27th Annual Minisymposium on Reproductive Biology

October 16, 2006
Hilton Garden Inn
1818 Maple Avenue
Evanston, IL 60201

Sponsored by:

Center for Reproductive Science

NORTHWESTERN UNIVERSITY
The offices of the President and Vice President for Research
About the Center for Reproductive Science

The Center for Reproductive Science (CRS) includes faculty whose research is relevant, in a broad sense, to reproductive biology and human medicine. The Center does not have a discrete physical location, but rather includes faculty from across the University who share common interests in research and training in the reproductive sciences.

CRS has three missions: 1. To enhance and coordinate research in the reproductive sciences at Northwestern, 2. To promote the application of this research toward human welfare, and 3. To promote training in reproductive sciences of future research, teaching and clinical investigators.

CRS includes 44 faculty from 13 departments in the Weinberg College of Arts and Sciences and the McCormick School of Engineering on the Evanston campus and Children’s Memorial Research Center and the Feinberg School of Medicine on the Chicago campus. Research areas of current faculty interest include: circadian rhythms; clinical fertility and infertility; gonadal development and function; growth and development; neural control of sexual function; neuroendocrinology; ovarian function; prostate biology; reproduction and society; and testis function.

CRS administers several multidisciplinary NIH grants in the reproductive sciences, including the T32 Training Program in Reproductive Biology (Jon Levine, P.I.), the P01 Program Project Hormonal Signals that Regulate Ovarian Differentiation (Kelly Mayo, P.I.) and the U54 Cooperative Center Structure Function Relationships in Reproductive Biology (Teresa Woodruff, P.I.). For further information about the center, please visit the CRS website at www.northwestern.edu/research/crs/

The CRS Newsletter entitled “Reproduction Matters” is a biannual publication available on the CRS website.

Center-Sponsored Awards

CRS “Go to Meeting” Award. This is a travel award for undergraduates working in the laboratories of CRS Members to accompany other lab members to professional meetings such as the annual meeting of the Endocrine Society, Society for the Study of Reproduction, Society for Neuroscience, etc.

Marcia L. Storch Scholarship for Undergraduate Women Dr. Storch practiced gynecology in New York City for a number of years and died at her home in Maine. She was always interested in the education of young women, and stipulated that donations in her name be made to the Center for Reproductive Science for the purpose of introducing undergraduate women to research. This scholarship is awarded to undergraduate women working in some aspect of ovarian research and provides a sum for supplies in the designated laboratory.

Go to www.northwestern.edu/research/crs/ for details on how to apply for the CRS “Go to Meeting” and the “Marcia L. Storch Scholarship for Undergraduate Women” awards. The deadline to apply for both awards is June 1, 2007.

Constance Campbell Memorial Research Award. The awards given at the Annual Minisymposium on Reproductive Biology are named after Constance Campbell. Connie, a Ph.D. in psychology from University of Illinois-Chicago, was hired as an assistant professor in the department of Biological Sciences at Northwestern in 1974. Her research was on the relationship of sexual and other behaviors to environmental cues. She was promoted to associate professor in the department of Neurobiology and Physiology and administered the undergraduate honors program before her untimely death in 1981. Connie cared deeply about student research. After her death, her family, friends and colleagues raised money which is now used to fund the Constance Campbell Memorial Research Awards for the Minisymposium on Reproductive Biology.

Cover Photograph: Ovulated cumulus cell oocyte complex stained for HA (red), versican (green) and cell nuclei (blue). Courtesy of Dr. JoAnne Richards.
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Minisymposium Overview

8:00 - 8:25 AM Registration/Continental Breakfast
8:25 – 8:30 AM Welcome/Announcements
8:30 - 8:45 AM Northwestern Alumni Speaker – Dr. Daniel Bernard
8:45 - 10:15 AM Oral Session I
10:15 - 10:30 AM Coffee Break
10:30-10:45 AM Northwestern Alumni Speaker – Dr. Richard Cutler
10:45-12:00 PM Oral Session II
12:00 -1:00 PM Lunch
1:00 - 2:15 PM Poster Session
2:15 - 2:30 PM Northwestern Alumni Speaker – Dr. Eileen Wang
2:30 - 3:45 PM Oral Session III
3:45 - 4:30 PM Wine and Cheese Reception
4:30 - 4:40 PM Intro to Keynote Address
4:40- 5:40 PM Keynote Address – Dr. JoAnne S. Richards
5:40 - 5:50 PM Awards Presentations
5:50 PM Closing Comments/Adjourn
Neena B. Schwartz Lectureship in Reproductive Science

This year is the 27th anniversary of the Minisymposium on Reproductive Biology. The idea for the Minisymposium, a day-long event focused on providing trainees with an early opportunity to present their research, came primarily from Dr. Neena B. Schwartz, William Deering Professor Emerita of Biological Sciences and founder of the Center for Reproductive Science.

Neena is a native of Baltimore who has spent most of her research and teaching career in the greater Chicago area. After obtaining her undergraduate degree from Goucher College, Neena completed her M.S. and Ph.D. degrees in physiology at Northwestern University. Her first faculty position was at the University of Illinois College of Medicine, where she rose to the rank of Professor before moving to Northwestern University in 1974 as Chair of the Department of Biological Sciences. Neena founded the Program in Reproductive Research, now the Center for Reproductive Science, in 1980, and developed the program into a premier training site for students and young investigators interested in reproductive endocrinology.

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

To honor Dr. Schwartz on the 25th anniversary of the Minisymposium, the Center for Reproductive Science named the keynote address the Neena B. Schwartz Lectureship in Reproductive Science. This year, Dr. JoAnne S. Richards, Professor of Molecular and Cellular Biology at Baylor College of Medicine will give the keynote address and receive the lectureship award.
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 -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.

For further information on Dr. Richards’ research visit: [http://www.bcm.edu/mcb/faculty/richards.htm](http://www.bcm.edu/mcb/faculty/richards.htm)
Northwestern Alumni Guests

Daniel J. Bernard, Ph.D.
Associate Professor
Department of Pharmacology and Therapeutics
McGill University

Northwestern University Postdoctoral Fellow, 1995-1998, Turek Lab
Northwestern University Research Associate, 1998-2001, Woodruff Lab

Richard E. Cutler Jr., Ph.D.
Senior Scientist
Exelixis, Inc.

Northwestern University Graduate Student, 1989-1994, Hunzicker-Dunn Lab

Eileen Wang, M.D.
Assistant Professor
Department of Obstetrics and Gynecology
University of Chicago

Northwestern University Postdoctoral Fellow, 1997-2000, Woodruff Lab
PROGRAM FOR
27th ANNUAL MINISYMPOSIUM
ON
REPRODUCTIVE BIOLOGY
October 16, 2006
Northshore and Lakeshore Rooms
Hilton Garden Inn

8:00-8:30 AM  Registration  Atrium
Continental Breakfast  Northshore Room
Run-through talks  Northshore Room
Set up Posters  Lakeshore Room & Atrium

8:25 AM  Opening Remarks – Melissa Chamberlin, Neurobiology & Physiology, Northwestern University, Evanston, IL

8:30 AM  Northwestern Alumni Speaker – Dr. Daniel Bernard, Associate Professor, Department of Pharmacology and Therapeutics, McGill University

ORAL SESSION I: GERM CELL DEVELOPMENT
Session Moderators:  Maxfield Flynn  Cell and Molecular Biology, Northwestern University, Chicago, IL and Christina Matulis, Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL

Abstract #  Presenting authors shown in bold

8:45 AM  1  Time Course of Spermatogonial Stem Cell Loss in ERM Knockout Mice: Critical Neonatal Effects of ERM, Heather N. Schlesser¹, Marie-Claude Hofmann², Kay Carnes¹, Kenneth M. Murphy³,⁴, Rex A. Hess¹ and Paul S. Cooke¹, ¹Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; ²Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO; ³Department of Pharmacology and Therapeutics, Washington University School of Medicine, St Louis, MO

9:00 AM  2  Sox3 is Necessary for Maintaining the Spermatogonial Stem Cell Phenotype, M.M. Laronda, G. Raverot, U. Jadhav, L. Pfaff, J. Weiss, J.L. Jameson, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.

9:15 AM  3  FGF-2 Regulates Expression of ERM and GDNF, Sertoli Cell Proteins Essential for Spermatogonial Stem Cell Maintenance, Liz Simon¹, Kenneth M. Murphy²,³, Rex A. Hess¹ and Paul S. Cooke¹, ¹Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; ²Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO; ³Howard Hughes Medical Institute
Institute

9:30 AM 4 Localization of RNA Binding Protein - Pumilo in Mouse Testis and Spermatozoa, **Chirag A. Shah**, Terrance Lee, Villian Naeem and Eugene Xu, Division of Reproductive Biology, OBGYN and Centre for Genetic Medicine, Northwestern University Feinberg School of Medicine, Lurie 7-250, 303 E Superior Street, Chicago, IL-60611 USA

9:45 AM 5 Neonatal Role of Ets-related Molecule (ERM) in Spermatogonial Stem Cell (SSC) Maintenance May Involve SSC Receptors for GDNF, **Gaurav Tyagi**1, 2, Kay Carnes2, Liz Simon2, Kenneth M. Murphy3, 4, Marie-Claude Hofmann5, Rex A. Hess2 and Paul S. Cooke6, Departments of 1Veterinary Pathobiology and 2Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; 3Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO; 4Howard Hughes Medical Institute and 5Department of Biology, University of Dayton, Dayton, OH.

10:00 AM 6 Notch Receptor and Ligand Expression Suggest Roles for Notch Signaling During Early Folliculogenesis, **Daniel Trombly** and Kelly E. Mayo, Department of Biochemistry, Molecular Biology & Cell Biology and Center for Reproductive Science, Northwestern University, Evanston, IL 60208

10:15-10:30 AM Coffee Break

10:30 AM Northwestern Alumni Speaker - Dr. Richard Cutler, Senior Scientist, Exelixis, Inc.

**ORAL SESSION II: DEVELOPMENT AND REGULATION OF OVARIAN FUNCTION**

*Session Moderators: Cheryl Park, Neurobiology & Physiology, Northwestern University, Evanston, IL and Zhen Zhou, Neurobiology & Physiology, Northwestern University, Evanston, IL*

Abstract #

10:45 AM 7 Matrix Stiffness Regulates Secondary Follicle Development and Tissue Differentiation in a Three-dimensional Culture System, **Erin R. West**1, Teresa K. Woodruff2, 3, Lonnie D. Shea1, 3, 1Department of Chemical and Biological Engineering, 2Department of Neurobiology and Physiology, 3Center for Reproductive Research, Northwestern University, Evanston, IL

11:00 AM 8 Dephosphorylation of the Actin Depolymerizing Factor, Cofilin, by hCG in Preovulatory Granulosa Cells, **Amelia B. Karlsson**1, Evelyn T. Maizels1, and Mary Hunzicker-Dunn1, 1Northwestern University, Feinberg School of Medicine, Chicago Illinois
11:15 AM  9  The Peroxisome Proliferator-Activated Receptor Gamma is a Downstream Target of Progesterone Receptor and Controls Ovulation in Mice, Jaeyeon Kim\(^1\), Marcey Sato\(^1\), John P. Lydon\(^3\), Francesco J. DeMayo\(^3\), Indrani C. Bagchi\(^2\), Milan K. Bagchi\(^1\). \(^1\)Department of Molecular & Integrative Physiology, \(^2\)Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, \(^3\)Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

11:30 AM  10  Prolactin Signaling through the Short Isoform of its Receptor Activates the Inflammatory Pathway, Sangeeta Devi, Aurora Shehu\(^1\), Julia Halperin\(^1\), Carlos Stocco\(^1,2\), Nadine Binart\(^3\), Geula Gibori\(^1\). \(^1\)University of Illinois at Chicago, Chicago, IL, \(^2\)Yale School of Medicine, New Haven, CT, \(^3\)Inserm U809 Faculté de Médecine Necker-enfants Malades, Paris, FRANCE.

11:45 AM  11  Increased Frequency of Natural Killer T (NKT) Cells in the Tumor Microenvironment of Ascites in Women with Ovarian Cancer, Shatavi SV\(^1\); Al-Harthi L\(^3\); Mitchell S\(^2\); Usha L\(^4\); Rotmensh J\(^2\); Landay A\(^3\); Luborsky JL\(^1,2\). \(^1\)Departments of Pharmacology, \(^2\)Obstetrics & Gynecology, \(^3\)Immunology and Microbiology and \(^4\)Internal Medicine, Section Medical Oncology and Hematology, Rush University Medical Center, Chicago, IL

12:00-1:00 PM  Lunch Break

1:00-2:15 PM  POSTER SESSION

Abstract #

P1  Maternal Administration of Leptin Hormone Stimulates the Postnatal Gonadal and Fetal Brain Development of the Syrian Hamster (*Mesocricetus auratus*), Alper Karakas\(^1\),\(^#\) and Bulent Gunduz\(^1\). \(^1\)Department of Biology, Faculty of Arts and Sciences, Abant Izzet Baysal University, 14280 BOLU-TURKEY [Current Address: \(^#\)Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611, USA]

P2  The Effects of Leptin Hormone on Reproductive System, Body Weight and Food Intake in Juvenile Male Syrian Hamsters (*Mesocricetus auratus*), Fatma P. Karakas, Bulent Gunduz, Department of Biology, Faculty of Arts and Sciences, Abant Izzet Baysal University 14280 BOLU-TURKEY

P3  A Nonclassical ER\(\alpha\) Signaling Pathway is Sufficient to Convey Estrogen Negative but not Positive Neuroendocrine Feedback of LH in the Female Mouse, C. Glidewell-Kenney\(^1\), L.A. Hurley\(^1\), L. Pfaff\(^3\), J. E. Levine\(^2\), J. Weiss\(^1\), J.L. Jameson\(^1\), Division of Endocrinology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL\(^1\), WCAS Neurobiology & Physiology, Northwestern University, Chicago, IL\(^2\)
The Role of Nonclassical Estrogen Receptor Signaling in the Sexual Receptivity of the Female Mouse, **Mariana A. Jimenez**¹, Christine Glidewell-Kenney², J. Larry Jameson² and Jon Levine¹

Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, USA. Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611-2908, USA.

LH-receptor Activation in Ovarian Granulosa Cells Promotes PKA-dependent Dephosphorylation of the AKAP MAP2D. **M.P. Flynn**¹, E.T. Maizels¹, M. Hunzicker-Dunn¹,² ¹Feinberg School of Medicine, Northwestern University, Chicago, Illinois, ²School of Molecular Biosciences, Washington State University, Pullman, Washington.

Posttranslational Modification of the NR5A Family of Nuclear Receptors in the Ovary, **Christina K. Matulis** and Kelly Mayo, PhD, Department of Biochemistry, Molecular Biology, and Cellular Biology, Center for Reproductive Science, Northwestern University, Evanston, IL

Human Aldehyde Dehydrogenase (ALDH) as an Autoantigen for Ovarian Autoimmune Disease, **Espinosa, S**¹; Shatavi, SV¹; Edassery, SL¹; Brucker, C³; Kunkel, JP⁴; Dias, JA⁴; Vasiliou, V⁵; Luborsky JL¹,² ¹Departments of Pharmacology and ²Obstetrics & Gynecology; ³Rush University Medical Center, Chicago, IL, Department of Gynecology and Obstetrics; ⁴Centre of Reproductive Medicine, University of Nuremberg, Nuremberg, Germany, Wadsworth Laboratories; ⁵New York State Department Health, Albany, NY, Center for Pharmaceutical Biotechnology; Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center

Synergistic Effect of Peptidoglycan (PGN) and Polyinosinic-polycytidylic Acid (poly(I:C)) on Expression of Toll-like Receptor (TLR)-dependent Genes, **Vladimir Ilievski, MD**¹ and Emmet Hirsch, MD¹,² ¹Department of Obstetrics and Gynecology, Evanston Northwestern Healthcare Research Institute, Evanston, IL; ²Feinberg School of Medicine, Northwestern University, Chicago, IL.

Epigenetic Modification of Eutopic Endometrium in a Baboon Model of Endometriosis, **Julie M Hastings**¹, Kevin S Jackson¹, Hugh S Taylor², Sun-Wei Guo³, Asgerally T Fazleabas¹. ¹Dept Ob/Gyn, University of Illinois at Chicago, Chicago, IL; ²Dept Ob/Gyn & Reproductive Sciences, Yale University, New Haven, CT; ³Dept Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) Expression in Baboon Endometrium and Endometriotic Lesions, **A. Braundmeier**¹, M. Nakai¹, A. Fazleabas² and R. Nowak¹, ¹University of Illinois, Urbana-Champaign and ²University of Illinois, Chicago, USA

Changes in the Cytoskeleton Organization during Decidualization In Vitro: The Role of Myosin Light Chain (MLC) Phosphorylation, **I. Ihnatovych**¹, W.Y. Hu², J.L.
P12 Notch1 Signaling in the Endometrium is Regulated by Human Chorionic Gonadotropin and Ovarian Steroids, **Yalda Afshar**¹, Adina Stanculescu², Lucio Miele², Jaewook Jeong³, Franco DeMayo³, Asgi Fazleabas¹, ¹Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL, ²Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL, ³Loyola University Medical Center, Maywood, IL.

P13 Signaling by Chorionic Gonadotropin (CG) through the CG Receptor (CGR) in Primate Endometrial Epithelial Cells is through a Gᵢ – independent MAPK Pathway, **Prajna Banerjee**¹, Paula Cameo¹, Santha Srisuparp², Zuzana Strakova¹, Asgerally T Fazleabas¹. ¹Dept. of Ob/Gyn, University of Illinois, Chicago, IL, USA; ²Rajavithi Hospital, Bangkok, Thailand.

P14 Estrogen Differentially Regulates the Expression of Cyclin-Dependent Kinase 1 (Cdk1) in Primary and Secondary Decidual Compartments During Implantation, **Athilakshmi Kannan**¹, Quanxi Li¹, Indrani C. Bagchi¹ and Milan K Bagchi², ¹Department of Veterinary Biosciences, College of Veterinary Medicine, and ²Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign.

P15 Regulation of 17β-hydroxysteroid Dehydrogenase Type 2 Expression in Human Placental Endothelial Cells. **Su EJ**¹, Cheng YH¹, Chatterton RC¹,², Lin ZH¹, Yin P¹, Reierstad S¹, Innes J¹, Bulun SE¹. ¹Department of Obstetrics and Gynecology, ²Department of Physiology, Northwestern University, Chicago, IL.

P16 Localization of RNA Binding Protein - Pumilo in Mouse Testis and Spermatozoa, **Chirag A. Shah**, Terrance Lee, Villian Naeem and Eugene Xu, Division of Reproductive Biology, OBGYN and Centre for Genetic Medicine, Northwestern University Feinberg School of Medicine, Lurie 7-250, 303 E Superior Street, Chicago, IL-60611 USA

P17 Characterization of Mammalian Boule Gene Isoforms and Developmental Expression Profiles, **N.L. Angeloni**, M. Bernhardt, E.Y. Xu, Division of Reproductive Biology, Department of Obstetrics and Gynecology, and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

P18 A Role for Altered Gli1 Signaling in Male Infertility in Quaking Viable Mutant Mice, **O.V. Lakiza**, M.L.G. Lamm, D.O. Walterhouse and P.M. Iannaccone, Children’s Memorial Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL

P19 Proliferation of Adult Sertoli Cells in a Conditional Knockout Mouse Lacking the Gap Junctional Protein Connexin 43, **Santhi Sridharan**¹, David E. Gutstein², Glenn I. Fishman² and Paul S. Cooke¹, ¹Department of Veterinary Biosciences, University of
P20 Apoptosis is Absent in Female Gonads of Fused Toes Mutant Mice During Development, **B. Kim**, Y. Kim, J.S. Jorgensen, Department of Veterinary Biosciences, University of Illinois, Urbana, IL.

P21 Subtilisin-like Proprotein Convertase Activity is Necessary for Ovarian Inhibin Secretion. **Monica Antenos**¹, Anjali Malipatil¹ and Teresa K. Woodruff¹,²,³, ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, IL, ²Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, ³Robert H Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL.

P22 Activin B Regulates FSHβ Promoter through Smad1 and Smad3 in LβT2 Cells, **Niti M. Jetly**¹, Magdalena I. Suszko¹, Monica Antenos¹, Teresa K. Woodruff¹,²,³, ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, USA, ²Department of Medicine, Northwestern University Medical School, Chicago, IL 60611 USA, ³Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL 60611, USA.

P23 Interactions Between Activin and Estrogen Signaling in the Mouse Ovary, **Jingjing Liu Kipp**¹,³, Signe Kilen¹,²,³, and Kelly Mayo¹,²,³, ¹Department of Biochemistry, Molecular Biology and Cell Biology; ²Department of Neurobiology and Physiology; ³Center for Reproductive Science. Northwestern University, Evanston, IL 60208.

P24 Activin and Estrogen Crosstalk Regulates Transcription and Cell Cycle Control in Human Breast Cancer Cells, **Joanna E. Burdette**¹ and Teresa K. Woodruff¹,²,³, ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208; ²Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, Illinois 60611; ³Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University Medical School, Chicago, Illinois 60611.

P25 Cyst Formation and Morphology from Superovulated Smad2 Dominant Negative Transgenic and Control Mice, **R. M. Oliver**¹,², J. E. Burdette¹, T. K. Woodruff¹, ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, IL; ²Department of Biochemistry, Beloit College, Beloit, WI.

P26 Expression of Selenium Binding Protein 1 in Ovarian Cancer in Women and Hens, **K. Stammer**¹, S. Edessary¹, A. Barua¹, S. Sharma¹, J. M. Bahr³, D. B. Hales⁴, J. L. Luborsky¹,², ¹Department of Pharmacology, ²Department of Obstetrics and Gynecology, Rush University Medical Center, Chicago, IL, ³Department of Animal Sciences, University of Illinois, Urbana-Champaign, IL, ⁴Department of Physiology and Biophysics, University of Illinois at Chicago.

P27 Mechanism of Regulation of PRAP/17βHydroxysteroid Dehydrogenase Type 7 Gene by Estradiol, **Aurora K Shehu**¹, Mike Risk², Jifang Mao¹, Constance Albarracin³.
and Geula Gibori1, 1Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL, 60612; 2Department of Urology, University of Washington, Seattle, Washington, 98195 and 3Department of Pathology, University of Texas, M.D. Anderson Cancer Center, Houston, TX, 77030.

P28 Application of Ultrasonography to Assess Ovarian Tumors in Laying Hens, A. Barua1, M. J. Braderic1, S. L. Edassary1, A. Dirk3, J. Abramowicz2, J.M. Bahr3 and J. L. Luborsky1, 2. 1Department of Pharmacology, 2Department of Obstetrics and Gynecology, Rush University Medical Center, Chicago, IL 3Department of Animal Sciences, University of Illinois, Urbana-Champaign, IL.

P29 Molecular Analysis of Spontaneous Ovarian Cancer in the Laying Hen Gallus Domesticus, Yan Zhuge1, Jo Ann Jaen Lagman1, Cassandra Mahon1, Animesh Barua2, Judy Luborsky2, Janice Bahr3, Dale Buchanan Hales1, 1Department of Physiology and Biophysics, University of Illinois-Chicago, Chicago, IL, 2Rush University Medical School, Chicago, IL, 3University of Illinois at Urbana-Champaign, Urbana-Champaign, IL.

P30 Gene Trap Mutagenesis: A Functional Genomics Approach towards Reproductive Research, Terrance Lee, Chirag A. Shah, Eugene Y. Xu, Division of Reproductive Biology, Northwestern University School of Medicine, Evanston, IL.

P31 Fluorescence Imaging of Inorganic Physiology in the Mammalian Oocyte, M. Alison Kim1, Thomas V. O’Halloran2,3, Teresa K. Woodruff1,3, 1Department of Neurobiology & Physiology, 2Department of Chemistry, 3Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.

2:15PM Northwestern Alumni Speaker- Dr. Eileen Wang, University of Chicago

ORAL SESSION III: PHYSIOLOGY & PATHO-PHYSIOLOGY OF THE UTERUS

Session Moderators: Unmesh Jadhav, Medicine - Endocrinology, Northwestern University, Chicago, IL and Theresa Peterson, Medicine - Endocrinology, Northwestern University, Chicago, IL.

Abstract #

2:30 PM 12 The CCAAT/Enhancer Binding Protein Beta is a Critical Regulator of Decidualization, Wei Wang1, Quanxi Li2, S. R. Mantena2, Robert Taylor3, Indrani C Bagchi1, Milan K Bagchi1 1Dept. of Mol. & Integrative Physiology, 2Dept. of Vet. Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, 3Dept. of Ob. & Gyn., Emory University, Atlanta, GA

2:45 PM 13 Conditional Knockout of Connexin 43 in Mouse Uterus Uncovers an Essential Role of Gap Junctions in Stromal Differentiation During Pregnancy, Mary Laws1, Francesco DeMayo3, John Lydon3, Milan K

Dynamic Steroid-Regulated Assembly and Disassembly of Lipid Droplets/Adiposomes During Implantation: Progesterone Promotes Assembly and Estrogen Triggers Disassembly in the Uterine Epithelium, **Srinivasa Raju Mantena**¹, Milan K Bagchi², and Indrani C. Bagchi¹, ¹Department of Veterinary Biosciences; ²Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign

The Bone Morphogenetic Protein 2 (BMP2) Signaling Pathway Plays a Critical Role in Uterine Stromal Differentiation in the Mouse and the Human, **Quanxi Li**¹, Athilakshmi Kannan¹, Wei Wang², Milan K. Bagchi², and Indrani C. Bagchi¹, ¹Department of Veterinary Biosciences. ²Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL

Wine and Cheese Reception

Introduction of Keynote Speaker – Erin Ward, Obstetrics and Gynecology, Northwestern University, Chicago, IL

Presentation of the Neena B. Schwartz Lectureship in Reproductive Science – Dr. Kelly E. Mayo, Director, Center for Reproductive Science, Northwestern University, Evanston, IL

Keynote Address – Ovulation: Novel Aspects of this Immune-Related Process, **JoAnne S. Richards**, PhD, Professor of Molecular and Cellular Biology, Baylor College of Medicine.

Presentation of Constance Campbell Research Awards -
Dr. Kelly E. Mayo, Director, Center for Reproductive Science

Closing Comments – Daniel Trombly, Biochemistry Molecular Biology and Cell Biology, Northwestern University, Evanston, IL

Please take down posters immediately following the Minisymposium
The transcription factor Ets-related molecule (ERM) is essential for self-renewal and maintenance of spermatogonial stem cells (SSCs). ERM is expressed in Sertoli cells, which establish the stem cell niche in seminiferous tubules. Mice with targeted disruption of ERM (ERM−/−) go through the first wave of spermatogenesis but all SSCs differentiate, causing a Sertoli cell only phenotype. This study sought to examine the temporal sequence of SSC loss in ERM−/− mice and also to determine if these animals are transiently fertile during the juvenile period. To quantify SSC loss, testis tubules from different aged wild-type (WT) and ERM−/− mice were used for wholemount immunohistochemistry with an antibody against GFRα1. GFRα1, a receptor for glial cell line-derived neurotrophic factor (GDNF), is a specific SSC marker used to visualize and quantitate SSCs. SSC density (SSCs/µm of seminiferous tubule) was highest at 4 days of age and there was no difference between WT (182 ± 50 x 10^-4) and ERM−/− (192 ± 30 x 10^-4) mice. By 12 days, SSC density was reduced in both genotypes compared to day 4 and ERM−/− testis had 36% less SSCs than WT(WT and ERM−/− = 107 ± 10 x 10^-4 and 69 ± 10 x 10^-4 SSC/µm, respectively; p < 0.05). The ERM−/− testis at day 28 had 77% less SSCs than WT (WT and ERM−/− tubules contained 4.34 ± 1.41 x 10^-4 and 0.98 ± 0.84 x 10^-4 SSC/µm, respectively). At this age, 86% of ERM−/− tubules were devoid of SSCs, while 100% of WT tubules had SSCs. At later ages (36 and 44 days), SSCs were absent in ERM−/− mice. ERM−/− mice (35-day-old) caged consecutively with three sets of two females for one week failed to produce litters, and no copulation plugs were observed, in contrast to WT males. Immunohistochemical staining showed strong ERM expression in epididymal epithelium of WT mice, suggesting ERM may play critical roles in this tissue. Consistent with these data, the 44-day ERM−/− mouse showed a marked decrease in epididymal sperm, and this decrease may result from loss of ERM in both testis and epididymis. In summary, loss of SSCs begins in ERM−/− mice between 4 and 12 days of age and SSCs disappear over a period of 3-4 weeks, with all SSCs being lost by 5 weeks. The early onset of SSC loss indicates that the neonatal period is critical for ERM actions in promoting stem cell maintenance. This is earlier than originally postulated (3-4 weeks) based on initial work on ERM expression, but is consistent with other data that ERM is expressed neonatally. ERM−/− mice have epididymal sperm during juvenile life, but may not be able to breed or may be infertile even during the transient period when epididymal sperm are present.

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Sox3 is Necessary for Maintaining the Spermatogonial Stem Cell Phenotype, M.M. Laronda, G. Raverot, U. Jadhav, L. Pfaff, J. Weiss, J.L. Jameson, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Stem cells are important for the development and survival of multicellular organisms. Understanding stem cell populations within the developing embryo and adult organism will provide investigators with information that will aid in the development of regenerative medicine, tissue repair and gene therapy. Spermatogonial stem cells (SSCs) are essential for the initiation and maintenance of spermatogenesis. It is unclear what molecular mechanisms are involved in the self-renewal, differentiation and pluripotency of a SSC. Several Sox proteins are important for developing many tissues and for maintaining the undifferentiated characteristics of precursor cells, such as neuronal precursors and chondrocytes. Sox3 expression is localized within the developing gonad and the type A spermatogonia population that contains SSCs. To determine the role of Sox3 in the male gonad, our lab has developed a Sox3 knockout mouse. Sox3- males exhibit a spermatogenic block by two weeks of age and display several tubules with no visible germ cells by eight weeks. Further investigations of Sox3- males, using whole testis RNA, revealed decreased expression of genes critical for early stages of spermatogenesis. Ngn3, a gene necessary for repopulating the testis, displays decreased levels of expression in Sox3- males compared to Sox3+ males. Expression of Plzf, a gene necessary for maintaining the epigenetic signature of undifferentiated spermatogonia, is also decreased with the removal of Sox3 expression. This evidence demonstrates the importance of Sox3 in spermatogenesis and leads to the hypothesis that Sox3 is necessary for maintaining the spermatogonial stem cell phenotype. We propose to isolate Sox3-expressing germ cells to better characterize their gene expression profile and to determine their ability to repopulate the testis and initiate spermatogenesis. Determining the functional and molecular characteristics of Sox3-expressing germ cells will provide a better understanding of undifferentiated spermatogonia and may describe a novel trait for SSCs that will aid in the isolation and further characterization of this unique population.

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FGF-2 Regulates Expression of ERM and GDNF, Sertoli Cell Proteins Essential for Spermatogonial Stem Cell Maintenance, Liz Simon¹, Kenneth M. Murphy²,³, Rex A. Hess¹ and Paul S. Cooke¹.¹Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; ²Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO; ³Howard Hughes Medical Institute.

The transcription factor Ets-related molecule (ERM) is required for maintenance and self-renewal of spermatogonial stem cells (SSCs) in mice. Targeted disruption of ERM, produced in Sertoli cells of the juvenile testis, resulted in total loss of SSCs despite a normal first wave of spermatogenesis. Fibroblast Growth Factor-2 (FGF-2) has been reported to regulate ERM expression, but there is no other information on factors that control ERM or the mechanism by which FGF-2 induces ERM production. In this study, we investigated the regulation of ERM using the TM4 murine Sertoli cell line. We observed that FGF-2 caused a dose-dependent increase in ERM mRNA expression, with maximum stimulation of 8.3-fold at 25 ng/ml, consistent with the 8- to 9-fold increase in ERM expression reported in the initial work. Western blotting showed an increase of similar magnitude in ERM protein, suggesting that FGF-2 effects on ERM are regulated transcriptionally. FGF-2 at the maximal stimulatory dose of 25 ng/ml showed a time-dependent increase in ERM, with a significant 3-fold increase at 3h, a maximal increase of 8-fold at 6h, then a decrease to basal levels by 48h. We then tested whether FGF-2 acted through the mitogen activated protein kinase (MAPK) and phosphatidyl inositol 3-kinase (PI3K) signaling cascades to regulate ERM. Specific inhibitors of MAPK (PD98059, 10 µM) and PI3K (wortmannin, 0.1µM) pathways inhibited ERM expression in FGF-2 treated TM4 cells by approximately 60%, suggesting that FGF-2 signals through these pathways to induce ERM mRNA expression, but the effects of PD98059 and wortmannin were not additive. In addition, FGF-2 stimulated a 5-fold increase in mRNA expression for glial cell derived neurotrophic factor (GDNF), another Sertoli cell protein that promotes stem cell maintenance and self-renewal; this is the first regulator of GDNF identified in Sertoli cells. PD98059 and wortmannin significantly inhibited expression of GDNF by approximately 25% suggesting that FGF-2 also signals through the MAPK and PI3K pathways to induce GDNF. Finally, we determined whether other hormones that regulate Sertoli cells have effects on ERM mRNA. FSH, testosterone and activin A did not affect ERM mRNA expression. These results indicate that FGF-2 regulates two Sertoli cell proteins, ERM and GDNF, that are essential for spermatogonial stem cell maintenance and self-renewal and that effects of FGF-2 on both proteins are partially mediated through the MAPK and PI3K pathways. Although classical hormones known to regulate Sertoli cells (FSH, testosterone, activin A) do not regulate ERM, FGF-2 is emerging as a key regulator of the Sertoli cell proteins that control spermatogonial stem cells.

Supported by Billie Field Endowment.
Localization of RNA Binding Protein - Pumilo in Mouse Testis and Spermatozoa, Chirag A. Shah, Terrance Lee, Villian Naeem and Eugene Xu, Division of Reproductive Biology, OBGYN and Centre for Genetic Medicine, Northwestern University Feinberg School of Medicine, Lurie 7-250, 303 E Superior Street, Chicago, IL-60611 USA

Post transcriptional regulations through RNA binding protein has been shown to play important roles in development and differentiation. The present study was undertaken to define the spatial and temporal expression of highly conserved RNA binding protein, Pumilo in mouse testis and spermatozoa. Pumilo gene isoforms were expressed in the cytoplasm of various spermatogenic cells, including secondary spermatocytes and immature testicular spermatids. The pattern of expression was heterogeneous and in most of the secondary spermatocytes the staining is in a typical arch shape at the perinuclear area within cell. In accordance, expression of Pumilo was found to be stage specific in mouse testis with typical increase in pattern of expression from stage II-III where detected as a dot like appearance, with increase in its shape of arch at stage VII-IX and having complete expression of Pumilo on the acrosomal surface of testicular spermatids at stage X. Pumilo protein was also localized on the acrosomal region of the caudal spermatozoa using the same set of antibodies. These results together suggest that RNA binding proteins like Pumilo may play important roles in sperm maturation, fertilization or/and early embryonic development.

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Neonatal Role of Ets-related Molecule (ERM) in Spermatogonial Stem Cell (SSC) Maintenance May Involve SSC Receptors for GDNF, Gaurav Tyagi¹,², Kay Carnes², Liz Simon², Kenneth M. Murphy³,⁴, Marie-Claude Hofmann⁵, Rex A. Hess² and Paul S. Cooke², Departments of ¹Veterinary Pathobiology and ²Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; ³Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO; ⁴Howard Hughes Medical Institute and ⁵Department of Biology, University of Dayton, Dayton, OH.

Ets-related molecule (ERM) is a transcription factor belonging to the Pea 3 family of proteins. ERM is expressed at high levels in mouse testis and is essential for maintenance and self-renewal of spermatogonial stem cells (SSCs). ERM knockout (ERM⁻/⁻) mice undergo a first wave of spermatogenesis then show total loss of SSCs and complete cessation of spermatogenesis by 10 weeks of age. ERM expression has previously been localized to Sertoli cells in juvenile mouse testes but has not been examined neonatally. This study sought to examine neonatal expression of ERM in mouse testis and test the hypothesis that loss of ERM could cause loss of SSCs by decreasing RET and/or GFRalpha1 in SSCs. Glial cell-derived neurotrophic factor (GDNF) is a TGF-beta family member produced by Sertoli cells that acts through the Ret/GFRalpha1 receptor complex at the surface of SSCs to promote self-renewal and maintenance of these cells. GDNF expression is normal in ERM⁻/⁻ mice but RET and GFRalpha1 have not been examined. Our immunohistochemistry results using a specific antibody showed that ERM is expressed at high levels both in Sertoli cells as well as in many SSCs from postnatal day 0 up to day 16, when staining was substantially reduced in both cell types. This was consistent with QPCR data showing high levels of ERM mRNA expression at days 0 and 4 in mouse testes. Although testicular histology in ERM⁻/⁻ and wild-type mice was similar up to 8 days of age, in ERM⁻/⁻ mice both RET and GFRalpha1 mRNA were down 60-75% as early as day 8. RET mRNA decreased more than 90% by day 22, consistent with a dramatic decrease in SSCs. Immunohistochemistry for RET in control and ERM⁻/⁻ testes at day 8 revealed both a marked reduction in SSC number as well as decreased RET staining intensity in the remaining SSCs of ERM⁻/⁻ mice. In summary, our results demonstrate that 1) ERM, initially described as a Sertoli cell protein, was expressed neonatally both in Sertoli cells and many SSCs, and ERM could have direct effects on SSCs as well as indirect effects mediated through Sertoli cell ERM during the early postnatal period; 2) the GDNF/RET/GFRalpha1 signaling pathway, which is critical for maintenance of undifferentiated SSCs, is disrupted in ERM⁻/⁻ testis and 3) decreased RET and GFRalpha1 expression could be contributory or even causative for SSC loss in ERM⁻/⁻ mice.

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Notch Receptor and Ligand Expression Suggest Roles for Notch Signaling During Early Folliculogenesis, Daniel Trombly and Kelly E. Mayo, Department of Biochemistry, Molecular Biology & Cell Biology and Center for Reproductive Science, Northwestern University, Evanston, IL 60208

Notch signaling directs cell fates during embryogenesis by influencing cell proliferation, differentiation, and apoptosis. Notch genes are expressed in the adult mouse ovary, and roles for Notch in regulating ovarian follicle maturation are beginning to emerge from mouse genetic models. We are investigating how Notch signaling might influence the formation and growth of primordial follicles. Our studies are centered on the interval between birth and day 4, the time period when germ cells are encapsulated by somatic cells (pre-granulosa cells) within mouse ovaries to generate primordial follicles. The expression of Notch receptors and ligands was determined in neonatal mouse ovaries through real-time PCR measurements and immunohistochemical methods. Multiple Notch family receptor and ligand transcripts were detected in day 3 postnatal ovaries, with Notch2 and Jagged1 mRNAs showing the highest expression. Further experiments therefore focused on this receptor-ligand pair. Immunolocalization studies demonstrated that Jagged1 is expressed in germ cells prior to follicle assembly and in oocytes of primordial and primary follicles. Jagged1 mRNA expression increases 2-fold between days 0 and 6 of postnatal life, the period when most follicles have formed. Notch2 receptor protein is localized predominantly in germ cells prior to follicle formation. Subsequently, primary and more advanced follicles express Notch2 chiefly in granulosa cells. The overlapping expression pattern of Notch2 and Jagged1 in germ cells prior to follicle assembly, and the expression pattern of this receptor-ligand pair in early follicles suggest that Notch activation may serve as a proliferation or differentiation signal during follicle formation and growth. Newborn mouse ovaries maintained in culture also express components of the Notch signaling pathway and form primordial follicles during the culture period. We are using this organ culture system in concert with Notch agonists and antagonists to assess the actions of Notch signaling during early follicle development.

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Matrix Stiffness Regulates Secondary Follicle Development and Tissue Differentiation in a Three-dimensional Culture System, Erin R. West¹, Teresa K. Woodruff²,³, Lonnie D. Shea¹,³
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An in vitro ovarian follicle culture system could provide reproductive options to cancer patients facing premature infertility by supporting the development of cryopreserved ovarian tissue. Three-dimensional culture systems maintain the morphology of the follicle while allowing for follicle expansion and the exchange of soluble factors with the surrounding culture medium. In our original culture system, follicles did not reach the size of follicles in vivo, and theca cell differentiation and antral cavity development were rarely observed. We hypothesized that growth and differentiation may be inhibited by the rigidity of the encapsulating alginate matrix, and therefore utilized radiation treatment, chemical oxidation, and varied solids concentration of alginate to create alginate gels exhibiting a range of mechanical characteristics. The resulting stiffness, measured by the shear elastic modulus, of alginate ranged from 203±13 Pa (0.7% alginate) to 3010±84 Pa (3% alginate). Both two-layered (100-130 µm diameter) and multilayered (150-180 µm diameter) secondary follicles were isolated from fresh murine ovaries, and cultured for 12 and 8 days, respectively. Follicles of both stages encapsulated in low elastic moduli conditions exhibited significantly greater growth than follicles grown in the high-modulus 3% alginate (final diameters of 248 µm vs. 138 µm for two-layered secondary follicles, and 411 µm vs. 202 µm for multilayered secondary follicles). In addition, antrum formation increased significantly in weaker alginate conditions, with 81% of multilayered secondary follicles forming an antrum in 0.7% alginate, and only 2% forming an antrum in 3% alginate. Theca cells were also observed exclusively in the low-moduli alginate conditions. Oocyte quality improved as a result of decreased alginate stiffness, as measured by in vitro oocyte maturation. Oocytes from follicles of both stages demonstrated increased rates of meiotic resumption with decreased alginate stiffness (e.g. 91% of oocytes cultured in 0.7% alginate vs. 46% of oocytes cultured in 3% alginate). In both follicle stages, estradiol levels increased with decreasing alginate stiffness, while trends for androstenedione and progesterone levels varied depending on follicle stage. Together, these results demonstrate that the mechanics of the encapsulating matrix direct cellular function and follicle and oocyte development, and are important considerations in optimizing the follicle culture environment.

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Abstract #8

**Dephosphorylation of the Actin Depolymerizing Factor, Cofilin, by hCG in Preovulatory Granulosa Cells**, Amelia B. Karlsson¹, Evelyn T. Maizels¹, and Mary Hunzicker-Dunn¹, ¹Northwestern University, Feinberg School of Medicine, Chicago Illinois

Activation of the luteinizing hormone receptor (LHR) on preovulatory granulosa cells (PO GCs) stimulates the cAMP/PKA pathway which has been shown to up-regulate target genes important for ovulation and follicle luteinization. However, the effect of these pathways on other cellular events, such as regulation of the actin cytoskeleton, is still roughly defined. Reorganization of the actin cytoskeleton is essential to cellular movement, adhesion, morphogenesis and cytokinesis and is often triggered in response to extracellular stimuli, such as binding of growth factors and chemoattractants to cell surface receptors. Cofilin, an actin depolymerizing factor, binds to actin and causes depolymerization and severance of actin filaments while also supplying actin monomers for polymerization. Cofilin’s severing activity is negatively regulated by phosphorylation at Ser3 and re-activated by dephosphorylation at this site. The Rho signaling pathway when activated causes phosphorylation and inactivation of cofilin. The purpose of our studies was to evaluate whether LHR signaling affects the phosphorylation status of cofilin. We demonstrated that under basal conditions cofilin phosphorylation at Ser3 is readily detected and in vitro stimulation with human chorionic gonadotropin (hCG), an LHR agonist, causes rapid dephosphorylation of cofilin at Ser3. This dephosphorylation of Ser3 is also seen in vivo, using PMSG-primed rats intraperitoneally injected with an ovulatory concentration of hCG. Using cell soluble pathway-selective cAMP analogs, we showed that activation of the cAMP/PKA pathway mimics hCG’s effect on cofilin phosphorylation. We also found that incubation of GCs with the Rho-kinase inhibitor, Y27632, reduces the extent of cofilin phosphorylation at Ser3 in vehicle treated cells, suggesting that the phosho-regulation of cofilin is mediated downstream of Rho-kinase. To determine whether hCG has a direct effect on the Rho signaling pathway, Rho activity assays were conducted. These assays indicate that hCG decreases Rho activity and suggests that hCG’s effect on cofilin may be regulated through the Rho signaling pathway. Taken together our results show that cofilin is a previously unrecognized downstream target of LHR signaling and suggest that by regulating the phosphorylation status of cofilin on Ser3, LHR activation likely modifies actin cytoskeletal dynamics. Studies are underway to directly link LHR-stimulated cofilin dephosphorylation to changes in the actin cytoskeleton in ovarian GCs.

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The Peroxisome Proliferator-Activated Receptor Gamma is a Downstream Target of Progesterone Receptor and Controls Ovulation in Mice, Jaeyeon Kim¹, Marcey Sato¹, John P. Lydon², Francesco J. DeMayo³, Indrani C. Bagchi², Milan K. Bagchi¹, ¹Department of Molecular & Integrative Physiology, ²Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, ³Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

Ovulation is a key female reproductive event, which involves the release of a fertilizable oocyte from an ovarian follicle. This process is initiated when follicular tissue is stimulated by a surge of pituitary gonadotropins, which induce a cascade of gene expression that culminates in the rupture of the follicle. The progesterone receptor (PR), a hormone-regulated transcription factor, plays a critical role in ovulation. Although mice heterozygous for PR knockout (PRKO) mutation are indistinguishable from wildtype, the homozygotes are anovulatory due to a failure in the rupture of the preovulatory follicles. The pathways that operate downstream of PR during ovulation are little understood. In this study, we performed global mRNA profiling to identify targets of PR regulation during the ovulatory process. We identified several potential PR-regulated genes that include PPARγ. Using real-time PCR analyses, we confirmed that this gene is induced in the mouse ovary at 5 h post human chorionic gonadotropin (hCG) following 48 h of pregnant mare serum gonadotropin (PMSG) treatment. In contrast, the expression of PPARγ was markedly reduced in the ovaries of PR null mice. Localization of PPARγ, using in situ hybridization, demonstrated that upon treatment with PMSG and hCG, PPARγ is expressed primarily in the granulosa cells of the preovulatory follicles. The LH/hCG-stimulated induction and PR regulation of PPARγ were also verified in vitro in primary cultures of mouse granulosa cells. To address the functional role of this gene during ovulation, we created a conditional knockout mouse model by crossing mice harboring “floxed” PPARγ gene with progesterone receptor Cre knockin (PR-Cre) mice. This resulted in the generation of females (PPARγflox/flox PRCre/+) in which the PPARγ gene undergoes Cre-mediated excision in the mural granulosa cells of the preovulatory follicles. When the conditional PPARγ knockout (KO) mice were subjected to gonadotropin-induced superovulation, we observed greater than 75% reduction in the number of released eggs compared to the PPARγflox/flox (control) mice. Upon histological examination of the ovaries of control and PPARγKO mice at 18-19 h post-hCG, numerous corpora lutea were seen in the control tissue, whereas only a few corpora lutea and a large number of unruptured preovulatory follicles were found in the mutant tissue. These results revealed that the loss of PPARγ signaling in the mural granulosa cells of the mutant ovaries leads to an impairment in follicular rupture. Using these mutant mice, we have identified endothelin 2 and cyclic GMP-regulated kinase II, known PR-regulated genes, as targets of regulation by PPARγ in the preovulatory follicle. Based on these results, we propose that (i) PPARγ and its downstream signaling molecules are critical regulators of ovulation, and (ii) they function as mediators of the biological actions of PR in the ovulatory pathway.

(Supported by NIH grants)
Prolactin Signaling through the Short Isoform of its Receptor Activates the Inflammatory Pathway, Sangeeta Devi, Aurora Shehu¹, Julia Halperin¹, Carlos Stocco¹,², Nadine Binart³, Geula Gibori¹, ¹University of Illinois at Chicago, Chicago, IL, ²Yale School of Medicine, New Haven, CT, ³Inserm U809 Faculté de Médecine Necker-enfants Malades, Paris, FRANCE.

Prolactin (PRL) has been shown to bind to two distinct receptors long (PRL-RL) and short (PRL-RS). PRL signaling through PRL-RL activates several pathways including STAT, PI3K and Src. However, PRL-RS mediated PRL signaling pathways have not been elucidated to date. PRLR null mice expressing only the PRL-RS have been generated and our analysis revealed premature follicular activation leading to premature ovarian failure and total absence of follicles due to massive granulosa and oocyte death. To determine how PRL signaling through PRL-RS causes such a severe phenotype, a transcription reporter array with consensus binding sequences for 354 transcription factors (TFs) was performed using ovaries from PRL-RS transgene mice. PRL signaling through PRL-RS affected profoundly DNA-binding activity of several TFs. These TFs are involved in developmental, glucose metabolism, and immune response pathways. Interestingly, anti-inflammatory gene such as E4BP4 was downregulated while the immune response gene, NFATc was stimulated. NFAT proteins are known to induce proinflammatory cytokines such as TNF alpha. Indeed TNF alpha was markedly stimulated both at the mRNA and protein levels in the PRL-RS expressing ovary upon PRL treatment. We also observed a severe inhibition of GSK3, a kinase which phosphorylates and inactivates NFATc, and whose deletion leads to massive granulosa and oocyte death. To determine how PRL signaling through PRL-RS causes such a severe phenotype, a transcription reporter array with consensus binding sequences for 354 transcription factors (TFs) was performed using ovaries from PRL-RS transgene mice. PRL signaling through PRL-RS affected profoundly DNA-binding activity of several TFs. These TFs are involved in developmental, glucose metabolism, and immune response pathways. 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Increased Frequency of Natural Killer T (NKT) Cells in the Tumor Microenvironment of Ascites in Women with Ovarian Cancer, Shatavi SV1; Al-Harthi L3; Mitchell S2; Usha L4; Rotmensch J2; Landay A3; Luborsky JL1,2, 1 Departments of Pharmacology, 2 Obstetrics & Gynecology, 3 Immunology and Microbiology and 4Internal Medicine, Section Medical Oncology and Hematology, Rush University Medical Center, Chicago, IL

**Background:** Natural killer T (NKT) cells are glycolipid reactive lymphocytes that are implicated in the resistance to pathogens and tumors. NKT cells are immune cells that could directly mediate tumor cell death. They are functionally capable of both adaptive (antigen driven) and innate (antigen independent) immune responses. Based on considerable in vitro and animal studies, they play a paradoxical role in modulating anti-tumor responses. Although it has been reported that NKT cells can have anti-tumor activity in a variety of cancers, there have been no reports concerning the frequency of NKT cells in ascites of women with ovarian cancer. Ascites, fluid which forms in the peritoneal cavity during ovarian cancer progression, contains viable tumor cells, host immune cells and soluble factors and reflects the local tumor microenvironment of women with ovarian cancer. Our overall objective was to determine the frequency of NKT cells in ascites and blood of women with ovarian cancer.

**Materials and Methods:** Ascites and peripheral blood mononuclear cells (PBMCs) were obtained from ovarian cancer patients (n = 9), under a protocol approved by the Institutional Review Board. PBMCs and peritoneal fluid from women with benign gynecological conditions were examined as controls (n = 3). Two primary ovarian cancer cell lines from ascites (HTB161, HTB77) were used to establish method and markers. NKT cells were identified using CD3-PercP/6B11-PE conjugated monoclonal antibody as markers (BD) by standard multi-color flow cytometry. Data was acquired and analyzed with CELLQUest software (BD).

**Results:** The frequency of NKT cells in PBMCs from healthy donors varied between 0.1- 0.5 %, with an average of 0.31 %. PBMCs from women diagnosed with ovarian cancer exhibited a lower frequency of NKT cells with 0.02% (range 0.01-0.03%). Characterization of immune cells in ascites of ovarian cancer patients revealed that NKT cells are abundant in ascites and represent 9% (range 7-11%) of all lymphocytes. In comparison only 0.3% (range 0.1-0.5%) of all lymphocytes expressed the NKT cell marker in peritoneal fluid from women with benign conditions (P≤0.05)

**Conclusion:** The frequency of NKT cells were ~100 fold increased in ascites of patients with ovarian cancer compared to women with benign gynecological conditions, which is a novel finding. Our long-term objective is to determine why NKT cells are attracted to this environment and why NKT cells do not appear to kill tumor cells. Both direct and indirect NKT-mediated anti-tumor responses will be evaluated in order to determine the functional relevance of NKT cell expansion within the tumor microenvironment.

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The CCAAT/Enhancer Binding Protein Beta is a Critical Regulator of Decidualization Wei Wang\(^1\), Quanxi Li\(^2\), S. R. Mantena\(^2\), Robert Taylor\(^3\), Indrani C Bagchi\(^2\), Milan K Bagchi\(^1\)\(^1\) Dept. of Mol. & Integrative Physiology, \(^2\) Dept. of Vet. Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, \(^3\) Dept. of Ob. & Gyn., Emory University, Atlanta, GA

During early pregnancy, the uterine stromal cells undergo extensive proliferation, differentiation and remodeling, known as decidualization. This hormonally induced cellular transformation is an essential prerequisite for embryo implantation. It is evident that perturbations in this process lead to dysfunctions in uterine receptivity that result in female infertility. We have recently identified C/EBP\(^\beta\) CCAAT box-binding transcription factor, as a unique regulator of decidualization. In pregnant mice, the uterine expression of C/EBP\(^\beta\) is observed in the proliferating as well as the decidualized stromal cells surrounding the implanted embryo. Most strikingly, the uteri of C/EBP\(^\beta\)-null mice exhibit a complete lack of response to a deciduogenic stimulus, indicating a critical role of this transcription factor in regulating the decidualization program. In order to analyze the molecular processes controlled by C/EBP\(^\beta\) during the decidualization program, we employed a primary culture system in which undifferentiated stromal cells undergo differentiation in vitro in a manner that mimics the in vivo decidualization program. To establish this system, we collected uteri from pregnant mice on day 4 (morning) of gestation. Stromal cells were isolated and plated in the presence of the steroid hormones estrogen (E) and progesterone (P). We monitored the differentiation program by analyzing the expression of alkaline phosphatase (ALP) and prolactin-related protein (PRP), two markers known to be associated with decidualization of stromal cells. We detected only low levels of these markers during the initial 48 h of culture, but their expression increased progressively and significantly at 72 and 96 h. To analyze the role of C/EBP\(^\beta\) in this decidualization program, we employed a loss-of-function approach using siRNAs targeted specifically to its mRNA. Administration of C/EBP\(^\beta\) siRNA to stromal cells led to a marked decrease in C/EBP\(^\beta\) mRNA and protein within 48 h of treatment. This intervention was accompanied by a significant loss of expression of ALP and PRP mRNA, indicating that C/EBP\(^\beta\) plays an essential role in the stromal decidualization program in mouse uterus. We also monitored the expression of C/EBP\(^\beta\) in a well established human stromal primary culture system in which decidualization is efficiently induced by a hormonal cocktail containing E, P, and a cAMP analog. We detected low level of C/EBP\(^\beta\) mRNA and protein expression in undifferentiated stromal cells isolated from late proliferative phase human endometrium. When these stromal cells were treated with the hormonal cocktail, we observed a marked enhancement in the expression of C/EBP\(^\beta\) mRNA and protein within 24 h of treatment. The C/EBP\(^\beta\) expression started to decline at 72 h but was maintained at a significant level up to 9 days of culture. We also observed the induction of classical decidualization marker genes, prolactin and IGFBP-1, during this in vitro decidualization process. The observation that the enhanced expression of C/EBP\(^\beta\) occurred early (12-24 h) during the decidual program raised the possibility that it is a critical regulator of this process. We will test this hypothesis by attenuating the expression of C/EBP\(^\beta\) by employing the siRNA strategy and determining the impact of this altered expression on the ability of the human stromal cells to undergo decidualization.

(Supported by NIH grants)
Conditional Knockout of Connexin 43 in Mouse Uterus Uncovers an Essential Role of Gap Junctions in Stromal Differentiation During Pregnancy, Mary Laws¹, Francesco DeMayo³, John Lydon³, Milan K Bagchi², and Indrani C. Bagchi¹. ¹Department of Veterinary Biosciences, ²Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, ³Baylor College of Medicine, Houston, TX

In most mammalian tissues, gap junction proteins connect cells by creating intercellular cytoplasmic channels clustered in the plasma membrane. These channels allow for communication between the cells by transferring ions and small molecules. Connexin 43 (Cx43) is one such gap junction protein that is present in multiple cell types. Our recent studies indicated that Cx43 is induced in the mouse uterus at the onset of implantation. The expression of Cx43 increased further during the decidualization phase of pregnancy and was localized in the proliferating as well as the decidualized stromal cells surrounding the implanted embryo. The Cx43 knockout mouse dies late in gestation or immediately following birth due to a developmental heart defect. Therefore, to address the functional role of Cx43 in the uterus during pregnancy, we created a conditional knockout mouse model by crossing transgenic mice harboring a “floxed” Cx43 gene (Cx43^fl/fl) with progesterone receptor Cre knockin (PR-cre) mice. This mating generated female mice in which the Cx43 gene undergoes Cre-mediated excision in the reproductive cell types expressing the PR. The PR-Cre X Cx43^fl/fl mice will be referred to as Cx43^d/d mice. The impact of Cx43 deletion on mouse reproduction was examined by comparing the fertility of female Cx43^d/d mice with that of female Cx43^fl/fl mice. Fertility was assayed by housing Cx43^fl/fl and Cx43^d/d female mice with an equal number of wild type male mice. Our study revealed that Cx43^d/d mice exhibited ~60% reduction in birth rate when compared to the Cx43^fl/fl (control) mice. To test whether or not this fertility defect is due to an impaired ovarian function, we subjected the mice to gonadotropin-induced superovulation. We observed that the number of released oocytes in Cx43^d/d mice was comparable to that of Cx43^fl/fl mice, suggesting that the reduced fertility in Cx43^d/d mice is not due to a functional defect of the ovary. Further reproductive analyses indicated that the Cx43^d/d mice were able to initiate embryo implantation and support pregnancy up to day 7 of gestation. Interestingly starting on day 8 of pregnancy, the implanted embryos in Cx43^d/d mice started to undergo resorption. In order to determine if the compromised fertility in the Cx43^d/d mice was due to the inability of the uterus to undergo decidualization, Cx43^fl/fl and Cx43^d/d female mice were subjected to an artificial decidual stimulation. Compared to control mice, we noted a significant reduction in the wet weights of uterine horns from Cx43^d/d mice. Histological analysis of uterine sections revealed a significant decrease in the number of decidual cells in the Cx43^d/d mice compared to controls. Collectively, our studies indicated that Cx43 plays an essential functional role in the process of uterine decidualization during pregnancy.

(Supported by NIH grants)

Endometrial carcinoma is the fourth most common malignancy among women in the developed world. In 30-80% of Type I endometrial cancers, the PTEN gene is inactivated by either mutations or the loss of heterozygosity. The loss of PTEN activity leads to constitutively active Akt, which inhibits several downstream targets such as, GSK-3, BAD, p27, and FOXO1A. FOXO1A is a member of the FOXO sub-family of Forkhead/winged helix family of transcription factors that is involved in cell cycle regulation, differentiation and apoptosis. It is highly expressed in the human endometrium. We propose that the deregulation of FOXO1A protein in the endometrium results in the aberrant expression of genes that regulate the cell cycle and apoptosis, thereby promoting tumorigenesis. Immunohistochemical staining for FOXO1A was done for normal, hyperplastic and endometrial cancer tissues. In normal endometrium, FOXO1A protein was highly expressed in both the glands and stroma. Hyperplastic tissue also exhibited strong staining for FOXO1A. In contrast, endometrial carcinoma showed very little to no detectable staining for FOXO1A. RT-PCR analysis of 4 endometrial cancer cell lines, Ecc1, Ishikawa, HeC1B and RL95 demonstrated that FOXO1A mRNA was expressed while Western blot analysis showed very little FOXO1A protein present. To further elucidate the mechanism for the decreased protein levels, the involvement of Skp2, the oncogenic subunit of the Skp1/Cul/F-box protein ubiquitination complex was studied. Skp2 was expressed in all four cell lines with the highest levels produced by the Ishikawa cells. The expression of FOXO1A was inversely correlated with Skp2 expression. When Ishikawa cells were treated with a siRNA specific to Skp2, levels of FOXO1A protein increased in Ishikawa cells, suggesting that Skp2 is involved in the ubiquitination of FOXO1A for protein degradation. Finally, since it has been shown by others that ubiquitination of FOXO1A by Skp2 requires phosphorylation of FOXO1A, we used an Akt inhibitor to inhibit the phosphorylation of FOXO1A. As a result, FOXO1A protein levels increased and the protein was localized to the nucleus. The loss of FOXO1A may be an important event in endometrial neoplasia and determining the mechanisms to reinstate this pathway will provide insights to the development of alternate therapies.

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Dynamic Steroid-Regulated Assembly and Disassembly of Lipid Droplets/Adiposomes During Implantation: Progesterone Promotes Assembly and Estrogen Triggers Disassembly in the Uterine Epithelium, Srinivasa Raju Mantena¹, Milan K Bagchi², and Indrani C. Bagchi¹, ¹Department of Veterinary Biosciences; ²Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign

Implantation of the embryo to the uterine wall is regulated by the concerted actions of maternal steroid hormones, progesterone (P) and estrogen (E). Previous studies have shown that lipids, acting as signaling molecules, play an essential role in this process. Enzymes involved in lipid metabolism, such as cyclooxygenase 2 and 12/15-lipoxygenase, are maximally expressed in the uterus at the time of implantation and critically regulate this process. Furthermore, mice lacking lysophosphatidic acid receptor 3, which signals via a small lipid moiety, exhibit defects in implantation. In the present study, we demonstrated that P and E facilitate the accumulation and dispersal of lipid droplets, also known as adiposomes, in the uterine epithelium at the time of implantation. We utilized a delayed implantation mouse model in which ovaries are removed in the preimplantation phase to prevent implantation of the embryos, which are then sustained in the uterus in a dormant state by administering P alone to these animals. In these delayed mice, we observed a significant accumulation of lipid droplets, containing neutral lipids, in the uterine epithelium as indicated by Oil-Red-O staining. Interestingly administration of E, which triggers initiation of implantation in the P-treated delayed mice, caused a dramatic degradation of these lipid droplets. E administration also led to a marked downregulation of TIP47, a Tail interacting protein of 47 KD, known to be involved in the assembly of the lipid droplets. To further analyze the steroidal regulation of this phenomenon, non-pregnant mice were subjected to ovariectomy and treated with P or E or a combination of P and E. We observed a remarkable accumulation of lipid droplets in the uterine epithelium of P-treated mice. This lipid accumulation was not evident in mice treated with E alone. However, when E was administered to P-treated mice, there was a rapid decline in both TIP47 immunostaining and Oil-Red-O staining in the uterine epithelium. Consistent with these findings, the patterns of TIP47 expression and lipid accumulation in the uterine epithelium of normal pregnant uterus precisely overlapped with each other. In response to the high level of P on day 3 of gestation, both accumulation of lipid droplets and expression of TIP47 were maximally stimulated in the uterine epithelium. Administration of RU486, a progesterone receptor (PR) antagonist, led to a marked reduction in both Oil-Red-O and TIP47 staining, indicating that the lipid droplet accumulation in response to P is mediated via PR. Strikingly, at the time of implantation at mid night of day 4 of pregnancy, we observed a concomitant downregulation of TIP47 and Oil-Red-O staining, presumably in response to a transient surge of E that precedes this period. Collectively, these results revealed, for the first time, that steroid hormones play an active and potentially important role in lipid droplet/adiposome assembly and disassembly in the uterine epithelium in the peri-implantation period and these effects are at least partly mediated via regulation of TIP47.

Supported by NIH grants.
The steroid hormones progesterone (P) and estrogen (E) act in concert to control uterine competency for embryonic implantation. A primary role of P is to induce differentiation of the endometrial stromal cells into decidual cells that maintain an environment conducive to the growth and development of the implanting embryo. Our recent studies have shown that BMP2, a member of the TGF beta superfamily, is rapidly induced in response to P and progesterone receptor (PR) in the mouse uterus during decidualization. We have analyzed the role of BMP2 in uterine stromal cell differentiation using primary cultures of these cells isolated from pregnant mouse uterus. We found that addition of recombinant BMP2 to undifferentiated stromal cultures markedly advanced the differentiation program by stimulating the Smad signaling pathway. Conversely, siRNA-mediated down-regulation of BMP2 expression in these cells efficiently blocked the differentiation process, as indicated by the loss of expression of selected markers of the decidual state. In more recent studies, we investigated the expression of BMP2 in human endometrial stromal cells during in vitro decidualization. Stromal cells isolated from human endometrial biopsies obtained from normal fertile women in the proliferative stage of the menstrual cycle were placed in culture and subjected to decidualization in response to a hormonal cocktail containing P, E, and 8-bromo-cAMP (a cAMP analog). We noted a clear morphological transition of the stromal cells from a fibroblastic to an epitheloid shape with the progression of decidualization by day 6 of the culture. We also observed the induction of classical decidualization biomarkers, PRL and IGFBP-1, during the in vitro decidualization process. We observed a marked enhancement in the expression of BMP2 in human stromal cells in response to the hormone cocktail. More importantly, addition of exogenous BMP2 along with P, E, and 8-bromo-cAMP to these stromal cultures led to a dramatic induction in the levels of PRL and IGFBP-1 mRNAs. Taken together, our results indicated that BMP2 is induced in the mouse and human endometrium during decidualization and plays a critical role in this differentiation program.

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Maternal Administration of Leptin Hormone Stimulates the Postnatal Gonadal and Fetal Brain Development of the Syrian Hamster (*Mesocricetus auratus*), Alper Karakas¹,# and Bulent Gunduz¹, ¹Department of Biology, Faculty of Arts and Sciences, Abant Izzet Baysal University, 14280 BOLU-TURKEY [Current Address: ¹Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611, USA]

Growth of the fetus in utero is the result of maternal nutrition, nutrient transfer and metabolism across the placenta, and fetal metabolism, regulated by growth factors. The interaction among these processes is very complex and varies throughout the gestation. Leptin hormone is a metabolic signal which informs the brain about fat and energy contents of the body. It has been suggested that leptin may act as a growth factor in the fetus, directing growth and development via central or peripheral actions. In the present study, the effects of exogeneous leptin administration throughout the gestation on fetal brain and prepubertal gonadal development of the Syrian hamster were investigated. In the first experiment, female hamsters were paired and injected with leptin (2 µg/kg), insulin (2 units/kg), melatonin (25 mg/kg) and saline (0.9% NaCl) from day 8 to day 15 of gestational period. After weaning, the prepubertal hamsters were examined for their body, testes, adipose, epididymis and seminal vesicle weights throughout eight weeks. All parameters examined were significantly higher in hamsters borned to leptin injected mothers at the end of 8 weeks (p<0.01). Histological examination of the testes showed that there was a complete spermatogenesis in hamsters borned to leptin injected mothers. These results suggest that leptin may accelerate the onset of puberty by stimulating GnRH from the hypothalamus. In the second experiment, pregnant hamsters were injected with leptin (2 µg/kg) or saline (0.9% NaCl) from day 8 to day 15 of gestational period. The newborn pups were decapitated at the third day after birth and their brains were examined for total weight, volume and number of neurons. Leptin accelerated the development of the brains. Brain weight, volume and number of neurons were significantly higher in the leptin treated groups than in control groups (p<0.05). These results demonstrated that leptin administration to pregnant female Syrian hamsters stimulates the prepubertal reproductive system and prenatal brain development.
Leptin hormone which is secreted from adipose tissue, beside its regulatory role of energy metabolism, it has an acceleratory effect on onset of puberty in some species. Melatonin hormone which is secreted rhythmically from pineal gland, can delay onset of puberty. In this study, the influences of pineal gland and leptin hormone on the onset of puberty of male juvenile Syrian hamster was examined. Adult Syrian hamsters (2-3 months of age) were mated at 14L:10D photoperiod. Male pups were weaned at the age of 21 days and and separated into 3 groups [control (n=10), leptin injection (n=10) and pinealectomy plus leptin injection (n=10)]. Daily food intake, weekly body and testis weights were measured throughout 8 weeks. Leptin (1µg/kg/day) and saline (0.9% NaCl) treatments were prepared fresh the day of treatment. Body and testes weight of leptin-treated pups showed a significantly faster increase than the control group (p<0.05). Leptin did not affect food intake in either control or pinealectomized pups (p>0.05). Leptin hormone accelerated the onset of puberty in juvenile male Syrian hamsters in the presence or absence of endogenous melatonin hormone. However, the onset of puberty in pinealectomized plus leptin injected pups occurred earlier than in the other groups (p< 0.05). These results show that leptin affects the time of puberty onset in male Syrian hamsters. In conclusion, although the leptin and melatonin hormones exert antagonistic effects, the hamster reproductive system appears to be more sensitive to leptin hormone.

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A Nonclassical ERα Signaling Pathway is Sufficient to Convey Estrogen Negative but not Positive Neuroendocrine Feedback of LH in the Female Mouse, C. Glidewell-Kenney¹, L.A. Hurley¹, L. Pfaff³, J. E. Levine², J. Weiss¹, J.L. Jameson¹, Division of Endocrinology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL¹, WCAS Neurobiology & Physiology, Northwestern University, Chicago, IL²

Estrogen feedback in the female hypothalamic-pituitary-gonadal (HPG) axis is of central importance in coordinating follicle development and ovulation to maximize reproductive success. Ovarian follicles produce estrogen that enters the circulation where it is detected by the hypothalamus and the pituitary. Throughout most of follicle development, estrogen exerts negative feedback on luteinizing hormone (LH) by suppressing hypothalamic gonadotropin releasing hormone (GnRH) release from hypothalamic GnRH neurons. However, at the preovulatory stage follicles secrete high levels of estrogen and, through an unknown mechanism, estrogen feedback switches from negative to positive resulting in an LH surge to initiate ovulation.

Estrogen feedback in the HPG axis can be influenced by stress, metabolic and, in some species, circadian pathways. Moreover, estrogen is thought to act indirectly on GnRH neurons through paracrine effects to decrease GnRH release. The complexity of estrogen feedback has largely restricted studies to physiological paradigms, making the identification of underlying cellular and molecular mechanisms difficult. Multiple isoforms of the ER have been identified (α and β) and recent studies using estrogen receptor knockout mouse models have identified ERα as the predominant isoform conveying estrogen negative feedback regulation of luteinizing hormone (LH). However, it is known that ERα signals through multiple cellular pathways, including rapid nongenomic as well as classical (ERE-dependent) and nonclassical (ERE-independent) genomic pathways. The relative contribution of the classical and nonclassical ERα signaling pathways to mediate estrogen feedback regulation of LH will further our understanding of the underlying cellular and molecular mechanisms.

Previously our lab created a Nonclassical Estrogen Receptor α Knock In (NERKI) mouse model using a mutant estrogen receptor (E207A/G208A) with disrupted classical but intact nonclassical activity. In this study, results confirm previous reports that the ERαKO female mouse has elevated serum LH (1.94 ± 0.40 ng/ml) versus wild-type (0.18 ± 0.02 ng/ml). Selectively restoring only the nonclassical aspects of ERα signaling, by breeding the NERKI allele onto the ERαKO background, results in significantly decreased serum LH (0.58 ± 0.26 ng/ml). Physiological paradigms demonstrate the sufficiency of the nonclassical ERα pathway to mediate estrogen negative feedback. In contrast, the classical ERα pathway is required for estrogen positive feedback and complete negative feedback. These results indicate distinct physiological roles for the classical and nonclassical ERα signaling pathways in the female neuroendocrine axis. The modulation of these two pathways may represent a cellular mechanism for the switch from estrogen negative to positive estrogen feedback.

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The Role of Nonclassical Estrogen Receptor Signaling in the Sexual Receptivity of the Female Mouse, Mariana A. Jimenez¹, Christine Gidewell-Kenney², J. Larry Jameson² and Jon Levine¹
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It is well established that estrogen (E₂) regulates female reproductive behavior by binding neural intracellular estrogen receptors (ERs). These ligand activated transcription factors bind specific estrogen response elements (EREs) in DNA with high affinity to transactivate gene expression in response to E₂. The genomic actions of E₂ on reproductive behaviors have been demonstrated in studies of ERα knock-out animals (ERαKOs), which lack the receptive lordosis response and display an increase in rejective behaviors toward stud males when tested for receptivity. However, the specific role of the nonclassical ER in mediating reproductive behaviors has not yet been demonstrated. Nonclassical ER knock-in (NERKI) animals contain a mutation that selectively eliminates binding to EREs, effectively disabling classical signaling, while preserving nonclassical signaling. This allows only protein-protein interactions between ERs and other transcription factors to alter gene transcription. The purpose of this study was to test the hypothesis that nonclassical ER signaling rescues the lack of sexual receptivity seen in ERαKO females. The NERKI model enabled us to distinguish the role of nonclassical ER signaling in female sexual receptivity. Ovariectomized WT, ERαKO and NERKI females were treated with two daily subcutaneous injections of estradiol benzoate (10μg in 0.1 ml sesame oil), and a single injection of progesterone (500μg in 0.1 sesame oil) the following day, 5 hours prior to behavioral receptivity testing. Each female was given two 30-minute tests at a 2-week interval, with a WT stud male. Overall, under these hormonal conditions, ERαKO females were unreceptive to stud males, in contrast to their WT littermates who were receptive on both tests. Female ERαKOs displayed significantly longer periods of rejective behaviors (e.g. kicking, fleeing, and standing in an upright posture) compared to WTs, resulting in the inability of studs to intromit. Although nonclassical signaling did not rescue the non-receptive phenotype of the ERαKOs, it did partially rescue the rejective and aggressive phenotypes; NERKIs were significantly less rejective to stud males when compared with ERαKO females. These findings are the first to demonstrate that nonclassical estrogen receptor signaling is important in mediating certain aspects of female sexual behavior.
LH-receptor Activation in Ovarian Granulosa Cells Promotes PKA-dependent Dephosphorylation of the AKAP MAP2D. M.P. Flynn¹, E.T. Maizels¹, M. Hunzicker-Dunn¹,², ¹Feinberg School of Medicine, Northwestern University, Chicago, Illinois, ²School of Molecular Biosciences, Washington State University, Pullman, Washington.

The actions of luteinizing hormone (LH) to induce ovulation and luteinization of preovulatory follicles are mediated principally by activation of cAMP-dependent protein kinase (PKA) in granulosa cells. PKA activity is targeted to specific locations in many cells by A-kinase anchoring proteins (AKAPs). We previously showed that follicle-stimulating hormone (FSH) induces expression of microtubule-associated protein (MAP) 2D, an 80 kD AKAP, in rat primary granulosa cells, and that MAP2D co-immunoprecipitates with PKA regulatory subunits in these cells. Neuronal MAP2 isoforms have been shown not only to function as AKAPs but also to serve as scaffolds and substrates for various kinases and phosphatases, including protein phosphatase (PP) 2A. Phospho-regulation of neuronal MAP2 by PP2A and other proteins at numerous sites has been shown to affect the affinity of interactions with microtubules and additional binding partners and, thus, is likely to influence signal transduction and microtubule dynamics. Here we demonstrate protein interactions between MAP2D and PP2A in preovulatory rat granulosa cells by co-immunoprecipitation and microcystin-agarose pulldown. We also report dephosphorylation of MAP2D at Thr256/259 after 10 minutes of treatment with hCG, an LH receptor agonist, as detected with a phospho-specific antibody. This event was blocked by the PP2A inhibitor okadaic acid, suggesting that this phospho-regulation may be mediated by PP2A. hCG-induced MAP2D dephosphorylation was mimicked by 10 minute treatment with forskolin or the cell permeable cAMP analog, 8-chlorophenylthio-cAMP. Moreover, dephosphorylation was blocked by the cell permeable PKA inhibitor myristoylated-PKI, indicating a role for cAMP and PKA signaling in the regulation of this event. Taken together, these results show that the phosphorylation status of the AKAP MAP2D is acutely regulated by LH receptor activation via a pathway dependent on PKA. The PKA-dependent dephosphorylation of MAP2D on Thr256/259 appears to be mediated by PP2A. Future studies will investigate the effect of dephosphorylation at Thr256/259 on MAP2D binding to the microtubule cytoskeleton.

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Posttranslational Modification of the NR5A Family of Nuclear Receptors in the Ovary, Christina K. Matulis and Kelly Mayo, PhD, Department of Biochemistry, Molecular Biology, and Cellular Biology, Center for Reproductive Science, Northwestern University, Evanston, IL

Liver receptor homolog 1 (LRH-1) and steroidogenic factor 1 (SF-1) are both members of the nuclear receptor subfamily NR5A. LRH-1 and SF-1 are expressed in several of the same tissues and bind to the same response element in target gene promoters. In the ovarian granulosa cell, LRH-1 and SF-1 both bind to and transactivate the gene encoding the alpha subunit of the protein hormone inhibin. Work published by others has shown that the transactivation ability of LRH-1 and SF-1 is modulated by posttranslational modifications including sumoylation and phosphorylation. Since LRH-1 and SF-1 are both expressed in the same cell type in the ovary and target the same binding site in promoters, we have investigated whether posttranslational modifications of LRH-1 and SF-1 can mediate differences in the transactivation of the same target promoter. LRH-1 and SF-1 are known to be sumoylated in the hinge region (K289 and K194 respectively). Sumoylation of transcription factors is a posttranslational modification often associated with repression of the target gene. In a mammalian 1 hybrid system, mutation or deletion of the sumoylation site of LRH-1 or SF-1 significantly increased the transactivation of a reporter gene compared a wild type construct. However, in transient transfection of GRMO2 cells (immortalized mouse granulosa cell line), the sumoylation site mutant constructs did not increase the transactivation of an inhibin alpha promoter reporter construct compared to wild type constructs. This result may be explained by the fact that the GRMO2 cell line has a high endogenous expression of LRH-1 and SF-1. A human ovary library was screened using a yeast two hybrid assay and several interactors were identified that differentially recognize wild type and sumoylation site mutant LRH-1 and SF-1 bait constructs. SF-1 has a known MAPK phosphorylation site (S203) that is located several amino acids downstream of the sumoylation site. When LRH-1 is aligned to SF-1, there is a serine in the position corresponding to SF-1 S203 with another serine adjacent to it (S298 and S299 respectively). To determine if phosphorylation of the NR5A family members has an effect on the transactivation of target genes, SF-1 S203 and LRH-1 S298 and S299 were mutated to either an alanine or a glutamate. Surprisingly, the mutant constructs did not transactivate the inhibin alpha promoter luciferase construct differently than wild type when transfected into GRMO2 cells. Thus, the importance of phosphorylation of these nuclear receptors may vary in a promoter-context or a cell type-specific fashion. We are currently investigating the various sumoylation and phosphorylation site mutants on simpler promoters and in cells that do not express endogenous SF-1 or LRH-1.

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Human Aldehyde Dehydrogenase (ALDH) as an Autoantigen for Ovarian Autoimmune Disease, Espinosa, S1; Shatavi, SV1; Edassery, SL1; Brucker, C3; Kunkel, JP4; Dias, JA4; Vasiliou, V5; Luborsky JL1,2, 1Departments of Pharmacology and 2Obstetrics & Gynecology; 3Rush University Medical Center, Chicago, IL, Department of Gynecology and Obstetrics; 4Centre of Reproductive Medicine, University of Nuremberg, Nuremberg, Germany, Wadsworth Laboratories; 5New York State Department Health, Albany, NY, Center for Pharmaceutical Biotechnology, Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center

Background: 50-70% of women with unexplained infertility or Premature Ovarian Failure (POF) have an autoimmune disease of the ovary associated with ovarian or oocyte autoantibodies. Identification of the major protein autoantigens associated with ovarian autoimmunity is in progress. We used a proteomics based approach to identify specific ovarian autoantigens (Shatavi et al, 2006, submitted). Antigens found in several other autoimmune diseases, such glyceraldehyde-3-phosphate dehydrogenase (GAPDH), vimentin and enolase, were identified and may represent general immunoreactions that are not disease specific. Aldehyde dehydrogenase 1 (ALDH1) was identified as a potential unique autoantigen for ovarian autoimmune disease. The objective of this study was to evaluate the direct reaction of patient sera with ALDH1A1 and a related family member, ALDH3A1.

Methods: Sera were obtained from patients with unexplained infertility (n=30), premature ovarian failure (n=10) and normal cycling control women (n=5, ProMedDx BioRepository), with approval from the Institutional Review Board. Sera were tested against recombinant ALDH1A1, ALDH3A1 (5µg final concentration) and purified GAPDH (25µg, Sigma) in one-dimensional Western blots using a multiscreen apparatus. Blots were incubated with horseradish peroxidase-conjugated goat anti-human IgA + IgG + IgM (H+L); (Jackson Immuno Research). Antibodies to each protein were used as a positive control. The reaction was visualized as a chemiluminescence product with Super Dura West substrate (Pierce) and the image analyzed using a Chemidoc XRS (BioRad). QuantityOne software (BioRad) was used for analysis of one-dimensional Western blots.

Results: 15/30 (50%) of women with unexplained infertility reacted with ALDH1A1 and 20/30 (67%) reacted with ALDH3A1. In women with POF, 4/10 (40%) reacted with ALDH1A1 and 2/10 (20%) reacted with ALDH3A1. In the control group only 1/5 (20%) reacted with ALDH1A1 and none reacted with ALDH3A1. For comparison, only 13% (4/30) of infertility sera, 30% (3/10) of POF sera and 20% (1/5) of control sera reacted with purified GAPDH. GAPDH and control sera reactions did not differ significantly. Overall, ovarian autoimmunity was significantly associated with ALDH1A1 but not with GAPDH reaction (p≤0.05).

Conclusion: There are several potential autoantigens associated with ovarian autoimmune disease. The results confirmed that aldehyde dehydrogenase is a potentially unique autoantigen in women with ovarian autoimmunity.

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Synergistic Effect of Peptidoglycan (PGN) and Polynosinic-polycytidylic Acid (poly(I:C)) on Expression of Toll-like Receptor (TLR)-dependent Genes, Vladimir Ilievski, MD¹ and Emmet Hirsch, MD¹,², ¹Department of Obstetrics and Gynecology, Evanston Northwestern Healthcare Research Institute, Evanston, IL; ²Feinberg School of Medicine, Northwestern University, Chicago, IL.

TLRs are receptors that recognize various structural components of bacterial and viral pathogens.

TLR2 mediates cellular responses to gram-positive organisms via their membrane lipoproteins, glycolipids and peptidoglycan (PGN, a major component of Gram-positive bacterial cell walls). TLR-2 then signals through the ‘MyD88-dependent pathway’ and induces the early expression of inflammatory cytokines via activation of NF-κB. TLR-3 is involved in the response to viral infections by recognizing double-stranded RNA, and signals via the ‘MyD88-independent pathway’. This pathway is responsible for induction of interferon β and interferon-dependent gene products. In addition to these products, the MyD88-independent pathway induces a late-phase NF-κB response.

METHODS: The mouse macrophage-like cell line RAW 264.7 was obtained from ATCC and cultured in DMEM with 10% fetal bovine serum (FBS), 1% streptomycin and 1% penicillin. Total RNA was isolated after 5-hour treatment with 1 µg/ml PGN and 10 µg/ml of poly(I:C) or a mixture of both reagents at 1 µg/ml of PGN and 5 µg/ml of poly(I:C). Multiplex RT-PCR was performed using ABI TaqMan reagents for inducible nitric oxide synthase (iNOS), interleukin 1-beta (IL-1β), interferon-beta (IFNβ), tumor necrosis factor-alpha (TNFα).

RESULT: Co-stimulation of cells with both PGN and poly (I:C) resulted in expression of iNOS, IL-1α and TNFα in a synergistic fashion compared with either PGN or poly (I:C) alone.

CONCLUSIONS: When cells are treated with both PGN and poly (I:C), a synergistic effect is noted on expression of iNOS, IL-1α and TNFα. This may have implications for superimposed bacterial and viral infections.

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Epigenetic Modification of Eutopic Endometrium in a Baboon Model of Endometriosis, Julie M Hastings1, Kevin S Jackson1, Hugh S Taylor2, Sun-Wei Guo3, Asgerally T Fazleabas4. 1Dept Ob/Gyn, University of Illinois at Chicago, Chicago, IL; 2Dept Ob/Gyn & Reproductive Sciences, Yale University, New Haven, CT; 3Dept Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

Introduction: Endometriosis is a gynecological condition associated with chronic pelvic pain and infertility. Reduced responsiveness to P4 has been demonstrated in the eutopic endometrium of women with endometriosis: it has been proposed that decreased expression of P4-regulated genes, including HOXA10, creates a uterine environment that is unreceptive to an implanting embryo. In an experimental model of endometriosis in the baboon, we have previously shown up-regulation of the E2 early response gene, FOS, in the eutopic endometrium in the early phase of disease progression. FOS-induced cellular transformation is associated with the induction of DNMT1, which causes DNA hypermethylation and subsequent gene silencing. We now hypothesize that increased FOS regulates the suppression of P4-regulated genes in the eutopic endometrium of baboons with endometriosis via epigenetic regulation of their DNA.

Methods: Endometriosis was induced in four cycling baboons by inoculation of menstrual endometrium into the peritoneal cavity. Eutopic endometrium was consecutively harvested by laparotomy at day 10 Post Ovulation (window of receptivity) from baboons at 3, 6, 9, 12, and 15 months post-inoculation. Control endometrium was similarly collected from disease-free, control animals. Endometrium was either snap frozen in liquid N2 for RNA and DNA extraction or fixed in buffered formalin for immunohistochemical analysis. Gene transcript levels were determined by quantitative real time PCR (Q-RT-PCR). Proteins were localized by immunohistochemistry (IHC). DNA methylation status was evaluated by bisulfite sequencing.

Results: Minimal levels of DNMT3a and DNMT3b mRNAs were detectable in control and endometriotic baboon eutopic endometria. Increased levels of DNMT1 mRNA were observed in endometria from baboons with disease compared to control, disease-free animals. Correspondingly, IHC analysis confirmed a reduction in the level of DNMT1 protein in endometria from baboon with endometriosis. The mRNA transcripts and protein levels of the P4-regulated genes, HOXA10 and CALC, were decreased in endometria from baboons with endometriosis. Bisulphite sequencing revealed significantly increased levels of methylation in the F1 region within the promoter of the HOXA10 gene.

Conclusion: These data suggest that in an induced model of endometriosis in the baboon, increased levels of FOS may induce up-regulation of DNMT1 and subsequent hypermethylation of HOXA10. We propose that the reduced fecundity associated with endometriosis is mediated, in part, by epigenetic modification of P4-regulated genes, resulting in a P4 resistance within the eutopic endometrium during the window of receptivity.

(U54 HD40093)
Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) Expression in Baboon Endometrium and Endometriotic Lesions, A. Braundmeier¹, M. Nakai¹, A. Fazleabas² and R. Nowak¹, ¹University of Illinois, Urbana-Champaign and ²University of Illinois, Chicago, USA

Extracellular matrix metalloproteinase inducer (EMMPRIN) regulates matrix metalloproteinases (MMPs) and is important for tissue remodeling in the uterus. Endometriosis is the attachment and invasion of uterine endometrial fragments into the peritoneal mesothelium. One consequence of endometriosis is that it leads to alterations in protein expression in the eutopic endometrium which may contribute to the infertility problems associated with this disease. We hypothesized that expression of EMMPRIN might be altered in a baboon model of endometriosis and that this could lead to changes in endometrial remodeling. The first study evaluated the pattern of EMMPRIN gene and protein expression in eutopic endometrium of cycling control animals. EMMPRIN mRNA levels were similar for tissues collected during menses and the proliferative phase but were significantly higher throughout the secretory phase (p<.05). EMMPRIN protein localized primarily to glandular epithelial cells during the proliferative phase whereas during the secretory stage expression was detected in both glandular and luminal epithelial cells and weakly in stromal cells. Menstrual tissues showed little if any immunostaining for EMMPRIN in epithelial cells. Our second objective compared EMMPRIN gene and protein expression in eutopic endometrium of diseased animals with controls during disease progression. At 1 and 3 months (m) of disease EMMPRIN mRNA levels were elevated in eutopic endometrium compared to controls (p<0.05), whereas mRNA levels at 8-10 m of disease were similar to controls. Diseased animals showed increased EMMPRIN protein expression in eutopic endometrium and protein localization was primarily increased in stromal cells compared to control eutopic endometrium. Between 3 and 6 m of disease progression EMMPRIN protein expression increased but then declined at 8-10 m of disease. EMMPRIN protein expression in ectopic endometrium closely resembled that of matched eutopic endometrium. Our data show that EMMPRIN mRNA and protein expression were altered in animals with endometriosis and suggest that the presence of endometriotic lesions alters gene expression in the eutopic endometrium. These data are similar to findings reported in women and validate the use of the baboon as a model for studying the pathogenesis of endometriosis.

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Decidualization of stromal cells into decidual cells represents a complex transformation requiring changes in cytoskeletal architecture. Cytoskeleton organization is determined primarily by the actin-myosin II interaction as regulated by the phosphorylation of MLC. In this study we have demonstrated that differentiation of human uterine fibroblasts (HuF) into decidual cells is accompanied by the following changes in overall internal cell architecture and ECM: downregulation of alpha smooth muscle actin (α-SMA) and β-tubulin, changes in focal adhesion kinase (FAK) phosphorylation and reorganization of vinculin. Moreover, cAMP (with hormones)-induced decidualization is caused by a 40% decrease in phosphorylation of MLC20 that correlates with a 55 % decline in the nonmuscle form of MLC kinase (MLCK). To provide evidence that MLC phosphorylation is essential for decidualization, HuF were infected with an adenovirus construct containing a constitutively active, truncated form of MLCK (A-tMK). Infection of HuF by A-tMK led to a 30% increase in MLC phosphorylation in comparison to HuF transfected with an empty construct (EA). HuF infected with A-tMK and EA were exposed to decidualization stimuli (IL-1β or cAMP with steroid hormones, 6 d) and the markers of decidualization, including insulin-growth factor binding protein-1 (IGFBP-1) and prolactin, were measured. Upregulation of MLC phosphorylation by A-tMK in HuF resulted in a 70% decrease in IGFBP-1 and prolactin in comparison to HuF with EA. On the other hand, destabilization of the cytoskeleton by myosin II or MLCK inhibitors had the opposite effect on decidualization induced by cAMP (with hormones) and IL-1β with hormones. Blebbistatin and ML-7 accelerated decidualization induced by the artificial stimulus (cAMP), but significantly delayed decidualization induced by the embryonic stimulus (IL-1β). Inhibition of the ERK pathway resulted in a similar effect: pretreatment of HuF with PD 98059 (ERK inhibitor) followed by treatment with decidualization stimuli led to a decrease in IGFBP-1 synthesis during decidualization induced by IL-1β (with hormones), but had no effect on the decidualization process induced by cAMP (with hormones). Taken together, despite the difference in mechanisms underlying in cAMP- and IL-1β (both in the presence of hormones)-induced decidualization, the cytoskeleton is crucial in this process.

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Notch1 Signaling in the Endometrium is Regulated by Human Chorionic Gonadotropin and Ovarian Steroids, Yalda Afshar\(^1\), Adina Stanculescu\(^2\), Lucio Miele\(^2\), Jaewook Jeong\(^3\), Franco DeMayo\(^3\), Asgi Fazleabas\(^1\), \(^1\)Department of Obstetrics and Gynecology, University of Illinois College of Medicine, Chicago, IL, \(^2\)Department of Pathology, Cardinal Bernadin Cancer Center, Loyola University Medical Center, Maywood, IL, \(^3\)Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas.

Synchrony and the spatio-temporal interactions between the genotypically different embryo and the uterine host are required for successful implantation. In rodents and primates, inhibition of stromal fibroblast apoptosis and their subsequent differentiation into decidual cells are two processes critical for implantation. We propose that the primate early embryonic signal, human chorionic gonadotropin (hCG) and Notch1 play critical roles in these processes. The Notch1 receptor transduces the extracellular signals responsible for cell fate determination during development and is recognized as a major mediator of survival. Cleavage of full-length Notch1 (NFL) releases an active intracellular (NIC) peptide, which is later translocated to the nucleus. Using both \textit{in vitro} and \textit{in vivo} models, we demonstrate that a combination of hCG (50IU), estrogen (E\(_2\); 36nM) and progesterone (MPA; 1\(\mu\)M) increases both RNA and protein NFL levels. Interestingly, E\(_2\) and MPA alone increase active NIC levels. Since the Notch1 cascade is one of the most important regulators of apoptosis and differentiation during development, we evaluated the role of Notch1 in the hCG induced decidualization response. Induction of either differentiation (E\(_2\), MPA, dbcAMP, 0.1mM) or apoptosis (cytochalasin D; 10 \(\mu\)M) results in a significant decrease of NIC levels. Significantly, treatment with hCG and E\(_2\) and MPA partially rescues this decrease in Notch1 following cytoskeletal disruption, which is also associated with the decrease in apoptosis. Additionally, we have utilized a transgenic Notch1 uterine specific knockout, using a Cre-Lox mouse model, to create a tissue-specific conditional deletion, overcoming the problem of embryonic lethality. When compared to control (/+Notch\(^{fr}\)) mice, \textit{PR}\textsuperscript{cre/+Notch}\(^{fr}\) mice demonstrate a significant decidualization defect, as seen by both gross morphological and histological changes, including reduced stroma and polyploidy, hallmarks of rodent decidualization. These data suggest a critical role of Notch1 during the window of uterine receptivity in the maternal endometrium. A direct correlation was observed between Notch1 and hCG protein levels during early and late pregnancy, suggesting that Notch1 might regulate decidualization by preventing apoptosis. Furthermore, we propose that Notch1 could be used as an early infertility marker in women.

\textit{(Supported by HD 42280)}
Signaling by Chorionic Gonadotropin (CG) through the CG Receptor (CGR) in Primate Endometrial Epithelial Cells is through a Gi – independent MAPK Pathway, Prajna Banerjee¹, Paula Cameo¹, Santha Srisuparp², Zuzana Strakova¹, Asgerally T Fazleabas¹, ¹Dept. of Ob/Gyn, University of Illinois, Chicago, IL, USA; ²Rajavithi Hospital, Bangkok, Thailand.

Introduction: Implantation involves a spatio-temporal and synchronous dialog between the embryo and the uterus. In the baboon infusion of CG, the primary embryonic signal, induces biochemical and morphological changes in the uterine endometrium. CG activates the MAPK pathway, phosphorylating extracellular regulatory kinase, ERK 1/2, in a cAMP/PKA independent manner. The objective of this study was to elucidate the signal transducers activated by CG downstream of the CGR and upstream of ERK 1/2. Additionally, to provide an experimental model to study the mechanism of CG signaling, we designed a system to silence the CGR using small interference RNAs (siRNAs).

Methods and Results: To determine the role of the Gi subunit of the CGR and verify transactivation of the epidermal growth factor receptor (EGFR) by CG, human endometrial epithelial cells (HES) were pre-treated with inhibitors against Gi, Pertussis Toxin (PTX), and EGFR, AG1478, before stimulation with 10IU/ml hCG. Protein extracts were subjected to Western Blot analysis with specific antibodies against ERK 1/2. But inhibition of Gi and EGFR by PTX and AG1478 respectively failed to decrease CG-induced ERK 1/2 phosphorylation, indicating that induction of ERK 1/2 by CG is independent of both Gi and EGFR. Furthermore, HEK293T cells were transiently co-transfected with a CGR-YFP (yellow fluorescent protein) construct together with siRNAs directed against specific regions of the CGR. The expression of the CGR was analyzed by fluorescent microscopy and flow cytometry. A phenomenal decrease in the expression of the receptor was seen in cells co-transfected with the siRNA sequences and the CGR-YFP construct in comparison to those transfected with the CGR-YFP construct alone.

Discussion: Unlike the classical cAMP-dependent G-protein signal transduction in gonadal cells, we have demonstrated a unique CG-induced MAPK pathway in HES cells that is mediated neither by the activation of the Gi subunit of the CG receptor nor through the transactivation of the EGFR. We propose that CG modulates the uterine endometrium in preparation for the implanting embryo via a Gi and EGFR independent MAPK pathway. Additionally, the development of the CGR-YFP construct presents a mechanism to express the CGR in any cell type and analyze its expression using the yellow fluorescent protein. Finally, silencing of the CGR using siRNAs provides a valuable tool to study the direct effects of CG on its receptor and its subsequent downstream signaling.
Estrogen Differentially Regulates the Expression of Cyclin-Dependent Kinase 1 (Cdk1) in Primary and Secondary Decidual Compartments During Implantation, Athilakshmi Kannan1, Quanxi Li1, Indrani C. Bagchi1 and Milan K Bagchi2, 1Department of Veterinary Biosciences, College of Veterinary Medicine, and 2Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign.

Implantation is regulated by a timely interplay of the steroid hormones, estrogen (E) and progesterone (P). A transient surge of E immediately preceding implantation is believed to trigger the expression of a unique set of genes that critically control embryo attachment and subsequent decidualization. To identify these E-induced genes, we utilized a delayed implantation mouse model in which embryo attachment to P-primed pregnant uterus is dependent upon the administration of E. We performed gene expression profiling using oligonucleotide micro arrays to identify several genes that are up regulated in the delayed uteri in response to E. One of these genes encoded the cyclin-dependent kinase 1 (Cdk1), which is known to regulate the G2-M phase of the cell cycle. Analysis by quantitative PCR of total RNA obtained from uteri of delayed mice treated with or without E indicated a significant increase in the level of Cdk1 mRNA within 1 h of E administration, confirming the E regulation of this gene. Immunohistochemical localization of Cdk1 protein during delayed implantation revealed its enhanced expression in the sub-epithelial stromal cells in response to E. Interestingly, the stromal cells in the immediate vicinity of the implanting embryo were devoid of Cdk1 expression. We further explored the expression of Cdk1 in the stromal compartment during normal pregnancy. While a robust expression of Cdk1 was localized in the stromal cells of the secondary decidual zone (SDZ) on days 5 to 7 of gestation, its expression was undetectable in the stromal cells of the primary decidual zone (PDZ). This pattern of Cdk1 expression precisely overlapped with that of ERα, consistent with our hypothesis that Cdk1 is a downstream target of regulation by E in the stromal cells at the time of decidualization. Localization of PCNA, a marker of cell proliferation, showed that the stromal cells of both PDZ and SDZ entered the cell cycle and proceeded to the S phase. However, immunohistochemical analysis of the mitosis marker phospho-histone 3 indicated the presence of mitotic activity only in the stromal cells of the SDZ but not in the PDZ. The expression of cyclin B2, the activating cyclin partner of Cdk1, was observed in both PDZ and SDZ. Collectively, these results provided novel insights into the mechanisms by which E exerts differential proliferating effects in the PDZ and SDZ during decidualization. While it promotes cell cycle entry and mitosis of the stromal cells in the SDZ via induction of Cdk1, it fails to stimulate the proliferation of the stromal cells of PDZ, which remain blocked at the G2-M phase, presumably due to a lack of ERα and Cdk1 expression. It is conceivable that these cell-type specific proliferative effects of E contribute to differential architecture, function and cell-fate of the PDZ and SDZ during early gestation.

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Regulation of 17β-hydroxysteroid Dehydrogenase Type 2 Expression in Human Placental Endothelial Cells. Su EJ, Cheng YH, Chatterton RC, Lin ZH, Yin P, Reierstad S, Innes J, Bulun SE. Department of Obstetrics and Gynecology, Department of Physiology, Northwestern University, Chicago, IL.

Background: During pregnancy, the placenta secretes progressively higher levels of progesterone and estrogens. 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) oxidizes estradiol to estrone, testosterone to androstenedione, and 20α-dihydroprogesterone to progesterone. It is highly expressed in human placental tissue where it is localized to placental endothelial cells lining the fetal compartment, suggesting that its presence protects the fetus from large levels of circulating maternal bioactive steroid hormones. The aim of this study was to investigate the effects of potential regulatory factors including progesterone, estradiol, and retinoic acid (RA) on 17β-HSD2 expression in primary placental endothelial cells in culture.

Methods: Term placentas were obtained immediately after delivery, and the deciduas and loose chorion and amnion were removed. An IV cannula was inserted into the umbilical vein, which was perfused with a collagenase/dispase solution. The perfusate was collected and subjected to Percoll density gradient centrifugation. Endothelial cells were found at a density of 1.05 g/mL and thereafter cultured in complete medium. After the initial passage of these cells, purity was confirmed by immunofluorescence studies using an endothelial-specific monoclonal antibody against CD146. Cells subsequently underwent treatment with either vehicle, progesterone (10^{-7} M), the progesterone agonist R5020 (10^{-7} M), estradiol (10^{-7} M), 9-cis RA (10^{-8} to 10^{-6} M), or all-trans RA (10^{-8} to 10^{-6} M). Quantitative real-time PCR, western blotting, RNA interference, and enzyme activity assays were performed thereafter.

Results: Placental endothelial cell purity was confirmed by immunofluorescence studies. Using real-time PCR, there was no regulation of 17β-HSD2 mRNA levels with progesterone, the progesterone agonist R5020, or estradiol treatment. Rather, RA significantly induced 17β-HSD2 mRNA levels and enzyme activity in a dose- and time-dependent manner. Maximal stimulation occurred at 48-hours with a RA concentration of 10^{-6} M (p<0.0005). Western blotting demonstrated readily detectable levels of retinoic acid receptor α (RARα) and retinoid X receptor α (RXRα). RNA interference experiments against either RARα or RXRα led to significantly reduced basal levels of 17β-HSD2 mRNA levels and significantly abolished RA-induced 17β-HSD2 mRNA levels (p<0.0005).

Conclusion: This is the first demonstration of a mechanism responsible for 17β-HSD2 regulation in the placenta. Our findings indicate that induction of 17β-HSD2 mRNA levels and enzymatic activity by RA is mediated by RARα and RXRα.

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Localization of RNA Binding Protein - Pumilo in Mouse Testis and Spermatozoa, Chirag A. Shah, Terrance Lee, Villian Naeem and Eugene Xu, Division of Reproductive Biology, OBGYN and Centre for Genetic Medicine, Northwestern University Feinberg School of Medicine, Lurie 7-250, 303 E Superior Street, Chicago, IL-60611 USA

Post transcriptional regulations through RNA binding protein has been shown to play important roles in development and differentiation. The present study was undertaken to define the spatial and temporal expression of highly conserved RNA binding protein, Pumilo in mouse testis and spermatozoa. *Pumilo* gene isoforms were expressed in the cytoplasm of various spermatogenic cells, including secondary spermatocytes and immature testicular spermatids. The pattern of expression was heterogeneous and in most of the secondary spermatocytes the staining is in a typical arch shape at the perinuclear area within cell. In accordance, expression of *Pumilo* was found to be stage specific in mouse testis with typical increase in pattern of expression from stage II-III where detected as a dot like appearance, with increase in its shape of arch at stage VII-IX and having complete expression of *Pumilo* on the acrosomal surface of testicular spermatids at stage X. Pumilo protein was also localized on the acrosomal region of the caudal spermatozoa using the same set of antibodies. These results together suggest that RNA binding proteins like Pumilo may play important roles in sperm maturation, fertilization or/and early embryonic development.

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Characterization of Mammalian Boule Gene Isoforms and Developmental Expression Profiles, N.L. Angeloni, M. Bernhardt, E.Y. Xu, Division of Reproductive Biology, Department of Obstetrics and Gynecology, and Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

The Boule gene is the founding member of human reproductive gene family—DAZ (Deleted in AZoospermia) family. It has been demonstrated to function as an important meiotic regulator in Drosophila spermatogenesis, and is highly conserved from fly to mice to humans. Our study used a combination of bioinformatics and molecular biology to search for different isoforms of this key meiotic regulator in mice and humans, and to determine developmental expression profile of mammalian Boule. After analyzing all the known EST, we have identified two additional exons suggesting that the mouse gene is larger than previously thought. RT-PCR analysis of mouse testis cDNA has confirmed the existence of two splicing variants, containing one or both of the additional exons. These extra exons may have important functions as 3’ untranslated regions. The expression pattern of Boule in male and female gonadal tissues from different developmental stages was also examined and we show that the Boule gene is not only expressed in male germ cells but also in female germ cells, raising the possibility that Boule is a meiotic regulator for both male and female meiosis. We are also comparing human and mouse Boule regulatory regions to identify potential conserved regulatory elements.
A Role for Altered Gli1 Signaling in Male Infertility in Quaking Viable Mutant Mice, O.V. Lakiza, M.L.G. Lamm, D.O. Walterhouse and P.M. Iannaccone, Children’s Memorial Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL

Quaking viable (qkv) mice are homozygous for a spontaneous deletion of about 1 Mb from the proximal end of chromosome 17 and show a phenotype that is characterized by tremors and infertility in males, the latter due to oligospermia, postmeiotic spermatogenetic arrest, and a flagellar defect. The deletion has been shown to affect the regulation of 3 nearby genes: Quaking, Parkin and Parkin-coregulated gene (Pacrg). Transgenic expression of Pacrg in the testis has been shown to partially restore spermatogenesis and male fertility in qkv mice. We previously demonstrated that Quaking down-regulates Gli1 translation and that up-regulation of Gli1 in male germ cells blocks spermatogenesis, raising the possibility that altered Quaking expression contributes to infertility in qkv males through altered expression/regulation of Gli1. Using quantitative RT-PCR we find, as expected, that Gli1 mRNA expression does not differ in the qkv homozygous mutant testis compared with wild type. However, expression of Patched, a Gli1 target gene, is altered, suggesting alterations in the function of the Gli1 protein. It has also been suggested, that Leydig cells represent important targets of Hedgehog-Gli1 signaling in the testis. Indeed, histological sections of the qkv mutant testis show mild Leydig cells hyperplasia and tight apposition of Leydig cell clusters to the basal lamina of the seminiferous tubules. Our findings suggest that alternations in Gli1 signaling may contribute to qkv male infertility.

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Proliferation of Adult Sertoli Cells in a Conditional Knockout Mouse Lacking the Gap Junctional Protein Connexin 43, Santhi Sridharan, David E. Gutstein, Glenn I. Fishman and Paul S. Cooke. 1Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; 2Division of Cardiology, New York University School of Medicine, New York, NY

Sertoli cells, the somatic cells of the seminiferous epithelium, are obligatory for testis development and spermatogenesis. Each Sertoli cell supports a fixed number of germ cells, so Sertoli cell number determines ultimate testis weight and sperm production. Sertoli cell proliferation is regulated by the cyclin-dependent kinase inhibitors p27 and p21. Cellular p27 and potentially p21 concentrations are regulated by the ubiquitin/proteosome system, rather than by transcriptional regulation. Skp2, the F-box protein that recognizes and targets p27 and p21 for ubiquitylation and degradation, is a major regulator of p27 and p21 concentrations. We have shown that Skp2, which is high neonatally then decreases as Sertoli cells mature, plays a critical inhibitory role in Sertoli cell proliferation and establishment of adult Sertoli cell population. A critical question that remains is what regulates Skp2 during Sertoli cell development. Gap junctional communication between adjacent Sertoli cells and Sertoli and germ cells is indispensable for spermatogenesis and connexin 43 (Cx43) is the predominant testicular gap junction protein. Furthermore, some data suggest Cx43 may decrease Skp2 and increase p27, and could therefore inhibit proliferation. Based on this, we hypothesized that lack of Cx43 would result in inhibited differentiation and an extension of the proliferative period in Sertoli cells. Cx43 knockout mice would be useful to test this hypothesis, but the Cx43 global knockout is embryonic lethal. Therefore, a Sertoli cell-specific Cx43 knockout was generated by using the Cre-lox system, which involved breeding mice expressing Cre driven by a Mullerian inhibiting substance promoter (MIS-Cre) with mice containing two floxed Cx43 alleles to study the role of Cx43 in Sertoli cell differentiation and proliferation. The resultant Cx43 conditional knockout (Cx43 KO) male mice lacked Cx43 only in Sertoli cells, but had normal Cx43 expression in other tissues. We compared testicular weight and histology in wild-type (WT) and Cx43 KO mice during development and also analyzed serial sections for immunohistochemical expression of Ki67 (marker for cell proliferation) and WT-1 (Wilm’s tumor-1, Sertoli cell specific marker). Body weights in WT and Cx43 KO mice were comparable. Testicular weight was reduced 28% and 74% in Cx43 KO mice at 20 and 60 days, respectively, compared to WT mice. Histologically, seminiferous tubules of Cx43 KO mice contained only Sertoli cells and early stages of spermatogonia; the spermatogonia were actively proliferating. Thus, Sertoli cell Cx43 is not essential for maintenance or proliferation of spermatogonia, but is necessary for spermatogenesis. Sertoli cells normally cease proliferation at two weeks of age in mice and become terminally differentiated. There were proliferating Sertoli cells in Cx43 KO, identified by co-expression of Ki67 and WT-1, at both 20 and 60 days of age (1.57% ± 0.59% and 1.31% ± 0.23%, respectively) in Cx43 KO mice. In contrast, proliferating Sertoli cells were never observed in 20- or 60-day WT mice (n=3 for all groups and p<0.05 for WT vs. Cx43 KO). In conclusion, we have successfully generated a Sertoli cell-specific Cx43 knockout and shown that Cx43 in Sertoli cells is essential for spermatogenesis but not for maintenance and proliferation of spermatogonia. Critically, Cx43 KO mice showed continued Sertoli cell proliferation long after Sertoli cell proliferation had ceased in WT mice, indicating that Cx43 plays a key inhibitory role in controlling Sertoli cell proliferation and is essential for normal maturation of Sertoli cells in mice.

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Apoptosis is Absent in Female Gonads of Fused Toes Mutant Mice during Development, B. Kim, Y. Kim, J.S. Jorgensen, Department of Veterinary Biosciences, University of Illinois, Urbana, IL

The goal of the present study is to investigate the functional significance of Irx3 in developing gonads. Irx3, a member of the Iroquois homeobox family, has been shown to be critical for axis and pattern formation during the development of several organisms. Affymetrix GeneChip and quantitative real-time PCR analysis have demonstrated an 8-fold increase in Irx3 expression that is specific to somatic cells of the female gonad during sex determination. Based on these findings, we hypothesize that Irx3 will have important functions for female gonad development. Our experiment utilizes a mutant mouse model called Fused toes (Ft) which lacks Irx3 as well as 5 other genes, including Irx5 and 6, Fto, Fts, and Ftm. Homozygote mutants die in development (E10-E12) because of severe malformation of the developing brain and loss of left-right asymmetry. All of these genes are present in developing gonads of both sexes except Irx3, which is female specific. Fused toes embryos were dissected at embryonic day 11.5 and gonads were cultured in vitro for 2-3 days. Embryonic sex and genotype were distinguished by PCR on genomic DNA isolated from embryo tails (wild type Ft/Ft, heterozygote Ft/-, and Ft null -/-). The cultured gonads were assessed for morphology by histological sections and for apoptosis or proliferation by Tunel, BrdU, and PCNA staining. In female gonads, gonad morphology and cell proliferation (BrdU and PCNA) were similar among gonads of all genotypes. However, in female gonads from Ft -/- embryos, we observed a significant decrease in cells positive for Tunel staining compared to wild type or heterozygote samples. This result suggests that the absence of these 6 genes induces abnormal gonad development in the female, and we are currently pursuing additional studies to address the impact of the Ft mutant on gonad development.

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Subtilisin-like Proprotein Convertase Activity is Necessary for Ovarian Inhibin Secretion, Monica Antenos¹, Anjali Malipatil¹ and Teresa K. Woodruff¹,²,³, ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, IL, ²Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, ³Robert H Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL.

Inhibin is an essential heterodimeric glycoprotein belonging to the transforming growth factor beta (TGFβ) superfamily that consists of both an alpha and beta subunit. The primary function of inhibin is to suppress follicle stimulating hormone (FSH) production by the pituitary gonadotrope. The production of inhibin is regulated during the reproductive cycle and requires the processing of the pro-ligand to mature hormone. The enzymes required for inhibin processing are currently unknown. Furin belongs to the subtilisin-like proprotein convertase family and activates precursor proteins by cleavage at basic sites during their transit through the secretory pathway and/or at the cell surface. These precursors include prohormones, proreceptors, growth factors, adhesion molecules, and viral glycoproteins. We hypothesized that furin-like proprotein convertases are central regulators of the inhibin alpha and beta subunits within the ovary. We have analyzed the expression of PC1, PC2, PACE4, furin, PC4, PC5, and PC7 in the rat ovary at various stages of the estrous cycle by both in situ hybridization and immunohistochemical techniques, as well as in rat tissues and various cell lines by quantitative reverse transcription-PCR. The data show that most proconvertase enzymes are expressed in ovarian granulosa cells, where furin and PC5 are the most abundant enzymes present. We also demonstrate that inhibiting proconvertase enzyme activity by chloromethylketone (CMK), a highly specific and potent competitive inhibitor of subtilisin-like proprotein convertases, significantly impedes both inhibin alpha and beta subunit maturation and secretion in granulosa cells. We have demonstrated that several subtilisin-like proprotein convertase enzymes are expressed within the rat ovary and that these enzymes play a critical role in inhibin processing. Our data support the role of proprotein convertases-mediated processing of inhibin in mammalian folliculogenesis and that these enzymes serve as key components of the reproductive process.

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Activin B Regulates FSHβ Promoter through Smad1 and Smad3 in LβT2 Cells, Niti M. Jetly¹, Magdalena I. Suszko¹, Monica Antenos¹, Teresa K. Woodruff¹,²,³ ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, USA, ²Department of Medicine, Northwestern University Medical School, Chicago, IL 60611 USA, ³ Robert H. Lurie Comprehensive Cancer center of Northwestern University, Chicago, IL 60611, USA

Activins are members of the transforming growth factor beta (TGFβ) superfamily of proteins that play a fundamental role in cell differentiation and development. Like other TGFβ ligands, activins are biologically active as dimers. Assembly of the various subunits into homodimers or heterodimers results in a nomenclature reflecting the subunit components such as activin A (βA-βA), activin B (βB-βB) and activin AB (βA-βB). Although Activin A and Activin B have 63% homology at the amino acid level and many of the functions of activin A are complemented by insertion of the β-B-gene into the β-A-locus of activin A knockout mice (Brown et al., 2000). Interestingly, these studies suggested that activin B knockin mice had a non-overlapping set of phenotypes, suggesting differential signal transduction pathways engaged by the two ligands. Activin A signaling is mediated through a combination of activin type II receptors and type IB receptor, ALK4. However, the precise signaling pathway for activin B needs to be determined. In these studies, we tested the hypothesis that activin B signals through additional Smad-regulated pathways in the pituitary gonadotrope. Activin A and activin B activated stably transfected FSHβ-luc promoter in mouse pituitary gonadotrophe cell line (LβT2 –338). Downregulation of ALK4 and ALK7 by siRNA resulted in significant reduction of FSHβ-luc promoter activation induced by activin A and B as determined by luciferase assay, suggesting that both these ligands signal through ALK4 and ALK7. Interestingly, it was also observed that activin B recruited both the Smad1 and Smad3 signaling pathway unlike activin A which induced only the Smad3 signaling pathway. Decreased expression of ALK4 mRNA level by siRNA resulted in diminished phosphorylation of Smad3 by both ligands. Activin B mediated phosphorylation of Smad1, however, was not affected by ALK4-specific siRNA. This suggested that phosphorylation of Smad1 by activin B is not dependent on ALK4 but both activin A and activin B mediated ALK4 dependent phosphorylation of Smad3. Our data suggest that the differences between these two ligands appear at the level of type I receptor activation and also at the level of intracellular propagation of the signal via the Smad pathway.

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Interactions Between Activin and Estrogen Signaling in the Mouse Ovary, Jingjing Liu Kipp¹,³, Signe Kilen¹,²,³, and Kelly Mayo¹,²,³, ¹Department of Biochemistry, Molecular Biology and Cell Biology; ²Department of Neurobiology and Physiology; ³Center for Reproductive Science. Northwestern University, Evanston, IL 60208

Activin and estrogen are important modulators of the female reproductive system. Multiple intra-ovarian roles for activin and estrogen in ovarian follicle formation and development have been described. Our laboratory has generated a transgenic mouse model that overexpresses inhibin α-subunit, which as a consequence has decreased activin expression. Studies on these transgenic mice have revealed a decreased response in the ovary to estrogen treatment, indicating that decreased activin levels may negatively impact estrogen signaling. We have also previously demonstrated that neonatal estrogen exposure suppresses activin expression in the mouse ovary, suggesting a regulation of activin expression by estrogen. Therefore, the purpose of this study was to examine the interactions between activin and estrogen signaling. We showed that expression of estrogen receptor-α and -β (ERα and ERβ) mRNA and protein was decreased in the ovaries from inhibin α-subunit overexpressing transgenic mice. This suggested that activin might be important for normal expression of estrogen receptors in the ovary. To test this, primary cultured granulosa cells were treated with activin A. Our results showed that activin A treatment significantly increased the mRNA levels of ERα and ERβ, and this induction of ER expression by activin was specific, since when activin was given together with excess follistatin this effect was abolished. Follistatin alone decreased the mRNA levels of ERβ, while inhibin A had no effect on the expression of either ERα or ERβ. In addition, activin A was able to increase estradiol-induced estrogen response element (ERE) promoter activity in transfected granulosa cells. Our study also revealed that estrogen could directly regulate activin subunit gene expression, as in GRMO2 cells E2 treatment decreased activin βA and βB subunit promoter activities and this effect was able to be neutralized by the anti-estrogen ICI182, 780. Overall the data suggest that activin is important for maintaining ERα and ERβ expression in the mouse ovary and estrogen can in turn negatively regulate activin gene expression.

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Activin and Estrogen Crosstalk Regulates Transcription and Cell Cycle Control in Human Breast Cancer Cells, Joanna E. Burdette¹ and Teresa K. Woodruff¹²³, ¹Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208; ²Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, Illinois 60611; ³Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University Medical School, Chicago, Illinois 60611.

The transforming growth factor beta (TGFβ) superfamily of growth factors is responsible for a variety of physiological actions. Activin is a member of the TGFβ superfamily that functions by interacting with its type I and type II receptors to induce phosphorylation of the intracellular signaling molecules Smad2 and Smad3. Activin slows the growth of breast cancer cells by inducing G0/G1 cell cycle arrest. Estrogen is a steroid hormone that stimulates the proliferation of mammary epithelial cells in development and oncogenesis. Both activin and estrogen act as transcription factors where the intracellular signaling molecules in each pathway interact. The crosstalk between estrogen and activin that regulates activin ligand expression, activin and estrogen signal transduction, and cell cycle arrest was investigated in this study. Estrogen inhibited activin B mRNA and protein. Estrogen combined with activin inhibited activin dependent transcription of the plasminogen activator inhibitor (PAI-1) promoter linked to luciferase. Inhibition from estrogen could be augmented by overexpression of estrogen receptor alpha (ERα). Estrogen receptor repression could be recapitulated in ERα negative cells when the receptor was overexpressed by transient transfection. Activin combined with estradiol inhibited estrogen dependent transcription of the estrogen response element promoter linked to luciferase. Overexpression of Smad3, but not Smad2, alone or in combination with activin further inhibited estrogen dependent transcription as did transfection with a constitutively active type I receptor. Previously, ERα negative cells were considered unresponsive to activin induced growth arrest. In order to directly test the requirement of ERα for activin induced growth inhibition, siRNA directed against ERα was transfected into T47D cells and treated with activin for 48 hours. Subsequent flow cytometry revealed that activin mediated cell cycle arrest was not dependent on estrogen receptor expression. Therefore, a loss of activin signaling from sustained estrogen production may contribute to the release of breast cells from controlled proliferation by directly down-regulating activin ligand production and activin transcriptional activity.

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Cyst Formation and Morphology from Superovulated Smad2 Dominant Negative Transgenic and Control Mice, R. M. Oliver\textsuperscript{1,2}, J. E. Burdette\textsuperscript{1}, T. K. Woodruff\textsuperscript{1}, \textsuperscript{1}Department of Neurobiology and Physiology, Northwestern University, Evanston, IL; \textsuperscript{2}Department of Biochemistry, Beloit College, Beloit, WI.

Ovarian cancer is the fourth most common cause of death from all cancers in women and the leading cause of death due to gynecologic malignancies. The OSE (ovarian surface epithelium) commonly gives rise to malignancies due to monthly ovulatory ruptures and exposure to ovarian hormones and growth factors responsible for inducing ovulation. Induced repetitive superovulation was applied to a transgenic mouse model deficient in Smad2 phosphorylation and its control mouse counterpart in order to analyze the relationship between ovulation and ovarian epithelial cyst formation. Though superovulation did not dramatically increase the incidence of cyst, ovarian surface invagination, or OSE proliferation rate, transgenic and superovulated mice did tend to differ from un-stimulated normal littermates. Superovulation affected cyst location and cyst size, whereas the transgenic animals tended to have larger cysts and chronic ovulation resulted in more cysts that measured less than 500 microns. Smad phosphorylation of ovarian cysts was also more abundant in transgenic animals indicating that growth factors, which stimulate Smad signaling, are activated in these cysts. Therefore, ovarian cyst formation in CD1 and Smad2 phosphorylation deficient animals is not significantly increased from chronic superovulation after six months, but ovulation does alter the type of cysts formed and their signaling properties.

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Expression of Selenium Binding Protein 1 in Ovarian Cancer in Women and Hens, K. Stammer¹, S. Edessary¹, A. Barua¹, S. Sharma¹, J. M. Bahr³, D. B. Hales⁴, J. L. Luborsky¹,², ¹Department of Pharmacology, ²Department of Obstetrics and Gynecology, Rush University Medical Center, Chicago, IL, ³Department of Animal Sciences, University of Illinois, Urbana-Champaign, IL, ⁴Department of Physiology and Biophysics, University of Illinois at Chicago

**Background:** Due largely in part to the lack of early detection methods, ovarian cancer (OVCA) is responsible for 15,000 cancer-related deaths annually, making it the foremost lethal gynecological cancer in the United States. A primary obstacle to the detection of early stage OVCA remains knowledge deficits in the areas of both diagnostic markers and disease etiology. Identification of specific protein markers is a means to observe the continuum of OVCA pathology, and markers have potential as predictors of treatment success and survival. Selenium binding protein 1 (SELENBP1) is one of the most down-regulated proteins in ovarian tumors and its down-regulation is associated with poor prognosis (Huang, et al.). Furthermore, androgen decreased and selenium increased SELENBP1 in normal cells but had the reverse effect in cancer cells, suggesting SELENBP1 regulation is altered. In addition, dietary selenium has anti-cancer effects.

**Objective:** The objective was to determine the presence of SELENBP1 in hen ovarian cancer in order to study the role of SELENBP1 in the etiology of OVCA. The laying hen is the sole animal model to exhibit spontaneous OVCA and provides the potential to examine very early events in tumor development.

**Methods:** A panel of normal and tumor tissues (ovary, oviduct, brain, spleen, muscle, heart, kidney, liver) was collected from egg laying hens age 2-3 years. Control tissues were obtained from women (pancreas, liver, muscle, ovary) and hens (muscle, kidney, heart, liver, spleen, brain, oviduct, ovary) with no history of cancer diagnosis. Tissue samples were homogenized by a standard method and the expression of SELENBP1 was assessed by Western Blot Analysis. In addition, RNA was prepared by a standard isolation using trizol reagent, cDNAse treatment, first strand synthesis and RTPCR, and SELENBP1 was visualized using agarose gel electrophoresis and ethidium bromide staining.

**Results:** SELENBP1 mRNA expression was observed in 100% normal human tissues tested and expression was reduced in 60% of human ovarian tumor tissues, similar to previous reports. In hens, SELENBP1 expression was observed in 100% of normal tissues as expected and was absent in 50% hen tumor tissues. Western blot analysis is consistent with these findings.

**Conclusion:** Our study confirms the expression of SELENBP1 in normal and tumor tissues in the hen. In addition, SELENBP1 showed a decrease in hen ovarian tumors, similar to that seen in humans. Thus it will be possible to examine SELENBP1 function in hen ovarian cancer to determine (1) if a switch in response to androgens occurs in hen tumors and (2) the role of SELENBP1 in tumor development.

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Mechanism of Regulation of PRAP/17βHydroxysteroid Dehydrogenase Type 7 Gene by Estradiol, Aurora K Shehu \(^1\), Mike Risk \(^2\), Jifang Mao\(^1\), Constance Albarracin\(^3\) and Geula Gibori\(^1\), \(^1\)Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL, 60612; \(^2\)Department of Urology, University of Washington, Seattle, Washington, 98195 and \(^3\)Department of Pathology, University of Texas, M.D. Anderson Cancer Center, Houston, TX, 77030.

Our laboratory has previously cloned and purified a novel ovarian 17βhydroxysteroid dehydrogenase enzyme, (PRAP/17βHSD7), that converts estrone to estradiol. In the present investigation, we have found this enzyme to be highly expressed in ductal carcinoma of human breast cancer and in breast cancer cell lines. We have found that while this enzyme acts to convert estrone to estradiol, its expression is strongly upregulated by estradiol suggesting a local positive feedback mechanism. Estradiol increases both PRAP/17βHSD7 mRNA and protein levels in the estrogen-responsive human breast carcinoma cell line, MCF-7. To examine whether this regulation is at the level of transcription, we isolated PRAP/17βHSD7 promoter and examined its activity. We found estradiol to markedly stimulate the transcriptional activity of PRAP/17βHSD7 promoter through ER\(\alpha\) alone. This steroid had no stimulatory effect in cells expressing only the ER\(\beta\) receptor. Either trans-hydroxytamoxifen or ICI 182,780(ICI) antagonized estradiol-induced promoter activity. Truncations of the PRAP/17βHSD7 promoter identified the estrogen responsive element to a region highly conserved amongst rat, mouse, and human and proximal to the transcriptional starting site. In summary, we have demonstrated, for the first time, a new type of 17βHSD enzyme expressed in breast cancer able to convert the weak form of estradiol to its more potent form. Once expressed, estradiol stimulates robustly PRAP/17βHSD7 at the level of transcription through the binding of ER\(\alpha\) receptor to the proximal region of PRAP/17βHSD7 promoter. The ability of estradiol to enhance the transcriptional activity of PRAP/17βHSD7 implicates this enzyme as an important factor that exacerbates the growth of the estrogen-dependent breast cancer. This could be an appealing target for the development of future therapeutic strategies against human breast cancer.

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Application of Ultrasonography to Assess Ovarian Tumors in Laying Hens, A. Barua¹, M. J. Braderic¹, S. L. Edassary¹, A. Dirk³, J. Abramowicz², J.M. Bahr³ and J. L. Luborsky¹, ², ¹Department of Pharmacology, ²Department of Obstetrics and Gynecology, Rush University Medical Center, Chicago, IL ³Department of Animal Sciences, University of Illinois, Urbana-Champaign, IL

Background: Ultrasonography has demonstrated usefulness in the detection of ovarian abnormalities including ovarian cancer (OVCA) in humans. The difficulty of analyzing the molecular and biological changes during the early stage of OVCA has impeded our understanding of ovarian cancer etiology and the development of early detection tests. Animal models have historically facilitated understanding of disease etiology. Laying hens are the only animal known to spontaneously develop ovarian cancer. The morphology, histology and protein expression of ovarian cancer are similar in hens and humans. The aim of this study was to determine the utility of ultrasonography in the non–invasive detection of ovarian abnormalities including ovarian tumors in laying hen, in order to evaluate the early events leading to OVCA.

Methods: Laying hens were reared under a light regimen of 14h light:10h dark with food and water provided ad libitum. Ultrasonography of their ovaries was performed using a commercially available instrument equipped with an endovaginal transducer (Z1, Zonare Medical Systems, CA). Transmission gel was applied to the surface of the transducer, a probe cover applied and endovaginal Bmode, as well as color and pulsed Doppler sonography were performed. The region surrounding the ovary was scanned and once a follicle had been located, the transducer was swept through the entire area for complete scanning of the ovary. Hens with a hierarchy of 3-5 developing preovulatory ovarian follicles were considered as hens with normal ovarian status. Blood flow indices (resistive index [RI] and pulsatility index [PI]) in blood vessels with the highest flow velocity were measured. Hens with lower RI or PI values than normal hens were considered to have abnormal ovarian status. Hens were euthanized following ultrasound examinations and gross ovarian status was compared to those of ultrasound results.

Results: During ultrasound analysis, it was possible to identify hens with multiple follicular hierarchy or hens without follicles or other ovarian abnormalities including solid masses and ascites. The RI and PI values obtained from hens with normal ovarian status (n=7) was 0.52 ± 0.09 (range: 0.41-0.68) and 0.72 ± 0.16 (range: 0.55-0.96), respectively. Under gross examinations following necropsy, hens with malignant masses and ascites (n=2) were associated with the lowest RI (0.33 ± 0.08, range: 0.27-0.38) or PI values (0.36 ± 0.02, range: 0.34-0.37). The RI (0.45 ± 0.14, range: 0.28-0.59) or PI (0.62 ± 0.19, range: 0.42-0.80) values of hens with non-tumor ovarian pathology (n=4) and no preovulatory follicles were in between nor mal or tumor hens. The RI and PI values in all groups of hen were significantly correlated (r = 0.98, p<0.01).

Conclusion: Transvaginal ultrasonography can be used to determine ovarian status including tumor progression in hens. This non–invasive tool offers the ability to make repeated examinations on the same hen to monitor and understand early changes in the ovarian morphology associated with ovarian tumorigenesis. Low resistance to blood flow as measured by the resistive or pulsatility indices used in color Doppler sonography may be positively correlated with the malignant ovarian tumors.

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Molecular Analysis of Spontaneous Ovarian Cancer in the Laying Hen *Gallus Domesticus*, Yan Zhuge¹, Jo Ann Jaen Lagman¹, Cassandra Mahon¹, Animesh Barua², Judy Luborsky², Janice Bahr³, Dale Buchanan Hales¹, ¹Department of Physiology and Biophysics, University of Illinois-Chicago, Chicago, IL, ²Rush University Medical School, Chicago, IL, ³University of Illinois at Urbana-Champaign, Urbana-Champaign, IL

Ovarian carcinoma is the leading cause of gynecologic malignancy and cancer death. There is a lack of experimental animal models for the study of this cancer, with the exception of the aging hen. The purpose of this study was to characterize the molecular and histopathological phenotype of ovarian carcinoma in the aging White Leghorn hen (*Gallus domesticus*) in order to identify factors important to the etiology and early detection of the disease. Age vs. disease dependent expression of cytochrome P4501B1 (CYP1B1), cyclooxygenase(COX) 1 & 2, markers of inflammation, oxidative stress and proliferation were examined in disease free hens aged 9 months to over 3 years, and in hens with cancer. Upon necropsy and gross examination, the presence of metastatic carcinoma, or non-metastatic tumors in the oviduct and ovary, or ovary only was determined. Tissue was either fixed in neutral buffered formalin or Bouin’s fixative, dehydrated, paraffin embedded and sectioned at 5um; preserved in RINalater, or flash frozen in liquid nitrogen. RNA was isolated by Trizol, treated with RQ1 DNase, and analyzed by end-point RT PCR. cDNA was then quantitated by Quant-iTTM and real-time PCR was performed. Oligonucleotide probes were designed by interrogating the *Gallus gallus* genome database and real-time PCR was performed using the ABI 7900HT. Tumors presented with endometrioid, clear cell, poorly differentiated and mixed histology in H&E stained sections. Immunohisto-chemistry of the normal ovary demonstrated that only the granulosa cell layer surrounding the follicle was strongly positive for proliferative cell nuclei antigen (PCNA) staining, whereas the majority of nuclei in ovarian tumors were strongly positive, consistent with the proliferative phenotype. Analysis of oxidative stress marker, CYP1B1 mRNA levels demonstrated little upregulation in tumor tissue. Inflammatory genes, COX-1 and COX-2, both show significantly higher expression in young chicken Post Ovulatory Follicles (POF1) versus old normal chicken POF1. However, by mRNA comparison of young, old normal and cancer chicken ovarian tissue, COX-1 shows obvious up-regulated expression in ovarian cancer vs. normal, but not COX-2. COX-1 has been previously shown to be markedly elevated in mouse model’s epithelial ovarian cancer and in the human disease. Our results provide further evidence that COX-1 over expression is a common feature in epithelial ovarian cancer (EOC). These results further support the use of the hen as a model of human ovarian cancer.

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Gene Trap Mutagenesis: A Functional Genomics Approach towards Reproductive Research, Terrance Lee, Chirag A. Shah, Eugene Y. Xu, Division of Reproductive Biology, Northwestern University School of Medicine, Evanston, IL

Biomedical research and our understanding of diseases have benefited tremendously from the use of animal models. Gene trapping is a method of generating murine embryonic stem cell lines containing insertional mutations. While gene trapping is a valuable resource in functional genomics, the efficacy and availability of gene trap lines is unclear. In the context of reproductive research, the database created by the International Gene Trap Consortium (IGTC) can be mined to identify cell lines corresponding to genes specifically expressed in reproductive tissue. To address these needs, we wrote a program in Visual Basic named SpiderGene that obtains cDNA sequences from many genes and utilizes the IGTC database to return corresponding gene trap cell lines. Using the program, we have been able to find gene trap lines for thousands of genes expressed in human and mouse reproductive tissue, including various parts of the sperm cell, testis, ovary, and premeiotic germ cells. The reliability of gene trapping for functional analysis was positively confirmed using a list of genes causing reproductive defects, which yielded gene trap lines for 41% of the genes. Further studies will proceed with the characterization of genes associated with identified gene trap cell lines. Moreover, because gene trapping is only effective with genes expressed in embryonic cells, using SpiderGene to ascertain the availability of gene trap cell lines for genes expressed in germ cells can elucidate the similarity between germ cells and ES cells. This can potentially yield new pathways in embryonic stem cell research and new discoveries in germ cell development.

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Fluorescence Imaging of Inorganic Physiology in the Mammalian Oocyte, M. Alison Kim\textsuperscript{1}, Thomas V. O’Halloran\textsuperscript{2,3}, Teresa K. Woodruff\textsuperscript{1,3}, \textsuperscript{1}Department of Neurobiology & Physiology, \textsuperscript{2}Department of Chemistry, \textsuperscript{3}Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL.

The genesis of an entire organism is dependent on the oocyte, a unique cell with the remarkable ability to transform its fate from terminally differentiated to totipotent. The health and quality of the oocyte is crucial for the fertility of the female and consequently her ability to reproduce. With nutritional studies continuing to highlight the importance of transition elements in development, we are interested in probing the inorganic physiology of the female germ cell. Initial studies have focused on zinc, which is one of the most abundant metals in nature. We have utilized live-cell fluorescence imaging to visualize zinc distribution in the mouse oocyte. The results show striking patterns of localization at various stages of oocyte maturation. In particular, oocytes arrested at metaphase II display a pool of chelatable zinc in the first polar body, which is often dismissed as a trash receptacle for unwanted chromatin. Additional studies are being completed with collaborators at the Argonne National Laboratory, using x-ray fluorescence spectroscopy to elucidate the oocyte’s elemental distribution.

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