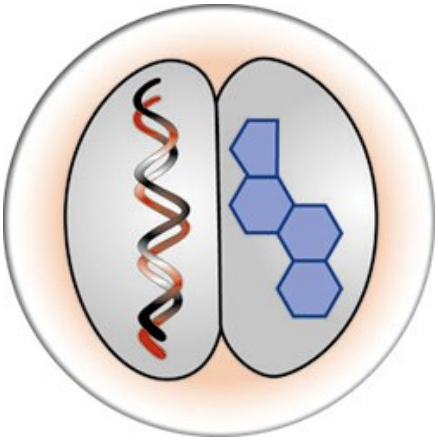
Annual Research Symposium

Endocrinology & Reproductive Physiology Program

Celebrating 50 Years of Graduate Training

1959-2009



April 14, 2009

Fluno Center for Executive Education, 601 University Ave.

Schedule of Events

10:00—10:30 AM **Welcome and Introductions**

10:30—12:00 PM **Trainee Presentations**

Derek Boeldt (PI: Ian Bird) - "Pregnancy-Specific Changes in VEGF Ca2+ Signaling in Uterine Artery"

Kathryn Guerriero (PI: Ei Terasawa) - "Developmental Changes in the Release Pattern of Kisspeptin-54 from the Stalk-Median Eminence of the Hypothalamus of Intact and Ovariectomized Female Rhesus Monkeys"

Jinwoo Lee (PI: Colin Jefcoate) - "Superoxide generation and oxidative stress in the regulation of StAR expression"

Celina Checura (PI: John Parrish) - "Use of a GnRH-antagonist to study the relationship between gonadotropins and intrafollicular free IGF1 at the expected time of deviation in mares"

12:00—1:00 PM **Lunch—Dining Room**

1:00—1:30 PM **Poster Session**

1:30—2:30 PM ***Keynote Presentation: Dr. Vasantha Padmanabhan,***
University of MI– Ann Arbor

"Developmental Programming of Reproductive and Metabolic Health"

2:30—4:00 PM **Trainee / MD Fellow / Alumni Presentations**

Justin Bushkofsky (PI: Colin Jefcoate) - "Multiple affects of Cyp1b1 deficiency in mouse development"

Maria Giakoumopoulos (PI: Ted Golos) - "The Effects of Spatial and Mechanical Interactions of Collagen I on Human Embryoid Body-Derived Trophoblast Differentiation"

Dr. Chanel Tyler: (PI: Manish Patankar) - "Phenotypic Expression of Natural Killer Cells: A Comparison of Normal Term Versus Preeclampsia"

Dr. Francisco Diaz, PhD (2003, Milo Wiltbank) - "Follicles in the Balance: Opposing Roles of Oocytes and FSH"

4:00—4:30 PM **50 Years of the ERP Program / Awards**

4:30 PM **Steering Committee Meeting with External Reviewer**

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Symposium Organizing Committee

Program Director	Dr. Ian Bird, PhD
Admissions Committee Chair	Dr. Manish Patankar, PhD
Student Services Coordinator	Tiffany Bachmann, MA
Abstract Book Designed by	Tiffany Bachmann, MA

Invited Guests

We are pleased to have the following invited guests participating in today's symposium.

Keynote Speaker	Dr. Vasantha Padmanabhan University of MI– Ann Arbor
Alumni Speaker	Dr. Francisco Diaz University of Pennsylvania, University Park
External Reviewer	Dr. Ronald Rosenfeld The Lucile Packard Foundation

Special Thanks To...

Funding for this year's annual event is generously provided by the following organizations

- UW Madison Lecture's Committee
- The Department of Obstetrics & Gynecology
- The Endocrinology & Reproductive Physiology Program Training Grant HD041921
- The Endocrinology & Reproductive Physiology Program

50 years ago....

In world history...Fidel Castro becomes President of Cuba; The Dalai Lama flees to India; Soviet Premier Nikita Khrushchev tours the United States, meeting with Eisenhower at Camp David.

In US history...Alaska and Hawaii become the 49th and 50th States in the Union; St. Lawrence Seaway opens to allow ocean vessels into the Midwest Great Lakes.

In Madison, WI…Chadbourne Hall opens on the UW-Madison campus; the Board of Regents vote to tear down the Armory/Red Gym to build an underground parking structure; UW Bio-chemist Karl Link receives prestigious award for his work on the blood thinner Warfarin

In Other News...Barbie made her debut selling for $4; Gunsmoke was the first weekly television show to be broadcast in color; Frank Lloyd Wright dies; Best Picture Oscar went to Gigi

Sources: Infoplease.com/year/1959.htm; http://www.thedailypage.com/daily/article.php?article=25480

Keynote Speaker

Presentation Title: " Developmental Programming of Reproductive and Metabolic Health"

Sponsored by the UW Lectures Committee

Dr. Vasantha Padmanabhan, PhD holds professorial appointments at the University of Michigan-Ann Arbor in the departments of Pediatrics, Obstetrics/Gynecology and Molecular and Integrative Physiology in addition to as serving as the Director of Pediatric Endocrine Research and Senior Research Scientist in the Reproductive Sciences Program. Dr. Padmanabhan received both her BS and MS degrees from the University of Mysore in Banaglore India. She earned her PhD in Cytogentics from the Indian Institute of Science in 1971 and completed her postdoctoral training in Microbiology/Genetics at the Ohio State University in Columbus, OH. Her current research at the University of Michigan—Ann Arbor is translational and centers on the understanding the fetal origin of pubertal and adult reproductive and metabolic disorders and the impact of steroids and estrogenic environmental pollutants in programming such defects. In addition to her active research laboratory which she has published 123 publications, Dr. Padmanabhan has chaired NIH Study Sections of Special Interest Panels, been a member of the Editorial Board for the Biology of Reproduction journal, member on multiple committee's within the Society for the Study of Reproduction (SSR) member of Sigma Xi, the Endocrine Society, Society for Advancement of Science, Society for Neuroscience and received two UROP Faculty Mentor Recognition awards. She also maintaines active research collaborations both nationally (Dr. David Abbott and Dr. Dan Dumesic—UW Madison) as well as internationally in the UK, Scotland, Australia, New Zealand, and Chile. Dr. Padmanabhan is active in training and mentoring undergraduate students, predoctoral and postdoctoral students including MD Fellows. She is a nationally and internationally sought after speaker in the areas of fetal programming, reproductive and metabolic health.

Alumni Speaker

Presentation Title: "Follicles in the Balance: Opposing Roles of Oocytes and FSH"

We are pleased to welcome **Dr. Fancisco Diaz, PhD (2003),** as our alumni guest speaker. Dr. Diaz earned his BS degree from the University of Vermont and his MS (1999) and PhD from the University of Wisconsin-Madison, ERP Program in Dr. Milo Wiltbank's laboratory. He completed his postdoctoral training at the Jackson Laboratory in Bar Harbor, ME from 2003-2007 under the direction of Dr. John Eppig. Dr. Diaz is currently an Assistant Professor of Poultry Science at the University of Pennsylvania—University Park campus. His current research focuses on three areas: 1) Interaction between oocytes and granulosa cells; 2) Mechanisms regulating the differentiation of early female germ cells in mammals and birds; and 3) Mechanisms regulating differences in reproductive capacity between broiler-breeder and laying hens. Career accomplishments include: Define changes in MAPK signaling associated with the preantral to antral transition in mouse ovarian follicles; Characterized the requirement for oocyte-secreted factors in specifying the cumulus granulosa cell phenotype and antagonizing the mural granulosa cell phenotype; Identified SMAD2 signaling as a key oocyte-stimulated pathway in cumulus cells; Characterized the differential regulation of the progesterone, estradiol, prostaglandin and AP-1 signaling pathways in porcine CL before and after acquisition of luteolytic capacity; and Determined that progesterone blocks PGF 2a - induced luteolytic responses in the porcine CL without luteolytic capacity. Dr. Diaz is also a faculty member in the Inter-college Graduate Degree Programs in Physiology and Cell and Developmental Biology.

External Reviewer

Ron G. Rosenfeld, M.D. is the Senior Vice-President for Medical Affairs at the Lucile Packard Foundation for Children's Health at Stanford University. He is Professor of Pediatrics at Stanford University, as well as Professor and Chair (emeritus) of Pediatrics and of Cell and Developmental Biology at Oregon Health and Sciences University. He has also served as President and CEO of ProteoGenix, Inc.

Dr. Rosenfeld received his B.A., summa cum laude, from Columbia University in 1968 and his M.D. with Honors from Stanford University in 1973. He stayed at Stanford as intern, resident and chief resident in Pediatrics and, thereafter, as a postdoctoral fellow in pediatric endocrinology. He joined the faculty at Stanford in 1980, was promoted to Associate Professor with tenure in 1985 and to Professor in 1989. In 1993, he left Stanford, to accept the position of Chairman of Pediatrics at Oregon Health Sciences University, where he served for nine years, before returning to Stanford in his current position.

Dr. Rosenfeld is an internationally renowned authority on the endocrine basis of growth and development and has been at the forefront of understanding the biology of growth hormone and growth factors for over 25 years. His work has elucidated physiological, pathological, cellular and molecular aspects of mammalian growth, as well as the role of growth factors and their receptors in the fetus, newborn, child and adult in health and disease.

The author of approximately 600 publications and 8 edited books, Dr. Rosenfeld has received numerous awards, including a Mellon Foundation Fellowship, Basil O'Connor Award from the March of Dimes, an NIH Career Development Award, and 30 years of continuous NIH grant support. He is the recipient of the Kaiser Award for Excellence in Teaching at Stanford, the Joseph St. Geme, Jr. Award from the Society for Pediatric Research for pediatric education, the Ross Award for Research from the Society for Pediatric Research, The Clinical Endocrinology Trust Medal from the Royal Society of Medicine, the Maureen Andrew Mentor Award from the Society for Pediatric Research, the 2008 Transatlantic Medal as the outstanding North American endocrinologist from the Society for Endocrinology, and the 2008 Robert H. Williams Distinguished Leadership Award from the Endocrine Society. He has been active on many editorial boards and study sections, and served as Chair of the Endocrinology Study Section of the NIH and as Chair of the Clinical Research Advisory Board of the March of Dimes. In 1997, he served as President of the Lawson Wilkins Pediatric Endocrine Society.

ERP Program History

The ERP Program at the University of Wisconsin-Madison was formally organized in 1959. In 1963 Dr's. L.E. Casida, Professor, Department of Genetics; W.H. McShan, Professor, Zoochemistry, Department of Zoology; R. K. Meyer, Chairman (ERP Program) and Marshall Professor of Zoology; B. M. Peckham, Chairman and Professor of Obstetrics and Gynecology; and R.C. Wolf, Associate Professor, Department of Physiology submitted an application to the Ford Foundation and received $1,500,000 to support the ERP Program for five years, which was then further extended to 1970. In addition to the Ford Foundation award, this group prepared a successful training grant application to the National Institute of Child Health and Human Development for $1,000,185. Funds from both grants enabled the ERP Program to increase the number of trainees in the program. In 1968 the program membership also expanded to include: Dr's. A.E. Colás, Professor of Obstetrics and Gynecology and Physiological Chemistry; N.L. First, Professor of Meat and Animal Science; O. J. Ginther, Assistant Professor of Veterinary Science; H. J. Karavolas, Assistant Professor of Physiological Chemistry; and K.W. Thompson, Professor of Obstetrics and Gynecology. The original group from 1963 and new faculty from 1968 proceeded with a renewal application of the National Institute of Child Health and Human Development training grant (above). This successful renewal no longer stipulated a specific number of trainees from developing countries be enrolled in the ERP Program. The ERP Program then continued along these lines and over the following decades developed a worldwide reputation as a program of excellence. In 1972 additional faculty were added to the ERP Program including Dr. J. Gorski, Professor of Biochemistry and Dr. D. Dierschke, Assistant Professor of Meat and Animal Sciences. Of note, during the next three decades, the program was awarded further support both alone (NIH Training Grants 1970-1972, 1978-1982, AGRICCSRS 1987-1992, US Army 1996-2001) and in conjunction with other notable programs (Cell and Molecular Biology Program, Developmental Biology, and University departments under the umbrella of a Gamete and Embryo Biology training grant through NICHD (1989-1993). Also by this time, the longstanding existence of the program was now shown by the prominent achievements of its early trainees, many of which have gone on to meritorious achievements.

Since 2000 the ERP Program has been led by Dr. Ian M. Bird, Ph.D., Professor, Department of Obstetrics and Gynecology. The ERP Training Grant Steering Committee was set up on his initiative and became the ERP Program's current steering committee. Dr. Bird has worked extensively with the Dean's of the Graduate School and School of Medicine and Public Health to achieve the ERP Program's realignment to the Medical School in 2006. Dr. Bird has the ability to seek new opportunities to fully integrate the ERP Program into the graduate education mission of both the Department of Obstetrics and Gynecology as well as the School of Medicine and Public Health (SMPH). During the past eight years, Dr. Bird has gained recognition at NIH as well as within the UW system as a long-term Program Director with valuable insights to graduate student training and program management. The ERP Program regained its NIH Training Grant Funding in 2004 and has been successfully renewed for another five year period (2014).

ERP Program Directors

Through the dedication, resolve and determination of these individuals who have served as Chair or Director of the ERP Program, we thank them for their leadership and service that has enabled us to celebrate 50 years of graduate training at the University of Wisconsin-Madison.

Faculty	Years	Tenure Dept.
Dr. Roland K. Meyer	1959-1968	Zoology
Dr. Richard C. Wolf	1968-1986	Physiology / Primate Center
Dr. Roy Ax	1986-1989	Dairy Sciences
Dr. Lewis Sheffield	1989– 1996	Dairy Sciences
Dr. Barry Bavister	1996-2000	Veterinary Sciences
Dr. Ian Bird	2000-Present	Obstetrics/Gynecology

ERP Program Administrators

It is also with great appreciation we recognize the talents of our three Program Administrators who have been instrumental in providing administrative and student support to our past, present and future trainees.

Program Administrator	Years	Directors Served With
Lois Frasier	1961—1996	Drs. Meyer, Wolf, Ax and Sheffield
Jeannette Rutschow	1996—2000	Dr. Bavister
Tiffany Bachmann	2000—Present	Dr. Bird

Pregnancy-Specific Changes in VEGF Ca2+ Signaling in Uterine Artery Endothelial Cells

Derek S Boeldt* , Mary A Gummer, Fu-Xian Yi, Ian M Bird

Pregnancy is a time of greatly increased blood flow in the uterus to meet the needs of the growing fetus. This is achieved through the mechanisms of vasodilation and angiogenesis. In diseased states, such as preeclampsia, proper flow to the fetus is not achieved, putting both the mother and fetus at risk. We have focused on vasodilation, specifically reprogramming of cell signaling leading to production of the potent vasodilator nitric oxide (NO) in pregnancy. While it has been observed that endothelial nitric oxide synthase (eNOS) expression levels drop to equivalent levels in uterine artery endothelial cells in primary culture, derived from pregnant ewes (P-UAEC) and non-pregnant ewes (NP-UAEC) by passage 4, eNOS activity remains elevated in P-UAEC. A variety of agonists can increase eNOS activity, including ATP and VEGF, the primary agonists used in our studies. Both of these agonists are capable of causing increased Ca2+ levels in cells, presumably leading to increased eNOS activity and therefore increased NO production. The ATP induced calcium signaling mechanism has been thoroughly studied in UAEC, but much is unknown about VEGF Ca2+ signaling. Furthermore, proper function of Connexin 43 (Cx43) gap junctions is essential for maintenance of the pregnancy-specific increased sustained phase of the ATP Ca2+ response. My studies are aimed at describing the VEGF signaling mechanism in P- and NP-UAEC as it relates to Ca2+ mobilization, as well as its potential impact on ATP signaling and proper Cx43 function. Recent work has validated previous reports from the lab that the VEGF Ca2+ response is mediated by VEGFR2, and not VEGFR1 through the use of the R2 and R1 selective agonists VEGF-E and PlGF, respectively. Analysis of Ca2+ data has also revealed pregnancy related differences in not only a greater number of NP-UAEC than P-UAEC responding to VEGF-E than VEGF-165 and PlGF, but also maintaining a longer elevated Ca2+ state. This data suggests that cell-signaling events are altered in the pregnant state UAEC not only to ATP, but also VEGF, and dysfunction of these signaling changes could underlie conditions such as preeclampsia.

* Supported by HD041921

Developmental Changes in the Release Pattern of Kisspeptin-54 from the Stalk-Median Eminence of the Hypothalamus of Intact and Ovariectomized Female Rhesus Monkeys

Kathryn A Guerriero*, Kim L Keen, Ei Terasawa

An increase in the pulsatile release of luteinizing hormone-releasing hormone (LHRH) from the hypothalamus is critical for the initiation of puberty. However, what triggers the pubertal increase in LHRH release remains unknown. Recently, because kisspeptin (KP) and its receptor, GPR54, have been proposed to play an important role in the pubertal increase in LHRH release and because our lab previously showed that mean KP release increases along with the pubertal increase in LHRH release in the stalk-median eminence (S-ME), in the present study we further examined the role of KP/GPR54 signaling in the pubertal increase in LHRH release. First, developmental changes in KP release pattern in the S-ME were assessed in prepubertal (15±2 months, n=4) and pubertal (33±3 months, n=6) intact female monkeys using a microdialysis method. KP levels in dialysates were measured with radioimmunoassay and peaks were identified with PULSAR algorithm. Results indicate that 1) KP was released in a pulsatile manner in both age groups, 2) similar to our previous observation, the mean KP level in pubertal monkeys (3.6±1.5pg/ml) was significantly ($p<0.05$) higher than in prepubertal monkeys (1.9±0.2 pg/ml), 3) interpulse interval in pubertal monkeys (46.2±4.9 min) was significantly ($p<0.001$) shorter than in prepubertal monkeys (86.4±5.0 min), and 4) pulse amplitude was not different between age groups (pubertal: 4.6±1.7 pg/ml vs. prepubertal: 4.5±1.0 pg/ml). These results are interpreted to mean that KP release increases at the time of puberty and KP frequency, rather than amplitude, changes at the time of the pubertal increase in LHRH release in primates. Second, developmental changes in GPR54 sensitivity to KP were examined by assessing the LHRH response to a KP agonist and antagonist, KP-10 and peptide 234, respectively. Preliminary data suggests that the LHRH response to neither KP-10 nor peptide 234 appeared to be different between the age groups, indicating that GPR54 sensitivity does not undergo developmental changes. Third, developmental changes in KP release were assessed before and after ovariectomy (OVX) in both age groups. Preliminary data suggests that because the mean KP level increased after OVX in pubertal, but not prepubertal monkeys, the developmental increase in KP is independent from the pubertal increase in ovarian steroid hormones. In conclusion, mean KP release and pulse frequency increases in an ovarian steroid independent manner similar to that in LHRH release at the onset of puberty, and the sensitivity of GPR54 does not appear to undergo developmental changes. Whether KP is critical for the pubertal increase in LHRH release remains to be investigated.

* Supported by HD041921

Superoxide generation and oxidative stress in the regulation of StAR expression

Jinwoo Lee and Colin Jefcoate

Generation of superoxide and oxidant stress is particularly prevalent in adrenal mitochondria. Metformin is used for the treatment of Type 2 diabetes and functions by activating AMPK (5'AMP-activated protein kinase). AMPK consists of three subunits (α, β, and Y) which regulate the balance between glucose metabolism, fatty acids metabolism and ATP utilization. AMPK is activated through phosphorylation by LKB1 and enhanced binding of AMP. Metformin (Met) enhances mitochondrial generation of superoxide, subsequent phosphorylation of LKB1 and then AMPK. Phenformin (Phen) functions similarly. A third drug AICAR is rapidly converted to a direct activator of AMPK. StAR expression in Y-1 cells is substantionally affected in two ways by these drugs. Low concentrations of Met and Phen increased the basal and cAMP induced StAR expression. Higher concentrations, on the other hand, decreased basal and cAMP induced StAR expression. AICAR, by contrast, failed to stimulate StAR expression, despite effectiveness in stimulating phosphorylation of AMPK and acetyl CoA carboxylase, an AMPK substrate. AICAR did suppress cAMP stimulation of StAR. We conclude that the steps derived from the Met/Phen induced superoxide generation rather than AMPK activation synergize with cAMP to stimulate StAR transcription. Suppression by AICAR, Met and Phen involves more extensive activation of AMPK and oxidative stress. These processes apply more generally to affects of cellular metabolism on StAR expression and the disruptive effect of xenobiotic induces oxidative stress.

Use of a GnRH-antagonist to study the relationship between gonadotropins and intrafollicular free IGF1 at the expected time of deviation in mares.

C. M. Checura 2,3,4, M. A. Beg 3, J.J. Parrish 4, O.J. Ginther 2,3,4 2Eutheria Foundation, Cross Plains, Wisconsin, 53528, USA. 3Department of Pathobiological Sciences, and 4Endocrinology and Reproductive Physiology Program, University of Wisconsin-Madison

In monovular species (women, horses, cattle), the selection of a single ovulatory follicle involves a distinct change in growth rates between the developing dominant follicle (F1) and the remaining (subordinates) follicles, known as follicle deviation. In mares, deviation begins when the most advanced follicle reaches a specific developmental stage at an approximate diameter of 22.5 mm, during the progressive decline in circulating FSH concentration and increasing LH concentrations. It has been proposed that free IGF1 is the main intrafollicular factor needed for the initiation of deviation in horses. The present experiment was conducted to test the hypothesis that the increase in free IGF1 in the follicular fluid of F1 at the expected beginning of deviation is dependent on gonadotropins. In addition, the relationships between gonadotropins and free IGF1 with other intrafollicular factors were considered. At Hour 0 (F1 $\geq$ 20 mm), mares were randomized into three groups: a) 3 ml vehicle for Acyline (a GnRH antagonist) (i.m.; control group, n = 8), b) 3 mg Acyline (i.m.; Acyline group, n = 8), or c) 3 mg Acyline (i.m.) and 250 µg rhIGF1 into F1 (Acyline/IGF1 group, n = 8). Diameter of F1, blood samples, and follicular-fluid samples were taken at Hours 24 and 48 after treatment. Averaged over hours, diameter of F1 for control group (26.5 $\pm$ 0.9 mm) was larger ($P < 0.01$) than for the Acyline group (22.4 $\pm$ 0.6 mm) and intermediate in the Acyline/IGF1 group (23.8 $\pm$ 0.5 mm). Plasma concentrations of FSH were higher ($P < 0.01$) in the controls (9.8 $\pm$ 1.3 ng/ml) than in the Acyline/IGF1 group (5.2 $\pm$ 0.3 ng/ml) and intermediate in the Acyline group (7.1 $\pm$ 0.7 ng/ml). Concentrations of LH were higher ($P < 0.01$) in the controls (2.6 $\pm$ 0.3 ng/ml) than in the Acyline (1.0 $\pm$ 0.1 ng/ml) and Acyline/IGF1 (1.2 $\pm$ 0.1 ng/ml) groups. For intrafollicular free IGF1, control (37.2 $\pm$ 7.8 ng/ml) and Acyline/IGF1 (65.0 $\pm$ 10.1 ng/ml) groups had higher ($P < 0.05$) concentrations than the Acyline group (13.2 $\pm$ 1.7 ng/ml). Concentrations of inhibin-A were higher ($P < 0.05$) in the control (724.7 $\pm$ 42.2 ng/ml) and Acyline/IGF1 (731.2 $\pm$ 48.4 ng/ml) groups than in the Acyline group (553.1 $\pm$ 54.9 ng/ml). Concentrations of estradiol were higher ($P < 0.005$) in the controls (1220.4 $\pm$ 129.0 ng/ml) than in the Acyline group (494.8 $\pm$ 86.2 ng/ml) and intermediate in the Acyline/IGF1 group (735.0 $\pm$ 101.1 ng/ml). Concentrations of progesterone were higher ($P < 0.005$) in the controls (134.7 $\pm$ 12.0 ng/ml) than in the Acyline group (71.5 $\pm$ 4.4 ng/ml) and intermediate in the Acyline/IGF1 group (93.1 $\pm$ 7.0 ng/ml). Estradiol and progesterone increased ($P > 0.01$) in controls from Hour 24 to 48, but were maintained or decreased ($P < 0.05$) in the other two groups. In conclusion, in the mare, the increase in intrafollicular IGF1 observed in F1 at deviation time was gonadotropin dependent. In the relative absence of gonadotropins, increased intrafollicular IGF1 concentrations supported follicle growth and intrafollicular inhibin-A concentration, but failed to stimulate intrafollicular estradiol and progesterone production.

Multiple affects of Cyp1b1 deficiency in mouse development.

Justin Bushkofsky*, Colin Jefcoate, Ph.D.

Recent work has demonstrated robust expression of Cyp1b1 in the neural crest of chick, quail and mouse embryos that has been linked to neural development (Chambers et al. 2007). The developmental deficiencies of Cyp1b1 inhibition can be reversed by retinoic acid and have been attributed to the observed low rate of conversion of retinaldehyde to retinoic acid by Cyp1b1. The Cyp1b1 deficient C57BL/6 mice are in most respects totally normal, suggesting that the standard route of synthesis provided by RALDHs can compensate. Although Cyp1b1 -/- mice exhibit similar eye defects to humans with functional CYP1B1 mutations who develop congentital glaucoma (Rautenstrauss et al. 2008). We have however seen other deficiencies when these mice are placed under exceptional conditions. The vascular recovery from hyperoxic damage is substantially inhibited in Cyp1b1-/- mice. The endothelial cells from Cyp1b1-/- mice are deficient in the tubular organization on Matrigel in vitro. This effect is due to oxygen-induced stress and is removed at low oxygen and with anti-oxidants but not with retinoic acid. We hypothesize that Cyp1b1 removes inhibitors of these adhesion processes that are produced by oxygenation of polyunsaturated fatty acids or other constitutive cell chemicals. Cyp1b1 is also expressed in adipocytes and exhibits adipogenic regulation. A high fat diet fails to increase fat deposition in Cyp1b1-/- C57BL/6 mice. Since this anti-obesity effect is also produced by RALDH 1 deficiency (Plutzky et al. 2007), local retinoid changes provide an explanation. However, fat pad development also depends on vascularization that may be deficient in Cyp1b1-/- mice. Additional roles for Cyp1b1 may explain this surprising developmental participation of Cyp1b1 in retinoic acid synthesis at a time when vascular development is starting.

* Supported by HD041921

The Effects of Spatial and Mechanical Interactions of Collagen I on Human Embryoid Body-Derived Trophoblast Differentiation

Maria Giakoumopoulos*, Mark Garthwaite, Thaddeus Golos

The first differentiation event in the human embryo is the formation of the trophectoderm at the blastocyst stage, committing to become the first layer of cells that will eventually form the placenta. A substantial number of pregnancies fail within the first weeks, at least in part, due to failure upon implantation of the embryo to properly form the placenta. During implantation, the interactions between the trophectoderm of the embryo and the uterus of the mother are difficult to investigate in humans because of ethical concerns regarding research with human embryos, as well as experiments with pregnant women. Our laboratory has established an in vitro model to study trophoblast differentiation (Gerami-Naini, et al., 2004). Human embryonic stem cells (hESC) will consistently differentiate to trophoblasts and secrete high levels of placental hormones when allowed to form embryoid bodies (EBs), and are transferred into a three-dimensional (3D) extracellular matrix (ECM) (Matrigel) environment. Hormone secretion was enhanced in this 3D system in comparison with planar trophoblast outgrowths in standard adherent culture. To explore the spatial and mechanical effects of 2D vs. 3D culture on trophoblast differentiation, EBs were cultured on top of the collagen I gel (2D) or were mixed with the gel (3D). The mechanical features of the gels were altered by allowing gels to either maintain adherence to the culture dish (rigid gel) or to float in the culture media (flexible gel). Culture media was assayed for hCG and progesterone secretion to determine trophoblast differentiation through 30 days of culture. A trend toward increasing hCG secretion with a peak on days 20-25 of culture was observed, however there were no statistical differences in the levels of hCG secretion between experimental groups. No statistical difference in progesterone secrection by EBs on days 10 and 25 of culture was found between experimental groups. Immunohistochemical staining revealed HLA-G and human placental lactogen positive cells within the groups indicating differentiation toward the extravillous trophoblast lineage. In conclusion, in collagen I gels, the cell-ECM contact rather than the spatial environment is important to obtain an extravillous trophoblast phenotype characteristic of advanced trophoblast differentiation. Further investigation of the cell signaling events as a result of the varying collagen I environments is under way. This EB model provides a novel platform to study early implantation failure and placental development.

* Supported by HD041921

MD Fellow Presentation

Phenotypic Expression of Natural Killer Cells: A Comparison of Normal Term Versus Preeclampsia

Chanel Tyler, MD, Christine Trautman, MD, Jennifer Belisle, Dinesh Shah, MD, Manish Patankar, PhD

Our study was designed to evaluate the role and presence of decidual natural killer (dNK) cells at term in human gestations. Our objectives were to first determine the presence and phenotypic expression of dNK cells at term using noninvasive approaches. The second objective was to assess the relationship between the dNK cells and MUC16, a mucin expressed in the decidua. The third objective was to then compare the normal phenotypic expression and MUC16 to preeclamptic dNK and peripheral NK cells at term. STUDY DESIGN: All subjects signed an informed consent, and the study was approved by the Institutional Review Boards at the University of Wisconsin-Madison and Meriter Hospital. The women were recruited at the time of admission to Labor and Delivery. Placental samples were obtained upon delivery from both full-term, uncomplicated pregnancies (n=5) and preeclamptics (n=5). Serum samples were collected as well uncomplicated pregancies (n=17) and preeclamptic (n=9). The decidua was micro-dissected and digested. The mononuclear cells were isolated using Histopaque. The phenotypic expression of the cells was characterized by multi-color flow cytometry using an LSR-II. RESULTS: NK cells are present in the deciduas at term. There are two subsets: CD56bright and CD56dim. The majority of these dNK cells are of the CD56bright phenotype. Both subsets of dNK cells were analyzed for the expression of CD158a, CD158b, CD158e, CD226, CD244, NKG2A, NKG2D, and NKp44 receptors. Both dNK subsets consistently express elevated levels of NKG2D. Expression of KIR (CD158a, CD158b, and CD158e) was variable across subsets. There was no difference between the normal and preeclamptic phenotypic receptor expression of dNK cells. Both decidual NK cells and peripheral NK cells carry MUC16 on their surface. MUC16 is present in a greater quantity on the surface of preeclamptic cells both in the decidua and serum. CONCLUSION: In previous studies, we have demonstrated that MUC16 attenuates the cytolytic capacity of peripheral blood derived NK cells. The MUC16 is highly expressed by ovarian tumors, likely as a strategy to evade NK cell mediated responses. This method of suppression likely originates from the reproductive system where MUC16 and other factors are specifically produced in the decidua to protect the growing fetus from maternal immune responses.

Poster Session Abstract

Poster # 1

Immunocytokine KS-IL2 increases natural killer (NK) cell immune synapse formation and conjugates effector and target cells via the IL-2 receptor.

Jennifer A. A. Gubbels*, Mildred Felder, Helen Holden, Zane Neal, Jackie Hank, Alexander Rakhmilevich, Paul Sondel, Manish S. Patankar, and Joseph Connor

Epithelial Cell Adhesion Molecule (EpCAM) is overexpressed by a majority of ovarian tumors. KSIL-2 is a chimera of the IL-2 molecule with the anti-EpCAm antibody, KS. The immunocytokine KS-IL2 is therefore currently being evaluated as an immunotherapeutic agent for the targeting of ovarian tumors. It is postulated that binding of KS-IL2 to the ovarian tumor cell surface will result in recruitment of natural killer (NK) and other cytotoxic cells via the Fc receptor (CD16). In addition, the chimeric IL-2 would facilitate recruitment of additional immune cells to the tumor microenvironment. Previous studies have shown that KS-IL2 enhances the cytolytic function of NK cells derived from the peritoneal fluid and peripheral blood of ovarian cancer patients. In the current study we demonstrate that KS-IL2 facilitates immune synapse formation between the ovarian cancer cell line, OVCAR-3 and NK cells derived from peripheral blood of healthy donors or ovarian cancer patients. KS-IL2 facilitated immune synapse formation is associated with polarization of LFA-1, CD2, perforin, and F-actin to the interface between NK and OVCAR-3 cells. An important factor to consider in immunotherapeutic strategies is the competence of the immune cells residing in the tumor microenvironment. NK cells residing in the peritoneal environment of ovarian cancer patients exhibit a severe downregulation of CD16. The peritoneal NK cells may therefore exhibit reduced ADCC following KS-IL2 treatment. It has previously been postulated, however, that KS-IL2 may facilitate NK cell-tumor cell conjugation via the IL-2 receptor. To address this possibility we utilized the CD16neg NK cell leukemia cell line NKL. These cells express high amounts of IL-2 receptor. Cell cytotoxicity experiments indicate that KS-IL2 significantly increases the ability of NKL cells to lyse OVCAR-3 targets. Flow cytometry based cell conjugation experiments show that NKL cells form basal level of conjugates with OVCAR-3 cells. However, in the presence of KS-IL2 a 2.4-fold increase in conjugate formation between NKL and OVCAR-3 cells is observed. This cell conjugation is inhibited by the anti-IL2 receptor antibody, Tac. Furthermore, 3 and five-fold excess IL-2 also blocks cell conjugation between NKL and OVCAR-3 cells. In conclusion, we demonstrate that KS-IL2 facilitates immune synapse formation between NK and target cells. KS-IL2 also bridges NK and target cells via IL-2 receptor. This is likely the first demonstration that the IL-2 receptor, in addition to its immune activating functions, may also serve an important role in enhancing the immunocytokine response by serving as a cell adhesion molecule.

*Supported by HD041921

Poster Session Abstract

Poster # 2

Reduced Serum Antimullerian Hormone (AMH) Levels Following Early Prenatal Androgenization in Adult Female Rhesus Monkeys Predict an Exaggerated Age-Related Loss of Ovarian Response to Recombinant Human (rh) Follicle-Stimulating Hormone (FSH) Therapy

Daniel A Dumesic, MD[1,2,3], Manish S Patankar, PhD[2,4], Beverly A Hutcherson, BS[4] and David H Abbott, PhD[1,2,4]. [1]National Primate Research Center, University of Wisconsin, Madison, WI, United States, 53715; [2]Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI, United States, 53792; [3]Reproductive Medicine and Infertility Associates, Woodbury, MN, United States, 55125 and [4]Endocrine-Reproductive Physiology Training Program, University of Wisconsin, Madison, WI, United States, 53706.

Early prenatal androgenization in adult female rhesus monkeys is followed by an exaggerated loss of ovarian response to rhFSH therapy with age, with greater amounts of rhFSH administered per mature oocyte retrieved (Dumesic DA et al. Endocrine Society, 2006, P1-388). The present study examines whether reduced AMH production, as a marker of premature ovarian aging, occurs in early-treated (E) prenatally androgenized (PA) adult female rhesus monkeys with such an exaggerated loss of ovarian response to rhFSH therapy for *in vitro* fertilization. Five E and 3 late-treated (L) PA females exposed to prenatal testosterone propionate beginning gestational days 40-44 or 100-115, respectively, and 6 normal (NL) monkeys underwent rhFSH injections with oocyte retrieval (OR) after human chorionic gonadotropin administration. Serum AMH levels were determined basally, after rhFSH therapy and at OR. Basal serum AMH levels also were measured in 11 EPA, 6 LPA and 12 NL females during early- (12-15 yrs), mid- (19-21 yrs) and late-reproductive (20-23 yrs) life and at perimenopause (22-25 yrs [menopause, 26-28 yrs]). Basal serum AMH levels were similar in all female groups until late-reproductive life, when serum AMH levels were significantly lower in EPA (0.4 ± 0.2) than LPA (1.2 ± 0.3) and NL (1.0 ± 0.2 ng/mL, $P<0.03$) females. In all late-reproductive aged females, serum AMH levels basally, after rhFSH therapy and at OR positively correlated with numbers of total oocytes (basal: $R^2=0.83$, $P<6x10^{-6}$; after rhFSH: $R^2=0.83$, $P<2x10^{-5}$; at OR: $R^2=0.78$, $P<6x10^{-5}$) and mature oocytes retrieved (basal: $R^2=0.76$, $P<4x10^{-5}$; after rhFSH: $R^2=0.80$, $P<4x10^{-5}$; at OR: $R^2=0.84$, $P<9x10^{-6}$). At perimenopause, serum AMH levels in all female groups decreased to similarly low levels. Thus reduced serum AMH levels predict premature ovarian aging in EPA adult female rhesus monkeys, suggesting that prenatal androgen excess adversely affects folliculogenesis in late reproductive life. Support: NIH U01 HD044650, P51 RR000167

Poster Session Abstract

Poster # 3

Effect of Organic Zinc, Manganese, Copper, and Cobalt On Follicular Growth, Embryo Quality, and Tissue Mineral Concentrations In Lactating Dairy Cows

K. S. Hackbart, R. M. Ferreira, A. A. Dietsche, M. T. Socha, R. D. Shaver, M. C. Wiltbank, and P. M. Fricke

Previous research has demonstrated a positive effect of organic trace minerals on dairy cow reproduction. We hypothesized that supplementing cows with organic trace minerals would improve reproduction as determined by an earlier postpartum return to cyclicity and improved embryo parameters and increase tissue concentrations of the supplemented minerals. In this experiment, cows were blocked by breed and randomly assigned at dry-off to receive either inorganic trace mineral supplementation (C; n=32) or have a portion of supplemented inorganic trace minerals replaced with an equivalent amount of organic trace minerals (T; n=31). Trace minerals were provided through C or T premixes fed at 100 g/cow/d. Premixes (C and T) were fed to dry cows (range = 40-72 d before calving) in 1.8 kg/cow/d concentrate pellets through a computer feeder and to lactating cows (range = 69-116 d after calving) in a TMR. Treatment affected endometrial tissue concentrations of Fe (C=57.0ppm, T=72.0ppm; P=0.059). Treatment did not affect (P>0.1) first-wave follicular dynamics (percentage of cows anovular at 50 d postcalving, days to first ovulation, number of ovulations, P4 peak, or length of the luteal phase), embryo parameters (percentage fertilized, percentage degenerated, percentage viable, quality, accessory sperm number, or cell number), liver trace mineral concentrations, or luteal trace mineral concentrations. It was also determined that animals with a rectal temperature equal to or greater than 102 F at the time of breeding had significantly lower percentage fertilized structures (C=36.8%, T=81.5%; P<0.001) but, of the structures that were fertilized, percentage of viable embryos, embryo quality, accessory sperm number, and embryo cell number were not affected (P>0.1). We conclude that replacing a portion of inorganic supplemented trace minerals with an equivalent amount of organic minerals did not affect postpartum ovarian dynamics, embryo quality, or liver and luteal trace mineral concentrations.

Poster Session Abstract

Poster # 4

Chronic Hypoxia Differentially Promotes VEGF- and FGF2-Stimulated Angiogenic Responses in HUVE Cells

YiZhou Jiang, MS, Kai Wang, PhD, Dong-bao Chen, PhD and Jing Zheng, PhD

During pregnancy, placental development occurs under physiological hypoxia (~2-8% O2), which is critical for placental angiogenesis. To examine mechanisms of chronic hypoxia governing placental angiogenesis, in this study, we determined if chronic hypoxia enhanced placental angiogenesis using human umbilical cord vein endothelial (HUVE) cells as a model. Methods: HUVE cells were isolated from umbilical cords (n=3) of normal term pregnant patients and cultured under hypoxia (37o C, 5% CO2, 3% O2) and normoxia (37o C, 5% CO2, 95% air). After cultured for 20-25 days, cell phenotypes were verified and cell proliferation and migration were evaluated using the BrdU ELISA kit and a BD migration system. After serum-starvation, cells derived under hypoxia & normoxia were treated with VEGF & FGF2 for 16 hr under hypoxia & normoxia, respectively. For cell proliferation, cells were labeled with BrdU for another 8 hr. Additional cells derived under HYPOXIA & NORMOXIA were pre-exposed to normoxia & hypoxia, respectively, for 24 hr, followed by treating cells in the same fashion. Results: The majority of cells exhibited positive uptake of Ac-LDL and CD31 expression. Both VEGF & FGF2 dose-dependently stimulated cell proliferation and migration under normoxia & hypoxia with a maximal dose at 10 ng/ml. Compared with chronic normoxia, chronic hypoxia further enhanced VEGF- (7.9 vs. 2.6 fold of control) & FGF2- (25.0 vs. 6.8) stimulated cell proliferation. Acute hypoxia moderately promoted FGF2-, but not VEGF-stimulated cell proliferation in normoxia-derived cells, whereas acute normoxia attenuated FGF2-, but not VEGF-stimulated cell proliferation in hypoxia-derived cells. Compared with chronic normoxia, chronic hypoxia further promoted VEGF- (6.3 vs. 3.1), but not FGF2- (1.8 vs. 1.3) stimulated cell migration. Acute hypoxia and normoxia did not alter VEGF- & FGF2-stimulated cell migration in cells derived under normoxia and hypoxia, respectively. Conclusions: Chronic hypoxia further enhances VEGF- & FGF2-stimulated cell proliferation, and VEGF-stimulated cell migration in HUVE cells. Acute changes in O2 levels differentially modulate VEGF- & FGF2-stimulated cell proliferation, whereas had no effect on migration. These data suggest that chronic and acute hypoxia differentially modulates different steps of VEGF- & FGF2-induced placental angiogenesis.

Poster Session Abstract

Poster # 5

Estradiol-17β, its Hydroxylated, and Methoxylated Metabolites Stimulate Proliferation of Uterine Artery Endothelial Cells from Pregnant Sheep

Sheikh O. Jobe1, Jill M. Koch1,3, Jayanth Ramadoss1, Yizhou Jiang1, Jing Zheng1, Ronald R Magness 1, 2, 3- 1 Perinatal Research Laboratories, Depts. of Ob/Gyn. 2 Pediatrics and 3 Animal Sciences, University of Wisconsin-Madison.

Introduction: Uterine blood flow is elevated during high estrogen states such as pregnancy. We have previously reported that endogenous estrogen results in the maintenance of vasodilatation and an increased in uterine blood flow during late gestation. However, its role in uterine angiogenesis is less well studied. Moreover, it is unknown if estradiol 17β (E2β) metabolites which are elevated during pregnancy and are synthesized by cytochrome (CYP) P450 1A1 (CYP1A1), 1A2 (CYP1A2), 1B1 (CYP1B1), and catechol-O-methyltransferase (COMT) to form 2-hydroxyestradiol (2-OHE), 4-hydroxyestradiol (4-OHE), 2-methoxestradiol (2-ME), and 4-methoxyestradiol (4-ME) respectively play a role in modulation of uterine angiogenesis and/or blood flow. In the present study, we tested the **hypothesis** that E2β, its hydroxylated as well as its methoxylated metabolites cause a greater angiogenic response in late gestation uterine artery endothelial cells (UAECs) vs. cells from nonpregnant sheep and that the respective enzymes that metabolize E2β are present in these cells. **Methods:** Validated UAECs from late pregnant (P) sheep (day 120-130; term= 147 days; n=5) and nonpregnant (NP) sheep (Luteal phase; n=3) were treated with vehicle (control, basal media) or increasing concentrations (0.1-100nM) of E2β, 2-OHE, 4-OHE and 2-ME. An In vitro index of angiogenesis was evaluated utilizing the BrdU Proliferation Assay technique following the subsequent 24 hrs of treatment. Furthermore, the presence of the CYP and COMT enzymes was evaluated by immunofluorescent staining and multi-photon confocal imaging. **Results:** E2β treatment significantly induced UAEC proliferation ($P < 0.0001$) in P more than in NP ($P=0.05$; n=3) animals. Pos-hoc tests revealed maximum response at a physiological E2β concentration of 0.1 nM (magnitude of response, 2.67 + 0.23 fold of control in pregnant vs. 1.27 ±0.13 fold of control in nonpregnant animals). Metabolite responses were also seen although these were less that E2β treatment alone. Treatment with 2-OHE, and 4-OHE resulted in equivalent significant increases in proliferation ($P < 0.0001$; maximal responses 1.79 + 0.05 fold of control) in P sheep vs. NP sheep ($P=0.05$; Maximum responses for 2-OHE and 4-OHE treatments at 1.27 ± 0.15 fold of control and 1.30 ± 0.12 fold of control respectively). In contrast, 2-ME resulted in only minor increases in UAEC proliferation levels. Post hoc tests revealed maximum response at 0.1 nM 2-ME on P animals ($P < 0.05$; maximal response at 1.30 + 0.02 fold of control) vs NP animals ($P=0.026$; maximal response at 1.07 ± 0.02). Multi photon confocal imaging revealed the presence of CYP1A2 and CYP1B1 in both cytoplasmic and nuclear domains of UAECs whilst COMT was found in only in the cytoplasmic domain of these cells. However, CYP1A1 was not detectable. **Conclusions:** These data demonstrate a potential role of E2β in concert with its metabolites in regulating uterine angiogenesis during gestation. These data also show for the first time, the presence of estrogen metabolizing enzymes in UAECs. Collectively, our findings are suggestive that E2β, its hydroxylated and methoxylated metabolites may play an important role in maternal uterine adaptation to pregnancy through participation in uterine angiogenesis. NIH HL49210, HD38843, HL87144 & HL64703

Poster Session Abstract

Poster # 6

Pregnane X receptor activation upregulates CYP 3A4 and MDR1 in murine granulosa cell line.

Arvinder K Kapur, Samet Albayrak, Sana Salih

Introduction: Many patients are cured of their initial cancer with improved cancer diagnosis and aggressive cancer therapeutics'. It is anticipated that 1:250 adults will be a survivor of childhood cancer in 2015. In addition, 4% (~50,000) of adults are under the age of 35 when diagnosed with invasive cancers in US annually. Cancer diagnosis and treatments result in gonadal failure and sterility. Currently, no drugs are available to ameliorate the effects of chemotherapy on the ovary. The nuclear pregnane X receptor (PXR; NR1I2) is an important component of the body's adaptive defense mechanism against toxic substances including foreign chemicals (xenobiotics). PXR is activated by a large number of endogenous and exogenous chemicals including steroids, antibiotics, antimycotics, bile acids, and the herbal antidepressant St. John's wort. PXR stimulate CYP3A4 and MDR1 gene expression in the mouse liver and ovary. Here we investigate the rule of PXR, MDR1, and CYP3A4 upregulation in protecting the ovary against chemotherapeutic toxicity. Methodology: KK15 murine granulosa cells were treated with SR12813 (PXR agonist). RNA and protein expression of PXR, MDR1, and CYP3A4 in KK15 murine granulosa cell lines was assessed following PXR agonist treatment (SR12813). Treated cells were exposed to chemotherapy and flow cytometry was used to assess cell viability. Results: SR 12813 agonist treatment resulted in a dose and time dependant increase in PXR, MDR1, and CYP3A4 RNA and protein expression in KK15 cell line. The MDR1 up regulation was confirmed by Luciferase assay. MDR1 over expression in KK-15 cells via up regulation of PXR and CYP3A4 might be a way to protect ovaries from xenobiotic toxicity. CONCLUSION: Upregulation of PXR, CYP3A, and MDR1 with PXR agonist treatment seems to protect granulosa cells against chemotherapy treatment in vitro. Molecular shielding of the ovaries presents a novel idea where by drug efflux transporter could protect against ovarian chemotoxicity.

Poster Session Abstract

Poster # 7

Over expression of MDR1 in KK-15 cells protects against Chemotherapeutic Toxicity.

Arvinder K Kapur, Samet Albayrak, Sana Salih

Fertility preservation in women and children has eluded scientists. The ovaries are easily accessible through transvaginal ultrasound guided techniques. This presents a unique opportunity allowing easy delivery of medicinal treatment directly to the ovaries. Thus molecular shielding of the ovary present a novel idea where by antiapoptotic, chemo resistance inducing, and angiogenic agents could be directly delivered to the ovaries to protect the ovaries against chemotherapy. We are investigating the potential of Multidrug resistance gene, which is a drug efflux transporter that naturally extrude chemotherapy out of the cells, to protect normal ovaries against chemotherapy. We established clonal PG13 packaging cells producing SF91m3 retrovirus (which carries cDNA for murine MDR1) by infection from amphotropic producer cells GP+envAM12 and subsequent sorting of single cells expressing MDR1, as defined by staining with monoclonal antibody UIC2. The KK-15were co-cultured with PG13 cells for four days, harvested, selected with colchicines and then plated. The cells were then treated with vehicle (water and DMSO), 5uM doxorubicin, 10uM Paclitaxel, 1mM Carboplatin, 5uM doxorubicin and 1mM carboplatin, 10uM Paclitaxel and 1mM carboplatin. Similarly we grew normal KK-15 cells in 12 well plates and treated them with chemotherapy just like the transduced cells. The cells were grown in the presence of chemotherapies for 72 hours. The cells were then harvested and analyzed using Flow cytometry to look for the live dead ratio. The mice were operated on to expose the ovaries, the ovaries were then injected with retrovirus carrying MDR gene. Another group of mice were operated, ovaries were exposed and injected with the media and third group of mice was used as a control. Twenty four hours later the mice were given single injection of paclitaxel 7.5mg/kg intraperitoneally. A week later mice were sacrificed and ovaries harvested to do immunohistochemistry and RNA isolation to look for message for MDR . RESULTS: The percentage of cells that were alive after chemotherapy treatment in control and transduced kk-15 cells. Control Carboplatin Doxorubicin Paclitaxel Dox/Carb Pacli/Carb kk-15 (con) 98 49 12 82 51 81 kk-15 (transduced) 95 92 61 93 79 91 The immunohistochemistry picture of ovaries from retroviral injection showed that MDR was over expressed in granulose cells and it appears that the MDR1 virus did not go to the cumulous cells, however there is a need to further validate the safety of this technique. The rtPCR for MDR in different group of mice showed that retroviral injection increased the message for MDR in the ovarian tissue. So potentially, the over expression of MDR in ovaries might protect them from chemo toxicity CONCLUSION: Transduction of KK-15 (murine granulose cells) with MDR1 seems to protect the cells against chemotherapy treatment in vitro. So potentially, the over expression of MDR in ovaries might protect them from chemo toxicity. Molecular shielding of the ovaries present a novel idea where by drug efflux transporter could protect against ovarian chemo toxicity.

Poster Session Abstract

Poster # 8

Involvement of epidermal growth factor-receptor (EGF-R) in rapid estrogen action mediated by GPR30 in primate luteinizing hormone releasing hormone (LHRH) neurons

Brian P. Kenealy, Kim L. Keen, James R. Lehman, and Ei Terasawa

Estrogen plays a pivotal role in the control of female reproductive function. Recent studies in our lab indicate that 17ß-estradiol (E2) causes a rapid, direct action in LHRH neurons in primates. This estrogen action is, in part, mediated by GPR30, as transfection with GPR30 siRNA abrogated the E2-induced changes in intracellular calcium ([Ca2+]i) oscillations (Noel et al., Mol Endocrinol 23: 349–359, 2009). However, the signal transduction mechanism of E2-induced GPR30 activation is still unclear. The present study examines the hypothesis that EGF-R plays a role in the direct rapid action of E2 in primate LHRH neurons, based on the observation in human breast cancer cells that rapid estrogen action through GPR30 involves an EGF-R mediated pathway (Filardo et al., Mol Endocrinol 16:70-84, 2002). First, the colocalization of EGF-R in LHRH neurons was investigated. Hypothalamic tissues from female rhesus monkeys were double immunostained for LHRH and EGF-R. Results indicate that LHRH neurons readily express EGF-R. Second, to determine whether EGF causes a stimulatory effect, similar to that caused by E2 in primate LHRH neurons, the effects of EGF on [Ca2+]i oscillations in cultured LHRH neurons, derived from the olfactory placode of rhesus monkey embryos, were examined. Application of EGF at 10 and 100 nM to cultures resulted in a rapid increase in the frequency of [Ca2+]i oscillations, number of stimulated cells, and the synchronization frequency in a dose responsive manner. Third, to ascertain whether EGF-R is involved in E2 action, the effects of E2 in the presence of AG1478, a selective EGFR blocker, were investigated. Results show that AG1478 at 100 nM completely abolished the E2-induced changes in [Ca2+]i oscillations, whereas AG1478 alone did not cause any significant changes. Collectively, the results suggest that EGF-R is involved in the GPR30-mediated E2 action. These data are further interpreted to mean that E2 action through GPR30 induces an increase in extracellular EGF concentration, which in turn, leads to a signal transduction mechanism in LHRH neurons. Whether E2 increases EGF concentration in our culture system remains to be determined.

Poster Session Abstract

Poster # 9

Abnormal Development of Female Gonads in Fused Toes Mutant Mice

B. Kim, Y. Kim, J.S. Jorgensen

The goal of the present study is to investigate the functional significance of Iroquois (Irx) genes in developing gonads. The Iroquois homeobox family has been shown to be critical for axis and pattern formation during the development of several organisms. Our experiment utilizes a mutant mouse model called Fused toes (Ft) which lacks 6 genes including the entire IrxB cluster, Irx3, 5 and 6, and 3 other genes, Fto, Fts, and Ftm. Homozygote mutants (Ft-/-) die early in development (E10-E12) because of severe malformation of the developing brain and loss of left-right asymmetry. Of the six genes involved in the Ft mutation, Irx3 and 5 are the only factors specifically expressed in female gonads; the others are not differentially expressed between male and female gonads. Based on these findings, we hypothesize that the IrxB cluster, especially Irx3 and Irx5, plays an important role in female gonad development. Fused toes embryos were dissected at embryonic day 12.5 and gonads were cultured in vitro for 3 days or transplanted in vivo for 2 weeks under the kidney capsule of castrated or ovariectomized recipient nude mice. The cultured gonads were assessed for morphology by histological sections and for apoptosis (Tunel) or proliferation (PCNA staining). Morphology and cell proliferation were similar among gonads of both sexes and of mutant and wild type genotypes after 3 days of in vitro explant culture. In female gonads from Ft -/- embryos, however, we observed a significant decrease in cells positive for Tunel staining compared to wild type or heterozygote samples in 3 days after culture. Embryonic gonads were subject to 2 weeks of transplantation under the kidney capsule of recipient mice to approximate development at postnatal day 5. In male gonads, wild type and mutant samples developed similarly. In contrast, we observed differences between the wild type and mutant female gonads. The wild type ovary produced primordial, primary and secondary follicles and structures consistent with maturation of a single oocyte. Ovaries derived from the Ft-/- embryo, however, did not appear to form primordial follicles and instead, maintained immature cyst-like structures. Together, these results suggest that the Ft mutation induces abnormal gonad development in the female. We are currently pursuing additional studies to address the impact of the Ft mutant on gonad development.

Poster Session Abstract

Poster # 10

Maternal Recognition of Pregnancy Programs Early Alterations in Ovine Uterine Endothelial Calcium and Nitric Oxide Regulation

Jill M. Koch[1,3], FuXian Yi[1], Gladys Lopez[1] and Ronald R. Magness[1,2,3]

Perinatal Research Labs, Departments of Ob/Gyn[1], Pedatrics[2] Animal Science[3], University Wisconsin-Madison;

Introduction: Maternal recognition of pregnancy is a critical period associated with many signals and physiologic changes required to maintain pregnancy. In ewes, a rise in uterine blood flow (UBF) is seen during maternal recognition of pregnancy. The rise and timing of the elevation in UBF has been proven critical to the survival of the embryo and without this rise in perfusion, the pregnancy is lost. The mechanism by which these early rises in UBF occur is unknown and is the aim of this research project. Previously we reported several characteristic changes in uterine artery endothelium during late ovine gestation, including rises in: 1) Nitric Oxide (NO) production 2) eNOS expression 3) level of eNOS phosphorylated at stimulatory sites in excess of total eNOS levels and 4) upon stimulation with ATP rises in the frequency of calcium bursts which are critical to maintain sustained NO production. We **hypothesized** that during Maternal Recognition of Pregnancy uterine artery endothelial cells begin to function more like late pregnant uterine artery endothelium and NO from these cells may play a role in this early conceptus-induced rise in UBF. **Methods:** Estrus was synchronized for all ewes before they were divided into a control nonpregnant group or an early pregnant group on day 14 or 16 of pregnancy. At surgery, UBF was measured, ewes were then euthanized, ovariohysterectomized and uterine flushings collected to confirm the presence of embryos. Uterine arteries were obtained, noting the exact branch generation which were used for the subsequent Fura-2 calcium imagining experiments (3rd generation) and endothelial isolation for eNOS expression studies (2nd-3rd generation). **Results**: UBF increased during early pregnancy and was greater than nonpregnant by day 16 of early pregnancy; nonpregnant control, day 14 and day 16 (16.1$\pm$2.0, 30.1$\pm$10.9, and 39.0$\pm$5.7 ml/min, respectively; $P<0.05$). In early pregnant vessels eNOS expression was elevated in day 16 compared to luteal and day 14 early pregnant (0.47$\pm$0.08, 0.24$\pm$0.04 and 0.19$\pm$0.03 OD , respectively; $P<0.05$). *Ex vivo* cellular imaging of tertiary uterine arteries, which upon stimulation with ATP allows for direct real time imaging of calcium within individual uterine artery endothelial cell, showed that the number of calcium burst per endothelial cell in the subsequent 30 min was greater ($P<0.05$) in day 16 pregnant compared to the nonpregnant controls, with day 14 pregnant vessels intermediate to day 16 pregnant and nonpregnant controls, but still significantly elevated ($P<0.05$). Previous reports from our laboratory also using ATP show that compared to nonpregnant sheep, uterine artery endothelium from late pregnant ewes also have increased numbers of calcium bursts per cell which appear to be similar to the day 16 early pregnant calcium responses we report herein. **Conclusion**: The increase in the number of ATP-associated calcium bursts and increasing eNOS expression demonstrates that even as early as maternal recognition of pregnancy the uterine vasculature is being programmed to a vasodilator producing phenotype. Programming of the uterine vasculature early in pregnancy maybe a key component to the rise in UBF associated with maternal recognition of pregnancy. Furthering our understanding of the mechanisms that lead to rise in UBF and programming of these vessels will further our understanding of early pregnancy loss at such a critical window of gestation. *NIH HL49210, HL87144, and HD38843.*

Poster Session Abstract

Poster # 11

Enhancing the Genetics of the Laboratory Rat through Assisted Reproductive Technologies

Kathy J. Krentz, James M. Amos-Landgraf and William F. Dove

We have recently enhanced our program in the genetics of colon cancer with the laboratory rat using several advances in reproductive biology. A rat model of familial human colon cancer was isolated at the McArdle Laboratory following germline ENU mutagenesis. These heterozygous carriers of a mutation in the Apc gene develop tumors primarily in the colon; thus, we have named the strain Pirc (Polyposis in the Rat Colon). The original mutant line was maintained on the Fisher 344 (F344) inbred background and was found to be serologically positive for TMEV as well as helicobacter positive. To derive the mutation on a series of genetic backgrounds, we outcrossed to other strains (Brown Norway, ACI and F344).Subsequently, we superovulated females, mated to males carrying the Pirc mutation and transferred embryos, from each line into specific pathogen free pseudopregnant recipients. In order to study genetic and genomic differences within our colon cancer rat model, it was necessary to obtain genetically modified rats from sources off the UW campus. Our collaborators at the Medical College of Wisconsin (MCW) and at the National Bioresource Center in Kyoto, Japan have produced embryos from multiple rat strains, including Long –Evans, Fawn-hooded and F344. Vitrified two-cell embryos were shipped from Kyoto and implanted into pseudopregnant females in our facility. Similarly, fresh embryos from MCW were brought from Milwaukee and implanted here. Both procedures have produced live SPF rats that are now in breeding. Recent advancements in rat sperm cryopreservation and in vitro fertilization (IVF) techniques have led us to establish these procedures in our facility. Initial experiments have shown success with IVF using fresh F344 sperm. Future experiments will address efficiency rates of IVF among different strains using both fresh and frozen sperm.

Poster Session Abstract

Poster #12

Uterine Artery Endothelial Bradykinin Receptor Levels and Their Activation of NO Production in During the Ovine Ovarian Cycle and Pregnancy

Ronald R. Magness[1,2,3], Fu-Xian Yi[1], Gladys Lopez[1] and Kimberly Vonnahme[4]

Perinatal Research Labs, Departments of Ob/Gyn[1], Peds[2] Anim Sci[3], Univ Wisconsin-Madison; Anim/ Range Sci, North Dakota State Univ Fargo ND[4]

Pregnancy increases uterine artery shear stress which is associated with higher NO production and eNOS expression by uterine artery endothelium (UAendo). This leads to increases of uterine blood flow (UBF) in order to meet the demands of the growing fetus. The Follicular (Fol) Phase of the ovarian cycle is characterized by elevations in UBF/shear stress and estrogen whereas Luteal Phase sheep have low UBF/shear stress and high progesterone. Bradykinin (BK) can be locally produced and is a very potent stimulator of endothelial cell NO production. We hypothesize that the predominate BK receptor on the endothelium, which is the BK2 receptor, has greater activation-coupling and expression levels in UAendo from Pregnant and Fol Phase sheep compared to Luteal Phase, which in part accounts for the greater stimulated NO production seen in these state of elevated perfusion. **Methods**: We recently have developed methodologies to directly monitor in real time NO production simultaneously with $[Ca^{2+}]_i$ in intact endothelium *ex vivo* on the surface of the UA. Using fluorescent microscopy to directly monitor NO production (DAF-2 DA as probe) and $[Ca^{2+}]_i$ (fura 2 as indicator) in individual endothelial cells of intact UAs we evaluated the effects of BK (1 uM; receptor mediated) and Ionomycin (2 uM; non-receptor mediated) stimulation of NO in UAs from Nonpregnant (Fol and Luteal; Days 0/periovulatory and 10-11, respectively) and Pregnant (120-130days) sheep (n= 4-6 per group). The protein levels of BK2 receptors and eNOS in endothelia isolated from Luteal and Fol Phase sheep (n=9/ group) and Late Pregnant sheep (n=6) were quantified using Western analysis. **Results**: BK2 receptor and eNOS were strongly localized by immunohistochemistry to the uterine artery endothelium. With Bradykinin and Ionomycin we observed the stimulated and sustained phase of $[Ca^{2+}]_i$ in UAendo from all three physiological states. However, direct imaging of UAendo showed that Bradykinin and Ionomycin each stimulated NO in Preg >>> Fol > Lut. Based on Western analysis, BK2 receptor levels in UAendo were similar in Luteal and Fol Phase sheep (P= 0.29). In contrast to our hypothesis, in Pregnancy the BK2 levels were unexpectedly reduced by 60% (P<0.001). Furthermore, when compared to the Luteal phase levels of eNOS run on the same Western blots, we observed the expected rise in UAendo eNOS expression in Preg >> Fol > Luteal (P<0.01). **Conclusion**: The Fol Phase and more so in Pregnancy is associated with elevations in BK and Ionomycin stimulated uterine artery NO production which is consistent with increased estrogen production, UBF, UA shear stresses, but especially elevations in UA eNOS protein expression (i.e. the capacity of the ECs to produce NO). By contrast, the BK effects during gestation were associated with an unexpected and quite substantial decrease in the BK2 receptor levels. These BK data suggest that this was due to either greater BK2 receptor activation-coupling of eNOS via a Ca2+ dependent mechanism and enhanced intracellular signaling mechanisms or the recruitment of another UAendo BK receptor subtype during gestation. *NIH HL49210, HL87144, HD50578, HD38843.*

Poster Session Abstract

Poster #13

Effects of a Novel Maternal Uterine Space Restriction Model on Fetal Kidney Development in Sheep

Katie M. Meyer1,2,3, Jill M. Koch3, Jason M. Habeck1, Sharon E. Blohowiak1, Jayanth Ramadoss3, Pamela J. Kling1, Ronald R. Magness1,2,3. Departments of Pediatrics1, Animal Scidences2, and Obstetrics/Gynecology, Perinatal Research Labs, University of Wisconsin, Madison WI.

Background: Fetal growth restriction during pregnancy has been shown to reduce fetal renal development and nephron allotment due to *in utero* nutrient restriction. Decreases in fetal kidney weight is further associated with altered renal function after delivery (e.g. hyperfiltration) and may result in adult hypertension and/or chronic renal failure as described by the Barker Hypothesis. Herein, a novel model of uterine space restriction was developed that may facilitate understanding of renal development. **Purpose:** Evaluate the effects of reduced uterine space on fetal growth and renal development in late ovine gestation. **Methods**: Multiparous ewes were studied as: Control singletons, twins, and triplets and Unilateral singletons, twins, and triplets. The unilateral surgery consisted of a single uterine horn ligated and completely disconnected via removal of all intercorneal connections at least 2 months prior to pregnancy. These 6 groups were combined into 2 based on placental attachment sites per fetus. The non-uterine space restricted group (non-USR) consists of placentome #/fetus of $\geq 53.2\pm2.4$ (Control singletons, Unilateral singletons, and Control twins) while the uterine space restricted group (USR) consists of placentome #/fetus of $\leq 27.6\pm1.7$ (Unilateral twins, Control triplets, and Unilateral triplets). These treatments were decided based upon a clear demarcation in the number of placentomes/fetus in each group. For this study, fetuses were evaluated at either 120 or 130 days of gestation (term 147). Fetal weight (wt) and kidney wt were measured. Kidney tissues were fixed, stained for collagen by Gomori Trichrome, and histology examined microscopically for glomerular generation number (as measured by branching patterns). Fetal umbilical arterial blood was analyzed for plasma creatinine. **Results**: In the USR group at 120 days gestation, fetal wts were not appreciably altered. At 130 days gestation, fetal wts were 18% lighter (3324 ± 152g) compared to the non-USR group (4073 ± 297;$P<0.03$). At 120 days gestation, kidney wts were 13% lighter ($P<0.03$) in the USR group (9.7 ± 0.5g) compared to the non-USR group (11.6 ± 0.4g). At 130 days gestation, kidney wts were 24% lighter ($P<0.01$) in the USR group (9.7 ± 0.2g) compared to the non-USR group (12.8 ± 0.4). The USR group had 12% less glomerular generations (13.3 ± 0.4) compared to the non-USR group (15.0 ± 0.4; $P<0.01$) at 120 days, while at 130 days, the USR group had 14% less glomerular generations (13.4 ± 0.2) compared to the non-USR group (15.5 ± 0.2; $P<0.01$). Fetal plasma creatinine levels were not appreciably altered at 120 days gestation, while at 130 days gestation, creatinine levels were elevated by 44% ($p<0.01$) in the USR group (2.2 ± 0.2 mg/dl) compared to the non-USR group (1.5 ± 0.1mg/dl). **Conclusion**: Ovine kidneys complete growth and development between 120 and 130 days gestation. IUGR became evident at 130 days gestation. The decreased, kidney wt, glomerular generations, and increased plasma creatinine in the USR group support the concept that IUGR may impair renal development. Space restriction did not alter calculated placental efficiency, as based on fetal wt/individual placental wt at either time point, but the ratio was greater at the later gestation. Further research needs to be done to examine the dynamic adaptation of the ovine placenta during rapid fetal growth. Additional studies should examine the role of the fetal kidney and the placenta on creatinine levels.

Poster Session Abstract

Poster # 14

Studies on the induction of Ptf1a in murine embryonic stem cells

Gopika Nair and Jon Odorico

Pancreatic development in mammals is controlled, in part, by the temporal expression of numerous genes encoding transcription factors. Yet, how these factors regulate each other and their target genes is not completely understood. Pancreas specific transcription factor 1a (ptf1a) is known be to be involved in pancreas specification in the foregut endoderm and transactivation of PDX1 promoter. A tetracycline-inducible murine ES cell line with temporally-controlled induction of ptf1a was established and it serves as a new tool to interrogate the role of PTF1A in directing differentiation of ES cells down the pancreatic lineage. Induction of ptf1a in plated Embryoid Bodies (EBs) resulted in extensive expression of PDX1 in EB derived cells. In particular, the EBs gave rise to PTF1A+PDX1+ bud-like structures reminiscent of in vivo pancreatic organogenesis. Additionally, cells of the differentiating PDX1+ epithelium expressed other pancreatic markers like CPA1 and NGN3, and became progressively post-mitotic with time. The expression of amylase and islet endocrine hormones at later time points indicated that these cells were capable of further differentiation into mature pancreatic cell types. This is the first study to show branching-like morphogenesis, with both acinar and islet formation, similar to in vivo pancreatic development in an ES derived culture.

Poster Session Abstract

Poster # 15

Islet Morphology in a Monkey Model with Diminished Glucoregulation.

Lindsey E. Nicol[1], Timothy D. O'Brien[2], Joshua Parker[2], David H. Abbott[3,4]

[1]Division of Pediatric Endocrinology, University of Wisconsin Hospital and Clinics; [2]Veterinary Population Medicine Department, University of Minnesota; [3]National Primate Research Center, Madison, WI; [4]Department of Obstetrics and Gynecology, University of Wisconsin School of Medicine and Public Health

Adult, prenatally androgenized (PA) female rhesus monkeys exhibit polycystic ovary syndrome (PCOS)-like phenotypes, as well as insulin resistance and impaired insulin response to glucose also associated with this metabolic disorder. Islet amyloid polypeptide (IAPP) and insulin are both normal products of the pancreatic beta cell, but IAPP deposition as amyloid has been linked to beta cell death in the pathogenesis of type 2 diabetes. Our aim was to determine whether the functional changes in glucose metabolism seen in PA female monkeys were associated with altered islet morphometry and/or differences in IAPP and insulin expression. Pancreatic tissue from adult female PA rhesus monkeys (n=6) exposed to androgen excess when their pregnant dams received daily subcutaneous injections of 10 mg testosterone propionate for 25-35 days starting on 40-44 days of gestation (term: 165 days), and from adult and age and BMI similar controls (C; n=7) were sectioned and stained with antibodies specific for IAPP or insulin. The tissue was examined histologically via Image Pro software to quantify immunostaining and calculate islet count, size, and area fraction. Islet count per mm^2 (IAPP: C 1.98±0.49 and PA 2.69±0.53; insulin: C 0.42±0.10 and PA 0.63±0.10 x 10^{-5}; mean±SEM) and islet fractional area (IAPP: C 1.67[0.75, 1.92] and PA 2.46[1.30, 5.97]; insulin: 0.18[0.14, 0.28] x 10^{-2}; median [25^{th}, 75^{th}%ile]) were similar to their respective controls. Total islet area per islet count, reflecting average islet size, with IAPP stained sections was increased (p=0.022) in PA (10.80[8.17, 16.00] x 10^2 mm^2) compared to C (6.09[4.91, 706] x 10^2 mm^2) females. A similar difference was not observed with insulin staining (C 5.23 [4.23, 6.58] and PA 5.73[4.79, 8.55] x 10^2 mm^2). Islet size distribution revealed further differences between female groups (small: <500, intermediate: 501-1000, large: > 1000, and very large: > 8,000 mm^2). While there were no differences in islet size distribution found for IAPP stained sections, size distribution analysis from insulin stained sections showed PA females with fewer (p=0.039) intermediate islets and increased (p=0.043) numbers of very large islets (C: 451.7±60.2; PA: 244.2±65.0 x 10^6). These results suggest that PA female monkeys with PCOS-like phenotypes, and disruption in glucose metabolism similar to that found in the progression toward type 2 diabetes, have a propensity to develop larger islets and greater expression of IAPP.

Poster Session Abstract

Poster # 16

Effects of Steroid Hormones Associated with Concentrated Animals Feeding Operations (CAFOs) on Embryonic Development in Fathead Minnows and Zebrafish

Bunlung Nuangsaeng, Xuejiao Tian and Terence P. Barry

Effects of Steroid Hormones Associated with Concentrated Animals Feeding Operations (CAFOs) on Embryonic Development in Fathead Minnows and Zebrafish Bunlung Nuangsaeng, Xuejiao Tian and Terence P. Barry Laboratory of Fish Endocrinology and Aquaculture, Department of Animal Science University of Wisconsin-Madison, WI Abstract As part of a larger project to determine the presence, persistence and biological effects of natural and synthetic hormones released into the environment from cattle, dairy, swine, and poultry CAFOs, we investigated the effects of testosterone (T), estradiol-17β (E2), progesterone (P4), 17α,20β-dihydroxyprogesterone (DHP), cortisol, 4-androstene-3,17-dione (A4), trenbolone acetate (synthetic androgen), and melengestrol acetate (synthetic progestin) on the early embryonic development of two fish species, the fathead minnow and zebrafish. Newly fertilized eggs were cultured individually in 96-well culture plates, and exposed to graded doses of the target steroids (5 to 5000 ng/ml). The developing embryos are observed once daily until hatching (3-6 days). Various developmental endpoints were measured or scored, including length, heart rate, degree of pericardial edema, degree of pigmentation, hatching time, and survival. Both species showed dose-dependent responses to every steroid tested, and there were significant species and steroid differences in responsiveness depending on the endpoint. The steroids E2, T and cortisol, for example, induced the highest mortality rates in both species. Unexpected observations included early hatching induced by E2 in both fathead minnow and zebrafish, and a delay in the onset of pigmentation induced by P4 in zebrafish. In general, fathead minnow embryos were much more sensitive to steroid exposure than zebrafish embryos for all endpoints evaluated suggesting that this species may be a superior sentinel species for assessing endocrine disruption. The relatively high throughput and sensitive fish embryonic development assay used in this study will be useful in efforts to rapidly screen potential endocrine disruptors in the environment, and aid in efforts to optimize management practices that mitigate environmental problems associated with hormones discharged from CAFOs. The experiments also lay the groundwork for future mechanistic studies designed to elucidate the roles of steroids in regulating various aspects of fish embryonic development. Funded by EPA STAR R833421

Poster Session Abstract

Poster # 17

The Partitioning and Domain-specific Multi-site Phosphorylation "Zip Codes" of Endothelial Nitric Oxide Synthase in Uterine Artery Endothelial Cells

Jayanth Ramadoss[1], Wu-xiang Liao[4], Dong-bao Chen[4], Manish S. Patankar[1], Ronald R. Magness[1, 2, 3]

[1]Department of Obstetrics & Gynecology Perinatal Research Labs, [2]Animal Sciences, [3]Pediatrics, University of Wisconsin-Madison 53715, [4]Department of Obstetrics & Gynecology, University of California Irvine, United States

Introduction: Pregnancy-associated rises in uterine blood flow occurs via changes in endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) production at the level of uterine arteries. In arterial endothelial cells, the eNOS protein has been associated with lipid-ordered microdomains called caveolae and is tonically inhibited by the major caveolar scaffolding protein caveolin-1 (cav-1). However, the spatial and temporal dynamics of eNOS, including domain-specific post-translational modification via multi-site phosphorylation that are critical for enzyme activation and NO production, in response to a physiologic calcium mobilizing agonist like ATP, especially during pregnancy are unknown. **Objective:** The aim of this study was to determine the spatial and temporal dynamics of eNOS in response to the calcium mobilizing physiologic agonist ATP in endothelial cells from the uterine arteries of pregnant ewes when the rises in UBF and NO are greatest. **Methods:** Primary uterine arteries of late gestation ewes were dissected and the endothelial cells were isolated and validated. Temporal and spatial dynamics of eNOS, and domain-specific multi-site phosphorylation were studied by density centrifugation, confocal microscopy, immunoisolation, native gel electrophoresis and Western blotting. **Results:** Under unstimulated conditions, eNOS was predominantly located in specialized caveolar microdomains. Treatment with ATP resulted in time-dependent re-partitioning of eNOS between the caveolar and non-caveolar domains. The abundance of cav-1 bound eNOS significantly decreased in response to ATP (one way ANOVA, $P = 0.039$); compared to the unstimulated state, the magnitude of cav-1 bound eNOS was significantly lower at 2.5 ($P = 0.002$), 5 ($P = 0.02$) and 10 ($P = 0.02$) minutes after ATP treatment. eNOS exhibited a domain-specific multi-site phosphorylation "zip code". In unstimulated cells, $^{P\text{-}Thr497}$eNOS but not $^{P\text{-}Ser635}$eNOS or $^{P\text{-}Ser1179}$eNOS was detected in the caveolar domain. In contrast, $^{P\text{-}Ser114}$eNOS was detected only in the non-caveolar pool. Upon ATP stimulation, $^{P\text{-}Ser1179}$eNOS was mainly detectable only in the caveolar domain whereas $^{P\text{-}Ser635}$eNOS was detectable in both the caveolar and the non-caveolar pools. Moreover with ATP stimulation, $^{P\text{-}Thr497}$eNOS was barely detectable in the caveolar domain and $^{P\text{-}Ser114}$eNOS was not detectable in any of the fractions. These observations were confirmed by non-denaturing gel electrophoresis where ATP receptor activation increased cav-1 free-dimerized eNOS by 54% ($F_{1,5} = 15.8$; $P = 0.011$). Further, ATP resulted in a significant increase in the magnitude of ^{P14}cav-1. Confocal microscopy utilizing double immunofluorescence staining showed the temporal and spatial partition of post-translationally modified eNOS and total eNOS between the caveolar and cytoplasmic domains to be consistent with immunoblots. **Conclusion:** This is the first study to systematically investigate the temporal and spatial partition of eNOS in uterine artery endothelial cells. We show the novel observation that multi-site phosphorylation state of eNOS acts as a "zip code" for the intracellular location of the enzyme, and gives direct clues to the state of the enzyme activity. These data suggest that the regulatory mechanisms pertaining temporal and spatial dynamics of eNOS may delineate a greater understanding of the etiologies of gestational diseases. *NIH HL49210, HD38843, HL87144, HL86939, HL70562 and HL74947.*

Poster Session Abstract

Poster # 18

The Effects of Leukocytes on Early Implantation in the Rhesus Monkey

A. Rozner, K. Vielhuber, R. Durning, S. Dambaeva, J. Drenzek, and T. Golos

At the time of implantation and throughout pregnancy, the primate uterus contains numerous leukocytes, consisting primarily of natural killer cells and macrophages. These cells are thought to play an important role in the maternal fetal immune response. The cytokine balance at the maternal fetal interface and the expression of non-classical MHC class I molecules (HLA-G in humans and Mamu-AG in the rhesus monkey) on the trophoblast cells of the growing embryo are thought to be important immune modulators that allow the semi-allogenic embryo to implant and optimize the environment for placental and fetal growth. However, study of the maternal fetal interface in human implantation is not feasible. We have established a co-culture system with rhesus monkey embryos to model implantation. Rhesus blastocysts stage embryos were derived by in vitro fertilization of oocytes. Embryos were cultured to theperi-implantation blastocyst stage, then were co-cultured with peripheral blood cells (NK cells, monocytes, or both NK cells and monocytes) obtained from the oocyte donor. Culture media were collected to determine cytokine and chorionic gonadotropin secretion and embryo growth was monitored. TNF and IL-6 were consistently detectable in the media of leukocyte co-cultured embryos, while INFgamma, IL-5, IL-4, and IL-2 secretion were below the limits of detection. Trophoblast outgrowths expanding from the embryo were noticeable after 6 days of co-culture in controls and with embryos co-cultured with NK cells. Embryos co-cultured with monocytes did not show signs of growth within the two weeks of co-culture, however embryos co-cultured with both monocytes and NK cells grew at similar rates to controls. Based on these results we conclude that the monocyte/NK cell balance and/or interaction is important and that using this rhesus model of implantation may lead to increased knowledge of the role of leukocytes during early implantation.

Poster Session Abstract

Poster # 19

Suppression of Protein Phosphatase 2 Does Not Affect VEGF- and FGF2-Stimulated Ovine Fetoplacental Artery Endothelial Cell Proliferation

Yang Song, Kai Wang, Dong-bao Chen, Ronald R. Magness, Jing Zheng

Vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) elicit cellular responses via activation of protein kinases and phosphatases. We have reported that the mitogen-activated protein kinase kinase 1/2 (MAP2K1/2)/mitogen-activated protein kinase 3/1 (MAPK3/1) and phosphoinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog 1 (AKT1) pathways are critical for VEGF- and FGF2-stimulated ovine fetoplacental endothelial (OFPAE) cell proliferation. Recently, we have further shown that protein phosphatase 3 (PPP3) differentially modulates VEGF- and FGF2-stimulated cell proliferation and activation of MAPK3/1 and AKT1 in OFPAE cells. Herein, we investigated if protein phosphatase 2 (PPP2) participated in VEGF- and FGF2-stimulated OFPAE cell proliferation via modulating activation of MAPK3/1 and AKT1. Small interfering RNA (siRNA) specifically targeting human PPP2 catalytic subunit α (PPP2CA) was applied to suppress PPP2CA expression in OFPAE cells. As compared with the scrambled siRNA, the PPP2CA siRNA decreased ($p < 0.05$) PPP2CA protein levels (~ 70%) and activity (~ 50%) without altering protein levels of PPP3 catalytic subunit α (PPP3CA), nitric oxide (NO) synthase 3 (NOS3), total MAPK3/1 and AKT1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Suppression of PPP2CA enhanced ($p < 0.05$) VEGF-induced AKT1, but not MAPK3/1 phosphorylation, whereas inhibited ($p < 0.05$) FGF2-induced MAPK3/1 and slightly attenuated FGF2-induced AKT1 phosphorylation. Suppression of PPP2CA did not significantly affect VEGF- and FGF2-stimulated OFPAE cell proliferation. Thus, suppression of PPP2CA alone did not alter VEGF- and FGF2-stimulated OFPAE cell proliferation, even though this suppression differentially modulated VEGF- and FGF2-induced MAPK3/1 and AKT1 activation. These data also suggest that signaling molecules other than MAPK3/1 and AKT1 could emerge as important mediators for VEGF- and FGF2-stimulated cell proliferation after PPP2CA suppression in OFPAE cells.

ERP Program Faculty

Name	Department	Email Address
Abbott, David Professor	Primate Center; OB/Gyn	abbott@primate.wisc.edu
Alarid, Elaine Associate Professor	Oncology	alarid@oncology.wisc.edu
Barry, Terence Senior Scientist	Animal Sciences; Aquaculture Program	tpbarry@wisc.edu
Bertics, Paul Professor	Biomolecular Chemistry	pbertics@wisc.edu
Bird, Ian (Program Director) Professor	OB/Gyn	imbird@wisc.edu
Bosu, William Professor	Medical Sciences	bosuw@svm.vetmed.wisc.edu
Downs, Karen Professor	Anatomy	kdowns@wisc.edu
Drezner, Marc Professor	Medicine	mkd@wisc.edu
Duello, Theresa Associate Professor	OB/Gyn	tmduello@wisc.edu
Cezar, Gabriela Assistant Professor	Animal Sciences	ggcezar@wisc.edu
Ginther, Oliver Professor	Pathobioloigical Sciences	ginther@svm.vetmed.wisc.edu
Golos, Thaddeus Professor	Comparative Biosciences; Primate Center	golos@primate.wisc.edu
Jefcoate, Colin Professor	Pharmacology	jefcoate@wisc.edu
Jorgensen, Jaon Assistant Professor	Comparative Biosciences	jsjorgensen@wisc.edu
Khatib, Hasan Associate Professor	Dairy Sciences	hkhatib@wisc.edu
Kessel, Julie Assistant Professor	Pediatrics	jmkessel@pediatrics.wisc.edu
Kling, Pamela Associate Professor	Pediatrics	pkling@wisc.edu
Kreeger, Pamela Assistant Professor	Biomedical Engineering	kreeger@wisc.edu

ERP Program Faculty

Name	Department	Email Address
Magness, Ronald Professor	OB/Gyn	rmagness@wisc.edu
Martin, Thomas Professor	Chemistry	tfmartin@wisc.edu
Ntambi, James Professor	Biochemistry; Nutritional Sciences	ntambi@biochem.wisc.edu
Odorico, Jon Associate Professor	Surgery	jon@surgery.wisc.edu
Parrish, John Professor	Animal Sciences	parrish@ansci.wisc.edu
Patankar, Manish Assistant Professor	OB/Gyn	patankar@wisc.edu
Peterson, Richard Professor	Pharmacy	repeterson@pharmacy.wisc.edu
Schuler, Linda Professor	Comparative Biosciences	schulerl@svm.vetmed.wisc.edu
Shah, Dinesh Professor	OB/Gyn	dmshah@wisc.edu
Shalev, Anath Associate Professor	Medicine	as7@medicine.wisc.edu
Sheffield, Lewis Professor	Dairy Science	lgsheffi@wisc.edu
Terasawa, Ei Professor	Pediatrics	terasawa@primate.wisc.edu
Thomson, James Professor	Anatomy; Primate Center	thomson@primate.wisc.edu
Watters, Jyoti Assistant Professor	Comparative Biosciences	jjwatters@wisc.edu
Wiltbank, Milo Professor	Dairy Sciences	wiltbank@wisc.edu
Xu, Wei Assistant Professor	Oncology	wxu@oncology.wisc.edu
Yu, Ziming Assistant Professor	OB/Gyn	zyu1@wisc.edu
Zheng, Jing Associate Professor	OB/Gyn	jzheng@wisc.edu

ERP Program Students

Student	Faculty Advisor
Jennifer Arens Gubbels	Dr. Manish Patankar
Derek Boeldt	Dr. Ian Bird
Justin Bushkofsky	Dr. Colin Jefcoate
Celina Checura	Dr. John Parrish
Luca Clemente	Dr. Paul Bertics
Marjorie Curet	Dr. Jyoti Watters
Lefric Enwall	Dr. John Parrish
Maria Giakoumopoulos	Dr. Ted Golos
Kathryn Guerriero	Dr. Ei Terasawa
Katherine Hackbart	Dr. Milo Wiltbank
Kentaro Hayashi	Dr. Craig Atwood
Beverly Hutcherson	Dr. David Abbott
Dr. Igor Iruretagoyena	Dr. Dinesh Shah
Yizhou Jiang	Dr. Jing Zheng
Omar Jobe	Dr. Ron Magness
Brian Kenealy	Dr. Ei Terasawa
Dr. Heather Kerrick	Dr. Ian Bird
Carly Kibbe	Dr. Anath Shalev

ERP Program Students

Student	Faculty Advisor
Jinwoo Lee	Dr. Colin Jefcoate
Samantha Lewis	Dr. Joan Jorgensen
Katie Meyer	Dr. Pam Kling
Gopika Nair	Dr. Jon Odorico
Ann Rozner	Dr. Ted Golos
Soraya Silva	Dr. Lewis Sheffield
Yang Song	Dr. Jing Zheng
Dr. Chanel Tyler	Dr. Manish Patankar

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Past Trainees

- Dr. Jacqueline Cale
- Dr. Behazd Gerami-Naini
- Dr. Nichole Korpi-Steiner
- Dr. J. Christina Pattison
- Dr. Amy Reeder
- Dr. Sekoni Noel
- Dr. Jessica Drenzek

Students In Training

- Jennifer Arens Gubbels
- Derek Boeldt
- Justin Bushkofsky
- Kathryn Guerriero
- Maria Giakoumopoulos

Notes