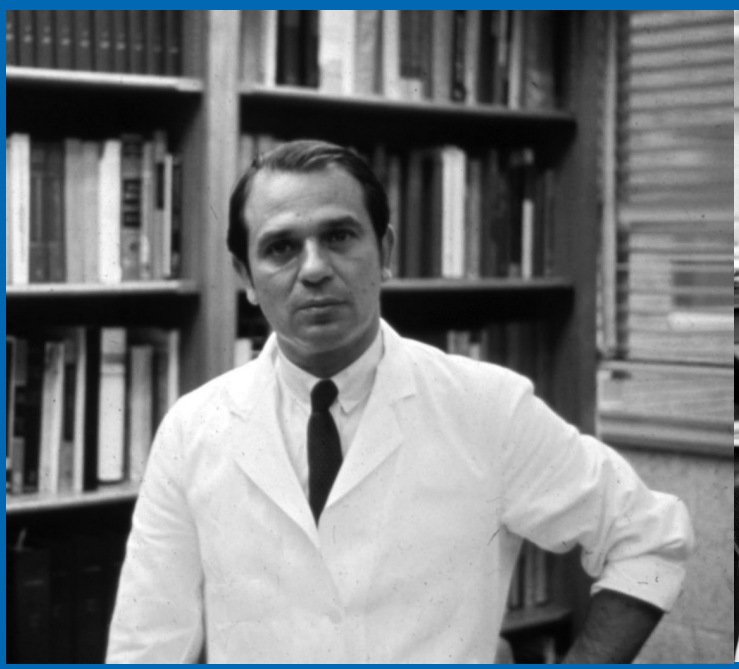
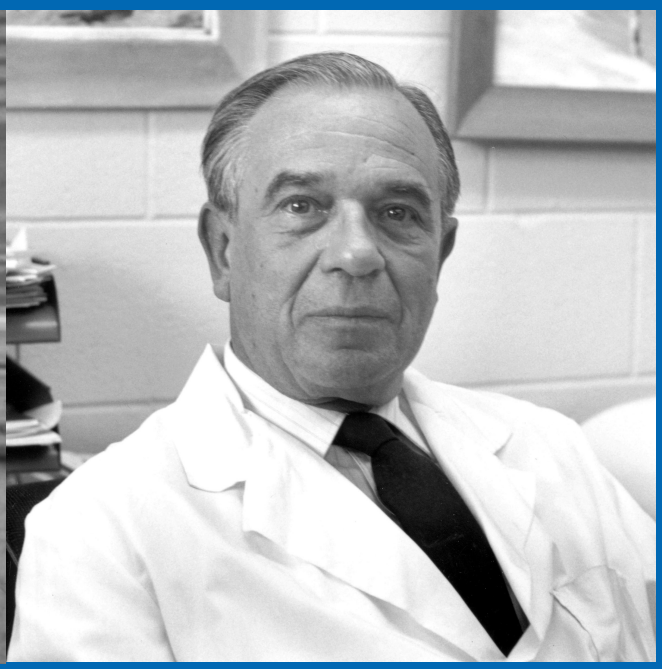
KU MEDICAL CENTER
The University of Kansas

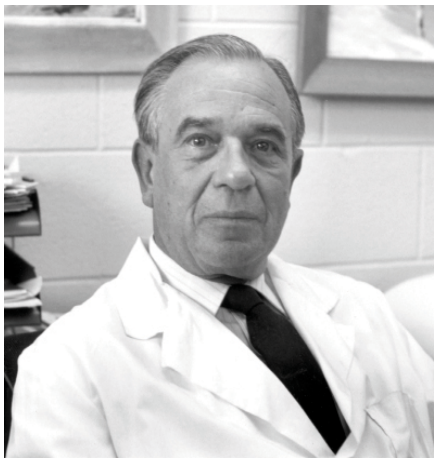
The 12th Annual Gilbert S. Greenwald Symposium on Reproduction and Regenerative Medicine

October 22-23, 2015





Biography - Gilbert S. Greenwald



The Institute for Reproductive Health and Regenerative Medicine at the University of Kansas Medical Center hosts the Annual Gilbert S. Greenwald Symposium on Reproduction in honor 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.

TABLE OF CONTENTS

Sponsors & Volunteers

Sincere thanks to our generous sponsors and volunteers for making this event possible.

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KU Cancer Center
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Susmita Jasti, PhD (Trainee Representative)
Senior Scientist, Anatomy and Cell Biology

EVENT SUPPORT STAFF: **Institute for Reproductive Health and Regenerative Medicine**
Lesley Shriver, Senior Administrative Assistant
Stacy McClure, Associate Director of Administration

Symposium History

Plenary Speakers

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
Evanston, IL

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

John Peluso, PhD
University of Connecticut
Farmington, CT

Miles Wilkinson, PhD
MD Anderson Cancer Center
Houston, Texas

Nasser Chegini, PhD
University of Florida
Gainesville, Fl

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

2009 *(continued)*

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

Symposium History

Plenary Speakers

2011

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

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

2012

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

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 Lecture

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

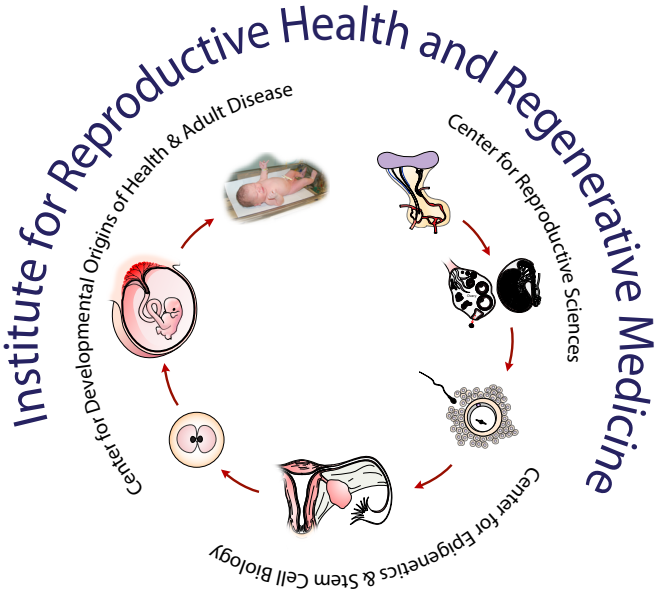
W. Lee Kraus, PhD, University of Texas Southwestern
Keynote Lecture

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



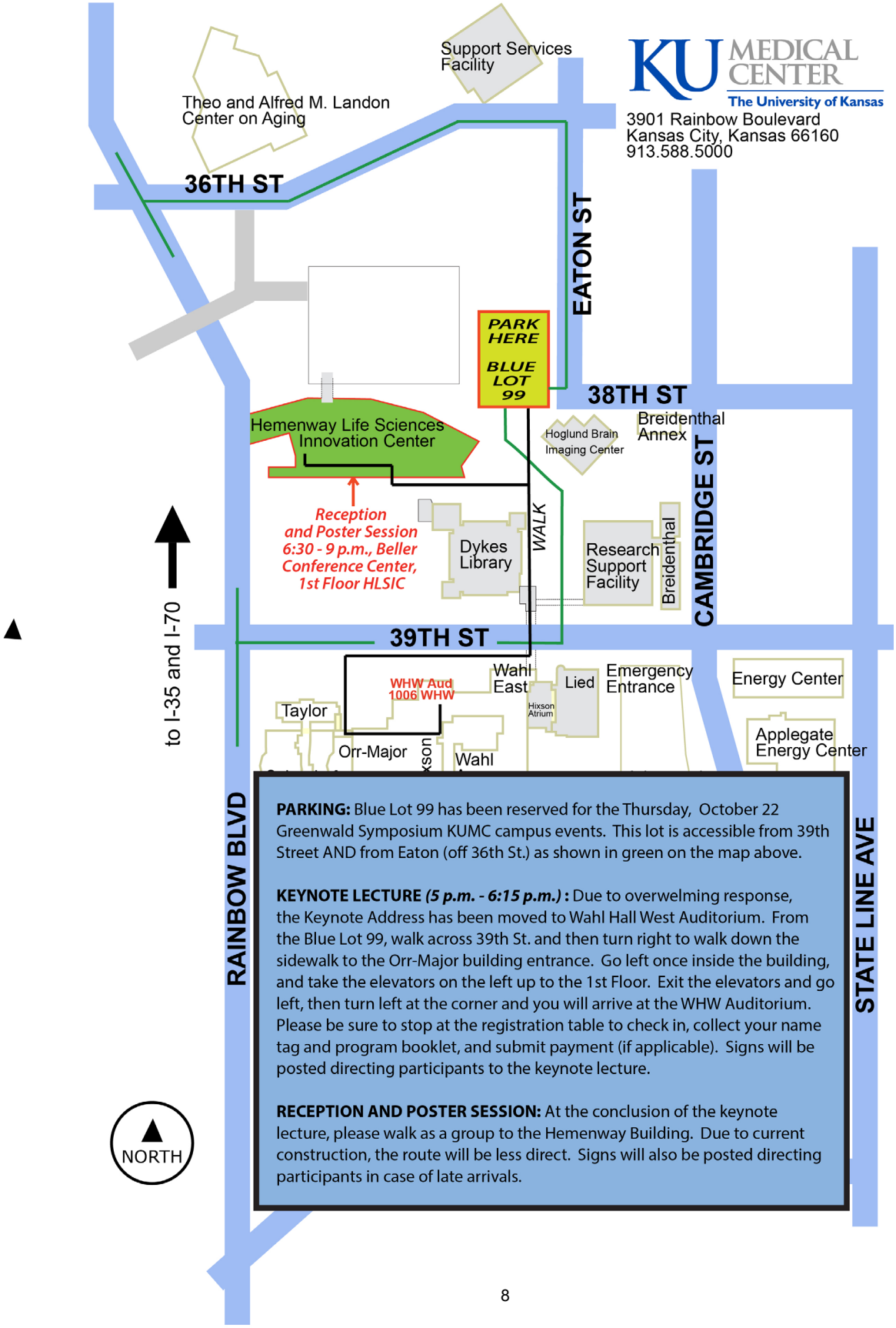
Program Schedule

THURSDAY, OCTOBER 22nd	**University of Kansas Medical Center** **3901 Rainbow Blvd., Kansas City, KS 66160**
4:30 - 5:00 p.m.	**Registration, Wahl Hall West Auditorium** (NOTE LOCATION CHANGE)
5:00 - 5:02 p.m.	**General Welcome, T. Rajendra Kumar, PhD, CRS Director**
5:02 - 5:05 p.m.	**Welcome/Opening Remarks, Peter Smith, PhD, Senior Associate Dean for Research**
5:05 - 5:08 p.m.	**Brief History of the Greenwald Symposium/Dr. Greenwald, Paul F. Terranova, PhD, Emeritus Professor**
5:08 - 5:10 p.m.	**Introduction of Keynote Lecturer, T. Rajendra Kumar, PhD, Center for Reproductive Sciences (CRS) Director**
5:10 - 6:15 p.m.	**Keynote Lecture - Bert W. O'Malley, MD, Baylor College of Medicine** *"Structure-Function Relationships of the Estrogen Receptor-Coactivator Complex"*
6:30 - 9:00 p.m.	**Reception**, Beller Conference Center, 1005-1009 Hemenway Building
7:00 - 9:00 p.m.	**Poster Session**, Beller Conference Center, 1001-1003 Hemenway Building
FRIDAY, OCTOBER 23rd	**Kansas City Public Library - Central (Downtown)** **14 West 10th St., Kansas City, MO 64108** **Helzberg Auditorium, 5th Floor** *(Parking garage located on NW corner of 10th & Baltimore, just West of library -* ***BRING IN YOUR PARKING TICKET****)*
8:00 - 8:30 a.m.	**Breakfast**
8:30 - 8:35 a.m.	**Introductory Remarks**, **T. Rajendra Kumar, PhD, CRS Director**
Session I	**GENETICS AND EPIGENETICS OF EARLY EMBRYO DEVELOPMENT**
8:35 - 9:20 a.m. *(Q&A 9:10-9:20 a.m.)*	**Scott Coonrod, PhD, Cornell University** *(Susmita Jasti, PhD, introducing)* *"Role of PAD Enzymes in Early Development, Estrogen Signaling, and Cancer Progression"*
9:25 - 9:55 a.m. *(Q&A 9:50-9:55 a.m.)*	**Amy Ralston, PhD, Michigan State University** *(Pavla Brachova, PhD, introducing)* *"Cell Fate Decisions in the Early Mouse Embryo"*

Program Schedule

10:00 - 10:15 a.m. *(Q&A 10:12-10:15 a.m.)*	**Lei Wang, PhD, Postdoctoral Fellow, University of Kansas Medical Center** *(Francesca Duncan, PhD, introducing)* *"The histone H3K9 methyltransferase Suv39h2 participates in the maintenance of the trophoblast stem cell state"*
10:15 - 10:35 a.m.	**Morning Break**
Session II	**MECHANISMS OF GONADAL DEVELOPMENT AND FUNCTION**
10:35 - 11:20 a.m. *(Q&A 11:10-11:20 a.m.)*	**Wei Yan, MD, PhD, University of Nevada School of Medicine** *(Khursheed Iqbal, PhD, introducing)* *"Roles of Small Noncoding RNAs in Gametogenesis and Transgenerational Epigenetic Inheritance"*
11:25 - 11:55 a.m. (Q&A 11:50-11:55 a.m.)	**James A. MacLean, PhD, Southern Illinois University** *(Sara Pearson, MS, introducing)* *"Recent Insights into RHOX Transcription Factor Action in Gonad Development"*
12:00 - 12:15 a.m. *(Q&A 12:12-12:15 p.m.)*	**Xiangmin Lv, MS, Graduate Student, University of Nebraska Medical Center** *(Warren Nothnick, PhD, introducing)* *"Yes- Associated Protein 1 (YAP) regulates ovarian follicle development and transformation of granulosa cells"*
12:15 - 1:30 p.m.	**Lunch**
1:30 - 1:40 p.m.	**Trainee Poster Award Presentation, T. Rajendra Kumar, PhD**
Session III	**MECHANISMS OF UTERINE DEVELOPMENT AND PATHOLOGY**
1:45 - 2:30 p.m. (*Q&A 2:20-2:30 p.m.)*	**Robert N. Taylor, MD, PhD, Wake Forest School of Medicine** *(Pramod Dhakal, DVM, PhD, introducing)* *"In the Beginning: Cellular Behavior of Nascent Endometriosis"*
2:35 - 3:05 p.m. *(Q&A 3:00-3:05 p.m.)*	**Qinglei Li, PhD, Texas A&M University** *(Zahraa Alali, MS, introducing)* *"New Insights into the Function of TGF-beta Signaling in the Uterus"*
3:10 - 3:25 p.m. *(Q&A 3:22-3:25 p.m.)*	**Zeenat Asghar, MS, Graduate Student, Washington University - St. Louis** *(T. Rajendra Kumar, PhD, introducing)* *"Maternal fructose consumption drives placental uric acid production and leads to adverse fetal outcomes"*
3:30 p.m.	***Thank you for participating - meeting adjourned***

KUMC Campus Map



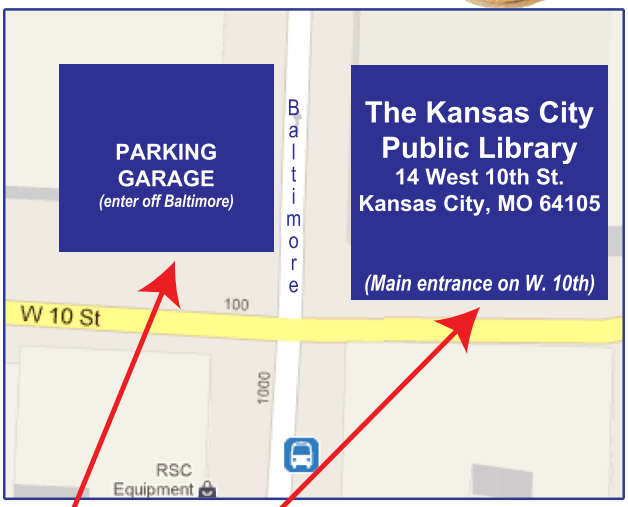
PARKING: Blue Lot 99 has been reserved for the Thursday, October 22 Greenwald Symposium KUMC campus events. This lot is accessible from 39th Street AND from Eaton (off 36th St.) as shown in green on the map above.

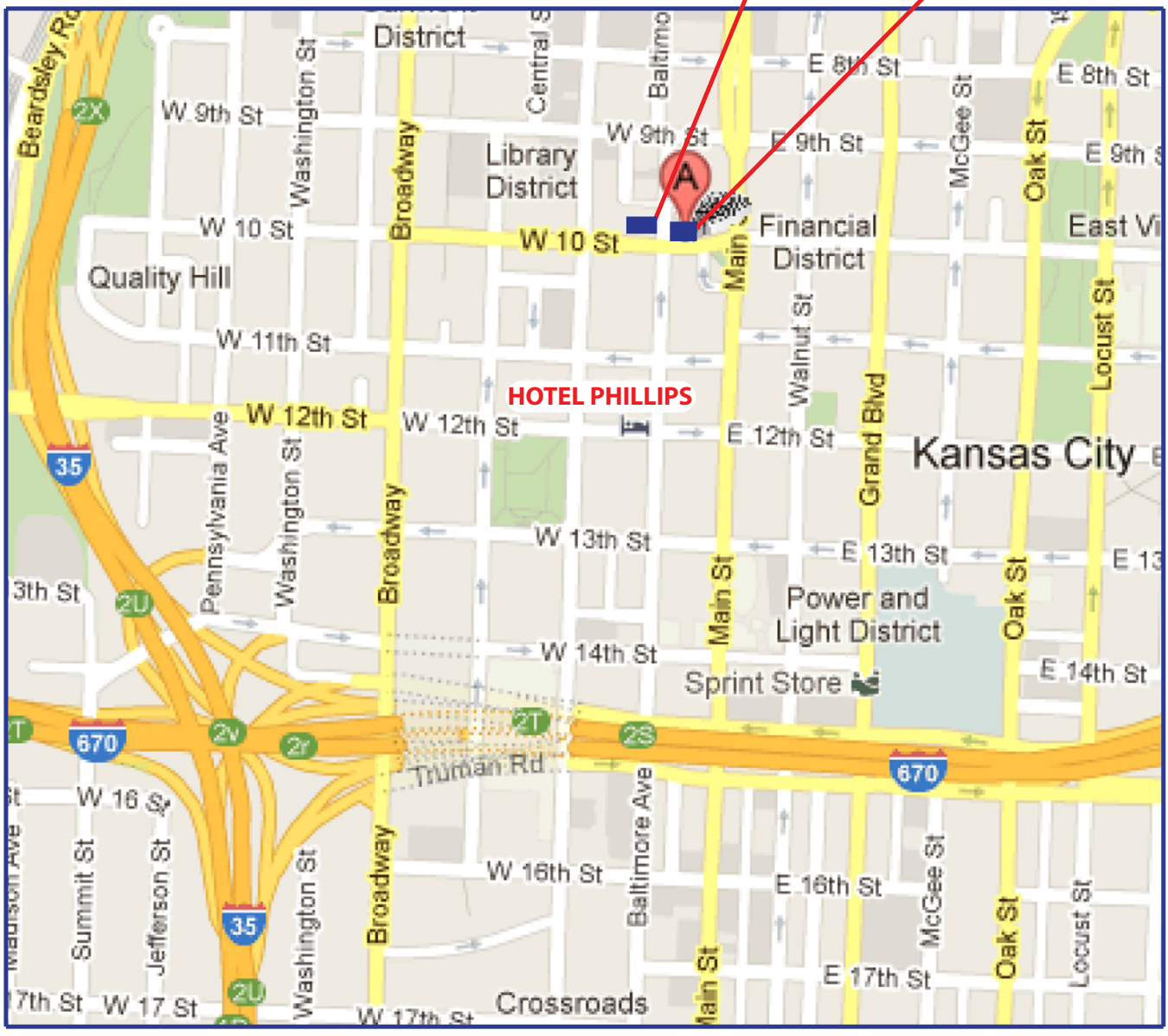
KEYNOTE LECTURE *(5 p.m. - 6:15 p.m.)*: Due to overwelming response, the Keynote Address has been moved to Wahl Hall West Auditorium. From the Blue Lot 99, walk across 39th St. and then turn right to walk down the sidewalk to the Orr-Major building entrance. Go left once inside the building, and take the elevators on the left up to the 1st Floor. Exit the elevators and go left, then turn left at the corner and you will arrive at the WHW Auditorium. Please be sure to stop at the registration table to check in, collect your name tag and program booklet, and submit payment (if applicable). Signs will be posted directing participants to the keynote lecture.

RECEPTION AND POSTER SESSION: At the conclusion of the keynote lecture, please walk as a group to the Hemenway Building. Due to current construction, the route will be less direct. Signs will also be posted directing participants in case of late arrivals.

Kansas City Map

The Kansas City Public Library - Central is located on the Northeast corner of W. 10th and Baltimore in downtown Kansas City, MO. The parking garage is West of the library, on the Northwest corner of W. 10th and Baltimore. **BE SURE TO BRING YOUR PARKING TICKET IN WITH YOU SO WE CAN VALIDATE IT FOR YOU.** Enter the library at the main entrance on W. 10th, and take the elevator to the Helzberg Auditorium on the 5th Floor.





Venue Information

The Kansas City Public Library - Central

The Kansas City Public Library system consists of a central library, nine branches, and an outreach services program serving a constituency of over 250,000 in Kansas City, Missouri. In addition to providing library services to residents, the Library also serves as a resource for the 1.7 million metropolitan residents of greater Kansas City.

In 2004, the Kansas City Public Library - Central moved into the former First National Bank building at 10th and Baltimore in downtown Kansas City, Missouri. The century-old building, a true masterpiece of craftsmanship with its marble columns, bronze doors and ornate mouldings, required remodeling and a fifth floor addition, but provided the framework for a modern and impressive urban library. The location features state-of-the-art technology, improved and increased services, meeting rooms, a screening room, a coffee shop and much more, all within the walls of a building originally constructed to convey a sense of strength and continuity. It is upon that foundation the Library places its vision for the next century to come.

Facts About the Library

•The Kansas City Public Library has ten locations.
•The Central Library is the largest facility, housing resources, special collections and administrative offices.
•More than 2,348,408 materials were checked out during the last fiscal year.
•The Inter Library Loan department loaned out 115,846 items last year to other libraries.
•2,492,118 customers used the Library system last year.
•The Library system counts 1,147,278 items in its holdings.

Helzberg Auditorium

The Library's most versatile meeting space, the Helzberg Auditorium is located on the 5th floor of the library. Helzberg is also aggressively styled with contemporary and clean lines for an energetic atmosphere, and features performance quality acoustics using cork flooring and specially designed ceiling elements, built-in AV system, and floor-to-ceiling windows on multiple sides that provide natural lighting.

Gladys Feld Helzberg

Gladys Feld Helzberg was the wife of the late Barnett C. Helzberg, Sr, of Helzberg Diamonds. Helzberg jewelry store was founded in 1915 by the late Morris Helzberg, in Kansas City, Kan., and expanded to a regional market by Barnett C. Helzberg, Sr. Gladys was an active member of the Kansas City Chapter of the Association for Women in Communications and one of the founders of Veterans' Voices. The Gladys Feld Helzberg Scholarship Fund was established in 1960 for talented journalism students and is administered by the University of Kansas endowment fund. She was also the founder of the Greater Kansas City chapter of the Brandeis Women's Committee.

Speaker Information

Keynote Lecture

Bert W, O'Malley, MD

Tom Thompson Distinguished Leadership and Service Professor and Chairman
Department of Molecular and Cellular Biology
Professor of Medicine, Neuroscience and Ob/Gyn
Co-Director, Baylor Center for Reproductive Biology
Director of the Alkek Center for Molecular Discovery
Associate Director of Basic Research, Dan L. Duncan Cancer Center
Baylor College of Medicine

"Structure-Function Relationships of the Estrogen Receptor-Coactivator Complex"

Dr. Bert O'Malley is the Tom Thompson Distinguished Leadership and Service Professor of Molecular and Cellular Biology at Baylor College of Medicine. He graduated medical school at U. Pittsburgh, followed by periods at Duke, NIH, and Vanderbilt. He was first to discover that nuclear receptors are transcription factors that regulate mRNA production in target cells in response to intracellular hormones. He uncovered mechanisms for activating steroid receptors, and discovered the existence of 'coregulators', the coactivators and corepressors of gene transcription. The coregulators turned out to be the long sought 'master regulators' of mammalian gene function. His work led to a molecular understanding of how hormonal antagonists work and had major importance to reproduction, genetic disease, metabolism, and especially cancers. Dr. O'Malley is the founding father of the field of Molecular Endocrinology and a member of the National Academy of Sciences and the Institute of Medicine, and received over 60 honors and awards for his work, including the National Medal of Science (White House, 2008). He trained over 250 scientists and published over 700 papers and holds 23 patents in the fields of Gene Regulation, Molecular Endocrinology and Steroid Receptor and Coactivator Action. He developed a later interest in the impressive cooperation of proteins in both processes or transcription and DNA replication. This interest was fueled by his recent studies of the entire coregulatsome of mammals detailing the crossover roles of many transcription/repair proteins. In very recent work, he developed the concept that small molecules can regulate coactivators to produce therapeutic outcomes for diseases such as cancer.

Session I

Scott Coonrod, PhD

The Judy Wilpon Associate Professor of Epigenetics and Cancer Biology
Baker Institute for Animal Health, College of Veterinary Medicine
Cornell University

"Role of PAD Enzymes in Early Development, Estrogen Signaling, and Cancer Progression"

Dr. Scott Coonrod is the Judy Wilpon Associate Professor of Cancer Epigenetics and Reproductive Biology at Cornell Universities' Baker Institute for Animal Health in Ithaca, New York. The Baker Institute for Animal Health is one of the oldest research centers dedicated to the study of veterinary infectious diseases, immunology, genetics, and reproduction. Prior to that, Scott was an Assistant Professor in the Department of Genetic Medicine at Weill Medical College of Cornell University in New York City. He received his B.S. in Veterinary Physiology (1984) and his Ph.D. in Veterinary Physiology (1995) from Texas A&M University. Dr. Coonrod then did his postdoctoral research at the University of Virginia under Dr. John Herr in the Department of Cell Biology. The Coonrod lab studies the role of peptidylarginine deiminase (PAD) enzymes in early development and in the epigenetics of cancer.

Early Development - Dr. Coonrod's lab discovered that the oocyte-restricted maternal protein, PAD6, is essential for female fertility and for the formation of a poorly characterized cytoskeletal structure, the oocyte cytoplasmic lattices. Surprisingly, mouse oocytes can grow and be fertilized normally in the absence of PAD6 and the lattices, but the resulting embryos arrest at the two-cell stage of development. This period of development is critical for early life because that is when the developmental program shifts from the mother to the embryo. Thus, PAD6 and the lattices are critical for the oocyte-to-embryo transition and his lab is trying to understand the nature of this requirement.

Cancer Epigenetics – More recently, the Coonrod lab has been investigating the role of PAD enzymes in chromatin structure, gene regulation, and cancer progression. In addition to packaging DNA into the nucleus, histones contain a wide array of posttranslational modifications that regulate chromatin structure and function. Several years ago his lab made the seminal discovery that a novel histone modification, termed citrulline, plays an important role in regulating the expression of estrogen receptor target genes in breast cancer cells. More recently, the lab has utilized mouse models of cancer to show that PAD enzymes, which catalyze the citrulline modification, play an important role in cancer progression.

Amy Ralston, PhD

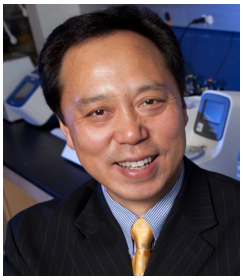
Assistant Professor, Department of Bioshemistry and Molecular Biology
Michigan State University

"Call Fate Decisions in the Early Mouse Embryo"

Amy Ralston is an assistant professor of Biochemistry and Molecular Biology at Michigan State University. Her research is focused on discovering how stem cell progenitors are established and used during development. Dr. Ralston received her B.A. in Biochemistry from Oberlin College in Oberlin, OH, and her Ph.D. in Drosophila developmental genetics from the University of Wisconsin in Madison, WI. For her postdoctoral studies, Dr. Ralston studied mouse embryology and stem cell biology with Dr. Janet Rossant at the Hospital for Sick Children in Toronto, ON.

Dr. Ralston started her own laboratory in 2009, which has been funded since 2010 by grants from the Ellison Medical Foundation and the National Institutes of Health. Studies from Dr. Ralston's lab have been published in Development, PLoS Genetics, Trends in Genetics, and Developmental Cell. Dr. Ralston's expertise, as well as her track record as an effective teacher and mentor, led to her selection as an organizer of the annual Cold Spring Harbor Laboratory course in Mouse Development, Stem Cells, and Cancer.

Session II



Wei Yan, MD, PhD

Professor, Department of Physiology and Cell Biology
University of Nevada School of Medicine

"Roles of Small Noncoding RNAs in Gametogenesis and Trangenerational Epigenetic Inheritance"

Wei Yan received his MD from China Medical University in 1990 and PhD from University of Turku, Finland in 2000. After his post-doc training with Dr. Martin M. Matzuk at Baylor College of Medicine, he started his own lab at the University of Nevada School of Medicine in 2004. He is currently Professor of Physiology, Molecular and Cellular Biology. He received the 2009 Young Investigator Award of the Society for the Study of Reproduction (SSR), the 2012 Young Andrologist Award of the American Society of Andrology (ASA) and the 2013 Nevada Healthcare Hero Award for Research and Technology. He serves as serve as an Associate Editor for Biology of Reproduction and Environmental Epigenetics. His research addresses the molecular mechanism of gametogenesis and its application in fertility control. He has published >100 peer-reviewed research articles and book chapters. His lab is currently working on coding and noncoding genes that regulate spermatogenesis, and epigenetic contribution of gametes to fertilization, early embryonic development and adulthood health.

James A. MacLean II, PhD

Associate Professor, Department of Physiology
Southern Illinois University

"Recent Insights into RHOX Transcription Factor Action in Gonad Development"

James MacLean is an Associate Professor of Physiology at Southern Illinois University where he has been since 2007. After receiving a BS in Biology, his PhD training in Biochemistry and Molecular Biology was with R. Michael Roberts at the University of Missouri-Columbia. He completed his postdoctoral training at the University of Texas MD Anderson Cancer Center in the laboratory of Miles Wilkinson. The initial goal of Dr. MacLean's postdoctoral work was to bring Dr. Wilkinson's gene of interest, Pem (now Rhox5) into the realm of female reproduction. However, while at MDACC, Dr. MacLean along with Chad Wayne, discovered that Pem was not alone on the X chromosome, but rather at the core of a novel large homeobox gene cluster that is preferentially expressed in reproductive-associated tissues. Dr. MacLean's current research involves dissection of the unique and overlapping actions of RHOX5 and RHOX8, the only somatic-cell specific members of the RHOX family, in Sertoli cells and granulosa cells. The long range goal of Dr. MacLean's research is to identify and characterize the transcriptional networks under the control of each Rhox gene and to determine how these genes synergize to promote gonad development, germ cell differentiation, and fertility.

Session III

Robert N. Taylor, MD, PhD

Professor and Vice Chair for Research
Department of Obstetrics and Gynecology
Wake Forest School of Medicine

"In the Beginning: Cellular Behavior of Nascent Endometriosis"

Robert N. Taylor, MD, PhD is Vice Chair for Research in the Department of Obstetrics and Gynecology and co-director, Molecular Medicine and Translational Science graduate program at Wake Forest School of Medicine. Previously, he was Professor of Obstetrics and Gynecology at Emory University and Director of the Center for Reproductive Sciences at the University of California, San Francisco (UCSF). Dr. Taylor received his undergraduate education at Stanford University and completed the combined MD-PhD program at Baylor College of Medicine. His graduate work on the molecular biology of estrogen action was performed under the supervision of Roy G. Smith, PhD and Bert W. O'Malley, MD. Dr. Taylor trained as a resident in Obstetrics and Gynecology and subsequently as a fellow in Reproductive Endocrinology with Robert B. Jaffe, MD at UCSF. He then completed a postdoctoral fellowship with Lewis T. ("Rusty") Williams, MD, PhD in the Howard Hughes Medical Institute at UCSF, where he studied the regulation of placental angiogenesis by growth factors and their receptors. He is a board-certified obstetrician-gynecologist and reproductive endocrinologist widely published in the areas of endometriosis, embryonic implantation and early pregnancy complications. Dr. Taylor serves on several influential international, national and state executive committees including the American Board of Obstetrics and Gynecology, NIH Reproductive Scientist Development Program and the California Institute for Regenerative Medicine. He is honorary secretary of the World Endometriosis Society and Past President of the Society for Gynecologic Investigation.

Qinglei Li, PhD

Assistant Professor
Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences
Texas A&M University

"New Insights into the Function of TGF-beta Signaling in the Uterus"

Qinglei Li is an Assistant Professor in the Department of Veterinary Integrative Biosciences at Texas A&M University. He received his PhD in reproductive physiology in Harbin Medical University in China. He completed his postdoctoral training in reproductive biology with Drs. George Smith (Michigan State University; 2003-2005) and Martin Matzuk (Baylor College of Medicine; 2005-2010). In 2011, Dr. Li joined Texas A& M University as an Assistant Professor.

Dr. Li's research interests are focused on understanding the role of TGF-beta signaling in reproduction and diseases. Using multiple cellular and molecular approaches and genetically modified mouse models, his lab has identified novel functions of TGF-beta signaling in reproductive development, function, and cancer. His work is funded by the Department of Defense and National Institute of Child Health and Human Development (NICHD). Dr. Li has received the Ralph E. Powe Junior Faculty Enhancement Awards in 2013. He serves on the board of reviewing editors of Biology of Reproduction and the editorial board of Reproductive Biology and Endocrinology.

Abstract Titles

1. **Epigenetic Regelation of DNA Damage Repair is facilitated by the H3K79 methyltransferase, Dot1L/DOT1L.** **Nehemiah S. Alvarez**[1], Patrick E. Fields[1] [1]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

2. **Role of Kdm3a in rat trophoblast differentiation.** **Keisuke Kozai**, Damayanti Chakraborty, Khursheed Iqbal, Pramod Dhakal and Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

3. **Maternal fructose consumption leads to impaired fetal-placental development: an underlying role of uric acid induced placental dysfunction.** **Zeenat Asghar**, Jessica Saben, Alysha Thompson, Maggie Chi, Andrew Cusumano, Kelle Moley[1] [1]Department of Obstetrics and Gynecology, Washington University - St. Louis School of Medicine.

4. **A hypoxia/HIF/Kdm3a pathway controls trophoblast stem cell lineage decisions and organization of the hemochorial placenta.** **Damayanti Chakraborty**, Wei Cui, Regan Scott, Pramod Dhakal, Stephen J. Renaud, Gracy X. Rosario, Adam J. Krieg, M.A. Karim Rumi, Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

5. **TEAD4 facilitates mitochondrial genome transcription and oxidative phosphorylation in trophoblast during blastocyst maturation.** **Kumar RP**[1] and Paul S[2] [1] Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA. [2] The University of Kansas Cancer Center, University of Kansas Medical Center, Kansas City, KS 66160, USA [2] Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA [3] Institute of Reproductive Health & Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA.

6. **Development of SCNT embryos derived from fibroblast donor-cell treatment or culture with CPI-613 and PS48.** **B.R. Mordhorst**, S.L. Murphy, L.D. Spate, J.A. Benne, B.K. Redel, R.M. Ross, K.D. Wells, J.A. Green, R.S. Prather; University of Missouri, Columbia, MO, United States.

7. **Extravillous trophoblast cells derived from iPS cells from preeclamptic patients identify a potential invasion defect in preeclampsia.** **Megan Sheridan**[2,4], Ying Yang[1,4], Penghua Yang[1], Aihua Dai[1], Sambasiva Brahmasani[1], Danny Schust[3], Laura Schulz[3], Toshihiko Ezashi[1], R. Michael Roberts[1, 2, *] [1]Division of Animal Sciences, Bond Life Sciences Center, [2]Department of Biochemistry, [3]Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, Missouri 65211 USA [4]Co-first authors *Correspondence: robertsrm@missouri.edu.

8. **The critical roles of retinaldehyde reductase DHRS3 in embryonic development.** **Suya Wang**,[a] Sara E. Billings,[a] Keely Pierzchalski,[b] Naomi E. Butler Tjaden,[cd] Xiaoyan Pang,[a] Paul A. Trainor,[cd] Maureen A. Kane,[b] Alexander R. Moise[a,1] [a] Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, Kansas, 66045, USA; [b] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland, 21201, USA; [c] Stowers Institute for Medical Research, Kansas City, Missouri, 64110, USA; [d] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas, 66160, USA.

9. **TEAD4 promotes proliferation and self-renewal of trophoblast progenitors: An implication in mammalian placental homeostasis.** **Biswarup Saha**, Ramkumar Parikhsan, Pratik Home, Avishek Ganguly, Soma Ray and Soumen Paul. Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA.

10. **Involvement of CITED2 in the regulation of trophoblast cell differentiation and placentation.** **Pramod Dhakal**[1], Kazuyoshi Imakawa[2], Khursheed Iqbal[1], Kaiyu Kubota[1], Damayanti Chakraborty[1], Kazuya Kusama[2], M.A. Karim Rumi[1], and Michael J. Soares[1] [1]Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS; [2]Laboratory of Theriogenology and Animal Breeding, The University of Tokyo, Tokyo, Japan.

11. **CRISPR/Cas9-mediated inactivation of glucocorticoid receptor (NR3C1): Effect on ovine embryo development.** **Kelsey Brooks**, Gregory Burns and Thomas E. Spencer. Department of Animal Sciences, University of Missouri-Columbia.

12. **Uterine natural killer cell contributions to hemochorial placentation and pregnancy outcome.** Stephen J Renaud, **Regan Scott,** Damayanti Chakraborty, MA Karim Rumi, and Michael J Soares. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA.

13. **LSD1 Regulates Trophoblast Syncytialization via a GATA2-dependent pathway.** **Jessica Milano Foster**, Soma Ray and Soumen Paul, Institute for Reproductive Health and Regenerative Medicine Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, Kansas City, Kansas.

14. **Aryl hydrocarbon receptor signaling: potential impacts on trophoblast development and placentation.** **Khursheed Iqbal,** Pramod Dhakal, Katherine F. Roby, and Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Departments of Pathology and Laboratory Medicine and Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

15. **The histone H3K9 methyltransferase Suv39h2 participates in the maintenance of the trophoblast stem cell state.** **Lei Wang***, Damayanti Chakraborty*, Shui Qing Ye‡, and Michael J. Soares* *Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, 66160, USA; ‡Departments of Pediatrics and Biomedical and Health Bioinformatics, Division of Experimental and Translational Genetics, Children's Mercy Hospitals and Clinics, University of Missouri-Kansas City School of Medicine, Kansas City, MO, 64108, USA.

16. **Diet induced obesity impairs mitophagic response to mitochondrial damage in oocytes.** **Anna L Boudoures**, Jessica Saben, Andrea Drury, Maggie Chi, Alysha Thompson, Wendy Zhang and Kelle H Moley, MD. Department of Obstetrics & Gynecology; Washington University in St. Louis, St. Louis MO.

17. **Follicular fluid extracellular vesicles (EVs) regulate cumulus-oocyte-complex (COC) expansion and gene expression in cumulus cells.** **Wei-Ting Hung,** Lynda K. McGinnis, and Lane K. Christenson. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160, USA.

18. **Localization and absolute quantification of *Dppa3* and *Pou5f1* mRNAs coupled to DPPA3 and POU5F1 protein expression profiles in individual GV and MII oocytes.** **Fang Xie** and Jennifer R. Wood. Department of Animal Science, University of Nebraska-Lincoln.

19. **Evidence of reproductive-age associated changes in nucleolar structure and function in the growing oocyte.** **Susmita Jasti**, Barbara Fegley, John M. Kelsh, and Francesca E. Duncan. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

20. **The effect of L-leucine supplementation on mammalian in vitro follicle growth.** **Sara Pearson** and Francesca E. Duncan. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

21. **The effect of human ovulatory follicular fluid on fallopian epithelial cell proliferation and genomic stability.** **Pavla Brachova***, Erika Munch, Donna Santillan, Bradley Van Voorhis, Lane Christenson*, Institute for Reproductive Health and Regenerative Medicine, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

22. **Yes-Associated Protein 1 (YAP) regulates ovarian follicle development and transformation of granulosa cells.** **Xiangmin Lv**[1, 2, *], Chunbo He[1, 2, 3, *], Guohua Hua[1, 2, 3], Jixin Dong[4], John S. Davis[1, 2, 4, 5], and Cheng Wang[1, 2, 4] [1]Olson Center for Women's Health, [2]Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE; [3]College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei province, China; [4]The Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE; [5]Nebraska-Northwest Iowa VA Medical Center, Omaha, NE; *Co-first author.

23. **Regulation of FoxM1 by p21 in cancer cells.** **Jill A. Madden** and Jeremy Chien. Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS.

24. **A single dose exposure to 0.1 Gy total body ionizing radiation induces ovarian follicle abnormalities.** **Shawn M. Briley**[1], Susmita Jasti[1], Bruce F. Kimler[2], Francesca E. Duncan[1] [1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS [2]Radiation Oncology, University of Kansas Medical Center, Kansas City, KS.

25. **A comparison of ovarian follicular and luteal cell gene expression profiles provides insight into cellular identities and functions.** **Sarah Romereim**[1], Jennifer Wood[1], Xiaoying Hou[2], Heather Talbott[2], John Davis[2], Andrea Cupp[1] [1]University of Nebraska–Lincoln; [2]University of Nebraska Medical Center.

26. **Reproductive age-associated fibrosis in the mammalian ovary.** Shawn Briley[1], Susmita Jasti[1], Jessica E. Hornick[2], Michele T. Pritchard[3], **Francesca E. Duncan**[1] [1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS [2]Biological Imaging Facility, Northwestern University, Evanston, IL [3]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

27. **Defining roles for ESR2 in the regulation of female fertility.** **Mohammad Rumi**, Katherine Roby, Xiao Zhao, Khursheed Iqbal, Anamika Ratri, Rafia Mir, Shaon Borosha, Tianhua Lei, Wei Cui, Kaiyu Kubota, Pramod Dhakal, Michael Wolfe, Jay Vivian and Michael Soares. Institute for Reproductive Health and Regenerative Medicine; Departments of Pathology and Laboratory Medicine, Anatomy and Cell Biology, Molecular and Integrative Physiology. University of Kansas Medical Center, Kansas City, KS.

28. **Identification of loci associated with fertility in holstein heifers.** **Joao G. N. Moraes**, Joseph Dalton, Thomas E. Spencer, Jennifer N. Kiser, Gregory W. Burns, Andrzej Wojtowicz, Mahesh Neupane, Holly L. Neibergs. Department of Animal Science, Washington State University, Pullman. Department of Animal and Veterinary Sciences, University of Idaho, Caldwell.

29. **A portion of heifers attaining "early puberty" do not display estrus, are anovulatory and have reduced sex hormone binding globulin concentrations.** **Sarah Tenley**[1], Renata Spuri-Gomes[1], Mohamed Ayoub Abedal-Majed[1], Jeff Bergman[1], Scott Kurz[1], Jennifer Wood[1], Robert Cushman[2], Andrea S Cupp[1] [1]University of Nebraska–Lincoln; [2]USDA, ARS, U.S. Meat Animal Research Center.

30. **Excess maternal fructose consumption leads to decreased progesterone, increased fetal loss and impaired endometrial stromal cell decidualization in mice.** **Jessica L. Saben**, Zeenat Asghar, Julie S. Rhee, Andrea Drury, Kelle H Moley. Department of Obstetrics and Gynecology, Washington University School of Medicine, Saint Louis, Missouri.

31. **Regulation of REST target genes and miRNA29 in uterine leiomyomas.** **Mina Farahbakhsh**, Faezeh Koohestani and Vargheese Chennathukuzhi. The Center for Reproductive Sciences, Institute for Reproductive Health and Regenerative Medicine. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

32. **Impact of uterine glands and uterine luminal fluid on endometrial receptivity in mice.** **Andrew M Kelleher** and Thomas E Spencer. Department of Animal Sciences, University of Missouri, Columbia.

33. **Loss of PRICKLE1 leads to alterations in WNT/PCP pathway in uterine fibroids.** **Faezeh Koohestani**[1], Kavya Shivashankar[2], Sornakala Ganesh Kumar[1], Wendy Jefferson[3], Carmen Williams[3] & Vargheese Chennathukuzhi[1] [1]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, [2]Columbia University, New York City, NY, [3] National Institute of Environmental Health Sciences Research Triangle Park, NC.

34. **Hormonal regulation of female reproductive cyclicity: a role for progesterone?** **Kaiyu Kubota**, Wei Cui, M.A. Karim Rumi, Pramod Dhakal, Michael W. Wolfe, Jay L. Vivian, Katherine F. Roby, and Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Departments of Pathology and Laboratory Medicine and Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

35. **Botanical inhibitors of endometriosis.** **Chun-Xia Meng**[1], Christopher D. Kassotis[2], Sadia Akter[3], Victoria D. Balise[1], Chiamaka J. Isiguzo[1], Michelle A. Williams[1], Katelyn M. Cinnamon[1], Jiahao Hu[1], Trupi Joshi[3,4,5] and Susan C. Nagel[1] 1. Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, MO; 2. Nicholas School of Environment, Duke University, Durham, NC; 3. MU Informatics Institute, University of Missouri, Columbia, MO; 4. Department of Molecular Microbiology & Immunology, University of Missouri, Columbia, MO; 5. Department of Computer Science, University of Missouri, Columbia, MO.

36. **Systems biology approach to understanding uterine receptivity and pregnancy loss.** **Gregory W. Burns**[1], Michael E. Wehrman[3], Thomas W. Geary[3], James I. Moss[4], Anna C. Denicol[4], Kyle B. Dobbs[4], M. Sofia Ortega[4], Peter J. Hansen[4], Andrzej Wojtowicz[2], Holly Neibergs[2], Thomas E. Spencer[1] [1]Department of Animal Sciences, University of Missouri, Columbia, MO [2]Department of Animal Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA [3]USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT [4]Department of Animal Sciences, University of Florida, Gainesville, FL.

37. **Gonadotropin-releasing hormone II receptor (GnRHR-II) knockdown reduces testis size and decreases testosterone secretion during pubertal development in swine.** **Amy T. Desaulniers**[1], Rebecca A. Cederberg[1], Ginger A. Mills[1] and Brett R. White[1] [1]University of Nebraska Lincoln, Lincoln, NE.

38. **Divergent VEGFA signaling determines spermatogonial stem cell fate.** **Kevin M. Sargent**, John R. Essink, Meredith L. Bremer, William E. Pohlmeier, Melissa M. Laughlin, and Andrea S. Cupp. University of Nebraska-Lincoln, Lincoln, NE.

39. **Regulation of metabolism by estrogen receptor alpha.** **Ashley Ward***, Paige Geiger*, Robert Rogers, Josh Wheatley, Kathleen White, M.A. Karim Rumi. *Institute of Reproductive Health and Regenerative Medicine, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Full Abstracts

1. **Epigenetic Regelation of DNA Damage Repair is facilitated by the H3K79 methyltransferase, Dot1L/DOT1L.** **Nehemiah S. Alvarez**[1], Patrick E. Fields[1] [1]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

Dot1L/DOT1L, a histone 3 lysine 79 (H3K79) methyltransferase, is an essential epigenetic factor require for embryogenesis. Dot1L$^{-/-}$ mice die at E10.5 and are smaller and paler than their wild-type littermates. The lethality of Dot1L$^{-/-}$ mice is attributed to anemia due to a loss of blood progenitors; however, the mechanism by which blood progenitor loss occurs remains to be elucidated. Using a BrdU/microscopy assay we observed that Dot1L$^{-/-}$ mouse embryonic fibroblasts (MEF) proliferate at a slower rate than their wild-type counterparts. These data indicate that Dot1L is required in more cells types and is not limited in function to hematopoietic lineages. Interestingly, we observed that Dot1L$^{-/-}$ MEF have increased micronuclei, an indicator of genomic instability. Furthermore, Dot1L$^{-/-}$ MEF fail to repair DNA lesions after irradiation. Correspondingly, Dot1L$^{-/-}$ MEF have temporal defects in the formation of DNA repair foci. Specifically, foci formation of factors associated with homologous recombination (HR) and non-homologous end joining (NHEJ) repair is delayed. We utilized the human embryonic kidney 293T cell line (HEK293T) to engineer a DOT1L knock out (KO) and a DOT1L methyltransferase-deficient cell line (Y312A) using CRISR/Cas9. We observed that both the KO and Y312A cell lines have reduced HR and NHEJ repair activity. All together, our data indicate that Dot1L/DOT1L is essential for the temporal recruitment of DNA repair factors to maintain genomic stability.

2. **Role of Kdm3a in rat trophoblast differentiation.** **Keisuke Kozai**, Damayanti Chakraborty, Khursheed Iqbal, Pramod Dhakal and Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

Development of the trophoblast lineage is a key event required for growth and survival of the embryo in the female reproductive tract. Transcriptional and epigenetic regulation is fundamental to cell differentiation and trophoblast development. Histone modifications, including histone H3K9 methylation, affect gene regulation and directly impact cell differentiation. Histone H3K9 methyl transferases and demethylases are potential contributors to the regulation of cell differentiation. In this study, we explore a role for Kdm3a, a histone H3K9 demethylase, in the regulation of trophoblast differentiation. We utilized rat trophoblast stem (TS) cell models (blastocyst derived rat TS cells and Rcho-1 TS cells) for in vitro analyses. We are also in the process of establishing a Kdm3a mutant rat model for in vivo experimentation. TS cell models can be induced to differentiate by mitogen withdrawal. Trophoblast differentiation was associated with robust expression of Kdm3a mRNA and protein. The differentiation-dependent increase in Kdm3a expression was dependent upon PI3K/AKT signaling. Increased Kdm3a expression was also observed in trophoblast cells developing within the placentation site. In parallel we have utilized clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-mediated genome editing of the Kdm3a gene to generate Kdm3a mutant rats. Cas9 mRNA and guide RNA (gRNA) targeted to exon 9 of Kdm3a gene were microinjected into single-cell rat embryos and transferred to pseudopregnant recipients. Two founders with monoallelic mutations, consisting of deletions of 419 and 508 bp, were generated. Each mutation specifically removed 79 bp from the 5' of exon 9, which results in a frameshift, the emergence of a stop codon, and an expected null mutation. Founders were backcrossed to wild-type rats and the genotypes of offspring analyzed. Kdm3a mutations were successfully transmitted through the germline. In summary, Kdm3a expression is directly linked to differentiation and is a potential contributor to the regulation of trophoblast development. Future work will be directed to identifying a role for Kdm3a and histone H3K9 methylation in trophoblast differentiation using rat TS cell models and a Kdm3a deficient rat model. (Supported by Lalor Foundation postdoctoral fellowship and NIH HD079363)

3. **Maternal fructose consumption leads to impaired fetal-placental development: an underlying role of uric acid induced placental dysfunction.** **Zeenat Asghar**, Jessica Saben, Alysha Thompson, Maggie Chi, Andrew Cusumano, Kelle Moley[1] [1]Department of Obstetrics and Gynecology, Washington University - St. Louis School of Medicine.

Maternal metabolic syndrome and diabetes as well as low birth weight increase offspring risk for developing obesity, cardiovascular disease, and diabetes in adulthood. Consumption of fructose is on the rise which may confer metabolic risks for both women and their offspring. Excess fructose consumption likely contributes to offspring health risks by impairing function of the placenta, the transitory organ that mediates exchange of nutrients and wastes between the mother and fetus. However, the mechanism by which consumption of a high-fructose diet impairs placental function and the *in utero* milieu is unknown. Fructose is metabolized to fructose-1-phosphate, leading to a reduction in intracellular ATP levels and activation of AMP deaminase, which catalyzes the conversion of AMP to xanthine. Xanthine is then converted to uric acid by xanthine oxidase. Excess intracellular uric acid can lead to lipotoxicity, oxidative stress and cellular dysfunction. Thus we hypothesized that excess fructose consumption leads to adverse effects in the mother and her offspring by driving placental uric acid synthesis and transport. To test this, we placed six-week old C57BL/6J female mice on a high fructose diet (HFrD) or standard rodent chow diet or six weeks prior to mating with chow fed C57BL/6J males. HFrD fed mice did not exhibit obesity, insulin resistance or dyslipidemia but were glucose intolerant. Maternal serum, fetal and placental data were collected on embryonic day 18.5 after a four hour fast. Fetuses from HFrD-fed mice were growth restricted ($P<0.01$) despite increases in placental weight and size ($P<0.001$). The fetal to placental weight ratio was significantly decreased, suggesting an inefficient placental growth environment. In addition, the placentas from HFrD-fed mice had decreased ATP levels, increased xanthine oxidase activity and significantly increased uric acid accumulation ($P<0.05$). Allopurinol inhibits xanthine oxidase and thereby inhibits uric acid production. In HFrD-fed mice that received allopurinol (150 mg/l) in their drinking water, the placental insufficiency phenotype was reversed and normalized as assessed by two-way anova analysis. Thus we conclude that fructose drives uric acid synthesis and transport within the placenta which likely contributes to the adverse fetal and placental phenotype.

4. **A hypoxia/HIF/Kdm3a pathway controls trophoblast stem cell lineage decisions and organization of the hemochorial placenta.** **Damayanti Chakraborty**, Wei Cui, Regan Scott, Pramod Dhakal, Stephen J. Renaud, Gracy X. Rosario, Adam J. Krieg, M.A. Karim Rumi, Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

The placenta develops from the coordinated multi-lineage differentiation of trophoblast stem cells with specific trophoblast cell lineages performing specialized functions. The invasive lineage remodels uterine spiral arteries, facilitating nutrient flow, failure of which is associated with pathological conditions such as preeclampsia, intrauterine growth restriction, and preterm birth. Low oxygen (hypoxia) signaling plays an instructive role in influencing trophoblast cell differentiation and regulating placental organization. In this study, we perform a series of in vitro and in vivo experiments delineating a hypoxia-activated regulatory pathway controlling hemochorial placentation. Key downstream events are delineated using rat trophoblast stem (TS) cells and tested *in vivo*, using trophoblast-specific lentiviral gene delivery and genome editing. DNA microarray analyses performed on rat TS cells exposed to ambient or low oxygen (0.5%) showed upregulation of genes characteristic of an invasive/vascular remodeling/inflammatory phenotype and a marked downregulation of stem state-associated genes. Among upregulated genes were a histone H3K9 demethylase (Kdm3a) and a matrix metalloelastase (Mmp12). We hypothesized that Kdm3a was a mediator of trophoblast cell lineage differentiation and that Mmp12 was a key downstream target. Consistent with the hypothesis, knockdown of Kdm3a in rat TS cells inhibited the expression of a subset of the hypoxia/HIF-dependent transcripts, including Mmp12, altered H3K9 methylation status and subsequently decreased hypoxia-induced trophoblast cell invasion in both *in vitro* and *in vivo* experiments. Mmp12 possesses the capacity to degrade elastin and modify the structure of arterial blood vessels. To further explore the functional importance of Mmp12 in trophoblast cell-directed uterine spiral artery remodeling, we generated an Mmp12 mutant rat model using TALEN-

mediated genome editing. A rat model was established with a 609 bp deletion targeting exon 2 of Mmp12. Homozygous mutant rats showed reduced hypoxia-dependent endovascular trophoblast invasion and impaired trophoblast-directed uterine spiral artery remodeling. In summary, we have discovered a hypoxia/HIF/Kdm3a pathway modulating trophoblast cell lineage development, leading to acquisition of the invasive trophoblast cell phenotype, including upregulation of the extracellular matrix-modifying enzyme Mmp12 and subsequent uterine spiral artery remodeling.

5. **TEAD4 facilitates mitochondrial genome transcription and oxidative phosphorylation in trophoblast during blastocyst maturation.** **Kumar RP**[1] and Paul S[2] [1] Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA. [2] The University of Kansas Cancer Center, University of Kansas Medical Center, Kansas City, KS 66160, USA [2] Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA [3] Institute of Reproductive Health & Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA.

Trophoblast stem cells (TSC) and the trophectoderm rely on mitochondrial activity for ATP production through oxidative phosphorylation. However, the molecular mechanism directly associated with energy metabolism has yet to be identified.

In this study, we for the first time convincingly demonstrate mitochondrial sub-cellular localization of TEAD4 and its direct association with the mitochondrial genome transcription and energy homeostasis. TEAD4 knock-down TSCs showed mitochondria with fewer cristae, reduced complex-I activity, basal metabolic rate, ATP production and spare capacity. Loss of TEAD4 results in reduced mitochondrial transcripts. Finally, we show loss of TEAD4 in TSCs results in impaired recruitment of mitochondrial RNA polymerase at and in proximity to the promoter.

Our results ascribe a novel role of TEAD4 as a positive regulator of the mitochondrial genome transcription, and thereby maintain energy homeostasis. This offers new insight into the regulatory mechanism for the mitochondrial genome during mammalian development.

6. **Development of SCNT embryos derived from fibroblast donor-cell treatment or culture with CPI-613 and PS48.** **B.R. Mordhorst**, S.L. Murphy, L.D. Spate, J.A. Benne, B.K. Redel, R.M. Ross, K.D. Wells, J.A. Green, R.S. Prather; University of Missouri, Columbia, MO, United States.

Recent evidence suggests that early embryos exhibit a Warburg Effect (WE)-like metabolism. A WE hallmark is predominate use of glycolysis for energy production as opposed to the tricarboxylic acid cycle (TCA) as used by differentiated cells. Increased signaling of the PI3K pathway is correlated with an increase in glucose metabolism consistent with the WE. PS48 stimulates the PI3K pathway and CPI-613 inhibits pyruvate dehydrogenase. Both compounds should decrease mitochondrial use of the TCA cycle and promote resources toward the pentose phosphate pathway. Day 35 porcine fetal fibroblasts were treated as controls (CON, 0 μM), or with CPI-613 (50 or 100 μM) or PS48 (5 or 10 μM) for 7 days and used for NT in enucleated oocytes. Additionally drug treatments were tested in the culture of SCNT embryos from non-drug-treated fibroblasts; as a vehicle control DMSO was added at the same concentration of the highest drug treatment. Cleavage and blastocyst percentages, blastocyst cell number, and TUNEL positive cell number were analyzed for treatment effects by using the GLM procedure of SAS 9.4 (Cary, NC). Cleavage was not affected by any donor cell or culture drug treatment ($P \geq 0.05$). Development to blastocyst stage was not impacted by donor cell treatment ($P \geq 0.99$); however was impacted by drug culture treatment for every treatment (CPI 100μM=10.3%, DMSO=19.3%, CPI 50μM=28.9%, and PS48 10μM=48.8%; Error ±2.12; $P<0.01$). Blastocyst cell number was not altered with any treatment ($P \geq 0.34$). Number of TUNEL positive cells was decreased with PS48 10μM culture treatment compared to CPI treatments (2.1±0.5 vs. ≥3.3±0.5; $P \leq 0.01$), but not DMSO control (3±0.5; $P=0.09$). Donor cell treatment did not affect number of TUNEL positive cells ($P \geq 0.08$). While blastocyst development and cell number were not impacted by donor cell treatment, future experiments will look at embryonic development from embryo transfers and may investigate embryonic metabolic programming. Funded by Food for the 21st Century and NIH R01HD080636.

7. **Extravillous trophoblast cells derived from iPS cells from preeclamptic patients identify a potential invasion defect in preeclampsia.** **Megan Sheridan**[2,4], Ying Yang[1,4], Penghua Yang[1], Aihua Dai[1], Sambasiva Brahmasani[1], Danny Schust[3], Laura Schulz[3], Toshihiko Ezashi[1], R. Michael Roberts[1, 2, *] [1]Division of Animal Sciences, Bond Life Sciences Center, [2]Department of Biochemistry, [3]Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, Missouri 65211 USA [4]Co-first authors *Correspondence: robertsrm@missouri.edu.

Preeclampsia (PE) is a pregnancy-specific disease affecting ~5 % of pregnancies and characterized by a shallow and poorly perfused placenta and onset of hypertension and proteinuria in the mother. In the early onset, more severe, form of the disease the infant is usually delivered prematurely by caesarian section, but the underpinnings of the disease remain unknown, although there is an ill-defined genetic component to it. There is presently no means for diagnosing PE in early pregnancy when the dysfunctional placenta becomes established and a lack of models relevant to progression of the disease. To address this problem, we derived induced pluripotent stem cells (iPSC) from umbilical cords of infants born to mother who had experienced early onset PE and from infants born after a normal pregnancy. These two kinds of iPSC were then converted to trophoblast by exposing them to BMP4 (**B**, 10 ng/ml) together with the ACTIVIN signaling inhibitor A83-01 (**A**, 1 μM) and the FGF2 signaling inhibitor PD173074 (**P**, 0.1 μM) (**BAP** conditions). This treatment leads to unidirectional commitment to the trophoblast lineage, and provides both syncytiotrophoblast (STB) and HLA-G-expressing extravillous trophoblast (EVT). In PE, the latter cell population has been suggested to be responsible for the shallow placentation and a failure to modify the spiral arteries, which supply maternal blood to the placenta. To compare the invasion efficiency of EVT from the PE and control pregnancies, the iPSC were plated on Matrigel-coated invasion chambers with 8.0 ㎛ pores and exposed to BAP treatment for 6 days to induce trophoblast, and, in particular, EVT. For each cell line, three independent invasion assays were performed under either 20 % or 5 % O2 conditions. Control embryonic stem cells (ESC) and iPSC, which remain undifferentiated in absence of BAP treatment, were used as treatment controls. After the cells remaining on the top of the membrane had been completely removed, the number of invaded cells present on the lower surface of the membrane was counted. The experiment confirmed earlier work that undifferentiated ESC and iPSC migrate poorly through Matrigel compared to after BAP treatment, when significantly more cells ($p < 0.05$) appeared on the lower surface of the membrane. Over 90% of these cells were KRT7-positive. Significantly, the invasiveness of EVT from all seven PE-iPSC lines was reduced under 20% O_2 compared to 5% O_2 ($p < 0.05$) while all four control iPSC lines exhibited similar invasiveness under both oxygen conditions. These results suggest that the abnormalities documented for EVT in PE pregnancies *in vivo* may be recapitulated in the phenotype of EVT generated from umbilical cord-derived iPSC *in vitro.* These experiments indicate that events of a past pregnancy can be recapitulated in this model system and suggest a means for studying the pathophysiology of preeclampsia *in vitro.*

8. **The critical roles of retinaldehyde reductase DHRS3 in embryonic development.** **Suya Wang**,[a] Sara E. Billings,[a] Keely Pierzchalski,[b] Naomi E. Butler Tjaden,[cd] Xiaoyan Pang,[a] Paul A. Trainor,[cd] Maureen A. Kane,[b] Alexander R. Moise[a,1] [a] Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, Kansas, 66045, USA; [b] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland, 21201, USA; [c] Stowers Institute for Medical Research, Kansas City, Missouri, 64110, USA; [d] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas, 66160, USA.

All-*trans*-retinoic acid (ATRA), a metabolite of vitamin A, retinol, plays an important role in embryonic development, whose homeostasis is sustained by a precise balance between its synthesis and degradation. Previous studies of the regulation of ATRA metabolism have focused on the enzymes involved in its synthesis, namely the oxidation of retinol and retinaldehyde and the degradation by cytochrome P450 enzymes. In addition, *in vitro* studies have identified several enzymes that catalyze the reduction of retinaldehyde to retinol. However, it is still unknown if such conversion affects ATRA levels *in vivo*. DHRS3 is a ubiquitously expressed

short chain dehydrogenase/ reductase (SDR) enzyme which reduces retinaldehyde to retinol and whose expression is controlled by ATRA. Here, we investigate the physiological role of *Dhrs3* by employing a *Dhrs3*-deficient mouse model. Ours studies reveal that *Dhrs3*-deficient embryos exhibit a statistically significant reduction in the levels of retinol and retinyl esters and an increase in ATRA, compared to wild-type embryos. Such an accumulation of ATRA in *Dhrs3*$^{-/-}$ mice leads to metabolic compensation through both upregulation of the expression of the ATRA catabolic enzyme *Cyp26a1* and reduction in the expression of genes involved in ATRA synthesis. Despite such compensation, *Dhrs3*$^{-/-}$ mice show altered ATRA signaling and developmental programming during embryogenesis. As the result of these alterations, *Dhrs3*$^{-/-}$ mice are not viable and present with multiple embryonic defects. These results provide evidence of the critical function of DHRS3 in reducing retinaldehyde to retinol to safeguard against excess formation of ATRA during embryonic development.

9. **TEAD4 promotes proliferation and self-renewal of trophoblast progenitors: An implication in mammalian placental homeostasis.** **Biswarup Saha**, Ramkumar Parikhsan, Pratik Home, Avishek Ganguly, Soma Ray and Soumen Paul. Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA.

In placental mammals, trophoblast cells are essential for embryo implantation leading to successful progression in pregnancy. During placental development, distinct trophoblast cell types are specified from trophoblast stem cells (TSCs) or TSC-like trophoblast progenitors. However, the molecular mechanisms that regulate self-renewal or differentiation of trophoblast stem/progenitor cells, are poorly understood. In our study, we show that transcriptional activity of TEAD4, a TEA domain containing transcription factor, plays a crucial role in promoting cell growth and proliferation in the trophoblast progenitors of developing placenta. In the early stage Ectoplacental cone (EPC) and at other stages in developing rodent placentas, TEAD4 is present within the nuclei of a small TSC-like progenitor and can directly regulate expression of TSC-specific genes. Our Global gene expression analysis (RNA-seq) in TEAD4-depleted mouse trophoblast stem cells (mTSCs) indicated that TEAD4-mediated gene regulation is important to promote proliferation and self-renewal of mTSCs. Furthermore, analyses with primary trophoblast progenitors from mouse ectoplacental cone and cytotrophoblasts (CTBs) from first-trimester human placenta confirmed that TEAD4 promotes self-renewal of trophoblast stem/progenitors cells by directly regulating expression of several *Cyclins/ CDKs* and other TSC-specific genes. In contrast to trophoblast progenitors of a developing human placenta, differentiated trophoblast cells within a matured human placenta generally lack TEAD4 transcriptional activity due to its absence in their nuclei. However, intriguingly, matured human placentas harbor a small number of CTB population, characterized by the presence of TEAD4 in their nuclei. Laser -capture micro-dissection followed by gene expression analyses revealed that TEAD4 expressing CTBs in matured placenta have higher expressions of TSC-specific genes and positive regulators of cell proliferation. Furthermore, a very small percentage of TEAD4 expressing CTBs in matured placenta also express Ki67, a marker for actively proliferating cells. Thus, in matured placenta, TEAD4 transcriptional activity marks a population of quiescent trophoblast progenitors that are poised for proliferation. Not surprisingly, higher expression of TEAD4 is associated with proliferating trophoblast cells in choriocarcinomas. Our study indicates that transcriptional activity of TEAD4 marks proliferating trophoblast progenitors during placental development where TEAD4 function could be important for placental homeostasis.

10. **Involvement of CITED2 in the regulation of trophoblast cell differentiation and placentation.** **Pramod Dhakal**[1], Kazuyoshi Imakawa[2], Khursheed Iqbal[1], Kaiyu Kubota[1], Damayanti Chakraborty[1], Kazuya Kusama[2], M.A. Karim Rumi[1], and Michael J. Soares[1] [1]Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS; [2]Laboratory of Theriogenology and Animal Breeding, The University of Tokyo, Tokyo, Japan.

The hemochorial placenta possesses a diverse set of responsibilities ensuring survival and growth of the embryo/fetus within the mother's reproductive tract. Execution of these functions requires coordinated temporal and spatial differentiation of stem and progenitor cells into specialized trophoblast cell types and their organization into a hemochorial placenta. cAMP-response element binding protein/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain-2 (CITED2) is a transcriptional co-regulator

implicated in the regulation of placentation. Our objective is to investigate the involvement of CITED2 in trophoblast development and placentation using both in vitro and in vivo approaches. The rat Rcho-1 trophoblast cell line was used to investigate the biology of CITED2 in trophoblast development. CITED2 transcript and protein showed a robust induction during Rcho-1 trophoblast cell differentiation. We next used a short hairpin RNA (shRNA) knockdown approach to disrupt CITED2 expression. Rcho-1 trophoblast cells were stably infected with lentiviral constructs containing shRNAs specifically targeting CITED2 or a control shRNA and exposed to differentiation culture conditions. CITED2 shRNAs effectively inhibited the expression of the differentiation-dependent upregulation of CITED2 mRNA and protein. Disruption of CITED2 expression did not interfere with trophoblast cell proliferation or the formation of trophoblast giant cells. RNA-sequencing was used to dissect the impact of CITED2 on trophoblast cell differentiation. CITED2 disruption affected the differentiating trophoblast cell transcriptome with links to several signal transduction pathways and to hypoxia-regulated (Tpbpa, Mmp12) and coagulation (Mmp12, Thbd, Apoa4, F3) processes. To further assess the role of CITED2 on placentation, we generated *Cited2* mutations in rats using the Crispr/Cas9 system. Two guide RNAs were designed to effectively delete the entire coding region of *Cited2*. Founders possessing desired deletions were identified and backcrossed to wild-type rats. *Cited2* null mutations were successfully transmitted through the germline. These rats will be used to generate pregnancies for further characterization. In summary, our findings indicate that CITED2 is a regulator of trophoblast differentiation. Our mutant rat model will provide an opportunity for in vivo investigation of the role of CITED2 in trophoblast cell development and hemochorial placentation. (Supported by HD020676, HD079363)

11. **CRISPR/Cas9-mediated inactivation of glucocorticoid receptor (NR3C1): Effect on ovine embryo development.** **Kelsey Brooks**, Gregory Burns and Thomas E. Spencer. Department of Animal Sciences, University of Missouri-Columbia.

In sheep, the elongating conceptus synthesizes and secretes interferon tau (IFNT), as well as prostaglandins (PGs) and cortisol. The enzymes, hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1) and HSD11B2, interconvert inactive cortisone and active cortisol. In sheep, *HSD11B1* is expressed and active in the conceptus trophectoderm as well as in the endometrial luminal epithelia; in contrast, *HSD11B2* expression is most abundant in conceptus trophectoderm. Cortisol is a biologically active glucocorticoid and ligand for the glucocorticoid receptor (NR3C1) and mineralocorticoid receptor (NR3C2). NR3C2 is not detectable in either the endometrium or conceptus of the ovine uterus during early pregnancy. In tissues that do not express NR3C2, HSD11B2 protects cells from the growth-inhibiting and/or pro-apoptotic effects of cortisol. Our previous study found that HSD11B1, but not HSD11B2, is essential for successful conceptus development *in vivo.* In the present study, we used the CRISPR/Cas9 system to test the hypothesis that NR3C1 has an essential role in conceptus development in sheep. Superovulated ewes were bred at estrus (n=5), and one-cell zygotes were recovered 36 hours post-mating. Zygotes were injected with either: (1) wildtype Cas9 RNA alone (Control); or (2) wildtype Cas9 RNA along with 4 guide RNAs targeting ovine NR3C (NR3C1-targeted). Injected zygotes were developed to the blastocyst stage in culture and then transferred to day 8 recipient ewes. The uterus was obtained 6 days post-transfer (Day 14). Elongating, filamentous type conceptuses (12-14 cm in length) were recovered from ewes gestating control embryos (n=7/7). Similarly, conceptuses recovered from ewes gestating NR3C1-targeted embryos were fully elongated and filamentous (n=6/7). DNA was isolated from recovered conceptuses, and the targeted region of NR3C1 was amplified and Sanger sequenced. All control conceptuses and one of the NR3C1 targeted conceptuses had no sequence alterations. However, the other 6 NR3C1 targeted conceptuses were edited, causing a frame shift mutation resulting in a premature stop (n=2/6) or deletion of the essential zinc finger binding domain (n=4/6). These results support the ideas that: (1) NR3C1 is dispensable for ovine embryo survival, blastocyst development, and peri-implantation conceptus elongation; and (2) the effects of HSD11B1-derived cortisol on conceptus elongation are indirectly mediated by the endometrium.

12. **Uterine natural killer cell contributions to hemochorial placentation and pregnancy outcome.** Stephen J Renaud, **Regan Scott,** Damayanti Chakraborty, MA Karim Rumi, and Michael J Soares. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA.

Natural killer (NK) cells comprise the predominant uterine leukocyte population during pregnancy. NK cells are recruited to the uterus soon after embryo implantation and are hypothesized to facilitate hemochorial placentation through their actions on uterine spiral arteries; activities performed in cooperation with invasive trophoblast. Pregnancy-dependent uterine spiral artery transformation is a key feature of hemochorial placentation and its failure is the root cause of major obstetric complications. The purpose of this study was to utilize a rat genetic model of NK cell deficiency to investigate the involvement of NK cells in placentation and pregnancy outcome. To accomplish this task, we utilized zinc finger nuclease (ZFN)-mediated genome editing of the interleukin 15 (*Il15*) gene. The *Il15* locus was targeted because it encodes a cytokine required for NK cell maturation and survival. ZFNs were microinjected into zygotes, and transferred to pseudopregnant dams. Following genotypic characterization of offspring, we identified a founder containing a seven base-pair deletion within exon 2 of the *Il15* gene. The deletion resulted in a frameshift and premature stop codon. The mutation was transmitted to progeny, and caused a loss of the IL15 transcript and protein. In comparison to wild-type pregnant female rats, whose uteri contained massive quantities of NK cells, uteri of females harboring a homozygous mutation at the *Il15* locus contained no detectable uterine NK cells. Implantation sites within these NK cell-deficient rats showed obvious macroscopic uterine blood vessel dilatation. Histological analysis revealed an early disruption in pregnancy-dependent uterine spiral artery development and compensatory increases in endovascular trophoblast invasion and an expansion of the junctional zone. These adaptive responses within the hemochorial placenta did not significantly impact pregnancy outcomes, including litter size or fetal weights at gestation day 18.5. Furthermore, other maternal adaptations (liver and spleen gravimetric responses) were not significantly affected by maternal NK cell deficiency. In summary, NK cells contribute to uterine spiral artery remodeling; however, at least on the short-term, their absence can be compensated through a redirection of the efforts of invasive trophoblast. The evolutionary drive for NK cell involvement in hemochorial placentation remains elusive. (Supported by NIH HD020676, HD082535)

13. **LSD1 Regulates Trophoblast Syncytialization via a GATA2-dependent pathway.** **Jessica Milano Foster**, Soma Ray and Soumen Paul, Institute for Reproductive Health and Regenerative Medicine Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, Kansas City, Kansas.

Proper placental development is essential for a successful pregnancy. Placental function is critically dependent upon proper development of syncytiotrophoblasts, which provides the exchange surface between the mother and the fetus. However, molecular regulation of syncytiotrophoblast development is poorly understood. In this study, we explore the regulatory pathway of lysine-specific demethylase 1 (LSD1), with transcription factor Gata2 and cellular signaling, in human trophoblast syncytialization. Using cell-culture models, we show that LSD1 is important for human trophoblast syncytialization. Cellular signaling, such as the activation of protein kinase A, together with LSD1 contribute to the recruitment of Gata2 at Syncytin1 and Syncytin2 loci. Loss of LSD1 in trophoblast cells impairs GATA2 function, histone modifications and RNA polymerase 2 recruitment at Syncytin1/2 loci leading to transcriptional suppression and impaired syncytialization. In summary, our data reveals a regulatory pathway demonstrating that LSD1 is essential for human trophoblast syncytialization.

14. **Aryl hydrocarbon receptor signaling: potential impacts on trophoblast development and placentation.** **Khursheed Iqbal,** Pramod Dhakal, Katherine F. Roby, and Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Departments of Pathology and Laboratory Medicine and Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

The aryl hydrocarbon receptor (AHR) is a member of the basic helix–loop–helix (bHLH) –PER–ARNT–SIM superfamily of transcription factors. AHR ligands include polycyclic aromatic hydrocarbons and dioxins. Upon ligand binding, AHR translocates into the nucleus and heterodimerizes with AHR nuclear translocator (ARNT) where it regulates target genes, including those encoding enzymes that are important in drug metabolism and detoxification of environmental pollutants. Hypoxia-inducible factor-1α (HIF1A) is closely related to AHR, also dimerizes with ARNT, mediates cellular adaptations to hypoxia, and regulates hemochorial

placentation. We hypothesize that exposures to environmental pollutants may activate AHR signaling during critical periods of gestation redirecting ARNT to distinct target loci and potentially interfering with the instructional actions of hypoxia-HIF on placentation. The purpose of this study was to explore AHR activation on placental development. We used 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; dioxin) as a model AHR ligand and examined its effects on rat trophoblast stem (TS) cells and on placentation in pregnant rats. TS cells express the relevant components of the AHR signaling pathway and when exposed to TCDD show a dramatic upregulation of CYP1A1, demonstrating AHR activation. To elucidate the effects of AHR activation on hemochorial placentation, pregnant rats were treated with TCDD or vehicle at gestation day 6.5 and exposed to atmospheric or hypoxic (10.5% oxygen) conditions until gestation day 13.5 when the animals were euthanized and placentation sites examined. TCDD treatment induced CYP1A1 in the uterine decidua and the labyrinth zone and also affected the organization of the gestation day 13.5 placentation site. These efforts were complemented through the generation of an AHR mutant rat model. To this end, we utilized Crispr/Cas9 technology for targeted genome editing. Exon 2 of Ahr gene that harbors the DNA binding bHLH domain was targeted. Founders possessing deletion mutations causing exon 2 disruptions were identified and backcrossed to wild-type rats. *Ahr* mutations were successfully transmitted through the germline and are being used to produce *Ahr* null rats. In summary, we are establishing the experimental tools, including in vitro and in vivo model systems, to examine the effects of AhR activation on trophoblast development and hemochorial placentation.

15. **The histone H3K9 methyltransferase Suv39h2 participates in the maintenance of the trophoblast stem cell state.** **Lei Wang***, Damayanti Chakraborty*, Shui Qing Ye‡, and Michael J. Soares* *Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, 66160, USA; ‡Departments of Pediatrics and Biomedical and Health Bioinformatics, Division of Experimental and Translational Genetics, Children's Mercy Hospitals and Clinics, University of Missouri-Kansas City School of Medicine, Kansas City, MO, 64108, USA.

Hemochorial placentation involves lineage specific development of trophoblast cell types, which orchestrate the efficient redirection of blood flow to the placenta and delivery of nutrients to the fetus. As pregnancy progresses, specialized trophoblast cell lineages arise through the precise expansion and differentiation of trophoblast stem (TS) cells. Failure of proper trophoblast lineage development is associated with pathological conditions such as preeclampsia, intrauterine growth restriction, and preterm birth. Thus we can gain insights into these disorders through elucidating regulatory mechanisms controlling trophoblast cell fate decisions. Epigenetic mechanisms, including histone modifications, have been demonstrated to possess key roles in controlling cell differentiation but little is known about their involvement in trophoblast development. In this investigation, we examined the contributions of a histone H3K9 methyltransferase, Suv39h2, in the regulation of TS cell differentiation. Suv39h2 catalyzes the dimethylation and trimethylation of histone H3K9, histone marks generally associated with transcriptional repression. Among the H3K9 methyltransferases, we targeted Suv39h2 because it exhibited the most robust decrease in its expression from stem to differentiation states. Suv39h2 transcript and protein expression is significantly elevated in the TS cell stem state and declines as TS cells differentiate. Suv39h2-specific shRNA delivery inhibited TS stem cell proliferation and promoted trophoblast cell differentiation. Initially, using a candidate approach we observed a significant upregulation of transcripts for markers of differentiation (Adm, Hand1, Prl3b1) in the Suv39h2-shRNA expressing TS cells. These results suggested that Suv39h2 participates in the maintenance of the TS cell stem state and led us to perform transcriptome analyses using RNA sequencing. Complementary DNA libraries from control shRNA and Suv39h2 shRNA knockdown TS cells were sequenced. Overall, 527 annotated transcripts were significantly different between the two groups ($P<0.05$). Of the differentially regulated transcripts, 216 were upregulated and 311 downregulated in the Suv39h2 knockdown TS cells. Pathway analysis indicated that Suv39h2 possesses a prominent role in the regulation of TS cell growth and proliferation. In summary, our findings indicate that Suv39h2 is a key epigenetic regulator controlling the TS cell stem state, impacting TS cell expansion, trophoblast cell differentiation, and ultimately the development of the hemochorial placenta. (Supported by NIH grants: HD20676, HD079363)

16. **Diet induced obesity impairs mitophagic response to mitochondrial damage in oocytes.** **Anna L Boudoures**, Jessica Saben, Andrea Drury, Maggie Chi, Alysha Thompson, Wendy Zhang and Kelle H Moley, MD. Department of Obstetrics & Gynecology; Washington University in St. Louis, St. Louis MO.

Obesity, a prevalent and persistent problem worldwide, affects not only adults but predisposes offspring to obesity and metabolic syndrome. Previous research has shown the oocyte itself is largely responsible for offspring health. Many studies from our lab and others have demonstrated the consequences of obesity for the oocyte: increased rate of meiotic spindle deformation, lipid accumulation, lipotoxicity, and endoplasmic reticulum stress. Oocytes from obese mice also have several hallmarks of mitochondrial damage, such as decreased mitochondrial membrane potential, increased production of reactive oxygen species, decreased TCA cycle activity, and more mitochondria, many of which appear damaged or dysfunctional. The accumulation of damaged mitochondria in oocytes from diet induced obese (DIO) mice suggests that these cells have an imbalance of mitochondrial fission, fusion, and mitophagy, processes involved in removing damaged mitochondria. DIO results in accumulation and changed distribution of the mitochondrial dynamics protein Dynamin related protein 1, but no other effects of DIO on mitochondrial dynamics or mitophagy are known. I hypothesize that damaged mitochondria accumulate in DIO oocytes as a result of impaired mitophagy.

17. **Follicular fluid extracellular vesicles (EVs) regulate cumulus-oocyte-complex (COC) expansion and gene expression in cumulus cells.** **Wei-Ting Hung,** Lynda K. McGinnis, and Lane K. Christenson. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160, USA.

Cumulus oocyte complex (COC) expansion is critical for ovulation. Expansion of the COC is accomplished by the cumulus cells creating a hyaluronan (HA)-rich extracellular matrix, which is under the regulation of prostaglandin-endoperoxide synthase 2 (*Ptgs2*), pentraxin-related protein 3 (*Ptx3*), tumor necrosis factor, alpha-induced protein 6 (*Tnfaip6*), and hyaluronan synthase 2 (*Has2*). The follicular fluid that separates these distinct granulosa cell types is a rich and complex fluid. Recent studies have shown that extracellular vesicles (EVs) are abundant within antral fluid, yet to date, no functions of EVs have been demonstrated. In our study, the effect of follicular fluid EVs on COC expansion was assayed. EVs were isolated from follicular fluid of early (3-5 mm) and late (>9 mm) antral bovine follicles and then characterized. Uptake of fluorescently labelled EVs by COCs was examined by confocal microscopy. To test EV bioactivity, mouse and bovine COC were cultured with or without EVs from different sized follicles. Expansion was measured and RNA was isolated. Quantitative RT-PCR was used to measure *Ptgs2*, *Ptx3*, *Tnfaip6*, and *Has2* expression. Electron microscopy indicated numerous bilipid membrane enclosed vesicles (50-150 nm) and negligible protein contaminants. Nanoparticle tracking analysis confirmed that EVs ranged in size (30-300 nm) with a mean diameter of 130 nm. The exosomal marker, CD81 was enriched in EV preparations. Uptake of EVs was evident within the cytoplasm of cumulus cells as numerous green punctate spots. Functional analysis indicated that EVs from early or late antral follicles stimulated COC expansion, by 20% or 15% compared with controls cultured without EVs ($p<0.05$). Expression of *Ptgs2* and *Ptx3* increased significantly upon treatment of EVs from early follicles but not late follicles. *Tnfaip6* expression followed the same trend while *Has2* expression did not change in response to EVs. In conclusion, EVs isolated from follicular fluid positively regulated COC expansion and marker genes expression, with a paradoxically greater effect of small follicle derived EVs than large follicle derived EVs. This study is the first to demonstrate a biological effect of follicular fluid EVs on COC expansion and gene expression, critical events in the process of ovulation.

18. **Localization and absolute quantification of *Dppa3* and *Pou5f1* mRNAs coupled to DPPA3 and POU5F1 protein expression profiles in individual GV and MII oocytes.** **Fang Xie** and Jennifer R. Wood. Department of Animal Science, University of Nebraska-Lincoln.

RNAs stored in the MII-arrested oocyte play important roles in successful embryonic development. Their abundance is defined by transcriptional activity during oocyte growth and selective degradation during oocyte meiotic maturation. In previous studies we showed that transcript abundance of two important maternal effect

genes, *Dppa3* and *Pou5f1,* was significantly increased in growing and mature oocytes from obese female mice suggesting altered transcriptional and post-transcriptional regulation of these mRNAs. However, normal coordinated regulation of mRNA synthesis, stability, and protein translation of these two transcripts in the oocyte remains unclear. Thus, the objective of the current study was to localize and quantify *Dppa3* and *Pou5f1* mRNA and protein expression in individual GV and MII oocytes. To attain this goal, we established single molecule RNA fluorescent *in situ* hybridization (FISH) technology in individual oocytes. We targeted *Dppa3* and *Pou5f1* RNAs using a gene-specific probe and the probe signal was subsequently amplified using sequential hybridization. FISH images were generated by confocal microscopy and stitched together in Image J allowing for localization and quantification of each mRNA species using the counting program Localize. Using this methodology, we demonstrated that individual oocytes from lean CD-1 female mice had higher numbers of both *Dppa3* and *Pou5f1* mRNA in GV compared to MII oocytes. In complementary immunohistochemistry assays, we showed that DPPA3 protein expression was higher compared to POU5F1 protein expression in both GV and MII oocytes. POU5F1 protein only surrounded the germinal vesicle in GV oocytes while it was evenly distributed in MII oocyte. Conversely, DPPA3 expression was localized to both the germinal vesicle and subcortical region of GV oocytes and was mainly distributed at the subcortical region in MII oocytes. Together, these data indicate that unique mechanisms are likely regulating the post-transcriptional storage and translation of these two transcripts in GV and MII oocytes. In future studies we will determine if female obesity disrupts this profile of mRNA and protein expression and thereby contributes to reduced developmental competence of oocytes from obese females.

19. **Evidence of reproductive-age associated changes in nucleolar structure and function in the growing oocyte.** **Susmita Jasti**, Barbara Fegley, John M. Kelsh, and Francesca E. Duncan. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

During its growth phase, the mammalian oocyte is one of the most translationally active cells in the body because it must accumulate critical stores of maternal proteins to support fertilization and early embryo development. Such maternal products define the cytoplasmic competence of the oocyte and are key determinants of gamete quality. Advanced reproductive age is associated with a significant decline in oocyte quality, and we hypothesized that this may be due to alterations in intra-oocyte protein synthesis. To address this hypothesis, we examined nucleolar architecture in isolated primary and secondary stage follicles from CB6F1 mice of young (6-12 weeks, n=5) and advanced (14-17 months, n=11) reproductive age. We focused on the nucleolus because it is the key organelle for ribosome biogenesis, and alterations in nucleolar architecture can influence protein synthesis. We assessed nucleolar architecture using Transmission Electron Microscopy-TEM and immunofluorescence for nucleolar components: Upstream Binding Transcription Factor (UBTF), fibrillarin, and nucleolin. These markers define the tri-partite nucleolar organization and are involved in rDNA transcription, rRNA processing, and ribosome subunit formation, respectively. Oocytes from young and old mice were compared for the following parameters: nucleolar number and diameter, pattern of UBTF (punctate/Giant Fibrillar Centers; GFC) staining, intensity of fibrillarin staining and pattern of nucleolin (homogeneous/rim) staining. Based on this analysis, two parameters revealed significant age-associated differences. First, GFCs, structures associated with rDNA transcription, were significantly larger in old compared to young oocytes (average diameter 1.88 ± 0.14 vs 1.39 ± 0.2 μm, respectively; p=0.03). Second, oocytes from old mice expressed 1.8-fold more fibrillarin compared to young counterparts (p=0.007). Fibrillarin catalyzes 2'-O-methylation of rRNA, and alterations in the rRNA methylation pattern generate ribosomes with reduced translational fidelity. In cancer cells, reduced p53 expression causes enhanced RNA Polymerase I activity and high fibrillarin expression which together lead to increased numbers of poor quality ribosomes. Quantification of cytoplasmic ribosomes in oocytes by TEM revealed a 1.87-fold increase between old and young mice (p=0.005), consistent with increased rDNA transcription. Studies are ongoing to determine whether reproductive aging, like cancer, is associated with ribosome heterogeneity that contributes to altered protein products due to reduced translation fidelity. This work was supported by the Center of Biomedical Research Excellence (P20 GM104936-8) and by the Kansas IDeA Network of Biomedical Research Excellence (P20 GM103418).

20. **The effect of L-leucine supplementation on mammalian in vitro follicle growth.** **Sara Pearson** and Francesca E. Duncan. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

In vitro follicle growth (IVFG) is a technique whereby immature follicles are isolated and grown outside the ovary to generate mature gametes. IVFG has important implications for fertility preservation especially for women who have had ovarian tissue cryopreserved but for whom ovarian tissue transplantation is contraindicated (i.e. ovarian cancer or bloodborne malignancies). For these women, IVFG could be used to obtain oocytes. The efficiency of IVFG, however, is low because of the inability to fully recapitulate coordinated oogenesis and folliculogenesis. High levels of transcription and translation are essential for generating a high quality oocyte because it must accumulate sufficient stores of maternal products for subsequent development. We, therefore, hypothesized that stimulating translation during IVFG could improve follicle and oocyte development outcomes. To test our hypothesis, we isolated primary and secondary follicles (100-115 μm in diameter) from CD-1 mice and grew them in 50mM L-Leucine (LEU), an amino acid that stimulates the mTOR pathway -- a central signaling cascade that regulates translation. Short-term (24 hour) exposure of follicles to LEU activated downstream components of the mTOR pathway (p70SK), and did not impact follicle morphology. We then used an alginate hydrogel-based cohort culture system to grow these early stage follicles *in vitro* ± LEU. LEU supplementation significantly attenuated follicle growth. For example, control follicles started at 109 ± 5.3 μm in diameter and grew to 141 ± 25.2 μm after 8 days in culture. In contrast, follicles cultured in LEU started at 103 ± 5.0 μm in diameter and reached 117 ± 15.2 μm. Interestingly, however, LEU treatment did not appear to grossly impact follicle morphology, and the zona pellucidas of oocytes from follicles cultured in LEU were more prominent than controls (5.2 ± 0.4 μm thickness vs. 4.2 ±0.5 μm thickness, P = 0.006), suggesting that translational programs were activated within the oocytes. However, the presence of such a high dose of LEU may compromise coordinated oocyte-follicle development by making the oocyte less reliant on the surrounding somatic cells for its growth. Dose response studies are, therefore, warranted and ongoing to optimize the concentration of LEU for IVFG. This work was supported by the Centers of Biomedical Research Excellence (P20 GM104936-8 and the Interdisciplinary Graduate Program in Biomedical Sciences.

21. **The effect of human ovulatory follicular fluid on fallopian epithelial cell proliferation and genomic stability.** **Pavla Brachova***, Erika Munch, Donna Santillan, Bradley Van Voorhis, Lane Christenson*, Institute for Reproductive Health and Regenerative Medicine, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Repeated ovulations throughout the reproductive lifespan of a woman exposes the fallopian epithelium to follicular fluid (FF), a complex biologic fluid, which may have pathologic consequences. Recent studies demonstrate that high grade serous ovarian carcinomas originate in the fallopian epithelium. We hypothesize that FF may contribute to the malignant transformation of the fallopian epithelium. To examine this, and to assess the role of compounds in FF that may have lipotoxic effects, we obtained FF and plasma from 8 patients that were undergoing infertility treatment. The effect of FF on cell proliferation and genomic stability in normal immortalized human fallopian epithelial cells was examined. Exposure of the fallopian epithelial cells to FF induces cell proliferation and DNA damage accumulation. Specifically, we observed a significant increase in cell proliferation in cells treated with FF but not with plasma, indicating an effect specific to the FF. Interestingly, we also observed an increase in DNA damage repair factor foci formation as well as an accumulation of total DNA damage in cells treated with FF. Increased cellular proliferation may lead to an accumulation of DNA replication errors and mutations which may contribute to genotoxic stress, which has the potential to lead to cancer progression. We are currently working to identify the major constituents of FF that induce these effects, to shed light on the pathways and molecular alterations that occur in early ovarian carcinogenesis. This research was supported by *R01 HD061580 (LKC).*

22. **Yes-Associated Protein 1 (YAP) regulates ovarian follicle development and transformation of granulosa cells.** **Xiangmin Lv**[1, 2, *], Chunbo He[1, 2, 3, *], Guohua Hua[1, 2, 3], Jixin Dong[4], John S. Davis[1, 2, 4, 5], and Cheng Wang[1, 2, 4] [1]Olson Center for Women's Health, [2]Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha,

NE; [3]College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei province, China; [4]The Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE; [5]Nebraska-Northwest Iowa VA Medical Center, Omaha, NE; *Co-first author.

Ovarian granulosa cells are essential for successful follicle development. Dysregulation of granulosa cell proliferation and differentiation may lead to impairment of female fertility or development of ovarian tumors. Although studies indicate that the Hippo signaling pathway plays critical roles in both development and tumorigenesis of several organs, its role in ovarian physiology and pathology remains largely unknown. The present study aims to investigate the role of Yes-Associated Protein 1 (YAP) in ovarian granulosa cell proliferation, differentiation and transformation. Immunohistochemical analyses showed that the active form of YAP (nuclear YAP) was highly expressed in proliferative granulosa cells, whereas the inactive form of YAP (cytoplasmic YAP) was detected mainly in terminally-differentiated luteal cells, indicating that YAP may be involved in proliferation and differentiation of ovarian granulosa cells. Knockdown of YAP or pharmacological inhibition of YAP activity in human granulosa cells suppressed cell growth and reduced cell survival in both traditional 2D cell culture system and a novel 3D hanging-drop culture system, suggesting that YAP is essential for the growth and survival of human granulosa cells. In addition, ectopic expression of wild-type or constitutively active YAP impaired gonadotropin-induced differentiation of granulosa cells and promoted the transformation of granulosa cells, findings which were reflected by increased colony formation in soft agar assay and growth of tumors in a xenograft mouse model. Injection of verteporfin (a selective antagonist of YAP) into female CD1 mice disrupted mouse ovarian follicle development, leading to severe subfertility. Consistently, mice with ovarian-specific expression of constitutively active YAP demonstrated disruption of granulosa cell differentiation and failure of ovarian follicle development. In conclusion, our studies demonstrate that homeostatic YAP expression and activation are essential for ovarian granulosa cell proliferation, differentiation and survival. YAP is a novel therapeutic target for treatment of ovarian diseases associated with granulosa cell dysregulation.

23. **Regulation of FoxM1 by p21 in cancer cells.** **Jill A. Madden** and Jeremy Chien. Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS.

The oncogenic transcription factor forkhead box M1 (FoxM1) is overexpressed in many cancers, including 84% of ovarian cancer and plays a role in DNA repair, mitotic checkpoint, cell proliferation, and cancer drug resistance. Similar to Her2 in breast cancer, the constitutive expression of FoxM1 makes it a plausible gene target for novel anti-cancer therapies, but the regulation of FoxM1 has yet to be elucidated. Evidence suggests FoxM1 up-regulation is a result of TP53 mutations, however, no TP53 response element has been found within the FoxM1 promoter, thus there is likely another molecule mediating p53-induced FoxM1 overexpression. We hypothesize that cyclin-dependent kinase inhibitor 1a, p21, is involved in the regulation of FoxM1 by p53 in ovarian cancer. To test this hypothesis, we knocked down p21 expression with siRNA and shRNA in two wild-type p53 cancer cell lines, NCI-H23 and A2780. Knock down of p21 resulted in a mirrored increase in FoxM1 protein level in both cell lines as detected by Western blot. The addition of nutlin-3 (10 μM), a p53 stabilizer, to these p21 knocked down cell lines restored some p21 expression 24 hour post-treatment, which led to a subsequent decrease in FoxM1 levels compared to the corresponding DMSO-treated p21 knock down. These results suggest p21 is a negative regulator of FoxM1 in cancer cell lines. To further investigate this pathway, the same two cancer cell lines were treated with the FoxM1 inhibitor thiostrepton (10 μM) and protein levels were measured over a time course of 48 hours. FoxM1 protein levels were reduced following 8 hours, a time point where p21 protein levels peaked. This finding was further support by qRT-PCR analysis of A2780 ovarian cancer cell mRNA collected 0, 6, 8, 9, 10, 11, and 12 hours following thiostrepton (10 μM) treatment. In this experiment, p21 mRNA levels peaked 6 hours post-treatment, with FoxM1 mRNA levels reduced at 8 hours post-treatment, relative to control. Although thiostrepton has been shown to directly bind and inhibit the transcription of FoxM1, these results suggest that thiostrepton may also induce p21, potentiating FoxM1 inhibition. Thiostrepton has previously been shown to induce cancer cell death and reduce tumor burden, therefore further supporting FoxM1 as a potential drug target as well as encouraging the need to understand regulators involved in this pathway.

24. A single dose exposure to 0.1 Gy total body ionizing radiation induces ovarian follicle abnormalities. **Shawn M. Briley**[1], Susmita Jasti[1], Bruce F. Kimler[2], Francesca E. Duncan[1] [1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS [2]Radiation Oncology, University of Kansas Medical Center, Kansas City, KS.

Oocytes within ovarian follicles are highly sensitive to radiation-induced DNA damage, which leads to apoptosis, premature depletion of the ovarian reserve, and accelerated reproductive aging. We are interested in determining the effects of radiation therapy on the ovarian stroma because damage to the microenvironment in which oocytes grow may also negatively impact reproductive function. Here we used a mouse model of total body irradiation to determine whether a single dose exposure to 0.1 Gy ionizing radiation induced ovarian damage in adult mice. Six-week old CD1 female mice were exposed to γ-radiation using a Cesium-137 irradiator, and sham-treated mice served as controls (N=3/group). Mice were sacrificed 2 weeks post-exposure, and their ovarian tissue was harvested and processed for immunohistochemistry with an antibody against the oocyte-specific protein, MSY2. This method allowed us to perform follicle counts and assess the ovarian reserve with increased objectivity and efficiency while simultaneously gaining insight into oocyte quality. We counted the number of morphologically normal primordial, primary, secondary, early antral, and antral follicles on every fifth section for each animal (≥10 total sections/animal). Although the average number of follicles per section was lower across all stages of follicle development in irradiated mice compared to controls, these differences were not statistically significant. We then classified and quantified abnormal follicles as follows: Class I (activated oocytes), Class II (MSY2-negative oocytes), Class III (cell infiltration within oocytes), Class IV (MSY2-positive stroma), and Class V (multi-oocyte follicles). There were on average 2.4 ± 0.7 total abnormal follicles/section in irradiated animals compared to 1.3 ± 0.3 in controls ($P < 0.01$). Class I-IV follicles were more prevalent in irradiated animals compared to controls, whereas Class V follicles were observed equally in both groups as expected because multi-oocyte follicles form neonatally and should not be impacted by adult radiation exposure. Our results indicate that 0.1 Gy radiation causes modest but significant damage to ovarian follicles without affecting the ovarian reserve. Ongoing studies will determine whether a longer time post-radiation exposure or higher doses of radiation cause more prominent phenotypes, which will then be followed up with a detailed analysis of the ovarian stroma.

This work was supported by the Center for Reproductive Health After Disease (P50 HD076188-02) from the National Centers for Translational Research in Reproduction and Infertility (NCTRI).

25. A comparison of ovarian follicular and luteal cell gene expression profiles provides insight into cellular identities and functions. **Sarah Romereim**[1], Jennifer Wood[1], Xiaoying Hou[2], Heather Talbott[2], John Davis[2], Andrea Cupp[1] [1]University of Nebraska–Lincoln; [2]University of Nebraska Medical Center.

After ovulation, somatic cells of the ovarian follicle (theca and granulosa cells) transform to become small and large luteal cells of the corpus luteum. Aside from known cell type specific receptors and steroidogenic enzymes, there is a lack of understanding of the differences in gene expression of these four cell types. We hypothesized that a full analysis of the gene expression patterns of these cells would yield new cell-specific genetic markers, functional insight into the behavior of each cell type, and potentially lineage markers to determine which luteal cell type arose from the theca or granulosa cells. To address this, a comparison of the RNA present in each cell type [theca cells (TC, n=3), granulosa cells (GC, n=4), large luteal cells (LLC, n=3), and small luteal cells (SLC, n=3)] as measured by Affymetrix microarray analysis was performed and the biological relevance interpreted via Gene Ontology Annotations and Ingenuity Pathway Analysis of the differentially expressed RNAs. Microarrays were performed using cells isolated from large antral follicles of estrous-synchronized cows or corpora lutea from cows from a local abattoir. Each cell type had genes that were differentially expressed compared to all three other cell types. The TC population had 166 differentially expressed RNAs (e.g. smooth muscle specific *actin gamma2, hydroxyprostaglandin dehydrogenase-15*); GC had 567 (e.g. *FSH receptor*, *inhibin beta*); LLC had 312 (e.g. *prostaglandin F receptor, prolactin receptor, lipoprotein lipase*); and SLC had 60 (e.g. *LH receptor, CD274)*. Select genes have been validated with qPCR, with strong correlations between microarray and qPCR results. Analysis of the gene ontology and predicted roles of these genes revealed expected functions (such as increased cell growth/hypertrophy and vascularization in luteal cells) and less expected functions (such as immune response/inflammation and

metabolism in large luteal cells). Thus, gene expression analysis revealed transcriptional markers for individual cell types and provided information on the regulation of cell behaviors relevant to ovarian function. This new information on gene expression in these four cell types may provide insight into the lineage and differentiation process that creates luteal cells from follicular cells. This research was supported by USDA/NIFA grants 2011-67015-20076 and 2013-67015-20965.

26. **Reproductive age-associated fibrosis in the mammalian ovary.** Shawn Briley[1], Susmita Jasti[1], Jessica E. Hornick[2], Michele T. Pritchard[3], **Francesca E. Duncan**[1] [1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS [2]Biological Imaging Facility, Northwestern University, Evanston, IL [3]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Under normal physiological conditions, tissue remodeling in response to injury leads to tissue regeneration without permanent damage. However, in the case of chronic pathogenic tissue remodeling, the homeostasis between synthesis and degradation of extracellular matrix components (ECM) is altered. Excessive ECM production – or fibrosis - disrupts tissue architecture and compromises its function. The mammalian ovary is unique in that it undergoes chronic wound healing for decades throughout a female's reproductive lifespan due to repeated ovulatory cycles. In this study we tested the hypothesis that fibrosis increases in the ovary with advanced reproductive age. To examine fibrosis in the mouse ovary, we used Picro Sirius Red (PSR) staining. PSR is a connective tissue stain used for the histologic detection of collagen I and III fibers and is frequently used to monitor fibrosis. We visualized PSR staining in the ovary using three methods: light microscopy, conventional fluorescence microscopy, and polarized light microscopy. Together these methods demonstrated specific staining of highly ordered collagen bundles and validated the use of this technique in the ovary. We next examined ovarian PSR staining in two mouse strains (CB6F1 and CD1) across an aging continuum. We found that PSR staining was minimal in reproductively young adult animals (<5 months) but increased in distinct foci within the ovarian stroma in animals of mid-to-advanced reproductive age (7-18 months). At an age associated with complete reproductive senescence (>22 months), PSR staining was prominent throughout the ovarian stroma. Importantly, fibrosis is associated with inflammation, and we noted a significant age-associated infiltration of immune cells (Fc receptor positive) that have a foamy macrophage appearance in the ovarian stroma. The presence of immune cells together with the observed increase in PSR staining strongly suggest that fibrosis is a hallmark of reproductive aging in the ovary. Whether such fibrosis is linked to ovulation and contributes to the well-established decline in oocyte quality that occurs with reproductive age is currently under investigation. This work was supported by the Center for Reproductive Health After Disease (P50 HD076188-02) from the National Centers for Translational Research in Reproduction and Infertility (NCTRI) and by the Centers of Biomedical Research Excellence (P20 GM104936-8).

27. **Defining roles for ESR2 in the regulation of female fertility.** **Mohammad Rumi**, Katherine Roby, Xiao Zhao, Khursheed Iqbal, Anamika Ratri, Rafia Mir, Shaon Borosha, Tianhua Lei, Wei Cui, Kaiyu Kubota, Pramod Dhakal, Michael Wolfe, Jay Vivian and Michael Soares. Institute for Reproductive Health and Regenerative Medicine; Departments of Pathology and Laboratory Medicine, Anatomy and Cell Biology, Molecular and Integrative Physiology. University of Kansas Medical Center, Kansas City, KS.

Estrogens are essential hormones in the regulation of fertility. Two nuclear receptors, estrogen receptor alpha (ESR1) and estrogen receptor beta (ESR2) transduce cellular responses to estrogens. In mouse and rat models, disruption of *Esr1* causes infertility in both males and females. However, the effects of *Esr2* disruption in the mouse range from subfertility to unexplained infertility. Such variability can be related to differences in the sites targeted for mutagenesis and potential inframe alternative splicing of *Esr2* transcripts. Among the splice variants is a transcript that lacks exon 4, which encodes part of the DNA binding domain. Using zinc finger nuclease (ZFN) mediated genome editing, we have generated two different mutant rat models; one with a targeted deletion of exon 3 (ΔE3) and the other with a targeted deletion of exon 4 (ΔE4) of *Esr2*. The ΔE3 results in a frameshift and a null mutation, whereas ΔE4 generates an allele that expresses ΔE4 ESR2 protein lacking the DNA binding domain. In both mutant rat models, we observed that males were

fertile but females were infertile. Homozygous ΔE3 or ΔE4 *Esr2* mutant females exhibited cyclic changes in vaginal cytology indicating intact estrous cycles. When cohabitated with adult wild type males, ΔE3 or ΔE4 mutant females mated but failed to become pregnant or pseudopregnant. In both of the rat models, ovaries were significantly smaller in eight week old mutant females. Wild type ovaries contained multiple corpora lutea, whereas ΔE3 or ΔE4 *Esr2* mutant ovaries were characterized by the absence of corpora lutea despite various stages of follicular development. Immature ΔE3 or ΔE4 *Esr2* mutant female rats and their wild type littermates were treated with gonadotropins and changes in ovarian morphology, ovarian weight, and oocyte yield were evaluated. Ovaries from both mutant models exhibited premature follicles, which failed to ovulate upon gonadotropin stimulation. Our findings indicate that the transcriptional function of ESR2 is critical for preovulatory follicle maturation and ovulation. Supported by pilot funds from the IRHRM and NIH (HD072100).

28. Identification of loci associated with fertility in holstein heifers. **Joao G. N. Moraes**, Joseph Dalton, Thomas E. Spencer, Jennifer N. Kiser, Gregory W. Burns, Andrzej Wojtowicz, Mahesh Neupane, Holly L. Neibergs. Department of Animal Science, Washington State University, Pullman. Department of Animal and Veterinary Sciences, University of Idaho, Caldwell.

Selection for higher milk production in United States dairy cattle has been very successful during the past 50 years, however modern lactating dairy cows exhibit a high incidence of subfertility and infertility with a national pregnancy rate of only 15 to 20%. The objective of this study was to identify genomic loci associated with fertility in nulliparous Holstein heifers. Breeding and health records of Holstein heifers (n=2,333) were analyzed from a commercial heifer raising facility in Southern Idaho. Of these, 1,114 heifers were classified as highly fertile (conceived on first AI service) and 209 were identified as subfertile (did not conceive until after the fourth AI service or culled due to failure to conceive). Blood samples were obtained from the fertility-classified heifers, and DNA was extracted from 497 high fertile and 196 subfertile heifers. The DNA was genotyped with the Illumina Bovine HD Genotyping BeadChip. Quality control consisted of removing SNPs with <90% call rate, and a MAF <1% and removing heifers with a genotyping rate <90%, leaving 581,918 SNPs and 468 fertile and 188 subfertile heifers for analysis. A genome wide association analysis (GWAA) and heritability estimate was conducted with the Efficient Mixed-Model Association expedited (EMMAX) software. This mixed model program empirically estimated a genomic relationship matrix and used it to model the correlation between the fertility phenotypes. Correction for population stratification was done by variance components and resulted in λ_{GC}=1.01. The GWAA identified 74 QTL defined by 91 SNPs associated with heifer fertility. Two QTL with strong association with fertility were identified on Bos taurus (BTA) 4 and BTA6. Seventy two QTL were identified with a moderate association with fertility on BTA1, BTA2, BTA3, BTA4, BTA5, BTA6, BTA7, BTA8, BTA9, BTA10, BTA11, BTA12, BTA15, BTA18, BTA21, BTA23, BTA25, BTA26, BTA27 and BTA28.The heritability estimate for fertility in Holstein heifers was 0.56. These results indicate that there is ample opportunity to make significant gains in fertility in nulliparous Holstein heifers with genomic selection. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2013-68004-20365 from the USDA National Institute of Food and Agriculture.

29. A portion of heifers attaining "early puberty" do not display estrus, are anovulatory and have reduced sex hormone binding globulin concentrations. **Sarah Tenley**[1], Renata Spuri-Gomes[1], Mohamed Ayoub Abedal-Majed[1], Jeff Bergman[1], Scott Kurz[1], Jennifer Wood[1], Robert Cushman[2], Andrea S Cupp[1] [1]University of Nebraska–Lincoln; [2]USDA, ARS, U.S. Meat Animal Research Center.

Cows with excess androstenedione (High A4) in the follicular fluid of dominant follicles attain puberty earlier than their low androstenedione counterparts. Furthermore, High A4 cows are anovulatory (chronic or sporadic) and have lower Sex Hormone Binding Globulin (SHBG) compared to Low A4 ovulatory cows. Thus, our objective was to investigate how endocrine profiles around puberty affect reproductive cycles, and pregnancy rates after artificial insemination (AI). Our hypothesis was that heifers with earlier puberty are predisposed to anovulation. Blood plasma was collected on heifers (female cows that have never had a calf) at 6 months of age until 1 year and estrous cycles were synchronized for AI at one year of age. Blood plasma was analyzed for progesterone (P4) with concentrations >1 ng/ml indicative of puberty attainment. Interestingly,

four different P4 profiles were identified in these 105 heifers: 1) Early Puberty Cycling- pubertal early (6-9 mo) and continued cyclicity with 48% pregnant to AI; 2) Typical Puberty Cycling- pubertal at 10-12 months of age with 42.5% pregnant to AI ; 3) Start -Stop Cycling- pubertal between 6-9 months of age but discontinued detection of P4 and no estrus detected resulting in no AI; and 4) No Cycling- no P4 > 1 ng/ml and no estrus thus no AI. Blood plasma was also analyzed for concentrations of SHBG. Heifers with P4 profiles 1&2 have greater concentrations of SHBG than 3& 4. Ten heifers (5 that were pubertal at <7mo and 5 pubertal at >7mo) had more intensive blood collection and ultrasound to determine follicular waves, and reproductive cyclicity including estrus and ovulation. Interestingly, heifers with P4 profiles similar to 1&2 had higher progesterone, greater SHBG, and normal reproductive cycles where follicles developed, and estrus and ovulation were detected. However, heifers with progesterone profiles similar to profiles 3&4 developed follicles; but, estrus and ovulation were not detected and these heifers also had reduced SHBG concentrations. Therefore, heifers with P4 profiles similar to 3&4 may have difficulty in establishing the hypothalamic-anterior pituitary-gonadal axis and could be a population that will be chronic or sporadic anovulatory. This research was funded through USDA grant 2013-67015-20965.

30. **Excess maternal fructose consumption leads to decreased progesterone, increased fetal loss and impaired endometrial stromal cell decidualization in mice.** **Jessica L. Saben**, Zeenat Asghar, Julie S. Rhee, Andrea Drury, Kelle H Moley**.** Department of Obstetrics and Gynecology, Washington University School of Medicine, Saint Louis, Missouri.

The most significant increase in metabolic syndrome over the previous decade occurred in women of reproductive age, which is alarming given that metabolic syndrome is associated with reproductive problems including subfertility and early pregnancy loss. Individuals with metabolic syndrome often consume excess fructose, and several studies have concluded that excess fructose intake contributes to metabolic syndrome development. Here, we examined the effects of increased fructose consumption on pregnancy outcomes in mice. Female mice fed a high-fructose diet (HFrD) for six weeks developed glucose intolerance and mild fatty liver but did not develop other prominent features of metabolic syndrome such as weight gain, hyperglycemia, and hyperinsulinemia. Upon mating, HFrD-exposed mice had lower pregnancy rates and smaller litters at mid-gestation than control chow (Con)-fed controls. To explain this phenomenon, we performed artificial decidualization experiments and found that HFrD consumption impaired decidualization. This appeared to be due to decreased circulating progesterone, as exogenous progesterone administration rescued the decidualization defect. Furthermore, HFrD intake was associated with decreased bone morphogenetic protein 2 expression and signaling that was rescued upon with addition of P4. Finally, decreased expression of Foxo1 and Sod2 proteins were associated with HFrD-feeding, suggesting that HFrD consumption promotes a pro-oxidative environment in the endometrium. In summary, these data suggest that excess fructose consumption impairs murine fertility by decreasing steroid hormone synthesis and promoting an adverse uterine environment.

31. **Regulation of REST target genes and miRNA29 in uterine leiomyomas.** **Mina Farahbakhsh**, Faezeh Koohestani and Vargheese Chennathukuzhi. The Center for Reproductive Sciences, Institute for Reproductive Health and Regenerative Medicine. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Uterine leiomyomas (ULs), also known as uterine fibroids, are smooth muscle cell tumors of the myometrium and are the most frequent reason for a hysterectomy. Patients with ULs suffer from symptoms of abnormal uterine bleeding, incontinence and infertility. Although ULs are the most common tumors of the female reproductive tract with an estimated annual cost of $34 billion to the US economy, there are currently no long-term therapies that will leave fertility intact. Hence, there is an urgent need to understand the pathogenesis of ULs in order to develop effective long-term drug therapies.

Atypical activation of the PI3K/AKT-mTOR pathway is thought to play a role in UL development. However, the mechanism of activation of such pathways in ULs is unknown. Our lab has shown the expression of RE1 suppressing transcription factor (REST), a known tumor suppressor, is lost in ULs. Analysis of gene

expression datasets indicated that many of the most abnormally expressed genes in ULs are known targets of REST. Using matched human leiomyoma and myometrial specimen, we analyzed the expression level of REST target genes, *SCG2, GRIA2, NEFH, SALL1, GRIN2A, STMN2, DCX and CBLN1* and found them to be overexpressed in ULs. Furthermore, silencing REST in primary myometrial cells led to an increase in the expression of REST-target genes, suggesting that the loss of REST leads to the activation of these genes in ULs.

Additionally, REST is known to regulate the expression of miRNAs including miR-29b, which plays an important role in the pathogenesis of ULs. Interestingly, the miRNA29 family has been shown to directly target the 3'UTR and to regulate the expression of *ADAM12*, (a-disintegrin and metalloprotease 12), ADAM12 is involved in the regulation of ECM and in the activation of growth factor receptor pathways. In leiomyoma specimen, we found an increase in *ADAM12* transcript levels along with a decrease in miRNA29 expression compared to that in matched myometrial specimen. Moreover, silencing *REST* in primary myometrial cells led to a loss in miRNA29 and a gain in *ADAM12* transcript levels.

In conclusion, our data suggests that myometrial loss of REST is involved in the pathogenesis of ULs. This research was supported by R01HD076450-01A1.

32. Impact of uterine glands and uterine luminal fluid on endometrial receptivity in mice. **Andrew M Kelleher** and Thomas E Spencer. Department of Animal Sciences, University of Missouri, Columbia.

Uterine glands and their secretions are essential for blastocyst implantation and stromal cell decidualization in mice, and likely humans. In mice, the uterus becomes receptive to implantation on day 4 post-mating and, by the afternoon of day 5, becomes refractory. Glandular epithelia (GE) synthesize and secrete or transport bioactive substances fundamental for the establishment of pregnancy. Treatment of newborn C57BL/6 mice with progesterone ablates postnatal differentiation of GE, resulting in uterine gland knockout (UGKO) phenotype. UGKO mice are infertile and exhibit defects in blastocyst implantation and uterine stromal cell decidualization. In Study One, adult control wild type (WT) and progesterone-induced UGKO (PUGKO) mice were mated to intact fertile males and flushed at 1600 h on day 5 post-mating. No blastocysts were recovered from WT females, whereas an average of 4 blastocysts were recovered from PUGKO uteri. In Study Two, WT and PUGKO mice were mated to vasectomized males, and uteri and uterine luminal fluid (ULF) obtained on days 3, 4 and 5 of pseudopregnancy (DOPP). Total RNA was isolated from the uteri and sequenced. Dynamic changes in gene expression occurred surrounding uterine attainment of receptivity in WT uteri. Proteomic profiling of the ULF was conducted using liquid chromatography-tandem mass spectrometry. This approach identified 1,111 proteins in WT ULF, but Lif was not among them. Study Three tested the hypothesis that apical secretions of the GE into the uterine lumen regulate uterine receptivity. Mice received injections of various ULF treatments into the lumen of one uterine horn on DOPP 4. Uteri were obtained on DOPP 5. None of the intraluminal treatments altered expression of uterine receptivity genes (*Abp1, Areg*, *Hdc1*, *Hegf1, Ihh, Lif, Msx1, Msx2, Muc1, Noggin, Sfrp4, Ptgs2, Wnt7a*) in the PUGKO uterus by q-PCR. Collectively, these results support the working hypothesis that blastocyst implantation defects in the PUGKO uterus stem from a lack of uterine receptivity due to alterations in gene expression and ULF homeostasis. Results also support that uterine glands secrete factors, such as Lif, in a basolateral direction and that paracrine crosstalk between the GE and other cell types is required for establishment of uterine receptivity.

33. Loss of PRICKLE1 leads to alterations in WNT/PCP pathway in uterine fibroids. **Faezeh Koohestani**[1], Kavya Shivashankar[2], Sornakala Ganesh Kumar[1], Wendy Jefferson[3], Carmen Williams[3] & Vargheese Chennathukuzhi[1] [1]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, [2]Columbia University, New York City, NY, [3] National Institute of Environmental Health Sciences Research Triangle Park, NC.

Uterine fibroids are the most common pelvic neoplasms in women of reproductive age with severe morbidity and an annual economic burden exceeding that of breast cancer. Due to our poor understanding of their pathogenesis, current treatment options are limited and not fertility-sparing. Therefore, there is an urgent need to understand the underlying mechanisms involved in fibroid formation for better disease prevention and management.

To address this need, we focused on our recent finding of the loss of PRICKLE1, a key player in the Planar Cell Polarity (PCP) pathway, in fibroids. PCP, part of the non-canonical WNT (ncWNT) pathways, regulates tissue architecture and bears significance in fibroids whose main feature is altered tissue architecture. Using healthy myometrial and fibroid tissues from patients (n=7), we discovered the expression of PRICKLE1 and its counterpart DISHEVELLED1 were significantly (P<0.05) altered in fibroids. Increased expression of collagens, a hallmark of fibroids, upon silencing *PRICKLE1* in myometrial cells as well as altered expression of PCP target genes further confirmed the involvement of PCP in fibroid pathogenesis. Due to the interdependent nature of ncWNT and canonical WNT (cWNT) pathways in tumorigenesis, we next examined the status of CTNNB1 as the key component of cWNT. *In silico* and *ex vivo* data showed cytoplasmic localization of CTNNB1 without significant changes in cWNT target genes in fibroids. Interestingly, the OFF state of cWNT and altered ncWNT was nicely mimicked in the uteri of *GPR10* transgenic model of fibroids generated in our laboratory.

Since environmental estrogens are known risk factors for fibroids, we investigated the modulatory role of these agents on the status of WNT/PCP pathways. Neonatal mice (n=6) were treated with 50 mg/kg genistein or 1 mg/kg diethylstilbestrol and uteri were collected on days 5 and 22. Immunohistochemical analysis showed a pattern similar to human fibroids' in mice treated with environmental estrogens as compared to control mice.

Taken together, our data suggest that WNT/PCP pathway is involved in the pathogenesis of fibroids and may be modulated by environmental estrogens. These findings link a major risk factor for fibroids to a crucial and neglected signaling pathway that controls tumorigenesis.

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34. Hormonal regulation of female reproductive cyclicity: a role for progesterone? **Kaiyu Kubota**, Wei Cui, M.A. Karim Rumi, Pramod Dhakal, Michael W. Wolfe, Jay L. Vivian, Katherine F. Roby, and Michael J. Soares. Institute for Reproductive Health and Regenerative Medicine, Departments of Pathology and Laboratory Medicine and Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.

The fundamental elements regulating the female reproductive cycle have been known for decades and include a hierarchy of control involving the hypothalamic/anterior pituitary/ovarian axis. At the core of the female reproductive cycle is a balance of sex steroid hormone feedback regulation of gonadotropin secretion. These concepts have been reinforced through phenotypic examination of mice possessing null mutations at either estrogen receptor alpha (*Esr1*) or progesterone receptor (*Pgr*) loci. However, characterization of rats with a null mutation at the *Pgr* locus has forced a reexamination of the role of progesterone in the regulation of the female reproductive cycle. We generated two different *Pgr* mutant rat models. A 136 bp deletion within exon 1 of the *Pgr* gene was produced using zinc finger nuclease-mediated genome editing. The other possesses a 984 bp deletion including all of exon 3 of the *Pgr* gene (the same exon targeted in *Pgr* null mice) using the CRISPR/Cas9 system. In both cases, the deletion results in a truncated protein lacking the DNA binding domain and ligand binding domain due to a nonsense frame-shift and the emergence of a stop codon. Similar to *Pgr* null mice, *Pgr* null rats were infertile due to deficits in sexual behavior, ovulation, and uterine endometrial differentiation. However, in contrast to the reported phenotype of female mice with disruptions in *Pgr* signaling (*Endocrinology* 138:4147-52, 1997), *Pgr* null female rats exhibit robust estrous cycles. Four to five day cycles in vaginal cytology, uterine histology, serum hormone levels (LH, FSH, estradiol, and progesterone), and wheel running activity were evident in *Pgr* null female rats similar to wild type controls. Furthermore, exogenous progesterone treatment inhibited estrous cycles in wild type female rats but not in *Pgr* null female rats. This phenotypic description of *Pgr* null rats resembles aspects of those previously described for a patient with progesterone resistance and normal menstrual cycles (*J Clin Endocrinol Metabol* 48:127-32, 1979). We conclude that in the rat, and possibly other species, progesterone signaling is not required for the establishment and maintenance of the female reproductive cycle. (Supported by AHA, JSPS, Lalor Foundation postdoctoral fellowships; NIH grants: HD066406, OD01478)

35. Botanical inhibitors of endometriosis. **Chun-Xia Meng**[1], Christopher D. Kassotis[2], Sadia Akter[3], Victoria D. Balise[1], Chiamaka J. Isiguzo[1], Michelle A. Williams[1], Katelyn M. Cinnamon[1], Jiahao Hu[1], Trupi Joshi[3,4,5] and Susan C. Nagel[1] 1. Department of Obstetrics, Gynecology and

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Endometriosis is an estrogen-dependent disease associated with chronic pelvic pain and infertility. While the etiology is unknown, immune dysfunction has been implicated. Hormonal interventions are somewhat effective but they have undesirable side effects, therefore, botanical treatments have been proposed with hopes of fewer side effects and for women desiring an alternative to pharmaceuticals. Two botanicals, quercetin and chlorogenic acid (CGA), both putative active ingredients of elderberry juice, have been shown to inhibit NF-κB signaling pathway, which has been shown to inhibit endometriosis *in vitro* and *in vivo*. Using an estrogen receptor reporter gene assay in human endometrial adenocarcinoma cells, we found all three exhibited anti-estrogenic activity. We hypothesized that elderberry juice, quercetin, and CGA would inhibit growth of endometriotic lesions in a mouse model of endometriosis. We administered three doses of elderberry juice (0.1%, 1%, 10%), quercetin, and CGA (5.7 µg/mL, 57 µg/mL, 571 µg/mL for both) via drinking water to 9-week-old C57BL/6 female mice (n=12/group) one week prior to surgical induction of endometriosis and four weeks after. After five weeks of treatment, mice were euthanized at estrus stage. We found that quercetin at 57.1 µg/mL (≈ 10 mg/kg/d) reduced the weight of endometriotic lesions, but it did not alter cell proliferation. Cell apoptosis and angiogenesis in the endometriotic lesion and eutopic endometrium and the proportion of immune cells in peritoneal fluid will be analyzed. RNA sequence analysis showed that compared to the vehicle, 93 and 40 genes were differentially expressed in the endometriotic lesions, but not in eutopic endometrium, from mice that received 57 µg/mL quercetin or 1% elderberry juice, respectively. The numbers of differentially expressed genes in the ectopic lesions versus eutopic endometrium were 1044, 534, 2668 in the vehicle, 57 µg/mL quercetin, 1% elderberry juice groups, respectively, and gene pathway analysis for differentially expressed genes is underway. Identification of the immune cell profile in the peritoneal fluid and the gene expression pathways will elucidate potential mechanisms of action for these possible botanical inhibitors of endometriosis.

36. Systems biology approach to understanding uterine receptivity and pregnancy loss.
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The majority of embryonic loss in domestic animals and humans occurs during the first month of gestation when pregnancy recognition and conceptus implantation are obligatory. The receptive endometrium in ruminants represents a temporary, but unique physiological state of the uterus when conceptus elongation and implantation is possible. Thus, inadequate uterine receptivity may compromise conceptus growth, signaling for pregnancy recognition, and pregnancy establishment. However, the critical genes and physiological pathways that mediate uterine receptivity and pregnancy success are not well understood. Predominantly Angus heifers were classified based on fertility using serial ET (n=4 rounds) to select animals with intrinsic differences in pregnancy loss. In each round, a single *in vitro*-produced embryo was transferred into synchronized heifers (n=228) and pregnancy determined on day 28. Heifers were classified as high fertile (HF; 100% pregnancy success) or subfertile (SF; ≤25%). Following fertility classification, a single *in vivo*-produced embryo was transferred into HF (n=29) and SF (n=32) heifers on day 7 post-estrus. Uteri of recipient heifers were flushed on day 14 to recover the conceptus. If present, the recovered conceptuses (n=35) were imaged and length and area were measured. The effect of recipient fertility classification on pregnancy rate, conceptus length and area were determined using embryo donor as a covariate. Pregnancy rate, conceptus length and area were not different (P=0.93, P>0.10, P>0.10) in HF and SF heifers. The HF (n=29) and SF (n=51) heifers were genotyped using blood sample DNA and Illumina's 778K SNP BovineHD Genotyping BeadChip. A genome-

wide association study (GWAS) was then conducted using the EMMAX mixed-model association test. Highly significant associations (n=20; $P<5X10^{-7}$) were detected on several chromosomes and queried against the cattle Quantitative Trait Loci (QTL) database which revealed 8 SNPs located in loci with at least one known fertility trait. Results of these studies indicate that pre-implantation conceptus growth is not compromised in SF heifers that are fertility-classified by ET. Further, they support the idea that the observed difference in capacity for pregnancy success is manifested between days 14 and 28 when pregnancy recognition signaling and conceptus implantation must succeed for the establishment of pregnancy. Supported by 1 R01 HD072898.

37. Gonadotropin-releasing hormone II receptor (GnRHR-II) knockdown reduces testis size and decreases testosterone secretion during pubertal development in swine. **Amy T. Desaulniers**[1], Rebecca A. Cederberg[1], Ginger A. Mills[1] and Brett R. White[1] [1]University of Nebraska Lincoln, Lincoln, NE.

The second mammalian isoform of gonadotropin-releasing hormone (GnRH-II) functions quite differently from the classical form (GnRH-I) as it is a poor stimulator of gonadotropin release. Unlike most species, a functional GnRHR-II has been identified in swine. Our laboratory detected GnRHR-IIs on Leydig cells and demonstrated that GnRH-II stimulates testosterone release as effectively as GnRH-I, despite minimal luteinizing hormone (LH) secretion. These data suggest that GnRH-II acts directly on Leydig cells to stimulate steroidogenesis. Therefore, we produced a knockdown (KD) swine line to further examine the function of GnRHR-II. Here, the objective was to evaluate testis size and serum hormone levels in transgenic males during pubertal development. GnRHR-II KD (n = 7) boars and littermate controls (n = 5) were monitored throughout development; blood was collected and testis size measured using calipers at 40, 100, 150, 190, 225 and 300 d of age. Predicted testis volume was calculated and serum was isolated for testosterone and LH radioimmunoassay. For testis volume, we observed an effect of line (P = 0.0006), time (P < 0.0001) and a tendency for a line by time interaction (P = 0.0731). By 300 d of age, testes from transgenics were smaller than littermate controls (559.9 ± 34.3 versus 772.6 ± 39.7 cm^3; P < 0.05) despite similar body weights (P > 0.05). We also observed an effect of line (P < 0.0001), time (P < 0.0001) and a line by time interaction (P = 0.0018) for serum testosterone concentrations. Testosterone levels were similar (P > 0.05) between lines from 40 -100 d of age but lower in transgenic boars at 150 (1.3 ± 0.9 versus 3.7 ± 1.0 ng/ml), 225 (6.9 ± 1.5 versus 15.2 ± 1.5 ng/ml) and 300 (12.2 ± 1.5 versus 21.3 ± 1.5 ng/ml) d of age. Notably, LH levels were similar (P = 0.91) between transgenic (0.34 ± 0.08 ng/ml) and littermate controls (0.32 ± 0.09 ng/ml) at 300 d of age, suggesting that LH is not mediating the diminished testosterone secretion observed in transgenics. Together these data indicate that in swine, GnRHR-II is involved in LH-independent steroidogenesis and postnatal testis development.

38. Divergent VEGFA signaling determines spermatogonial stem cell fate. **Kevin M. Sargent**, John R. Essink, Meredith L. Bremer, William E. Pohlmeier, Melissa M. Laughlin, and Andrea S. Cupp. University of Nebraska-Lincoln, Lincoln, NE.

Treatment with or testis-specific elimination of vascular endothelial growth factor A (VEGFA) isoforms alters spermatogonial stem cell (SSC) maintenance *in vivo*. Therefore, we hypothesized that testis-specific elimination of neuropilin-1 (NRP1), a co-receptor that only binds VEGFA angiogenic isoforms, would impair SSC maintenance. We generated *Nrp1 x Amhr2-Cre* male mice and collected them both at 3 and 6 months of age. Whole testis mRNA abundance for genes that regulate SSC maintenance were downregulated in the testes of 3 month-old *Amhr2-Cre;Nrp1*$^{-/-}$ (KO) males including: *Gdnf* (P < 0.05); the GDNF receptor *Ret* (P < 0.09, tendency), *Foxo1* (P = 0.003); *Sin3a* (P < 0.04), *Kitl* (P < 0.1, tendency), and *Neurog3* (P < 0.01). These findings suggest that NRP1 loss reduced SSC self-renewal. Surprisingly, there was an ***increase*** in the amount of *Zbtb16* mRNA in KO testes (P < 0.001) and an increase in ZBTB16-positive staining per tubule (P < 0.002), suggesting there were more undifferentiated spermatogonia in KO testes. This was accompanied by a 19% increase in caput epididymal sperm (P < 0.03). NRP1 also co-expressed with ID4 in undifferentiated spermatogonia, and these numbers were fewer per tubule in KO testes (P < 0.04). While the number of ZBTB16+ spermatogonia was increased per tubule, the number of ID4+/ZBTB16+ cells was reduced in KO males (P < 0.05). KO males had reduced phosphorylation of RET (P = 0.04) at 3 months. KO and control males were mated with control females from 2 to 6 months of age. Compared to controls, KO males sired

fewer pups at the first litter ($P = 0.0001$) and in all litters ($P = 0.03$) in addition to tending to take longer to impregnate females ($P < 0.1$). KO males were subfertile by 6 months, and their epididymides contained 36% *fewer* sperm ($P < 0.05$), and whole testis *Gdnf* tended to be reduced ($P < 0.08$). RET and KDR, the primary VEGFA receptor, physically interact in the testis of mice at both time points. These data demonstrate that loss of NRP1 and reduction of VEGFA angiogenic signaling impairs SSC maintenance, resulting in fewer potential SSCs and impaired fertility.

39. **Regulation of metabolism by estrogen receptor alpha.** **Ashley Ward***, Paige Geiger*, Robert Rogers, Josh Wheatley, Kathleen White, M.A. Karim Rumi. *Institute of Reproductive Health and Regenerative Medicine, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Postmenopausal estrogen deficiency increases the risk for metabolic syndrome, obesity, and Type 2 Diabetes. Estrogen treatment improves metabolic function in postmenopausal women, and estrogen receptor α (ERα) is thought to be the primary player in these protective metabolic effects. However, direct molecular targets and tissue-specific roles of ERα have not been elucidated. The purpose of this study was to investigate the impact of ERα loss on whole body metabolism in a rat model. 14 wk-old female Wildtype (WT) and ERα -/- rats were exercised to exhaustion and one week later a glucose tolerance test was performed. At sacrifice, body weight and adipose tissue depot weights (subcutaneous, retroperitoneal and periuterine) were measured. Adipocyte cell size and mitochondrial protein content were assessed by immunohistochemistry and western blot, respectively. Intramuscular triglyceride storage was also assessed. ERα deficient rats had increased body weight and subcutaneous adipose tissue mass compared with WT rats. Although there was no increase in retroperitoneal adipose tissue mass, adipocyte size was increased in ERα deficient rats. HOMA-IR values were also increased in ERα -/- animals indicating increase insulin resistance. ERα deficiency also decreased exercise time to exhaustion when compared with WT rats. Mitochondrial proteins Complex IV and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) were significantly decreased in skeletal muscle from ERα deficient rats. There was also increased intramuscular triglyceride storage in skeletal muscle of ERα deficient rats. This data reveals the potential regulatory role of ERα to enhance oxidative capacity and improve energy metabolism. This research was supported in part by an NIH grant (R01 AG031575) and an IRHRM Pilot grant.

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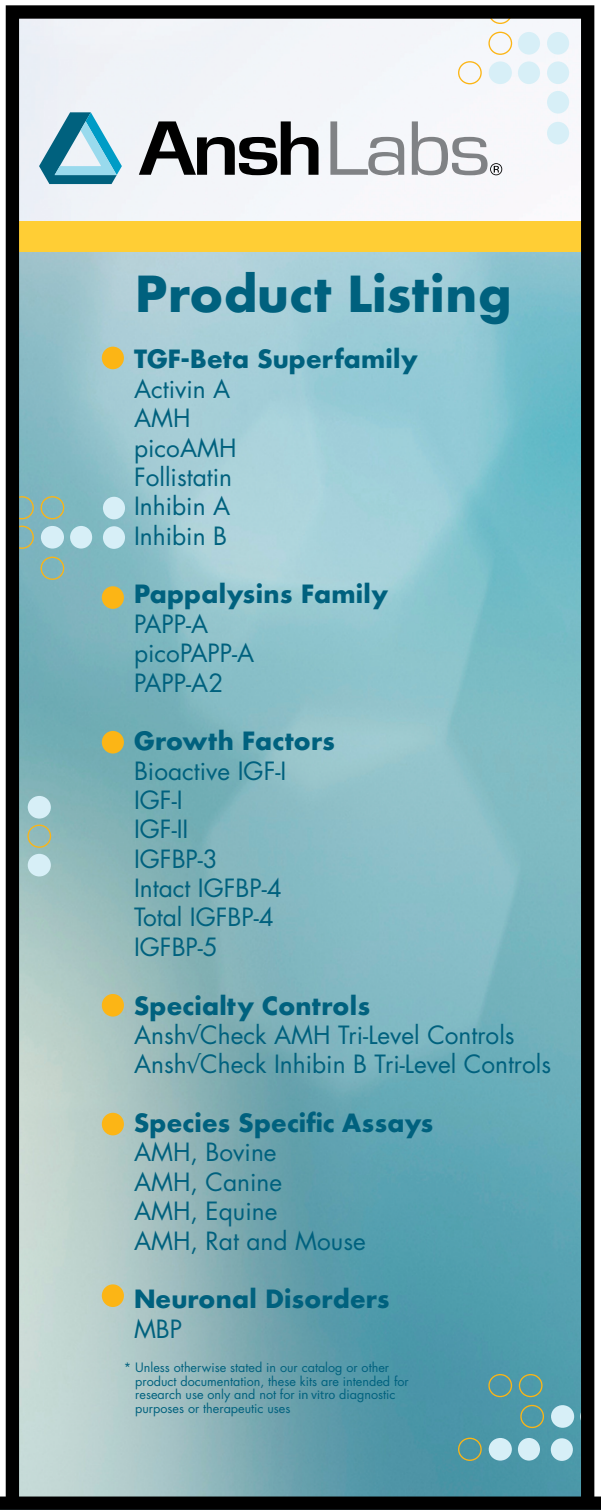

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