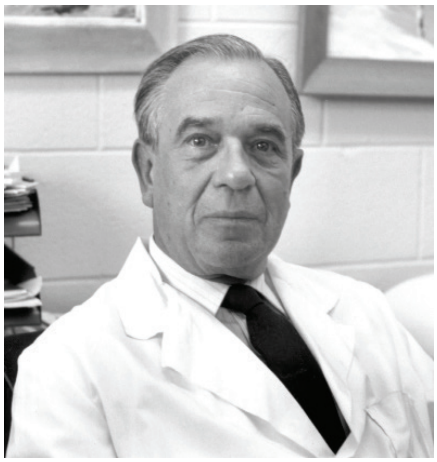
KU MEDICAL CENTER
The University of Kansas

The 11th Annual Gilbert S. Greenwald Symposium on Reproduction and Regenerative Medicine

November 6-7, 2014

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.

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IRHRM: Institute for Reproductive Health & Regenerative Medicine

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

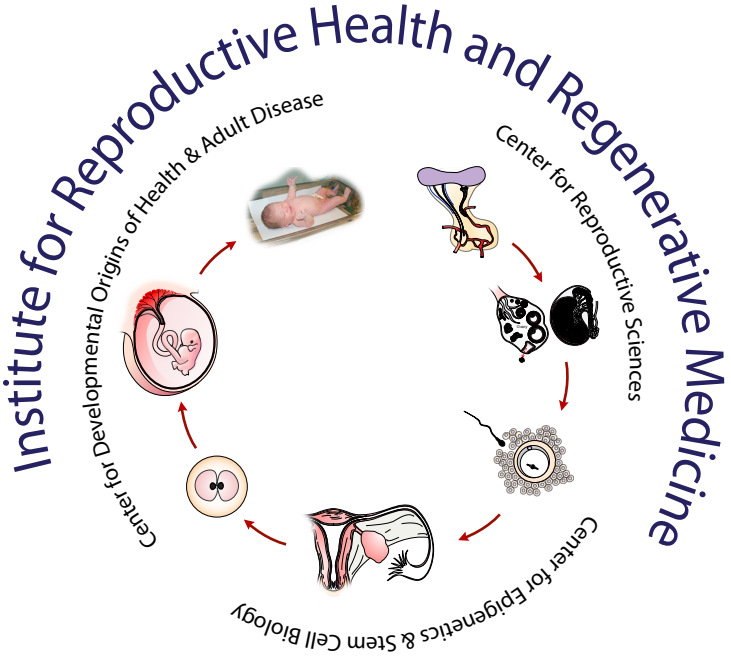
Frederick vom Saal, PhD, University of Missouri-Columbia

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



Program Schedule

THURSDAY, NOVEMBER 6th

University of Kansas Medical Center
3901 Rainbow Blvd., Kansas City, KS 66160

4:30 - 5:00 p.m.	Registration, G013 School of Nursing (SON)
5:00 - 5:05 p.m.	**Introductory Remarks, Paul F. Terranova, PhD**
5:05 - 5:08 p.m.	**Welcome from the CRS Director, T. Rajendra Kumar, PhD**
5:08 - 5:10 p.m.	**Introduction of Keynote Lecturer, Katherine F. Roby, PhD**
5:10 - 6:15 p.m.	**Keynote Lecture - W. Lee Kraus, PhD, University of Texas-Southwestern** *"Characterization of the Estrogen-Regulated Transcriptome in Breast Cancer Cells"*
6:30 - 9:00 p.m.	**Reception**, Beller 1005-1009, Hemenway Building
7:00 - 9:00 p.m.	**Poster Session**, Beller 1001-1003, Hemenway Building

FRIDAY, NOVEMBER 7th

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)

7:30 - 8:00 a.m.	**Breakfast**
8:00 - 8:05 a.m.	**Introductory Remarks** ***(Katherine F. Roby, PhD)***
Session I	
8:05 - 8:50 a.m. *(Q&A 8:40-8:50 a.m.)*	**Marisa S. Bartolomei, PhD, University of Pennsylvania** ***(Pavla Brachova, PhD, introducing)*** *"Genomic Imprinting: ART and Science"*
8:50 - 9:20 a.m. *(Q&A 9:15-9:20 a.m.)*	**Sundeep Kalantry, PhD, University of Michigan** ***(Pratik Home, PhD, introducing)*** *"Novel Mechanisms of X-chromosome Inactivation"*
9:20 - 9:35 a.m. *(Q&A 9:32-9:35 a.m.)*	**Trainee Oral Presentation: Zhen Zhang, MS, Graduate Student** ***(Adam Krieg, PhD, introducing)*** *"O-GlcNAcylation Regulates γ-Globin Transcription"*
9:35 - 9:55 a.m.	**Morning Break** (Refreshments)
Session II	
9:55 - 10:40 a.m. *(Q&A 10:30-10:40 a.m.)*	**Suzanne Moenter, PhD, University of Michigan** ***(Kaiyu Kubota, PhD, introducing)*** *"New Insights into Reproductive Neuroendocrine Development"*

Program Schedule

10:40-11:10 a.m. *(Q&A 11:05-11:10 a.m.)*	**Melinda E. Wilson, PhD, University of Kentucky *(Faezeh Koohestani, PhD, introducing)*** *"Dynamic Regulation of Estrogen Receptors by Epigenetic Mechanisms"*
11:10 - 11:25 a.m. *(Q&A 11:23-11:25 a.m.)*	**Trainee Oral Presentation: Damayanti Chakraborty, PhD, Postdoctoral Fellow *(Michael Wolfe, PhD, introducing)*** *"Epigenetic Regulation of MMP12 by Histone H3K9 DemethylaseKDM3A Modulates Trophoblast Stem Cell Adaptations to Hypoxia"*
11:25 a.m. - 12:45 p.m.	**LUNCH (and Trainee-Speaker Lunch Interaction)**

Session III

12:45 - 1:30 p.m. *(Q&A1:20-1:30 p.m.)*	**Kathy Sharpe-Timms, PhD, University of Missouri-Columbia *(Michelle McWilliams, MS, introducing)*** *"Transgenerational Endometriosis: The Missing Link"*
1:30 - 2:00 p.m. *(Q&A 1:55-2:00 p.m.)*	**Jae-Wook Jeong, PhD, Michigan State University *(Pramod Dhakal, PhD, introducing)*** *"Progesterone Signaling in Endometrium"*
2:00 - 2:15 p.m. *(Q&A 2:12-2:15 p.m.)*	**Trainee Oral Presentation: Michelle McWilliams, MS, Graduate Student *(Warren Nothnick, PhD, introducing)*** *"The Role of PRICKLE-1 in the Pathogenesis of Uterine Leiomyoma"*

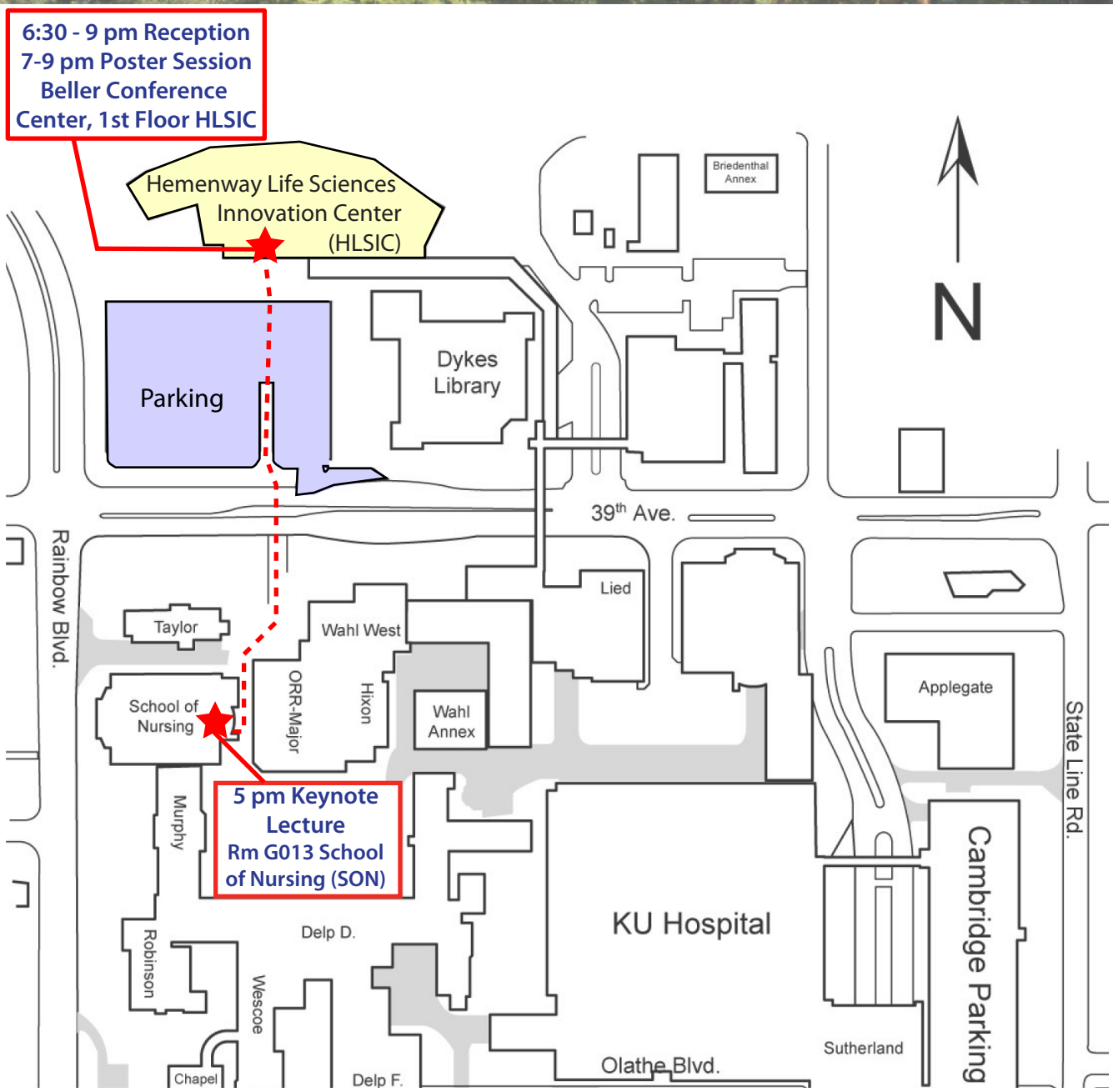
Session IV

2:15 - 3:00 p.m. *(Q&A 2:50-3:00 p.m.)*	**David Zarkower, PhD, University of Minnesota *(Mina Farahbakhsh, MS, introducing)*** *"Keeping Sex Signaling Safe: DMRT1 and Gonadal Transdifferentiation"*
3:00 - 3:20 p.m.	**Afternoon Break**
3:20 - 4:05 p.m. *(Q&A 3:55-4:05 p.m.)*	**Amander Clark, PhD, University of California-Los Angeles *(Damayanti Chakraborty, PhD, introducing)*** *"Methylation Reprogramming in the Human Germ Line"*
4:05 - 4:20 p.m. *(Q&A 4:17-4:20 p.m.)*	**Trainee Oral Presentation: Prabuddha Chakraborty, PhD, Postdoctoral Fellow *(Matthew C. Goering, PhD, HCLD, introducing)*** *"Bone Morphogenetic Protein-2 (BMP2) Uses ALK2/3 to Mediate Estradiole-17β Effect Towards Primordial Follicle Formation in Hamster Ovary by Promoting Germ Cell and Somatic Cell Differentiation"*
4:20 - 4:30 p.m.	**Trainee Award Presentations & Closing Remarks/Adjourn**

KUMC Campus Map

The University of Kansas Medical Center is located at 39th & Rainbow in Kansas City, Kansas. Parking is available in the HLSIC Parking Lot, shown below in lavendar. You can only enter the parking lot coming from the east (heading West) on 39th St., so if you take Rainbow to 39th St., you will need to drive East on 39th St. then turn around to head West on 39th.

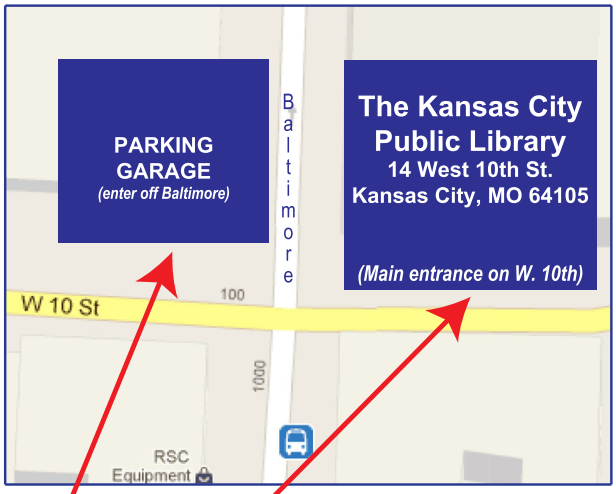
Hemenway Life Sciences Innovation Center (HLSIC)
2146 E. 39th Street
Kansas City, KS 66160

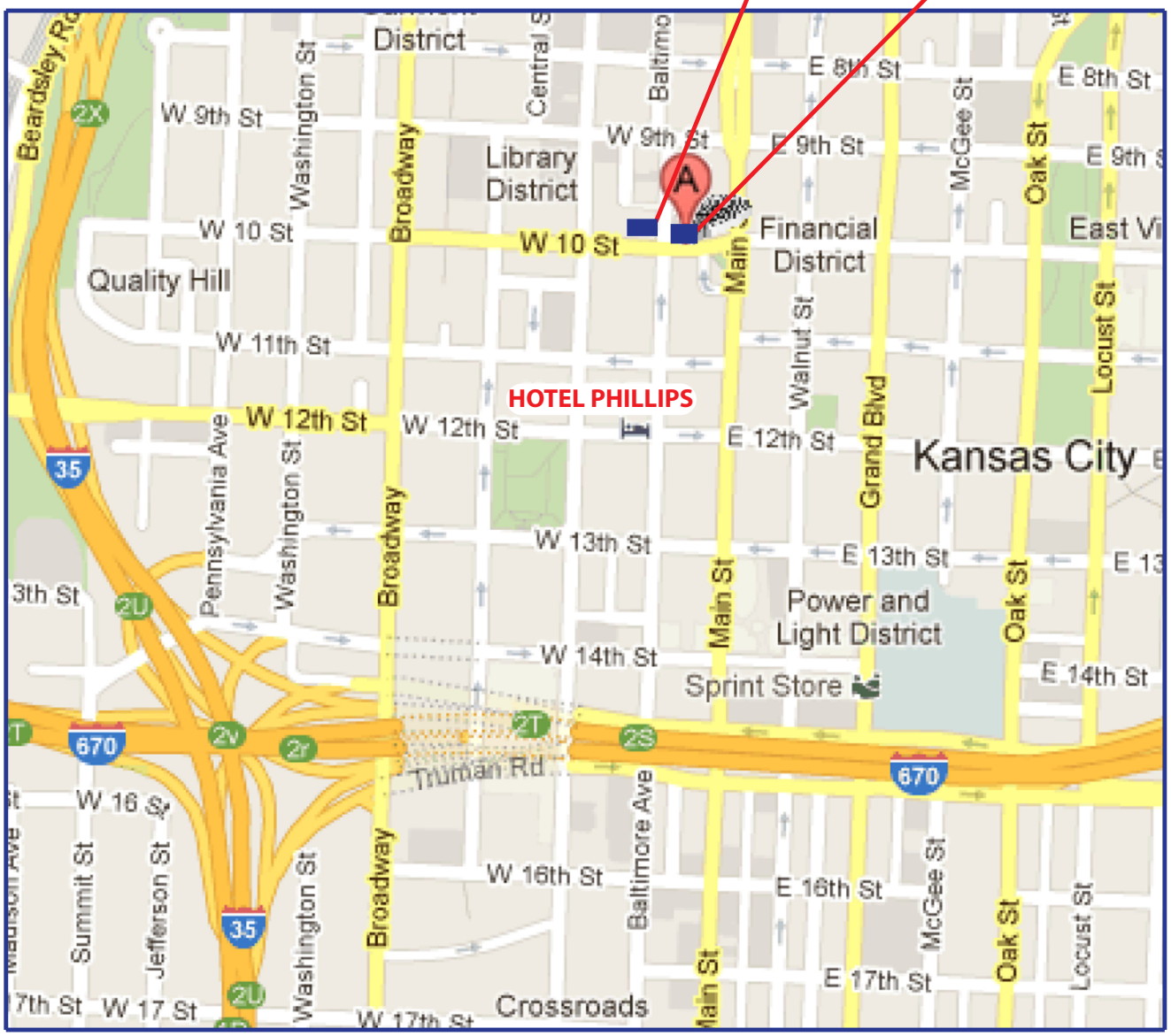


Kansas City Map

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. Parking for our event is free - let the attendant know you're with the Greenwald Symposium.

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

W. Lee Kraus, PhD

Cecil H. and Ida Green Distinguished Chair in Reproductive Biology Sciences
Professor and Vice Chair for Basic Sciences
Departments of Obestetrics and Gynecology
University of Texas Southwestern

"Characterization of the Estrogen-Regulated Transcriptome in Breast cancer Cells"

Dr. W. Lee Kraus is the Director of the Cecil H. and Ida Green Center for Reproductive Biology Sciences at University of Texas South Western Medical Center. He is also Professor and Vice Chair for Basic Sciences in the Department of Obstetrics and Gynecology, and Professor of Pharmacology. Dr. Kraus received his Ph.D. in 1994 from the University of Illinois, Urbana-Champaign, with Dr. Benita S. Katzenellenbogen, where he studied gene regulation by steroid hormone signaling pathways. He completed his postdoctoral work at UC San Diego in 1998 with Dr. Jim Kadonaga, where he studied the mechanisms of estrogen-regulated transcription from chromatin. Dr. Kraus was on the faculty at Cornell University in Ithaca, NY from 1999 to 2010, rising through the ranks to full professor. Since July 2010, he has been at UT Southwestern.

Dr. Kraus' research has led to new information about the connections between hormone-regulated gene expression and the gene-regulating effects of chromatin, which has implications for understanding and treating breast cancers. His recent work has helped to characterize the estrogen-regulated transcriptome and identify thousands of novel non-coding RNAs. His recent work has also led to some surprising new conclusions about the activity of poly(ADP-ribose) polymerase-1 (PARP-1), an NAD+-regulated nuclear factor that connects cellular NAD+ levels to nuclear signaling, chromatin structure, and gene expression.

Dr. Kraus is an editor for *Molecular Endocrinology* and *Molecular Cancer Research*. He is the founding organizer of the Cold Spring Harbor Laboratory meeting on the PARP family and has been an organizer of two Keystone Conferences on nuclear receptors. He has been recognized for his outstanding research by the Endocrine Society with the Richard E. Weitzman Memorial Award for research excellence is 2007 and the Ernst Oppenheimer Award for research excellence in 2014. Dr. Kraus holds the Cecil H. and Ida Green Distinguished Chair in Reproductive Biology Sciences at UT Southwestern Medical Center.

Session I

Marisa S. Bartolomei, PhD

Professor of Cell and Developmental Biology
University of Pennsylvania
"Genomic Imprinting: ART and Science"

Marisa S. Bartolomei received her BS in Biochemistry at the University of Maryland and then obtained her PhD from the Johns Hopkins University School of Medicine under the guidance of Dr. Jeffry Corden. She trained as a postdoctoral fellow with Dr. Shirley Tilghman at Princeton University. In 1993, Dr. Bartolomei was appointed as an Assistant Professor of Cell and Developmental Biology at the University of Pennsylvania Perelman School of Medicine and was promoted to Associate Professor with tenure in 1999 and Professor in 2006. In 2006, Dr. Bartolomei received the Society for Women's Health Research Medtronics Prize for Contributions to Women's Health. In 2011, Dr. Bartolomei received the Jane Glick Graduate School Teaching Award for the University of Pennsylvania School of Medicine and a MERIT award. Dr. Bartolomei participates extensively graduate and medical education, having trained numerous pre- and postdoctoral students, clinicians, and other health care professionals. She is a member of the Human Molecular Genetics and Molecular and Cellular Biology editorial boards and is an Associate editor for PLOS Genetics. Dr. Bartolomei's research addresses the epigenetic mechanisms of genomic imprinting and X inactivation, as well as the impact of adverse environmental insults on epigenetic gene regulation using the mouse as a model.

Sundeep Kalantry, PhD

Assistant Professor, Department of Human Genetics, University of Michigan
"Novel Mechanisms of X-chromosome Inactivation"

Sundeep Kalantry is an assistant professor in the Department of Human Genetics at the University of Michigan Medical School. He received his PhD from Weill Graduate School of Medical Sciences of Cornell University in 2001, where he trained under Elizabeth Lacy at the Sloan-Kettering Institute on mouse embryology and cloned the gene underlying the Amnionless mouse mutation. In 2002, he moved to UNC Chapel Hill for a post-doctoral fellowship with Terry Magnuson on epigenetic regulation, including X-chromosome inactivation, by the Polycomb group proteins and long non-coding RNAs. In 2009, Dr. Kalantry was recruited to the University of Michigan as a Biological Sciences Scholar.

Dr. Kalantry's laboratory studies X-inactivation as well as other epigenetic processes that characterize the early embryo using mouse models. Dr. Kalantry's honors include an NIH Pathway to Independence Award (K99/R00), an NIH Director's New Innovator Award (DP2), an Ellison Medical Foundation New Scholar in Aging Award, and a March of Dimes Basil O'Connor Starter Scholar Research Award. He is also a previous recipient of an American Cancer Society post-doctoral fellowship.

Session II

Suzanne Moenter, PhD

Professor, Departments of Molecular and Integrative Physiology,
Obstetrics and Gynecology and Internal Medicine
University of Michigan

"New Insights into Reproductive Neuroendocrine Development"

Suzanne M. Moenter, PhD, is a Professor of Molecular and Integrative Physiology, Internal Medicine and Obstetrics and Gynecology at the University of Michigan. She received her Ph.D. from the University of Michigan, performed postdoctoral research at University of California San Francisco and began her academic career at the University of Virginia before moving back to Michigan in 2010. Her studies focus on the neuroendocrine regulation of reproduction, specifically electrophysiological and molecular characterization of gonadotropin-releasing hormone (GnRH) and kisspeptin neurons in four broad thematic areas: 1) the development and generation of episodic GnRH release, 2) the central mechanisms of negative and positive estradiol feedback, 3) metabolic and stress regulation of fertility, and 4) models for infertility associated with hyperandrogenemia, such as polycystic ovary syndrome. She has a strong record of mentoring predoctoral trainees and was the first recipient of the Robert J. Kadner Award for Outstanding Graduate Mentoring at the University of Virginia, where she also directed the Neuroscience Graduate Program. She is currently Director of the NIH-funded Career Training in Reproductive Biology Program, and of the Physiology Graduate Program, and co-director of the Reproductive Sciences Program at the University of Michigan. Dr. Moenter has served on review boards for the NIH, NSF and USDA, the editorial board of Endocrinology, the Journal of Neuroscience and the Journal of Neuroendocrinology, and as Associate Editor for Biology of Reproduction. She has also served on the Trainee Affairs Committee, Annual Meeting Steering Committee and Basic Science Meeting Chair of the Endocrine Society.

Melinda E. Wilson, PhD

Associate Professor, Department of Physiology
University of Kentucky

"Dynamic Regulation of Estrogen Receptors by Epigenetic Mechanisms"

Melinda Wilson received her Ph.D. in Molecular Biology from Loyola University Chicago in 1997. She completed postdoctoral training at the University of Kentucky in the laboratory of Dr. Phyllis Wise. She was appointed to the faculty in the Department of Physiology at the University of Kentucky as an Assistant Professor in 2002. She was promoted to Associate Professor in 2008.

Dr. Wilson's research is focused on molecular mechanisms that regulate estrogen receptor expression in the brain. She has discovered that the estrogen receptor gene is regulated by epigenetic mechanisms during brain development and this can be modulated by injury in the adult brain. Furthermore, she has identified sex differences in this expression in the adult. As many of the actions of estrogen require the estrogen receptor, appropriate expression during development, aging and disease are all critical for estrogen action.

Session III

Kathy Sharpe-Timms, PhD

Professor and Director, Division of Reproductive and Perinatal Research
Director, MU Assisted Reproduction Labs
University of Missouri-Columbia

"Transgenerational Endometriosis: The Missing Link"

Dr. Timms joined the faculty of the Department of Obstetrics, Gynecology and Women's Health at the University of Missouri in 1990. In addition to serving as the Director and Embryologist for the Missouri Center for Reproductive Medicine and Fertility, she is the Director for the Division of Reproductive and Perinatology Research in her department and conducts her own federally funded research (NIH) in endometriosis. She received her PhD from The University of Tennessee in Reproductive Pathophysiology and completed a postdoctoral fellowship in the Department of Obstetrics and Gynecology at the University of Kentucky where she worked under the tutelage of Professor Michael M. Vernon. It was here that she first learned of endometriosis and of a novel rat model developed in that lab to study the disease. She identified proteins synthesized and secreted by endometrium and endometriotic lesions from this rat model and translated them to human tissues and cells, a field which would carry her forward into a lifelong career studying the pathogenesis and pathophysiologies of endometriosis.

Dr. Timms' current research interests include mechanisms causing subfertility in endometriosis. Most recently, Timms has shown the aberrant ovarian and embryo phenotype and altered gene expression in endometriosis persists transgenerationally; mechanisms by which this occurring are the current focus of her laboratory.

Dr. Timms has written 84 peer-reviewed publications, 19 book chapters and 96 abstracts in Professional Society Proceedings. She has served as an associate editor for Human Reproduction and Human Reproduction Update and reviews ~50 manuscripts per year ad hoc for more than 30 journals. She is an active member of the World Endometriosis Society, where she serves as a representative to the World Endometriosis Society Board of Directors. She is also active in the American Society for Reproductive Medicine (ASRM) where she served as a member of the ASRM Research Board, as a co-founder of the ASRM Endometriosis Special Interest Group (EndoSIG) where she held office from 2007 to 2010 and chaired the ASRM Reproductive Biology Professional Group.

Jao-Wook Jeong, PhD

Associate Professor, Departments of Obstetrics, Gynecology and Reproductive Biology
Michigan State University

"Progesterone Signaling in Endometrium"

Jae-Wook Jeong received his PhD in Microbiology from the Korea University, Seoul, South Korea. Following his post-doctoral training at the Department of Molecular and Cellular Biology, Baylor College of Medicine in Houston, Texas, he held the rank of Assistant Professor at Baylor College of Medicine until November 2010. He is currently Associate Professor in the Department of Obstetrics, Gynecology and Reproductive Biology at Michigan State University.

Dr. Jeong's laboratory has special interests in research relating to women's health, particularly infertility and endometrial cancer using genetically engineered mouse models. Current studies are aimed at the application of genetic animal model systems to further study and understand the role of steroid hormone in infertility and endometrial cancer. Dr. Jeong's group is primarily funded through grants from the NICHD-NIH and American Cancer Society. Dr. Jeong's research has resulted in the publication of 72 peer-reviewed scientific articles and other invited publications.

Session IV

David Zarkower, PhD

Professor, Department of Genetics, Cell Biology and Development
Director, Developmental Biology Center
University of Minnesota

"Keeping Sex Signaling Safe: DMRT1 and Gonadal Transdifferentiation"

David Zarkower is a Professor of Genetics, Cell Biology and Development and Director of the Developmental Biology Center at the University of Minnesota in Minneapolis where he has been since 1995. His PhD training was with Marvin Wickens at the University of Wisconsin-Madison, where he studied mRNA polyadenylation and his postdoctoral training was at the MRC Laboratory of Molecular Biology in Cambridge (UK) where he worked with Jonathan Hodgkin studying nematode sex determination.

Dr. Zarkower's research interests are centered on understanding the molecular and genetic basis of sexual development. His lab uses mainly nematodes and mice as models to address this question and focuses largely on the DMRT transcription factor family of conserved animal sex regulators. More recently the lab has studied evolution of vertebrate sex determining mechanisms and sex chromosomes using geckos as a model clade.

Dr. Zarkower's research program has been consistently funded by the NIH since 1996, and is currently also funded by NSF. Dr. Zarkower's work has been published in prestiegous journals, such as Nature, PNAS and Development.

Amander Clark, PhD

Associate Professor, Department of Molecular, Cell and Developmental Biology
UCLA Broad Stem Cell Research Center
University of California - Los Angeles

"Methylation Reprogramming in the Human Germ Line"

Amander Clark, PhD, is Associate Professor and Vice Chair of the Department of Molecular Cell and Developmental Biology at the University of California Los Angeles. She is a key member of the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and Co-Director of the Embryonic Stem Cell Derivation laboratory. She is also a member of the Jonsson Comprehensive Cancer Center and the Molecular Biology Institute at UCLA. Dr. Clark's work is focused on the use of pluripotent stem cells to understand the cell and molecular basis of human reproduction and embryo development with a focus on germline epigenetic reprogramming. Dr. Clark's laboratory is funded by the National Institute of Child Health and Human Development, National Institute of General Medical Sciences and the California Institute for Regenerative Medicine. Dr. Clark is a recipient of Young Investigator Award from the Lange Armstrong, a Research and Career Development award from STOP Cancer, and a Young Investigator Award from the International Society for Stem Cell Research. Dr. Clark is a member of the Hinxton Group, an International consortium of scientists, ethicists and policy makers for the use of pluripotent stem cell derived gametes.

Abstract Titles

1. **Space Flight for 30d Alters the Expression of Testis- and Epididymis-specific Genes in Male Mice.** **Lesya M Holets-Bondar**1, S Gunewardena2, Jennifer Knapp2, Joseph S Tash1 Department of Molecular & Integrative Physiology, 2Kansas Intellectual and Developmental Disabilities Research Center (KIDDRC), University of Kansas Medical Center, Kansas City, KS

2. **Development of a Non-obese Mouse Dietary Model of Gestational Diabetes.** **Kathleen A Pennington**, Kelly E. Pollock, Laura C. Schulz, Department of Obstetrics, Gynecology, and Women's Health, University of Missouri-Columbia

3. **Chronic Hypoxia Triggers Fetal Brain Injuries Mediated by Dephosphorylated Cofilin-1 Through Bax Activation.** **Wei Wang** and Yafeng Dong. Institute for Reproductive Health and Regenerative Medicine, Department of OB/GYN & Pathology, University of Kansas, Kansas City, KS

4. **The Effect of Osteogenesis Imperfecta in Implantation and Maternal Development.** **Arin Kettle Oestreich**, Janae Judon, Kathleen Pennington, Laura C. Schulz, PhD & Charlotte L. Phillips, PhD, Department of Obstetrics, Gynecology and Women's Health, Department of Biochemistry, and Department of Child Heath, University of Missouri-Columbia

5. **Development of a Pregnancy Associated Glycoprotein Assay to Accurately Detect Late Embryonic Mortality in Cattle.** **Ahmed Gatea**, Jon A. Green, Tina Egen, Ky G. Pohler, and Michael F. Smith, Division of Animal Sciences, University of Missouri-Columbia

6. **Blood glucose affects embryonic growth between d33 and d45 of pregnancy in lactating dairy cows.** **Tyler J. Stratman**, Scott Poock, Duane Keisler, and Matt Lucy. Division of Animal Sciences, University of Missouri Columbia, MO

7. **Regulation of REST Target Genes in Uterine Fibroids.** **Mina Farahbakhsh** a, b, Faezeh Koohestani a, b and Vargheese Chennathukuzhi a, b The Center for Reproductive Sciences a, Department of Molecular and Integrative Physiology b, University of Kansas Medical Center, Kansas City, KS.

8. **Generation and Characterization of Natural Killer Cell Deficient Rats Via Targeted Genome Editing of the Interleukin-15 Locus.** **Stephen J Renaud**, 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, KS.

9. **Halofuginone Suppresses Growth of Human Uterine Leiomyoma Cells in a Mouse Xenograft Model.** **Faezeh Koohestani, Ph.D.** a , Wenan Qiang, Ph.D. b,c,d, Amy MacNeill, D.V.M, Ph.D. e, Stacy A. Druschitz, B.S. b ;Vanida A. Serna, M.S. b,Takeshi Kurita, Ph.D. b, and

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10. **Neonatal Progesterone Programs Adult Uterine Responses to Progesterone and Susceptibility to Uterine Disease. Pramod Dhakal**, M.A. Karim Rumi, Kaiyu Kubota, Damayanti Chakraborty, Katherine F. Roby, Jeremy Chien, and Michael J. Soares, Institute for Reproductive Health and Regenerative Medicine, Departments of Pathology and Laboratory Medicine, Anatomy and Cell Biology, and Cancer Biology, University of Kansas Medical Center, Kansas City, KS 66160

11. **The Role of PRICKLE-1 in the Pathogenesis of Uterine Leiomyoma. Michelle McWilliams,** Faezeh Koohestani1, Riley Wertenberger1, Carmen Williams , Sumedha Gunewardena1, T. Rajendra Kumar1, Vargheese Chennathukuzhi1 1Department of Molecular and Integrative Physiology, University of Kansas Medical Center. 2Reproductive Medicine Group, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences.

12. **Diet-Induced Obesity Increases of Pou5f1 and Dppa3 mRNAs in Growing and Mature Oocytes is Due to Increased STAT3 Signaling. Fang Xie**, Kelsey Timme, Jenna L. Rifer, and Jennifer R. Wood. Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE.

13. **Comparison of Global Levels of DNA Methylation and Mecp2 Between Fully Grown GV Oocytes of Young and Aged Female Mice. Kira L. Marshall**, Sarah R. Huffman, Rocio Melissa Rivera, Division of Animal Sciences; University of Missouri; Columbia, MO USA

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15. **Hormonal Regulation of Female Reproductive Cyclicity. Kaiyu Kubota**, M.A. Karim Rumi, Pramod Dhakal, Wei Cui, Jay L. Vivian, Michael W. Wolfe, 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

16. **Characterization of Global Loss-of-imprinting in Fetal Overgrowth Syndrome Induced by Assisted Reproduction. Zhiyuan Chen** a, Darren Erich Hagen a, Christine G. Elsik a, Tieming Ji b, Laura Emily Moon c, Collin James Morris d, and Rocío Melissa Rivera a, aDivision of Animal Sciences, University of Missouri, Columbia, MO 65211, bDepartment of Statistics, University of Missouri, Columbia, MO 65211, cDepartment of Bioengineering, University of Missouri, Columbia, MO 65211, dDepartment of Biological Sciences, University of Missouri, Columbia, MO 65211

17. **TEAD4 Transcriptional Activity Regulates Proliferation of Trophoblast Progenitors During Mammalian Placental Development.** **Biswarup Saha**, Pratik Home, Arindam Paul, Biraj Mahato, Soma Ray and Soumen Paul, Department of Pathology & Laboratory Medicine, Institute of Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA.

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19. **Assessing Neural Differentiation with Rat Induced Pluripotent Stem Cells.** **Ganeshkumar Rajendran**, A. Paul, D. Agbas, J.L. Vivian, P. Smith and S. Paul, Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS

20. **Impacts of Reprogramming Method and Culture Conditions in Trophoblast Differentiation of Induced Pluripotent Stem Cells.** Mitsuyoshi Amita1, Bhanu VL Telugu1,3, Andrei Alexenko1, Laura Schulz2, Danny Schust2, R. Michael Roberts1, **Toshihiko Ezashi**1 1Division of Animal Sciences, Bond Life Sciences Center, 2Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, MO. 3current address: Animal and Avian Sciences, University of Maryland, College Park, MD.

21. **O-GlcNAcylation Regulates γ-Globin Transcription.** **Zhen Zhang**1, Ee Phie Tan1, Nathan Bushue1, Flavia C. Costa1, Kenneth R. Peterson1, 2, 3, and Chad Slawson1, 3. Departments of 1Biochemistry and Molecular Biology, 2Anatomy and Cell Biology, and 3Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS 66160.

22. **GATA Switch Regulates FLK1 Transcription During Erythroid Differentiation.** **Avishek Ganguly**, Soma Ray and Soumen Paul, Department of Pathology & Laboratory Medicine, Institute of Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, Kansas

23. A**denosine A3 Receptor Signaling Inhibits Stem-like Properties of Osteosarcoma.** **Swathi V. Iyer**, Harold K. Elias, Atul Ranjan, Alejandro Parrales, and Tomoo Iwakuma, Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS 66010, USA.

24. **Epigenetic Regulation of MMP12 by Histone H3K9 Demethylase KDM3A Modulates Trophoblast Stem Cell Adaptations to Hypoxia.** **Damayanti Chakraborty**, Wei Cui, M.A. Karim Rumi, Gracy X. Rosario, Pramod Dhakal, Kaiyu Kubota, Stephen J. Renaud, Jay L. Vivian, 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

25. **Regulation of Mitochondrial Function and Cellular Energy Metabolism by Protein Kinase C-λ/ι: A Novel Mode of Balancing Pluripotency.** Biraj Mahato1, **Ram P Kumar**1, Russell H. Swerdlow3 and Soumen Paul1, 2 1Department of Pathology & Laboratory Medicine, Kansas City, Kansas, USA, 2Institute of Reproductive Health and Regenerative Medicine, Kansas City, Kansas, USA, and 3Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas, USA.

26. **SATB Homeobox Gene Targets in Trophoblast Stem Cells. Khursheed Iqbal**, Shaon Borosha, Anamika Ratri, Tianhua Lei, Kazuo Asanoma, Michael J. Soares and MA Karim Rumi. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

27. **KDM4B Regulates Expression of Angiogenic Genes in Ovarian Cancer Cells. Cailin Wilson**1,2, Lei Qiu1,2, Yan Hong1, Adam J. Krieg1,2. 1Department of Obstetrics and Gynecology, 2Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA;

28. **O-linked β-N-Acetylglucosamine Cycling Regulates Acetylation on Mitochondrial Proteins. Ee Phie Tan**, Christopher Lanza, Maria T. Villar, Lezi E, Jianghua Lu, Eva Selfridge, Antonio Artigues, Russell Swerdlow and Chad Slawson. Institute for Reproductive Health and Regenerative Medicine, Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS.

29. **The Histone Demethylase KDM4B Promotes Peritoneal Dissemination of Ovarian Cancer. Lei Qiu**1,2, Cailin Wilson1,2, Yan Hong1, Tejashree Karnik2, Giurgius Tadros2, Brian Mau2, Tammy Ma2,Ying Mu1, Jacob New3, Raymond J. Louie4, Denise A. Chan4, Sumedha Gunewardena5, Andrew K. Godwin2, Ossama W. Tawfik2, Katherine F. Roby6, Adam J. Krieg1,2 1Department of Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS 66160, USA, 2Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA, 3Medical Scholars Program, University of Kansas Medical Center, Kansas City, KS 66160, USA, 4Department of Radiation Oncology, University of California, San Francisco, San Francisco, CA 94115, USA 5Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA, 6Department of Anatomy and Cell biology, University of Kansas Medical Center, Kansas City, KS, 66160

30. **Differential Regulation of Cell Cycle and Immune Response Networks in Bovine Granulosa Cells with Excess Intrafollicular Androstenedione. Sarah Romereim**1, Adam Summers2, Bill Pohlmeier1, Renee McFee1, Renata Spuri-Gomes1, Scott Kurz1, Tony McNeel3, Bob Cushman3, John Davis4, Jennifer Wood1, Andrea Cupp1 1University of Nebraska–Lincoln; 2New Mexico State University, 3US-Meat Animal Research Center, 4University of Nebraska Medical Center

31. **Granulosa Cell Proliferation Differentially Regulated by Follicular Fluid Extracellular Vesicles (EVs) From Early and Late Antral Follicles. Wei-Ting Hung**, Raphatphorn Navakanitworakul, Lynda K. McGinnis, Sumedha Gunewardena and Lane K. Christenson, Department of Molecular and Integrative Physiology, University of Kansas Medical Center,

Full Abstracts

1. **Space Flight for 30d Alters the Expression of Testis- and Epididymis-specific Genes in Male Mice.** **Lesya M Holets-Bondar**1, S Gunewardena2, Jennifer Knapp2, Joseph S Tash1 Department of Molecular & Integrative Physiology, 2Kansas Intellectual and Developmental Disabilities Research Center (KIDDRC), University of Kansas Medical Center, Kansas City, KS

The BION M1 flight was the first opportunity to directly test the hypothesis that prolonged spaceflight negatively affects testicular function and spermatogenesis. Seven mature male mice were flown in orbit for 30d. Asynchronous diet and age-matched ground control (GC) mice were maintained in the same BION hardware on the ground for 30d. For histology, testis and epididymis were fixed in Bouin's solution, washed in 70% ETOH, paraffin-embedded and processed for hematoxylin and eosin staining. For RNA and protein extraction tissues were harvested in RNAlater and stored at -80°C. RNA was subjected to real-time RT PCR. Next Generation sequencing was used to compare gene expression profiles between flight and GC mouse testes. Remarkably, only 43 genes showed significant changes ($q \geq .05$) of 14,360 expressed genes in flight, and 14374 genes in GC (FPKM>1).Among space flight up-regulated genes in testis most were reproductive tissue genes, but with canonical impacts in other systems. Significantly down-regulated genes involved in metabolic and inflammatory control and confirmed by quantitative RT PCR included FABP4 (over 20 fold), CFD (5- fold), and CA3 (2-fold) in the flight testis. Remarkably, our results in testis of male mice from BION M1 were consistent with identical changes of the same genes in uteri of female mice flown 12d in orbit on Space Shuttle flight STS-131. Ingenuity Pathway Analysis of physiological networks demonstrated that space flight significantly affected pathways involved in immune responses, metabolic, and endocrine regulation. This finding is important since the testis is normally an immunologically privileged site maintained by the blood-testis barrier (BTB). Preliminary assessment of the testis histopathology suggests severe changes in integrity /organization of the seminal epithelium in the space flight testes.

Finally, our overall hypothesis that space flight negatively effects gonadal functions are supported by this newly obtained NextGen transcriptome analysis and corresponding histopathological data.

2. **Development of a Non-obese Mouse Dietary Model of Gestational Diabetes.** **Kathleen A Pennington**, Kelly E. Pollock, Laura C. Schulz, Department of Obstetrics, Gynecology, and Women's Health, University of Missouri-Columbia

Gestational diabetes mellitus (GDM) is defined as diabetes that begins during pregnancy, and it usually resolves after parturition. It has both immediate and long term impacts on metabolic and cardiovascular health of the mother and fetus. GDM is one of the most common obstetrical complications, affecting 7-18% of all pregnancies. Despite its high prevalence, there is no widely accepted animal model. Previous studies in rodents have shown that a high fat diet (HFD) for 1 month before pregnancy induces obesity and type II diabetes. Other laboratories have used the heterozygous leptin receptor mutant (Leprdb/+) as a model of GDM, but our laboratory and others were unable to replicate this. We therefore set out to develop a mouse model of GDM. We used both wild type (Wt) C57/B6/J and Leprdb/+ mice placed on either a control or 45% HF diet from one or three weeks prior to mating and throughout pregnancy. At d17.5 glucose tolerance tests were performed. Regardless of genotype, mice placed on a HFD one week prior to mating developed GDM [Area under the curve 12474 + 721(Wt CD) vs. 23921 + 2902 (Wt 1WkHFD), 13657 + 435 (Leprdb/+ CD) vs. 23033 + 933 (Leprdb/+ 1WkHFD)]. Weights differed by genotype, but not diet: Wt CD (31.26±0.93g) vs. WT HFD (31.13±0.78g) and Leprdb/+ CD (36.53±0.80g) vs. Leprdb/+ HFD (38.22±0.80g). Glucose tolerance was not significantly impaired in mice placed on a HFD 3 weeks prior to mating (AUC WT 3WkHD 15578 + 1429 vs. Leprdb/+ 3WkHFD 17454 + 2471), suggesting adaptation to the HFD over time. In

conclusion, we have developed a novel model of GDM with no obesity and no diabetes prior to pregnancy. Future studies will use this model to characterize the effects of GDM on offspring and maternal health.

3. **Chronic Hypoxia Triggers Fetal Brain Injuries Mediated by Dephosphorylated Cofilin-1 Through Bax Activation.** **Wei Wang** and Yafeng Dong. Institute for Reproductive Health and Regenerative Medicine, Department of OB/GYN & Pathology, University of Kansas, Kansas City, KS

Epidemiological studies suggest a quarter of the 4 million neonatal deaths are associated with asphyxia at birth. However, the precise mechanism for the fetal chronic hypoxia brain injury has not been adequately identified. Utilizing Nissl staining, and immunostaining, we illustrated that hippocampus neuron density and astrocytes activation is significantly altered in fetal brain by 12% 02, 10.5% 02, respectively; 2-D gel and MALDI-TOF study indicated that Cofilin-1, an actin binding protein, was induced by chronic hypoxia in a dose response manner; the mass spectrometry data was confirmed by western blot, we demonstrated that the dephosphorylated cofilin-1 and Bax are both up-regulated by hypoxia, respectively; using double fluorescence label staining, we illustrated that Bax co-localized with dephosphorylated cofilin-1 rather than phosphorylated cofilin-1. These findings confirm that chronic hypoxia induces histological brain damage in fetus after 14 days of 10.5% O2 and 12% O2; fetal hypoxic injury is mediated in part by cofilin-1 modification leading to apoptosis gene activation in the fetal brain.

4. **The Effect of Osteogenesis Imperfecta in Implantation and Maternal Development.** **Arin Kettle Oestreich**, Janae Judon, Kathleen Pennington, Laura C. Schulz, PhD & Charlotte L. Phillips, PhD, Department of Obstetrics, Gynecology and Women's Health, Department of Biochemistry, and Department of Child Heath, University of Missouri-Columbia

Osteogenesis Imperfecta (OI) is an inherited connective tissue disorder characterized by short stature along with extreme bone fragility, and is caused by alterations in the structure or synthesis of type I collagen. The advancement of medical technologies have allowed patients with OI to live longer and posed many questions about adult pathologies, including fertility and reproductive health. There is a marked paucity in the clinical literature concerning the effect of OI on fertility and maternal development.
Purpose: The purpose of this study was to determine whether or not the deficiency of type I collagen affects fertility and reproductive tract development in an animal model of OI.
Procedure: In this study virgin and pregnant wildtype (+/+) and recessive osteogenesis imperfecta (oim/oim) females at 10 weeks of age were bred to Wt stud males and followed for 2 litters. Implantation was further evaluated at E6.5 and E10.5 of pregnancy in females that were four months of age. Mice were sacrificed and histological comparisons of picrosirius red stained reproductive tissues were evaluated in regular and polarized light.
Results: The reproduction tests show a significant decrease in the litter size ($p= 0.042$) and total litter weight of the OI mice ($p=0.016$). OI uteri stain faintly with picrosirius red indicating that they contain less type I collagen than the WT mice in both the myometrium and at the base of the luminal epithelium.
Conclusion: These preliminary results suggest that deficiency of type I collagen negatively impacts reproductive function.

5. **Development of a Pregnancy Associated Glycoprotein Assay to Accurately Detect Late Embryonic Mortality in Cattle.** **Ahmed Gatea**, Jon A. Green, Tina Egen, Ky G. Pohler, and Michael F. Smith, Division of Animal Sciences, University of Missouri-Columbia

Pregnancy associated glycoproteins (PAGs) appear in maternal serum between days 24 and 28 post insemination and peak during the last week of gestation. Beef cows that lost an embryo after day 28 (late embryonic mortality) had lower circulating concentrations of PAGs in maternal serum compared to cows that maintained pregnancy (Pohler et al 2013; J. Anim Sci 91:4158). The long term goal of this work is to utilize maternal circulating concentrations of PAGs to accurately predict which cows will either undergo late embryonic mortality or maintain the pregnancy. Some PAG members may be a better predictor of late embryonic mortality compared to others. Three monoclonal antibodies (A6, J2, and L4) have been

shown to bind bovine PAG and can be used in ELISAs to quantify PAGs. The objectives were as follows: 1) Determine whether circulating concentrations of PAGs differ during early gestation when measured by monoclonal antibodies (A6, J2, and L4) individually or in combination and 2) to determine if there is a specific monoclonal antibody or combination thereof that will serve as an accurate predictor of late embryonic mortality in cattle. There was an effect ($P < 0.0001$) of time and antibody (A6, J2, L4, or a mixture) on serum concentrations of PAGS on days 7, 24, 31, 45, 60 and 104. PAGs were increased ($P< 0.0001$) gradually from day 24 to day 31 and decreased by day 45. In addition there was an effect of antibody with a combination of the three monoclonal antibodies detecting higher ($P<0.0001$) circulating concentrations of PAGs then the L4 antibody alone. However, there was no difference in serum concentrations of PAGs on day 28 post insemination in cows that maintained or lost a pregnancy by day 100 of gestation when the specific monoclonal antibodies or their combination were employed in the ELISA.

6. **Blood Glucose Affects Embryonic Growth Between d33 and d45 of Pregnancy in Lactating Dairy Cows.** **Tyler J. Stratman**, Scott Poock, Duane Keisler, and Matt Lucy. Division of Animal Sciences, University of Missouri Columbia, MO

The objective was to examine the potential factors that affect embryonic growth from d 33 to 45 of pregnancy. Lactating Holstein and Guernsey cows (n=108) were examined by transrectal ultrasonography on d 33, 35, 38, 40, 42, and 45 of pregnancy. Length (l) and width (w) of the embryo and amnionic vesicle were measured. The volumes for the embryo (e_vol) and amnionic vesicle (a_vol) were calculated [volume = $4/3*\pi*(0.5*l)*(0.5*w)*(0.5*w)$]. E_vol and a_vol were plotted for each animal and a second order polynomial regression was fitted for the plot of volume versus day of pregnancy. The coefficients for the regression (volume = b2*x2 + b1*x + b0) were analyzed as dependent variables. Regression coefficients (b2, b1, and b0) were analyzed by using backward elimination (PROC GLMSELECT; SAS Inst., Cary, NC) with a cut-off of $P = 0.15$. The initial model included days in milk, blood glucose concentration, lactation, year, uterine horn, fetal sex, breed, number of inseminations, and body condition score. For e_vol, glucose status was the only variable remaining in the model in the analysis of b2 ($P < 0.001$), b1 ($P < 0.001$), and b0 ($P< 0.001$) by using backward elimination. Glucose status did not affect the coefficients for a_vol. The e_vol polynomial coefficients were used to output daily estimates for e_vol on d 33, 35, 37, 39, 41, 43, and 45. The daily estimates were analyzed by using a mixed model (PROC MIXED; SAS Inst., Cary, NC) with a model that included status (either low or high for blood glucose), day, and status by day. The e_vol was greater on d 37, 39, and 41 for cows with blood glucose above the population median. Conclusions were that blood glucose concentration affects embryonic growth between d 37 to 41 of pregnancy in lactating dairy cows.

7. **Regulation of REST Target Genes in Uterine Fibroids.** **Mina Farahbakhsh** a, b, Faezeh Koohestania, b and Vargheese Chennathukuzhi a, b The Center for Reproductive Sciencesa, Department of Molecular and Integrative Physiologyb, University of Kansas Medical Center, Kansas City, KS.

Uterine leiomyomas (ULs), also known as uterine fibroids, are benign smooth muscle cell tumors of the myometrium. ULs are the most common pelvic tumors in women with symptoms ranging from abnormal uterine bleeding to recurrent pregnancy loss and infertility. Currently there are no long-term and cost-effective treatments that will leave fertility intact. Therefore, there is an urgent need to understand the mechanism of tumor growth and pathogenesis in order to develop safe and effective therapeutics for ULs.

Dysregulation of growth factor-mediated signaling, leading to PI3K/AKT-mTOR activation is thought to play a role in UL development. However, the mechanism of activation of such pathways in ULs is currently unknown. Our lab has recently shown the expression of RE1 suppressing transcription factor /neuron-restrictive silencing factor (REST/NRSF), a known tumor suppressor, is lost in ULs. REST is involved in long term silencing of many genes in the periphery. Analysis of gene expression datasets from GEO database indicates that many of the most atypically expressed genes in ULs are known targets of REST. Using human leiomyoma tumor specimen, we investigated the expression level of REST target genes such as GRIA2, GRIN2A, NEFH, STMN2, SCG2, DCX, SALL1, CBLN1 and ADAM12 and found that compared

to matched normal myometrium, these genes are overexpressed in ULs. To analyze the role of REST in this upregulation, we silenced REST in primary myometrial cells using REST-specific siRNA and saw a significant increase in the expression of the above-mentioned genes. Remarkably, ADAM12, one of the REST target genes upregulated in ULs is known to promote tumor growth by activating IGF1R and EGFR pathways. We hypothesize that the loss of REST leads to altered gene expression and improper activation of cell signaling pathways, resulting in the pathogenesis of ULs.

8. **Generation and Characterization of Natural Killer Cell Deficient Rats Via Targeted Genome Editing of the Interleukin-15 Locus.** **Stephen J Renaud**, 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, KS.

Uterine natural killer (uNK) cells are the most prevalent uterine leukocyte population during early pregnancy. They are recruited to the uterus soon after embryo implantation, and “prime” the uterine spiral arteries in preparation for the increased demand on these vessels later in pregnancy. A second wave of spiral artery modification occurs by invasive trophoblast cells. This “two-wave” transformation of the uterine vasculature by both NK cells and trophoblast cells occurs in humans and rats. We have previously shown a robust enhancement of trophoblast invasion in NK cell-immunodepleted rats (PNAS 108:16295-300, 2011). The purpose of this study was to generate and characterize a genetic model of uNK cell deficiency in rats, and observe the resulting consequences on reproductive outcomes. 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 pups, we identified a founder animal containing a seven base-pair deletion within exon 2 of the Il15 locus. The deletion resulted in a frameshift within the coding sequence of Il15, and caused a functional loss of IL15 protein. Breeding of heterozygous Il15 rats resulted in generation of Il15-wildtype, heterozygous, and null pups in expected Mendelian ratios. In comparison to wild-type pregnant female rats, whose uteri contained massive quantities of perforin-positive NK cells, uteri of females harboring a homozygous mutation at the Il15 locus contained no detectable uNK cells. In uNK cell-deficient rats, scarce decidual angiogenesis during early pregnancy was evident. At midgestation, an expansion of the junctional zone of the placenta was observed, as well as depth and quantity of trophoblast cells invading within the spiral arteries. Accordingly, the internal luminal diameters of these vessels were substantially larger in rats lacking uNK cells. In summary, we have generated a novel NK cell-deficient rat by targeting the Il15 locus using ZFNs. Further studies will characterize the effect of NK cell deficiency on uteroplacental adaptations and fetal growth and survival later in pregnancy. These studies will enhance our understanding of the role of NK cells on uterine spiral artery remodeling, trophoblast invasion, and hemochorial placental development. (Supported by NIH HD020676).

9. **Halofuginone Suppresses Growth of Human Uterine Leiomyoma Cells in a Mouse Xenograft Model.** **Faezeh Koohestani, Ph.D.** a , Wenan Qiang, Ph.D. b,c,d, Amy MacNeill, D.V.M, Ph.D. e, Stacy A. Druschitz, B.S. b ;Vanida A. Serna, M.S. b,Takeshi Kurita, Ph.D. b, and Romana A. Nowak, Ph.D. a a Department of Animal Sciences, University of Illinois, Urbana, IL, 61801, USA; b Department of Obstetrics and Gynecology-Division of Reproductive Science in Medicine, c Department of Pathology, and d Robert H. Lurie Comprehensive Cancer Center , Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, USA; e Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL, 61801, USA

Uterine leiomyomas (ULs) are the most common pelvic tumors in reproductive-aged women, arising from the clonal proliferation of myometrial smooth muscle cells and subsequent fibrosis. Despite being benign, these tumors are associated with high morbidity such as pelvic pain and pressure, bleeding, anemia, and complications in fertility and pregnancy. Hysterectomy and hormonal therapy are the leading management options for ULs. However, hysterectomy leads to infertility, and there is a high rate of tumor recurrence and major side effects after hormonal therapies. In order to identify an alternative therapeutic treatment for ULs

with good efficacy but fewer side effects, we evaluated the potential of the anti-fibrotic drug halofuginone (HF) in reducing fibrosis and the growth of ULs.

Primary leiomyoma and myometrial cells from patients undergoing hysterectomy were grafted under the kidney capsule of female, ovariectomized mice carrying hormone pellet implants. Treatment of xenografted mice with HF at 0.25 or 0.50 mg/kg body weight for four weeks resulted in a 35-40% ($p < 0.05$) reduction in tumor volume. The HF-induced volume reduction was accompanied by increased apoptosis and decreased cell proliferation. In contrast, there was no significant change in the collagen content either at the transcript or protein level between UL xenografts in control and HF groups as assessed by quantitative RT-PCR analysis and collagen I and III immunofluorescence staining, respectively. HF treatment did not change either the serum levels of ovarian steroid hormones or the expression level of their associated receptors. Furthermore, there were no pathological side effects on major mice organs. In conclusion, HF was found to be effective in reducing UL tumor growth by interfering with the main cellular processes regulating cell proliferation and apoptosis and can be considered as an alternative therapeutic option for women suffering from ULs.

10. **Neonatal Progesterone Programs Adult Uterine Responses to Progesterone and Susceptibility to Uterine Disease.** **Pramod Dhakal**, M.A. Karim Rumi, Kaiyu Kubota, Damayanti Chakraborty, Katherine F. Roby, Jeremy Chien, and Michael J. Soares, Institute for Reproductive Health and Regenerative Medicine, Departments of Pathology and Laboratory Medicine, Anatomy and Cell Biology, and Cancer Biology, University of Kansas Medical Center, Kansas City, KS 66160

Progesterone possesses a crucial role in female reproduction. Perturbations in progesterone signaling, especially those leading to uterine progesterone resistance, are at the core of a range of uterine diseases. Progesterone has also been shown to possess critical organizational actions on the development of the uterus. In this investigation, we developed a rat model of neonatal progesterone exposure and examined its consequences on adult uterine function. Female pups were subcutaneously injected with vehicle or progesterone from postnatal days 3 to 9. Early progesterone exposures negatively affected endometrial gland biogenesis, decidualization, and fertility. Since pregnancy success is directly linked to progesterone action on the uterus we investigated the responsiveness of the adult uterus to progesterone using RNAseq. At eight weeks of age, females were ovariectomized, rested for two weeks, and then treated subcutaneously with progesterone or vehicle. Nine h post-injection, rats were sacrificed, uteri harvested, and prepared for RNAseq. A subset of differentially expressed transcripts was identified and validated using qRT-PCR. Female rats treated neonatally with progesterone or vehicle were similarly prepared as adults and progesterone-responsive uterine transcripts identified from the RNAseq experiment were monitored by qRT-PCR. Several progesterone responsive genes exhibited significantly dampened responses in neonatally progesterone-treated females when compared to vehicle treated controls, while other progesterone responsive transcripts did not differ between female rats exposed to vehicle or progesterone as neonates. The organizational actions of progesterone on the uterus were dependent upon signaling through the nuclear progesterone receptor. To summarize, neonatal progesterone exposure leads to disturbances in endometrial gland biogenesis, progesterone resistance, and uterine dysfunction. Neonatal progesterone effectively programs adult uterine responsiveness to progesterone. The overall importance of aberrations in progesterone signaling during critical windows of uterine development as etiologic factors in uterine disease remains to be determined. (Supported by NIH, Lalor Foundation, Japan Society for the Promotion of Science)

11. **The Role of PRICKLE-1 in the Pathogenesis of Uterine Leiomyoma.** **Michelle McWilliams,** Faezeh Koohestani1, Riley Wertenberger1, Carmen Williams , Sumedha Gunewardena1, T. Rajendra Kumar1, Vargheese Chennathukuzhi1 1Department of Molecular and Integrative Physiology, University of Kansas Medical Center. 2Reproductive Medicine Group, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences.

Uterine leiomyoma (UL) are benign tumors arising in the smooth muscle tissue of the myometrium. UL

are present in over 77% of women, often causing severe pain, bleeding and discomfort. The molecular pathogenesis of UL is poorly understood. It is well established that UL have amplified estrogen signaling and that the growth of UL is dependent on ovarian steroid hormones. Extensive evidence has also indicated the central role of PI3K/AKT-mTOR pathways, which lead to cell growth, proliferation and survival in UL. Importantly, REST (repressor element silencing transcription factor) is a tumor suppressor whose loss in UL leads to aberrant expression of genes that activate PI3K/AKT-mTOR pathway. We report a novel critical link between environmental estrogen signaling, PRICKLE-1 (an interacting partner of REST required for its nuclear localization) and the loss of REST in UL. We found that PRICKLE-1 is severely under expressed in UL, and that silencing of PRICKLE-1 significantly down regulates REST protein levels. Similarly, overexpression of PRICKLE-1 results in increased REST protein expression in primary uterine cells. Next, we report the novel regulation of PRICKLE-1 by estrogen through ERα. Lhb null mice lacking endogenous estrogen show increased PRICKLE-1 expression, and subsequent estradiol treatment rescues PRICKLE-1 to wild-type levels. Further, Esr1 null mice express high levels of PRICKLE-1 in the uterus. Environmental estrogen exposure is a major risk factor for UL. We have found mice exposed neonatally to environmental estrogens express strikingly low levels of PRICKLE-1. Furthermore, we identify a novel mechanism that involves EZH2, (Enhancer of Zeste Homolog 2) in the regulation of PRICKLE-1 by estrogen. We found that EZH2 is overexpressed in UL and that silencing of EZH2 by siRNA results in an increase in PRICKLE-1 expression. Taken together, our data identifies a novel link between environmental estrogen exposure and downstream tumorigenic signaling pathways in UL.

12. **Diet-Induced Obesity Increases of Pou5f1 and Dppa3 mRNAs in Growing and Mature Oocytes is due to Increased STAT3 Signaling.** **Fang Xie**, Kelsey Timme, Jenna L. Rifer, and Jennifer R. Wood. Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE.

Transcript abundance which is increased in the ovulated oocyte from obese female mice is dependent on both transcriptional and post-transcriptional regulation of mRNAs during growth and maturation. To examine mechanisms of increased Dppa3 and Pou5f1 abundance, five-week-old female C57BL/6J mice were placed on normal diet (ND) or high fat diet containing either 45% (45HFD) or 60% (60HFD) of kcal from fat. At 17 weeks of age, ovulation was induced which removed all fully grown and therefore transcriptionally quiescent oocytes from the ovary. While abdominal adipose tissue was increased in 45HFD and 60HFD females, there were no differences in ovary weight or morphology which included similar numbers of corpora lutea and growing follicles.. QPCR analyses demonstrated increased abundance of both Pou5f1 and Dppa3 in ovulated oocytes and whole ovaries of 60HFD compared to ND females suggesting a transcriptional mechanism of increased mRNAs. Indeed ChIP analyses using an antibody against acetylated histone H3 (i.e. H3K9Ac) which is a marker of transcriptionally active promoters showed a 4-fold and 2-fold increase in H3K9Ac associated with the Dppa3 and Pou5f1 promoter regions, respectively. Leptin mRNA was increased in abdominal adipose tissue and phosphorylated STAT3 levels were increased in whole ovaries from 60HFD females. To determine if STAT3 regulated the transcription of Pou5f1 and/or Dppa3, ChIP was carried out using the phospo-STAT3 antibody. There was a 175-fold increase in phospho-STAT3 association with the Pou5f1 promoter in 60HFD vs. ND ovaries but no difference in its association with the Dppa3 promoter. These data indicates that the obese phenotype increases transcription of both Dppa3 and Pou5f1. Furthermore, increased expression of Pou5f1 is mediated in part by Leptin dependent increases in STAT3 phosphorylation.

13. **Comparison of Global Levels of DNA Methylation and Mecp2 Between Fully Grown GV Oocytes of Young and Aged Female Mice.** **Kira L. Marshall**, Sarah R. Huffman, Rocio Melissa Rivera, Division of Animal Sciences; University of Missouri; Columbia, MO USA

In mammals, fertility and chronological age show an inverse correlation. Oocyte quality is a contributing factor to this multifactorial phenomenon. DNA methylation (DNAm) is an epigenetic modification that functions primarily to repress genes and promote chromatin compaction. We previously demonstrated that introducing high levels of FSH by exogenous administration of equine chorionic gonadotropin (eCG) leads to decreased levels of global DNAm in mouse oocytes. Follow-up studies demonstrated that even though

aged female mice have higher levels of serum FSH, global levels of DNAm were higher in oocytes from aged females than levels seen in oocytes from young females. Results also demonstrated a dissimilar pattern of chromatin configuration between oocytes of young and old mice, suggesting the increased level of DNAm may be involved in this phenomenon. Chromatin remodeling occurs through epigenetic modifiers that act on the DNA and associated histone proteins. Methylated DNA attracts methyl DNA binding domain proteins such as Mecp2 (Methyl-CpG binding protein 2). We hypothesized that levels of Mecp2 would be increased in oocytes of aged mice. To test this, fully grown oocytes were retrieved from the ovaries of 10 aged (85-88 weeks [n=3], 77-79 weeks [n=7]) and 7 young (12-16 weeks [n=5], 6-8 weeks [n=2]) females and Mecp2 detected by confocal microscopy. Settings were maintained for all image acquisitions. Image J software was used to obtain values (pixel intensity) for Mecp2 in the germinal vesicle (GV) and averaged for comparison. Results show that Mecp2 is distributed across the genome but does not colocalize with heterochromatin, chromocenters, or the nucleolar ring. No significant differences in levels of Mecp2 were detected between oocytes from any age group (n=17, n=23, n=13, and n=7 respectively). From these results we conclude that Mecp2 is not involved in the apparent difference in chromatin arrangement between oocytes of aged and young mice.

14. **Bone Morphogenetic Protein-2 (BMP2) Uses ALK2/3 to Mediate Estradiole-17β Effect Towards Primordial Follicle Formation in Hamster Ovary by Promoting Germ Cell and Somatic Cell Differentiation.** **Prabuddha Chakraborty**1 and Shyamal K. Roy1,2. Department of Cellular and Integrative Physiology1, and Obstetrics and Gynecology2, University of Nebraska Medical Center, Omaha, NE

Primordial Follicles (PF) are formed when undifferentiated ovarian somatic cells differentiate into flattened granulosa cells, which invaginate into the meiotic-oocyte nest and encircle individual oocytes. Estradiol-17β (E2) promotes PF formation in the hamster. BMP2 plays an important role in cellular differentiation. We hypothesize that BMP2 promotes the entry of the oogonia into the meiotic prophase and induces differentiation of somatic cells into flattened-granulosa cells resulting in their assembly to form PFs. RT-PCR and immunofluorescence analysis revealed the presence of BMP2 mRNA and protein in the oocytes and somatic cells of the developing hamster ovary. Based on this information, E15 hamster ovaries were cultured for 8 days (C8, corresponding to postnatal day 8, P8), with or without rhBMP2, and PF formation was examined. BMP2 significantly increased PF formation ($p<0.01$). A combined treatment with E2 and BMP2 resulted in further increase in PF formation ($p<0.05$). Whereas LDN 193189, an ALK2/3 inhibitor completely inhibited PF formation, DMH-1, a specific inhibitor of ALK2 had partial effect ($p<0.05$) suggesting that both ALK2 and ALK3 participate in BMP2-mediated PF formation. LDN193189 pretreatment also partially blocked ($p<0.05$) E2-induced PF formation indicating that ALK2/3 mediates at least part of the E2 effect. ICI 182,780 (a classic ESR antagonist) pretreatment did not alter the effect of BMP2 ($p>0.05$) suggesting that BMP2-mediated PF formation does not involve classic ESR/E2 mechanism. Furthermore, E2 treatment in vivo increase BMP2 mRNA expression ($p<0.05$) on P8, indicating that BMP2 might act downstream of E2 in the ovary. BMP2 exposure in vitro leads to increased meiotic entry of oogonia compared to untreated controls, and the effect was most prominent on C3 ($p<0.05$). In summary, the results suggest that E2-stimulated ovarian somatic cell differentiation may be mediated by BMP2 and ALK2/3 mechanisms.

15. **Hormonal Regulation of Female Reproductive Cyclicity.** **Kaiyu Kubota**, M.A. Karim Rumi, Pramod Dhakal, Wei Cui, Jay L. Vivian, Michael W. Wolfe, 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 negative and positive feedback regulation of gonadotropin secretion. These concepts have been reinforced through phenotypic examination of mice possessing null mutations at either Esr1 or Pgr loci. ESR1 and PGR encode estrogen receptor alpha and progesterone receptor, respectively. Analysis of rats with an ESR1 deficiency has further strengthened

the importance of estrogen and ESR1 in regulating cyclicity; 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 a Pgr mutant rat model using ZFN-mediated genome editing. A 136 bp deletion within exon 1 of the Pgr gene was produced, resulting in a truncated protein lacking the DNA-binding and ligand-binding domains 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 differentiation. However, in contrast to the reported phenotype of female mice with disruptions in Pgr signaling, 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 the wild type female rat but not in the Pgr null female rat. We conclude that in the rat progesterone signaling is not required for the establishment and maintenance of the female reproductive cycle. (Support: AHA, JSPS, Lalor Postdoctoral Fellowship, NIH)

16. **Characterization of Global Loss-of-imprinting in Fetal Overgrowth Syndrome Induced by Assisted Reproduction.** **Zhiyuan Chena**, Darren Erich Hagena, Christine G. Elsika, Tieming Jib, Laura Emily Moonc, Collin James Morrisd, and Rocío Melissa Riveraa, aDivision of Animal Sciences, University of Missouri, Columbia, MO 65211, bDepartment of Statistics, University of Missouri, Columbia, MO 65211, cDepartment of Bioengineering, University of Missouri, Columbia, MO 65211, dDepartment of Biological Sciences, University of Missouri, Columbia, MO 65211

Embryos generated with the use of assisted reproductive technologies (ART) can develop overgrowth syndromes. In ruminants, the condition is referred to as large offspring syndrome (LOS) and exhibits variable phenotypic abnormalities including overgrowth, enlarged tongue, and abdominal wall defects. These characteristics recapitulate those observed in the human loss-of-imprinting (LOI) overgrowth syndrome Beckwith-Wiedemann (BWS). We have recently shown LOI at the KCNQ1 locus in LOS, the most common epimutation in BWS. Although the first case of LOS was reported in 1995, studies have not yet determined the extent of LOI in this condition. Here, we determined allele-specific expression of known imprinted genes in day 105 Bos taurus indicus X Bos taurus taurus F1 hybrid control and LOS fetuses using RNAseq. Our analysis allowed us to determine the monoallelic expression of 21 genes in tissues of control fetuses. LOS fetuses displayed variable LOI when compared to controls. Biallelic expression of imprinted genes in LOS was associated with tissue-specific hypomethylation of the normally methylated parental allele. In addition, a positive correlation was observed between bodyweight and the number of biallelically expressed imprinted genes in LOS fetuses. Further, not only was there loss of allele-specific expression of imprinted genes in LOS, but we also observed differential transcript amounts of these genes between control and overgrown fetuses. In summary, we characterized previously unidentified imprinted genes in bovine and identified misregulation of imprinting at multiple loci in LOS. We concluded that LOS is a multi-locus loss-of-imprinting syndrome, as is BWS.

17. **TEAD4 Transcriptional Activity Regulates Proliferation of Trophoblast Progenitors During Mammalian Placental Development.** **Biswarup Saha**, Pratik Home, Arindam Paul, Biraj Mahato, Soma Ray and Soumen Paul, Department of Pathology & Laboratory Medicine, Institute of Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA.

In placental mammals, trophoblast cells are essential for embryo implantation and successful progression of the pregnancy. During placental development, distinct trophoblast cell types are specified from trophoblast stem cells (TSCs) or TSC-like trophoblast progenitors. However, molecular mechanisms, which regulate proliferation and differentiation of trophoblast stem/progenitor cells, are poorly understood. In this study, we show that transcriptional activity of TEAD4, a TEA domain containing transcription factor, plays a crucial role in promoting cell proliferation in trophoblast progenitors of both rodent and human placenta. In the early stage of mouse and human placentas, TEAD4 is present within the nuclei of TSC-like progenitors and directly regulates expression of TSC-specific genes. Our genetic analysis with mouse TSC lines and primary cytotrophoblasts from first-trimester human placenta also confirmed that TEAD4 promotes proliferation

of trophoblast stem/progenitors cells by regulating expression of several Cyclins/CDKs. In contrast to trophoblast progenitors of a developing placenta, differentiated trophoblast cells within matured rodent and human placentas generally lack TEAD4 transcriptional activity due to its absence in their nuclei. However, intriguingly, both matured mouse and human placentas harbor a small number of trophoblast population, characterized by the presence of TEAD4 in their nuclei and higher transcription of TEAD4-regulated genes. Thus, TEAD4 transcriptional activity marks a population of trophoblast cells that are actively proliferating or poised for proliferation in matured mammalian placenta. Our study indicates that transcriptional activity of TEAD4 balances progenitor vs. differentiated state of trophoblast population during mammalian placental development.

18. **Cells With Features of Totipotency Derived From Human ESC and iPSC**. Ying Yang1,4, Katsuyuki Adachi2,4, **Megan Sheridan**3, Andrei Alexenko1, Danny Schust2, Laura Schulz2, Toshihiko Ezashi1, Michael Roberts1, 3, * 1Division of Animal Sciences, Bond Life Sciences Center, University of Missouri, Columbia, Missouri 65211 USA, 2Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, Missouri 65211 USA, 3Department of Biochemistry, University of Missouri, Columbia, Missouri 65211 USA, 4Co-first authors

Human pluripotent stem cells (hPSC) exposed to BMP4 (B) in absence of FGF2 differentiate into colonies primarily comprised of trophoblast (TB), a process enhanced if inhibitors of ACTIVIN signaling (A83-01; A) and FGF2 (PD173074; P) are also present (BAP conditions). In an attempt to isolate TBSC, hESC and hiPSC were exposed to BAP for 24-36 h, trypsin-dissociated, and cultured on gelatin. Organized CDX2+/ KRT7- colonies emerged within a few days. These self-renewing cell lines were not TBSC but met standard in vitro criteria for pluripotency. They formed well-differentiated teratomas, but, unlike ones from H1 and H9 cells, contained TB. The cells differed from the progenitor hPSC in morphology, ability to be clonally propagated on gelatin, and transcriptome profile (including higher expression of NANOG, EOMES, TFAP2C and LEFTY1 & 2). In absence of FGF2 and other added factors they spontaneously differentiated along multiple lineages, including TB. The cells responded to PD173074 in absence of BMP4 in vitro by robust conversion to syncytioTB, while an A83-01/ PD173074 combination favored increased expression of HLA-G, a marker of extravillous TB. Together, these data suggest that the cell lines are totipotent and that BMP4 is not a requirement for TB emergence. Instead, BMP4 primes hPSC to a state where they can be readily differentiated along both embryonic and extra-embryonic lineages. This work was supported by NIH Grants R01HD067759 and R01HD077108.

19. **Assessing Neural Differentiation with Rat Induced Pluripotent Stem Cells.** **Ganeshkumar Rajendran**, A. Paul, D. Agbas, J.L. Vivian, P. Smith and S. Paul, Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS

Despite advances in our understanding and research of induced pluripotent stem cells (iPSCs), their use in clinical practice is still limited due to lack of preclinical experiments. Rat is an excellent animal model to study human diseases owing to its physiological and behavioral resemblances with the human. Similar to human iPSC, rat iPSCs can differentiate into many cell types and are important for regenerative medicine. However, further work is needed to reliably differentiate riPSC into neural-restricted multipotent derivatives or specialized cell types, to be used for transplantation in Spinal Cord Injury (SCI) models. Towards this goal, we reprogrammed rat embryonic fibroblast to rIPSC with facilitation of inhibiting PKC signaling by a selective PKC inhibitor, 3-[1-[3-(dimethylamino)- propyl]-5-methoxy-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2, 5-dione(Go6983, henceforth mentioned as PKCi), further tested the transition of rIPSC line towards the neuronal lineage using different substrates such as Matrigel, Laminin and ultra-low attachment plates and several different cocktails of inhibitors. The differentiation was evaluated by immunoreactivity to V-Glut2, NKX6.1, Tuj, nestin, BDNF and GFAP; and the expression of Olig2, Tuj1, PGP9.5, Nkx 2.2, V-Glut 2 and Neurog 3 was studied using RT-PCR. Further to check the efficacies of rIPSC-differentiation model to improve the SCI, we transplanted rIPSC-derived neuronal progenitors in rat SCI models and evaluated the BBB score. Our study provides a new approach to generate riPSc and riPSC-derived neuronal progenitors

to assess efficacy for neuronal regeneration.

20. **Impacts of Reprogramming Method and Culture Conditions in Trophoblast Differentiation of Induced Pluripotent Stem Cells.** Mitsuyoshi Amita1, Bhanu VL Telugu1,3, Andrei Alexenko1, Laura Schulz2, Danny Schust2, R. Michael Roberts1, **Toshihiko Ezashi**1 1Division of Animal Sciences, Bond Life Sciences Center, 2Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, MO. 3current address: Animal and Avian Sciences, University of Maryland, College Park, MD.

Human induced pluripotent stem cells (iPSC) and embryonic stem cells (ESC) share many common features including their requirement for directed differentiations into various cell types. Trophoblast differentiation from ESC has been achieved by exposing the cells to BMP4 with or without supplementation of ALK4/5/7 inhibitor (A83-01) and FGF2 signaling inhibitor (PD173074) (BAP). Here the two differentiation conditions, BMP4 and BAP were applied to two sets of iPSC lines that were generated by means of two reprogramming methods, DOX-inducible lentiviral (V) and episomal plasmid (P) transductions of umbilical cord mesenchymal cells derived from three individuals. Both V- and P-iPSC expressed pluripotent markers and have similar phenotypes with hESC in their undifferentiated state. The V-iPSC showed residual transgene expressions from the viral vectors in DOX-free culture condition while P-iPSC were transgene free. When the both iPSC lines were differentiated simultaneously, similar time dependent morphological changes were observed but BMP4 treated V-iPSC showed a minor yet consistent lag in the differentiation progression compared to BMP4 treated P-iPSC and hESC. Although both differentiated P- and V-iPSC showed dominant trophoblast phenotypes, the BMP4 treated V-iPSC also expressed gene markers consistent with the presence of mesoendoderm. The BAP condition provided more efficient differentiation than BMP4 alone, and the BAP-differentiated iPSC and ESC never expressed mesoendoderm markers. Methods for iPSC generation and differentiation must be carefully selected if induced pluripotent stem cells are to be used to study the basis of placental diseases such as preeclampsia, where it may be possible to recapitulate trophoblast differentiation from a previous pregnancy in a culture dish. Supported by NIH grants R01HD067759 and R01HD077108.

21. **O-GlcNAcylation Regulates γ-Globin Transcription.** **Zhen Zhang**1, Ee Phie Tan1, Nathan Bushue1, Flavia C. Costa1, Kenneth R. Peterson1, 2, 3, and Chad Slawson1, 3. Departments of 1Biochemistry and Molecular Biology, 2Anatomy and Cell Biology, and 3Institute for Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, KS 66160.

Patients with Sickle Cell Disease (SCD), caused by mutation of adult β-globin gene, are phenotypically normal if they carry compensatory mutations that result in continued expression of fetal γ-globin genes. Thus, a logical clinical goal for treatment of SCD is to up-regulate γ-globin synthesis. One mode of γ-globin silencing occurs at the GATA binding sites located at -566 or -567 relative to the Aγ-globin or Gγ-globin CAP sites respectively, and is mediated through GATA-1 binding and its co-repressor partners, FOG-1 and Mi-2. Post-translational modification (PTM) of repressor proteins can regulate their activity; one such modification is O-GlcNAcylation. The O-GlcNAc PTM is the attachment of a single N-acetyl-glucosamine moiety to either a serine or threonine residue on nuclear and cytoplasmic proteins. O-GlcNAc is added to proteins by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA). We hypothesized that O-GlcNAc plays a role in regulating γ-globin gene transcription. We induced human erythroleukemia K562 cells to differentiate toward the erythroid lineage as measured by an increase in γ-globin gene transcription. Interestingly, we found even more increase of γ-globin gene transcription after pharmacological inhibition of OGA. Furthermore, Mi-2 was modified with O-GlcNAc and interacted with both OGT and OGA. Knocking down OGA or OGT in K562 cells decreased γ-globin transcription during induction. We also performed chromatin immunoprecipitation (ChIP) analysis and found both OGT and OGA associated with the -566 region of the Aγ-globin promoter. In addition to the cell model, ChIP experiments demonstrated that Mi-2 and OGT were recruited to the -566 Aγ-globin GATA silencer site in day E18 fetal liver when γ-globin is repressed, but not in day E12 fetal liver when γ-globin is expressed. Our data demonstrate that O-GlcNAc cycling is a novel γ–globin regulatory mechanism, which might be modulated to increase HbF.

22. **GATA Switch Regulates FLK1 Transcription During Erythroid Differentiation.** **Avishek Ganguly**, Soma Ray and Soumen Paul, Department of Pathology & Laboratory Medicine, Institute of Reproductive Health and Regenerative Medicine, University of Kansas Medical Center, Kansas City, Kansas

Vascular endothelial growth factor A receptor 2 (FLK1) is essential for both hematopoietic and vascular endothelial cell lineages. Gene targeting studies revealed severe defect in both the hematopoietic and endothelial lineage in mice lacking this receptor. Although the role of FLK1 is well established in the context of endothelial cell biology, the relationship of Flk1 with hematopoietic differentiation is poorly understood. In particular, How FLK1 expression is temporally regulated during hematopoietic differentiation is poorly understood. Therefore, present study explores the temporal regulation of Flk1 expression in hematopoietic differentiation. Using global DNase 1 hypersensitive site mapping and reporter gene analysis, we have identified a regulatory element at -88Kb upstream of the mouse Flk1 gene. We also found that a switch in chromatin occupancy of GATA factors at the -88kb region temporally regulates Flk1 transcription during hematopoietic development. Using embryonic stem cell (ESC) differentiation model along with primary erythroid progenitors from mouse and human, we show that GATA2 positively regulate Flk1 transcription in hematopoietic stem and progenitor cells by directly occupying the -88kb region. In contrast, transcriptional repression of Flk1 during erythroid differentiation is associated with replacement of GATA2 by GATA1 at the -88kb element. Gene-knockout studies in GATA2-null ESCs and genetic complementation studies in GATA1-null erythroid progenitors confirmed functional importance of the GATA2 and GATA1 in dynamic regulation of Flk1 transcription. Moreover, chromosomal conformation capture (3C) analysis demonstrated GATA factor-dependent altered chromatin conformation at the Flk1 loci during hematopoietic differentiation. Therefore, this study uncovers a novel GATA factor-dependent mechanism at a distal regulatory element that dictates dynamic expression of Flk1 during hematopoiesis.

23. **Adenosine A3 Receptor Signaling Inhibits Stem-like Properties of Osteosarcoma.** **Swathi V. Iyer**, Harold K. Elias, Atul Ranjan, Alejandro Parrales, and Tomoo Iwakuma, Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS 66010, USA.

Osteosarcoma (OS) is the second leading cause of cancer-related death in children and young adults. Evidence indicates the presence of a defined population possessing stem-like properties that are responsible for the aggressive attributes of the tumor. This subpopulation is capable of self-renewal and reinitiates tumors, hence referred to as tumor initiating cells (TICs). TICs grow in serum- and anchorage-independent conditions to form spheres, and the ability of cancer cells to form spheres is well correlated with stem-like malignant properties. Our previous data showed that a limited number of cancer cells derived from spheres efficiently gave rise to tumors in immunocompromised mice and possessed high proportion of cells expressing stem cell-associated markers. However, the factors that regulate sphere formation in OS remain unknown. To identify crucial players that regulate stem-like properties in OS, we performed sphere assays using OS cells harboring poor sphere-forming potential infected with a human whole-genome shRNA library. This screening led to the identification of adenosine A3 receptor (A3AR), whose down-regulation significantly increased sphere formation in multiple OS cell lines. We hence hypothesized that A3AR signaling regulates stem-like properties in OS. Our in vitro and in vivo experiments successfully revealed that A3AR down-regulation significantly increased the self-renewability, tumor initiation and metastatic potential of OS cells. A3AR down-regulation also increased the expression of stem cell transcription factors Oct-4 and Sox2, as well as the activity of stem cell-related marker aldehyde dehydrogenase (ALDH). A3AR down-regulation showed an increase in nuclear NF-kB, a downstream target of A3AR signaling in cells. Analysis of human OS tissues revealed low expression of A3AR which correlated with high expression of ALDH. These data strongly suggest that A3AR signaling plays a crucial role in the malignancy and stem-like properties of OS, justifying using A3AR signaling as an attractive therapeutic target towards the treatment of human OS.

24. **Epigenetic Regulation of MMP12 by Histone H3K9 Demethylase KDM3A Modulates Trophoblast Stem Cell Adaptations to Hypoxia.** **Damayanti Chakraborty,** Wei Cui, M.A. Karim Rumi, Gracy X. Rosario, Pramod Dhakal, Kaiyu Kubota, Stephen J. Renaud, Jay L. Vivian, 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

The placenta develops as a result of a coordinated expansion and differentiation of trophoblast stem (TS) cells. As pregnancy progresses, specific trophoblast cell lineages develop and are organized within the placentation site. The invasive lineage remodels uterine spiral arteries facilitating the flow of nutrients to the placenta and fetus. Failure of trophoblast invasion and vascular remodeling is associated with pathological conditions such as preeclampsia, intrauterine growth restriction, and preterm birth. Matrix metallopeptidase 12 (MMP12) degrades elastin and modifies the structure of arterial blood vessels. MMP12 is expressed in human trophoblast and has been implicated in uterine spiral artery remodeling. In this project, we explore the regulation of MMP12 in trophoblast cells and establish a genetic model for investigating MMP12 actions during hemochorial placenta formation. Our initial observation indicating a linkage between MMP12 and the invasive trophoblast lineage was derived from DNA microarray analysis of uterine mesometrial compartments of pregnant rats exposed to atmospheric and hypoxic environments. Hypoxia stimulates trophoblast cell invasion into the uterine mesometrial compartment. MMP12 was dramatically upregulated in the hypoxic state and expressed exclusively in endovascular invasive trophoblasts. This observation was effectively modeled in vitro using rat trophoblast stem cells. The hypoxia driven response was hypoxia-inducible factor dependent and associated with KDM3A mediated H3K9 demethylation at the Mmp12 locus. To explore the functional importance of MMP12 in trophoblast invasion, we generated a mutant rat model using TALEN-mediated genome editing. Mmp12 mutant rats showed impaired hypoxia dependent endovascular invasion and vascular remodeling . In summary, MMP12 is an intriguing extracellular matrix-modifying enzyme induced during activation of the invasive trophoblast lineage. The availability of Mmp12 mutant rats will provide an effective experimental tool for evaluation of the involvement of MMP12 in uterine spiral artery remodeling and hemochorial placentation. (Supported by AHA, JSPS, KUMC-BRTP, and Lalor postdoctoral fellowships and NIH grants: HD020676, HD079363)

25. **Regulation of Mitochondrial Function and Cellular Energy Metabolism by Protein Kinase C-λ/ι: A Novel Mode of Balancing Pluripotency.** Biraj Mahato1, **Ram P Kumar**1, Russell H. Swerdlow3 and Soumen Paul1, 2 1Department of Pathology & Laboratory Medicine, Kansas City, Kansas, USA, 2Institute of Reproductive Health and Regenerative Medicine, Kansas City, Kansas, USA, and 3Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas, USA.

Pluripotent stem cells (PSCs) contain functionally immature mitochondria and rely upon high rates of glycolysis for their energy requirements. Thus, altered mitochondrial function and promotion of aerobic glycolysis are key to maintain and induce pluripotency. However, signaling mechanisms that regulate mitochondrial function and reprogram metabolic preferences in self-renewing versus differentiated PSC populations are poorly understood. Here, using murine embryonic stem cells (ESCs) as a model system, we demonstrate that atypical protein kinase C isoform, PKC lambda/iota (PKCλ/ι), is a key regulator of mitochondrial function in ESCs. Depletion of PKCλ/ι in ESCs maintains their pluripotent state as evident from germline offsprings. Interestingly, loss of PKCλ/ι in ESCs leads to impairment in mitochondrial maturation, organization, and a metabolic shift toward glycolysis under differentiating condition.
Our mechanistic analyses indicate that a PKCλ/ι-hypoxia-inducible factor 1α-PGC1α axis regulates mitochondrial respiration and balances pluripotency in ESCs. We propose that PKCλ/ι could be a crucial regulator of mitochondrial function and energy metabolism in stem cells and other cellular contexts.

26. **SATB Homeobox Gene Targets in Trophoblast Stem Cells.** **Khursheed Iqbal**, Shaon Borosha, Anamika Ratri, Tianhua Lei, Kazuo Asanoma, Michael J. Soares and MA Karim Rumi. Institute for Reproductive Health and Regenerative Medicine, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.

SATB homeobox proteins (SATB1 and SATB2) are global chromatin organizers and master regulators of gene expression. Trophoblast stem (TS) cells and the placenta during early gestation express high levels of SATB1 and SATB2. Using TS cell culture models, we demonstrated that SATB proteins play a key role in TS cell renewal and the regulation of trophoblast differentiation. We have observed that disruption of both SATB1 and SATB2 expression affected the growth and development of the placenta and the fetus. However, Satb1-null or Satb2-null embryo and placenta exhibited normal development. This study investigated the gene regulatory function of SATB1 in mouse TS cells established from day 3.5 blastocysts. We performed genome wide expression analysis on Satb1-null and wild-type TS cells using RNA-seq and compared their transcriptome profiles. Global analysis of the transcriptomes demonstrated that Satb1-null and wild-type TS cells present different expression patterns. Statistical analysis indicated that 1,177 genes were differentially expressed (adjusted $P< 0.05$ and fold change >2), of which 326 genes were upregulated and 851 were downregulated in Satb1-null TS cells. Among the downregulated genes, 202 genes were trophoblast core genes that are vital for trophoblast development. Gene ontology (GO) analysis revealed that biological processes enriched by the genes upregulated in Satb1-null TS cells are related to transcriptional regulation and nucleotide metabolism, whereas genes downregulated in Satb1-null cells are related to cellular transport, cell adhesion and placental development. Remarkably, expression of the known TS-specific transcription factors such as Cdx2, Eomes, Gata3, Tead4, Elf5, Esrrb, Tcfap2c, Ets2, Id1, and Id2 was not altered, which explains why the self-renewal and growth potential of Satb1-null TS cells remained unaffected. Our findings indicate that SATB2 and/or one or more of the deregulated genes might compensate for SATB1 deficiency during trophoblast development. (Supported by HD020676, HD072100, HD079363 and KUMC-Illumina NGS Pilot Grant)

27. **KDM4B Regulates Expression of Angiogenic Genes in Ovarian Cancer Cells.** **Cailin Wilson**1,2, Lei Qiu1,2, Yan Hong1, Adam J. Krieg1,2. 1Department of Obstetrics and Gynecology, 2Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA;

The hypoxia-inducible histone demethylase, KDM4B is upregulated during the progression of ovarian cancer, connecting the hypoxic tumor microenvironment to epigenetic activation of cancer promoting genes and tumor progression. Knockdown of KDM4B with shRNA reduces tumor growth and metastasis in peritoneal models of ovarian cancer. These results suggest that KDM4B promotes an invasive tumor phenotype. Angiogenesis is an essential component of the metastatic cascade. Analysis of secreted proteins like VEGF provides a way to identify functional contributors to the development of angiogenesis. In order to test the contribution of KDM4B in promoting an angiogenic phenotype, SKOV3ip.1 and OVCAR8 cells expressing shRNA to KDM4B or an irrelevant control (GFP) were cultured in 2% fetal bovine serum and exposed to 21% or 0.5% oxygen for 16 hours. Conditioned media was harvested at this time point for analysis. Using an antibody array with affinity for 55 angiogenesis-related proteins, 11 secreted factors from SKOV3ip.1 and 16 from OVCAR8 cell lines were identified. An example is IGFBP1, insulin growth factor binding protein 1, which was knocked down with loss of KDM4B in OVCAR8 under normal growth conditions (21%) ($p<0.05$). These results suggest that KDM4B may contribute to angiogenesis through these targets. Future work will include ChIP and HUVEC assays to continue dissecting the contribution of KDM4B to angiogenesis in ovarian cancer models.

28. **O-linked β-N-Acetylglucosamine Cycling Regulates Acetylation on Mitochondrial Proteins.** **Ee Phie Tan**, Christopher Lanza, Maria T. Villar, Lezi E, Jianghua Lu, Eva Selfridge, Antonio Artigues, Russell Swerdlow and Chad Slawson. Institute for Reproductive Health and Regenerative Medicine, Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS.

O-linked N-acetylglucosamine (O-GlcNAc) is a post-translational modification. It is a modification involving an addition of a single N-acetylglucosamine residue to serine and threonine in nuclear and cytoplasmic. The O-GlcNAc modification is processes by O-GlcNAc transferase (OGT) (addition), and O-GlcNAcase (OGA) (removal). Recent evident have demonstrated links between mitochondrial proteins with O-GlcNAcylation such as OGT was showed targeted to cytochrome c in the mitochondria. In our previous work, by using a SILAC (Stable Isotope labeling of Amino Acids in Cell Culture) based proteomics screen, we found that altering the expression of OGT and OGA altered numerous mitochondrial proteins expression, including proteins involve in the respiratory chain and TCA cycle. Furthermore, mitochondrial morphology was altered in these cells. Both cellular respiration and glycolysis was impaired in the OGT/OGA gain of function cells. Acetylation is a common post-translational modification for numerous mitochondrial proteins. This modification is proved tightly regulating the metabolic pathway. Elevation of acetylation can impair mitochondrial functions, while increased expression of Sirtuin 3 (SIRT3), the mitochondrial deaceytylase improves mitochondrial functions and increases lifespan. Herein, we demonstrate that by altering the expression of OGT and OGA, respectively, changes the total mitochondrial acetylation, as well as altering the expression of SIRT3. These data demonstrate that alterations to the cellular homeostasis of O-GlcNAc levels may have a profound effect on the metabolic regulatory pathway.

29. **The Histone Demethylase KDM4B Promotes Peritoneal Dissemination of Ovarian Cancer.** **Lei Qiu**1,2, Cailin Wilson1,2, Yan Hong1, Tejashree Karnik2, Giurgius Tadros2, Brian Mau2, Tammy Ma2,Ying Mu1, Jacob New3, Raymond J. Louie4, Denise A. Chan4, Sumedha Gunewardena5, Andrew K. Godwin2, Ossama W. Tawfik2, Katherine F. Roby6, Adam J. Krieg1,2 1Department of Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS 66160, USA, 2Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA, 3Medical Scholars Program, University of Kansas Medical Center, Kansas City, KS 66160, USA, 4Department of Radiation Oncology, University of California, San Francisco, San Francisco, CA 94115, USA 5Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA, 6Department of Anatomy and Cell biology, University of Kansas Medical Center, Kansas City, KS, 66160

Epithelial ovarian cancer (EOC) has poor prognosis and rapid recurrence due to widespread dissemination of peritoneal metastases at the time of diagnosis. Multiple pathways contribute to the aggressive growth of ovarian cancer, including those mediated by the Hypoxia Inducible Factors (HIFs). In this study, we have determined that the hypoxia-inducible histone demethylase KDM4B is expressed in high grade serous adenocarcinoma and EOC lines, and regulates expression of metastatic genes and pathways. KDM4B interacts with and demethylates promoter regions of target genes, linking the hypoxic microenvironment to epigenetic mechanisms regulating metastasis. Suppressing KDM4B in vitro inhibits ovarian cancer cell invasion, migration and spheroid formation under both atmospheric and hypoxic conditions. Adding the KDM4B-WT construct in the background of shRNA knockdown rescued SKOV3ip.1 cell proliferation and invasion in collagen I, whereas the KDM4B-mut construct showed dominant-negative effect. KDM4B is also crucial for peritoneal metastasis in vivo. These experiments establish that KDM4B is a potent contributor to peritoneal metastasis, one of the predominant factors affecting prognosis of EOC, making KDM4B or the genes it regulates attractive targets to improve existing therapies.
Significance: The hypoxia-inducible histone demethylase KDM4B contributes to peritoneal metastasis, making KDM4B and the genes it regulates potential therapeutic targets for improved treatment of epithelial ovarian cancer.

30. **Differential Regulation of Cell Cycle and Immune Response Networks in Bovine Granulosa Cells with Excess Intrafollicular Androstenedione.** **Sarah Romereim**1, Adam Summers2, Bill Pohlmeier1, Renee McFee1, Renata Spuri-Gomes1, Scott Kurz1, Tony McNeel3, Bob Cushman3, John Davis4, Jennifer Wood1, Andrea Cupp1 1University of Nebraska–Lincoln; 2New Mexico State University, 3US-Meat Animal Research Center, 4University of Nebraska Medical Center

Anovulatory infertility (either chronic or sporadic anovulation) affects up to 40% of infertile women. In fact, sporadic anovulation in humans may often go undetected. Recent literature has reported that 8-13% of normally menstruating women (250 total, two reproductive cycles) exhibit sporadic anovulation. To gain a greater understanding of anovulation, our lab has described a naturally-occurring bovine model system which includes a sub-population of cows with a 17% reduction in calving rate, suggesting that they are subfertile, and endocrine profiles that are similar to women with chronic or sporadic anovulation. These cows exhibit excess androstenedione (A4) accumulation in their follicular fluid (10%; >30 fold higher) and lack an increase in estrogen production of similar magnitude (only 2 to 4 fold higher). In order to better understand the mechanism underlying this phenotype, our lab performed microarray analysis on the granulosa cells from control Low A4 (n=4) and High A4 (n=5) cows. Overall, there were 1229 upregulated genes and 255 downregulated genes. With Ingenuity Pathway Analysis, we found that granulosa cells from the High A4 population exhibit a strong inhibition of the cell cycle and an activation of many immune regulators. The cell cycle genes negatively affected include many G1/S checkpoint proteins (e.g. cyclins and cyclin-dependent kinases), regulators of chromosome alignment and segregation (e.g. kinesins and related molecules), and other cell cycle regulators. There was also a striking increase in cytokines, interleukins, and other immune regulators (SELL, TAC1, prolactin, etc.). Arrest of the cell cycle and increased recruitment of immune cells may indicate dysfunctional or premature differentiation of granulosa cells to large luteal cells. These preliminary results are intriguing and may provide insights into aberrant mechanisms resulting in anovulation in both cows and humans.

31. **Granulosa Cell Proliferation Differentially Regulated by Follicular Fluid Extracellular Vesicles (EVs) From Early and Late Antral Follicles.** **Wei-Ting Hung**, Raphatphorn Navakanitworakul, Lynda K. McGinnis, Sumedha Gunewardena and Lane K. Christenson, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160, USA

Follicular fluid is a rich and complex fluid containing numerous proteins, nucleic acids, and other macromolecules. Recent studies have shown that EVs are abundant within follicular fluid. To date, no functions of EVs from follicular fluid have been demonstrated. To determine if EVs from antral follicles can stimulate cell proliferation, primary granulosa cells were treated with EVs isolated from early and late antral follicles and proliferation was assayed by BrdU incorporation. Isolation of EVs was completed using a ultracentrifugation protocol, followed by nanoparticle tracking analysis (NTA), electron microscopy and Western blot analysis using established exosome markers to determine purity, concentration and sizes of EVs. To test EVs bioactivity, primary granulosa cells were plated as 5 x 104 cells/well and treated with 10 µg protein/well of different EVs and cultured for 24h. Electron microscopy indicated that the EV pellets contained numerous bilipid membrane enclosed and the size of EVs ranged in size from 50 to 200 nm under NTA analysis. Immunoblots indicated that EVs were enriched in these known exosomal marker genes. Concentration of EVs decreased from 20 x 1012 particles/ml in early antral follicles to 2.8 x 1012 particles/ml in late antral follicles (~10-fold decrease) as determined by NTA. Analysis of cell proliferation indicated that EVs from early antral follicles increased BrdU incorporation 2.6-fold compared to control (no EVs treatment). In contrast, EVs from late antral follicles elicited reduced 1.4-fold increase in cell proliferation compared to control. In conclusion, we observed that the number of EVs changed as follicular development proceeded. Lastly, these studies demonstrate that EVs can differentially affect cell proliferation, dependent on whether they were derived from early or late antral follicles. The increased cell proliferation following early antral EV treatment is consistent with higher levels of granulosa cell proliferation in early antral follicles. Funded by NIH-HD061580.

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