. Using this system, the OSE mimic ovulation-induced wound repair by proliferating and encapsulating the ovary. In order to study early transformative events in the OSE, the ovary organoids were cultured with chemical carcinogens to induce spontaneous DNA damage, as would be observed after repeated ovulation. Culturing organoids with H2O2, which recapitulates the oxidative stress that occurs during ovulation, causes an increase in OSE proliferation. As an additional indication of transformation, OSE enzymatically removed from organoids cultured in the presence of H2O2, n-methylnitrosourea, or dimethylbenzanthracene form colonies in a soft agar contact inhibition assay. As an alternative model of early events in the development of ovarian cancer, we have transfected organoids to express SV40 T antigen to evaluate transformation of OSE after targeted genetic disruption. OSE isolated from organoids transformed by spontaneous mutagenesis or targeted genetic disruption can then be analyzed by transcriptional array for changes in genes involved in cancer initiation and progression to identify new targets for the treatment and prevention of ovarian cancer. Supported by NIH BIRCWH grant number K12HD055892, NIH grant C06RR15482, and UIC Cancer Center Pilot Grant (J.E.B.).

P16: Regeneration of normal prostate gland and prostate cancer tissue using normal and tumorigenic prostate progenitor cells, Wen-Yang Hu, Guang-Bin Shi, Dan-Ping Hu, Ikenna Madueke, Gail Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

Background: A small subpopulation of prostate progenitor cells (PPCs) has recently been documented. PPCs may be the direct targets for prostate cancer initiation. Prostate cancer progression and recurrence are increasingly being attributed to cancer PPCs. Therefore, there is a special need in developing useful animal models to study cancer PPCs and their therapies. In the current study, we have investigated and attested the self-renewal as well as the regenerative abilities of PPCs in both normal and tumorigenic prostate epithelial cells, and consequently developed a useful cancer PPCs-derived prostate cancer model. Methods and Results: NRP152 (nontumorigenic) and NRP154 (tumorigenic) were two rat prostate epithelial cell lines isolated and selected from carcinogen-treated Lobund/Wistar rats. In an in vitro 3-D culture system, both cell lines formed prostaspheres as evidenced by the increasing size of cellular spheres over time, indicating a rare subpopulation of PPCs with self-renewal ability in both cell lines. The cell differentiation of these PPCs was evaluated by forming ductal branches in prostasphere culture system. The regeneration capacity was demonstrated by the generation of organized prostate ductal structure in vivo following tissue recombination with rat urogenital-sinus mesenchyme (UGM) tissue and grafting under the renal capsule of nude mice for 1~4 months. Regenerated grafts were further characterized by immunohistochemistry staining using differentiation markers (p63, CK5, CK14, CK8/18 and AR). NRP152 PPCs were able to generate normal prostate structure in vivo, whereas, NRP154 PPCs generated prostate tissues with localized PIN lesion at one month, and progressed into invasive cancer at 2-4 months, indicating the tumorigenic origin and the cancer initiating cells exist in this cell lines. Immunostaining showed positive AR expression in NRP152 cells and the generated grafts tissues but not in NRP154 cells and the cancer grafts, implying that the NRP154 cells generated prostate cancer is androgen independent. Conclusion: The isolation of
PPCs and the regeneration of prostate gland from both normal and tumorigenic prostatic epithelial cells as well as the success in developing prostate cancer, will provide a useful model to study prostate carcinogenesis originating from reprogrammed prostate cancer initiating cells and subsequently enable the screening and selection of the chemotherapeutic agents targeting cancer initiating cells. This study was supported by NIEHS grant RC2 ES 018758.

**P17: Follicle-stimulating hormone and luteinizing hormone induce expression of oncogenes in a 3D model of normal ovarian surface epithelium.** T. S. Hilliard, J. E. Burdette, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL.

Ovarian cancer is the most lethal gynecological malignancy in the U.S. Epithelial ovarian carcinomas (EOC) accounts for 90% of all ovarian cancers and are believed to originate from the ovarian surface epithelium (OSE), a single layer of epithelial cells surrounding the ovary. Early signs or symptoms of ovarian cancer are often subtle and nonspecific, which are frequently ignored. The etiology of ovarian cancer is poorly understood and understanding the events leading to the initiation and progression are critical for detecting early stages of ovarian cancer. Ovarian surface proliferation, associated with ovulation, and the gonadotropins that regulate ovulation are factors in ovarian surface transformation and cancer progression. Based on the gonadotropin theory, we hypothesized that follicle-stimulating hormone (FSH) and luteinizing hormone (LH), play a role in the pathogenesis of EOC. This theory states the excessive levels of FSH and LH related to the surge during ovulation, and the loss of gonadal negative feedback from menopause and premature ovarian failure may play a role in the development and progression of EOC. A three-dimensional alginate-hydrogel organ culture system, developed in our lab, supported the growth of normal OSE and responds to the gonadotropins. Cultures treated with FSH and LH proliferated more on day 8 when compared to control tissues, while control tissues encapsulated more damaged surface by day 8. Using this culture system, the OSE can be removed with collagenase and studied further by isolating RNA to determine pathways regulated by FSH and LH using transcriptional array analysis. FSH transcriptionally up-regulates Mdm2 (double minute 2) and LH transcriptionally up-regulates c-Met and c-Myc which can increase proliferation. FSH and LH both transcriptionally up-regulates CHK2 (checkpoint 2) and PI3K (Phosphatidylinositol-3-kinase) pathways that contribute to increased proliferation. The role of these pathways can be validated by using knockdown and overexpression analysis and quantified by IHC, qPCR and western blot analysis. A novel 3D ovarian organ culture identified key cancer pathways regulated by gonadotropins and can help uncover how these hormones contribute to OSE tumorigenesis. Supported by UIC Cancer Center Pilot Grant, K12HD055892, and CO6RR15482

**P18: DNA Methylation Analysis of Human Uterine Leiomyomas.** Antonia Navarro, Ping Ying, Jianjun Wei, Pan Du, Simon Lin, Serdar E. Bulun. Department of Reproductive Biology Research, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Uterine leiomyomas (fibroids) are the most common benign tumors of the female genital tract and become symptomatic in 30% of women and up to 70% of African American women of reproductive age. The incidence of epigenetic marks such as DNA methylation in human uterine leiomyoma remains largely unknown, and to our knowledge, a genome-wide profile of DNA methylation in these benign tumors has not yet been performed. In this analysis to determine genome-wide analysis of differential DNA methylation patterns, we have quantified the DNA methylation levels of 27,578 CpG dinucleotides spanning 14,587 genes in 18 pairs of human uterine leiomyoma (ULM) and matched normal myometrium (NMM) samples using bisulfite-modified gDNA screening beadarrays. Supervised cluster analysis demonstrated significant differential DNA methylation in ULM compared to NMM. Approximately 1300 genes were
P19: MEK1 and 2 are essential for the survival of the adult population of Leydig cells in the mouse, Soichi Yamashita1, Jean Charron2, CheMyong Ko3, and Mario Ascoli1, 1Department of Pharmacology, The University of Iowa, Iowa City, IA, 2Centre de Recherche en Cancérologie de l’Université Laval, Québec, Canada and 3Division of Clinical and Reproductive Sciences, Department of Clinical Sciences, University of Kentucky, KY.

Previous studies done with primary cultures indicated that the LHR-sensitive ERK1/2 pathway is an important mediator of the proliferative and anti-apoptotic effects of LH on Leydig cells. To examine the importance of this pathway in a more physiological setting we set out to conditionally delete MEK1 and MEK2 (the kinases that phosphorylate ERK1/2) from Leydig cells. To accomplish this goal we crossed Mek1ff;Mek2-/- (henceforth referred to as -2M) and Cyp17iCre mice. Males of the appropriate genotype (Mek1ff;Mek2-/-;iCre+), henceforth referred to as -4MHM, show recombination of the floxed Mek1 allele in the testes but not in the kidney or brain. Leydig cells from -4MHM mice show ~80% decrease in MEK1 expression and a substantial decrease in ERK1/2 phosphorylation when stimulated with EGF, c-Kit ligand or hCG. -4MHM males housed with -2M females produced a total of 19 pups/pair during a 6 month period, whereas -2M males housed with -2M females produced a total of 35 pups/pair. At 6 months of age, -4MHM males show a 15%, 10% and 55% decrease in body, testes, and seminal vesicle weight, respectively. Testicular histology of -4MHM mice show a decrease in the number of Leydig cells withouth a change in the shape, size and cellular composition of the seminiferous tubules. This decrease in the density of Leydig cells is paralleled by a decrease in the testicular expression of several Leydig cell markers such as Cyp11a, StAR, Cyp17, and Estrogen Sulphotransferase. The expression of Sertoli or germ cell markers, such as Clusterin and VASA, respectively, was not affected. Testosterone synthesis in primary cultures of Leydig cells with or without stimulation (hCG, Bt2cAMP) or with or without added substrate (22OH cholesterol or pregnenolone) is almost undetectable in -4MHM mice. Our study demonstrates that MEK1/2 is an essential mediator of the survival of the adult population of Leydig cells in the mouse. Further studies are currently on the way to investigate the mechanism of this decrease in the number of Leydig cells. Financial support: CA-40629.

P20: Extracellular matrix-mediated regulation of proliferation in leiomyoma smooth muscle cells Faezeh Koohestani and Romana Nowak. University of Illinois Urbana-Champaign, Urbana, IL

Uterine leiomyomas are the most common pelvic neoplasms in women. Current treatments are limited to surgical procedures or hormonal therapy, which are associated with high costs and significant side effects. Since one of the main characteristics of these tumors is excessive collagen deposition, we decided to examine the effects of collagen matrix on leiomyoma smooth muscle cell (LSMC) proliferation. Primary LSMCs were cultured on monomorphic and fibrillar collagen as well as plastic dishes. While cells grown on plastic and monomorphic collagen had a spindle-like morphology, cells on fibrillar collagen had extended surface areas with numerous projections and showed decreased pFAK localization. Using thymidine incorporation assay, we measured proliferation of these cells in the absence or presence of PDGF. In comparison to the rate of growth on plastic, monomorphic collagen induced up to a 45% increase in LSMC proliferation while fibrillar collagen acted as a non-conducive matrix decreasing the rate of proliferation by as much as 75%. Treatment of LSMCs cultured on monomorphic collagen with 10 ng/ml PDGF for 24 hours increased
proliferation by up to 60% over similarly treated cells on plastic, suggesting a synergistic effect between extracellular matrix and growth factors. We next investigated the effect of two major anti-fibrotic drugs, trichostatin A (TSA) and halofuginone (HF), on expression of collagens I and III and proliferation of LSMCs cultured on different collagen matrices. Both of these drugs were able to down-regulate the expression of collagens I and III by 95% within 6 hours. Dose response studies on these two drugs, showed that 50 nM HF and 100 nM TSA decreased the rate of proliferation by 50% in cells cultured on plastic whereas in cells cultured on monomeric collagen higher concentrations of these drugs were required to achieve 50% inhibition. Proliferation of LSMCs cultured on plastic and treated with PDGF plus one of these anti-fibrotic drugs was reduced to a greater extent than when the cells were cultured on monomeric collagen. These results indicate that the monomeric collagen matrix can partially compensate for the inhibitory effects of TSA and HF on cellular proliferation. Taken together our findings demonstrate an important role for different forms of collagen in the morphology and proliferation of LSMCs and how antifibrotic agents might be used to overcome the proliferative effects of specific matrix proteins. Supported by NICHD #PO1HD057877.

**P21: Identification and Characterization of AP3 Binding Proteins in Luteal Cells**, Deepika Talla and Carlos Stocco, Department of Physiology and Biophysics, College of Medicine, University of Illinois at Chicago, Chicago, IL.

The aromatase enzyme (Cyp19) is required for the biosynthesis of estrogens, steroid hormones crucial for both male and female fertility as well as several non-reproductive functions. In the ovary, Cyp19 expression is regulated by the proximal promoter, which is highly conserved between humans and rodents. In the rat, luteal Cyp19 expression increases progressively during pregnancy and falls before parturition; however, the mechanisms that control these changes in Cyp19 expression remain unknown. Using gel shift assays, we sought to identify the promoter regions that control Cyp19 expression in the rat corpus luteum (CL). Nuclear extracts were obtained from CL of rats on days 4, 15, and 23 of pregnancy and from the ovaries of immature rats treated with PMSG, a hormone that induces Cyp19 expression in follicles. An cAMP like site was active in the ovaries of PMSG-treated rats but inactive in the CL, whereas binding to nuclear receptor and GATA sites was correlated with Cyp19 expression in both tissues. Strikingly, binding to an AP-3 response element was present only in the corpus luteum. AP3 binding was higher on d15 of pregnancy when compared with d4 and d23. Ovaries of immature or PMSG-treated rats did not show AP3 binding. Induction of luteinization in PMSG-treated rats increased AP3 binding activity. These results suggest that in luteal cells aromatase expression is regulated by binding of unknown proteins to the AP3 region of the aromatase proximal promoter. Using DNA-affinity chromatography we attempted to purify and characterize the proteins that bind to the AP3 region. Moreover, several genes that contain an AP3 region in their regulatory region were identified suggesting a role of AP3 proteins in gene expression. The identification of AP3 binding proteins provides new insights into the regulation of aromatase expression in corpus luteum leading ultimately to our understanding of molecular mechanisms that control estradiol production in various tissues. RO1 HD057110.

**P22: The granulosa cell inositol phosphate cascade is essential for fertility**, S.M. Breen, S. Offermans, J.A. Gossen, and M. Ascoli, Department of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA, University of Heidelberg, Heidelberg, Germany, Target Discovery Oss, Schering-Plough Corporation, Molenstraat, Oss, The Netherlands.

It is well established that the LHR activates the cAMP and inositol phosphate (IP) cascades. However, little is known about the involvement of the IP cascade in fertility. To examine this issue
we generated mice with a granulosa cell specific deletion of Gaq/11 (the G proteins that activate the IP cascade) by crossing Gaqf/f;Ga11-/ mice with Cyp19-iCre mice. Granulosa cells from mice of the appropriate genotype (Gaqf/f;Ga11-/iCre+, referred to as q11KO) injected with PMSG bind hCG normally but have undetectable levels of Gaq/11 and do not respond to hCG with increased IP accumulation. Fertility in these mice is severely disrupted but not completely lost. Follicular growth and other markers of FSH actions (expression of Cyp19a1 and Lhcgr) are normal in PMSG-injected mice and cumulus expansion after an injection of hCG are normal in the ovaries of q11KO mice. Ovulation, fertilization, and implantation are all reduced. Although, the histology of the ovaries of q11KO mice injected with PMSG is normal. After an injection of hCG, there is a higher incidence of corpora hemorrhagica or cysts and in spite of the normal morphology of the luteal cells the overall morphology of the corpora lutea seems more disorganized. The expression of several ovarian genes involved in luteinization such as Lhcgr, Cyp11a1, Star, HSD3b1 and Prlr are normal or slightly elevated in the ovaries of PMSG-primed q11KO mice injected with hCG for 24 or 48 hours. The expression of Akr1c18, the enzyme that inactivates progesterone to 20α hydroxyprogesterone, and Sfrp4, a prolactin target, appear much higher in the ovaries of PMSG-primed q11KO mice injected with hCG for 48 hours. Overall these data suggest that the LHR-induced activation of the granulosa cell inositol phosphate cascade is essential for fertility, but it remains to be determined if this is entirely due to an ovulatory defect or a combination of ovulation and luteinization defects. Supported by NIH-NRSA Postdoctoral Fellowship 1F32HD065410-01

**P23: The Forkhead transcription factor, FOXP3, is essential for normal reproductive function**, Deborah O. Jung, David B. Schwartz, Corrie L. Farris, Elaina Rathbun, and Buffy S. Ellsworth, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL.

The hypothalamic-pituitary-gonadal (HPG) axis is crucial for the normal development and function of the reproductive system. Many forkhead transcription factors are important in reproduction. The forkhead factor, FOXP3, is essential role for normal development and function of T regulatory cells. FOXP3 mutations in humans cause an autoimmune disorder referred to as IPEX. Phenotypically similar, the scurfy mouse possesses a two base pair insertion in the coding region of Foxp3. Interestingly, scurfy mice are also hypogonadal and sterile. To better understand their infertility, we measured seminal vesicle weights, which directly correlate with testosterone production. Weights test significantly lower in scurfy mice compared with wild type mice. Histologically, we do not observe spermatids in scurfy mice, indicating spermatogenesis is arrested during meiotic division. To determine if loss of testicular function is due to the absence of pituitary stimulation, we measured LHβ production in pituitary using immunohistochemistry (IHC) and real-time RT-PCR. LHβ is greatly reduced at 6 weeks in the scurfy mouse. We do not detect Foxp3 expression in the pituitary during development or in the adult, suggesting that the loss of LHβ we see is secondary to loss of Foxp3 in another organ. Treatment of mice with a GnRH analog rescues LHβ production, indicating that the pituitary is capable of producing LHβ. GnRH levels are reduced in the scurfy hypothalamus by nearly half, relative to wild type, indicating that reduced pituitary gonadotropin levels in these mice are due, at least in part, to loss of GnRH stimulation. Foxp3, however, is not expressed in the hypothalamus, suggesting that the decrease in Gnrh expression is likely a secondary defect due to loss of Foxp3 in immune cells. In light of these findings, we conclude that FOXP3 is essential for normal pituitary function in spite of the fact that Foxp3 is not expressed in the hypothalamic-pituitary-gonadal axis. Supporting financial information: SIU School of Medicine General Academic Instruction - Startup
P24: The role of the Forkhead transcription factor, FOXO1, in pituitary gland development, Sreeparna Majumdar, Corrie L. Farris, Deborah O. Jung, Buffy S. Ellsworth, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL.

Congenital hormone deficiencies are common, occurring in approximately one in 4,000 live births. Pituitary hormone deficiency can consist of loss of a single hormone (isolated hormone deficiency) or several hormones (combined pituitary hormone deficiency). Absence of anterior pituitary hormones does not interfere with fetal viability, but are required for survival after birth, gonadal differentiation, and maturation of the fetal thyroid. Lesions in the transcription factors PITX1, PITX2, HESX1, LHX3, LHX4, TPIT, PROP1 and PIT1 lead to combined pituitary hormone deficiency in mice and humans. However mutations in these transcription factors account for only a fraction of congenital hormone deficiencies in humans. To identify additional factors that contribute to human congenital hormone deficiencies, we are investigating the forkhead transcription factor, FOXO1, which is important for the normal development of several organs. We find that FOXO1 is present in nuclei of non-dividing pituitary cells, suggesting that it inhibits cell proliferation. We also find that FOXO1 is present in a fraction of differentiated cells. FOXO1 is expressed in four-fifths of somatotrope cells, three-fourths of gonadotrope cells, one-third of thyrotrope cells in both e18.5 and adult mice pituitary cells; whereas, FOXO1 is expressed in one-third of corticotrope cells in e18.5 and half of corticotropes in adult mice. These data suggest that FOXO1 is not specifically required for differentiation of any one cell type, but rather is important for regulating cell cycle progression in pituitary cells regardless of cell type. Mouse knockout models for Foxo1 result in embryonic lethality at embryonic day (e)10.5 due to vascular defects. To circumvent this problem, we have eliminated FOXO1 specifically in the pituitary gland. We are currently investigating the phenotype of the developing mouse pituitary in the absence of FOXO1.

P25: Identification of developmental competence related genes in mature porcine oocytes, Y. Yuan, R. Krisher, Department of Animal Sciences, University of Illinois, Urbana, IL.

Oocyte competence impacts embryonic development, fetal growth and health of offspring. Many in vitro matured (IVM) oocytes, as well as oocytes derived from prepubertal animals, can complete meiosis, but then fail to produce viable embryos. This has been explained as poor oocyte cytoplasmic maturation, although the underlying causative mechanisms are still unclear. The objective of this study is to examine the molecular mechanisms of oocyte cytoplasmic maturation, by comparing candidate gene expression profiles in IVM and in vivo matured (VVM) prepubertal and adult porcine oocytes (4 treatment groups). A panel of 9 genes that are correlated with oocyte competence suggested by previous microarray and real-time PCR studies was examined in the current study (PERV, TL10, SFRS1, TNF-alpha, HMGR, ACSL3, ACDAL, 4ALD, and LDH-C). IVM oocytes were cultured in defined Purdue Porcine Medium (PPMmat; containing 2mM glucose, 6mM lactate, 0.5mM cysteamine, 100ng/ml EGF, 0.01units/ml LH, 0.01units/ml FSH and supplemented with 1% recombinant and 0.2% fetuin) in 5% CO2 in air at 39°C for 44h. VVM oocytes were recovered via retrograde flush of the oviducts from synchronized and superstimulated prepubertal and adult animals. Oocytes with a visible polar body were frozen at -80°C in pools of 20. mRNA from 3 pools of oocytes in each group were used to quantify the relative expression of the candidate genes by reverse transcription followed by linear amplification using the MEGAscript High Yield Transcription Kit (Ambion), and real-time PCR was performed in duplicate. Data was analyzed by REST 2005 version 1.9.12 software (Corbett Research); GAPDH expression was used as a reference gene. The comparison between IVM prepubertal and adult oocytes revealed 5 genes (TL10, SFRS1, TNF-alpha, ACSL-3 and LDH-C) were differentially expressed. The comparison between IVM and VVM prepubertal oocytes revealed 5 genes (TL10, TNF-alpha, HMGR, ACDAL and LDH-C) were differentially expressed, and the comparison between IVM and VVM adult
oocytes revealed 8 genes (PERV, TL10, SFRS1, TNF-alpha, ACSL-3, ACADL, 4ALD and LDH-C) were differentially expressed. None of the genes was differentially expressed between VVM prepubertal and adult oocytes. The differential expression patterns of the genes suggested that proteins encoded by these genes may be functionally relevant to oocyte competence.

P26: 17β-estradiol increases the expression of the oxidative stress response protein apurinic/apyrimidinic endonuclease or redox factor-1 (Ape1) in the cerebral cortex, Alicia K. Dietrich and Ann M. Nardulli, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign.

Stroke is the third leading cause of death in the United States as well as a major source of permanent disability. The fact that postmenopausal women are at greater risk of suffering a stroke than premenopausal women suggests that circulating 17 β-estradiol (E2) may be required to maintain optimal brain cell function, reduce the risk of stroke in premenopausal women, and help protect postmenopausal women from stroke-induced brain injury. In support of this idea, previous studies have shown that E2 attenuates ischemia-induced damage in animal models of stroke. Interestingly, our laboratory has shown that E2 increases the expression of specific oxidative stress response proteins in MCF-7 breast cancer cells. We believe that one way E2 may mediate its protective effects in the brain is through increasing oxidative stress response protein expression. The current study focuses on the oxidative stress response protein apurinic/apyrimidinic endonuclease or redox factor-1 (Ape1). Ape1 is a multifunctional protein involved in base excision repair and reduction of transcription factors. Importantly, overexpression of Ape1 has been shown to reduce DNA fragmentation and infarct volume in mice after an ischemic event. To examine the effect of E2 on Ape1 expression, we used brain slice cultures, which maintain many of the architectural features and cellular networks found in the intact brain. Immunofluorescent studies demonstrated that Ape1 and estrogen receptor β were expressed in the nuclei of cortical neurons and that E2 treatment of brain slice cultures increased Ape1 expression in the cerebral cortex. Additionally, Western blot analysis using extracts from brain slice cultures confirmed that E2 increased Ape1 expression. These studies suggest that the E2- induced expression of Ape1 protects the brain from an ischemic insult and define a novel mechanism by which E2 brings about its neuroprotective effects. This research was supported by NIH grant R01 DK 53884.

P27: Testosterone, not 5α-dihydrotestosterone stimulates LRH-1 leading to FSH independent expression of Cyp19 and P450scc in granulosa cells, Yan-Guang Wu, Jill Bennett, Deepika Talla, and Carlos Stocco. Department of Physiology and Biophysics, College of Medicine, University of Illinois at Chicago, Chicago, IL.

Androgens are crucial for normal folliculogenesis and female fertility as evidenced in androgen receptor null and granulosa-cell conditional knockout mice. It is thought, however, that the multiple effects of androgens in the ovary are mainly complementary to the actions of gonadotropins. Using primary rat granulosa cells, we demonstrate that in the absence of gonadotropins, testosterone increases aromatase (Cyp19) and P450 side change cleavage 30 (P450scc) expression, two enzymes crucial for normal ovarian function. Testosterone can be converted into estradiol, a classical estrogen, by aromatase and into 5α-dihydrotestosterone (DHT), a pure androgen, by 5α-reductase. However, inhibition of aromatase and/or 5α- reductase did not prevent the stimulatory effects of testosterone. In contrast, the effect of this steroid was potentiated by blocking 5α-reductase. Additionally, testosterone, not DHT stimulates 35 liver receptor homolog-1 (LRH-1) expression; whereas the expression of steroidogenic factor-1 (SF-1) was not affected by either steroid. LRH-1 and SF-1 are transcription factors known to be involved in the regulation of aromatase. Accordingly, small interference RNA against LRH-1 prevented aromatase
and P450scct up-regulation whereas anti-SF-1 siRNA had no effects. Chromatin immunoprecipitation demonstrated that testosterone stimulation of LRH-1 leads to 40 the recruitment of LRH-1 to the native aromatase promoter, which was not affected by cotreatment with 5α-reductase and aromatase inhibitors. These results provide novel evidence that testosterone has a direct effect on the expression of genes involved in granulosa cell differentiation. This further implies the existence of ovarian pathways activated primarily by testosterone but not DHT. This work was supported by NIH grant R01HD057110.

P28: Sertoli cell specific Rhox8 knockdown via siRNA, Matt Davis, Raquel Brown, Kanako Hayashi, and James A. Maclean II, Department of Physiology, Southern Illinois University, Carbondale, IL.

Rhox genes are Reproductive homeobox genes found on the X chromosome. These genes encode transcription factors that are important for fertility, specifically for germ cell development and survival. The discovery of this 33 gene cluster in mice resulted from studies characterizing the founding member of the cluster Rhox5, previously known as Pem. This gene is expressed exclusively in the testis, epididymis, placenta, and ovary. In the male, RHOX5 is thought to regulate both spermatogenesis and sperm maturation. This hypothesis is supported by the finding that Rhox5-null mice are hypofertile or subfertile. This is due to increased apoptosis of meiotic germ cells in the testis. Additionally, Rhox5 and Rhox8 are the most highly expressed Rhox genes in the testes. The expression pattern of RHOX8 in the gonads overlaps that of RHOX5. Thus, we have shifted our focus of the Rhox gene cluster toward the Rhox8 gene. Also, RHOX8 shows expression in Sertoli cells at time intervals similar to that of RHOX5. These findings support the hypothesis that RHOX5 and RHOX8 may exhibit redundant functions in the testes, and potentially why Rhox5-null animals are subfertile and not infertile. Therefore, successful knockdown or knockout of Rhox8 is key to answering this question. However, due to the proximity of Rhox5 and Rhox8 on the X chromosome, it will be difficult to produce a double knockout by using traditional knockout strategies. Recently, a tissue specific RNAi mechanism has been successful in gene targeting in vivo. This model uses the Rhox5 proximal promoter which contains regulatory elements for expression of the transgene under the control of androgens in Sertoli cells. The pMAN vector containing the siRNA step loop structure with Rhox8 targeting sequences, as well as a 5’ UTR and 3’ BGH UTR for detection was introduced into the animal by standard methods and produced 11 independent founder lines which are being characterized for fertility as well as breeding with Rhox5-null animals to produce double knockouts. Supported by NIH/NICHD 55268 and SIU-ORDA Faculty Seed Grant 2009.

P29: Rhox homeobox gene cross-regulation in granulosa cells, Raquel Brown, Matthew Davis, Kanako Hayashi, and James A. MacLean II, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

Rhox homeobox gene cluster has been identified as a group of transcription factor genes clustered on the X chromosome. In the mouse there are 33 Rhox genes divided into three subclusters. Rhox gene expression is normally found only in reproductive tissue, therefore they are good candidates to control reproductive development. In the male reproductive tract several Rhox genes have been significantly studied, however, in the females’ gonad Rhox gene expression and function are unclear. In females, preliminary studies have shown that the mRNA expression of Rhox genes during the ovarian cycle falls into three groups. The first grouping revealed peak expression prior to ovulation, the second grouping revealed peak expression during ovulation and the final grouping revealed peak expression after ovulation. The significance in the expression also showed that Rhox gene cluster are collinear regulated. During testicular development, Rhox genes exhibited
temporal and quantitative colinear expression. Similar results are seen in the ovary, but collinearity seems to cease when ovulation begins. *Rhox5* and *Rhox8* have been shown to be the most highly expressed *Rhox* genes in testis and their temporal patterns are super imposable. In *Rhox5*-null mice, the expression of *Rhox8* significantly decreased in the immature and preovulatory phases and stayed the same when ovulation occurred. The most unique expression pattern is that the expression of *Rhox8* is the only *Rhox* gene significantly expressed during ovulation. This suggests that *Rhox8*’s role is important for the periovulatory phase. *Rhox8* promoter was created and characterized to identify the putative transcription factor sites within its promoter. These results showed that the deletion of PRE significantly decreased *Rhox8* promoter activity, which suggested that *Rhox8* is also regulated by progesterone receptor. Interestingly, loss of RHOX5 resulted in reduced expression of *Rhox8* during the window which *Rhox5* is highest, but no significant change in *Rhox8*’s expression 8h post hCG when progesterone signaling dominates. Supported by NIH/NICHD 55268 and SIU-ORDA Faculty Seed Grant 2009.

**P30: Functional mechanism of WNT7A in ovarian carcinomas**, Shin Yoshioka¹, Sophia Ran², Hiroshi Okuda², James A. MacLean II¹, Mary E. McAsey³, Kouonsuke Watabe² and Kanako Hayashi¹, Department of Physiology¹, Medical Microbiology, Immunology and Cell Biology², Obstetrics and Gynecology³, Southern Illinois University School of Medicine, Carbondale, IL.

The WNT system is known to play an important role in the regulation of developmental events where they govern cellular proliferation and differentiation. WNT signaling plays a key role in the normal embryonic development of the ovary as well as ovarian function. Abnormal activation the WNT/β-catenin signaling pathway has been associated with ovarian tumorigenesis. It is likely that the WNT signaling pathway is involved in ovarian cancer development via multiple, diverse mechanisms such as gene mutations and changes in pathway components such as extracellular inhibitors and nuclear transcription cofactors. To date, the ligand partners associated with WNT signaling have not been examined in ovarian cancers. The present study investigated the potential role of WNT7A in the ovarian carcinomas including its functional mechanisms. Although *WNT7A* has not been detected in normal ovary, *WNT7A* became abundantly expressed in ovarian malignant tumors, and patients had worse overall survival rate in the group with high expression of *WNT7A*. To determine whether WNT7A activates the canonical CTNNB1-TCF/LEF and/or the non-canonical JNK signal transduction pathways in ovarian tumors, SKOV3 cells were transfected with the TOP-FLASH reporter or pFR-Luc reporter with pFA2-c-Jun, then overexpressed WNT7A. The activity of the TOP-FLASH reporter was significantly stimulated by cells transiently transfected with WNT7A. WNT7A significantly activated JNK by a 2.3-fold induction of luciferase activity compared with control cells. Overexpression of WNT7A stimulated the expression of *MMP7*, *MMP9* and *CDH1* in the SKOV3 cells compared to control. MMP7 promoter activity was conferred by 237 bp 5’-flanking sequence which contains 2-TCF binding sites. Further, stable overexpression of WNT7A in SKOV3 cells increased cell adhesion and migration. The directional cell migration decreased by 60% in *WNT7A* knocked down SKOV3.ip1 cells, which are highly expressed *WNT7A*, using siRNA. Therefore, these results suggest that WNT7A is a critical regulator of ovarian tumorigenesis and progression via the canonical WNT/β-catenin pathway. Supported by ACS-IL 139038.

**P31: Targeting PTB (polypyrimidine tract-binding protein): promising therapeutic tool for ovarian cancer**, A. D. Arslan¹, X. He¹,², W. T. Beck¹,², ¹Department of Biopharmaceutical Sciences, College of Pharmacy and Cancer Center, University of Illinois at Chicago, Chicago, IL; ²Gynecologic Oncology Group (GOG) Core Laboratory for Molecular Pharmacology, Chicago, IL.
PTB is an RNA binding protein belonging to the heterogeneous nuclear ribonucleoprotein family (also known as hnRNP I). This multifunctional protein participates in pre-mRNA splicing, internal ribosome entry site (IRES)-mediated translation, mRNA polyadenylation, RNA localization and mRNA stabilization. We observed overexpression of PTB in human epithelial ovarian tumors compared to matched-normal ovarian tissues. Knockdown of PTB expression by vector based-shRNA impaired ovarian tumor cell growth and malignant properties in vitro, and decreased tumor growth in a xenograft mouse model of ovarian cancer (unpublished). These data suggest that PTB is a novel therapeutic target. We have designed a cell-based high-throughput screening (HTS) system that will permit fast and reliable detection of small molecule inhibitors of PTB protein activity that have potential to alter mRNA splicing and exhibit antitumor activity. Our approach is based on differential splicing of a PTB target gene (identified by microarray analysis) at different levels of PTB activity in the cell. We have constructed two reporter minigenes using fluorescent markers EGFP and dsRED. We further verified that the splicing of the minigenes was dependent on the PTB level in the cells. We are presently validating their compatibility for HTS assay by measuring the stability of fluorescence protein expression over time and response to known PTB inhibitory stimuli (e.g. shRNA). Optimization for HTS includes testing cell seeding density, DMSO tolerance, pilot experiments with specific chemical libraries, data analyses, and verification of “hits” in vitro, followed by pilot HTS with small chemically diverse libraries of small molecules to determine if any of these agents will block the activity of PTB. Since PTB plays an important role in maintaining ovarian tumor cell growth and malignant properties, identification of small molecule PTB inhibitors by this approach may uncover novel drugs for the treatment of ovarian cancer. Support in part by grants RO1 CA40570 and RO1 CA138762 (to WTB), by OCRF (to XH), and by UIC.

P32: Evolution and patterns of reproduction in Philippine mammals, Vince FitzPatrick, Department of Mammals, Field Museum of Natural History, Chicago, IL.

In most groups of animals, body size and litter size are allometrically related: the smaller the animal, the larger its litter size. This is typically true of mammals and of rodents specifically, which usually have large litters. However, anecdotal evidence obtained during field work in the Philippines has suggested that several speciose endemic clades of Philippine murid rodents (rats and mice) differ significantly from this pattern. A departure of this large set of species could have interesting implications for theories of life history and evolutionary aspects of island biogeography. Using field notes, published materials, and reproductive autopsies of about 600 specimens, we seek to document the reproductive characteristics of the Philippine murid fauna. This includes the two highly diverse “Old Endemic” clades (the cloud rats and the earthworm mice) which first arrived in the Philippines 12-20 million years ago, as well as several clades that arrived in the more recent geologic past, and some introduced by humans. Several species from Palawan, an area biogeographically distinct from the rest of the Philippines, were also included. Initial results suggest that the Old Endemics produce 1-2 offspring per litter, regardless of body size. This is much lower than “exotic pest” species of similar size, which are representative of continental species and have litters of 5-10 offspring. Some of the endemic clades which arrived in the Philippines more recently than the “Old Endemics” show intermediate litter sizes of 3-4 offspring. These data raise many currently unanswered questions about predation, longevity and reproductive strategies. Supported by a National Science Foundation REU grant.

P33: Mice lacking two sperm serine proteases, ACR and PRSS21, are subfertile, but the mutant sperm are infertile in vitro, Woojin Kang1, Natsuko Kawano1, 3, Misuzu Yamashita1, Yoshitaka Koga1, Taiga Yamazaki1, Tamako Hata2, Kenji Miyado3, Tadashi Baba1, 1 Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki,
Although sperm serine protease has long been believed to play an important role(s) in the fertilization process, the molecular mechanism remains controversial. In this study, we have produced double-knockout mice lacking two sperm serine proteases, ACR and PRSS21, to uncover the functional role of the trypsin-like activity in fertilization. The double-knockout male mice were subfertile, likely owing to the incompleteness of fertilization in the oviductal ampulla. Despite male subfertility, the mutant epididymal sperm exhibited the inability to undergo acrosomal exocytosis on the zona pellucida (ZP) surface and to traverse the ZP, thus resulting in the failure in fertilization in vitro. The double-knockout epididymal sperm were also defective in penetration through the cumulus matrix to reach the ZP. To account for the discrepancy between the in vivo and in vitro functions of double-knockout sperm, we characterized ejaculated sperm that were recovered from the uterus after mating (termed “uterine sperm”). Importantly, double-knockout uterine sperm revealed a low but significant rate of IVF (approximately 20%). Following artificial injection of the mutant epididymal sperm into the uterus, the 2-cell embryos were recovered from the oviduct at the similar level. The mutant epididymal sperm were also capable of fertilizing the oocytes in the presence of uterine fluids in vitro. These data demonstrate that the trypsin-like protease activity of ACR and PRSS21 is essential for the process of sperm penetration through the cumulus matrix and ZP in vitro, and that the sperm protease activity is still important but not essential for fertilization in vivo in the mouse.

P34: Possible involvement of SDK2 in sperm/oviductal epithelium interaction, Yusuke Suzuki, Masayo Ogawa, Ekyune Kim, Keiko Tsumura, Misuzu Yamashita, Woojin Kang, Fumi Tanaka, Tadashi Baba, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki, Japan.

Mammalian oviduct plays an important role in fertilization and early embryonic development. In particular, sperm adhesion to the oviductal epithelium is thought to be essential for migration of sperm into the oviduct and preservation at the isthmus. Although some molecules are reported to participate in the process, the details remain unclear. In this study, we carried out GST pull-down assays of oviductal extracts to identify novel proteins involved in binding to ADAM3 that is a sperm membranous protein essential for both sperm migration into the oviduct and sperm binding to the egg zona pellucida. MALDI-TOF MS analysis of ADAM3-binding proteins revealed that SDK2 is one of the candidate proteins. SDK2 is known to belong to the transmembrane immunoglobulin superfamily, and mediate cell-cell adhesion of neuronal cells. RT-PCR indicated that Sdk2 is expressed in all tissues examined. The protein abundance in the testis, brain, uterus, and oviduct was demonstrated by immunoblot analysis. In addition, when sections of female reproductive tracts were immunohistochemically examined, SDK2 was localized in the apical region of uterine and oviductal epithelia. To identify the binding domains between SDK2 and ADAM3, we carried out GST pull-down assays. ADAM3 associated with SDK2 through the cysteine-rich domain, and this domain was recognized by the third and fourth Ig domains of SDK2. These data suggest that SDK2 may function as a binding partner of ADAM3 in the process of sperm migration from the uterus to the unfertilized eggs.

P35: Endogenous EMMPRIN expression by human uterine fibroblast cells regulates metalloproteinase production, proliferation and decidualization, Andrea Braundmeier, Jiajia Bi, Pavni Mehrotra, and Romana A. Nowak, Department of Animal Sciences, University of Illinois, Urbana, IL.
The uterine endometrium undergoes extensive proliferation and remodeling in preparation for embryo implantation. Tissue remodeling is orchestrated by matrix metalloproteinases (MMPs). Extracellular matrix metalloproteinase inducer (EMMPRIN) stimulates MMP production in a number of cell lines including human uterine fibroblast (HUF) cells. We have shown in previous studies that human uterine epithelial cells (HES) secrete full length EMMPRIN via microvesicle shedding. This study’s goals were to determine 1) does soluble EMMPRIN from HES cells stimulate MMP production by HUF cells; 2) is EMMPRIN expression in HUF cells necessary for MMP stimulation by HES cells; and 3) is EMMPRIN expression in HUF cells important for cell proliferation and decidualization. Treatment of HUF cells with unconcentrated or 50-fold concentrated HES-cell conditioned medium (p<0.05) increased MMP-1, -2, and -3 mRNA levels. Removal of EMMPRIN from HES-cell conditioned medium by immunodepletion reduced MMP stimulation (7.7 fold for MMP-1, 4.2 fold for MMP-2 and 12.4 fold for MMP-3 (p<0.05)). Transfection of HUF cells with EMMPRIN siRNAs resulted in a dramatic reduction in the stimulatory effect of HES-cell conditioned medium on MMP-2 mRNA levels but a marked increase in MMP-3 mRNA levels, it did not affect MMP-1 levels. Thus, endogenous expression of EMMPRIN by HUF cells is important for MMP stimulation by HES-cell conditioned medium. Treatment of uterine fibroblasts with EMMPRIN siRNAs also decreased the proliferative response of these cells to serum by 40% as measured by DNA synthesis assays. Effects of EMMPRIN siRNA on decidualization of stromal cells were determined by treating cells for 10 days with estradiol, progesterone and cAMP in the presence or absence of EMMPRIN siRNAs. Immunoblotting showed that EMMPRIN protein levels were significantly reduced by day 4 of treatment and nondetectable on days 6-10. Levels of prolactin mRNA, a marker of decidualization, increased 150-200 fold in control cells by days 6-8 but were increased less than 50 fold in EMMPRIN siRNA transfected cells. These data indicate that EMMPRIN expression by uterine fibroblast cells is important not only for regulation of MMP production but also appears to play a role in regulating proliferation and decidualization of these cells. Future studies will focus on the role of EMMPRIN in cell cycle regulation in uterine stromal cells. Supported by SCCPRR U54HD20093.

P36: Effects of levonorgestrel and mifepristone on endometrial receptivity and embryo implantation in a 3-dimensional in vitro model system, C.X. Meng1, 2, P.G.L. Lalitkumar1, F. Hambiliki1, K. Gemzell-Danielsson1, 2Department of Women’s and Children’s Health, Karolinska Institute, Stockholm, Sweden, 2Department of Obstetrics, Gynecology & Reproductive Biology, Michigan State University, MI.

Levonorgestrel or mifepristone can be administered orally for emergency contraception, preventing unwanted pregnancy after an unprotected intercourse with an efficacy ranging from 57%-95%. However, the mechanisms of action of these two steroids remain incomplete, mainly because their effects on the endometrial-embryo interaction are impossible to be studied in vivo due to ethical concerns. This project was designed to study the effects of these two steroids on endometrial receptivity markers and embryo implantation in vitro. These studies were approved by the local ethics committee at Karolinska University Hospital, Sweden. Endometrial biopsies were obtained from fertile women between days 4 and 5 after the luteinizing hormone surge. The endometrial cells were isolated and co-cultured in 3-dimensional constructs consisting of stromal cells underlying the epithelial cells. Surplus embryos in blastocyst stage from an IVF clinic were cultured on the epithelial cell layer. All cultures were exposed to either levonorgestrel or mifepristone, while the controls were treated with progesterone. Immunostaining revealed that estrogen receptor (ER)-α and β, progesterone receptor (PR)-(A+B), vascular endothelial growth factor (VEGF), leukemia inhibitory factor, interleukin-1β, and cyclooxygenase-2 were present in both cultured epithelial and stromals cells, whereas the expression of PR-B, androgen receptor,
integrin αvβ3, and mucin 1 were confined to epithelial cells. Treatment with levonorgestrel did not change the expression of any markers studied or impair blastocyst attachment to the endometrial construct. In contrast, mifepristone up-regulated the ER-β and PR-B expression, whereas it down-regulated the expression of stromal VEGF, epithelial integrin αvβ3 and mucin 1. Mifepristone also inhibited blastocyst attachment in vitro. These studies indicate that levonorgestrel is unlikely to have any post-ovulatory effect whereas mifepristone has an inhibitory effect. This finding is consistent with the conclusion drawn by a Cochrane Database of Systematic Review 2008 that mifepristone has a higher efficacy than levonorgestrel when used for emergency contraception. [Supported by grants from UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, WHO, Geneva, Switzerland; Swedish Medical Research Council (2003- 3869, K2007-54X-14212-06-3), Karolinska Institute.

P37: FOXM1 is expressed in actively proliferating cells during pituitary gland development, Adam Ploegman, Buffy S. Ellsworth, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL.

POX1 is a member of the forkhead family of transcription factors whose DNA-binding domain exhibits a “winged-helix” structure. FOXM1 exists as three different isoforms: FOXM1a, FOXM1b, and FOXM1c. FOXM1b and FOXM1c are transcriptionally active, while FOXM1a is inactive, and therefore may serve as a dominant negative form. Forkhead factors are important in many different cellular processes. The chief role of FOXM1 is to regulate the cell cycle and mitosis. FOXM1 acts on genes that push the cell into mitosis through G1/S phase and G2/M phase checkpoints by regulating the expression of targets such as Cyclin D, Cyclin B and Cdc25b. FOXM1 is necessary for cell proliferation associated with organ development. The central goal of our studies is to understand the molecular mechanisms that govern pituitary gland development. We have found that FOXM1 co-localizes with proliferation markers such as BrdU in mouse embryos at ages e12.5 through e16.5 as well as a subset of cells that express Ki67 at ages e14.5 and e16.5, but not with the cell cycle inhibitor p57. We have acquired Foxm1b knockout mice in order to address the role of FOXM1 during pituitary gland development. These studies are critical to determine whether FOXM1 is required for normal pituitary gland development. This work was supported by startup funds from SIU School of Medicine.

P38: Regulation of the inhibin alpha subunit gene by the NR4A orphan nuclear receptors in ovarian granulosa cells, K.M. Meldi, A.D. Burkart, PhD, S.M. Thomas, W.B. Pearse, and K.E. Mayo, PhD, Department of Molecular Biosciences, Center for Reproductive Science, Northwestern University, Evanston, IL.

Nuclear receptors play important roles in regulating gene expression in response to hormonal ligands. Some nuclear receptors exhibit ligand-independent binding and regulation of gene expression. One such family is the NR5A nuclear receptors steroidogenic factor 1 (SF-1) and liver receptor homolog 1 (LRH-1), which play key roles in regulating many genes in the ovary and are important for fertility. One such gene is the inhibin α subunit gene, which is dynamically regulated by follicle-stimulating hormone and luteinizing hormone during the rodent estrous cycle. We previously found that the NR4A orphan nuclear receptor nerve growth factor inducible B (NGFI-B) can act to repress inhibin α expression. In this study, we sought to more broadly investigate roles for the NR4A receptors nuclear receptor related 1 (NURR1) and neuron-derived orphan receptor 1 (NOR-1) in inhibin α regulation. We demonstrate that all three NR4A mRNAs are rapidly and robustly induced in the rat ovary in response to hCG treatment in vivo. This upregulation coincides with the down-regulation of the inhibin α mRNA. Transient transfections in
GRMO2 cells show that the NR4A factors are capable of repressing inhibin α promoter activity via the proximal promoter containing binding sites for the NR5A nuclear receptors and CREB flanked by upstream (5') and downstream (3') GATA factor binding sites. Electrophoretic gel mobility shift assays and chromatin immunoprecipitation failed to reveal binding of the NR4A factors to this region, suggesting the repression is independent of DNA-binding. Furthermore, DNA-binding domain mutants of NGFI-B, NURR1, and NOR-1 were still capable of repressing the inhibin α promoter. Mutation of the 5' GATA site inhibited repression by the NR4A factors. Co-immunoprecipitation assays show that GATA-4 interacts with the NR4A receptors when overexpressed in HeLa cells. Ongoing experiments are aimed at determining where the proteins interact and the functional consequences of the interactions. This work is funded by the NIH Specialized Cooperative Centers Program in Reproductive Research (U54 HD41857) to KEM and the NICHD Reproductive Biology Training Grant T32 HD07068 to KMM.

**P39: Differential and Interactive Effects of Ligand-Bound Progesterone Receptor A and B Isoforms on Tyrosine Hydroxylase Promoter Activity, Philip J. Jensik and Lydia A. Arbogast,** Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

Progesterone receptors (PRs) are expressed in hypothalamic and brainstem catecholaminergic neurons. PRs are highly expressed in tuberoinfundibular dopamine neurons and ovarian steroids influence prolactin release in part by regulating dopamine synthesis. Tyrosine hydroxylase (TH) is the rate-limiting enzyme and progesterone can exert both stimulatory and inhibitory actions on TH activity and TH mRNA levels in these dopamine neurons. The aims of this study were to: 1) determine the independent transcriptional effects of PRA and PRB on the TH promoter, 2) map the location of progesterone’s action using TH promoter deletion constructs and 3) evaluate interactions of PRA and PRB on TH promoter activity. TH positive neuronal mouse CAD cells were transiently transfected with 1) CMV-rPRA and/or CMV-rPRB, 2) TH promoter luciferase constructs and 3) a CMV-Renilla luciferase (normalization) construct. Transfected cells were treated for 20 hours with vehicle or 200 nM progesterone and then TH promoter activity was assessed. When PRA was transfected alone, progesterone treatment did not alter TH-9000 promoter activity above basal levels, but progesterone treatment of cells transfected with PRB alone caused a 6 fold increase in TH promoter activity. Progesterone treatment of PRB transfected cells increased TH-9000 through TH-1400 construct promoter activities around 7 fold, but did not alter TH-1300 or smaller deletion construct promoter activities. When PRA and PRB were transfected at a 1:1 ratio, the fold activation of the TH-9000 promoter was about 50% of the activation of PRB alone after 20 hours of progesterone treatment. These data indicate that ligand bound PRB may act as a transcriptional activator of the TH gene and that its site of action lies between the -1400 and -1300 positions of the TH promoter, whereas PRA may inhibit the activities of PRB on the TH promoter. Taken together, these data suggest that progesterone actions on the TH promoter are determined by the PR isoform expressed, and changes in the ratio of PRA to PRB may affect the ability of progesterone to increase TH expression. Supported by NIH grants HD045805 and HD048925.

**P40: FOXO1 expression is reduced in human pituitary adenomas, Corrie Farris, Deborah Jung, Buffy S. Ellsworth,** Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

FOXO1 is a forkhead transcription factor that inhibits proliferation and promotes apoptosis. It regulates genes that are involved in many diverse processes, including regulation of cell proliferation and cancer. The goal of our research is to understand FOXO1 and the role that it plays in human pituitary adenomas. In order to characterize FOXO1’s role in pituitary cell proliferation, we performed immunohistochemical staining with FOXO1 and bromodeoxyuridine (BrdU) on
mouse pituitaries at various ages. We hypothesized that since FOXO1 acts as a tumor suppressor by inhibiting cell cycle progression, FOXO1 will be expressed in non-proliferating cells. Consistent with our hypothesis, we find that FOXO1 does not co-localize with BrdU in actively proliferating cells in the mouse pituitary at ages e12.5, e14.5, and e16.5. Because of the role that FOXO1 plays as a tumor suppressor in many tissues, we hypothesized that FOXO1 expression in human pituitary tumors would be at lower levels than in normal (non-tumor) human pituitary tissue. To determine FOXO1 expression levels in human pituitary adenomas, real time RT-PCR was performed. Gonadotropinomas and null cell adenomas were examined. Gonadotropinomas are characterized by the oversecretion of the reproductive hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH). Null cell adenomas do not secrete any hormones but are related to gonadotropes. Real time RT-PCR data shows that FOXO1 levels are significantly reduced by approximately 16.5 fold in gonadotropinomas and by 14 fold in null cell adenomas as compared to normal human pituitary. These data suggest that FOXO1 may act as a tumor suppressor in pituitary gonadotrope cells. This work was supported by startup funds from SIU School of Medicine.

**P41: Analysis of protein expression differences in MCF-7 breast cancer cells due to exposure with varied concentrations of acetochlor and chlorpyrifos,** Jessica D. Rich and Jennifer R. Schultz-Norton, Department of Biology, Millikin University, Decatur, IL.

Acetochlor, a highly toxic herbicide most commonly used for weed prevention in field crops, is primarily a protein inhibitor in plants, but has also shown adverse effects towards mammalian systems. Chlorpyrifos, a moderately toxic insecticide also used on field crops, has also been thought to have negative effects on such systems. Transient transfections were performed with each of these two chemicals in order to determine their effects on both the MCF-7 breast cancer and U-2 OS osteosarcoma cell lines. Dimethyl sulfoxide (DMSO) was used as the negative control and 10 nM estradiol as the positive control. Acetochlor and chlorpyrifos were tested for estrogenicity in concentrations ranging from 10 nM to 10 µM. While no significant differences from the control were obtained for either acetochlor or chlorpyrifos, trends indicating activation for low levels of acetochlor were observed. Cytotoxicity assays were performed to determine the effect of acetochlor and chlorpyrifos on cell growth. After a 48-hour treatment with 10 nM to 10 µM hormone, differences in the toxicity of these compounds were observed when compared to DMSO control. Taken together, these studies will help to elucidate the transcriptional effects and toxicity of acetochlor and chlorpyrifos in cancer cells. Funding provided by Millikin University.


A genome-wide mutagenesis screen employing ENU in mice identified five mutant lines with male urogenital defects. Whole genome analysis using a highly parallel SNP assay mapped each mutation to a 20-90 Mb region. Candidate genes in these regions were identified and sequenced, allowing identification of a mutation responsible for the line with hypogonadism and patchy germ cell loss in Polo-like kinase 4 (Plk4).

Plk4 encodes a serine/threonine kinase involved in centriole formation and cell cycle progression however, the specific role of PLK4 in spermatogenesis is unknown. The ENU mutation in PLK4 resulted in an amino acid alteration from isoleucine to asparagine at residue 242 (I242N), which resides within the kinase domain. Homozygous mice (Plk4 I242N /I242N) were embryonic lethal, but heterozygous mice (Plk4 + /I242N) exhibited hypogonadism and patchy germ cell loss. The I242N mutation did not affect sperm counts, motility or fertility. Genetic complementation analysis using a gene-trap Plk4 allele (Plk4GT) confirmed causality of the I242N mutation. The patchy
germ cell loss phenotype was not due to a defect in the hypothalamic-pituitary axis, as LH, FSH and testosterone were mainly unaffected. Analysis of the seminiferous tubules at 6 weeks of age did not show a stage-specific defect in spermatogenesis however, the number of defective tubules in the Plk4+/I242N mice compared to Plk4+/+ mice was greater. Earlier time points were then analyzed (P10, P15 and P21) and the defect was present throughout. Analysis of testis cord formation at E13.5 is ongoing but appears normal. To determine tissue expression of Plk4, organs were harvested from E18.5 Plk4+/GT embryos, which contain a β-geo gene, and were stained with X-gal and compared to Plk4+/+ littermates. The testis, epididymis, kidney, heart, thymus and brain showed staining in the Plk4+/GT mice but not in the Plk4+/+ littermates. To date, analysis of cellular expression of PLK4 within the testes using Plk4+/GT mice has shown that PLK4 is expressed within specific germ cell populations in a stage-specific manner. To determine the functional consequences of the I242N mutation, optimization of a kinase assay is underway that will compare wildtype, mutant (I242N), kinase dead and constitutively active PLK4. Further investigation into the underlying etiology of the defect, the mechanism of PLK4 action in the testis and potential downstream effector molecules is ongoing. Funding: U01 HD043425.

P43: GATA-4 Role in ovarian granulosa cell function and female fertility Jill Bennett and Carlos Stocco, Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL.

Two potential transcription factors involved in folliculogenesis are members of the GATA family. GATA-4 and GATA-6 are highly expressed in granulosa and theca cells, which are the two main cellular components of the ovarian follicle. However, the role of GATA factors in ovarian function in vivo has remained elusive due to embryonic lethality of knockout animals. To investigate the function of GATA-4 and GATA-6 in the ovary GATA-4 and GATA-6 were knocked down in granulosa cells both in vivo and in vitro. The in vitro results show that the response of primary rat granulosa cells to follicle stimulating hormone (FSH) is attenuated by GATA-4 knockdown (siGATA-4). We found that aromatase expression was significantly reduced in the presence of siGATA-4 but P450scc and StAR expression remained unaffected. In addition, in vivo silencing of GATA-4 expression in granulosa cells using a Cre-Lox model resulted in reduced fertility. Granulosa cell specific GATA-4 null animals have a significant decrease in the number of pups per litter when compared to wild-type animals. Interestingly, GATA-6 knockdown in granulosa cells have no effects on litter size. Moreover, knockdown of GATA-4 leads to a significant decrease in the size of the ovary when compared to control animals. Accordingly, granulosa cell proliferation was significantly reduced after GATA-4 knockdown when compared to wild-type cells. These results show that GATA-4 is an important regulator of normal ovarian function. The ovarian actions of GATA-4 are in part mediated by the stimulation of granulosa cell proliferation and differentiation. Funding by NIH RO1 HD057110.

P44: p53 Smad Crosstalk in Ovarian Cancer, Roshan Ahmed, Kari Inoue, Joanna E. Burdette, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL

Eighty percent of ovarian cancers have p53 mutations, which are also associated with primary tumor size, serous stage III cancers, and abnormal accumulation of p53 protein. Cooperation between smads, the downstream transcription factors of the TGFβ pathway, and tumor suppressor p53 has been documented. Mutations in both TGFβ signaling and p53 cause aberrant cell behavior, disease, and cancer, making their interaction critical to the understanding of cancer progression. The role of TGFβ in cancer changes as the disease progresses; in the early stages TGFβ suppresses tumor growth, however after tumor formation TGFβ promotes metastasis. Our
hypothesis is that TGFβ signaling prevents tumor progression in ovarian cancer p53 wildtype cell lines, while p53 mutants exhibit TGFβ-dependent migration, proliferation and tumorogenesis. To determine the role of p53 in the TGFβ pathway, a panel of six ovarian cancer cell lines was chosen based on p53 status (OVCA420, HEYC2 - p53WT; OVCA432, OVCAR3 - p53 Mutant; SKOV3, OVCAR5 - p53 null). Proliferation, apoptosis, migration, and protein expression were measured in these cell lines after stimulation with TGFβ (10ng/mL) and activin (20ng/mL). In response to TGFβ, p53 wildtype ovarian cancer cells were arrested in G1 and p21 was induced. Tumor suppressor maspin is absent in mutant p53 cell lines portraying a gain of function that could account for increased metastatic properties. MapK/Erk expression was upregulated only in p53 wildtype cell lines, and MapK/Erk encourages p53 phosphorylation and promotes p53/Smad interaction. Stable transfection of p53 in the null SKOV3 cell line allowed re-expression of p21, MapK/Erk, and maspin. We have identified p53 as a modifier of smad signaling that encourages cell cycle arrest in its wildtype form. Mutant p53 cell lines demonstrated a gain of function by eliminating the tumor suppressor maspin. Further studies will help determine whether the loss of maspin accounts for increased metastasis in p53 mutant cells and how maspin is regulated by the TGFβ pathway. Ultimately these experiments will determine whether p53 is a molecular switch modulating TGFβ induced smad signaling and tumor progression in ovarian cancer.

**P45: Effects of negative energy balance on kisspeptin and gonadotropin-releasing hormone in the rat hypothalamus.** A.E. Flowers1, J.E. Levine2, T.H. Horton2, B.G. Mann2, C.M. Muller2, 1Master of Biotechnology Program and 2Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL.

Energy homeostasis profoundly affects the hypothalamic-pituitary-gonadal (HPG) axis; studies thus far have shown that specific areas of the hypothalamus convey metabolic inputs to the reproductive system. Kisspeptin (KP) neurons in the arcuate nucleus (Arc) and anteroventral periventricular nucleus (AVPV) extend fibers directly to gonadotropin-releasing hormone (GnRH) neurons in the median eminence (ME); KP directly affects GnRH expression via its receptor, GPR54. Serum luteinizing hormone (LH) concentrations and Kiss-1 mRNA expression in the AVPV are suppressed by a 48h fast. We hypothesize that long-term food restriction similarly inhibits KP secretion, suppressing GnRH release, and thereby suppressing serum LH. To test this hypothesis, newly-weaned Sprague-Dawley rats were fed ad libitum until they reached 75g body weight, then were grouped into (1) ad libitum fed (AL), (2) food restricted (FR, 5.7g food daily) for 4 wk, or (3) food restricted as in (2), then re-fed ad libitum (RF) for 24h prior to sampling. Three to six days prior to sampling, a guide cannula was implanted into the ME; on sampling day, a microdialysis probe was inserted in this cannula. Rats were sampled for 6h at 5m intervals. Terminal trunk blood samples were collected for LH measurement, uterine and paired ovarian weights were measured, and brains were examined to verify cannula placement. FR significantly reduced uterine (p=0.03) and paired ovarian (p<0.001) weights. The uteri and ovaries of FR and RF animals resembled those of pre-pubertal, but not post-pubertal AL animals, indicating that puberty was prevented in these groups. RIA will be used to measure GnRH (hours 1-3) and KP (hours 4-6) in the microdialysates and LH in the serum samples. The program ULTRA will be used to identify pulses of GnRH and KP secretion. ANOVA will be applied to test significance of treatments. A reduction in the frequency and/or amplitude of GnRH and KP secretion and a reduction in serum LH concentrations in FR relative to AL animals, and partial or complete restoration of these measures to AL levels in RF animals would support our hypothesis. Supported by HD21921 from NIH to JEL.
P46: Germ cell loss, development delay and globozoospermia in mice lacking Pumilio, Y.H. Shih¹, W. Qiang², E. Y. Xu², ¹Master of Biotechnology Program, Northwestern University Robert R. McCormick School of Engineering and Applied Science, Evanston, IL, ²Division of Reproductive Biology research, Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL.

Pumilio belongs to a conserved RNA binding protein family-PUF family, which contain a conserved RNA binding domain and regulate germ cell development, stem cell maintenance and cell cycle in diverse species. Human contains two PUF proteins—PUMILIO 1 (PUM1) and PUMILIO 2 (PUM2), whose function remains unknown. We generate mouse models with the deletion of both Pum1 and Pum2 genes in order to understand the roles of mammalian Pumilio proteins during development. Mice lack both Pumilio 1 and Pumilio 2 in the testis is completely infertile with smaller testis and germ cell developmental defects. The developmental defects include extensive germ cell loss, developmental delay, giant round spermatid, vacuous spermatid, and rounded-head spermatozoa (globozoospermia). The globozoospermia is a human infertile syndrome characterized by acrosome malformation and rounded spermatozoa head. The reason of globozoospermia is not well known and only a few genes have been implicated, also we found some genes relate to this syndrome have Pumilio binding site on its mRNA 3’ UTR. Further study on the interaction between Pumilio and these mRNAs will be our next step. All together, Pumilio mutant animals represent a great animal model to understand this human infertility syndrome and also provide a foundation to dissect mechanisms of posttranscriptional regulators in mouse and human development. This work was supported by NIH, NICHD U01 HD045871.

P47: Sonic Hedgehog is dispensable for mouse gonad development, Jeff Huang, Paul Cooke and Humphrey Yao. Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, IL.

In mammals, three Hedgehog (Hh) orthologs have been identified: Desert hedgehog (Dhh), Indian hedgehog (Ihh), and Sonic hedgehog (Shh). The Hh ligands are produced by the earliest differentiated Steroidogenic factor 1 (SF1)-positive cells such as adrenocortical cells in the adrenal, Sertoli cells in the testis, and granulosa cells in the ovary. These tissue-specific ligands then act upon Hh-responding cells located mainly in the mesenchyme, including capsular cell in the fetal adrenal, fetal Leydig cells in the testis, and theca cells in the adult ovary. Although these three Hh genes have diverse expression patterns and functions, they are thought to induce a common signal transduction pathway in the target cells. We investigated the function of Shh in adrenals and gonads by producing a conditional knockout model where Shh is inactivated in the SF1-positive cells in adrenals and gonads as early as at embryonic day 11.5. Inactivation of Shh in the SF1-positive cells affected fetal adrenal growth but yielded no ovarian/testicular phenotypes in fetal as well as in adult life. It was reported that other Hh ligands are present in gonads: Dhh in the testis and Ihh and Dhh in the ovary. Our results suggest that loss of Shh could be compensated by other Hh ligands in the gonads. This study was supported by Billie Field Graduate Fellowship and NIH-HD059961.

P48: Impact of estrogen receptor alpha phosphorylation site mutations on hormone responsiveness and endocrine resistance in breast cancer, Kyuri Kim and Benita S. Katzenellenbogen, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana IL.

The estrogen receptor alpha (ER) contains multiple serine residues capable of undergoing post-translational modification by phosphorylation. In order to understand the role of phosphorylation status in affecting the response of receptor to the natural hormone estradiol, the selective estrogen receptor modulator, tamoxifen, and the selective estrogen receptor down-
regulator, ICI182,780 (Fulvestrant), we generated multiple combinations of ER phospho-mutants, at residues serine 104, 106, 118, 167, 236, and 305, and examined their impact on receptor half-life, the agonist and antagonist balance of SERMs and SERDs, the regulation of ER transcriptional activity, and stimulation of proliferation in response to estradiol and SERMs/SERD. ERα mutants were generated by substituting serine residues with either alanine or glutamic acid, and of the sixteen mutants screened, half were selected for further analysis. The mutant receptors were generated into U2OS osteosarcoma-tetracycline regulated- ERα stable cell lines for characterization. These phospho-ER mutant receptors were also expressed in MCF-7 breast cancer cells with concomitant knock-down of endogenous ERα. Receptors with changes at Ser-118 and Ser-167 showed altered responses to the antiestrogens tamoxifen and ICI182,780, i.e. strong agonistic stimulation and weak estrogen antagonistic activity of tamoxifen and ICI182,780 on gene regulation and differential stimulation of cell proliferation. Other mutant ERs showed increased protein stability in the presence of estradiol or ICI182,780. Hence, changes in ERα affecting the phosphorylation status of the receptor greatly impact receptor function and differential SERM and SERD modulated cellular responses that could contribute to resistance to endocrine therapies in breast cancer. Supported by grants from the Breast Cancer Research Foundation and NIH.

P49: Capacitation regulates oviduct glycan receptors on boar sperm. Kadirvel Govindasamy, S. Machado and David J. Miller, Department of Animal Science, University of Illinois, Urbana-Champaign, IL.

Prior to fertilization, mammalian spermatozoa bind to the lower oviduct to form a sperm reservoir (SR) that enhances the fertile lifespan of sperm, regulates capacitation and ensures availability of fertile sperm at the site of fertilization. This binding is proposed to be due to an interaction between glycans (ligand) in the oviductal epithelium and protein (receptor) on the sperm. Our earlier experiment using an array of 377 glycans showed that many sperm binding glycans contained a structural motif known as the LewisX trisaccharide (LeX). The present study was carried out to detect the carbohydrate receptor on the plasma membrane of boar sperm and carbohydrate binding changes during capacitation. Pooled semen from different boars was used for the study. Spermatozoa were washed through a Percoll cushion in non-capacitating medium (NCM) and sperm concentration was adjusted to 20 million/ml. Washed sperm were incubated either in capacitation medium (CM) or in NCM (negative control) for 4h. The uncapacitated and capacitated spermatozoa were incubated with fluorescein conjugated sulfated LeX and sulfated Lewis A (LeA) attached to an agarose polymer to simulate a glycoprotein at final concentration of 50µg/ml respectively in NCM and CM for 30min. Before incubation, sperm were immobilized with 0.01% formaldehyde. At least 100 sperm in each group were observed using fluorescence microscopy. The results revealed that sulfated LeX and sulfated LeA bound to the anterior surface of the sperm head. Most of the live sperm were labeled over the anterior acrosome, forming a crescent. In non-capacitating conditions, 58.4±1.6 % of sperm were labeled with sulfated LeX and only 4.7±0.8 % of sperm were labeled with sulfated LeA. After capacitation, there was a reduction in sulfated LeX labeling and only 8.6±1.4 % of sperm bound sulfated LeX. However, sulfated LeA labeling was increased after capacitation and 22.6±1.3 % of capacitated sperm bound to sulfated LeA. We concluded that the sulfated LeX recognizing molecule is located on the anterior surface of sperm head and available on uncapacitated boar spermatozoa, but few capacitated spermatozoa. Thus, sulfated LeX receptor expressed on uncapacitated sperm may be modified during the capacitation process. This study further supports the hypothesis that uncapacitated sperm bind to glycans in the isthmus region of oviduct to form a sperm reservoir and release may be coincident with capacitation. Supported by BOYSCAST and USDA-NIFA.
P50: Antioxidant enzyme activity in reproductive tract fluids of tropically-adapted rams, J.P.A. Rêgo, C.E. Souza, D.M.F. Gondim, J.T.A. Oliveira, A.A.A. Moura, 1Department of Animal Science, 2Department of Biochemistry, Federal University of Ceará, Fortaleza, Brazil.

Spermatozoa are an important source of reactive oxygen species (ROS), especially superoxide anion, produced by the sperm-bound NADPH oxidase. Before ejaculation, ROS play a role during epididymal maturation. After ejaculation, they are implicated in membrane changes during sperm capacitation. However, excessive ROS production can lead to oxidative stress and sperm damage, which could impact its ability to fertilize the egg, and embryo development as well. Cauda epididymal fluid (CEF) remains in prolonged contact with sperm before ejaculation. Accessory sex gland fluid, in turn, constitutes most of seminal plasma, and is composed by different proteins that interact with sperm immediately after ejaculation. These fluids display different scavenging strategies against ROS, including iron binding proteins and antioxidant enzymes. Particularly, superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anions in hydrogen peroxide (H2O2), and catalase (CAT), which converts H2O2 in water, play important role in this line of defense, and their activities in the male reproductive fluids have been related to reproductive success. However, few is known about their activities and roles in the ovine reproductive tract, especially in tropically-adapted sheep. Therefore, the purpose of this research is to characterize SOD and CAT activities in the CEF, vesicular gland fluid (VGF) and seminal plasma (SP) of tropically-adapted hairy rams. SP samples were obtained from 15 rams by artificial vagina before slaughter. CEF was obtained by epididymal perfusion, while VGF was collected by milking vesicular glands. Total protein and the activities of SOD and CAT were measured using a spectrophotometer, and expressed per milligram of protein in the respective fluid. SOD activity was 2.5-fold higher in the CEF compared to the VGF, and 1.8-fold compared to SP. On the other hand, CAT activity was much higher in the VGF, compared to both CEF (10-fold) and SP (7-fold). These results were not unexpected, since CEF must scavenge excess ROS produced during epididymal maturation and storage, specially superoxide anion. On the other hand, VGF, which comprises most of the AGF in ruminants, also seems to modulate ROS production during sperm capacitation, and scavenge excessive H2O2 resulting from SOD action. These findings suggest that antioxidant strategies in the different regions of the reproductive tract are complementary, to yield the best protection to spermatozoa.

P51: Murine homologs of highly conserved germline stem cell factors-Pum1 and 2 work redundantly to regulate growth and cell proliferation in dosage-sensitive manner, W. Qiang, Y. Chen, Y. Shih, T. Kurita, and E. Y. Xu, Division of Reproductive Biology Research, Department of OB & GYN, and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.

Pumilio (Pum) belongs to a highly conserved RNA binding protein family –PUF (Pumilio and FBF) family with diverse functions in development, cellular stress and aging in many species. In several divergent metazoan lineages Pumilio homologs function as key posttranscriptional regulator during germ cell development and differentiation. However function of mammalian Pumilio proteins (Pum1 and Pum2) remains poorly understood. We have previously shown that Pum2 is dispensable in mouse development and growth, but is essential for normal testis size and normal sperm count. Here we reported the phenotypic characterization of the other mammalian Pumilio homologue- Pum1. Loss-of-function mutation in Pum1 resulted in smaller but otherwise normal mice. The mutant mice were smaller than control littermates from birth to adulthood. Body weight reduction was detectable as early as embryonic stage E10.5. To determine the cellular basis of the growth defect, specifically the reduction in brain and thymus weight, we compare the cell number and cell size in postnatal wild type and Pum1 null mice. The cellularity of forebrains and
thymus in *Pum1* mutant mice was significantly lower than that of their wild-type littermate controls, while cell size was not reduced. Thus, the small body/organs size of Pum1 null mutant mice was a result of reduced cell number. In neurosphere assay, both numbers and sizes of neurospheres were significantly reduced in *Pum1* null brain cells, suggesting that the reduced brain size in Pum1 null mice reflects defects in self-renewal ability of neural stem cells. Furthermore, to address the functional redundancy of Pum1 and Pum2, the compound mutants were generated. The body weight was further reduced in Pum1 or Pum2 compound knockout mice in a dose dependent manner. While mice with a single copy of Pum1 or Pum2 gene can survive, Pum1 /2 double null mice were never recovered. These observations indicate the essential roles of Pum proteins in embryonic development as well as the functional redundancy between mammalian Pumilio homologues. This work was supported by NIH, NICHD U01 HD045871.

**P52: Growth hormone potentiates estrogen action on proliferation and gene expression in spontaneous dwarf rat mammary gland and in T47D human breast cancer cells**

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Mammary gland (MG) development is controlled by both ovarian and pituitary-derived hormones. Estrogen (E2) and Growth Hormone (GH) in particular are required for full mammary development, as evidenced by many studies in rodents. The Spontaneous Dwarf Rat (SDR) is a particularly useful model for studying GH, as these animals are deficient in GH yet have intact, functional pituitary glands. Previous studies in SDR have shown that both GH and E2 are required for mammary development as well as for sustaining carcinogen-induced mammary tumors. In this study, we used ovariectomized SDR to study crosstalk between GH and E2 in the MG. A 5-day treatment with GH induced slight ductal development (as shown by MG whole mounts) and a small amount of epithelial cell proliferation (shown by IHC staining of Ki67). The effect of E2 on these markers was greater than GH alone. Notably, GH+E2 treatment resulted in more extensive ductal development and proliferation than with E2 alone, suggesting GH could potentiate the effects of E2. Therefore we looked at whether GH could affect ER-α expression and activity. Indeed, not only did GH up-regulate ER-α mRNA expression in the MG, but also potentiated expression of ER target genes (PR, ABCG2, and AREG) induced by E2, even though GH alone could not stimulate transcription. In addition to normal MG development, GH is emerging as an important player in hormone-dependent breast cancer so we also investigated GH+E2 crosstalk in T47D human breast cancer cells. Similarly to the SDR MG, 5 days of GH treatment stimulated proliferation only slightly, while E2 had a more substantial effect. Again, GH+E2 treatment resulted in significantly more T47D cells compared to either hormone alone. In contrast to the SDR, GH had no effect on ER-α expression in the T47D cells, yet was still able to potentiate E2-induced transcription of the ER target genes PS2 and AREG. This potentiation of ER activity is gene-specific, since GH did not enhance transcription of all observed ER target genes. Together, these results suggest that the increased proliferation which occurs in the presence of both E2 and GH can be at least partly explained by the ability of GH to potentiate E2 action. The mechanism(s) by which GH elicits these effects is currently under investigation. A better understanding of the crosstalk between GH and E2 will contribute to the development of novel or improved breast cancer therapies with enhanced efficacy and fewer off-target effects. Supported by: NIH T32-HL07692-20 (D.L.F.); R01 CA099904 (S.M.S.); R01 CA130932-2 (J.F.); the Department of Veterans Affairs Merit Review Program (T.G.U.); American Cancer Society RSG-10-187-01.
P53: Pro-inflammatory cytokines influence estrogen activity at the promoter of BIRC3, a member of the inhibitor of apoptosis family, by facilitating recruitment of the estrogen receptor (ER) to a latent ERE, Madhumita Pradhan, Leslie Bembinster, Jonna Frasor, Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL.

ER and the pro-inflammatory transcription factor NFκB both play important roles in cell proliferation and survival. Repression of NFκB activity by ER is a widely studied phenomenon whereas how NFκB affects ER action is largely unknown. A previous microarray study from our lab has identified numerous genes in breast cancer cells where the NFκB pathway positively influences estrogen action. One such gene from this signature is BIRC3, a member of the inhibitor of apoptosis family that is known to confer protection against apoptosis in many cancer cell types. Interestingly, our lab has recently shown that although estrogen cannot regulate BIRC3 on its own, it can potentiate expression of BIRC3 induced by the pro-inflammatory cytokine TNFα, leading to prevention of apoptosis in breast cancer cells. However, the mechanism by which E2 potentiates TNFα action is not yet known. TNFα is known to regulate BIRC3 expression via NFκB recruitment to two functional NFκBREs in the gene promoter. Here, we show that E2 enhances TNFα activity through ER recruitment to a near-consensus ERE in the BIRC3 promoter. Intriguingly, presence of TNFα along with E2 is required for the ERE of BIRC3 to become functionally active, with ER recruitment to the gene occurring in an NFκB-dependent manner. Mutation of the ERE sequence to consensus as well as binding of ER to a naked template of the BIRC3 promoter indicate that the sequence of the ERE is not responsible for its latency in the presence of E2 alone. Interestingly, however, TNFα alone can cause enhanced histone H3 and H4 acetylation in the region around the ERE. This may contribute to the accessibility of the ERE which in turn may facilitate recruitment of ER in the presence of both E2 and TNFα. Once recruited, ER stabilizes NFκB on the promoter, leading to formation of an ER/NFκB complex at adjacent response elements, further increase in histone acetylation, and a substantial increase in transcription. These findings indicate that inflammation is capable of enhancing estrogen activity by facilitating ER occupancy at novel EREs which are previously inaccessible for ER binding; this phenomenon may be important for pathologies of hormone-dependent tissues that have an inflammatory component. Supported by NIH RO1 CA130932-01A2.

P54: Gene dosage of the coregulator REA is critical of uterine function and fertility, Sunghee Park1, Sangyeon Yoon1, Seongeun Park1, Yuechao Zhao1, Jianming Xu2, John P. Lydon2, Francesco J. DeMayo2, Bert O’Malley2, Milan K. Bagchi1, and Benita S. Katzenellenbogen1, 1Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL; and 2Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.

The sex steroid hormones- estrogen and progesterone -via their cognate receptors play critical roles in the regulation of uterine function. Although the effectiveness of hormone-receptor complexes is known to depend on coregulator partner proteins, little is known about the roles of coregulators in uterine development, early stages of pregnancy, and implantation. We previously identified a coregulator, repressor of estrogen receptor activity (REA), which is essential for proper cellular response to estrogen, and found that its conventional genetic deletion was embryonic lethal. In order to define the roles of REA in post-embryonic stages and in a tissue-specific manner, we generated REA conditional knockout mice (REA/f/fPRCre/+) by cre-loxP recombination in which REA function was abrogated only in progesterone receptor (PR)-expressing tissues. Conditional homozygous mutant mice were found to develop to adulthood, but females were completely infertile due to severely compromised uterine development and function, resulting in failure of implantation and decidualization. Deletion of REA in uterine cells led to cell cycle arrest and
apoptosis, phenotypic features indicating that REA is necessary for normal growth and maturation of the uterus. Interestingly, female mice heterozygous for REA (REA+/PRCre/+) had a uterine phenotype very different from that of homozygous knockout mice. While the uterine morphology of conditional REA heterozygous females was similar to that of wild type mice, treatment with estradiol (E2) resulted in abnormally large uteri showing increased proliferation of luminal epithelial cells. Taken together, our findings reveal that REA is essential for normal uterine function and successful implantation, and provide new insights in understanding the physiological roles of REA impacting reproduction and fertility and gene dosage-dependent actions. This research was supported by NIH grant U54HD055787.

P55: Health of primate ovarian tissue after long distance transport, J.E. Hornick, F.E. Duncan, M. Xu, and T.K. Woodruff. Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

A majority of reproductive-aged women faced with a cancer diagnosis survive their illness due to medical advances in cancer treatment. Returning to a normal lifestyle post-cancer is a priority for these survivors, and oftentimes, this involves having children. However, both chemotherapy and radiation can cause infertility and even sterility, leading patients to seek out fertility preservation options prior to the start of cancer treatment. Ovarian tissue cryopreservation (OTC) is becoming increasingly common as a fertility preservation treatment for female cancer patients because it does not delay the onset of cancer treatment, does not require hormonal stimulation, and can also be used for females of all ages. Yet, OTC is a specialized technique and not all cancer treatment facilities have this capability. To ensure OTC is an option for female patients across the country, transportation of ovarian tissue across long distances may be required. It is known that human ovarian tissue can be transported at 4°C for up to 4 hours and is still suitable for cryopreservation (Schmidt et al. 2003). However, this transportation time may be too short for patients who live at a distance from oncofertility centers. We tested whether primate ovarian tissue could survive transport for more than 12 hours at 4°C. Both rhesus macaque and human ovarian tissue were cut into quarters or strips, respectively, and transported overnight on ice in SAGE holding medium (ingredients). We assessed follicle morphology by histology. Follicles within the tissue appeared intact by standard hematoxylin and eosin staining. There were low levels of apoptosis in both stromal cells and follicles as indicated by TUNEL staining. We cultured tissue as 500 µm thick cortical strips to assess viability of follicles. Follicle morphology and levels of apoptosis were assessed throughout the culture period. Follicles remain intact after short-term culture and apoptosis in both stromal cells and follicles remains low. These data suggest that simple processing and transport protocols allow for same day or overnight (e.g. <12 hour) transport of ovarian tissue. Unlike traditional IVF where patients must live in an urban region, this emerging technology may reduce the disparity for patients living in regions where centers of excellence have not been established. Supported by NIH/NICHD R01C and T32 CA080621.

P56: Progesterone and prostaglandins in periovulatory leukocyte infiltration in the rat ovary, Oliver R. Oakley, HeyYoung Kim, Ismail El-Amouri, Po-Ching Patrick Lin, Jongki Cho, Mohammad Bani-Ahmad, Thomas Muse, and CheMyong Ko, Center of Excellence in Reproductive Sciences, Department of Clinical Sciences, College of Health Sciences, University of Kentucky, Lexington, KY.

Ovulation is preceded by intraovarian inflammatory reactions that occur in response to the preovulatory gonadotropin surge. As a main inflammatory event, leukocytes infiltrate the ovary and release proteolytic enzymes that degrade the extracellular matrix weakening the follicular wall, a required step for follicle rupture. In a recent study, we showed that similar numbers of leukocytes
reside in the ovary throughout the estrous cycle (approximately 500,000/ovary), except proestrus 2400 when 2-fold higher numbers of leukocytes were found (approximately 1.1 million/ovary). A similar trend of periovulatory rise in leukocyte numbers was seen in the superovulation-induced immature rat model, recapitulating a dramatic increase in leukocyte numbers upon gonadotropin stimulation. Both macrophage/granulocytes and lymphocytes were among the infiltrating leukocytes and were localized in the theca and interstitial tissues, where platelet-endothelial cell adhesion molecule-1 and intercellular adhesion molecule-1 may play roles in the transmigration of leukocytes, because their expressions correlates spatiotemporally with the infiltrating leukocytes. Surprisingly, we saw a strong inverse relationship between leukocyte numbers in the ovary and spleen, as well as significant reduction of leukocyte infiltration in the splenectomized rats, indicating that the spleen may serve as an immediate supplier of leukocytes to the periovulatory ovary. In the present study, we aimed to quantitatively measure the effect of blocking of prostaglandin and progesterone receptor signaling pathways in leukocyte trafficking from spleen to ovary during periovulatory period. Twenty-four day old immature rats were treated for superovulation by injecting PMSG followed by hCG injection. At the time of hCG injection, the rats were additionally injected with either RU 485 (progesterone receptor antagonist; 1 mg/kg body weight), Indomethacin (cyclooxygenase inhibitor; 1 mg/kg body weight) or vehicle. One, three and six hours later (hCG 1, 2 and 3h), leukocytes numbers were counted in the spleen, blood and ovary. Data analysis revealed that the spleen released similar numbers of leukocytes (10 millions/spleen) in all of the rats by hCG 6h. However, during this period, only vehicle-treated rats showed increased numbers of leukocytes infiltrating the ovary (1 million/ovary). These results show that neither progesterone nor prostaglandins affect splenic leukocyte release, but these hormones play critical role in recruiting leukocytes in the ovary.

P57: Role of Wnt4 in the prostate development, Wen-Yang Hu, Ikenna Madueke, Lynn Birch, Dan-Ping Hu, Guang-Bin Shi, Gail S. Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

Branching morphogenesis of the prostate is tightly regulated by common and tissue-specific morphoregulatory genes. Wnt genes encode a large family of secreted glycoproteins which are morphoregulatory factors important in controlling tissue patterning, cell fate and proliferation during development. Currently, little is known regarding the role(s) of Wnt genes during prostate gland development. We have previously shown that noncanonical Wnt5a is essential for normal prostate development where it regulates bud outgrowth, ductal elongation, branching, cell polarity and lumenization. The present study examines the role of another important noncanonical Wnt protein - Wnt4 during prostate gland development in rat and murine models. In the rat prostate, Wnt4 mRNA is expressed by epithelial cells during the budding stage. While Wnt4 expression is high during active morphogenesis in all prostate lobes, ventral prostate (VP) and lateral prostate (LP) expression declines rapidly following morphogenesis while dorsal prostate (DP) expression remains high into adulthood. Steroids modulate prostatic Wnt4 expression during early development with neonatal estrogen increasing wnt4 expression in VP and decreasing expression in LP and DP. In vitro organ culture of new born rat VP treating with Wnt4 protein resulted in significantly increase in branching morphogenesis. Immunostaining of incorporated BrdU indicated that Wnt4 treatment increased the prostate epithelial cell proliferation. In vivo and ex vivo analyses of developing mouse prostate was used to assess the functional roles of Wnt4. Wnt4 knockout (Wnt4−/−) mice prostates rescued by renal grafts revealed a role of Wnt4 in lumenization of the ducts. Analysis of day 30 prostates in Wnt4 heterozygous mice as compared to the wild type mice showed a significant decrease of branch tip number in Wnt4+/− mice. In summary, the present finds demonstrate that Wnt4 is essential for normal prostate development where it regulates ductal
branching and lumenization. These findings contribute to the growing body of knowledge on regulatory mechanisms involved in prostate gland development which are keys to understanding abnormal growth processes associated with aging. This study was supported by NIH/NIDDK R01 DK40890-18.

P58: Characterization and estrogen modulation of human adult prostate progenitor cells, Guang-Bin Shi, Wen-Yang Hu, Ikenna Madueke, Dan-Ping Hu, Jason L. Nelles, Hung-Ming Lam, Gail S. Prins, Department of Urology, University of Illinois at Chicago, Chicago, IL.

Adult stem cells are involved in normal tissue regeneration in most, if not all, organs of the human body. In the prostate, these adult stem cells are termed prostate progenitor cells (PPCs) and are better characterized in rodents in comparison to human counterparts. Reasons for the discrepancy are vast and include difficulty in isolation and enrichment of human PPCs. Applying recent advances made in stem cell research, we have been able to isolate and expand human PPCs from primary human prostate epithelial cells (PrECs) in vitro. Using a 3-D culture system, PPCs were differentially selected and increased with about 0.2% of PrECs forming spheroid structures referred to as prostaspheres (PS). Live video imaging was used to capture formation and increase in size of individual PS. By day 4, PS were solid and non-canalized with diameter averages of 30 mm. Day 7 PS increased to an average diameter of 80 mm and by day 10 between 100-150 mm. Immunohistochemical analyses of day 10 PS revealed double-layered PS in which the periphery consisted primarily of p63-positive cells and the inner-core contained more differentiated CK8/18-positive cells. The outer layer p63-positive cells were also positive for the prostate stem cell markers CD117 and CD133 indicating higher self-renewal capacity. Continuing the culture up to day 30, ductal branch formation was induced from the PS buds and lumen-like cavity confirmed by histology. Steroid receptor status of early stage PS showed lack of androgen receptor (AR) but elevated expression of all known estrogen receptors (ER) including ERα, ERβ1, GPR30 and progesterone receptor (PR) when compared to LnCaP cells. Investigating effects of estrogen on PS formation, PrECs were treated with 17β-estradiol (E2) at doses of 1 to 1000 nM for 7 days and there was a significant dose-dependent increase in both the number and size of formed PS. In summary, adult PPCs are a rare population that reside in human prostate tissue and by using an in vitro 3-D culture system PPCs can self-renew and form PS. With the prostate being a hormonally responsive gland, we present evidence that even at an early stage such as at PPC, there is some regulation by estrogen. Our lab has previously presented evidence for the increased predisposition of adult prostate to disease when neonatally exposed to estrogens and this new model of exposure to PPCs promises to offer better insight into the mechanisms of estrogen induced prostatic disease. This study was supported by NIEHS grant RC2 ES 018758.

P59: Regulation of Granulosa Cell Proliferation by Retinoic Acid and Cyp26b1 in the Mouse Ovary. Guadalupe Rodriguez¹, Ann Golebiowski¹, Michael Demczuk¹, Eemahn Haralelli¹, Kelly Mayo²,³, and Jingjing Kipp¹. ¹Department of Biological Sciences, DePaul University, Chicago, IL 60614; ²Department of Molecular Biosciences; ³Center for Reproductive Science, Northwestern University, Evanston, IL 60208

Cyp26b1 is a member of the cytochrome P450 family and encodes an enzyme that degrades the potent morphogen retinoic acid (RA). The importance of Cyp26b1 on limb, nervous system and bone development has been documented. It has also been shown to function as a meiosis inhibitor in the male gonad. Recently, we have revealed expression of Cyp26b1 mRNA and protein in granulosa cells of ovarian follicles at all postnatal developmental stages and demonstrated that it is the gene that is most strongly inhibited by activin in microarray studies. To further understand functions of Cyp26b1 in the mouse ovary, this study was designed to investigate the roles of
Cyp26b1 and RA in regulating granulosa cell proliferation. Both RA and the Cyp26 inhibitor R115866 stimulated granulosa cell proliferation in a dose-response manner, suggesting that RA is a proliferation stimulating factor for mouse granulosa cells and that Cyp26b1 may play an opposite role than RA. A similar effect was observed with activin in stimulating granulosa cell proliferation. A pan-retinoic acid receptor (RAR) inhibitor, AGN194310, abolished the stimulatory effect of both RA and activin on granulosa cell proliferation, indicating an involvement of RAR-mediated signaling. Consistent with this hypothesis, expression of RARα and RARγ mRNA was detected in granulosa cells. RA concentrations were increased in granulosa cells treated with activin A and decreased in those treated with Activin A plus its antagonist follistatin or with follistatin alone. This observation is consistent with the suppressive effect of activin on Cyp26b1 expression. Overexpression of Cyp26b1 decreased granulosa cell numbers as compared to controls, consistent with our data indicating that retinoic acid increases granulosa cell proliferation. Overall, this study provides evidence that suggests Cyp26b1 is a novel candidate in regulating the development and function of the ovary. We conclude that RA and activin stimulate while Cyp26b1 inhibits granulosa cell proliferation. The activin and RA pathways may interact to regulate ovary development. Supported by URC Grant, Genius Grant, Summer Research Grant, Minority Student Research Grant and Undergraduate Student Research Grant from DePaul University as well as by Program Project Grant HD21921.

**P60: A novel role for prolactin signaling through the short form of its cognate receptor in the ovary.** Y. Sangeeta Devi, Anita Seibold, Aurora Shehu, Julia Helparin, Jamie Le, Lei Bao, Evelyn Maizels and Geula Gibori. Dept of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL 60612

Prolactin (PRL) is essential for normal reproduction and signals through two types of receptors, the short (PRL-RS) and long (PRL-RL) form. We have previously shown that transgenic mice expressing only PRL-RS (PRLR-/-RS) display abnormal follicular development and premature ovarian failure. Here, we report that MAPK, essential for normal follicular development, is critically inhibited by PRL in reproductive tissues of PRLR-/-RS mice. Consequently, the phosphorylation of MAPK downstream targets are also severely inhibited by PRL without affecting immediate upstream kinases, suggesting involvement of MAPK specific phosphatase(s) in this inhibition. Similar results are obtained in a PRL responsive ovarian derived cell line (GG-CL) that expresses only PRL-RS. However, we found the expression/activation of several known MAPK phosphatases not to be affected by PRL, suggesting a role of unidentified phosphatases(s). We detected a 27KDa protein that binds to the intracellular domain of PRL-RS and identified it as dual specific phosphatase DUPD1. PRL does not induce expression of DUPD1 but represses its phosphorylation on T155. We also show a physical association of this phosphatase with ERK1/2 and p38 MAPK. Using an in vitro phosphatase assay and overexpression studies, we established that DUPD1 is a MAPK phosphatase. Dual specific phosphatase inhibitors as well as siRNA to DUPD1, completely prevent PRL mediated MAPK inhibition in ovarian cells. Our results strongly suggest that deactivation of MAPK by PRL/PRL-RS contributes to the severe ovarian defect in PRLR-/-RS mice and demonstrate the novel association of PRL-RS with DUPD1 and a role for this phosphatase in MAPK deactivation.

**P61: ZPBP2 is one of SBTI-binding proteins during the acrosome reaction of mouse sperm.** Yuichi Izumi, Misuzu Yamashita, Woojin Kang, and Tadashi Baba, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Science City, Ibaraki

Mammalian fertilization involves a complex set of molecular and cellular events, including penetration of sperm through the cumulus layer, adhesion and binding of sperm to the egg zona
pellucida (ZP), and acrosomal exocytosis of sperm. After the acrosome reaction on the ZP surface, sperm pass through the egg coat, and reach the egg plasma membrane. The acrosome reaction plays a crucial role in the fertilization process, because only acrosome-reacted sperm are capable of fusing with the egg plasma membrane. Soybean trypsin inhibitor (SBTI) is widely used as a tool for assessing the acrosome reaction. Although SBTI is thought to bind acrosomal component acrosin, the details still remain unclear. In this study, we attempted to identify SBTI-binding molecules in the acrosome of mouse epididymal sperm. Immunohistochemical analysis using fluorescent dye-conjugated SBTI indicated that the target protein(s) of SBTI are localized in the acrosome of acrosin-deficient sperm. The distribution of SBTI-binding protein(s) moved from the acrosome to the equatorial region during spontaneous acrosome reaction. Thus, SBTI may bind the other acrosomal component(s), in addition to acrosin. To identify the SBTI-binding protein(s), sperm protein extracts released by calcium ionophore A23187-induced acrosome reaction were purified by ion-exchange chromatography. Far-Western blot analysis of purified fractions revealed that SBTI binds ZP-binding protein ZPBP2 more tightly than acrosin. Moreover, pull-down assays demonstrated that the N-terminal immunoglobulin-like domain of ZPBP2 functions in binding to SBTI. These results suggest that SBTI is predominantly targeted to ZPBP2 during the acrosome reaction. We also describe the fertility of SBTI-treated epididymal sperm.
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