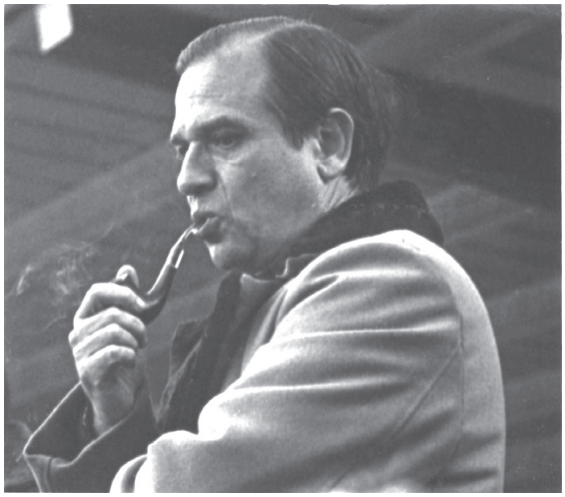
Gilbert S. Greenwald

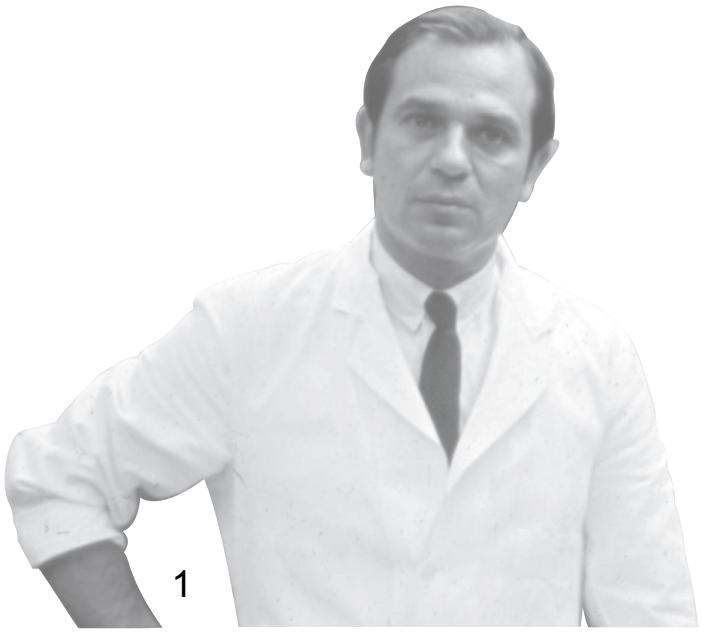


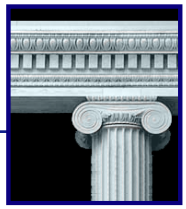
The Reproductive Biology Group at the University of Kansas Medical Center hosts the **Gilbert S. Greenwald Symposium on Reproduction** in honor and as a memorial to the life and research career of Gilbert S. Greenwald, PhD. Professor Greenwald had an illustrious career as a Distinguished Professor at 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 for 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.





Sponsors

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Smith Intellectual and Developmental Disabilities Research Center

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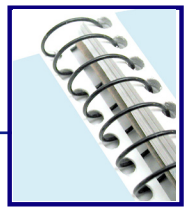

Titles of Abstracts

1. Pituitary Adenylate Cyclase Activating Protein (PACAP) Regulates Expression, Intracellular Localization And Function Of Early Growth Response Protein 1 (Egr1) In Gonadotropes.

Michael W. Wolfe, Gaurav Chaturvedi, Sara Turk and Emily McDonald, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160.

2. Genetic Analysis of Somatic-Germ Cell Interactions In The Mouse Testis.

T. Rajendra Kumar, Huizhen Wang, Phillip Stevenson.

3. Regulation Of Vascular Endothelial Growth Factor (VEGFA) Isoforms May Be A Mechanism To Regulate Follicle Progression Within The Ovary.

Andrea S. Cupp, Robin A Ten Broeck, Renee Pohlmann, and Debra T Clopton.

4. Anti-Spermiogenic Efficacy Of Single Oral Daily Dose Administration Of Iminosugars NB-DNJ And NB-DGJ Coincides With Their Relative Potency As Inhibitors Of Testicular Glucosyltransferase In C57Bl/6J Male Mice And Rats.

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7. Smad2 Signaling In Embryonic Stem Cells And Early Endoderm Development.
Katherine E. Galvin1, Della Yee2, Terry Magnuson2, and Jay L. Vivian1.

8. The Aryl Hydrocarbon Receptor Agonist 2,3,7,8-Tetrachloro-Dibenzo-P-Dioxin (TCDD) Inhibits Early Embryonic Development Following In Vitro Fertilization In Female Sprague Dawley Rats.
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24. Characterization Of Conditional Dicer Knock-Down In The Mouse Ovary.
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Lacey J. Luense, **Alison Ting, and Brian K. Petroff.**

THE 5TH ANNUAL
GILBERT S. GREENWALD
Symposium
on Reproduction

Center for Reproductive Sciences
University of Kansas Medical Center

2008 GREENWALD SYMPOSIUM ORGANIZING COMMITTEE:

MEMBERS:

T. Rajendra Kumar, PhD
Assistant Professor
Molecular & Integrative Physiology

Gustavo Blanco, MD, PhD
Associate Professor
Molecular & Integrative Physiology

Lane Christenson, PhD
Assistant Professor
Molecular & Integrative Physiology

William Kinsey, PhD
Co-Director, CRS
Professor
Anatomy & Cell Biology

Leslie Heckert, PhD
Co-Director, CRS
Professor
Molecular & Integrative Physiology

ADMINISTRATORS:

Linda Spears
Center for Reproductive Sciences

Stanton Fernald
Imaging Core, ICMCRD

General Information

THE RAPHAEL HOTEL
KANSAS CITY'S ELEGANT "LITTLE" HOTEL
325 WARD PARKWAY
THE COUNTRY CLUB PLAZA
KANSAS CITY MO 64112
816-756-3800 OR 800-821-5343
www.raphaelkc.com

Voted one of the World's Best Places to Stay by Condé Nast's Travelers, this one of a kind boutique hotel is patterned after Europe's finest small hotels. The Raphael is designed to offer a uniquely Kansas City experience, combining the intimacy and charm of a locally significant landmark with highly individualized service. Originally constructed in 1927 as the Villa Serena Apartments, the building was transformed into the Raphael Hotel in 1975 following a renovation that faithfully preserved historically significant features while providing for the needs of contemporary travelers. The hotel features 88 suites and 35 guest rooms furnished in traditional styles. The Raphael Restaurant, with its cozy bar area, is noted as one of the city's most romantic restaurants. The rotating dinner menu, which changes weekly, features "creative Continental" and "new American" cuisine. It always includes a variety of beef, seafood, lamb, poultry and vegetarian dishes.

THE INTERNCONTINENTAL
401 WARD PARKWAY
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Welcome to the Intercontinental Kansas City at the Plaza, where you will find impeccable service and all the luxuries you have come to expect from an Intercontinental Hotel. Our luxuriously appointed guest rooms and superb meeting facilities are all just steps away from the Country Club Plaza entertainment district. The Plaza is an outdoor museum of romantic Spanish architecture and European art, boasting more than 180 stores and distinctive boutiques and an eclectic mix of over two dozen restaurants. Plan your next meeting or event at the Intercontinental Kansas City at the Plaza where you will find our 29,000 square ft. of well appointed meeting space. Our professional onsite meeting planners will help you ensure every detail exceeds your expectations.

Symposium Shuttle Schedule

CONTACTS:
Shuttle Driver, Stan Fernald (816) 721-2517
Administrative Assistant, Linda Spears (816) 807-1840

Thursday, October 9

Shuttle service is available between the Raphael Hotel and the Welcome Reception (KUMC Campus).

5:45 p.m. Raphael Hotel to Reception – 1st Trip
6:15 p.m. Raphael Hotel to Reception – 2nd Trip

8:00 p.m. Reception to Raphael Hotel

Additional KUMC faculty and trainees will be available to drive guests back to the hotel as needed.

Friday, October 10

Shuttle service is available between the Raphael Hotel and the Kauffman Foundation Center, as well as between the Raphael and the banquet location, The Intercontinental.

7:45 a.m. Raphael Hotel to Kauffman – 1st Trip
8:00 a.m. Raphael Hotel to Kauffman – 2nd Trip
8:15 a.m. Raphael Hotel to Kauffman – 3rd and **Final Trip**

5:15 p.m. Kauffman to Raphael Hotel – 1st Trip
5:30 p.m. Kauffman to Raphael Hotel – 2nd Trip

Saturday, October 11

Shuttle service is available from the Raphael Hotel to the Kauffman Foundation Center (please check out of the hotel before coming to the Saturday morning session). For those of you who'll be dining at the Raphael for lunch, you can ask the hotel to hold your luggage for you.

7:45 a.m. Raphael Hotel to Kauffman – 1st Trip
8:00 a.m. Raphael Hotel to Kauffman – 2nd Trip
8:15 a.m. Raphael Hotel to Kauffman – 3rd and **Final Trip**

11:45 a.m. Kauffman to Raphael Hotel

PLEASE NOTE: If you are staying at the Intercontinental Hotel and you would like to take the shuttle, please walk over to the Raphael Hotel to be picked up.

Symposium History

2004 Speakers

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

Harry Weitlauf, MD
Texas Tech University
Osborn Address

Neena Schwartz, PhD
Northwestern University
Banquet Address

2005 Speakers

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

Shyamal K. Roy, PhD
University of Nebraska
Osborn Address

2006 Speakers

Robert Braun, PhD
University of Washington

Guela Gibori, PhD
University of Illinois at Chicago
Osborn Address

Susan Fisher, PhD
University of California-San Fransisco

Fred Karsch, PhD
University of Michigan

John Schimenti, PhD
Cornell University

John Schimenti, PhD
Cornell University

Teresa Woodruff, PhD
Northwestern University

Poster Competition Awardees (2006)

1st Place—
Lynda McGinnis
University of Kansas Medical Center

2nd Place—
Elizabeth Taglauer
University of Kansas Medical Center

3rd Place—
Toshihiro Konno, PhD
University of Kansas Medical Center

2007 Speakers

John J. Eppig, PhD
The Jackson Laboratory

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

Poster Competition Awardees (2007)

Damayanti Chakraborty
University of Kansas

Barbara J. Lutjemeier
Kansas State University

Cheng Wang
University of Nebraska Medical Center

Kansas City Map

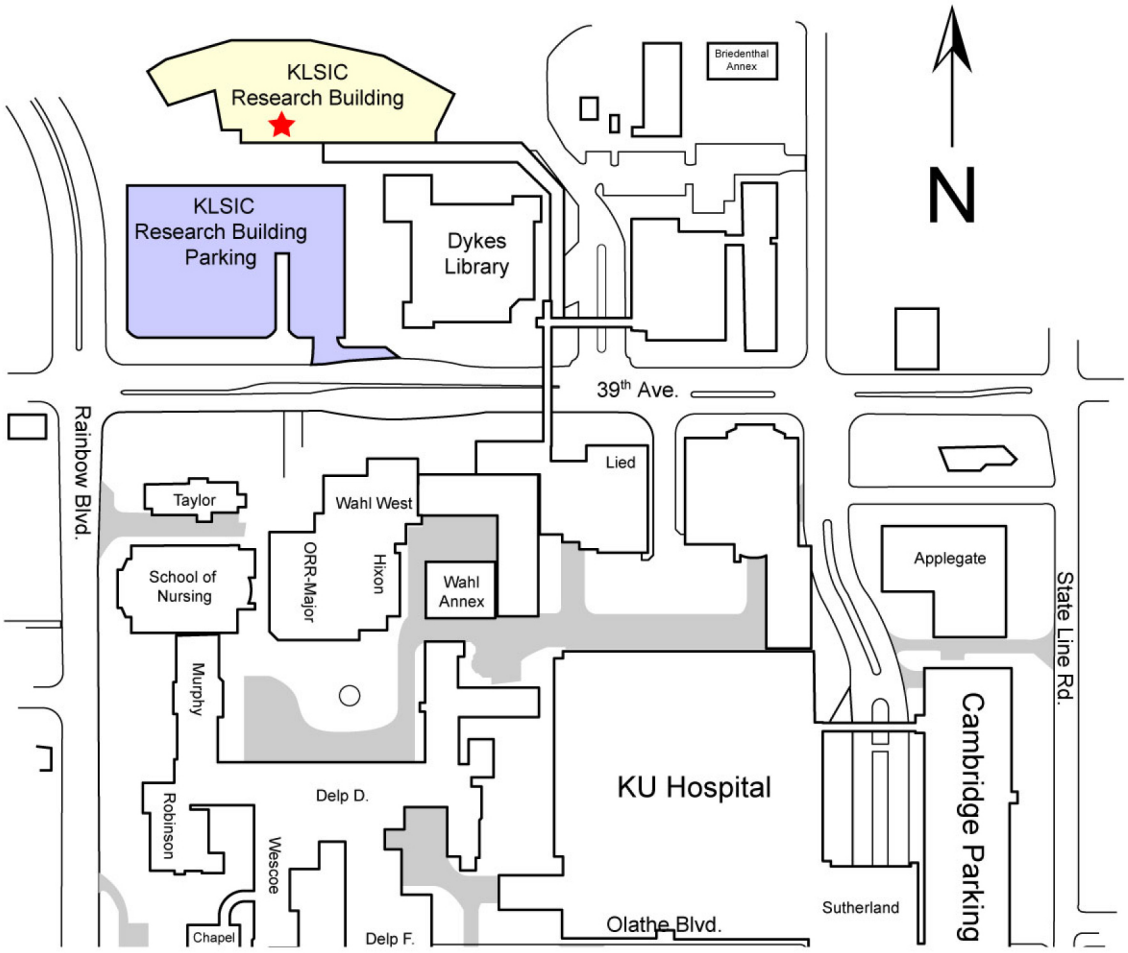
1 University of Kansas Medical Center—Kansas Life Sciences Innovation Center; 39th & Rainbow Boulevard: Thursday night reception

2 Banquet—The Intercontinental 401 Ward Parkway Kansas City, MO

3 The Raphael Hotel & Intercontinental Hotel; Ward Parkway

4 The Kauffman Foundation Conference Center: The Gilbert S. Greenwald Symposium on Reproduction

KUMC Campus Map



Schedule

THURSDAY, OCTOBER 9
6:00–8:00 PM

WELCOME RECEPTION
University of Kansas Medical Center—KLSIC Building
Beller Conference Center, Rooms 1005, 1007 and 1009 Northwest Corner of 39th Street & Rainbow Boulevard, Kansas City, KS, 66160.

FRIDAY, OCTOBER 10

5th ANNUAL GILBERT S. GREENWALD SYMPOSIUM

8:00–9:00 AM

REGISTRATION AND BREAKFAST
4801 Rockhill Rd, Kansas City, MO, 64110

900–9:15 AM

Introductory Remarks – Leslie Heckert, PhD, & William Kinsey, PhD

9:15–10:15 AM

Osborn Address – David Page, MD
"Choosing Sex: How Germ Cells Take the Road Less Traveled"

10:15–11:45 AM

Session I:
Session Chair, T. Rajendra Kumar, PhD

Platform Speaker – Jon Levine, PhD
"Feedback and Fitness: The Importance of Non-Classical Estrogen Receptor Signaling in Brain"

Short Presentation – Michael Wolfe, PhD
"Pituitary Adenylate Cyclase Activating Protein (PACAP) Regulates Expression, Intracellular Localization And Function Of Early Growth Response Protein 1 (Egr1) In Gonadotropes" (ABSTRACT #1)

11:45 – 1:45 PM

Lunch and Poster Presentations

1:45 – 3:15 PM

Session II:
Session Chair, - William Kinsey, PhD

Platform Speaker – Ina Dobrinski, Dr.med.vet., M.V.Sc., Ph.D., Dip. ACT
"Germline Stem Cells and Mammalian Spermatogenesis -Different Approaches to Study a Complex System"

Short Presentation – T. Rajendra Kumar, PhD
"Genetic Analysis of Somatic-Germ Cell Interactions In The Mouse Testis" (ABSTRACT #2)

3:15 – 3:30 PM

Break

3:30 – 5:00 PM

Session III:
Session Chair, Lane Christenson, PhD

Platform Speaker - John Peluso, PhD
"Progesterone Membrane Receptor Component 1 as a Mediator of Progesterone's Actions in Normal and Neoplastic Ovarian Cells"

Short Presentation – Andrea Cupp, PhD
"Regulation Of Vascular Endothelial Growth Factor (VEGFA) Isoforms May Be A Mechanism To Regulate Follicle Progression Within The Ovary" (ABSTRACT #3)

6:00 PM

Banquet – Intercontinental at the Plaza
401 Ward Parkway
Phone: (816) 756-1500

Presentation by: Paul Terranova, PhD
Vice Chancellor for Research, KUMC

Poster Awards Presentation

SATURDAY, OCTOBER 11

8:00 – 8:30 AM

Breakfast

8:30 – 10:00 AM

Session IV:
Session Chair, T. Rajendra Kumar, PhD
Co-Chair, Gustavo Blanco, MD, PhD

Platform Speaker – Miles Wilkinson, PhD
"Hormones, Homeoboxes, and Transcriptional Control"

Short Presentation – Viju Gupta, PhD
"H2-Gamendazole, A Promising Candidate For Reversible, Non-Hormonal Oral Male Contraceptive Development" (ABSTRACT #4)

10:00 – 11:30 AM

Session V:
Session Chair, Warren Nothnick, PhD
Co-Chair, Gustavo Blanco, MD, PhD

Platform Speaker – Nasser Chegini, PhD
"Female Reproductive Tract Disorders: Potential Regulatory Function of microRNAs"

Short Presentation – Warren Nothnick, PhD
"Conditional Deletion of Dicer1 Implicates a Functional Role for MicroRNAs in Uterine Development and Function" (ABSTRACT #5)

11:45 AM

Adjourn – Thank you for attending!

2008 Speaker Information

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

"Choosing Sex: How Germ Cells Take the Road Less Traveled."
Dr, Page has conducted fundamental studies of mammalian sex chromosomes and their roles in germ cell development, with special attention to the function, structure, and evolution of the Y chromosome. His laboratory recently completed the sequencing of the human Y chromosome in conjunction with the Washington University Genome Sequencing Center. Page's laboratory first reported DNA-based deletion maps of the Y chromosome in 1986, comprehensive clone-based physical maps of the chromosome in 1992, and systematic catalogs of Y-linked genes in 1997.

Jon Levine, PhD
Northwestern University
Evanston, IL

"Feedback and Fitness: The Importance of Non-Classical Estrogen Receptor Signaling in Brain."
Dr. Levines laboratory studies the synthesis, secretion, and actions of gonadotropin-releasing hormone (GnRH), a brain peptide that governs secretion of reproductive hormones from the anterior pituitary gland. We are particularly interested in the cellular mechanisms that mediate the physiological regulation of GnRH neurosecretion during the female ovulatory cycle. Our studies utilize a variety of experimental approaches and animal models to ascertain the molecular processes by which gonadal steroids, diet, stress, and neuroendocrine signals for sexual maturation can exert their effects on GnRH release.

Michael Wolfe, PhD
Molecular & Integrative Physiology, KUMC

"Pituitary Adenylate Cyclase Activating Protein (PACAP) Regulates Expression, Intracellular Localization And Function Of Early Growth Response Protein 1 (Egr1) In Gonadotropes."
Expression of luteinizing hormone (LH) and follicle-stimulating (FSH) in pituitary gonadotropes and expression of chorionic gonadotropin (CG) in placenta are essential to mammalian reproduction. These hormones are composed of two subunits - a common alpha-subunit and distinct beta-subunits. Research in my laboratory is directed towards understanding the cellular and molecular mechanisms involved in tissue-specific and hormonal regulation of the genes encoding the beta-subunit of these hormones. This involves studying the mechanisms regulating cell differentiation, elucidation of transcription factors regulating basal expression, and identifying the signal transduction pathways and associated transcription factors responsible for gonadotropin-releasing hormone (GnRH) regulation of gene expression. A variety of methodological approaches are utilized in these investigations including in vitro DNA-protein binding assays, cell culture and transient transfections, assays evaluating gene transcription and protein expression, and the use of transgenic mice.

Ina Dobrinski, Dr. med.vet., M.V.Sc., Ph.D.
Large Animal Reproduction / Animal Transgenesis and Germ Cell Research
University of Pennsylvania

"Germline Stem Cells and Mammalian Spermatogenesis -Different Approaches to Study a Complex System."
The long-term objective of my research program is to establish a system to study the biology of male germ line stem cells in non-rodent mammalian species. Recently, we succeeded in establishing the technique or germ cell transplantation in pigs and goats. This system will serve as a bioassay for stem cell potential of a given germ cell population, and will allow the manipulation of different aspects of spermatogenesis. Furthermore, it will lay the foundation for manipulation of the male germ line as an alternate approach to generate germ line transgenic animals. Present strategies to generate transgenic domestic animals are limited to pronuclear microinjection or nuclear transfer technology. Unfortunately, both these approaches are hampered by low efficiency due to excessive pregnancy wastage and perinatal losses, making the technology expensive and requiring large numbers of animals. Therefore, there is keen interest in the industry to find alternate, potentially more efficient ways of generating transgenic animals in species where embryonic stem cell technology is not available. Transgenesis through modification of the male germ line is one such promising approach. In addition, germ cell transplantation will serve to preserve valuable genetic material of animals and can be clinically applicable to restore fertility in cancer patients undergoing cytotoxic therapy.

T. Rajendra Kumar, PhD
Molecular & Integrative Physiology, KUMC

"Genetic Analysis of Somatic-Germ Cell Interactions In The Mouse Testis."
My laboratory studies development and regulation of the reproductive axis using both gain-of-function (transgenic) and loss-of-function (gene knockout) approaches. These unique genetic models mimic many of the human diseases and thus enable us to experimentally track them both in time and space. Specific projects include understanding human pituitary null cell adenoma, mechanisms of secretion of pituitary gonadotropins, and delineating mechanisms of gonadotropin regulation of testis and ovarian development and function. These studies are clinically relevant and have significant impact in understanding the physiology and pathology of the mammalian reproductive axis including abnormal reproductive tract development, infertility, and cancer of the pituitary and gonads.

John Peluso, PhD
University of Connecticut
Farmington, CT

"Progesterone Membrane Receptor Component 1 as a Mediator of Progesterone's Actions in Normal and Neoplastic Ovarian Cells."
Dr. Peluso's laboratory is involved in investigating the cellular and molecular mechanisms by which hormones and growth factors regulate ovarian follicular growth, differentiation, and atresia. At present there are three specific aspects of ovarian function being studied: (1) The role of cell contact in inhibiting ovarian cell apoptosis, (2) The identification and characterization of a novel progesterone receptor that possesses GABAA receptor-like properties and (3) The elucidation of the mechanism through which steroidogenic factor-1, an orphan nuclear receptor, negatively regulates mitosis and positively promotes steroidogenesis. The last project is being conducted in collaboration with Dr. Bruce White.

Andrea Cupp, PhD
University of Nebraska
Lincoln, Nebraska

"Regulation Of Vascular Endothelial Growth Factor (VEGFA) Isoforms May Be A Mechanism To Regulate Follicle Progression Within The Ovary."
My lab is interested in studing the effects of vascular development on gonadal morphogenesis, Effects of vascular development on follicle progression and ovulation, (Collaborative efforts)-Effects of nutrition on reproductive function in cattle, Collaborative efforts)- Effects of hormones and environment on oocyte quality.

Miles Wilkinson, PhD
MD Anderson Cancer Center
Houston, Texas

"Hormones, Homeoboxes, and Transcriptional Control."
My laboratory primarily works on two topics: (1) transcriptional regulatory pathways that control embryonic stem cell and germ cell development in vivo, and (2) RNA surveillance pathways that serve as quality-control mechanisms to degrade or "correct" aberrant transcripts that would otherwise express truncated proteins causing developmental defects and/or cancer.

Viju Gupta, PhD
Department Molecular & Integrative Physiology, KUMC

"H2-Gamendazole, A Promising Candidate For Reversible, Non-Hormonal Oral Male Contraceptive Development."
I received Bachelor's and Master's degree in Microbiology from University of Mumbai, India, PhD in Biochemistry specializing in Male Reproductive Biology from National Institute for Research in Reproductive Health,Mumbai India.
Joined Dr. Bill Kinsey in the dept of Anatomy and cell Biology at KU Med center in 2006 on a Postdoctoral fellowship and worked on Role of Protein kinase in mouse sperm capacitation for a year.
Currently, I am pursuing postdoctoral research in Dept of Molecular and Integrative Physiology with Dr.Joseph Tash and working on development of non-hormonal oral male contraceptives.
Research interest: Male reproductive biology, sperm proteins involved in fertilization and development of male contraceptives.

Nasser Chegini, Ph.D.
University of Florida
Gainesville, Fl

"Female Reproductive Tract Disorders: Potential Regulatory Function of microRNAs"
The long-term goal of our research group is to elucidate the implication of pro-inflammatory and profibrotic cytokines/chemokines in **A:** human reproductive tract tissues in normal and disease status (leiomyoma, endometriosis and dysfunctional uterine bleeding) and **B:** peritoneal tissue repair and adhesion formation and prevention. One team of investigators works on elucidating the menstrual cycle dependent expression of specific cytokines/chemokines and their receptors in normal uterine tissues, and in women with leiomyomas and endometriosis undergo hormone therapy (i.e. GnRHa) as part of their medical management and in progesterone only contraceptive users experiencing irregular bleeding. Using in vitro cell culture models we are investigating the regulation of these genes by ovarian steroids, GnRHa, SERM and SPRM, and identify signal transduction pathways activated by their receptors and cross-talk with cytokine/chemokine receptors signaling, more specifically transforming growth factors beta (TGF-β) and downstream gene expression whose products influence the outcome of these abnormalities.

Another team studies gene and microRNA expression profiling, antisense oligonucleotides, viral vectors and SiRNA technology and proteomic to identify specific genes and their products that may be responsible for pathogenesis of leiomyomas, endometriosis, and dysfunctional uterine bleeding and gene silencing are used as an alternative tool for medical management of these disorders as compared to GnRHa, SERM and SPRM.

Warren Nothnick, PhD, HCLD
Obstetrics & Gynecology and Molecular & Integrative Physiology, KUMC

"Conditional Deletion of Dicer1 Implicates a Functional Role for MicroRNAs in Uterine Development and Function."
My research focuses on the role of tissue remodeling factors in the female reproductive tract. Our major area of interest is the role of the matrix metalloproteinases (MMPs) and their tissue inhibitors of metalloproteinases (TIMPs) in uterine growth, development and function. Using TIMP-1 deficient mice, we have demonstrated that absence of the TIMP-1 gene product results in alterations in uterine development and reduced reproductive lifespan. Current studies are aimed at determining the mechanisms by which TIMP-1 regulates uterine development and growth and employ a variety of molecular, cellular and biochemical techniques.

A second project in my laboratory focuses on the role of TIMP-1 in corpus luteum physiology and function. The corpus luteum produces progesterone which is essential for the establishment of pregnancy. We have demonstrated that TIMP-1 deficient mice are subfertile and this subfertility is associated with reduced progesterone production and systemic levels of this steroid. We are currently examining the mechanisms by which TIMP-1 influences corpus luteum progesterone production using both in vivo and in vitro methodologies.

A third research interest of my laboratory involves the study of the female disease endometriosis. Endometriosis occurs in as many as 10% of all women of reproductive age and is defined as the presence of endometrial tissue in ectopic locations. We are currently examining the role of cytokines, particularly tumor necrosis factor alpha, in the pathophysiology of the disease. Understanding the mechanisms by which this inflammatory mediator may contribute to the development of endometriosis will lead to the development of novel treatments for the disease which would offer benefits over current therapies.

Abstracts

SHORT PRESENTATIONS: (1-5)

1. Pituitary Adenylate Cyclase Activating Protein (PACAP) Regulates Expression, Intracellular Localization And Function Of Early Growth Response Protein 1 (Egr1) In Gonadotropes.

Michael W. Wolfe, Gaurav Chaturvedi, Sara Turk and Emily McDonald, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160.

The transcription factor early growth response protein 1 (Egr1) is an early response gene that is rapidly induced upon stimulation of cells by growth factors, cytokines and various other extracellular factors. Targeted disruption of Egr1 has revealed the essential role it plays in gonadotrope cells of the anterior pituitary. Luteinizing hormone (LH) is synthesized and secreted by gonadotropes and is composed of an alpha- and LH beta-subunit. Expression of these subunits and secretion of LH is exquisitely controlled by the hypothalamic neuropeptide, gonadotropin-releasing hormone (GnRH). Studies in our laboratory have implicated Egr1 as being regulated by GnRH. Furthermore, it binds to and transactivates the LH beta-subunit promoter. Mutation of the Egr1 response elements within the promoter renders it unresponsive to GnRH. Pituitary adenylate cyclase activating protein (PACAP) has also been reported to regulate gonadotropin synthesis and secretion and to potentiate GnRH-induced gonadotropin secretion. Therefore, we evaluated the ability of PACAP (20 nM, 15 min pulse) to regulate Egr1 in the alphaT3-1 gonadotrope cell line. Following administration of PACAP, Egr1 mRNA levels were increased 4.0-, 3.5- and 1.6-fold at 25, 50 and 75 min, respectively. In contrast, stimulation with GnRH resulted in a 40-, 33-, and 20-fold increase in Egr1 mRNA at these same time points. Thus, GnRH results in a more robust induction of Egr1 as compared to PACAP. Due to the fact that pituitary gonadotropes are exposed to both GnRH and PACAP, we treated cells simultaneously with both hormones. Combinatorial treatment elicited a 24-, 26- and 15-fold increase in levels of Egr1 mRNA. Thus, PACAP appeared to dampen GnRH-induced expression of Egr1. This somewhat antagonistic effect of PACAP was further confirmed by western blot analysis of nuclear extracts. One hour following stimulation (the point of peak Egr1 levels), Egr1 protein was most abundant in cells treated with GnRH alone. Exposure of cells to both GnRH and PACAP resulted in reduced nuclear levels, while treatment with PACAP alone led to extremely limiting amounts of Egr1 being present in nuclear extracts. We also evaluated the total amount of Egr1 protein present within the cell (whole cell lysates) as well as its cellular localization (immunocytochemistry). Surprisingly, PACAP stimulation increased cytoplasmic Egr1 at 30 and 60 min; however, negligible amounts of Egr1 were observed within the nucleus at the time points examined (30-120 min). Furthermore, both western blot and immunocytochemistry revealed an increase in the rate of Egr1 degradation following co-stimulation with GnRH and PACAP, as compared to GnRH alone. In summary, these data indicate that PACAP can induce Egr1 levels in gonadotropes and more importantly, PACAP has the ability to influence cellular localization as well as degradation rate of Egr1 following GnRH stimulation. Thus, PACAP can influence Egr1 function as a gene transactivator and implicates a role for PACAP in regulating Egr1-dependent activation of the LH beta-subunit promoter. This research was supported by NIH DK67347.

2. Genetic Analysis of Somatic-Germ Cell Interactions In The Mouse Testis.

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Male germline stem cells colonize testis tubules and are positioned on the basement membrane. These have the capacity to self-renew and commit to spermatogonial progenitors which undergo mitotic and meiotic divisions to finally produce spermatozoa. The male germline stem cells are surrounded by somatic Sertoli cells that from a microenvironment or niche. The interstitial Leydig cells have also recently been shown to influence male germline stem cell development. Sertoli and Leydig cells are exquisitely sensitive to pituitary gonadotropins, FSH (acts on Sertoli cells) and LH (acts on Leydig cells) that are heterodimers consisting of a common α, and a hormone-specific β subunit. We have generated mice lacking either *Fshb* or *Lhb*, and hence these mice lack the functional heterodimers FSH or LH, respectively. Fshb null mice are hypogonadal, have reduced number of germline stem cell progenitors, qualitatively normal, but quantitatively reduced spermatogenesis and display normal fertility. Our previous analyses of FSH-responsive genes in the mouse testis identified that cyclinD2 is a key cell cycle regulator critical for FSH action in the gonads. Male mice lacking cyclin D2 phenocopy Fshb null mice. However, genetic removal of the cyclin D2 encoding gene *Ccnd2*, over the *Fshb* null background caused more severe hypogonadism, aberrant Sertoli cell maturation and defects in spermatogenesis and complete infertility. In contrast, *Lhb* null mice are hypogonadal, have low testosterone, defective steroidogenesis and are infertile. Defects in Leydig cells, Sertoli cells, Sertoli-germ cell junctions and a block in spermiogenesis are observed in the testes of *Lhb* mutants. Thus, these mouse models allow us to developmentally track the properties of somatic cell niche in the testis, and provide genetic approaches to investigate how the niche influences male germline stem cell self-renewal and differentiation. Our studies have clinical implications for both diagnosis and treatment of male infertility cases as well as identifying gonadotropin-regulated novel targets for male contraception. (Supported by HD043945 and RR024214 and a KUMC Biomedical Research Training Grant)

3. Regulation Of Vascular Endothelial Growth Factor (VEGFA) Isoforms May Be A Mechanism To Regulate Follicle Progression Within The Ovary.

Andrea S. Cupp, Robin A Ten Broeck, Renee Pohlmann, and Debra T Clopton, Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE.

The Vegfa gene contains 8 exons and is alternatively spliced to produce pro-angiogenic (Vegfaxxx) and anti-angiogenic isoforms (Vegfaxxxb). Our laboratory is interested in determining how VEGFA isoforms may regulate follicle development. We have amplified and sequenced the major anti-angiogenic isoform, Vegfa165b, during rat ovarian development. The Vegfa165b isoform is present earlier in the rat ovary versus the testis at embryonic day 13 (E13; 4 fold more) while Vegfa165b peaks at E18 (100 fold more) to decline at birth prior to oocyte nest breakdown and primordial follicle formation. In contrast to the testis, ovarian VEGFAxxxb isoform proteins are widely expressed in the developing genital ridge, oocyte nests and pre-granulosa cells, and in somatic cells of developing follicles. Inhibition of VEGFR signal transduction in postnatal day 3 (P3) rat ovarian cultures, dramatically reduced vascular development and inhibited follicle progression resulting in greater numbers of primordial follicles and reduced transitional stage 3 and 4 follicles ($P < 0.05$; 0=primordial, 1=early primary, 2=late primary, 3=transitional, 4=pre-antral, 5=antral). In addition, inhibition of KDR-specific signal transduction did not alter vascular development but arrested follicles at the primary follicle stage reducing numbers of follicles that progress to stage 3. Specific inhibition of VEGFA anti-angiogenic isoforms through a VEGFAxxxb antibody or addition of pro-angiogenic VEGFA isoforms induced follicle progression increasing the numbers of follicles at stage 3 ($P< 0.05$). The VEGFAxxxb antibody also depleted follicles at the primordial and early primary stages ($P< 0.05$). Preliminary data from transgenic mice using the MMTV promoter to overexpress VEGFA165b in the mammary glands and in the ovary indicate that VEGFA165b inhibits follicle progression in vivo since these mice had reduced litter sizes with decreased number of antral follicles on the ovary. Thus, regulation of the Vegfa gene to produce pro-angiogenic or anti-angiogenic isoforms may be a mechanism to manipulate follicle progression within mammalian gonads. This research was supported in part by grants from NIH, Nebraska Tobacco Funds for Biomedical Research and a NSF Women in Science Summer Support grant.

4. Anti-Spermiogenic Efficacy Of Single Oral Daily Dose Administration Of Iminosugars NB-DNJ And NB-DGJ Coincides With Their Relative Potency As Inhibitors Of Testicular Glucosyltransferase In C57Bl/6J Male Mice And Rats.

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Worldwide 87 million (42%) of pregnancies are unintended and 46 million pregnancies are terminated by abortion. Thus, the development of new safe, effective and reversible non-hormonal contraceptive methods for men is important. N-butyldeoxynojirimycin (NB-DNJ) was shown to be a potent reversible oral male contraceptive agent when tested in mice. However, it does not work in any other species than C57Bl/6J mice, yet it remains a good lead compound to develop analogues that overcome species-specificity. Previous studies have only administered the compound by mixing in the food. Our studies show that daily single oral administration of NB-DNJ and N-butyldeoxygalactonojirimycin (NB-DGJ) caused anti-spermiogenic effect in C57Bl/6J.NB-DNJ at 15, 25 and 50 mg/kg daily single oral dose over 35 consecutive days caused acrosomal disruption in 100% sperm population. On the other hand NB-DGJ showed dose-dependent acrosomal disruption. Histological analysis of testis showed abnormal spermatozoa with both iminosugars. However spermatogonial cells remained healthy indicating the reversibility potential. This finding coincides with the inhibition of mice and rat testicular glucosyltransferase by both these iminosugars, thereby validating the in-vitro enzyme assay standardized in our laboratory to shortlist novel iminosugar analogues to assess their anti-spermiogenic efficacy in an animal model.

5. Conditional Deletion of Dicer1 Implicates A Functional Role For MicroRNAs In Uterine Development And Function.

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The RNAse III endonuclease, Dicer1, is essential for the synthesis of the 19-25 nucleotide non-coding RNAs known as microRNAs (miRNA). These miRNAs associate with the RNA-induced silencing complex to regulate gene expression post-transcriptionally by base pairing with 3'untranslated-regions of complementary messenger RNA targets. While miRNAs are vital for normal development of several organ systems, misexpression of miRNAs are associated with several diseases with cancer being the most well explored. Previous studies from our laboratories have established that miRNAs are expressed in the reproductive tract, however their functional role and effect on reproductive disease remains unknown. To begin to examine the role of Dicer1 and subsequent miRNAs within the female reproductive tract, we generated female mice with conditional deletion of Dicer1. This was accomplished by mating mice with loxP insertions in the Dicer1 gene (Dicer1 (fl/fl)) and crossing them with mice expressing Cre-recombinase driven by the anti-Mullerian hormone receptor 2 promoter (Amhr2(Cre/+)). Adult female Dicer1 (fl/fl);Amhr2(Cre/+) mice displayed normal mating behavior, but failed to produce offspring when exposed to fertile males. Morphological and histological assessments of the reproductive tracts of immature and adult mice indicated that the uterus and oviduct were hypotrophic and the oviduct was highly disorganized. Further examination of the uterus revealed that uterine gland development was absent to severely compromised. In addition, estrogenic action upon the uterus (water imbibition, induction of estrogen-responsive genes) was also severely diminished. Analysis of estrogen regulated uterine miRNA profiles identified individual miRNA whose expression was modulated by estrogen and mis-expressed in the uteri of Dicer1 deficient mice. These miRNAs are putative regulators of several uterine factors which are necessary for uterine cell proliferation, differentiation and tissue remodeling. Collectively, these studies implicate Dicer1 and miRNAs as important factors in mediating post-transcriptional gene regulation in reproductive somatic tissues which are critical for the normal development and function of these tissues and for female fertility.

POSTERS: (6-31)

EMBRYO DEVELOPMENT: (6-9)

6. Expression of Neuropilin-1 and -2 May Indicate A Role In Sex-Specific Vascular Development And Germ Cell Viability In The Rat Gonads.

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Neuropilin-1 and 2 act as a co-receptor to Fms-like tyrosine-kinase 1 (FLT1) and Kinase Domain Region (KDR) receptor which is regulated by Vascular Endothelial Growth Factor A (VEGF). Neuropilin-1 serves to stabilize the interaction with VEGF receptors and bind specifically to VEGF isoforms which are critical in establishment of vasculature in most organs in the body. The function of Neuropilin-2 is not yet established. Our laboratory has determined that pro-angiogenic and anti-angiogenic VEGF isoforms are differentially expressed during gonadal development and may be critical to sex-specific vascular development. Therefore, the objective of this experiment was to characterize the expression of Neuropilin-1 and Neuropilin-2 during development in the rat testis and ovary. Gonadal tissue was collected from testes and ovaries at embryonic (E) and postnatal (P) days: E13, E13.5, E14, E16, E18, P0, P3 and P5. RNA from these tissues was extracted and reverse transcribed to produce cDNA pools (n = 6-10 gonads/age/pool). PCR primers for Neuropilin-1 and -2 were optimized for RT-PCR and each developmental age was examined for the Neuropilins. Neuropilin-1 was expressed at E13, E13.5 and E14 in the male at the time of endothelial cell migration during testis development. Furthermore, Neuropilin-1 was expressed after birth at P3 in the male with some expression in the ovary at E14 and P3. During sex specific vascular development endothelial cell migration occurs from the adjacent mesonephros into the testis while no migration occurs in the ovary. Thus, presence of Neuropilin-1 at this time in the male (and not the female) may aid KDR and VEGF interactions to elicit male-specific endothelial cell migration and vascular development. Neuropilin-2 was expressed in both the male and female gonads around gonadal differentiation, E13, E13.5, E14 and E16. Neuropilin-2 was also expressed after birth at P0 in both genders, and again at P3 in the male and P5 in the female. It is difficult to speculate what role Neuropilin-2 has during early male and female gonadal development. Our laboratory has demonstrated that VEGF is involved in both follicle progression and male germ cell survival, thus, after birth both Neuropilins may be involved in follicle progression in the female and germ cell viability in the male.

7. Smad2 Signaling In Embryonic Stem Cells And Early Endoderm Development.

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During early vertebrate development, Activin/Nodal signaling controls diverse developmental processes, including embryo patterning, cell proliferation, and cell-fate specification. To further understand the functions and regulation of Smad2, a downstream Activin/Nodal transcription factor, mouse embryonic stem (ES) cells were used to study Smad2 signaling targets and to model definitive endoderm formation. Gene expression analyses from ES cells treated with Activin and a pharmacological Activin inhibitor revealed a cohort of developmentally regulated genes. In particular, pathway inhibition strongly down-regulated Lefty1, Lefty2, Mixl1, and Fgf8 while Gata2, Mash2, and Id genes increased expression. Pathway stimulation resulted in moderate changes in gene expression, likely due to endogenous Nodal production. Long-term Activin stimulation under culture conditions permissive to differentiation induced gene expression indicative of differentiating endoderm. Furthermore, six days of Activin exposure doubled the population of definitive endoderm cells (E-cadherin^{+}/Cxcr4^{+}). These gene and protein expression assays for definitive endoderm development will be used to analyze the differentiation potential of mutant Smad2m1Mag ES cells. Mutant cells in Smad2m1Mag chimeric embryos failed to contribute to foregut, no-

tochord, and other midline structures. As the Smad2m1Mag allele contains only a single amino acid substitution yet produces a dramatic hypomorphic phenotype and differentiation defects, analysis of Smad2m1Mag ES cells will potentially reveal new roles, functional domains, and protein interactions of Smad2. Biochemical analyses of Smad2m1Mag cells demonstrated that the mutant cells express Smad2 and retain the ability to post-translationally modify the mutant protein. However, protein interactions analyzed through co-immunoprecipations revealed that the Smad2m1Mag mutant protein has decreased ability to form homodimers with wild-type and mutant Smad2. Further work is necessary to determine if other protein interactions, potentially novel, are also affected. Overall, our research aims to more fully understand the role of Smad2 in the pluripotency and differentiation of mouse ES cells and in the development of the early embryo.

8. The Aryl Hydrocarbon Receptor Agonist 2,3,7,8-Tetrachloro-Dibenzo-P-Dioxin (TCDD) Inhibits Early Embryonic Development Following In Vitro Fertilization In Female Sprague Dawley Rats.

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The aryl hydrocarbon receptor (AhR) mediates effects of many environmental toxicants. AhR ligands, including 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), accelerate reproductive senescence and one proposed target is the oocyte. To discriminate between direct effects on the oocyte versus those that would be mediated by complex ovarian interactions with TCDD, a rat in vitro fertilization (IVF) model was developed to examine AhR related functions soon after fertilization and early cleavage. Exposure to AhR ligands was limited in these studies to the fertilization period and beyond to evaluate fertilization and early cleavage. Accordingly, IVF was carried out in the presence of TCDD (0, 10, 100, 1000 nM) and the AhR antagonist CH-223191 (0.1 μM) combined factorially. In vivo matured cumulus ooycte complexes were collected from 31-day old female Sprague Dawley rats following superovulation, and incubated with sperm. After 8 h of co-culture, oocytes were examined for fertilization. A subset of 2-cell embryos and morula were collected 22 and 96h after insemination for analysis of Cyp1a1 mRNA expression, and whole mount fluorescence microscopy analysis of chromatin and f-actin microfilaments. Fertilization rates ranged from 56-84%. Development to the 2-cell stage was 86% for the control treated group. Treatment with 1 μM TCDD decreased this to 37%. TCDD-induced Cyp1a1 mRNA expression was absent in 2-cell embryos; however morulae exhibit dose-dependent Cyp1a1 expression. Treatment with 100 nM TCDD tended to decrease the proportion of morphologically normal 2-cell embryos and morula. Addition of CH-223191 restored these rates back to control levels. To summarize, TCDD-induced AhR activation may increase the incidence of abnormalities in the early embryo in an IVF system, suggesting acute exposure to TCDD has direct effects on oocytes and early cleavage in the rat. This model will aid in identifying specific targets of TCDD in the embryo that link cell cycle control with cytoskeletal and nuclear remodeling.

9. SF-1 Expression Is Mostly Directed By Distal Regulatory Elements.

Ravichandiran Kumarasamy and Leslie L. Heckert. Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, 66160.

Steroidogenic factor 1 (SF-1/Ad4BP/NR5A1) is a nuclear hormone receptor that has a pivotal role in endocrine regulation and development of the adrenal glands and gonads. Considering the critical nature of SF-1, many studies have focused on uncovering transcriptional mechanisms that drive SF-1 expression. Despite these efforts, little has been revealed regarding the mechanism that direct SF-1 to its appointed cell types, a result largely due to its requirement for distal regulatory sequences in directing tissue-specific expression. Previous transgenic studies identified a 153kb region, containing Nr5a1, that was sufficient for proper SF-1 expression, which provided an important initiation point for the identification of essential regulatory elements. Comparative genomics through the web-based tool ECR browser (http://ecrbrowser.dcode.org/) was performed and identified within the Nr5a1 locus several evolutionarily conserved non-coding regions called “ECR”s. Four of the most conserved ECRs

were examined for transcriptional regulatory activity. The ECRs were cloned into a vector that contained SF-1 promoter sequence from -734 to +60 upstream of a luciferase reporter. Transcriptional activity of each ECR was tested individually using transient transfection analysis in different SF-1-expressing cell types. Our results identified one ECR (ECR3) with activity that increased transcription in alpha T3(gonadotrope) and Y-1(adrenal)) cells and decreased it in MA-10(Leydig), Myoid, MSC-1(Sertoli), and primary Sertoli cells. The data indicate that ECR3 contains control elements necessary for SF-1 transcriptional induction in the pituitary and adrenal cells and repression in the testis cells. Mutagenesis and DNA/protein interaction studies identified sequences within ECR3 that were important for activity in alpha T3 cells. One such sequence contains an E-box that bound the class A basic-helix-loop-helix (bHLH) protein E2A, suggesting studies will reveal a class B bHLH, which exhibit tissue-specific expression, as its dimeric partner. This Research was supported by a Center Grant in Reproductive Sciences (U54 HD33994 from NICHD to LLH) and a KUMC Biomedical Research Training Grant to R.K.

PLACENTA: (10-17)

10. Differentiation of Rat TrophoblastT Stem Cells: Involvement of The Phosphatidylinositol 3-Kinase Signaling Pathway and Fos-Like Antigen 1.

Lindsey N. Canham, Mohammad A.K. Rumi, Kazuo Asanoma and Michael J. Soares. Institute of Maternal-Fetal Biology, Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, 66160.

A unique stem cell population is present at the blastocyst stage and within the extraembryonic ectoderm that gives rise to trophoblast cell lineages of the placenta. Trophoblast giant cells are essential for the establishment and maintenance of pregnancy and are a major endocrine and invasive cell of the placenta. Trophoblast giant cell differentiation in the rat can be studied in vitro using the Rcho-1 trophoblast stem (TS) cells. Rcho-1 TS cells have trophoblast stem cell properties and can be maintained in a proliferative (stem cell) state or induced to differentiate into trophoblast giant cells. We are interested in differentiation induced changes in gene expression as well as the role of Phosphatidylinositol 3-kinase (PI3K) signaling in trophoblast giant cells. PI3K activity is known to increase during trophoblast differentiation. To evaluate changes in gene expression that occur as a result of differentiation or PI3K signaling we profiled mRNA expression in trophoblast cells using an Affymetrix DNA microarray. The impact of PI3K inhibition on the steroidogenic and invasive phenotype of Rcho-1 TS cells was also measured. A potential mediator of PI3K signaling, Fos like antigen 1 (Fosl1), was evaluated by decreasing expression using lentiviral delivered Fosl1 shRNAs in Rcho-1 TS cells. Knockdown of Fosl1 was confirmed and the effect of Fosl1 knockdown on PI3K regulated genes and the invasive ability of trophoblast was evaluated. In summary we found that differentiation of Rcho-1 TS cells results in dramatic changes in gene expression and that PI3K signaling regulates the expression Fosl1(transcription factor), Cyp17a1 (steroidogenesis) and Mmp9 (invasion) and is involved in steroidogenesis and invasion in trophoblast cells. A potential mediator of PI3K, Fosl1, regulates the expression of a sub-set of PI3K-regulated genes including Mmp9 and the invasiveness of trophoblast cells.

11. Antigen-Specific Tolerance To The Fetus In Murine Pregnancy.

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Tolerance of the maternal immune system is believed to be important for successful pregnancy as the fetus is semi-allogeneic. We examined maternal T cell tolerance in mice using a system in which a model antigen, ovalbumin (OVA), is expressed exclusively in the fetus. This is achieved by breeding females that lack OVA to male mice that transgenically express membrane-bound OVA under the control of the β-actin promoter. By employing T cell receptor transgenic mice specific for a MHC class II-restricted epitope of OVA (OT-II) as mothers, we

investigated the fate of fetus-specific CD4+ T cells during gestation. OVA-specific CD4+ T cells displayed an activated phenotype in the spleen and lymph nodes of OVA-bred OT-II mice, consistent with their encounter of fetal antigen in peripheral lymphoid tissues. The interaction of OVA-specific CD4+ T cells with fetal antigen in the periphery of OVA-bred OT-II mice resulted in downregulation of the T cell receptor in a small percentage of T cells and a diminished proliferative capacity. Interestingly, T cell receptor downregulation was also observed in the thymus of OVA-bred OT-II mice, suggesting that fetal antigen may also affect central tolerance. These preliminary data indicate that fetal antigen has a mild effect on tolerance induction of maternal CD4+ T cells.

12. Placentation In The Rat Is Specifically Modulated By Regulatory Information On Chromosome 17.

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The rat possesses a hemochorial form of placentation, which is characterized by pronounced intrauterine trophoblast cell invasion and vascular remodeling. Strain-specific patterns of placentation are evident in the rat. The Holtzman-Sprague Dawley outbred and ACI, Fischer 344 and Dahl Salt Sensitive (DSS) inbred strains exhibit robust placentation, including extensive intrauterine trophoblast invasion. In contrast placentation in the Brown Norway (BN) is more restrictive. The strain-specific placentation differences can be quantified using structural criteria and gene expression profiles. The purpose of this project was to utilize these quantitative traits as tools toward the discovery of genetic mechanisms controlling placentation. The genetic control of physiologic processes can be investigated with chromosome-substituted inbred strains of rats. Chromosome-substituted rat strains have been generated with BN chromosomes introgressed into the DSS inbred strain. We surveyed 21 DSS-BN consomic strains and the parent DSS and BN strains. Pregnancies were established and animals sacrificed at gestation day 18.5. Placentation sites were dissected and prepared for histological or biochemical analyses. Litter size, placental weights, and fetal weights were not significantly different among the consomic strains. Expression profiles were performed on the DSS-BN consomic panel for genes exhibiting parent strain-specific patterns in the junctional zone (prolactin family 5, subfamily a, member 1, Prl5a1 or PLP-L). Consomic strains possessing Chromosome 17 introgressed into the DSS inbred rat strain displayed PLP-L transcript level that were significantly different from the DSS pattern and more closely resembling the BN pattern. Furthermore, in situ placental distribution of PLP-L mRNA also mimicked that observed for the BN rat. In conclusion, we have identified Chromosome 17 as possessing potential regulatory information controlling placentation. The existence of DSS-BN17 consomic rats will facilitate the generation of DSS-BN17 congenic strains and ultimately discovery of genetic loci controlling placentation. (Supported by NIH: HD20676, HD49503, and the Hall Family Foundation)

13. Differentiation-Induced Posttranscriptional Control Of B7-H1 In Human Trophoblast Cells.

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Trophoblast expression of immunomodulatory proteins in the human placenta is among the mechanisms that are critical for ensuring lymphocyte tolerance to the semi-allogeneic fetus. High levels of B7-H1 on trophoblast cells together with the known role of this protein in establishment of peripheral tolerance suggest that B7-H1 mediates immunological protection of the placenta during gestation. In this study we investigated the molecular mechanisms of regulation of B7-H1 in trophoblast cells by epidermal growth factor (EGF), a key regulator of trophoblast cell differentiation. EGF increased B7-H1 protein levels within 24 hours and mRNA levels within 4 hours of the initiation of treatment; by 24 hours B7-H1 mRNA levels were similar between control and EGF-treated cells. Analysis of two different potential promoter regions revealed strong promoter activity in response to IFN-γ.

In contrast, no promoter activity could be induced by EGF, suggesting that this cytokine regulates B7-H1 expression post-transcriptionally in trophoblast cells. EGF-induced B7-H1 protein expression was completely blocked in the presence of inhibitors of the PI3Kinase/Akt/mTOR pathway, a pathway known to regulate gene expression at the translational level. Finally, analysis of monosomal and polysomal mRNA fractions of untreated and EGF-treated term trophoblast cells revealed that EGF induces a shift towards the translatable fractions and away from the untranslated fractions. These results highlight a novel mechanism for regulation of B7 family proteins in the placenta and suggest that B7-H1 gene expression can be controlled by at least two separate mechanisms within the same cells.

14. Uterine Natural Killer Cells: Homing And Effects On Rat Placentation.

Damayanti Chakraborty, Toshihiro Konno, and Michael J. Soares, Institute of Maternal-Fetal Biology, Division of Cancer & Developmental Biology, Departments of Pathology & Laboratory Medicine and Obstetrics & Gynecology, University of Kansas Medical Center, Kansas City, Kansas

Decidualization of the uterine stroma recruits a population of lymphocytes called natural killer (NK) cells in women, non-human primates, and rodents. These uterine NK (uNK) cells are phenotypically distinct, characterized by lower killing activity and higher cytokine production. In rodent as well as in human pregnancy, the uNK cells peak around mid gestation after which they decline considerably and disappear during the later part of gestation. They are associated with the uterine mesometrial vasculature at midgestation. In order to study the in vivo role of NK cells during pregnancy, gestation stage-specific NK cell immunodepletion was performed in Holtzman Sprague Dawley (HSD) rats.
Methods: HSD rats were injected intra-peritoneally with anti asialo GM1 (αA-GM1) antibodies or normal rabbit serum (control) on gestation days 4.5, 6.5, and/or 9.5. Placentation sites were collected on different gestation days and sectioned for histological and immuno-histochemical analyses.
Results: A single injection of αA-GM1 at gestation d4.5 successfully depleted NK cells from placentation sites at gestation d9.5. Delaying the injection to gestation d6.5 was ineffective in depleting uNK cells. A combination of two injections (one at gestation d4.5 and the second at d9.5) of αA-GM1 was effective in depleting uNK cell numbers at gestation d13.5. Robust trophoblast invasion was observed in NK depleted animals. Uterine mesometrial blood vessels in these animals were lined by large cuboidal cells that were positive for cytokeratin and Prl7b1, a phenotype characteristic of invasive endovascular trophoblast cells. Significant effect of dosage and timing of αA-GM1 treatment on endovascular trophoblast invasion was also observed. NK cell immunodepletion affected mesometrial vasculature at gestation d9.5 and altered the organization of chorioallantoic placenta at gestation d13.5. The results indicate that there is a critical developmental window for NK cell homing to the uterus and that uNK cells modulate pregnancy associated uterine mesometrial vascular changes and trophoblast cell invasion.
(Supported by grants from the NIH: HD20676, HD48861, and the Hall Family Foundation)

15. A Differentiation State-Specific Promoter For Tracking Trophoblast Cell Fate Decisions.

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Trophoblast stem cells have the capacity for self renewal and differentiation along a multilineage pathway. Promoters of differentiation-lineage-specific genes can be potentially utilized to trace cell fate choices after induction of differentiation. Expression of placental lactogen-I (PL-I; Prl3d1) and PL-II (Prl3b1) are specifically induced as trophoblast stem cells differentiate into trophoblast giant cells. We constructed viral and nonviral reporters by cloning different lengths of rat PL-II promoter sequence upstream of green fluorescent protein (GFP) or firefly luciferase. Rcho-1 trophoblast cells were transfected with the luciferase reporter vectors containing 0.6, 1.6, 2.0, 3.0, 3.6 or 4.6 kb of the promoter and luciferase assays were performed after induction of differentiation. Results indicated existence of trophoblast specific enhancer(s) between -1620 to -1975 and additional upstream regulatory sequences. Rcho-1 cells transduced with lentiviral PL-II promoter-GFP constructs (0.6 or 3.6 kb) and maintained

in proliferative conditions did not express detectable GFP. However, differentiating Rcho-1 trophoblast cells transduced with the 3.6 kb PL-II promoter-GFP reporter exhibited activity associated with trophoblast giant cells. The 0.6 kb PL-II promoter-GFP reporter construct was inactive under similar conditions. Denuded rat blastocysts were also transduced with the reporter lentiviral particles expressing GFP. Transduced blastocysts outgrowths of trophoblast giant cells showed GFP expression from 3.6 kb PL-II promoter but not from the 0.6 kb promoter. For in vivo reporter assay, denuded blastocysts were transduced with the lentiviral 3.6 kb PL-II promoter construct and transplanted into pseudopregnant recipients. We observed that PL-II promoter specifically directed expression in trophoblast giant cells in gestation day 13.5 or 18.5 placenta. Thus PL-II promoter represents a useful tool for tracking and targeting trophoblast giant cells and lentiviral promoter-reporter constructs can be effectively utilized for monitoring trophoblast stem cell fate after induction of differentiation in vitro or trophoblast lineage decisions in vivo.

16. Isolation Of Stem Cells From Rat Blastocysts Possessing The Capacity To Differentiate Along The Trophoblast Cell Lineage.

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The placenta is comprised of specialized trophoblast cell lineages, which organize into a structure that facilitates nutrient delivery from the maternal uterine to the developing embryo. These differentiated trophoblast cells arise from a putative trophoblast stem cell population. The objective of this investigation was to establish trophoblast stem cell populations from rat blastocysts. Blastocysts were recovered from uteri of gestation d4.5 Holtzman Sprague (HSD) rats and from transgenic rats expressing the enhanced green fluorescence protein (EGFP) driven by a chicken beta actin promoter (chBA-EGFP). Blastocysts were cultured individually on a feeder layer of rat embryonic fibroblasts (REFs) in RPMI culture medium supplemented with 20% fetal bovine serum, fibroblast growth factor-4 (FGF4), and heparin. Once the cell lines were established on the REF feeder layers could be replaced with REF conditioned medium. Established rat blastocyst-derived cell lines were characterized by their morphology, flow cytometry, RT-PCR, and following in vivo transplantation. In the proliferative state the cells expressed established markers of trophoblast stem cells, including Cdx2 and Eomes. Cells ceased proliferation and differentiated when FGF4, heparin, and REF conditioned medium were removed. Differentiation was characterized by the decline of Cdx2 and Eomes expression, the appearance of large polyploid cells, and the expression of trophoblast differentiation-associated genes, including Ascl2, connexin 31, members of the prolactin gene family, Tpbpa, and Gcm1. The blastocyst-derived cell lines, in either proliferative or differentiated states, did not express genes, which are specific to ICM-derived tissues. The chBA-EGFP blastocyst derived cell lines were used for in vivo injection into renal transplantation analyses. Collectively, the data indicate that the rat blastocyst-derived cell lines possess characteristics of trophoblast stem cells. It is expected that these rat trophoblast stem cell lines will be powerful new in vitro and in vivo models for analyses of mechanisms controlling trophoblast stem cell renewal and differentiation.

17. Trophoblast-Specific Gene Transfer In The Rat Using Lentiviral Vector Delivery.

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The placenta is critical for ensuring proper intrauterine embryo development. A number of genes participating in the regulation of placental development have been identified from primarily serendipitous observations using mouse gene targeting approaches. More recently a lentiviral gene delivery approach has been utilized to specifically modify the mouse trophoblast cell lineage (Georgiades et al. BioTechniques 42:317-325, 2007; Okada et al. Nat Biotechnol 25:233-237, 2007). While the mouse is an effective tool for investigating some aspects of hemo-

chorial placentation, it is somewhat limited for studying others, including development of invasive trophoblast cell lineages. In contrast, rat placentation is characterized by extensive intrauterine trophoblast invasion and associated uterine vascular remodeling. However, to date strategies to genetically manipulate the rat trophoblast lineage have not advanced. In this study, we have adapted a lentiviral gene delivery technique for genetic modification of rat trophoblast cell lineages. Blastocysts were obtained from gestation day 4.5 rats, incubated with lentiviral particles containing specific gene constructs, washed, and transferred into the uteri of day 3.5 pseudopregnant female rats, harvested at various times during gestation, and then analyzed histologically. Two test systems were evaluated: 1) delivery of enhanced green fluorescent protein (EGFP) gene constructs under the control of ubiquitin or phosphoglycerate kinase promoters to wild type Holtzman Sprague-Dawley (HSD) rat blastocysts; 2) delivery of EGFP short hairpin RNA (shRNA) or scrambled control constructs to blastocysts obtained from a transgenic rat strain constitutively expressing EGFP driven by a chicken beta actin promoter (chBA-EGFP). We observed that the lentiviral packaged gene constructs were efficiently and specifically delivered to all trophoblast cell lineages. No evidence of gene delivery to inner cell mass-derived cell lineages was observed. Additionally, lentiviral mediated transfer of shRNAs was an effective strategy for modifying the expression of genes expressed in trophoblast cell lineages. Thus we have adapted a gene modification strategy for creating both 'gain of function' and 'loss of function' mutations in the rat trophoblast cell lineage that can be evaluated in vivo. The approach will permit a systematic analysis of genes controlling trophoblast development during hemochorial placentation in the rat. (Supported by NIH020676).

OVARY/UTERUS: (18-27)

18. Comparative Analysis Of The Metaphase II Spindle Of Human Oocytes Through Polarized Light And High Performance Confocal Microscopy.

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Human oocytes are a precious commodity and are prone to meiotic dysfunction during aging and by the use of assisted reproduction technologies (ART) to treat various forms of human infertility. Mounting evidence suggests that defects in the meiotic spindle presage errors in chromosome segregation which manifest during oocyte maturation and the early cleavage stages of human preimplantation development. The objective of this sudy was to determine the viability of using the Polscope® as a clinical tool for accessing oocyte spindle morphology by comparing fresh material on the Polscope comapred to the same samples after fixation by high resolution confocal microscopy. Fresh and frozen-thawed mature MII oocytes were analyzed through the Polscope and, immediately afterward, fixed for successive confocal microscopy assessment. Comparison of retardance values, derived from Polscope analysis, between spindles with different microtubule and chromosome configurations, defined by confocal microscopy evaluation were made in addition to measurements of the spindle longitudinal axis through both Polscope and confocal microscopy. In fresh and frozen thawed oocytes, the mean retardance of different categories of spindle configuration were not statistically significant ($P>0.123$). In spindles with a clear bipolar organization, the Polscope produced measurements of the spindle main axis which were in all cases statistically smaller ($P<0.001$) in comparison to the ones derived from confocal microscopy evaluation. In conclusion retardance measurements made using the Polscope are not indicative of the degree of microtubule organisation of the meiotic spindle. In addition, morphometric evaluation of the spindle through the Polscope is not consistent with confocal analysis. This questions the viability of the Polscope as an efficient method for assessing the MII spindle and, as a result, for non-invasive oocyte selection.

19. Human Meiotic Spindle Dynamics In Oocytes Following Slow-Cooling Cryopreservation.

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The demand for cryopreservation of human oocytes is increasing in assisted reproduction clinics for a several reasons: 1) it overcomes unwanted side-effects of exogenous gonadotropin administration, 2) it avoids ethical implications associated with embryo freezing and 3) it may provide an alternative for patients undergoing ovotoxic cancer therapy seeking to restore/preserve their fertility. Oocyte cryopreservation remains an experimental procedure largely because of the adverse effects it has on chromosome balance due to spindle disruption. The goal of these studies was to evaluate the process of meiotic spindle reassembly and chromosome alignment following cryopreservation of human metaphase-2 oocytes. Unfrozen control oocytes were compared to frozen oocytes that were fixed at 0, 1, 2, and 3 hours after thawing. Incomplete microtubule (MT) de-polymerization was observed in oocytes at 0 hr post thawing. Although no bipolar spindles with aligned chromosomes were seen at 0 hr, both bipolar (59.1%) and disorganized meiotic spindles (40.9%) with non-aligned chromosomes were observed. Following a one hour thaw, a significant proportion of spindles recover bipolarity with chromosomal alignment (71.4%) however, only a small fraction of oocytes retain this recovery configuration at 2 (31.6%) and 3 (22.7%) hours post-thaw. Interestingly, there was a significant positive correlation between spindle length and the number of displaced chromosomes at all time points examined. These findings challenge the current dogma that spindle formation uses the chromosomes as the primary point of MT polymerization due to the recovery of spindle bipolarity prior to chromosomal alignment. These studies should allow physicians to set new guidelines for achieving greater embryo quality by using cryopreserved oocytes one hour post-thaw instead of the traditional three hours protocol that has been linked to higher aneuploidy and spontaneous abortion rates in ART clinics. Supported by the Hall family Foundation and the ESHE Fund.

20. Differential Expression Of N- and E-cadherin In The Hamster Ovary During Perinatal Development: Potential Regulation By FSH.

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Factors controlling the size of the initial primordial follicle pool and the development of primordial follicles are largely unknown. Gestational neutralization of FSH attenuates the formation of primordial follicles, but the mechanisms are still unclear. Cadherins mediate homophilic, calcium-dependent cell adhesion; however, whether they are involved in FSH-regulation of primordial follicle formation remains unclear. The objective of the present study was to determine the expression and hormonal regulation of N- and E-cadherins in developing hamster ovaries with special reference to primordial follicle formation. Hamster N- and E-cadherin cDNA and amino acid sequences were highly similar to those of the mouse, rat and human. Both N- and E-cadherins were located mainly in the oocytes during early neonatal life. With the formation of primordial follicles on P8, N-cadherin expression shifted to the pregranulosa cells juxtaposed to the oocytes; however, E-cadherin expression in the oocytes decreased significantly. Subsequently, intense N-cadherin expression was restricted to granulosa cells of growing follicles, whereas E-cadherin signal in the oocytes almost disappeared. Levels of N-cadherin mRNA decreased from E13 to P6, but increased markedly on P7, the day before the onset of primordial follicles in the hamster ovary, followed by a decrease on P10. E-cadherin mRNA decreased from E13 through P3 and then remained low. N- and E-cadherin protein levels were consistent with mRNA levels. Exposure of E12 fetuses to an FSH-antiserum in utero resulted in a significant decrease in N-cadherin mRNA levels on P8 (1.7±0.1 ng/μg of total RNA vs. 0.8±0.09 ng/μg of total RNA), which coincided with a block in primordial follicle formation, but the decrease was prevented by a single injection of eCG on P1. A completely opposite result was obtained for E-cadherin mRNA. These results provide evidence for a differential spatio-temporal expression of N- and E-cadherins in perinatal hamster ovaries. Further, the expression is differentially regulated by FSH. The decrease in N-cadherin expression coinciding with the block in primordial follicle formation in FSH antiserum-treated animals and its reversal by eCG suggest that N-cadherin may facilitate the oocyte and somatic cell interaction during the formation of primordial follicles.

21. Vascular Endothelial Growth Factor (VEGFA) Inhibitory Isoforms Regulate Follicle Formation and Progression In The In Vivo Perinatal Rat Ovary.

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The objective of this experiment was to determine the effects of angiogenic and inhibitory (b) Vascular Endothelial Growth Factor (VEGFA) isoforms on follicle formation and progression in vivo. Based on in vitro data, it is our hypothesis that treatment with angiogenic VEGFA isoforms or removal of inhibitory isoforms would promote, while treatment with VEGFA inhibitory isoforms would inhibit follicle formation and progression. Postnatal day 0 (P0) female rat pups were injected IP with one of five treatments daily from P0 until P2; VEGFA164 (0.5ug; n= 4), VEGFA164b (0.5ug; n= 2), VEGFAxxxb antibody (1ug; n= 5), IgG control (1ug; n=4), and PBS control (0.5ug; n= 2). All pups were euthanized at P10 and ovaries were collected, embedded, and stained for hematoxylin and eosin. Three ovarian sections at least 10 microns apart, were counted per ovary by two different individuals for follicle development stages 0-5; (0= primordial, 1= early primary, 2= primary, 3= transitional, 4= pre-antral, and 5= antral). In the VEGFA164b treatment group, one ovary had multi-oocytic follicles (MOF's) while 3 of 5 ovaries contained MOF's in the VEGFAxxxb antibody treatment. Neither control (PBS or IgG) ovaries had MOF's, therefore; alteration of inhibitory isoforms may impair normal follicle formation. No differences in follicle morphology or stages were detected in the VEGFA164 treatment at the 0.5ug dose. However, the VEGFA164b treatment had more stage 2 follicles compared to the PBS control (18.8 vs. 10.7; $P<0.001$). Furthermore, the VEGFAxxxb antibody treatment had a greater number of stage 3 (13.7 vs. 7.5; $P<0.01$), stage 4 (25.6 vs. 13.5; $P<0.01$), and stage 5 (4.3 vs. 1.8; $P<0.001$) follicles than the IgG control. Thus, treatment with VEGFA164b may inhibit progression past stage 2, while removal of inhibitory isoforms (by the VEGFAxxxb antibody) accelerates follicle progression to later stages of development. Taken together, our results indicate a role for VEGFA inhibitory isoform regulation of both follicle formation and progression in the in vivo rat ovary.

22. Transcriptional Regulation Of miRNA-212/132 Expression In Murine Periovulatory Granulosa Cells.

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MicroRNAs (miRNAs) are 19-21 nucleotide non-coding RNAs that bind the 3`-untranslated region of target mRNAs to regulate translation. We have recently shown the mature forms of miRNA-212 and 132 are rapidly upregulated in periovulatory granulosa cells (GCs). Moreover, in vitro studies have indicated that 8-Br-cAMP treatment of GCs mimics LH-induced in vivo expression of miRNA-212 and 132. These two miRNAs share identical seed sequences and are positioned within 203 bases of each other. This study sets out to determine the identity of the nascent miRNA-212 and 132 transcript(s) within periovulatory GCs and the mechanisms regulating their expression. To establish whether LH affects the expression of miRNA processing factors, murine mural GCs were collected over a 12-hr time period following hCG treatment. No significant difference was observed in the expression of key proteins involved in the miRNA biogenesis pathway when analyzed by quantitative RT-PCR, suggesting the induction of mature miRNA-212 and 132 occurs as a result of increased miRNA transcription rather than increased miRNA processing. To determine whether these miRNAs are co-expressed as a single pri-miRNA, a 477 bp transcript was amplified using primers designed to span a region 5` to pre-miRNA-212 through the 3` end of pre-miRNA-132. Confirmation of a pri-miRNA-212/132 transcript suggests miRNA-212 and 132 are coordinately regulated by an LH/cAMP dependent mechanism. Several CRE sites located 5` to the pre-miRNA sequences have been implicated in the regulation of miRNA-212/132 expression. Additional studies are necessary to understand the role of these CRE sites as they relate to miRNA-212/132 expression.

23. The Expression And Function Of MicroRNA-21 In Periovulatory Granulosa Cells.

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The pituitary release of luteinizing hormone (LH) initiates ovulation as well as the differentiation of ovarian granulosa cells into luteal cells, a necessary event for the maintenance of pregnancy. This granulosa to luteal cell transition depends on coordinated changes in protein expression, and because microRNAs regulate protein translation we propose that microRNAs are important for this transition. MicroRNAs are ~21 nt RNA molecules that bind to the 3'UTR of specific messenger RNA transcripts to enhance or prevent their translation. Here we show that microRNA-21 is upregulated in murine granulosa cells following the LH surge using microRNA microarray analysis and qRT-PCR. To examine the function of miR-21, murine granulosa cells were cultured and treated with a 2'-O-methyl RNA oligonucleotide complementary to miR-21 (anti-miR21) to knockdown miR-21 expression, or a nonspecific antimir control. Protein from treated cells were subjected to 2-DIGE to identify potential miR-21 direct or downstream targets. Interestingly, elongation factor 2 (EF2), a protein involved in control of global translation, exhibited a significant increase in phosphorylation in response to anti-miR21. This increase in EF2 phosphorylation resulted in a decrease in EF2 activity, and therefore a decrease in the global translation of the cell. We are currently examining the EF2 pathways to identify the miR-21 target(s) that are responsible for this change in EF2 phosphorylation. In addition, cells transfected with anti-miR21 showed a 40% increase in apoptosis compared to cells transfected with control antimir. Therefore, our results indicate a novel function for miR-21 in stimulating global translation and preventing apoptosis, demonstrating it may play a critical role in the cellular growth and development associated with the granulosa to luteal cell transition.

24. Characterization Of Conditional Dicer Knock-Down In The Mouse Ovary.

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MicroRNAs (miRNAs) are 19-22 nucleotide long, non-coding RNA molecules that regulate gene expression post-transcriptionally. In animal cells, hundreds of miRNAs have been identified and reported to play a role in a number of processes including embryonic development, cellular differentiation and proliferation, and apoptosis. MicroRNAs are synthesized through a multi-step process that includes the formation of 70 to 110 nucleotide-long precursor stemloop RNA transcript referred to as pre-miRNA. These pre-miRNAs are cleaved by the RNase III enzyme, Dicer, to generate mature miRNA that regulate gene expression. Dicer is expressed by both the somatic and germline components within the ovary, as are its miRNA products. Previous work from our laboratory has found that LH induces the expression of specific miRNAs in ovarian granulosa cells. To determine if ovarian somatic cell miRNAs play a role in female fertility, a mouse line with Dicer knocked-down specifically in granulosa cells was established. Mice with loxP insertions in the Dicer gene (DicerFl/Fl) were crossed with mice expressing Cre-recombinase driven by the granulosa cell-specific Amhr2 promoter (Amhr2Cre). In breeding attempts, female DicerFl/Fl; Amhr2Cre mice failed to reproduce. To determine whether DicerFl/Fl; Amhr2Cre mice exhibit estrus, females (n=4) were exposed to fertile males and mating was confirmed. Another group of DicerFl/Fl; Amhr2Cre female mice (n=3) were administered 5 IU of eCG alone, or treated with 5 IU eCG for 46hr followed by 5 IU of hCG (n=3). Ovaries of the naturally mated DicerFl/Fl; Amhr2Cre mice had luteal tissue and oocytes/early embryos were present in the oviducts. However, fewer corpora lutea were observed in DicerFl/Fl; Amhr2Cre mice when compared to wild-type littermates. In mice administered eCG alone the ovaries contained follicles at all stages of follicular development, while the eCG+hCG treated DicerFl/Fl; Amhr2Cre mice exhibited luteinized antral follicles containing trapped oocytes, 19hr after hCG treatment. Further study using this conditional Dicer knock-down mouse line should provide important insight concerning the role of miRNAs in granulosa cell function.

25. Profibrotic Actions Of Transforming Growth Factor-β1 (TGFβ1) On The Endothelial Cells Of the Bovine Corpus Luteum.

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During involution of the bovine corpus luteum, transforming growth factor-β1 is produced in response to prostaglandin F2alpha (PGF2α). TGFβ1 has been shown to reduce progesterone secretion by steroidogenic cells. In other tissues TGFβ1 is well known for its pro-fibrotic effects on the extracellular matrix (ECM) and ECM remodeling proteins. The role of TGFβ1 on other cells present in the corpus luteum is unknown. Endothelial cells comprise about 50% of the cells present in the corpus luteum are involved in corpus luteum regression. In the present study we isolated microvascular endothelial cells from the bovine corpus luteum (CLENDO cells) in order to investigate the effects of TGFβ1 on endothelial cell proliferation and ECM production. Based on ELISA we found that CLENDO cells produce TGFβ1 and RT-PCR and western blot analysis demonstrated that CLENDO cells express the TGFβ1 receptors TBRI and TBRII. Western blot analysis revealed that Smad2 is phosphorylated when CLENDO cells are treated with TGFβ1 (1 ng/ml) with initial activation occurring by 5 min post-treatment and persisting through 240 min. Based on western blot analysis TGFβ1 treatment of CLENDO cells increased the expression of collagen I, fibronectin, MMP-2 and αSMA, but not collagen IV. Analysis of CLENDO cell-conditioned media by western blot showed that these cells express MMP-2 and TIMP-2, but not MMP-1, MMP-9 and TIMP-1. Gelatin zymography revealed that TGFβ1 treatment resulted in an increase active MMP-2 in CLENDO cell-conditioned media. Cell proliferation studies showed that TGFβ1 reduced DNA synthesis in a dose dependent manner and reduced the proliferative effect of calf serum; however TGFβ1 did not reduce CLENDO cell viability. These studies demonstrate that TGFβ1 acts directly on CLENDO cells to limit endothelial cell growth and induce pro-fibrotic changes in ECM and the ECM remodeling protein MMP2. Supported by the VA Medical Center and Olson Center.

26. Altered Gene Expression Patterns During The Initiation And Promotion Stages Of Neonatal Diethylstilbestrol-Induced Dysplasia/Neoplasia In The Hamster Uterus.

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Treatment of hamsters on the day of birth with the synthetic estrogen and prototypical endocrine disruptor, diethylstilbestrol (DES), results in a 100% incidence of uterine hyperplasia/dysplasia in adulthood and a large proportion of the disrupted organs progress to neoplasia (endometrial adenocarcinoma). Further investigation of this pathological phenomenon at the histomorphological level determined that, in accordance with the classic two-stage model of carcinogenesis, the neonatal DES exposure event directly and permanently disrupts the developing hamster uterus (initiation stage) so that it responds abnormally when it is stimulated with the natural ovarian estrogen, estradiol (E2), in adulthood (promotion stage). To identify candidate molecular elements involved in progression of the disruption/neoplastic process, we performed microarray experiments (Affymetrix Gene Chip System) and immunoblot analyses on at least triplicate sets of uterine RNA and protein extracts, respectively, from 5-day old animals (initiation stage) and from 2-month old animals that were chronically stimulated with E2 (promotion phase).

At the RNA level, the number of genes whose expression were at least 2-fold and significantly (t-test p-value < 0.05) different (U = upregulated, D = downregulated) in DES-exposed vs. control uteri were:
-Initiation Stage: 143-U, 86-D
-Promotion Stage: 20-U, 48-D

Examples of immunodetected proteins whose relative expression levels matched those measured at the RNA level are as follows:
-Initiation Stage: Connexin-43, U; Progesterone Receptor, U; and Signal Transducer and Activator of Transcription (Stat) 5A, U.

-Promotion Stage: Interferon Regulatory Factor-1 (IRF-1), U; Insulin Receptor Substrate-1 (IRS-1), D; Progesterone Receptor, D; and Specificity Protein 1 (Sp1), U.

These results demonstrate that progression of the neonatal DES-induced disruption/neoplasia process in the hamster uterus involves a spectrum of gene expression alterations. However, the complex of genes and their manner of altered expression was quite different during the initiation vs. promotion stages of the phenomenon.

27. Intrauterine Pathways Impacting Pregnancy-Dependent Adaptations To Physiological Stressors.

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The mouse possesses an expanded prolactin (PRL) gene family that encodes hormones/cytokines. These hormones and cytokines are associated with pregnancy and are produced by the anterior pituitary, uterine decidua, and/or trophoblast cells. Thus far, their biological activities appear to be associated, at least in part, with modulating uteroplacental adaptations to physiological stressors. Decidual prolactin-related protein (dPRP) is abundantly expressed in the uterine decidua. DPRP deficiency interferes with pregnancy-dependent adaptations to hypoxia resulting in pregnancy failure. The purpose of this study was to identify candidate targets for dPRP action within the uteroplacental compartment. Wild-type and dPRP null mice were mated and sacrificed on gestation d7.5. Decidual-placental-embryonic tissues were dissected from implantation sites, homogenized, and RNA extracted. Affymetrix DNA microarray analysis was performed for RNA samples from wild-type (n=3) and dPRP null (n=3) tissues. Genes exhibiting a minimum level of expression (>1,000 units, MAS5 signal intensity) and a significant difference between the two groups were further analyzed by quantitative RT-PCR and in some cases by northern blot analysis. Transcripts were localized by in situ hybridization. Six genes were identified, which exhibited a significant upregulation in dPRP null tissues, including: Derl3, Herpud1, Creld2, Tra1, Ddit3, and Hspa5, reflecting an increased endoplasmic reticulum (ER) stress response. Nine genes were confirmed with decreased expression in dPRP null tissues (e.g. Klk7, Ccl27, Calm4, Prl4a1, Lipg, Sprr2h, etc). These include potential decidual, endothelial, and trophoblast cell targets positively regulated by dPRP. In summary, potential dPRP targets have been identified, which will permit dissection of intrauterine regulatory pathways controlling pregnancy-dependent adaptations to physiological stressors. (Supported by HD055523)

TESTIS: (28-29)

28. The Na,K-ATPase α4 Isoform Maintains Membrane Potential, Intracellular Ca2+ and pH to Sustain Sperm Motility.

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The Na,K-ATPase is a plasma membrane enzyme essential to numerous cell processes that depend on the transcellular gradients of Na^+ and K^+. The Na,K-ATPase is expressed as multiple isozymes, each composed of the association of different isoforms of two polypeptides, the α and β subunits. Among the α polypeptides, α4 is a testis specific isoform found in spermatozoa. We have previously shown that α4 has a number of biochemical and functional characteristics that are highly unique and essential to sperm physiology. Here, we have explored the mechanisms by which α4 influences sperm function. For this, we have taken advantage of the high sensitivity of α4 to ouabain. Ouabain, in concentrations that selectively inhibit α4, affected total motility of rat sperm in a time dependent manner. Using computer assisted sperm analysis; we have found that different parameters of sperm movement, such as progressive motility, straight line, curvilinear and average path velocity, lateral head displacement, beat cross frequency, and linearity are also decreased. Inhibition of α4 was sufficient to produce

these effects and additional inhibition of α1, the other α isoform present in sperm, with higher ouabain doses did not result in further changes in the cell motility parameters studied. In addition, ouabain inhibition of α4 increased intracellular Na+ and affected cell membrane potential, causing depolarization of the cells. Moreover, ouabain interference of α4 activity increased calcium and diminished the pH of the sperm cytoplasm. Altogether, these results suggest that the α4 isoform is important in primarily controlling sperm Na+ gradient, and secondarily the Ca^{2+} and H^{+} levels in the cells. The relevance of these ions for sperm motility suggests that their control may be the mechanism by which α4 maintains the function and fertility of the male gametes. [Supported by NIH grants HD043044 and HD055763].

29. H2-Gamendazole, A Promising Candidate For Reversible, Non-Hormonal Oral Male Contraceptive Development.

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World Health Organization statistics show that 122 million planned pregnancies occur worldwide per year. Despite of the availability of many different female contraceptive methods and of condoms, an additional 87 million pregnancies were unintended (representing 42% of all pregnancies), and 46 million pregnancies were terminated by abortion. In the United States, the unintended pregnancy rate is 49% of all births, and about half of these are terminated by abortion. Surprisingly, in 50% of unintended pregnancies, the women reported having used a contraceptive. Thus, the development of novel reversible oral male contraceptive agents has been identified by the National Institutes of Health, Institute of Medicine, and World Health Organization as being a major advance needed to address this worldwide reproductive health issue.

Recent success in our efforts to develop a reversible non –hormonal, male contraceptives have been our study on h2-gamendazole which was identified as an orally active antispermatogenic compound with antifertility effect. H2-gamnedazole produced a reversible contraceptive effect with a single oral dose of 6 mg/kg in rats. Preliminary data showed that using weekly low doses of only 1 or 2 mg/kg shows complete loss of spermatids, but excellent retention of spermatocyte and spermatogonial populations with no change in Inhibin B production. In rabbits 3mg/kg single oral dose showed anti-spermatogenic response with a potential of reversibility, but 6 mg/kg seems to affect spermatocytes and spermatogonial cells too, which doesn't satisfy the reversibility criteria. Mice showed a statistically significant effect on testicular weight at 12 mg and 25 mg/kg single oral dose. The cellular mechanism(s) through which these effects occur and the molecular target(s) of h2-gamendazole action are currently unknown. Using membrane binding assay with rat testicular microsomes, we were able to identify 2 binding proteins (~135 and ~ 65kDa respectively).While the mechanism of action is still not clearly understood, the efficacy of h2-gamendazole in mice, rat and rabbit model positively paves way for h2- gamendazole towards study in non-human primates and eventually humans.

CANCER: (30-31)

30. GSK-3-Modulated Autophagy Act As A Switch Between Necrosis And Apoptosis.

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Glycogen synthase 3(GSK-3) is a protein serine/threonine kinase involved in multiple cellular function, and its activity in cell increases dramatically under the condition of serum starvation. Autophagy is a conserved lysosomal degradation pathway for cytoplasm and organelles during the stress conditions such as starvation or growth factor deprivation. In the present study, we sought to understand if GSK-3 controls autophagic activities after serum depletion. Consistent with previous reports, serum starvation led to typical autophagic responses including LC-3 processing and autophagosome formation in prostate cancer PC-3 cells. Treatment of serum-starved cells with GSK-3 inhibitors, peptide inhibitor L803mts, caused a profound necrostic cell death indicated by YO-PRO/PI staining in flow cytometry assay. Its culture supernatant exhibited significant increased activities of LDH and HMGB1. However, autophagy suppressor 3-methyladenine did not block L803mts-induced cell death but shift the necrostic cell death response to a dramatic apoptotic response, as evidenced by cleavage of caspase-3 and PARP. In the further study, we found that the intracellular ATP levels of PC-3 cells decreased obviously after treatment with L803mts and/or 3MA. Moreover, the phosphorylation of AMPK, P70 s6 kinase and P27kip1, involved in energy sensing LKB1-AMPK pathway, were significantly changed in response to the treatment. Taken together, our data suggest that GSK-3 activity is critical for autophagy-dependent survival under the condition of serum starvation, and this GSK-3-modulated autophagy can act as a switch to determine the cell death fate by necrosis or apoptosis. This study opened a novel field regarding GSK-3 control of cell survival.

31. A Meta-Analysis To Identify Shared Biomarker Genes And Prevention targets for human breast and ovarian cancer.

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Women at high risk for breast cancer are often at risk for ovarian cancer as well. Shared risk factors, similar pathobiologies as well as logistical and economic issues all suggest a combined approach to breast and ovarian cancer prevention. Towards this end, our laboratory has recently developed a preclinical model to test dual target breast and ovarian cancer prevention strategies. In this ancillary study, Oncomine (University of Michigan), an online depository for cancer microarray data, was used to identify common potential targets and shared biomarkers of cancer progression for breast and ovarian cancers. Microarray studies using the Affymetrix Human Genome U133A or U133A Plus 2.0 chips (n=4 ovary and n= 2 breast databases) to compare breast or ovarian carcinoma (not sorted by tumor subtype) with associated normal tissue were selected for meta-analysis. These combined datasets identified fewer than 80 genes dually and consistently upregulated in breast and ovarian cancers ($P<0.01$). BMP7, CCND1, and FOXP1 were among the positively regulated genes identified. These and other genes will be further analyzed and validated using our animal model of simultaneous breast and ovarian cancer. This study will assist in the identification of suitable biomarkers for early detection and development of dual-target prevention of breast and ovarian cancer.

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