THE 18TH ANNUAL GILBERT S. GREENWALD VIRTUAL SYMPOSIUM ON REPRODUCTION AND PERINATAL RESEARCH

OCTOBER 13-15 2021

KU MEDICAL CENTER
The University of Kansas

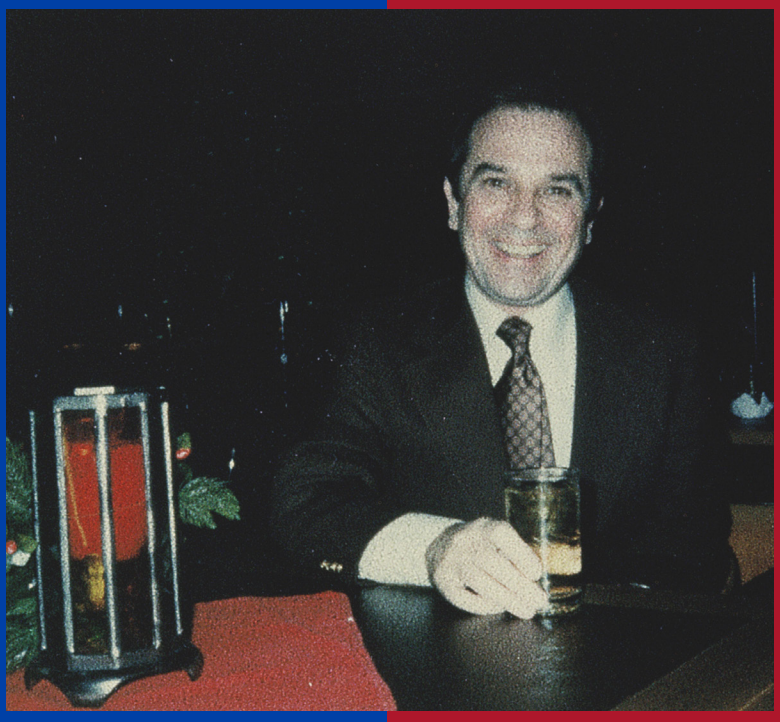
Gilbert S. Greenwald Biography

The Institute for Reproduction and Perinatal Research at the University of Kansas Medical Center hosts the Annual Gilbert S. Greenwald Symposium on Reproduction and Perinatal Research in honor of and as a memorial to the life and research career of Gilbert S. Greenwald, PhD. Professor Greenwald had an illustrious career as a Distinguished Professor at the Medical Center and as an internationally recognized reproductive biologist.

Professor Greenwald received his doctorate from the University of California at Berkeley, followed by postdoctoral studies at the Carnegie Institute of Embryology in Baltimore. He then moved to his first faculty appointment in the Department of Anatomy at the University of Washington. He joined the Departments of Obstetrics & Gynecology and Anatomy at the University of Kansas Medical Center in 1961 where he held an endowed chair in Research in Human Reproduction. He also served as chair of the Department of Physiology at the Medical Center for 16 years (1977-1993).

Professor Greenwald received numerous awards for his outstanding research accomplishments from several scientific societies. Among these is the Distinguished Service Award from the Society for the Study of Reproduction for his work as one of the founding members and early president of the Society, as well as Editor-in-Chief of its journal, Biology of Reproduction. Professor Greenwald also received the Carl Hartman Award for a career of outstanding scientific contributions to the field of reproductive biology.

The National Institutes of Health supported his research over his entire career. Professor Greenwald trained more than 50 graduate students and postdoctoral fellows and was instrumental in the career development of numerous faculty, including several currently holding leadership positions at the University of Kansas Medical Center and at other academic institutions throughout the world. He was a true scholar, a superb mentor, and a generous friend. Professor Greenwald passed away on August 26, 2004.



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September Numata, Graduate Student, Molecular and Integrative Physiology

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SYMPOSIUM HISTORY - PLENARY SPEAKER LIST

2004

Harry Weitlauf, MD
Texas Tech University
Osborn Address

James Cross, PhD
University of Calgary

B. Anne Croy, DVM, PhD
University of Guelph

Mary Hunzicker-Dunn, PhD
Northwestern University
Feinberg School of Medicine

Kevin Osteen, PhD
Vanderbilt University

Richard Stouffer, PhD
Oregon Health & Science University

Neena Schwartz, PhD
Northwestern University

2005

Shyamal K. Roy, PhD
University of Nebraska
Osborn Address

Sally Camper, PhD
University of Michigan

Thaddeus Golos, PhD
Wisconsin Regional Primate Center

Matthew Hardy, PhD
Population Council

Joy Pate, PhD
Ohio State University

John Robinson, PhD
Ohio State University

2006

Geula Gibori, PhD
University of Illinois at Chicago
Osborn Address

Robert Braun, PhD
University of Washington

Susan Fisher, PhD
University of California-San Fransisco

Fred Karsch, PhD
University of Michigan

John Schimenti, PhD
Cornell University

Teresa Woodruff, PhD
Northwestern University

2007

John J. Eppig, PhD
The Jackson Laboratory
Osborn Address

Indrani Bagchi, PhD
University of Illinois-Champaign

E. Mitchell Eddy, PhD
National Institute of Environmental Health & Safety

Patricia Hunt, PhD
Washington State University

Mark S. Roberson, PhD
Cornell University

Carole R. Mendelson, PhD
The University of Texas Southwestern Medical Center

Bruce D. Murphy, PhD
University of Montreal

2008

David Page, MD
Howard Hughes Medical Institute
MIT, Boston, MA
Osborn Address

Jon Levine, PhD
Northwestern University

Ina Dobrinski, M.V.Sc., PhD
University of Pennsylvania

John Peluso, PhD
University of Connecticut

Miles Wilkinson, PhD
MD Anderson Cancer Center

Nasser Chegini, PhD
University of Florida

2009

Jerome Strauss III, MD, PhD,
Virginia Commonwealth University
Osborn Address

Alberto Darszon PhD
National Autonomous University of Mexico

Louis DePaolo, PhD
Eunice Kennedy Shriver NICHD, NIH

Keith Latham, PhD
Temple University

Ajay Nangia, MD
University of Kansas Medical Center

Stephanie Seminara, MD
Massachusetts General Hospital, Harvard Medical School

Thomas Spencer, PhD
Texas A&M University

2010

Marco Conti, MD
University of California-San Fransisco
Osborn Address

Romana A. Nowak, PhD
University of Ilinois

Susan S. Suarez, MS, PhD
Cornell University

John Davis, PhD
University of Nebraska Medical Center

Sergio R. Ojeda, DVM
Oregon National Primate Research Center

Stephen A. Krawetz, PhD
Wayne State University

Gil G. Mor, MD, MSc, PhD
Yale University

2011

Kenneth S. Korach, PhD,
NIEHS/NIH
Keynote Lecturer

Blanche Capel, PhD, Duke University Medical Center

Aaron J.W. Hsueh, PhD,
Stanford University School of Medicine

Asgi T. Fazleabas, PhD,
Michigan State University

Yaacov Barak, PhD,
University of Pittsburgh

Tony M. Plant, PhD,
University of Pittsburgh

SYMPOSIUM HISTORY - PLENARY SPEAKER LIST

2012

R. Michael Roberts, PhD, University of Missouri-Columbia, Keynote Lecturer

Kyle Orwig, PhD, University of Pittsburgh

Bruce D. Murphy, PhD, University of Montreal

Francesco DeMayo, PhD, Baylor College of Medicine

Yoel Sadovsky, PhD, University of Pittsburgh

2013

Martin M. Matzuk, MD, PhD, Baylor College of Medicine and Ben Taub General Hospital, Keynote Lecturer

Frederick vom Saal, PhD, University of Missouri-Columbia

Mary Hunzicker-Dunn, PhD, Washington State University

Louis J. Muglia, MD, PhD, University of Cincinnati College of Medicine

Derek Boerboom, DVM, PhD, University of Montreal

Shoukhrat Mitalipov, PhD, Oregon Health and Science University

2014

W. Lee Kraus, PhD, University of Texas Southwestern, Keynote Lecturer

Marisa S. Bartolomei, PhD, University of Pennsylvania

Suzanne Moenter, PhD, University of Michigan

Kathy Sharpe-Timms, PhD, University of Missouri-Columbia

David Zarkower, PhD, University of Minnesota

2015

Bert W. O'Malley, MD, Baylor College of Medicine Keynote Lecturer

William Kinsey, PhD, University of Kansas Medical Center

Amy Ralston, PhD, MIchigan State

Wei Yan, MD, PhD, University of Nevada School of Medicine

James A. MacLean, PhD, Southern Illinois University

Robert Taylor, MD, PhD, Wake Forest School of Medicine

Qinglei Li, PhD, Texas A&M University

2016

David A. Haig, PhD, Harvard University Keynote Lecturer

Alex Bortvin, MD, PhD, Carnegie Institution for Science

Jon D. Hennebold, PhD, Oregone National Primate Research Institute

Sarah Kimmis, PhD, McGill University

Donald F. Conrad, PhD, Washington University

Deborah M. Sloboda, PhD, McMaster Univeristy

Kathleen M. Caron, PhD, University of North Carolina

2017

Kent Thornburg, PhD, Oregon Health and Science University - Keynote Lecturer

Hugh Clarke, PhD, McGill University

Diana Laird, PhD, University of California - San Francisco

Liang Ma, PhD, Washington University - St. Louis

James Pru, PhD, Washington State University

Eric Greer, PhD, Harvard Medical School, Boston Children's Hospital

Mellissa Mann, PhD, Magee-Womens Research Institute

2018

Sandra T. Davidge, PhD, University of Alberta Keynote Lecturer

Paul S. Cooke, PhD, University of Florida

Rebecca A. Simmons, MD, University of Pennsylvania

Satoshi H. Namekawa, PhD, Cincinnati Children's Hospital Medical Center

Todd. S. Macfarlan, PhD, NICHD
Ramakrishna Kom magani, PhD, Washington University, St. Louis

Joan S. Jorgensen, DVM, PhD, University of Wisconsin

2019

Serdar E. Bulun, MD, Northwestern University - Keynote Lecturer

Brian P. Hermann, PhD, University of Texas, San Antonio

Monica P. Colaiacovo, PhD, Harvard Medical School

Thomas R. (Tod) Hansen, PhD, Colorado State University

Ov D. Slayden, PhD, Oregon Health & Science University

Kaylon L. Bruner-Tran, PhD, Vanderbilt University Medical Center

David Natale, PhD, Queen's University

VIRTUAL SYMPOSIUM SCHEDULE

Wednesday, October 13, 2021 (DAY 1)

Moderators: ***Kaela Varberg, PhD and Marija Kuna, PhD, Postdoctoral Fellows, KUMC***

10:00 a.m. **DAY 1 OPENING REMARKS: Katherine Roby, PhD,** Research Professor and Greenwald Symposium Chair, and **Kaela Varberg, PhD**, Postdoctoral Fellow, University of Kansas Medical Center

10:03 a.m. **Keynote Speaker Introduction** by **Regan Scott, MS,** Graduate Student, University of Kansas Medical Center

10:05 a.m. **KEYNOTE LECTURE, Günter P. Wagner, PhD, Alison Richard Professor Emeritus of Ecology and Evolutionary Biology, Yale University, *"Evolution of the Fetal-Maternal Relationship: Why and how is mammalian viviparity special?"***

11:05 a.m. **Constantine Simintiras, PhD,** Postdoctoral Fellow, University of Missouri-Columbia, Spencer Lab, ***"Uterine Lumen Fluid is Metabolically Semi-Autonomous."***

11:18 a.m. **SHORT BREAK**

11:30 a.m. **Fatimah Aljubran, MS**, Graduate Student, University of Kansas Medical Center, Nothnick Lab, ***"Cyclin A2 Deficiency is Associated with Elevated Protein Kinase Inhibitor-alpha in Endometrial Stromal Cells from Women Who Fail to Achieve in Vitro Fertilization-Assisted Pregnancy."***

11:45 a.m. **Alison Ermisch, MS**, Graduate Student, University of Nebraska-Lincoln, Wood Lab, ***"Ovarian Inflammation Increases Oocyte Maternal mRNAs During Maturation and Alters Expression of Cumulus Regulatory Genes Resulting in Reduced Developmental Competence. "***

12:00 p.m. **Marija Kuna, PhD**, Postdoctoral Fellow, University of Kansas Medical Center, Soares Lab, ***"CITED2 Regulates Placental Development and Adaptations to Environmental Stressors."***

12:13 p.m. **Day 1 Closing Remarks - Kaela Varberg, PhD**

12:15 p.m. **Transition to Keynote Speaker - Trainee Breakout Session**

12:30 p.m. **Keynote Speaker - Trainee Breakout Session**

VIRTUAL SYMPOSIUM SCHEDULE

Thursday October 14, 2021 (DAY 2)

Moderators: ***Kaela Varberg, PhD and Marija Kuna, PhD, Postdoctoral Fellows, KUMC***

10:00 a.m. **DAY 2 OPENING REMARKS: Kaela Varberg, PhD**, Postdoctoral Fellow, University of Kansas Medical Center

10:05 a.m. **Sue Hammoud, PhD,** Assistant Professor, Department of Human Genetics, University of Michigan ***"Modern Approaches to Revisit Unsolved Questions in the Spermatogenesis Program", September Numata, BS,*** *Graduate Student, KUMC, providing introduction*

10:35 a.m. **Jessica Kincade, MS**, Graduate Student, University of Missouri-Columbia, Balboula Lab, ***"Central Nucleus Positioning in Murine Oocytes is Achieved by F-actin and Maintained by Microtubules to Avoid Erroneous Chromosome Segregation and Aneuploidy."***

10:50 a.m. **Pablo J. Ross, DVM, PhD,** Professor and Vice Chair, Department of Animal Science, University of California, Davis, ***"Reprogramming and Pluripotency in the Bovine Preimplantation Embryo", Fatimah Aljubran, MS,*** *Graduate Student, KUMC, providing introduction*

11:20 a.m. **SHORT BREAK**

11:30 a.m. **Sean (Seok-Yeong) Yu, PhD,** Postdoctoral Fellow, University of Nebraska Medical Center, Kim Lab, ***"Metabolic and Microenvironmental Features of Granulosa Cell Tumors in PIK3CA* Mice."***

11:45 a.m. **Stephanie Pangas, PhD,** Associate Professor and Roger Rossen Endowed Professorship of Reproductive Pathology, Department of Pathology & Immunology, Baylor College of Medicine, ***"SUMOylation Regulates Key Stages of Mammalian Oocyte Development", Wendena Parkes, BS,*** *Graduate Student, KUMC, providing introduction*

12:15 p.m. **Day 2 Closing Remarks - Kaela Varberg, PhD**

12:17 p.m. **Transition to Day 2 Speaker - Trainee Breakout Sessions**

12:45 p.m. **Day 2 Speaker - Trainee Breakout Sessions (Drs. Hammoud, Ross and Pangas)** - *3 simultaneous sessions*

VIRTUAL SYMPOSIUM SCHEDULE

Friday October 15, 2021 (DAY 3)

Moderators: ***Kaela Varberg, PhD and Marija Kuna, PhD, Postdoctoral Fellows, KUMC***

10:00 a.m. **DAY 3 OPENING REMARKS: Kaela Varberg, PhD**, Postdoctoral Fellow, University of Kansas Medical Center

10:05 a.m. **Lisa Joss-Moore, PhD,** Professor, Department of Pediatrics, Division of Neonatology, and Department of Nutrition and Integrated Physiology (adjunct), University of Utah, ***"The Ins and Outs of Developmental Lipid Signaling", Kaela Varberg, PhD,*** *Postdoctoral Fellow, KUMC, providing introduction*

10:35 a.m. **Regan Scott, MS,** Graduate Student, University of Kansas Medical Center, Soares Lab, ***"Single Cell Interrogation of the Uterine-Placental Interface."***

10:50 a.m. **Isabella Caniggia, MD, PhD,** Senior Investigator, Lunenfeld-Tanenbaum Research Institute of Sinai Health System, Professor of Obstetrics and Gynaecology and Physiology, University of Toronto, ***"Preeclampsia and the Oxygen Response: Mechanisms, Consequences, and Targeting Approaches", Vinay Shukla, PhD,*** *Postdoctoral Fellow, KUMC, providing introduction*

11:20 a.m. **SHORT BREAK**

11:30 a.m. **Pooja Popli, PhD,** Postdoctoral Fellow, Washington University - St. Louis, Kommagani Lab, ***"The Autophagy Protein Beclin 1 is required for Uterine Development and Receptivity during Pregnancy."***

11:45 a.m. **Thorold Theunissen, PhD,** Assistant Professor of Developmental Biology, Washington University - St. Louis, ***"Understanding Human Pluripotent States and Their Applications in Reproductive Science", Ananya Ghosh, MS,*** *Graduate Student, KUMC, providing introduction*

12:15 p.m. **Day 3 Closing Remarks - Kaela Varberg, PhD and Katherine Roby, PhD,** Research Professor and Greenwald Symposium Chair

12:17 p.m. **Transition to Day 3 Speaker - Trainee Breakout Sessions**

12:45 p.m. **Day 3 Speaker - Trainee Breakout Sessions (Drs. Joss-Moore, Caniggia and Theunissen) -** *3 simultaneous sessions*

Speaker Biographies

KEYNOTE LECTURER
Günter P. Wagner, PhD

"Evolution of the Fetal-Maternal Relationship: Why and how is mammalian viviparity special?"

Günter Wagner is the Alison Richard Professor Emeritus of Ecology and Evolutionary Biology at Yale University with a joint appointment at the Department of Obstetrics, Gynecology and Reproductive Sciences at the Yale Medical School. He is a chemical engineer by training and studied zoology and mathematics at the University of Vienna where he earned a Ph. D. in zoology. Postdoctoral training involved work in developmental neurobiology at the Max-Plank-Institute for Biophysical Chemistry in Göttingen, Germany, and at the Max-Planck-Institute for Developmental Biology in Tübingen. From 1985 till 1991 he was at the Department of Zoology at the University of Vienna, Austria and in the fall of 1991 joined Yale's Department of Biology. In 1997 he became the first chair of Yale's EEB Department and in 2010 he founded the Yale Systems Biology Institute. His research interests include the evolution of gene regulation, the evolution of pregnancy, and the evolutionary biology of cancer and female sexuality. GPW is a Mac Arthur Fellow, member of the US National Academy of Sciences, the American Academy of Arts and Sciences, Fellow of the American Society for the Advancement of Science, the Connecticut Academy of Science and Engineering and a corresponding foreign member of the Austrian Academy of Sciences. He is also an adjunct faculty of the Department of Obstetrics and Gynecology at Wayne State University in Detroit, Michigan.

Sue Hammoud, PhD

"Modern Approaches to Revisit Unsolved Questions in the Spermatogenesis Program"

Sue Hammoud is an Assistant Professor at University of Michigan in the Department of Human Genetics. Dr. Hammoud received her Ph.D. at the University of Utah. As a graduate student working with Drs. Brad Cairns and Douglas Carrell, Sue demonstrated that the paternal contribution to the embryo extends far beyond paternally imprinted genes and the genomic DNA sequence in sperm – encompassing histone modifications and small RNAs. As a postdoctoral Helen Hay Whitney fellow in the Cairns and Jones lab at the Huntsman Cancer Institute she has explored how chromatin regulates germline stem cell development and tissue homeostasis. Currently, Dr. Hammoud's lab is investigating the cellular and genetic factors required to make a healthy and developmentally competent gamete.

Speaker Biographies (cont.)

Pablo J. Ross, DVM, PhD

"Reprogramming and Pluripotency in the Bovine Preimplantation Embryo"

Pablo Ross is the Chief Scientific Officer at STgenetics, a company specialized in livestock genetics and reproductive services, including sex-sorted semen, embryo production and genomic testing. Dr. Ross was born and raised in Argentina, where he received a Degree in Veterinary Medicine from La Plata National University and an MS in Animal Science from Mar del Plata National University/ INTA Balcarce. He obtained a PhD in Animal Science from Michigan State University in 2007 and held a Research Assistant Professor position at that institution. Between 2010 and 2020, Dr Ross was a Professor of Reproductive Biology at the Animal Science Department in the University of California, Davis. He was also department Vice-Chair for 3 years. Dr. Ross academic work was aimed at understanding the mechanisms of epigenetic remodeling and transcriptional reprogramming that occur during preimplantation embryo development. Work at the Ross laboratory also related to developing gene editing approaches and embryo and pluripotent stem cell technologies for use in agriculture and biomedicine.

Stephanie Pangas, PhD

"SUMOylation Regulates Key Stages of Mammalian Oocyte Development"

Dr. Pangas is a reproductive biologist focusing on female reproductive health. She received her PhD with Dr. Teresa Woodruff at Northwestern University in Evanston, IL, then trained as a postdoctoral fellow with Dr. Martin Matzuk at Baylor College of Medicine in Houston, TX prior to establishing her research laboratory. She is currently an Associate Professor and holds the Roger Rossen Endowed Professorship of Reproductive Pathology in the Department of Pathology & Immunology at Baylor College of Medicine. Over the past 19 years, her laboratory has focused on the functional analysis of genes involved in female ovarian function primarily by generating novel mouse genetic models. Her laboratory has been funded by the Burroughs Wellcome Foundation, the National Cancer Institute, and the Eunice K. Shriver National Institute of Child Health and Human Development. In addition, she directs the NIH/NICHD-funded training grant, Comprehensive Mechanisms of Reproductive Sciences, and is co-director of the NIGMS-funded Initiative for Maximizing Student Development (IMSD) at Baylor College of Medicine.

Speaker Biographies (cont.)

Lisa Joss-Moore, PhD

"The Ins and Outs of Developmental Lipid Signaling"

Lisa Joss-Moore, PhD is a Professor in the Department of Pediatrics, Division of Neonatology, and Department of Nutrition and Integrated Physiology (adjunct), at the University of Utah. Dr Joss-Moore's focus is research and education. The Joss-Moore Lab studies how perinatal insults affect essential fatty acid driven molecular mechanisms predisposing to neonatal disease. The emphasis of the Joss-Moore lab is to understand the placental contribution to alterations in essential fatty acids in the fetus, and the subsequent epigenetic programing of the lung and adipose tissue during development. With an overall goal of informing therapeutic developments involving long chain fatty acids. Studies in the Joss-Moore Lab use molecular, morphometric, physiologic and genomic approaches, and are supported by the National Institutes of Health and Primary Children's Hospital Foundation. Dr Joss-Moore is also the Director of the Pre-Award Office for the Department of Pediatrics 'Pediatric Research Enterprise', and Past President and Chair of the Perinatal Research Society Subcommittee on Trainee Education. She teaches grant and manuscript writing locally and nationally.

Isabella Caniggia, MD, PhD

"Preeclampsia and the Oxygen Response: Mechanisms, Consequences, and Targeting Approaches"

Dr. Isabella Caniggia, MD, PhD is a Senior Investigator at the Lunenfeld-Tanenbaum Research Institute of Sinai Health System and a Professor of Obstetrics and Gynaecology and Physiology at the University of Toronto. Dr. Caniggia is internationally recognized for her work on molecular mechanisms regulating normal placental development and diseases including preeclampsia, IUGR and gestational diabetes. She has received numerous honors and awards including the Ontario Women's Health CIHR Mid-Career Award, the Castellucci Award from IFPA and, more recently, the Canada Research Chair (Tier 1) in Placental Biology in Pregnancy and Disease for her innovative research. Her work has been funded by CIHR, NIH and NSERC.

Speaker Biographies (cont.)

Thorold Theunissen, PhD

"Understanding Human Pluripotent States and Their Applications in Reproductive Science"

Thor Theunissen grew up in The Netherlands and received his A.B. in Biology from Harvard in 2007. He became interested in stem cells and developmental biology during his undergraduate work in the laboratories of Christine Mummery (Hubrecht Institute) and Stuart Orkin (Harvard Medical School). He completed his graduate studies in José Silva's laboratory in the Wellcome Trust Center for Stem Cell Research and Department of Biochemistry at the University of Cambridge in 2011. His doctoral thesis focused on the role of the homeodomain transcription factor Nanog in epigenetic reprogramming. As a Sir Henry Wellcome Postdoctoral Fellow in Rudolf Jaenisch's laboratory at the Whitehead Institute/MIT, Thor developed methods to isolate naïve human pluripotent stem cells. He was appointed Assistant Professor in the Department of Developmental Biology and Center of Regenerative Medicine at Washington University School of Medicine in 2017. He is a recipient of the NIH Director's New Innovator Award (DP2), the Edward Mallinckrodt Jr New Investigator Award, and the Shipley Foundation's Program for Innovation in Stem Cell.

Abstracts

Full abstracts are included on the following pages.

Cyclin A2 deficiency is associated with elevated protein kinase inhibitor-alpha in endometrial stromal cells from women who fail to achieve in vitro fertilization-assisted pregnancy.

Fatimah Aljubran, Amanda Graham, Courtney Marsh, Warren B. Nothnick. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Cyclin A2 (CCNA2) is a critical regulator of the cell cycle that also elicits noncanonical functions including regulation of estrogen and progesterone signaling in some cell types. Proper estrogen (E2) and progesterone (P4) signaling within the endometrium is essential for fertility. Previously, we have shown that CCNA2 expression is significantly reduced in the stromal compartment of endometrial tissue obtained from women with infertility, yet the role of Cyclin A2 in endometrial physiology is not well understood. To explore the mechanism by which Cyclin A2 regulates endometrial function, *CCNA2* expression was knocked down in human endometrial stromal cells (t-HESC) using *in vitro* siRNA transfection assay. RNA-seq experiment was performed to determine the differentially expressed genes using a 1.5-fold change as a cut-off between non-targeting (NT) and *CCNA2* siRNA transfected cells. One of the most differentially upregulated genes is protein kinase inhibitor-alpha (*PKIA*; 2.02-fold increase, p=3.59E-07). PKIA is a potent inhibitor of PKA pathway which is required for proper endometrial decidualization. Immunohistochemistry staining was performed to assess the expression and localization of PKIA in endometrial tissue from women undergoing in vitro fertilization (IVF). In comparison with women who achieved pregnancy, PKIA was robustly expressed in the stroma of women who failed to achieve pregnancy (n=5). To evaluate the expression of *PKIA* in an *in vitro* stromal cell culture model, we treated t-HESCs with decidualization media (E2 + P4 + 5% horse serum) and assessed *PKIA* expression at day 0 (prior to decidualization) and every 2 days for 10 days total. *PKIA* mRNA expression decreased at days 2 and 4 of *in vitro* decidualization then returned to higher levels for the remaining time points. To confirm our findings from the RNA-seq data, we transfected t-HESC cells with *CCNA2* or NT-siRNA, then treated the cells 24h later with decidualization media. *PKIA* mRNA expression was significantly higher in *CCNA2* knocked down t-HESC cells at day 0, 2 and 4 of decidualization compared to NT-siRNA transfected cells. Moreover, *CCNA2* knockdown was associated with significant reduction in decidualization markers *PRL* and *IGFBP1* compared to NT-siRNA. In summary, CCNA2 deficiency is associated with elevated expression of the protein kinase-A inhibitor (PKIA) both *in vivo* and *in vitro*. PKIA could represent one novel pathway by which CCNA2 deficiency contributes to impaired fertility at the level of the endometrium.

Corn-cob bedding impairs the development and long-term function of murine testes. M.L. Burns[1], A.P. Petty[1], L.E. Clifton[1], K.W. Lovercamp[1] and A.T. Desaulniers[2]. [1]School of Natural Sciences, University of Central Missouri, Warrensburg, MO 64093 USA. [2]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583 USA

In developed countries, sperm counts in men have declined by 50 – 60% in the last 50 years. Rodents are a prominent biomedical model in which to address rising male infertility. However, bedding choice influences reproductive outcomes in rodents which may confound reproductive research efforts. Corn-cob bedding is the most commonly used housing substrate due to its low cost and high absorptive capacity. However, corn-cob bedding contains compounds with estrogenic properties [e.g., linoleic acid-derived tetrahydrofuran (THF)-diols] that impair rodent sexual behavior and estrous cyclicity. Specifically, corn-cob bedding reduces estrogen receptor-α expression within the brain and stimulates proliferation of breast and prostate cancer cell lines. Notably, corn is a major food source for humans and contains THF-diols; thus, humans are directly exposed to endocrine disrupting components in corn which may impede fertility. Accordingly, the objective of this study was to assess the effects of corn-cob bedding on testis development and function in a murine model. Prepubescent littermate CD-1 male mice were randomly assigned to treatment in cages containing either corn-cob (n = 8; 700 ml) or shredded paper (n = 8; Alpha-Dri; control; 700 ml) bedding for 7 weeks spanning the attainment of puberty. At maturity (10 – 12 weeks), males were paired with 2 non-littermate females and the animals remained together on aspen bedding for the duration of the trial. Plugs were detected daily, and females were euthanized at d 14 of gestation to assess reproductive traits [e.g., gravid uterine weight, litter size, number of mummies, number of reabsorptions, ovulation rate (number of corpora lutea)]. Pregnancy rate as well as early and late embryonic survival were calculated. Males were subsequently euthanized (~1 month after removal from treatment) and measurements of body weight, anogenital distance, paired testis weight, and paired seminal vesicle weight were recorded. Blood was collected via intracardiac puncture for free testosterone analysis. Spermatozoa were isolated from cauda epididymides for morphological and computer-assisted semen analysis. Results demonstrated that males housed on corn-cob bedding sired heavier conceptuses (fetus + placenta; 0.91 ± 0.08 g) compared with control males (0.83 ± 0.09 g; P = 0.0424). Progeny from males housed on corn-cob bedding tended to have a reduced early embryonic survival rate (90.6 ± 3.2%) compared with the control group (98.3 ± 2.9%; P = 0.0840). Further, corn-cob males tended to have smaller seminal vesicles (358.0 ± 26.7 mg) compared with control males (425.0 ± 55.4 mg; P = 0.0984) which was likely due to impaired testosterone biosynthesis. Serum testosterone concentrations tended to be reduced by 72% in males reared on corn-cob (0.132 ± 0.127 ng/ml) versus control (0.470 ± 0.127 ng/ml) bedding. Taken together, these results suggest that exposure to corn-cob bedding impairs the development and long-term function of the murine testis. These results highlight the importance of cage bedding selection in reproductive research and have implications for human health.

Gut microbiota-derived short-chain fatty acids protect against the progression of endometriosis

Sangappa B. Chadchan[1&2], Pooja Popli[1&2], Chandrasekhar R. Ambati[5], Eric Tycksen[4], Sang Jun Han[5], Serdar E. Bulun[6], Nagireddy Putluri[5], Scott W. Biest[1&3], and Ramakrishna Kommagani[1&2]*

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Worldwide, approximately 196 million are afflicted with endometriosis, a painful disease in which endometrial tissue implants and proliferates on abdominal peritoneal surfaces. Although, theories on the origin of endometriosis remained inconclusive, a well-accepted theory is that endometriosis occurs when endometrial tissue enters the peritoneal cavity via retrograde menstruation and implants onto pelvic organs and peritoneal surfaces. However, whereas up to 90% of women experience retrograde menstruation, only 10% develop endometriosis, suggesting that factors that alter peritoneal environment might contribute to endometriosis. Herein, we report that whereas some gut bacteria promote endometriosis, others protect against endometriosis by fermenting fiber to produce short-chain fatty acids (SCFAs). Specifically, we found that altered gut microbiota drives endometriotic lesion growth in a mouse model of the disease. Additionally, feces from mice with endometriosis contained less of SCFA n-butyrate (n=9-10; $P \leq 0.05$; Student's t-test) than feces from mice without endometriosis. Treatment with n-butyrate reduced growth of both mouse endometriotic lesions and human endometriotic lesions (n=5; $P \leq 0.05$; Student's t-test) in a pre-clinical mouse model. Mechanistic studies revealed that n-butyrate inhibited human endometriotic cell survival (n=3; $P \leq 0.05$; Student's t-test) and lesion growth through G-protein-coupled receptors, GPR43 and GPR109A, and inhibit histone deacetylase (HDAC) (n=5; $P \leq 0.05$; ANOVA). Finally, we identified that the RAP1 GTPase activating protein (RAP1GAP), which was upregulated by n-butyrate possibly through inhibition of HDAC1, contributed to the ability of n-butyrate to inhibit cellular proliferation (n=3; $P \leq 0.05$; Student's t-test). Our findings will enable future studies aimed at developing diagnostic tests (e.g., gut bacteria metabolites) and treatment strategies (e.g., dietary supplements, n-butyrate analogs, or probiotics) for endometriosis.

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Conditional loss of ADAR in murine granulosa cells leads to altered gene expression and subsequent delay in ovulation

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Adenosine deaminases acting on RNA (ADAR) comprise a family of RNA editing enzymes that specifically catalyze adenosine to inosine (A-to-I) editing. A granulosa cell (GC) specific *Adar1*-depleted mouse model was utilized to evaluate the role of ADAR during ovulation. Loss of *Adar1* in GCs leads to delayed ovulation, decreased ovulation counts, and subfertility. We have performed transcriptome analyses of granulosa cells from $ADAR^{FL/FL}$/Aromatase-Cre and littermate control mice stimulated with gonadotropin (PMSG 48h+ 4h hCG). RNA-sequencing analyses identified 963 differentially expressed genes with an absolute fold change of 1.5 and FDR value <0.1. Of those, 373 genes were downregulated, and 590 genes were upregulated. Ingenuity pathway analyses of the differentially expressed genes showed numerous downstream targets of estrogen, progesterone, and EGF1 signaling pathways. Whereas multiple genes involved in inflammatory responses were upregulated in the $ADAR^{FL/FL}$/Aromatase-Cre GC. Analysis of the top twenty downregulated genes revealed a set of genes, namely *Ovgp1*, *Gprc6a*, *Klk1*, *Dnah5*, *Atg4c*, which are known to be involved in the final stages of ovulation and fertilization. Analysis of the top twenty upregulated genes showed genes *Ifit3*, *Ifit3b*, *Ifi44*, *IFit1*, *Oas1a*, *Oas1g*, *Ly6a*, *Usp18*, many of which are critical to inflammatory pathways. Ongoing analyses are aimed at determining the mechanism by which the loss of ADAR results in dyssynchronous ovulation and reduced fertility. Due to ADAR's key role in sensing dsRNA and downstream events, the induction of these interferon associated genes highlights importance of ADAR and its impact on ovulation.

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Role of TFAP2C in trophoblast cell and placental development

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The trophoblast cell lineage represents the first cell lineage to differentiate from the mammalian embryo. Trophoblast stem (TS) cells expand and differentiate to form the placenta and contribute to its functional properties. Specialized trophoblast cells can produce hormones, facilitate bi-directional nutrient/waste transfer, evade maternal immune attack, and/or invade and transform the uterus. Invasive/extravillous trophoblast (EVT) cells restructure uterine spiral arteries to facilitate blood delivery to the placenta. Disruptions in uterine spiral artery remodeling are connected to pregnancy related diseases such as preeclampsia, intrauterine growth restriction, and pre-term birth. Trophoblast cell lineage development is dependent on the orchestrated regulation of gene networks. Transcription factor AP-2 gamma (TFAP2C) is among a group of transcription factors known to be pivotal in trophoblast cell lineage development in the mouse and human. We hypothesize that the invasive trophoblast cell lineage is a target for the actions of TFAP2C. The goal of this investigation was to assess the role of TFAP2C in human TS cell and invasive/extravillous trophoblast cell development and to evaluate the role of TFAP2C in the establishment of the rat uterine-placental interface. TFAP2C transcripts are prominently expressed in stem and progenitor cell populations of the human placenta and in the in the EVT column. In addition, *Tfap2c* transcripts are also expressed in the junctional zone of the rat placentation site, which represents the origin of the invasive trophoblast cell lineage, and in invasive trophoblast cells at the uterine-placental interface. TFAP2C transcripts and protein were detected in human TS cells maintained in the stem state and following differentiation into EVT cells. Two shRNAs were identified as effective in silencing TFAP2C expression at the transcript and protein levels. TFAP2C knockdown in human TS interfered with trophoblast cell development, including effects on the stem state, proliferation, differentiation of the EVT cell phenotype, and the TS cell transcriptome. TFAP2C disruption resulted in the dysregulation of transcripts associated with both the stem state and EVT cell differentiation. Genome editing was used to generate a TFAP2 mutant rat model. A global 308 bp out-of-frame rat *Tfap2c* deletion was generated using *CRISPR*/Cas9 gene editing, which resulted in a germline null mutation and prenatal lethality. In conclusion, we have identified TFAP2C as a key player in the regulation of human and rat TS cell lineages and placental development. [Supported by Lalor Foundation fellowships to EMD and AM, NIH HD20676, HD099638, and the Sosland Foundation]

Ovarian Inflammation Increases Oocyte Maternal mRNAs During Maturation and Alters Expression of Cumulus Regulatory Genes Resulting in Reduced Developmental Competence.

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Obese women are sub-fertile and have reduced ART success, which may be due to inflammation-induced reductions in oocyte developmental competence. The hypothesis of this study is that exposure to inflammation during oocyte cytoplasmic maturation leads to altered mRNA stability and subsequent early embryonic loss. To test this hypothesis, we employed a comparative mouse model using C3H/HeJ (C3H), which have a low inflammatory response due to lack of a functional TLR4 receptor, and C57BL/6 (B6) with a normal inflammatory response. Eight-week-old C3H and 10-week-old B6 mice were randomly placed on normal chow (ND) or HFHS diet (42% kcal fat, 42% kcal sucrose chow; 20% sucrose water) for 4 weeks. DEXA scans confirmed that B6-HFHS and C3H-HFHS animals had greater ($p<0.01$) body fat percentage (28.0±1.8%, 29.1±1.2%, respectively) compared to ND counterparts (16.9±0.6%, 15.1±0.7%, respectively). Likewise, blood glucose levels following a 12h fast were increased ($p<0.01$) in B6-HFHS and C3H-HFHS (125.4±6.6 mg/dL, 127.9±6.1 mg/dL, respectively) compared to ND (93.9±5.6 mg/dL, 97.9±6.9 mg/dL, respectively). While both developed similar metabolic phenotypes, Western blot analysis demonstrated that only B6-HFHS females had increased ($p<0.01$) ovarian CD68, a marker for activated macrophages, indicating an expected localized inflammatory response in B6-HFHS but not C3H-HFHS. Females were also superovulated with PMSG and hCG (5IU each), and cumulus oocyte complexes (COCs) were collected 16-18h post-hCG. Following fertilization, there was no difference in the percentage of cleaved embryos, however there were decreases ($p<0.05$) in embryos that progressed to blastocyst (96h) and hatching blastocyst (116h) per cleaved embryo from B6-HFHS (54%, 38%, respectively) females compared to B6-ND (72%, 55%, respectively), C3H-ND (82%, 80%, respectively), and C3H-HFHS (76%, 73%, respectively). Interestingly, there were no differences in the number of ovulated oocytes, percentage of degenerate oocytes, or spindle abnormalities between ND and HFHS females within both strains. Therefore, we sought to explore the impact of inflammation on oocyte cytoplasmic maturation, particularly maternal mRNA expression. Collected COCs were denuded, and oocytes were pooled in groups of 20 for RNA extraction, reverse transcription and qPCR. There were diet dependent increases ($p<0.05$) in transcript numbers of maternal effect genes involved in transcriptional regulation (*Gdf9, Figla, Nobox, Pou5f1, Sebox*), genomic imprinting (*Dnmt1, Dppa3*), and the sub-cortical maternal complex (*Nlrp5, Ooep, Padi6*) in B6 mice; however, abundance of these mRNAs were not different between C3H-ND and C3H-HFHS. To further elucidate the role of cumulus cells in mediating the effects of inflammation on oocyte mRNAs, post-ovulation cumulus cell RNA from B6-HFHS and C3H-HFHS were analyzed using the Nanostring nCounter Metabolism panel to detect differences in transcript abundance of metabolic genes. There was significant downregulation (>two-fold) of genes that regulate COC expansion and communication (*Ptgs2, Alox15, Itgb2, Itgam, Il7, Il6, Il2ra, Tlr2, Cd14*) in B6-HFHS cumulus cells compared to C3H-HFHS. Taken together, these results suggest that diet-induced ovarian inflammation leads to aberrant cumulus cell-mediated oocyte maturation and/or directly alters mechanisms by which maternal mRNA are stored and cleared within the oocyte during the maternal to zygotic transition, which are subsequently contributing factors to early embryonic loss.

Investigating the secretory phase changes and decidual hormone response of human endometrial epithelial organoids. **Harriet C. Fitzgerald**, Andrew M. Kelleher, Danny J. Schust, Thomas E. Spencer. Division of Animal Sciences, University of Missouri, Columbia, MO; Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, MO

In humans, an unreceptive uterus and disrupted maternal-conceptus interactions can cause infertility due to pregnancy loss or later pregnancy complications. Uterine glands and, by inference, their products and secretions impact uterine receptivity, blastocyst implantation, stromal cell decidualization, and placental development. Investigating uterine gland-stromal cell and uterine gland-embryo interactions in vivo is challenging and as such endometrial epithelial organoids (EEO) present a promising avenue for research into these processes in vitro. Changes in gland function across the menstrual cycle are largely dependent on steroid hormone signaling; however, the direct impact of these hormones on EEO gene expression, proteomic profile and secretory products remains unclear. Our aim was to investigate changes in EEO function in response to steroid hormones which may impact stromal cell decidualization. EEO were derived from three patients and following passaging were allowed to grow for four days in expansion medium. The EEO were treated for two days with estradiol-17β (E2) and then either with nothing (vehicle control), E2, medroxyprogesterone acetate (MPA), E2+MPA or MPA and prostaglandin E2 (PGE2) in base medium, which lacks the WNT activating factors found in expansion medium, for an additional 6 days. Immunofluorescence analysis found that EEO contained both FOXA2 positive and negative cells, suggesting that some cells within an EEO are gland-derived. E2 treatment increased the localization of PGR, while a decrease in localization was seen with MPA treatment. Immunofluorescence analysis of acetylated alpha tubulin (ac-TUB), a marker of ciliated cells, showed that the EEO cells were both ciliated and unciliated, where the abundance of ac-TUB positive cells increased following E2 treatment. Real-time qPCR analysis revealed that E2 increased *OLFM4* expression, E2+MPA increased *PAEP*, *HSD17B2*, *OLFM4* and *ENPP3* expression, MPA alone increased *ENPP3* and *OLFM4* expression, while MPA+PGE2 increased *ENPP3* and *PAEP* expression. Collectively, these results indicate that EEO remain hormone responsive when removed from expansion medium and exhibit secretory phase changes in response to MPA+PGE2 which has not been shown before in this model system. Understanding the direct effect of hormonally driven EEO changes will enable critical investigations into the impact of uterine glands on stromal cell decidualization and pregnancy establishment in humans. Supported by NIH Grants R01 HD096266 and R21 HD087589.

Title: Conserved transcriptional regulation by GATA2 AND GATA3 in syncytiotrophoblast development during mammalian placentation.

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The placenta is an indispensable organ for intrauterine growth and development in mammals. It establishes an exchange interface that is essential for nutrient and gas exchange between the mother and the developing fetus. The establishment of this exchange interface is essential for the successful progression of pregnancy and mammalian reproduction. Trophoblast cells from the developing fetus interacts with the decidual cells of the mother and together form a functional placenta. The trophoblast cells also assist in establishing a vascular connection between the mother and the fetus and produce hormones essential for the successful progression of pregnancy. A critically conserved step in the establishment of the maternal fetal exchange surface in mammals is the fusion of trophoblast cells into a multinucleated syncytiotrophoblast layer (SynT).

Our lab discovered that the transcription factors, GATA2 and GATA3, are essential for trophoblast development and differentiation. In the early stages of mammalian development when GATA factors are selectively expressed in the trophoblast cells, simultaneous deletion of *Gata2* and *Gata3* genes in mice (**GATA-DKO**) severely impair placentation leading to embryonic death at embryonic day 8.0 (**E8.0)**, a stage similar to first-trimester human pregnancy. The abnormal phenotype was accompanied by severe blood loss in the GATA-DKO placenta and a reduction in the labyrinth layer that is composed mainly of syncytiotrophoblast cells.

I, therefore, hypothesize **that GATA2 and GATA3 are essential to establish a developmental stage-specific conserved transcriptional program in trophoblast cells at the maternal-fetal interface.** To address this, I have used SynT progenitor-specific marker Gcm1 as the promoter driving the Cre recombinase in mouse models (**Gcm1-Cre)** that would specifically ablate *Gata2* and *Gata3* genes in these labyrinth progenitor trophoblast cells. The goal is to study the effect of this cell type specific deletion of these transcription factors on overall placental and embryonic development. **Gcm1-Cre mice** were crossed with mice having LoxP sites flanking specific exons in *Gata2* allele and *Gata3* allele (**GATA-Floxed mice) to generate a conditional GATA-knockout mouse model.** The overall changes because of the knockout on the placental development were analysed using single cell transcriptomics and confirmed via immunohistochemistry and in-situ hybridization.

Our study shows that in $Gcm1^{Cre}$ mediated dual knockout of *Gata2* and *Gata3* arrest the growth of the embryos at E9.5 due to developmental abnormalities observed in the placenta particularly in the SynT layers. This was further confirmed by immunostaining for the specific SynT markers. Using single-cell transcriptomics analyses, we also show that the loss of GATA factors in the SynT progenitors result in the loss of differentiated trophoblast populations.

Based on the observations we infer that GATA2 and GATA3 play an important role in the commitment of progenitor trophoblasts to the SynT lineage and are essential for establishing a functional maternal-fetal interface for exchange.

Role of ALKBH1 in Regulating Hemochorial Placental Development.

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The development of the placenta is central to favorable pregnancy outcomes for both the mother and the fetus. Complex pathways of cellular proliferation, migration and differentiation must occur for this organ to function properly. DNA methylation is an essential component of epigenetic regulation and plays a vital role in regulating gene expression. An N6-deoxymethyladenosine (6mA) DNA modification was initially discovered in prokaryotes and more recently identified in mammalian genomes. The 6mA DNA modification is established through the actions of methyltransferase N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) and removed via the demethylase AlkB homolog 1 (ALKBH1). *Alkbh1* belongs to a family of 2-oxoglutarate and α-ketoglutarate-dependent oxygenases. They are evolutionarily conserved and implicated in nucleotide demethylation. Importantly, 6mA DNA modifications contribute to regulation of the epigenetic landscape. Inactivation of the *Alkbh1* gene in the mouse has provided evidence for the involvement of ALKBH1 in placental and fetal development. *Alkbh1* is widely expressed in trophoblast cell lineages. The rat has proven to be an effective model for investigating hemochorial placentation associated with deep intrauterine trophoblast invasion, as is also observed in the human. A mutant rat model was generated using CRISPR/Cas9-mediated genome edited deletion of part of Exon 3, resulting in a frame-shift mutation. Germline transmission was confirmed by genotyping offspring of the founder rat. Homozygous mutant rats were generated by mating homozygous mutant females and males. Fetal and placental weights were obtained from gestation days 15.5, 18.5 and 20.5. Placentas were dissected into their respective components (junctional and labyrinth zones) and independently weighed. *Alkbh1* null fetal and placental weights were significantly smaller, as were junctional and labyrinth zones, when compared to wild type littermates at each gestation day examined. Histological and transcriptomic analyses further highlighted the involvement of ALKBH1 in placentation. These findings implicate *Alkbh1* as a key regulator of hemochorial placenta development and provide a new tool for evaluating the involvement of the 6mA DNA modification in regulating the trophoblast cell lineage in an important experimental model of deep placentation. (Supported by a Lalor Foundation fellowship to AM, AHA fellowships to KK, NIH grants (HD020676, HD099638, ES028957), and the Sosland Foundation)

Two distinct syncytiotrophoblast types are revealed by single nucleus RNA sequence (snRNAseq) analysis of trophoblast derived from primed-type human pluripotent stem cells

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Syncytiotrophoblasts (STB) are multinuclear cells in the human placenta that are responsible for gas and nutrient exchange, but also produce a range of hormones essential for pregnancy maintenance and, during implantation, have an invasive phenotype. We have been studying STB generated from human pluripotent stem cells treated with **B**MP4 in the presence of **A**83–01 and **P**D173074, inhibitors for ACTIVIN/TGFB and FGF2 signaling, respectively (BAP exposure). Within 48 hours the cells are almost entirely KRT7-positive indicating an efficient conversion to trophoblast, and by days 5-7 STB emerges, which we have hypothesized resembles the syncytium that encases the early human conceptus as it implants. We have employed single nuclei RNAseq (snRNAseq) on BAP-exposed H1 human embryonic stem cells (hESCs) to study the heterogeneity of trophoblast cell types that emerge during the first 8 days of differentiation. Our hypothesis was that the STB would have a gene signature similar to that of STB from villous placenta during the first trimester of pregnancy. snRNA seq is particularly powerful in this regard because the fragility and size of STB precludes single cell RNAseq from being employed. On days 7 and 8 BAP-exposed cells were producing large quantities of hCG as measured by ELISA, confirming the presence of STB. Nuclei were isolated after dissociating the colonies and employing the 10X Genomics protocol from which cDNA libraries were constructed. RNAseq provided data from a total of 5,355 nuclei, which, after subtracting sequences representing ribosomal genes and contaminating mitochondria, provided between 200 and 7,500 unique sequences per nucleus. Seurat analysis revealed a high degree of heterogeneity among the nuclei representing different trophoblast cell types. Specifically, at least eight distinct clusters of nuclei were evident, of which four were likely different kinds of mononucleated cytotrophoblasts (clusters 1,4,7,8), two (clusters 2, 3) weakly resembled extravillous trophoblast, and two (clusters 5, 6) were strongly enriched with distinctive STB transcripts found also in villous STB. There was no evidence that other extra-embryonic lineages, including amnion, were represented among any of the eight clusters. Further analysis determined that, of the distinctive STB transcripts, 18 were common to clusters 5 and 6, whereas 57 were enriched in cluster 5, and 55 enriched in cluster 6. Immunostaining with cluster-specific markers has validated the presence of two STB subtypes in the colonies of BAP-exposed ESCs. Our data demonstrate that at least two distinct subtypes of STB, distinct from but carrying many of the transcriptional hallmarks of first trimester villous STB, emerge when ESCs are exposed to the BAP differentiation regimen. Present studies are aimed at following the events that accompany the emergence of these lineages and whether villous STB is also comprised of homologous sublineages.

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Central Nucleus Positioning in Murine Oocytes is Achieved by F-actin and Maintained by Microtubules to Avoid Erroneous Chromosome Segregation

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A majority of aneuploidy (the leading genetic cause of miscarriage and congenital abnormalities) arises due to mistakes in the first meiotic division, making it critical to understand the mechanisms involved in oocyte meiosis. We previously showed that increased aneuploidy occurs in oocytes that experience NEBD near the oocyte cortex and thus form a cortically located spindle. Histological analysis of nucleus positioning in ovaries from mice of varying ages revealed that a majority of oocytes in 2 or 3-week old mice exhibited a peripherally located nucleus. Mice aged 5 weeks showed similar proportions of oocytes with either a centrally or peripherally located nucleus, only 8-week old mice exhibited a majority of oocytes with a centrally located nucleus. *In vitro* collection of oocytes from mice aged 2, 3, 5, and 8 weeks reflected trends seen *in vivo,* suggesting that nucleus positioning in mouse oocytes is a dynamic process throughout development. Regulation of nucleus positioning during oocyte development is not known. Histological analysis of ovaries from naturally ovulated, super-ovulated, and FSHβ knockout mice showed similar proportions of central *vs.* peripheral GV oocytes, indicating that nucleus positioning is gonadotropin independent. At the cellular level, nucleus positioning in almost all cells is regulated by cytoskeletal components. However, F-actin or microtubules (MTs) patterns throughout oocyte development are largely unknown. We assessed F-actin and MTs in oocytes from mice of varying ages. F-actin levels were higher in oocytes collected from mice aged 2 and 3 weeks, compared to those collected from mice aged 5 weeks, 8 weeks. MTs, however, displayed patterning that decreased throughout oocyte development. These findings raise the question whether both F-actin and MTs are responsible for dynamic nucleus positioning during development. In full-grown GV oocytes, the movement of the nucleus from the periphery to the oocyte center is largely controlled by F-actin. It is suggested that MTs play a lesser role, as MT perturbation results in erratic nucleus movement. We thus hypothesized the proposal of two separate mechanisms governing nucleus positioning in mouse oocytes: one acting to move the nucleus to the center and another acting to maintain central positioning of the nucleus. Observing nucleus movement during a GV-arrested incubation perhaps provided the most insight into understanding these mechanisms. Oocytes were sorted based on nucleus positioning into either central or peripheral and observed for nucleus movement using time-lapse live imaging. Central oocytes treated with DMSO (control) remained central, while nearly all peripheral oocytes treated with DMSO achieved centralization of their nucleus, indicating that the centralization and the maintenance mechanisms are functional. F-actin-inhibited oocytes (cytochalasin-D) were able to maintain central positioning of their nucleus but were unable to relocate the nucleus from the periphery to the center. Oocytes in which MTs were depolymerized (nocodazole) were able to maintain central positioning of the nucleus in only 30% of central oocytes, while 70% of peripheral oocytes were able to recenter their nucleus from the periphery. Importantly. oocytes in which MTs were stabilized (taxol) were able to maintain central nucleus positioning 50% of the time, while peripheral oocytes were completely unsuccessful at any recentering of the nucleus. These results together indicate that while centering of the nucleus is controlled mainly by F-actin, there is an opposing mechanism ensuring controlled movement and positioning that is controlled by an optimum level of polymerized MTs. This research was supported by laboratory start-up funding from the University of Missouri to AZB.

PI3K/AKT/STAT1 signaling regulates human extravillous trophoblast cell lineage development.

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Remodeling of uterine spiral arteries is a key step for the establishment of normal pregnancy. Invasive/extravillous trophoblast (EVT) cells are central to directing uterine spiral artery remodeling. Dysregulated EVT cell lineage development is associated with abnormal pregnancies including early pregnancy loss, preeclampsia, and intrauterine growth restriction. Phosphoinositide 3-kinase (PI3K)/AKT signaling has been implicated in many biological processes including cell differentiation, migration, and invasion. However, our understanding of the role of PI3K/AKT signaling on EVT cell lineage development is limited. In this project, we utilized a human trophoblast stem (TS) cell model system and evaluated a potential role for PI3K/AKT signaling in the regulation of EVT cell lineage development. Human TS cells were treated with vehicle, a PI3K/AKT inhibitor (LY294002), or a STAT1 inhibitor (Fludarabine) throughout the EVT differentiation process. Effects on trophoblast development were determined by assessing TS cell morphology, differentiation, and transcriptomic analysis using RNA-sequencing (RNA-seq). Results of RNA-seq were validated by RT-qPCR. Immunoprecipitation of phospho-AKT substrate followed by mass spectrometry was performed to identify AKT substrates associated with EVT differentiation. Following differentiation of human TS cells into EVT cells, phosphorylation of AKT and its substrates were increased. Inhibition of PI3K/AKT signaling interfered with the acquisition of the classic EVT cell morphology and transcriptome indicative of disruptions in EVT cell differentiation. Among differentially expressed genes, some classic interferon-responsive genes, including *STAT1*, *ISG15*, *IFITM1*, *MX1*, *IFI35*, *OAS2*, and *OAS3* were downregulated. Inhibition of STAT1 altered EVT cell morphology and resulted in downregulation of *ISG15*, *IFITM1*, *MX1*, and *OAS2*. Mass spectrometry of AKT substrates in EVT cells identified candidate downstream mediators of AKT action. These results suggest that PI3K/AKT/STAT1 signaling is required for EVT cell lineage development. Identification of AKT substrates may shed light on AKT as a hub of the regulatory network critical for EVT cell development. (Supported by fellowship from AHA (KK), KUMC BRTP and K-INBRE programs (VS), NIH grants HD020676 and HD099638, and the Sosland Foundation)

CITED2 Regulates Placental Development and Adaptations to Environmental Stressors

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Hemochorial placentation involves the development of specialized trophoblast cells with the ability to enter and transform the uterine environment. Cells engineering these pregnancy-dependent adaptations are referred to as invasive trophoblast cells in the rat and extravillous trophoblast (EVT) cells in the human. CBP/p300-interacting transactivator with glutamic acid/aspartic acid-rich carboxyl terminal domain 2 (CITED2) regulates the recruitment of CBP/p300 to transcription factors and contributes to the regulation of trophoblast cell differentiation. In this project, we examined the involvement of CITED2 in the regulation of placental development and placental adaptations to environmental stressors.

CITED2 distribution was assessed in rat and human placentation sites using in situ hybridization. The role of CITED2 in placentation was investigated using a rat model generated by genome-editing, using CRISPR-Cas9 technology, and trophoblast cell-specific knockdown (KD) using lentiviral delivery of *Cited2*-specific shRNAs. Morphological, biochemical, and molecular parameters of placentation were determined. Roles for CITED2 in placentation site specific adaptations were evaluated following exposure to hypoxia and polyinosinic:polycytidylic acid.

CITED2 is expressed in the junctional zone (JZ) and invasive trophoblast cells of the rat placenta and in the EVT cell column of the human placenta. CITED2 deficiency in the rat resulted in intrauterine placental and fetal growth restriction and postpartum lethality. These CITED2 actions were conserved between the rat and mouse. Trophoblast-specific lentiviral CITED2 knockdown resulted in a similar growth arrested placental phenotype. CITED2-deficiency affected trophoblast invasion. The first wave of endovascular trophoblast cell invasion occurring at midgestation was dependent upon CITED2; however, subsequent trophoblast cell invasion was independent of CITED2. CITED2-deficient invasive trophoblast cells displayed a transcript profile distinct from wild type invasive trophoblast cells. The junctional zone transcriptome and chromatin landscape were profoundly affected by CITED2, as were placental adaptations to hypoxia and exposure to viral mimetics.

We conclude that CITED2 directs transcriptional decisions contributing to the adaptation of trophoblast cells to the uterine environment.

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Oocyte stage-specific roles of ERβ in primordial follicle activation. V. Praveen Chakravarthi, **Eun B. Lee**, Saeed Masumi, Hindole Ghosh, Katherine F. Roby, Michael W. Wolfe, and M. A. Karim Rumi. Departments of Pathology & Laboratory Medicine, Molecular & Integrative Physiology, and Anatomy & Cell Biology, Institute for Reproduction and Perinatal Research, University of Kansas Medical Center, Kansas City, KS.

Primordial follicle activation, the first step in ovarian folliculogenesis, steadily recruits a small number of dormant follicles out of the ovarian reserve. Loss of estrogen receptor β (ERβ) results in excessive activation of primordial follicles and depletion of the ovarian reserve leading to premature ovarian senescence. We recently observed that targeted depletion of ERβ in the oocytes of *Erβ*$^{fl/fl}$:*Gdf9-Cre* mice increases primordial follicle activation to a similar extent of *Erβ* knockout (*Erβ*^{KO}) mice that lack ERβ in all cell-types. This novel finding underscores an essential role of ERβ in the oocytes for the regulation of primordial follicle activation. We detected an abundant expression and nuclear localization of ERβ in both oocytes and granulosa cells of primordial follicles as well as primary follicles. The nuclear localization of phosphorylated ERβ (pERβ, Ser105) suggests that ERβ is transcriptionally active in oocytes and granulosa cells of either the dormant or the activated follicles. As *Gdf9-Cre* depletes the expression of ERβ in oocytes starting from the primordial stage, we examined the effect of targeted ERβ depletion selectively in the activated follicles using a *Zp3-Cre* line (*Erβ*$^{fl/fl}$:*Zp3-Cre*). Interestingly, we did not observe any increased primordial follicle activation in *Erβ*$^{fl/fl}$:*Zp3-Cre* mice, which suggests that the presence of ERβ is essential in primordial oocytes but not in activated oocytes for the regulation of primordial follicle activation. (Supported by K-INBRE and KUMC SOM)

PAQR6 upregulation is associated with AR signaling and unfavorite prognosis in prostate cancers

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Progesterone-induced rapid non-genomic signaling events have been confirmed through several membrane progesterone receptors (mPR). Some mPRs were reported to correlate with cancer progression and patient prognosis. In this study, we conducted a comprehensive analysis of all mPR and PGR-related genes in prostate cancer tissues and examined the correlations of their expression levels with disease progression and patient survival outcomes. We utilized multiple RNA-seq and cDNA microarray datasets to analyze gene expression profiles and performed logistics aggression and Kaplan-Meier survival analysis after stratifying patients based on TNM categories and Gleason scores. We also used NCBI GEO datasets to examine gene expression patterns in individual cell types of the prostate gland and to determine the androgen-induced alteration of gene expression. Spearman coefficient analysis was conducted to access the correlation of target gene expression with treatment responses and disease progression status. The classic PGR was mainly expressed in stromal cells and PAQR6/7/8 genes were the predominant genes in prostate epithelial cells. PGRMC1 was significantly higher than PGRMC2 in all prostate cell types. In prostate cancer tissues, PAQR6 expression was significantly upregulated while all other genes were largely downregulated compared to normal prostate tissues. Although both PAQR6 upregulation and PAQR5 downregulation were significantly correlated with tumor pathological TNM categories, only PAQR6 upregulation was associated with Gleason score, fPSA/tPSA ratio, and patient overall survival outcomes. In addition, PAQR6 upregulation and PGR/PGRMC1 downregulation were significantly associated with a quick relapse. Conversely, in NEPC tissues, PAQR6 expression was significantly lower but PAQR7/8 expression was higher than CRPC tissues. PAQR8 expression was positively correlated with AR score and AR-V7 expression levels but inversely correlated with NEPC score in metastatic CRPC tumors. This study provides detailed expression profiles of membrane progesterone receptor genes in primary cancer, CRPC, and NEPC tissues. PAQR6 upregulation in primary cancer tissues is a novel prognostic biomarker for disease progression, overall, and progression-free survival in prostate cancers. PAQR8 expression in CRPC tissues is a biomarker for AR activation.

The underlying mechanism of cyclophosphamide-reduced ovarian reserve

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Background: Radiotherapy and cytotoxic chemotherapy cause detrimental side effects while advanced cancer therapies have increased 5-year survival rates of cancer survivals. Due to the high sensitivity of the ovary, cancer treatment causes ovarian damage and follicle depletion which leads to premature ovarian insufficiency (POI) in young female cancer survivals. POI, presented as endocrine dysfunction and infertility, becomes a critical status in prepubertal girls and premenopausal women. Cyclophosphamide (CPA) forms DNA crosslinks and leads to apoptosis in rapidly proliferating tumor cells. However, the underlying mechanism of the CPA-caused death in dormant oocytes from the ovary remains controversial. One group proposed that CPA induces apoptotic pathway in oocytes of primordial follicles through DNAPK>rH2AX>CHK2>p53/TAp63a>PUMA and ABL kinase inhibitor, GNF2, prevents oocyte death against CPA. The other group suggested that CPA activates dormant oocytes of primordial follicles via the PI3K pathway, leading to burnout of follicle pool. Meanwhile, another group presented that p63 is not involved in the apoptotic pathway of oocyte death. In this study, we used oocyte-specific *p63* and *Abl* conditional knockout mouse models to clarify the elemental mechanism regarding the CPA-induced POI in the ovary.
Experimental Design: We generated oocyte-specific *Abl1* and *p63* knockout mouse models using *GDF9-iCre* to investigate the role of ABL and p63 in the oocyte death with CPA treatment. Postnatal day 7 (PD7) female mice were i.p. injected with 100 mg/kg CPA and ovaries were harvested day 3 post-treatment for further analysis. We also tested GNF2 in PD7 mice *in vivo* to examine its feasibility of protecting oocytes of primordial follicles against CPA.
Results: We found that CPA reduced the number of primordial follicles without accelerating the number of growing follicles. The quantification of surviving follicles validated that 90% of the primordial follicles from oocyte-specific *Abl1* knockout mice were lost following CPA treatment *in vivo* and *in vitro*, with most of the growing follicles maintained inside of the ovary. Accordingly, GNF2 did not prevent loss of primordial follicles after CPA treatment. However, ovarian morphology and follicle quantification revealed significant follicles were rescued in oocyte-specific *p63* knockout mouse with CPA treatment. Consistently, high expression of CHK2 was detected in the oocytes of the ovary cultured with the 4-hydroperoxy cyclophosphamide (4HC, CPA effective metabolite) *in vitro.*
Conclusions: The number of primordial follicles rather than growing follicles dramatically declines post CPA treatment, suggesting that dormant follicles are more sensitive to CPA compared to growing follicles. ABL tyrosine kinase is dispensable for CPA-induced oocyte death in the mouse ovary. Most of all, this study suggests that TAp63 is the master regulator to induce follicle depletion caused by CPA. CHK2 inhibitors can be further tested as fertoprotective reagents to preserve follicles against CPA as the CHK2-p63 pathway regulates primordial follicle death without activating dormant follicles.

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Placenta Specific 1 Promotes Deep Intrauterine Trophoblast Cell Invasion

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Hemochorial placentation occurs in many mammalian species including primates and rodents and is the pivotal event in the establishment of pregnancy. We use the rat as an experimental model since it exhibits deep intrauterine trophoblast invasion and extensive uterine spiral artery remodeling similar to human placentation. In this study, we investigated the expression and actions of placenta specific 1 (PLAC1) in the developing rat placentation site. PLAC1 is a highly conserved X-linked gene implicated in the regulation of placental development. PLAC1 expression was assessed in placentation sites during gestation using RT-qPCR and *in-situ* hybridization. A *Plac1* mutant rat model was generated using CRISPR/Cas9 genome editing. Wild type and *Plac1* mutant placentation sites were examined histologically and through biochemical and molecular analyses. A role for PLAC1 in rat trophoblast stem (TS) cell differentiation as well as evaluation of the PLAC1 interactome were assessed using both loss-of-function and gain-of-function approaches. *Plac1* transcripts were prominently expressed in the junctional zone of the rat placenta and in intrauterine invasive trophoblast cells. *Plac1* homozygous and hemizygous null and *Plac1* maternally inherited heterozygote conceptuses exhibited placentomegaly. Enlarged placentas were characterized by an expanded junctional zone, an irregular junctional zone-labyrinth zone boundary, and a prominent depletion of intrauterine invasive trophoblast cells. In addition to structural irregularities, PLAC1 mutant and wild type junctional zone tissues exhibited prominent transcriptomic differences indicative of underlying disruptions in trophoblast cell lineage differentiation. PLAC1 knockdown inhibited in vitro TS cell differentiation. Ectopic PLAC1 expression in rat TS cells promoted differentiation and facilitated the identification of the PLAC1 interactome. These findings implicate PLAC1 as an important regulator of hemochorial placentation and provide new tools for evaluating the involvement of PLAC1 in regulating the trophoblast-uterine interface and deep placentation. [Supported by a Lalor Foundation postdoctoral fellowship to AM, NIH HD20676 and HD099638, and the Sosland Foundation]

Effects of bovine pregnancy-associated glycoproteins on Chemokine and related gene transcripts in bovine endometrial explants.

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Pregnancy-associated glycoproteins (PAGs) are a complex gene family expressed by trophoblasts of ruminants and other even-toed ungulates in the Cetartiodactyla order. In cattle, the PAGs accumulate at the trophoblast-uterine interface. Many of these can enter the maternal circulation. However, very little is known about their exact function in pregnancy, although preliminary results suggest that PAGs at the placenta-uterine interface play roles involving immune modulation and matrix turnover. This study was designed to provide further insight into the biological roles of bovine PAGs by measuring changes in endometrial transcript abundance for some matrix metalloproteinases (MMPs) and chemokines/cytokines. PAGs for these experiments were purified from mid-gestation bovine placental extracts by affinity chromatography. Heifers were synchronized and bred by artificial insemination. Heifers were slaughtered at day 18 post-insemination and the reproductive tracts were obtained and flushed to determine if a conceptus was present. Endometrial explants were collected and split between 4 groups: pregnant with and without 15 µg/ml PAG (n=10) and non-pregnant with and without 15 µg/ml PAG (n=9). Endometrial explants were cultured for up to 96 hours at 37°C and 5% CO_2 and samples were harvested at 24 h intervals for RNA extraction. Transcript abundance for target genes was analyzed in the endometrial tissue by quantitative PCR, with PPIA being used as the normalization control transcript. Significant increases in CXCL1, CXCL2 and CXCL5 as well as MMP1, MMP3 and MMP13 were measured in the PAG-treated endometrium from pregnant and non-pregnant animals ($P<0.05$). There were also significant decreases in message for CCL2, CCL8, CCL16, CXCL10 and CXCL12 in the PAG-treated groups from pregnant and non-pregnant animals ($P<0.05$). Immuno-histochemical analysis of tissues supports data found during quantitative PCR analysis. Further Testing may explore transcript abundance for chemokines related to angiogenesis. These results suggest that PAGs are capable of inducing changes in transcript abundance in bovine endometrial explants, which suggests that this model system might be useful to assess PAG function at the placenta-uterine interface. Funded by the MU Research Board.

Leukemia inhibitory factor receptor signaling in pregnancy and placentation.

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Placental development involves coordinated expansion and differentiation of trophoblast cell lineages possessing specialized functions. Among the differentiated lineages, are invasive trophoblast cells, which migrate into the uterus and direct the remodeling of uterine spiral arteries. Failure of trophoblast cell-guided uterine vasculature remodeling is associated with pathological conditions such as preeclampsia, intrauterine growth restriction, and preterm birth. Knowledge of molecular mechanisms underlying development of the invasive trophoblast cell lineage is a key to understanding the etiology of pregnancy-associated diseases. A suitable animal model can facilitate the discovery regulatory pathways controlling development of the uterine-placental interface. Unlike mouse, the rat exhibits deep intrauterine trophoblast cell invasion, a feature shared with human placentation. Leukemia inhibitory factor (LIF)-LIF receptor (LIFR) signaling has been implicated in regulating events at the uterine-placental interface. LIFR protein belongs to the type I cytokine receptor family and heterodimerizes with interleukin 6 cytokine family transducer (IL6ST; also called gp130) to form a functional receptor complex. To investigate the involvement of LIF-LIFR signaling in placental development we generated an in vivo rat model of LIFR deficiency. CRISPR-Cas9 gene editing was used to produce deletions at the *Lifr* locus. A founder with an 88 bp deletion within Exon 2 was used to establish an LIFR mutant rat model. The deletion resulted in a frameshift, the acquisition of a premature stop codon, and a null mutation. The *Lifr* mutation was effectively transmitted through the germline. Global disruption of the *Lifr* resulted in prenatal lethality. *Lifr* null placentas exhibited evidence of structural abnormalities. In summary, we have established a model system for the evaluation of LIFR signaling in hemochorial placentation. These efforts implicate LIFR in the regulation of placental development and its importance in successful pregnancy outcomes. (Supported by a Lalor Foundation fellowship to AM, NIH grants HD020676, HD099638, ES028957, and the Sosland Foundation)

The Sodium-Glucose Cotransporter 1 (SGLT-1) is expressed in the mouse sperm flagellum, where it contributes to energy production and motility.

September Numata, Jeffrey McDermott, Gladis Sanchez, Amrita Mitra, and Gustavo Blanco. Department of Molecular and Integrative Physiology, The University of Kansas Medical Center, Kansas City KS.

Sperm energy production is essential for fertilization and male fertility. Although sperm can utilize a variety of substrates, glucose has been shown to be crucial for supporting sperm metabolism. Previous studies have demonstrated that sperm glucose uptake is facilitated by several isoforms of the glucose transporters (GLUT). Here, we report that sperm also express the Na^+-dependent glucose transporter (SGLT), a secondary active transport system that cotransports glucose and Na^+ into the cytoplasm. This was revealed by our observation that genetic deletion of the testis-specific Na,K-ATPase α4, which disrupts the plasma membrane Na^+ gradient, reduces sperm glucose uptake and ATP production. Immunoblot analysis of sperm from wild type (WT) mice identified the presence of isoform 1 of the transporter (SGLT-1), but not of isoform 2 (SGLT-2). Immunocytochemical analysis showed that SGLT-1 was localized primarily to the mid- and principal piece of the sperm flagellum. Inhibition of SGLT-1 with the isotype-specific inhibitor, Phlorizin, significantly reduced glucose uptake in both non-capacitated and capacitated sperm. Metabolic determinations using the Seahorse XF Analyzer indicated that Phlorizin significantly decreased glycolytic activity and ATP production in sperm from WT mice. These effects were more pronounced in capacitated versus non-capacitated sperm. In addition, Phlorizin reduced total and progressive sperm motility, as well as other parameters of sperm movement. In contrast, inhibition of SGLT-1 did not affect sperm capacitation, as measured by protein tyrosine phosphorylation, hyperactivation, and acrosomal reaction. Altogether, these results demonstrate that mouse sperm express a functional SGLT-1 transport system, which is important for supporting sperm energy production and motility.

Supported by NIH R01 HD080423 award.

Has3 deficiency does not alter ovarian stromal hyaluronan synthase or hyaluronidase transcript content relative to wild-type mice. **Wendena S. Parkes[1]**, Farners Amargant[2], Francesca E. Duncan[2], Michele T. Pritchard[1]. [1]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS, [2]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago IL

Hyaluronan's biological activity is regulated by its synthesis and degradation. With advanced reproductive age, ovarian stromal hyaluronan (HA) declines which could be due to decreased synthesis or increased degradation. HA synthase 3 (*Has3*) is the most abundant HA synthase in the ovarian stroma and it undergoes an age-related reduction while hyaluronidase 1 (*Hyal1*), an HA degrading enzyme, undergoes an age-related increase in 14 - 17 month old CB6F1 (WT) mice. These changes are associated with increased ovarian collagen content and increased stiffness. Consistently, ovaries from 5-month old *Has3* KO mice show increased stiffness relative to C57BL/6J (WT) mice, phenocopying physiologic aging. Because HA is regulated by the balance of HA synthase and hyaluronidase activities, the goal of this study was to determine how loss of *Has3* function impacts this enzymatic network. Therefore, we performed an aging study and collected ovarian stromal tissue from *Has3* KO and C57BL/6J mice in three age cohorts: reproductively young (7 weeks), mid (9 months), and old (17 months). Using real-time-PCR, we profiled the three HA synthases (*Has1, Has2, and Has3*) and the four somatic hyaluronidases (*Hyal1*, *Hyal2*, *Tmem2*, *Kiaa1199*) responsible for HA synthesis and degradation, respectively. Similar to what we recently observed in CB6F1 mice, *Has3* transcripts were the most abundant in ovarian stroma from C57BL/6J mice and they decreased with reproductive age. In contrast to what we observed in CB6F1 mice, *Has2* transcripts declined with reproductive age in C57BL/6J and *Has3* KO mice, but this reduction was not different between genotypes. *Has1* was not different between genotypes at any age. Comparing the four hyaluronidases, *Tmem2* was the least abundant and *Kiaa1199* was the most abundant hyaluronidase transcript in C57BL/6J mice, paralleling what we observed in CB6F1 mice, and this was also true in *Has3* KO mice. Additional assessment showed that there was no difference in any of the hyaluronidases with age, in contrast to increased *Hyal1* mRNA observed in CB6F1 mice, or between genotypes. Collectively, these data demonstrate that, with age, HA synthase and hyaluronidase regulation differ in mice with different genetic backgrounds, but age-associated changes in *Has3* are conserved, suggesting that HA synthesized by Has3 is critically important for maintaining ovarian HA content and tissue mechanics. *This work was supported by: R01HD093726, P20GM103549, and P30GM118247.*

[Association of serum kisspeptin levels and pregnancy outcomes in assisted reproductive technology]

Authors: Elaine Phillips, Macie Bokelman, Courtney Marsh, M.D. MPH, Michael Wolfe, Ph.D.

Background and Purpose:

Kisspeptin is a hypothalamic neuropeptide produced by the KISS-1 gene. During the early stages of pregnancy, Kisspeptin is produced by the syncytiotrophoblast and has been hypothesized to play a part in inhibiting invasion into the endometrium during implantation. Several studies have examined the effect of Kisspeptin levels on pregnancy outcomes in second and third trimester pregnancy, but trends in early pregnancy have not been well documented. Here, we examined how Kisspeptin levels correlate to serum beta human chorionic gonadotropin (bHCG), progesterone, and pregnancy outcomes in the first trimester of pregnancy in cisgender females undergoing reproductive technology.

Methods:

Serum samples were obtained from females undergoing assisted reproductive technology with viable first trimester pregnancy (n=3), not pregnant (n=3), biochemical pregnancy (n=3), first trimester complete abortion measured when bHCG is rising (n=3), and complete abortion measured when bHCG is falling (n=2). Progesterone and bHCG levels were measured by sequential two step immunoenzyme assay (Beckman Counter DXI 800) and kisspeptin levels were measured by Kisspeptin-10 EIA Kit (Phoenix Pharmaceuticals, Inc. Catalog No EK048056).

Results:

Our preliminary data for this small sample size shows no statistically significant differences between kisspeptin values and sampled groups. ANOVA correlation between kisspeptin and type of outcome showed a non-significant p-value of 0.73. T-test to correlate kisspeptin to pregnant vs. nonpregnant groups was not statistically significant with a p value of 0.25. A calculated correlation coefficient between kisspeptin and progesterone showed an R-value of 0.28.

Conclusions:

Due to the small sample size, no statistically significant correlations have emerged. With continued data collection and a larger sample size we hope to produce more robust data.

The Autophagy Protein Beclin 1 is required for Uterine Development and Receptivity during Pregnancy

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Successful establishment of pregnancy relies on the proper development of uterine endometrium, myometrium, and glands, as well as hormone-driven changes in endometrium. Several lines of evidence indicate that the cellular recycling pathway autophagy is necessary for endometrial decidualization, a differentiation process required for embryo implantation. Here, our objective was to determine whether the autophagy protein Beclin-1 was likewise required for endometrial decidualization and implantation. To do so, we generated mice in which Beclin-1 was conditionally knocked out (*Becn1* cKO) in the female reproductive tract by expression of Cre recombinase under control of the progesterone receptor. In six-month breeding trials, *Becn1* cKO females delivered no litters, while wild-type females delivered an average of 8.2 pups per litter every month ($P \leq 0.05$; n=4-6; Student's t-test). To understand the cause for this infertility defect, we first looked for the ovarian functions and found that infertility observed in *Becn1* cKO mice was not due to an ovarian defect, as they had similar serum ovarian hormone levels, follicle counts, and ovulation rates as wild-type mice. Instead, infertility appeared to be due to uterine defects as analysis of the uterus on Day 5 of pregnancy showed disrupted blastocyst implantation with altered expression of implantation markers; Lif and Muc1. *Becn1* cKO mice also failed to show induced uterine receptivity when given a controlled steroid hormone regimen with an extensive reduction in endometrial stromal cell proliferation ($P \leq 0.05$; n=4-6; Student's t-test). Additionally, in artificial decidualization experiments, uterine decidual response was severely curtailed in *Becn1* cKO mice compared to controls, as evidenced by reduced deciduoma size and decreased expression of the decidualization markers *Wnt4* and *Bmp2* ($P \leq 0.05$; n=6; Student's t-test). Interestingly, we noticed smaller gross uteri and lack of glands in matured uteri from *Becn 1* cKO mice, suggesting improper uterine maturation might underlie the reproductive defects in *Becn 1* cKO mice. At three weeks of age, uteri from *Becn1* cKO mice had smaller stromal compartment areas (marked with vimentin) than uteri from wild-type mice, and their myometrium compartment area (marked with smooth muscle alpha-actin) increased with age. At three weeks of age, uteri from *Becn1* cKO mice had ~4-fold more proliferative (Ki-67 positive) cells than uteri from age-matched controls ($P \leq 0.05$; n=4-6; Student's t-test). This result suggested that the epithelial and stromal cells in *Becn1* cKO mice continued proliferating and failed to differentiate. Consistent with this idea, we saw no evidence of gland development (as assessed by Foxa-2 staining) in uteri from *Becn1* cKO mice. Molecular analysis of cKO uterine tissues from mice at two and three weeks of age showed dysregulation of several Wnt pathway signaling molecules including *Wnt7a*, *Wnt 4*, *Wnt5*, *Wnt11*, *Wnt16*, *Fzd6*, and Fzd10 ($P \leq 0.05$; n=5-8; Student's t-test). More importantly, uteri from *Becn1* cKO mice at two and three weeks of age expressed ~7-8-fold less of stem cell marker *Lgr5* than uteri from control mice ($P \leq 0.05$; n=5-8; Student's t-test). Together, these data indicate that *Becn1* plays a crucial role in uterine development by regulating Wnt-dependent stem cell maintenance, which is essential for establishing successful pregnancy.

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Sentinel Event Analysis: A Comprehensive Model for Responding to Reproductive Surgical Emergencies.

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Sentinel events are defined as a critical incident that results in a severe negative outcome for the patient. Literature shows us that these events are more likely in situations with inherent increased stress. Items such as resident overtime, emergent obstetric presentations, and multiple lives at risk, are just a few examples of the intense nature of the field. For this reason, we developed a checklist for the most common cases that were studied to result in sentinel events, specific to reproductive incidents. First, we analyzed the top three most common cases in neighborhood health systems that caused sentinel events. From there, we used the Plan, Do, Study, Act Model to create checklists of preventable mistakes to be approached differently among future presentations. Distribution of these checklists is proposed to be critical in reducing patient morbidity and mortality in an evidence-based fashion. We hope to conduct future studies regarding not only the outcome measure changes post-checklist but also what other sentinel events are common and can be prevented with a similar approach. We utilized an interdisciplinary team and strongly believe in healthcare advocacy.

Conditional knockout of ADAR causes uterine atrophy and infertility in mice

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Adenosine deaminases acting on RNA (ADAR) are a family of RNA-editing enzymes responsible for the conversion of adenosine to inosine in dsRNA. Embryonic knockout of ADAR is lethal, and disruption of the enzyme in murine cardiomyocytes and hepatic tissue include apoptosis and fibrosis. While ubiquitous in both somatic and germline cells, the role of *Adar* in fertility is not well defined. Progesterone receptor (PR) is constitutively expressed in mouse uterine tissue whereas its expression in the ovary, specifically in the granulosa cells of the ovulatory follicle, peaks after the LH-surge. We used age-matched female *Adar*$^{FL/FL}$/PR$^{Cre/+}$ (ADAR cKO) mice and their wild-type (WT) littermates to evaluate the effects of diminished ADAR function on PR-expressing tissues of the reproductive tract. Ovulation rate was assessed following superovulation by PMSG (46hr) and subsequent hCG injection. Cumulus-oocyte complexes were collected from the oviduct and counted 16hr after hCG administration. Uterine histology and morphometric analyses of myometrial and endometrial areas were determined. At tissue collection, ADAR cKO mice had notably thin, thread-like uterine horns compared to WT mice, which had enlarged and fluid-filled uteri. ADAR cKO and WT ovaries generally appeared similar. Ovulation rates between ADAR cKO (n=5, 21.2±4.66) and WT (n=8, 16.88±2.76) were not significantly different (p=0.409). Analyses of uterine tissue showed reduction in cross-sectional areas consistent with attenuated PMSG-stimulation. An ongoing breeding trial (day 60) of ADAR cKO mice (n=5) with WT males indicates the mice as infertile despite mating, as evidenced by seminal plugs. These results suggest that the dsRNA-editing functions of ADAR appear to be critical to fertility, specifically in its impact on uterine hormonal responses. This project is supported by NICHD HD094545 to LKC.

Loss of *PRICKLE1* promotes endometrial epithelial disorganization and reduced fertility in mice.

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Our lab has previously shown that the loss of the tumor suppressor REST promotes the activation of PI3K/AKT-mTOR pathway in in uterine leiomyoma. Our recent results indicate that epigenetic silencing of *PRICKLE1,* a mediator of non-canonical WNT planar cell polarity (PCP) pathway, results in the loss of REST protein expression in the uterus. Furthermore, the loss of REST alters progesterone receptor function through a novel interaction. Changes in epithelial cell polarity play an important role during embryo implantation as well as in the development of endometrial cancer. However, PRICKLE1's involvement in planar cell polarity as well as apical-basolateral polarity organization of the endometrium has yet to be investigated. In order to study the effect of PRICKLE1's loss *in vivo,* we recently generated *Prickle1*$^{f/f}$ *Ltf*$^{+/icre}$ (*Prickle1*$^{d/d}$) conditional knockout mouse model where *Prickle1* is ablated in the endometrial epithelium. Histological analysis of uteri from *Prickle1*$^{d/d}$ mice showed changes to cell organization, extracellular matrix composition, increased atypia, and endometrial cell polarity. Expression and localization of E-cadherin was altered significantly in the cKO mice with prominent basal localization instead of the normal apico-lateral pattern. In addition, fertility of *Prickle1*$^{d/d}$ female mice was significantly reduced with litter size of 5.70 (n=23) compared to 9.36 (n=14) in controls ($p<0.0001$). Moreover, *Prickle1*$^{d/d}$ mice show activation of the PI3K-AKT/mTOR signaling pathway in the uterus, consistent with observations from human samples of endometrial hyperplasia. Lastly, using single-cell RNA sequencing analysis, we provide evidence for alterations in endometrial epithelial gene expression in the absence of *PRICKLE1.*

Modeling Trophoblast Development in Trisomy 21 using Patient-Specific Human Trophoblast Stem Cell lines (human TSCs). Avishek Ganguly[1], Ananya Ghosh[1], Soma Ray[1], **Abhik Saha[1]**, Angela Martin[2], Charles Gibbs[2] and Soumen Paul[1]. [1]Department of Pathology and Laboratory Medicine and Institute for Reproduction and Perinatal Research, [2]Department of Obstetrics and Gynecology, University of Kansas Medical Center, 3901 Rainbow Boulevard Kansas City, KS 66160, USA

Trisomy 21 is the most prevalent genetic abnormality associated with pregnancy. The outcome of pregnancy with Trisomy 21 is heterogenous. Many of those pregnancies need medical termination, whereas babys born with trosomy 21 suffer from Down Syndrome, associated with physical and mental disabilities and shorten life expectancy. However, how Trisomy 21 affects trophoblast development/function and placentation is almost unknown. Fortunately, the recent success in deriving true human trophoblast stem cells (**human TSCs**) from villous cytotrophoblast cells (**CTBs**) have opened up new strategies for direct assessment of early human placentation process in pregancieis that are associated with genetic abnormalities. The Trisomy 21 of a developing fetus is often tested via chorionic villous sampling (**CVS)**. In this study, we used a novel strategy, in which we isolated CTBs from residual tissues from CVS and establish patient-specific human TSC lines to study how Trisomy 21 affects trophoblast development. We used congenic (**from same parent**) euploid TSC lines as control thereby limiting the genetic variance in TSC lines to better understand the effect of Trisomy 21 on CTB self-renewal vs. their differentiation to syncytiotrophoblast (**STB**) or extravillous trophoblast (**EVT**). Intriguingly, our analyses with TSC lines revealed that Trisomy 21 affects both self-renewal and EVT differentiation in first-trimester CTBs. Furthermore, our unbiased gene expression analyses show that Trisomy 21 induces expression of human leucocyte antigen-A (**HLA-A**) in human trophoblast cells, thereby making them a target for maternal immune system.

In conclusion, our study made following advancements. First, we provide evidence that residual tissues from CVS, a common medical practice to test genetic abnormalities in a developing fetus, could be utilized to establish patient-specific TSC lines for studying trophoblast development. Second, we show that Trisomy 21 could affect trophoblast development by inhibiting CTB proliferation and their differentiation to EVT. Finally, we also show that Trisomy 21 could induce induction of HLA-A, thereby, altering the maternal immune tolerance to the developing placenta/fetus.

Saturated free fatty acids induce trophoblast lipoapoptosis mediated by endoplasmic reticulum stress. **Prakash K. Sahoo** and Sathish Kumar Natarajan. Depart of Nutrition and Health Sciences, University of Nebraska-Lincoln, NE.

Maternal obesity, a metabolic condition, has become a major public health concern among the women of reproductive age owing to an increased risk for the development of pregnancy complications, including gestational diabetes, pre-eclampsia, maternal inflammation, intrauterine growth retardation and large-for-gestational-age infants. Studies have also shown that maternal obesity could potentially impact the metabolic health of newborns and could increase the risk of future development of metabolic syndromes like obesity, diabetes and cardiovascular diseases in offspring. Studies in our lab showed that increased saturated free fatty acids in maternal circulation as a result of increased adipose tissue lipolysis induced trophoblast apoptosis, commonly known as lipoapoptosis. Trophoblasts exposed to physiological concentration of Palmitic (PA) and Stearic acid (SA) showed a concentration dependent increase in cell cytotoxicity and caspase 3/7 activity. While PA and SA increased trophoblast cytotoxicity, Oleic acid (OA), a monounsaturated fatty acid protected cell against PA and SA induced cell cytotoxicity. We showed increased cleaved caspase 3 and PARP levels in cells treated with PA and SA. To further characterize the mechanism behind cell cytotoxicity, we hypothesize that upon exposure to trophoblasts, free fatty acids induces organelle stress, in particular endoplasmic reticulum stress (ER stress) which could potentially mediate cell cytotoxicity by apoptosis. Initial results show that PA induces phosphorylation of IRE1α, an important mediator of ER stress at different time points in JEG-3 cells.

Single Cell Interrogation of the Uterine-Placental Interface

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During a healthy pregnancy, a subset of trophoblast cells exit the placenta and invade into the uterus where they restructure the uterine parenchyma and facilitate remodeling of the uterine spiral arteries. Invasive trophoblast cells help anchor the placenta, modulate immune cell populations, and facilitate nutrient delivery to the fetus. These trophoblast-directed uterine modifications are essential for a healthy pregnancy. Insufficient trophoblast invasion and abnormal cross-talk at the uterine-placental interface are major contributors to obstetrical complications, such as early pregnancy loss, preeclampsia, intrauterine growth restriction, and pre-term birth. In vitro analyses can provide insights into trophoblast cell potential but fall short as tools to understand the physiology of the invasive trophoblast cell lineage. Implementation of in vivo models to test hypotheses regarding mechanisms underlying the development and function of the invasive trophoblast cell lineage are essential to advance the field. Rodents exhibit hemochorial placentation similar to humans. While the mouse displays shallow trophoblast invasion, the rat exhibits deep intrauterine trophoblast invasion and extensive uterine spiral artery remodeling, comparable to what is observed in the human.

In this study, we sought to phenotype cells at the uterine-placental interface of the rat with the goal of identifying conserved candidate regulators of the invasive trophoblast cell lineage. Single cell RNA sequencing (scRNA-seq) and single nuclei assay for transposase-accessible chromatin sequencing (snATAC-seq) were used to interrogate cells at the uterine-placental interface on gestation days 15.5 and 19.5 of the Holtzman Sprague-Dawley rat. Single cell/nuclei suspensions were prepared by enzymatic digestion of the uterine-placental interface. Libraries were then constructed using the 10X Genomics platform and sequenced with an Illumina NovaSeq 6000 system. Computational analysis led to the identification of a number of unique cell clusters defined by their transcript profiles, including trophoblast, endothelial, vascular smooth muscle, natural killer, and macrophage cell clusters. To validate our findings, we performed in situ hybridization for transcripts within the rat uterine-placental interface. Spatial transcript distributions indicated the existence of subpopulations of some cell populations, including invasive trophoblast cells. Distributions of endothelial cell, macrophage, natural killer, and smooth muscle cells within the uterine-placental interface were also determined, including their positional relationships with invasive trophoblast cells. Analysis of scATAC-seq data identified unique clusters based on chromatin accessibility, including trophoblast, endothelial cell, vascular smooth muscle, natural killer cell, and macrophage clusters. ASCL2, AP1, and TFAP2C DNA binding motifs were most abundant in accessible regions of the trophoblast cell clusters.

These findings provide a foundational dataset to identify and interrogate key conserved regulatory mechanisms essential for development and function of an important compartment within the hemochorial placentation site that is essential for a healthy pregnancy. [Supported by HD020676, HD096083, HD099638, HD104495; Pew Charitable Trust, Sosland Foundation]

Importance of Aryl Hydrocarbon Receptor Signaling in Trophoblast Cell Differentiation

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The primary interface between mother and fetus, the placenta, serves numerous functions, including transport of nutrients and waste products, hormone secretion, and immunoprotection of the genetically distinct fetus. Aryl hydrocarbon receptor (AHR) signaling is activated by environmental and endogenous compounds that can affect cellular and molecular processes, including those controlling the development and function of the hemochorial placenta. In this study, we investigate the impact AHR activation on the development and function of human trophoblast stem (TS) cells. Human and rat TS cells were used to investigate the effects of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), a classic exogenous AHR ligand, on TS cell lineage development. Activation and loss-of-function manipulations were used to investigate the role of TCDD in AHR signaling. Transcriptional activation of CYP1A1 and CYP1B1 were used as an indicator of AHR signaling. Effects on trophoblast development were determined by assessing TS cell morphology, differentiation (extravillous trophoblast and syncytiotrophoblast), and transcriptomic analysis using RNA-sequencing (RNA-seq). TCDD induced CYP1A1 and CYP1B1 expression in human TS cells maintained in the stem cell state or induced to differentiate into extravillous trophoblast cells or syncytiotrophoblast. Activation of AHR signaling was dependent on the concentration of TCDD. Exposure to TCDD did not interfere with the maintenance of the stem state or trophoblast cell differentiation but did impact the trophoblast cell transcriptome. Inhibition of AHR expression using specific short hairpin RNAs disrupted TCDD-dependent activation of CYP1A1 and CYP1B1 in human TS cells. TCDD also induced the 2-methoxy estradiol (2ME) production in TS cells. TCDD was ineffective in activating CYP1A1 or CYP1B1 in rat TS cells. The latter findings are consistent with the lack of TCDD responsiveness of trophoblast cells within the rat placenta. The results of this study expand our understanding of the functions of AHR signaling in trophoblast cell development. Activation of AHR signaling does not have profound direct effects on trophoblast cell development; however, in a physiological context AHR activation does regulate the production of ligands (e.g. 2ME) with potential actions on the uterine interface, including the vasculature. [Supported by the KUMC BRTP and K-INBRE (VS), Sosland Foundation and NIH: HD020676, ES028957, ES029280, and HD099638]

Uterine lumen fluid is metabolically semi-autonomous.

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Proper uterine lumen fluid (ULF) biochemical composition regulation is central to successful pregnancy establishment and maintenance, in addition to offspring wellbeing during adulthood. The current dogma is that ULF culminates from endometrial glandular epithelial cell secretions, luminal epithelial cell turnover, transudate from the underlying vasculature, and substrate turnover by the embryo during pregnancy. Our hypothesis is that an additional mechanism is at play – that select metabolic pathways are active *within* ULF owing to enzymatic activities, rendering ULF composition metabolically semi-autonomous. To test this, ULF was collected from cyclic dairy heifers on Days 12 (n=5) and 16 (n=4) of the estrous cycle by lavage with phosphate-buffered saline, aliquoted, and incubated for various durations, prior to high-throughput untargeted semi-quantitative metabolomic profiling. Indeed, metabolite flux was observed in ULF on both days, confirming the hypothesis, that the biochemical makeup of ULF is semi-autonomously dynamic. Cumulatively, the concentrations of 17 metabolites (5.2% of total) changed ($P \leq 0.05$) during incubation, with a further 16 trending ($P \leq 0.10$) toward significant flux. Subsequent integrative and bioinformatic pseudotime analyses of these, and existing ULF proteomic and metabolomic, data were performed to predict the specific reactions underpinning this phenomenon. Key reactions revolving around glucose, energy substrate, polyamine, and cofactor metabolism were identified. Moreover, within the ULF from each heifer, the *total* concentration of metabolites remained unchanged across all incubation durations, and the mean (± S.D.) total protein across ULF aliquots was 2.0 ± 1.6 $\mu g \cdot ml^{-1}$ (n=9) – orders of magnitude lower than would be expected from cell lysis; cumulatively confirming that the observed semi-autonomy of ULF is independent of external influences. Thereafter, to test whether this metabolic semi-autonomy of ULF may facilitate maternal-embryo dialogue, *in vitro*-produced embryos were transferred into the uteri of the same heifers from which lavages were previously obtained, and pregnancies diagnosed by transrectal ultrasonography on Day 30. Comparing the original metabolomic profiles of ULF from animals subsequently exhibiting pregnancy loss *vs*. those with posterior healthy pregnancies by hierarchical cluster analysis, suggests that the degree of ULF metabolic semi-autonomy is causally linked to fertility. These data enhance our understanding of the biochemical mechanisms leading to pregnancy establishment, with implications for improving fertility and pregnancy outcomes in agriculturally relevant species as well as women. This research was supported by The NICHD (R01-HD096266), USDA-AFRI (2019-67015-28998), and USDA (2019-38420-28972).

Involvement of FSTL3 in trophoblast cell lineage development and placentation.

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Maternal and extraembryonic cells interact at the uterine-placental interface during gestation to facilitate critical adaptations for fetal growth and development. Extraembryonic trophoblast stem (**TS**) cells differentiate and invade into the uterus where they critically contribute to uterine vascular remodeling. Failure of invasive trophoblast cells to invade and remodel uterine spiral arteries leads to significant obstetrical complications including early pregnancy loss, preeclampsia, intrauterine growth restriction, and preterm birth. Little is known about the mechanisms driving invasive trophoblast cell lineage development. The goal of this study was to investigate the biology of follistatin like 3 (**FSTL3**) as a putative, conserved regulator of invasive trophoblast cell lineage development and placentation. FSTL3 is a known regulator of the actions of a subset of transforming growth factor beta (**TGFB**) cytokines. In our experimentation, we utilized human TS cells and the rat as in vitro and in vivo models, respectively. Human TS cells can be manipulated to differentiate into invasive trophoblast cells, which are known as extravillous trophoblast (**EVT**). The rat was selected as a model because, like the human, it exhibits deep intrauterine trophoblast cell invasion. To identify candidate regulators of the invasive trophoblast cell lineage, our lab performed single-cell RNA sequencing (**scRNA-seq**) of the rat uterine-placental interface. We identified FSTL3 as a transcript uniquely expressed in invasive trophoblast cells. These findings were confirmed by in situ hybridization of the rat placentation site, where we also found the labyrinth zone positive for *Fstl3*. In first trimestser human placentation sites, FSTL3 was expressed in the EVT cell column, the site of invasive trophoblast progenitors. In vitro, human TS cells showed a robust induction of *FSTL3* expression following differentiation into invasive EVT cells. FSTL3 loss of function models were generated in human TS cells using short hairpin RNAs and in the rat using CRISPR/Cas9 genome editing. Structural and functional parameters of EVT cell development were assessed in control and FSTL3 knockdown human TS cells. In the rat, a 3243 bp deletion affecting Exons 1-3 in the *Fstl3* locus resulted in a frameshift and a null mutation. The *Fstl3* mutation was successfully transmitted through the germline. *Fstl3* null placentas exhibited evidence of structural abnormalities. In summary, we have established model systems for the evaluation of FSTL3 in trophoblast cell lineage development and hemochorial placentation. These efforts implicate FSTL3 in the regulation of placental morphogenesis. [Supported by a Lalor Foundation fellowship (AM), an NRSA predoctoral F31HD104495 (RLS), an NRSA postdoctoral F32HD096809 (KMV), HD020676; HD096083, HD099638, Pew Charitable Trust, and the Sosland Foundation]

Autophagy promotes DNA damage repair machinery in mouse oocytes. **Fei Sun**, Ahmed Z. Balboula. Division of Animal Science, University of Missouri, Columbia, MO

DNA damage, specifically DNA double strand breaks (DSBs), is a major problem that leads to mutagenesis and premature aging in somatic cells and aneuploidy in female gametes (oocytes). In mitotic cells, the exposure to mild DNA damage is sufficient to induce cell cycle arrest, necessary to provide enough time for DNA damage repair. In contrast, mammalian oocytes can progress through meiosis while having a moderate level of DNA damage, leading to the development of aneuploid gametes. Surprisingly, the DNA damage response is further weakened in oocytes from aged females *vs.* young females, raising the necessity to understand this major problem in mammalian oocytes whose maturation is notoriously prone to mistakes. Using time-lapse live imaging and super-resolution microscopy we investigated the consequences of DNA damage during meiosis I. Induction of DNA DSBs in full-grown germinal vesicle (GV) oocytes by etoposide (50 µg/ml) or UV-radiation increased γ-H2AX (phosphorylated H2AX, a marker of DNA damage) relative to controls. Further analyses revealed that inducing DNA damage in GV oocytes did not affect spindle morphology, but perturbed meiotic progression and increased chromosome misalignment. Importantly, we found that metaphase I chromosome morphology is altered in DNA-damaged oocytes. In contrast to the normal bivalent structure of chromosomes in controls, etoposide-treated oocytes had chromosomes that were abnormally compact (83.4% *vs* 0% in controls, $p<0.02$) with clear signs of DNA fragmentation (77.05 % *vs* 0% in controls, $p<0.01$). In mitotic cells, reduced ubiquitinated histones leads to chromatin architecture alteration and the inability of DNA damage repair proteins to access damaged DNA foci. Recent evidence suggests that autophagy promotes chromatin ubiquitination and modulates several events to regulate DNA damage repair machinery in mitotic cells. Interestingly, we found that autophagy activity is significantly reduced in DNA-damaged oocytes. Combined DNA DSBs induction and autophagy inhibition (by Spautin-1) increased γ-H2AX levels and exaggerated chromosome compactness and DNA fragmentation phenotypes when compared to control or etoposide-treated groups. Importantly, induction of autophagy (using rapamycin) in DNA-damaged oocytes (by etoposide or UV-radiation) significantly increased ubiquitinated H2A, decreased γ-H2AX, and subsequently, rescued the aforementioned phenotypes (perturbed meiotic progression, DNA fragmentation and compact chromatin) when compared to DNA-damaged groups. Recent data suggest that the level of DNA damage in oocytes from aged mice is higher than that in oocytes from young mice. Given that autophagy is a hallmark of aging and decreased in oocytes from aged mice (our data), we hypothesized that aging-associated reduced autophagy is the cause, at least in part, of increased DNA damage levels in aged oocytes. Expectedly, oocytes obtained from aged mice had more DNA damage (γ-H2AX signals) compared to those obtained from young mice. Importantly, autophagy induction, but not autophagy inhibition, greatly rescued elevated DNA damage in aged oocytes. To our knowledge, these results provide the first evidence that autophagy plays a critical role against DNA damage in mouse oocytes and its induction is a promising approach to ameliorate DNA damage-induced alterations in aged oocytes.

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Global Epigenome and Transcriptome Mapping of Human Trophoblast Cells Across Lineage Development

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Placental trophoblast cells, specifically extravillous trophoblast (**EVT**), are essential for placentation, spiral artery remodeling, and successful pregnancy. However, our knowledge of transcriptional regulation driving EVT cell development is limited. We applied short- and long-read sequencing to map the transcriptome and epigenome of human trophoblast stem (**TS**) cells and their transition into the differentiated EVT cell lineage. Using this approach, we identified that chromatin accessibility in the promoter region was more robust in human TS cells in the stem state then following their differentiation to EVT cells. Motif enrichment performed within accessible regions identified transcription factor binding motifs common to each developmental cell state and other transcription factor binding motifs unique to the stem state or to EVT cells. Higher order three-dimensional organization of the genome and gene regulatory interactions was achieved through Hi-C chromatin conformation sequence analysis. Integration of chromatin accessibility and conformation, transcriptomic, DNA methylation, and transcription factor binding motif analyses in human TS and EVT cells enabled identification of transcription factors and regulatory mechanisms associated with EVT cell lineage development. Collectively, we have revealed activation of a dynamic regulatory network that provides a framework for understanding EVT cell specification in trophoblast cell lineage development and human placentation.

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Dynein regulates cytoplasmic microtubule organizing centers and spindle positioning during oocyte meiosis

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The fidelity of oocyte meiosis is critical for generating euploid eggs. Any error during oocyte meiosis I (MI) can result in aneuploidy, the leading genetic cause of miscarriage and congenital abnormalities. The oocyte at the diplotene stage of prophase I undergoes a lengthy arrest. After puberty and upon meiotic resumption, the nucleus membrane is degraded (nuclear envelope breakdown, NEBD) and the spindle is formed. The meiotic spindle lacks centrosome-containing centrioles, the main regulator of spindle organization in mitotic cells. Alternately, mouse oocyte contains numerous MT organizing centers (MTOCs) that are clustered together at spindle poles to build a bipolar spindle. We recently identified a new subset of MTOCs that are located in the cytoplasm of metaphase I oocytes and never contributed to spindle pole formation; termed metaphase cytoplasmic MTOCs (mcMTOCs). We found that depleting or perturbing mcMTOCs in mouse oocytes results in abnormal spindle positioning and aneuploidy. Importantly, these mcMTOCs nucleate MTs that connect the spindle to the cortex, necessary for spindle positioning and timely spindle migration. These results indicate that MTs play an important role in regulating meiotic spindle positioning, challenging the current paradigm that F-actin is the only cytoskeletal component responsible for regulating spindle positioning in mouse oocytes. However, the underlying molecular mechanism by which MTs regulate spindle positioning remains largely unknown. MT motors are ATPase-associated proteins that move along the length of MTs and play important roles in regulating MT dynamics. There are two main MT motor classes: kinesins which are mainly MT plus-end directed and dynein which is MT minus-end directed. Using immunocytochemistry, we found that dynein localizes to mcMTOCs, polar MTOCs and MT connections in metaphase I oocytes. Treating oocytes at NEBD with dynein inhibitors (dynarrestin or dynapyrazole-A) inhibited dynein-MT binding and increased the number of mcMTOCs relative to DMSO-treated oocytes. Importantly, time-lapse tracking of the spindle revealed that dynein inhibition perturbs spindle positioning, induces precocious spindle migration and causes early polar body extrusion, compared to controls. Notably, dynein inhibition during MI resulted in an increased incidence of aneuploidy. These results implicate dynein as a novel regulator of mcMTOCs and spindle positioning in mouse oocytes. This research was supported by laboratory start-up funding from the University of Missouri to AZB.

Effects of PD-1 blockade on ovarian follicles in a prepubertal female mouse.

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Immunotherapy has emerged at the forefront of cancer treatment. Checkpoint inhibitor pembrolizumab (KEYTRUDA), a chimeric antibody which targets programmed cell death protein 1 (PD-1), has been approved by the FDA for use in pediatric patients with relapsed or refractory classical Hodgkin's lymphoma. However, there is currently no published data regarding the effects of pembrolizumab on the ovary of female pediatric patients. In this study, prepubertal immunocompetent and immunodeficient female mice were injected with pembrolizumab or anti-mouse PD-1 antibody. The number of primordial follicles significantly decreased post-injection of both pembrolizumab and anti-mouse PD-1 antibody in immunocompetent mice. However, no changes in follicle numbers were observed in immunodeficient nude mice. Superovulation test and vaginal opening experiments suggest that there is no difference in the number of COCs and the timing of puberty onset between the control and anti-mouse PD-1 antibody treatment groups, indicating that there is no effect on short-term fertility. Elevation of pro-inflammatory cytokine TNF-α following COX-2 upregulation was observed in the area that primordial follicles generally occupy inside the ovary. $CD3^+$ T-cell infiltration was detected within some ovarian follicles and between stromal cells of the ovaries in mice following treatment with anti-mouse PD-1 antibody. Thus, PD-1 immune checkpoint blockade affects the ovarian reserve through a mechanism dependent on inflammation following $CD3^+$ T-cell infiltration.

Metabolic and microenvironmental features of granulosa cell tumors in PIK3CA* mice. **Seok-Yeong Yu**, Yi Luan, Pauline Xu, and So-Youn Kim**.** Olson Center for Women's Health, Department of Obstetrics and Gynecology, College of Medicine, University of Nebraska Medical Center, Omaha, NE

Granulosa cell tumor (GCT) in prepubertal girls and adults results from the abnormal proliferation of granulosa cells in preovulatory follicles and exhibits increased lipid accumulation, fibrosis and low macrophage infiltration. However, little has been appreciated regarding whether such microenvironmental and metabolic disturbances support GCT development due perhaps to few *in vivo* models available that resemble human GCT. PIK3CA* female mice (*Gdf9-iCre+;Pik3ca*/w*, Cre+) spontaneously develops GCT in postnatal day 65 (PD65). Here, we investigated the temporal expression of genes related to GCT and metabolism, employing transcriptome analyses with ovaries at PD8, PD50 and PD65 using *Gdf9-iCre-;Pik3ca*/w* (Cre-) and Cre+ mice. We found that Cre+ mice had significantly higher gene expression related to anti-inflammatory macrophages (*Arg1* and *Chil3*), fibrosis (*Timp1*, *Fn1* and *Col1a1*), and cell proliferation (*Cdk4* and *5*) between PD8 and PD65 compared to Cre-. In addition, when compared to Cre- at PD65, Cre+ mice had lower fasting blood glucose levels, and oral glucose tolerance test revealed that glucose utilization was quicker in Cre+ mice at PD80. Concomitantly, the expression of glycolysis-related genes (*Hk2*, *Pfkl* and *Idh*a) was remarkably higher in Cre+ mice than Cre- at PD65. Further, genes related to oxidative phosphorylation were notably upregulated in Cre+ mice when compared with Cre-. Regarding lipid metabolism, lipid oxidation-related genes were significantly downregulated in Cre+ mice, but the expression of genes related to lipid uptake and synthesis (*Cd36* and *Dgat2*) was considerably upregulated at PD65. Consistently, the protein expression of PPARγ and DGAT2 was increased in Cre+ at PD65, and the nuclear localization of PPARγ immunofluorescence corroborates that lipid metabolism of GCTs in this model favors lipid synthesis over oxidation. Our data indicate that PIK3CA* GCT mouse model exhibits the upregulation of genes for fibrosis, cell proliferation, glycolysis and lipid accumulation as evidenced in GCT patients. Such abnormalities, thus, would be considered for potential therapeutic targets for GCTs, supported by our PIK3CA* GCT mouse model which shows the progressive development of GCT.

Maternal *Smc3* protects the integrity of the zygotic genome through DNA replication and mitosis

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Aneuploidy is frequently observed in oocytes and early embryos, begging the question of how genome integrity is monitored and preserved during this critical period. Structural maintenance of chromosomes protein 3 (SMC3) is a subunit of the cohesin complex that supports genome integrity, but its role in maintaining the genome in this window of mammalian development is unknown. Here we hypothesize that maternal SMC3 supports genome integrity and chromosome segregation in oocytes and early embryos. Using mouse genetics, we discovered that although depletion of *Smc3* in oocytes following meiotic S phase did not impact meiosis, adult *Smc3* conditional knockout female mice were infertile. While other cohesin related genes are necessary for epigenetic reprogramming and gene expression during embryogenesis, we provide evidence that SMC3 is a novel maternal effect gene with essential functions in protecting the integrity of chromosomes in the zygote. DNA lesions accumulated following S phase in SMC3-deficient zygotes, followed by mitosis with lagging chromosomes, elongated spindles, micronuclei, and arrest at the 2-cell stage. Remarkably, although centromeric cohesion was defective, the dosage of SMC3 was sufficient to enable embryogenesis in juvenile mutant females. Our findings demonstrate that despite of high rates of aneuploidy are tolerated in early embryos, chromosome missegregation at 2-cell stage prevents embryogenesis and maternal SMC3 serves as a key protector of the zygotic genome.

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