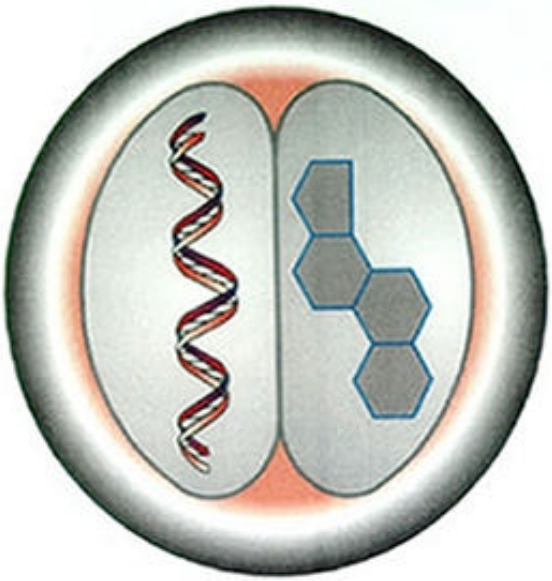
Endocrinology-Reproductive Physiology



2001 Annual Symposium

September 13, 2001

Memorial Union

ENDOCRINOLOGY-REPRODUCTIVE PHYSIOLOGY PROGRAM
University of Wisconsin-Madison
260 Animal Sciences
1675 Observatory Dr.
Madison, WI 53706
http://www.erp.wisc.edu

Dear Symposium Participant:

On behalf of the graduate students of the Endocrinology-Reproductive Physiology (ERP) Program, we welcome you to the 2001 Symposium. We are pleased to welcome Dr. Jon E. Levine from Northwestern University as this year's keynote speaker. Today's event will also feature Mr. Bryan Renk from the Wisconsin Alumni Research Foundation in addition to a panel discussion by ERP Program Faculty.

The Endocrinology-Reproductive Physiology Program at the University of Wisconsin is a multidisciplinary degree-granting program designed to encourage research in endocrinology and reproductive biology, to provide basic training and experience for pre- and post-doctoral students interested in these fields, and to provide training in problems of reproduction in animals and humans. The Program trains Ph.D. candidates for teaching and research careers in the broad field of endocrinology and reproductive physiology--basic, clinical and technical.

The Endocrinology-Reproductive Physiology Program has 24 participating faculty from 12 departments. The multidisciplinary research and the diverse interests of the faculty make possible many approaches to the study of reproduction, providing the individual student with a wide selection for his/her research training experience.

We hope that you take the opportunity to attend today's presentations by our guest speakers, students and faculty. Posters will available for viewing throughout the day in the Great Hall.

The Symposium Committee would also like to thank Beckman-Coulter, Fryer Company, Inc., the University Lectures Committee and the Department of Animal Sciences for their financial contributions for today's event. Also, we thank Dr. Ian Bird, the ERP Program Director for his continued support of the symposium as well as Dr. John Parrish the faculty advisor.

We hope you find today's symposium helpful in your quest for knowledge and in your research activities.

Jacqueline Cale, Committee Co-Chair Kinarm Ko, Committee Co-Chair
James Haughian Gabriela Cezar Xavier Donadeu Yuh-Lin Wu

Endocrinology-Reproductive Physiology Program
Annual Symposium

Schedule of Events for September 13, 2001

8:00 A.M.	Registration, Poster Set-up, Great Hall, Memorial Union, Coffee and Doughnuts provided
9:30 A.M.	Introduction of ERP Program and Symposium by Dr. Ian Bird, Program Director, Class of '24 Reception Room
10:00 A.M.	Invited Speaker Bryan Renk, WARF "Wisconsin Alumni Research Foundation and Intellectual Property "
11:00 A.M.	Scientific Presentations by Graduate Students and Staff 11:00—Shannon Koehler, Dr. Bird's Lab 11:15—Adam King, Dr. Magness's Lab 11:30—Dr. John Parrish 11:45—Dr. Ronald Magness
12:00 P.M.	Lunch, Great Hall, Memorial Union
1:00 P.M.	Keynote Speaker, Dr. Jon E. Levine, Northwestern University. "You've Got Male: Understanding the Neuroendocrinology of Male Reproductive Development and Behavior." Class of '24 Reception Room.
2:00 P.M.	Poster Break, Great Hall
3:00 P.M.	Panel Discussion: Dr. Ronald Magness, OB-GYN; Dr. Ei Terasawa, Pediatrics; Dr. Milo Wiltbank, Dairy Science "NIH and USDA: Insights into Funding Differences between Granting Agencies."
4:30 P.M.	Closing Remarks and Adjournment to the Union Terrace (weather permitting)

Keynote Speaker: Dr. Jon E. Levine, Northwestern University

Jon Levine is a Professor in the Department of Neurobiology and Physiology at Northwestern University in Evanston, IL. He is a neuroscientist and reproductive biologist whose special research interests are in the area of neuroendocrinology - particularly the mechanisms through which hormones affect brain function. Dr. Levine attained his B.A. in Biology at Oberlin College in 1976 and his Ph.D. in Neuroscience at the University of Illinois in 1982. After completing a postdoctoral fellowship at the Oregon Regional Primate Research Center, Levine joined the faculty at Northwestern in 1984 and became an Associate Professor in 1990, and a full Professor in 1995. Dr. Levine has been the Director of a NIH-supported Training Program in Reproductive Biology at Northwestern University for the past nine years. He has also been served as Director of Northwestern's Undergraduate Program in Biological Sciences since 1999.

Dr. Levine's research has led to a greater understanding of the basic mechanisms by which brain cells function to control reproductive hormone secretions, and how reproductive hormones, in turn, act within the brain to regulate neuronal activity. His work has shed much light on the processes of puberty, ovulatory cyclicity, and seasonal reproduction in animals. Levine's research has also contributed significantly to our understanding of certain types of infertility, as well as the actions of contraceptive steroids. He has served on advisory panels at the NICHD, and has been a member of the editorial boards of *Endocrinology*, *Neuroendocrinology*, *Endocrine*, and the review journal *Frontiers in Neuroendocrinology*. He is currently an Associate Editor for *Endocrinology*, and has been named as the new Editor-in-Chief of *Frontiers in Neuroendocrinology*.

YOU'VE GOT MALE: UNDERSTANDING THE NEUROENDOCRINOLOGY OFMALE REPRODUCTIVE DEVELOPMENT AND BEHAVIOR.

Sex hormone secretions and many types of behaviors are sexually dimorphic in most, if not all mammalian species. In adult male rodents, for example, gonadotropin-releasing hormone (GnRH), gonadotropins, and testosterone are produced in a "tonic" mode; in adult female rodents, by contrast, secretions of GnRH, gonadotropins, and sex steroids occur in cyclic patterns that sustain cyclic ovarian function. Similarly in rodents, male reproductive behaviors, e.g. mounting and intromission, and female reproductive behaviors such as the lordosis posture, are rarely displayed by the opposite sex. Species- and context-specific sex differences in aggression and parental behaviors have likewise been demonstrated in many animal species. How do these sex differences in hormone secretions and behaviors arise? It is generally held that neural circuitries governing GnRH secretion, as well as those regulating the display of many sexually differentiated behaviors, are "programmed" as such during fetal and/or neonatal development. Fetal androgen secretions have been implicated as the primary "organizer" of neural circuitries in the preoptic area and elsewhere that mediate male-type behaviors in adulthood; these same neural substrates are subsequently "activated" by androgens in adulthood to control hormone secretions and masculine behavior. In this presentation, I will describe and discuss new findings on the mechanisms by which the organizational actions of steroids may confer male-specific GnRH pulse generator activity, reproductive behavior, and non-reproductive behaviors. A new hypothesis will be advanced, in which it will be proposed that the progesterone receptor – long overlooked as a potentially important feature of the male hypothalamus – may in fact play a critical role in the masculinization of neuroendocrine function.

Invited Guest Speaker: Mr. Bryan Renk, Wisconsin Alumni Research Foundation

Bryan Renk is currently the Director of Patents and Licensing at the Wisconsin Alumni Research Foundation (WARF) n Madison, Wisconsin. He joined the Foundation in 1995 as a Licensing Manager specializing in agriculture and biotechnology in addition to covering a broad range of intellectual property including medical and pharmaceutical technologies.

Bryan is also a member of Broad of Directors for WiSys and serves as its Vice President. WiSys is a wholly owned subsidiary of WARF which handles the technology transfer duties for all of the UW System campuses outside of UW-Madison.

WARF is the designated manager of intellectual property of the University of Wisconsin-Madison and manages a comprehensive intellectual property program including receipt of new invention disclosures, patent prosecution, marketing and licensing of patented technology and development of relationships with commercialization partners. Currently, WARF has in excess of 3500 intellectual property cases under management coupled with over 300 active license agreements. This includes a start-up company portfolio numbering twenty-three (23) companies.

Bryan is a graduate of the University of Wisconsin-Madison where he earned his Bachelor of Science and a Master of Science in Meat and Animal Science and Muscle Biology/Business.

Bryan is also currently active in the Association of University Technology Managers (AUTM), the Licensing Executive Society (LES) and Wisconsin Biotechnology Association.

Wisconsin Alumni Research Foundation and Intellectual Property

A presentation on the Wisconsin Alumni Research Foundation (WARF) will be given. It will cover WARF's formation, mission, current makeup, and licensing activities. Hopefully, it will provide a good overall idea of WARF and it services to the U.W. Madison campus in regards to Intellectual Property and how it is handled.

Abstracts for Oral Presentations

Effects of Cell Culture on Agonist Stimulated ERK-1/2 and Ca^{2+} Signaling in UAEC.

Shannon M Gifford 1*, Tao Di 1*, Jeremy A Sullivan 1*, Ronald R Magness 1 and Ian M Bird 1.

We have previously described that ovine uterine artery endothelial cells maintained in culture to passage 4 retain pregnancy-specific increases in vasodilator production. In addition, these studies revealed that both ERK-1/2 phosphorylation and Ca2+ are necessary for the activation of eNOS. Furthermore, it was shown that AII, ATP, and the growth factors bFGF, EGF and VEGF can all couple to ERK-1/2 phosphorylation in cells from pregnant ewes (P-UAEC), while all agonists except EGF show much reduced/insignificant coupling to ERK-1/2 in cells from non-pregnant ewes (NP-UAEC). However, only ATP can stimulate increases in intracellular Ca2+ in P-UAEC and NP-UAEC. One question that remains is whether the responses seen at passage 4 accurately reflect the state of the cells at the time of isolation. An obvious limitation of the study of acutely isolated cells is the limited number available. Yet, small groups of eNOS positive cells from both pregnant ewes and non-pregnant luteal phase ewes could be isolated using the same isolation technique used for cell culture preparation. These groups of cells were used to image changes in the intracellular Ca2+ concentration using Fura 2 as well for immunocytochemistry to detect ERK-1/2 phosphorylation. Acutely isolated cells loaded with Fura 2 and stimulated with each agonist (AII 100 nM, ATP 100uM, EGF 10 ng/ml, bFGF 10 ng/ml and VEGF 10 ng/ml) showed no Ca2+ elevation except in response to ATP, which is in full agreement with passage 4 P-UAEC and NP-UAEC data. This response to ATP was dose-dependent (1-100 M) and entirely due to the release of an intracellular pool. In addition, it was of interest to note that phospho-ERK was detected by immunocytochemistry in the cytosol of ex vivo cells from pregnant and non-pregnant animals. This response was significantly higher for pregnant freshly isolated cells then non-pregnant freshly isolated cells when stimulated with ATP or bFGF. This data is also in agreement with passage 4 data. In addition, in each case staining could be totally blocked by the MEK inhibitor UO126 (20 M). Our data suggests the findings in cultured cells are largely in agreement with those from freshly isolated cells, and that pregnancy reflects a time of increased coupling of ERK signaling in vivo. Supported by USDA 002159, HL 64601 and HL49210.

Estrogen Increases Vascular Endothelial Growth Factor in Uterine Artery Endothelial Cells from Pregnant Sheep

King, AG[1], Zhang, J[1], Bird, IM[1,2], Magness, RR[1, 2] Perinatal Research Labs, Departments of Obstetrics/Gynecology[1], and Animal Science[2], UW-Madison Medical School, Madison, WI 53715

Angiogenesis is increased in the ovine uterus during the follicular ovarian phase and during early pregnancy. These physiologic states, when estrogen ($E_2\beta$) is elevated, suggests that estrogen may act as a modulator of growth factors within reproductive vascular beds. Vascular endothelial growth factor (VEGF) is a potent regulator of vascular permeability, and angiogenesis. Its predominant isoforms are VEGF-206 and VEGF-189 which are heparin binding variants bound to proteoglycans on the extracellular matrix, and VEGF-165 and VEGF-121 which are non-heparin binding excreted isoforms. This study's aim is to show a correlation between doses of $E_2\beta$ and cellular-binding and secreted expression of VEGF. **Method:** Uterine artery endothelial cells from pregnant sheep were grown in 20% charcoal-stripped MEM and treated with or without 0.1, 1.0, 5.0, 10, or 100 nM $E_2\beta$, 1.0 μM ICI-182780, or 5.0, 10, 100 nM $E_2\beta$+ICI for 24hrs in 0.1% BSA with serum free MEM. Free VEGF protein was quantified in the cell culture media via ELISA, and cellular-bound VEGF was quantified via western immuno-blot. **Results:** VEGF was dose dependently increased in response to $E_2\beta$, with maximal response at 10 nM. Cellular-bound and secreted VEGF was expressed up to 2-fold from control at 10nM $E_2\beta$, and was blocked to control levels by ICI. **Conclusion:** $E_2\beta$ may play a vital role in the regulation of VEGF expression within the UAEC. Because ICI blocks this estrogenic increase of VEGF expression, it is likely this increase is through estrogen receptor α or β. However, it is not currently clear which one.

Calcium Increases in the Anterior Bovine Sperm Head during Capacitation.

J.J. Parrish and J.L. Susko-Parrish. Department of Animal Sciences, University of Wisconsin-Madison.

Bovine sperm were incubated 4.5 hr under capacitating conditions with heparin (10 µg/ml) or non-capacitating conditions with no additions (control). Fura2-AM was loaded into sperm at 3.5 hr to measure intracellular calcium (Ca_i). At 4.5 hr, sperm were imaged at 0, 2.5, 5, 10 and 15 min after addition of solubilized zona pellucida proteins (ZP, bovine, 50 ng/ml). Epifluorescent (340nm and 380 nm excitation) and phase contrast images were evaluated to determine Ca_i (nM) and acrosome reaction (AR) status respectively. The Ca_i was determined in the anterior (ANT) and posterior half (POST) of each sperm head. Since the nucleus failed to accumulate Fura2, the POST represented cytoplasmic Ca_i and the ANT represented the Ca_i in both the cytoplasm and acrosome. The experiment was repeated with semen from 6 bulls and a total of 387 sperm were evaluated. Three percent of the 224 control sperm and 37% of the 163 heparin-treated sperm underwent an AR in response to ZP. There was no difference ($p>0.05$) between the control sperm that did or did not undergo an AR in the mean±sem Ca_i within the ANT (85±8 vs. 74±5) or POST (58±5 vs. 46±6). In contrast, the Ca_i was higher ($p<0.05$) for heparin-treated sperm undergoing an AR both in the ANT (378±51 vs. 129±13) and POST (216±34 vs. 114±18). The Ca_i was also greater ($p<0.05$) in the ANT vs. the POST for heparin-treated sperm which underwent an AR but was not different ($p>0.05$) for those that did not undergo an AR. The Ca_i at time 0 in the ANT of heparin-treated sperm undergoing an AR at 2.5, 5, 10 or 15 min after ZP addition was 540±202, 408±110, 367±98, and 192±49. The higher the Ca_i at the time of ZP addition, the faster heparin-treated sperm acrosome reacted. The Ca_i (time 0) in the ANT of sperm which did not acrosome react in response to ZP was greater ($p<0.05$) in heparin-treated sperm (141±22) than in control sperm (74±5). Results suggest calcium is accumulated in the acrosome during capacitation and the more calcium accumulated the faster sperm acrosome react in response to ZP. Supported by NIH 29HD31120 and USDA 97-35203-4612

Circulating Levels of Nitric Oxide (No) and VEGF during Ovine Pregnancy.

Ronald R. Magness1,2 PhD, Kimberly A. Vonnahme3 MS, Yun Li1 MS, Heidi L. Rupnow1 MS, Terrance M. Phernetton1 BS, and Stephen P. Ford3PhD, Perinatal Research Labs, Depts Ob/Gyn1 & Ani Sci, Univ of WI-Madison, Madison WI2, Dept. Ani Sci, Univ of Wyoming, Laramie, WY3.

Introduction: During the last third of pregnancy in the ewe, dramatic elevations in uterine blood flow (UBF), NO production and eNOS expression in uterine and placental artery endothelium coincide with maximal fetal growth and substantial cotyledonary neovascularization. VEGF has been reported to increase eNOS expression and NO production in endothelial cell cultures. We therefore hypothesized that circulating VEGF levels will be elevated in association with increased circulating NO levels during ovine gestation. **Methods**: Circulating NO measured as Nitrates/Nitrites (NOx) and VEGF concentrations were analyzed in systemic plasma obtained from 47 pregnant sheep ranging from 36 days of gestation until one week postpartum. Uterine venous-arterial NOx and VEGF concentrations were also analyzed from 5 additional chronically instrumented late gestation sheep. **Results**: Circulating levels of NOx were unaltered between 36 and 89 days of gestation averaging 4.7±0.44 uM (n=27), but rose progressively ($P<0.01$) to 10.3±1.3 uM (n=39) and 18.8±0.88 uM (n=149) at 90-109 and 110-145 days of gestation, respectively. During the postpartum period, NOx returned to initial basal levels (5.7±1.3 uM; n=16). Systemic NOx levels were approximately 30-40% higher in pregnancies with multiple fetuses (twin and triplets) versus singletons ($P<0.05$). Moreover, the rise in NOx levels were observed earlier in gestation in multiples (>90 days) as compared to singleton pregnancies (>110 days). In contrast to NOx, circulating levels of VEGF were not substantially altered from 36-145 days of gestation or into the postpartum period. Although multiples tended to have slightly greater VEGF levels then singletons ($P<0.05$), this was only observed from 75-110 days of gestation. No uterine venous-arterial concentration differences of either NOx or VEGF were observed. **Conclusion**. The pattern of the rise in NO in circulating plasma was not directly associated with changes in VEGF regardless of the number of fetuses present. Support: NIH HL49210, HL57653, HD33255, HL56702, and HD38843.

Abstracts for Poster Presentations

Poster Titles

1. **Donor Cell Passage Number, Starvation Period and Fusion-Activation Interval Affect Preimplantation Development of Bovine Nuclear Transfer Embryos.** Z. Beyhan, M. Mitalipova, T. Chang, N.L. First

2. **Dysregulation of Glucose and Oxygen Metabolic Pathways May Initiate Changes in Placental Villous Function, Which in Turn Contributes to the Pathophysiology of Fetal Growth and Maternal Well-Being Associated with the Type I Diabetic Pregnancy.** David Burleigh

3. **Temporal and Prostaglandin F2a Induced Changes in The mRNA or Steroid Hormone Receptors in the Porcine Corpus Luteum.** Francisco Diaz

4. **Expression and Activity of Ovine Endothelial Nitric Oxide Synthase (eNOS) Expressed in COS-7 Cells.** Jacqueline Cale and Ian M. Bird.

5. **Estrogen Receptor Expression and Regulation in Ovine Uterine Arteries.** MJ Byers, AL Zangl1, IM Bird1, G Lopez, Yun Li 1, RR Magness

6. **Development of a Model to Study UBF Changes in Synchronized Ovarian Cycles in Sheep.** Tiffini C. Gibson, Terrance M. Phernetton1, Milo C. Wiltbank, and Ronald R. Magness

7. **Lobe-Specific Effects of In Utero and Lactational 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (Tcdd) Exposure on Branching Morphogenesis in Mouse Prostate.** K Ko, R W Moore and R E Peterson

8. **Immortalized Human Umbilical Vein Endothelial Cell Characterization.** Shervon A. Pierre, Shannon M. Gifford, and Ian M. Bird

9. **Insulin Stimulates Akt But Not ERK 1/2 or Nitric Oxide (NO) in an Ovine Uterine Artery Endothelial Cell Culture Model (UAEC).** Jeremy A Sullivan, Jacqueline M Cale, Ronald R Magness, Ian M Bird.

10. **Generational Expression of COX-1, cPLA2, and PGIS Protein Expression in Large versus Small Uterine Arteries During the Ovine Ovarian Cycle and Mid- and Late Pregnancy.** JM Joyce, TM Phernetton, CE Shaw, and RR Magness.

11. **Effects of Shear Stress, Angiogenic Factors, and MAPK on Nitric Oxide Synthase (eNOS) Expression by Ovine Fetoplacental Artery Endothelial Cells.** Y Li, J Zheng, IM Bird, and RR Magness.

12. **Endothelial Vasodilator Production by Uterine and Systemic Arteries: Estrogen and Progesterone Effects on cPLA2, COX-1, and PGIS Protein Expression**. Heidi L Rupnow, Terrance M Phernetton, Mary L Modrick, Milo C. Wiltbank, Ian M Bird, Ronald R Magness.

13. **Basic Helix-Loop-Helix (bHLH) Transcription Factors and E Boxes in Chorionic Gonadotropin (CG) Transcription in Trophoblasts.** Yi-Ping Liu, D. Burleigh, R. Weinberger, M. Durning, and T. Golos.

14. **The Production and Regulation of Leptin in Bovine Mammary Epithelial Cells.** JL Smith and LG Sheffield

15. **An Efficient Method to Express Transgenes in Nonhuman Primate Embryos Using a Stable Episomal Vector.** M.J. Wolfgang, V.S. Marshall, S.G. Eisele, L. Knowles, M.A. Browne, M.L. Schotzko, J.A. Thomson and T.G. Golos

16. **Co-expression of VEGF and Neuropilin-1 (NP-1) in Ovine Feto-Placental Artery Endothelial (OFPAE) Cells.** Jing Zheng, Stephen C.M. Tsoi , Yunxia Wen, Dong Bao Chen, Ronald R. Magness.

1. Donor Cell Passage Number, Starvation Period and Fusion-Activation Interval Affect Preimplantation Development of Bovine Nuclear Transfer Embryos

Z. Beyhan[1], M. Mitalipova[2], T. Chang[1], N.L. First[1], [1]University of Wisconsin-Madison, Madison, WI 53706 U.S.A.,[2]BresaGen Inc., Athens, GA 30604 U.S.A

The objective of this study was to examine the effect of certain nuclear transfer parameters on preimplantation development of bovine nuclear transfer embryos using four different donor cell types (bovine adult and fetal fibroblasts, chondrocytes and ovarian cumulus cells).

Donor cell types were isolated by follicular aspiration, from live and tissue sampling from slaughtered animals for cumulus cells, fibroblasts and chondrocytes respectively. All cell types were cultured for 1-16 passages before nuclear transplantation. Nuclear transfer experiments were conducted as described by Dominko et al. (Biol Reprod 1999;60(6):1496-502). Recipient oocytes were matured in vitro, stripped of cumulus cells and enucleated at 18-20 hours post maturation (hpm). Enucleation was performed under ultraviolet light and oocytes were stained with Hoechst 33342 (5μM) to confirm complete removal of chromatin. Donor cells were starved 1-26 days, disaggregated by trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA•Na), individual cells were placed into oocyte perivitelline space and fused with enucleated oocytes by using two electrical pulses of 1.4 -1.8 KV/cm for 15 μs. Oocytes were activated chemically with 5μM ionomycin for 4 min. and 1.9 mM 6-dimethyl-aminopurine (6-DMAP) in CRIaa culture medium for 4 hours. Time interval between fusion and activation was 50-325 minutes. Reconstructed embryos were cultured at 38.5 degrees C in 5% CO_2in 50 μl CRIaa drops for 7-8 days. A total of 3123 NT embryos were produced in 117 trials using one type of donor cell in each trial. Data were analyzed by ANOVA and means were compared by protected LSD. Results are summarized in Table 1.

Table 1: The effect of donor cell passage number, starvation period and fusion-activation interval on pre-implantation development of bovine nuclear transfer embryos.

Parameter	Treatment	n	Fusion % (N)	Cleavage % (N)	Blastocyst % (N)
Passage Number	1-5 Days	1899	87.2±1.7 (72)	64.9±1.9 (73)	23.8±1.4 (73) [a]
	6-10 Days	866	84.4±2.6 (32)	65.5±2.9 (32)	22.1±2.1 (32) [a]
	11-16 Days	358	80.4±4.0 (13)	63.9±4.6 (13)	15.9±3.3 (13) [b]
Starvation Period	0 (monolayer)	588	89.3±3.2 (21)	61.6±3.6 (21)	20.1±2.6 (21) [a,b]
	1-5 Days	1306	86.2±2.0 (52)	65.3±2.3 (53)	22.8±1.6 (53) [a,b]
	6-10Days	736	79.4±2.8 (26)	66.2±3.2 (26)	26.7±2.3 (26) [a]
	11-15 Days	375	89.6±3.8 (13)	63.0±4.4 (13)	19.2±3.2 (13) [b]
	16-26 Days	118	88.0±6.4 (05)	74.8±7.4 (05)	15.3±5.3 (05) [b]
Fusion-activation interval	50-150 min.	270	75.3±4.3 (11)	76.4±4.9 (11) [a]	21.5±3.6 (11)
	151-250 min.	2454	86.9±1.5 (91)	63.7±1.7 (92) [b]	22.0±1.3 (92)
	251-325 min.	399	86.1±3.7 (15)	64.5±4.2 (15) [b]	26.2±3.1 (15)

Values with different superscripts within the same column differ significantly ($p<0.05$)

Our results indicate that nuclear transfer parameters such as donor cell passage number, starvation period and fusion-activation interval affect the outcome of preimplantation development of these embryos. Although preimplantation development is not a direct indicator of cloning efficiency in nuclear transfer experiments, considering these parameters in a given cell line may improve the overall efficiency of the nuclear transfer procedures.

2. Dysregulation of Glucose and Oxygen Metabolic Pathways May Initiate Changes in Placental Villous Function, Which in Turn Contributes to the Pathophysiology of Fetal Growth and Maternal Well-Being Associated with the Type I Diabetic Pregnancy.

David Burleigh, UWPRC, UW-Madison

We hypothesize further that the control of placental vasculogenesis and angiogenesis is also impacted by these changes. To better understand how alteration of glucose and oxygen tension may affect placental villous and vascular development, we have used immunohistochemical techniques to examine FGF-2 expression, cell proliferation (Ki67), and apoptosis (Apo-Tag; Oncor) in placentae fromterm diabetic and nondiabetic pregnancies. We have previously observed in nondiabetic placentae that FGF-2 was localized almost exclusively to cells located in the trophoblast compartment of chorionic villi.Immunostaining for FGF-2 in diabetic placentae demonstrated a consistent increase in both intensity and extent with in the placental villous. Associated with this change FGF-2 expression, preliminary results suggest that the numbers of Ki67-positive nuclei are similar between nondiabetic and diabetic placentae, but the number of apoptotic stromal nuclei is greater in the nondiabetic placenta. The ratio of Ki67:ApoTag-positive stromal nuclei show a trend towards decreased nuclei turnover in diabetic placentae. It has recently been reported from other laboratories that FGF-2 can inhibit the induction of apoptosis. These data would suggest that increased FGF-2 expression and decreased stromal nuclei turnover in the diabetic placenta may be a compensatory mechanism in response to the adverse effects of diabetes on placental function. Additional studies are in progress to correlate the extent of these placental changes with the classification of maternal diabetes.

3. Temporal and Prostaglandin F2a Induced Changes in The mRNA or Steroid Hormone Receptors in the Porcine Corpus Luteum

Francisco Diaz, ERP, UW-Madison

The pig corpus luteum acquires the capacity to regress in response to PGF2a treatment (luteolytic capacity) at a relatively late stage of the estrous cycle (day 12). Two estradiol 17b receptors, estrogen receptor alpha (ER-a) and estrogen receptor beta (ER-b) and progesterone receptor (PR) have been found in the corpus luteum of several mammalian species. This study examines the basal and PGF2a induced changes in the steady state mRNA concentration for the steroid hormone receptors, ER-b, ER-a and PR in pig CL before (day 9) and after (day 17 pseudopregnant) acquisition of luteolytic capacity. Porcine CL were surgically collected from animals on day 9 or day 17 of pseudopregnancy after saline treatment or 0.5 h and 10 h after PGF2a treatment. The mRNA for ER-a, ER-b and PR were evaluated by semi-quantitative RT-PCR. In the absence of exogenous treatment PR mRNA was greater ($p<0.05$) in day 9 CL (1.22±0.16) than in day 17 CL (0.66±0.07), whereas ER-a mRNA was greater ($p<0.05$) in day 17 CL (1.95±0.11) than in day 9 CL (1.07±0.21). Basal ER-b mRNA was similar in both day 9 and day 17 CL. Treatment with PGF2a did not alter PR or mRNA in either day 9 or day 17 CL. PGF2a decreased ER-a mRNA at 10 h after treatment only in day 17 (control 1.85±0.16; PGF2a 1.23±0.15. $p<0.05$) and not in day 9 (control 1.14±0.10; PGF2a 1.18±0.19) CL. Unexpectedly, PGF2a was found to acutely (0.5 h) increase ER-b mRNA in day 17 (control 0.34±0.09; PGF2a 0.87±0.08. $p<0.05$), but not day 9 (control 0.42±0.09; PGF2a 0.37±0.11) CL. At 10 h after PGF2a treatment ER-b remained unchanged in day 9 (control 0.33±0.01; PGF2a 0.43±0.09), but further increased in day 17 CL (control 0.24±0.04; PGF2a 1.34±0.04. $p<0.05$). Thus, a decrease in basal PR and an increase in basal ER-a is associated with a corpus luteum more susceptible to PGF2a induced luteolysis (luteolytic capacity). The shift in the predominant mRNA for estrogen receptor from ER-a to ER-b after PGF2a treatment may indicate a change in signaling required for the completion of luteolysis.

4. Expression and Activity of Ovine Endothelial Nitric Oxide Synthase (eNOS) Expressed in COS-7 Cells.

Jacqueline M Cale and Ian M Bird, Dept of OB/GYN, UW-Madison

Background: We have described the isolation and cloning of a putative full-length cDNA encoding ovine endothelial nitric oxide synthase that has high homology (>99%) to the published bovine and human sequences. In vivo, eNOS is constitutively expressed and appears to be negatively regulated by caveolin and positively regulated by the chaperone Hsp90. In addition, eNOS regulation is thought to be sensitive to intracellular calcium concentration and multiple phosphorylation events. **Objective:** This study demonstrates immunoreactivity, activation and protein interactions when the putative eNOS cDNA is transfected and expressed in an alternative cellular environment. **Methods and Results:** Subsequent to propogation in XL-1 Blue, endotoxin-free plasmids were isolated for transfection into COS-7 cells (ATCC) using the GeneJammer Transfection Reagent (Stratagene). The amount of DNA transfected was optimized for maximal cell survival and protein expression. Following transient expression of eNOS for 24 hours, we showed that the calcium ionophore, A23187, increases eNOS activity as measured by 3H-arginine to 3H-citrulline conversion in COS-7 cells. This sensitivity to A23187 is dose-dependent and can be blocked with the specific eNOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME). Treatment with the less active enantiomer NG-nitro-D-arginine methyl ester (D-NAME) was unable to block eNOS activity. We also treated the transfected cells with TPA, a well-known activator of PKC and ERK1/2, which resulted in no change in eNOS activity. Finally, we coimmunoprecipitated both Hsp90 and caveolin-1 with eNOS transiently expressed in the COS-7 cells. **Conclusion:** We have cloned and expressed a full-length ovine eNOS cDNA that is functional in the alternative environment of a COS-7 cell. This not only verifies the identity of the eNOS cDNA, but also provides a model system that will allow further study of the hormone-dependent activation of eNOS by protein kinases.

5. Estrogen Receptor Expression and Regulation in Ovine Uterine Arteries.

MJ Byers1*, AL Zangl1, IM Bird1, G Lopez1, Yun Li 1, RR Magness1,2. Perinatal Res. Labs, Dept of Ob/Gyn1 & Animal Sciences2, Univ. of WI-Madison.

The pregnancy-associated rise in estrogen is associated with a dramatic increase in utero-placental blood flow, and therefore laminar shear stress, a response required for normal fetal development. Estrogen is believed to exert its effect by binding to its specific estrogen receptors (ER) in target cells, resulting in increased expression and activity of endothelial nitric oxide synthase (eNOS). However, ER expression and cellular origin in uterine arteries (UAs) are currently unknown. **Objective**: To examine the expression, cellular localization, and regulation of ERs in ovine UAs. **Methods**: UAs were fixed and mounted for mRNA and protein analysis. ER mRNA localization was determined by in situ hybridization (ISH) using [35S]-labeled riboprobes. UA endothelial protein (UAendo) was isolated and the presence of ER protein was determined via Immunoblotting. Further verification of the in vivo experiments was obtained by utilizing uterine artery endothelial cell (UAEC) lines created in our lab by Dr. Bird. UAECs were cultured and treated with exogenous steroids, and analyzed using Immunoblotting, RT-PCR, and Immunocytochemistry (ICC). The effects of shear stress on receptor expression were also studied. UAECs were grown on specialized cartridges and subjected to physiologic shear stress (15 dynes/cm2) in the presence (10nM) or absence of E2β. **Results**: We observed by IHC and ISH that ERα and ERβ protein and mRNA were present in ovine UAendo. Immunoblotting and RT-PCR showed expression of ERα and ERβ protein and mRNA in UAECs. Furthermore, laminar shear stress as well as the type of serum used during cell culture could be manipulated to differentially regulate ERα and ERβ levels. Moreover, in contrast to static UAEC cultures, 10nM E2β dramatically increased the expression of eNOS protein in the presence of laminar shear stress. **Conclusion**: Taken together, we show that ER protein and mRNA are expressed in UA endothelium and are regulated both by exogenous treatment with ovarian steroids, indicating UA endothelium is a target for estrogen action in vivo, and by novel signaling mechanisms probably initiated through serum and shear stress responsive elements (Supported by NIH HD33255, HL57653, HL49210, HD38843, and HL57602).

6. Development of a Model to Study UBF Changes in Synchronized Ovarian Cycles in Sheep

Tiffini C. Gibson1, Terrance M. Phernetton1, Milo C. Wiltbank2, and Ronald R. Magness1,3 Departments of Obstetrics and Gynecology Perinatal Research Laboratories1, Dairy Sciences2 and Animal Sciences3, University of Wisconsin-Madison, Madison, WI 53715

The majority of uterine blood flow (UBF) studies to date have utilized ovariectomized sheep with pharmacological doses of exogenous estrogen. However, cyclical changes and UBF will need to be studied in order to evaluate the effects of endogenous estrogen of ovarian follicular origin. Because sheep are photoreceptive breeders, we have developed a model for studying UBF in sheep using vaginal progesterone implants (CIDR) and PGF2α; in order to maintain cyclicity throughout the year. **Purpose**: To develop a model for studying UBF changes during synchronized ovarian cycles regardless of season. **Methods**: Nonpregnant, cycling sheep were surgically instrumented with uterine artery blood flow transducers and uterine and femoral artery catheters. All sheep were implanted with a CIDR for 7 days. On Day -1, animals received I.M. 7.5mg of PGF2α; (Lutalyse) 2x- 4 hours apart. On Day 0, the CIDR was removed and animals received either 500 IU (n=6) or 1000 IU (n=5) of Pregnant Mare Serum Gonadotrophin (PMSG) and UBF was monitored continuously for 55-75 hours. **Results**: A rise in UBF was observed in all sheep regardless of photoperiod using this treatment regimen. The 500 IU group increased UBF between 10 and 15 hours with a gradual rise from baseline flows of 5-10 ml/min to maximum flows of about 75-80 ml/min at 45-55 hours. The 1000 IU group exhibited a delay in the time to increase UBF (30-35 hours). However, the rise in UBF with the 1000 IU was much more rapid and reached maximal flow (180-220 ml/min) significantly higher than the 500 IU group. **Conclusion**: The use of CIDR's and PGF2α; will extend the cycling season and allow continued study of UBF in response to endogenous estrogen throughout the year. We believe that the 500 IU dose is a more accurate representation of a true physiologic response to endogenous estrogen as compared to the 1000 IU dose which is a more pharmacological dose.

7. Lobe-Specific Effects of In Utero and Lactational 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (Tcdd) Exposure on Branching Morphogenesis in Mouse Prostate.

K Ko, R W Moore and R E Peterson. Univ. Wisconsin, Madison, WI, USA.

Branching morphogenesis is an essential component of prostate development. Previously we reported that in utero and lactational TCDD exposure inhibits ventral, dorsolateral, and anterior prostate growth. This study was conducted to test the hypothesis that TCDD inhibits branching morphogenesis in mouse prostate. Pregnant C57Bl/6 mice were given TCDD (5 mg/kg, po) or vehicle on Gestation Day 13 and their pups examined at 1, 7, 14, 21, 35 and 90 days of age. Prostate lobes were microdissected after incubation in 0.5% collagenase and the numbers of main ducts, branch points, and ductal tips determined by examining photographs of microdissected, whole-mount specimens. Ductal canalization was determined using histological sections. TCDD inhibited branching morphogenesis in all prostate lobes. The ventral prostate was extremely small throughout development and never developed any ductal structure. TCDD reduced the numbers of main ducts, branch points, and ductal tips in the dorsal prostate and the lateral prostate, but reductions in branch point and ductal tip numbers appear to be due entirely to reductions in the number of main ducts. Duct length in the dorsal and lateral prostates also appeared to be shorter. Dorsolateral prostate weights were slightly reduced by TCDD, but there did not appear to be any effect on ductal canalization in either the dorsal or lateral lobes. TCDD had no effect on main duct number in the anterior prostate but weight, branch point number, and ductal tip number were substantially reduced. These results demonstrate that the severe inhibition in ventral prostate development caused by in utero and lactational TCDD exposure is accompanied by complete absence of branching morphogenesis, and that inhibitions in dorsal, lateral, and anterior prostate development are associated with obe-specific inhibitions in branching morphogenesis. (Supported by NIH Grant ES 01332)

8. Immortalized Human Umbilical Vein Endothelial Cell Characterization

Shervon A. Pierre, Shannon M. Gifford, and Ian M. Bird, Dept OB/GYN, UW-Madison

HUVEC-C are spontaneously immortalized human umbilical vein endothelial cells. They are an important potential endothelial cell model because they are widely available through ATCC, however, little research has been done to characterize these cells. The purpose of this study is to determine if the HUVEC-C maintain endothelial cell characteristics and to ascertain if they utilize the same cell signaling pathways as the uterine artery endothelial cells (UAEC). An AcLDL uptake assay as well as immunostaining for eNOS revealed that the cells are indeed endothelial cells. Furthermore, localization of PCNA and KI67 within the nucleus of the cells showed that they were healthy and proliferating. After establishing that the cells were a dividing population of endothelial cells, they were treated with ATP to reveal if it could initiate an increase Ca2+ concentration within the cytosol of the cell. As in the UAEC, these cells increase their intracellular concentration of Ca2+ after ATP stimulation. Furthermore, like UAEC, it appears that ATP binding to P2Y receptors rather than P2X receptors causes the increase in Ca2+. This was established by treating the cells with thapsigargin; after thapsigargin had emptied all of the internal stores of Ca2+, ATP was unable to initiate a Ca2+ response. However, unlike the UAEC, L-type voltage gated channels may also play a role in the response. Verapamil but not nifedipine caused a significant decrease in the ATP response. In addition, BAYK8644 caused a significant but minor increase in the cytosolic Ca2+ concentration above the basal level. More experiments need to be performed to determine the exact role of the L-type channels in the ATP induced response. Yet, our data suggests that the HUVEC-C is a valid endothelial cell model that behaves very similar to the UAEC cell model in regards to calcium signaling.

9. Insulin Stimulates Akt But Not ERK 1/2 or Nitric Oxide (NO) in an Ovine Uterine Artery Endothelial Cell Culture Model (UAEC).

Jeremy A Sullivan, Jacqueline M Cale, Ronald R Magness, Ian M Bird Ob/Gyn, University of Wisconsin, Madison, WI

Concomitant with pregnancy is a normal increase in maternal insulin resistance so as to allow an increase in glucose delivery to the fetus. Although the exact cause of the pregnancy associated insulin resistance is not understood, alterations in insulin signal transduction pathways have been implicated as possible causes. Also concomitant with pregnancy, there is a dramatic increase in blood flow to the utero-placental unit so as to supply the fetus with the increase in nutrients it requires for a healthy gestation. The increase in blood flow to the utero-placental unit during pregnancy is dependent on angiogenesis of surrounding vessels as well as local, endothelial production of vasoactive substances such as NO. The regulation of vascular tone has been shown to involve many different factors. We are investigating whether insulin is capable of increasing NO production in UAEC and activating two intracellular signaling pathways associated with a multitude of cellular responses including vasodilator production, mitogenesis, glucose homeostasis, etc. The activation of eNOS, the hormone sensitive enzyme in NO production, has been shown to be sensitive to phosphorylation by the protein kinase Akt, rendering the enzyme active without an increase in intracellular calcium. Akt has also been identified as a factor responsible for regulating cellular glucose uptake. A second kinase implicated in the activation of eNOS is ERK 1/2. Herein, we report that insulin (10-7M) treatment does not increase the production of NO in UAEC, in contrast to that observed in other endothelial cells. We have observed that a large, 20 minute, insulin treatment (10-5 and 10-6M) in UAEC from Pregnant (P-UAEC) and Nonpregnant ewes (NP-UAEC), exhibits a sizeable increase (2.5-3 fold) in Akt activity as measured by phosphorylation state dependent antibodies. Insulin treatment in both P and NP-UAEC does not lead to a detectable increase in ERK 1/2 activation. We conclude that insulin does not appear to be involved in the production of NO in our UAEC model, nor does it appear to signal through ERK 1/2 under the conditions observed here in both P and NP-UAEC. Insulin has been shown to bind not only the classical insulin receptor, but at superphysiological insulin concentrations, it also binds IGF receptors. The presence of both the insulin and IGF-I receptor mRNA, as demonstrated by the use of genetic arrays, and the observation that insulin stimulates Akt, leads us to believe insulin does function in some capacity in our cell model, but not in the production of NO. Supported by USDA 0002159, NIH-HL56702, HL49210.

10. Generational Expression of COX-1, cPLA2, and PGIS Protein Expression in Large versus Small Uterine Arteries During the Ovine Ovarian Cycle and Mid- and Late Pregnancy.

JM Joyce 1, TM Phernetton 1, CE Shaw 1, and RR Magness 1,2. Department of Ob/Gyn 1 and Animal Sciences 2, UW-Madison, Madison, WI 53715.

Introduction: We previously reported that the follicular phase and pregnancy-associated rises in uterine blood flow (UBF), via elevations in shear stress (SS), appear to regulate eNOS protein expression in the smaller diameter uterine arteries (UAs). Prostacyclin (PGI2), like NO and eNOS, is also increased by SS, presumably via elevations in either COX-1, cPLA2 and/or PGIS. Therefore, we tested the hypothesis that regional vascular differences in responses of the three enzymes involved in PGI2 biosynthesis will be observed with both decreases in vessel diameter and during the ovarian cycle and pregnancy, since it is known that SS-mediated mechanism(s) will be altered with elevations in UBF. **Methods**: Western analysis was performed on endothelial-isolated proteins from the primary (UA 1), secondary (UA 2), and tertiary (UA 3) branches of the UA in luteal (LUT; n=6), follicular (FOL; n=6), mid pregnant (MP; 80-90 days; n=6) and late pregnant (LP; 120-130 days; n=6) sheep. Data were analyzed and expressed as % of UA 1. **Results**: Generational differences in UA COX-1 protein expression was observed during the LUT, but not FOL phase (UA 1 >/= UA 2 > UA 3; $P < 0.05$). Furthermore, COX-1 expression was significantly down-regulated in the smaller diameter UAs in both pregnant groups (UA 1 > UA2 = UA 3; $P < 0.05$). Consistent with COX-1 expression, a similar pattern was noted for LUT phase cPLA2 protein expression. In contrast, both FOL phase and MP were associated with up-regulated cPLA2 expression, although this did not reach statistical significance. LP cPLA2 generational expression was unaltered compared to UA 1. Generational differences in UA PGIS expression was also observed during the ovarian cycle (LUT and FOL) and MP (UA 1 >/= UA 2 > UA 3; $P < 0.05$). This pattern for PGIS expression was no longer observed in the LP sheep, which is consistent with LP cPLA2expression. **Conclusion**: COX-1, cPLA2 and PGIS protein expression are differentially regulated during the ovarian cycle and pregnancy. Thus, heterogeneity of COX-1, cPLA2 and PGIS in the uterine vasculature may play an important role in the control of UBF and SS-mediated changes in UA PGI2 in the sheep. Support by NIH grants HL49210, HL57653, HD33255, HD38843,and HL56702.

11. Effects of Shear Stress, Angiogenic Factors, and MAPK on Nitric Oxide Synthase (eNOS) Expression by Ovine Fetoplacental Artery Endothelial Cells.

Y Li1, J Zheng1, IM Bird1, and RR Magness1,2. Departments of Ob/Gyn1 and Ani Sci2, University of Wisconsin-Madison, Madison, WI 53715.

Placental blood flow, eNOS expression, and angiogenic factors (bFGF and VEGF) secretion increase during human and ovine pregnancy. Shear stress is the frictional force exerted on endothelial cells with increasing blood flow. ERK1/2 mediates bFGF stimulated eNOS expression in ovine fetoplacental artery endothelial cells (OFPAEC). Our hypothesis is that MAPK pathways are involved in shear stress induced eNOS expression in OFPAEC. **Methods**: OFPAEC were grown at 3dynes/cm2 in CELLMAX modules, after the cells reach confluence, they were exposed to various shear stresses, then NO production or eNOS expression (Western and RT-PCR) from eluted cells were analyzed. Pretreatment of drugs on OFPAEC were performed also at 3dynes/cm2 as follow: bFGF(10ng/ml) or VEGF(10ng/ml) for 30 min, MEK inhibitor UO126(10uM) or P38 MAPK inhibitor SB203580(10uM) for 1 hour. **Results**: eNOS protein was elevated by 10(1.8-fold) and 25(3-fold) dynes/cm2 from 6 to 24hr. eNOS mRNA level was increased after OFPAEC were exposed to 25 dynes/cm2 for 12hr. Although bFGF and VEGF alone increased eNOS expression, there was no synergy with addition of shear stress of 15dynes/cm2. The level of eNOS elevated after 24hr exposure to 15dynes/cm2, and it remained the same in the presence of UO126 or SB203580. **Conclusion**: Shear stress increases NO production, and promotes eNOS protein level which is unlikely mediated through MAPK pathways in OFPAEC. Angiogenic factors do not have synergistic effects with shear stress on eNOS expression.

12. Endothelial Vasodilator Production by Uterine and Systemic Arteries: Estrogen and Progesterone Effects on cPLA2, COX-1, and PGIS Protein Expression.

Heidi L Rupnow1, Terrance M Phernetton1, Mary L Modrick1, Milo C. Wiltbank2-, Ian M Bird1, Ronald R Magness1,3 Departments of Obstetrics and Gynecology1 Perinatal Research Laboratories, Dairy Science2, and Animal Sciences3, University of Wisconsin-Madison, Madison, WI 53715

During ovine pregnancy, when both estrogen and progesterone are elevated, PGI2 production by uterine arteries and the key enzymes for PGI2 production, cPLA2, COX-1, and PGIS, are increased. Purpose: To determine if exogenous Estradiol-17B (E2B) +/- progesterone (P4) would increase cPLA2, COX-1, and PGIS protein expression in ovine uterine, mammary, and systemic (renal, omental, and coronary) arteries. Methods: Nonpregnant ovariectomized sheep received either Vehicle (V; n=10), P4 (0.9g CIDRs vaginal implants; n=13), E2B(5ug/kg bolus followed by 6ug/kg/d; n=10), or P4 + E2B(n=12). Arteries were procured on day 10 and cPLA2, COX-1, and PGIS protein was measured by Western immunoblot analysis in endothelial isolated proteins and VSM. Results: cPLA2 was increased in uterine artery endothelium in P4 + E2B treated ewes, but was unaltered by treatment in renal and coronary artery endothelium and VSM of the uterine artery. Furthermore, cPLA2 was below detectable amounts in mammary and omental artery endothelium as well as mammary, omental, renal, and coronary VSM. COX-1 was increased with P4 + E2B treatment in uterine artery endothelium, unaltered with steroid treatment in mammary, renal, omental, and coronary artery endothelium and was not detectable in VSM of any artery type. PGIS was increased in uterine artery endothelium with E2B and unaltered in coronary, renal, and omental artery endothelium. P4 increased PGIS expression in the uterine, mammary, omental, and renal artery VSM, while E2B and P4 + E2B increased PGIS expression in the uterine and omental artery VSM. Conclusions: Both E2B and P4 treatments differentially alter protein expression key enzymes in PGI2 production in different artery types and may play a role in the control of blood flow redistribution during hormone replacement therapy. Support: NIH HL49210, HL56702, HD33255, HD38843.

13. Basic Helix-Loop-Helix (bHLH) Transcription Factors and E Boxes in Chorionic Gonadotropin (CG) Transcription in Trophoblasts.

Yi-Ping Liu, D. Burleigh, R. Weinberger, M. Durning, and T. Golos, Department of Obstetrics and Gynecology and Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715-1299.

Developmentally appropriate and cell-specific gene transcription is regulated in many systems by transcription factors binding to the E box motif, typically bound by bHLH factors. The activities of bHLH factors are often modulated by interactions with Id proteins. To begin to define the potential E box-binding factors within placental cells, we sreened a human placental cDNA library with a yeast 2-hybrid approach utilizing the negative regulator Id2 as bait. We isolated a variety of cDNAs, including multiple lones for the bHLH transcription factor E2-2. E2-2 (also called SEF-2 or ITF-2) has been reported to be expressed as several splice variants with different activities, dependent on the cell context.

We were interested in determining whether E2-2 or other E box-binding proteins play a role in placental hormone gene transcription. Examination of the human chorionic gonadotropin alpha and beta subunit 5'-flanking DNA revealed three E box-like motifs at -274 (A box), -135 (B box), and –126 (C box) relative to the start site of transcription in the beta subunit gene, and two E boxes at -51 and -21 in the alpha subunit gene. To examine whether these elements play a role in gene transcription, all E boxes were mutated individually or in combination to sequences known to abolish transcription factor binding and transcriptional activation in other E box elements. Transient transfection of JEG-3 choriocarcinoma cells with luciferase constructs controlled by 466 bp of 5'-flanking CG beta DNA (-362/+104) or 232 bp of CG alpha DNA (-187/+44) demonstrated that all E boxes contributed to basal transcriptional activation. Mutations in the A box as well as the B/C boxes individually reduced basal (but not cAMP-stimulated) transcription by 65% and 40%, respectively. Mutation of CG alpha E box 1 or 2 likewise resulted in a similar loss in basal transcriptional activation, whereas the effect on fold-induction by 8-Br-cAMP was

much more modest. The effects of E box mutation on basal transcription was not additive for either gene, suggesting interaction between the elements. In initial co-transfection experiments with an E2-2 expression vector in JEG cells, transcriptional activity of the wild-type CG alpha and CG beta promoters described above were decreased by up to 66%. These results demonstrate that bHLH transcriptional networks and E box-binding proteins may contribute to the transcriptional control of placental hormone gene expression. Supported by NIH grants HD26458 and HD36324.

14. The Production and Regulation of Leptin in Bovine Mammary Epithelial Cells.

JL Smith and LG Sheffield, UW-Madison, Dept of Dairy Science

Western blot analysis indicated the presence of leptin in bovine milk, while reverse transcription-polymerase chain reaction (RT-PCR) indicated the presence of leptin mRNA in mammary tissue and cultured bovine mammary epithelial cells (MAC-T cell line). A real time RT-PCR method was developed that allowed quantitative assessment of bovine leptin mRNA over approximately 3 orders of magnitude. Time course studies indicated a rapid increase in leptin mRNA in response to insulin or IGF-I. When normalized against bovine GAPDH as an endogenous control, 30 minute or 1 hr treatment with 10 ng/ml insulin gave 39±4 and 64±2 fold increase in leptin mRNA compared with 0 hr control. Leptin mRNA was increased 257±9 and 75±23 fold by 30 minute or 1 hr treatment with 10 ng/ml IGF-I. Dose response studies indicated significant increases in leptin mRNA in response to as little as 1 ng/ml insulin or 0.1 ng/ml IGF-I. Maximum increase in leptin mRNA was observed in response to 10 ng/ml insulin and 10 ng/ml IGF-I. These results indicate that production of leptin by bovine mammary epithelial cells can be regulated by factors known to alter mammary function and nutrient partitioning. This suggests that leptin may be an autocrine/paracrine signal in the bovine mammary gland.

15. An Efficient Method to Express Transgenes in Nonhuman Primate Embryos Using a Stable Episomal Vector.

M.J. Wolfgang, V.S. Marshall, S.G. Eisele, L. Knowles, M.A. Browne, M.L. Schotzko, J.A. Thomson and T.G. Golos. Wisconsin Regional Primate Research Center and the Departments of Anatomy, and Obstetrics and Gynecology, University of Wisconsin Medical School, Madison WI 53715, USA.

Trangenesis in the nonhuman primate can enhance the study of human physiology, pathophysiology and embryonic development by providing animal models with enhanced significance for the study of human disease processes. However, progress with this technology has been hindered by the inefficiency of transgenesis coupled with the availability of animals and practical restrictions on the production of sufficient pronuclear stage nonhuman primate zygotes. We have developed a novel technique using an Epstein Barr virus (EBV) based episomal vector to produce rhesus monkey (Macaca mulatta) embryos expressing a marker transgene. Plasmid DNA containing the latent origin of replication, oriP, and Epstein Barr Nuclear Antigen-1 (EBNA-1) of EBV, as well as a CMV IE eGFP expression cassette were introduced into rhesus embryos by direct pronuclear microinjection. We have been able to detect eGFP in early cleavage stage embryos (4-8 cell) and throughout the duration of culture (day 8-9). Expression of eGFP was monitored by epifluorescent microscopy. A 50% (24/49) transduction efficiency was obtained with the EBV-based vector. Microinjected embryos expressed eGFP and retained their developmental capacity as evidenced by development to the blastocyst stage. EBV-based vectors present a novel means to deliver transgenes both for the study of the molecular control of primate embryonic development, and may provide an efficient means to deliver transgenes to primate embryos. This work was supported by NIH grants RR00167 (W.R.P.R.C.) and HD26458, RR14040 (T.G.G.).

16. Co-expression of VEGF and Neuropilin-1 (NP-1) in Ovine Feto-Placental Artery Endothelial (OFPAE) Cells.

Jing Zheng1 Stephen C.M. Tsoi 1, Yunxia Wen1, Dong Bao Chen1, Ronald R. Magness1,2. Depts of Ob/Gyn, Perinatal Research Laboratories1 and Animal Sciences2, Univ of Wisconsin-Madison, Madison, WI 53715.

VEGF is a key regulator for placental angiogenesis and vascular functions via binding and activating two high affinity tyrosine-kinase receptors, VEGF receptor-1 (VEGFR-1) and -2 (VEGFR-2). Recently, a specific $VEGF_{165}$ receptor, neuropilin-1 (NP-1), was also identified in endothelial cells and upon binding, NP-1, synergistically with VEGFR-2, enhances VEGF-induced cell proliferation and migration. To evaluate the role of VEGF and NP-1 in regulating fetoplacental angiogenesis and endothelial function, an ovine fetal placental artery endothelial (OFPAE) cell cDNA library was constructed. In this study, we identified partial 3' VEGF and NP-1 sequences from this cDNA library. The VEGF sequence had 98% homology with reported ovine VEGF (GenBank accesssion # X89506). The partial NP-1 cDNA sequence included portion of the protein coding region and complete 3' untranslated region (UTR) and was 90% homology to human NP-1 (GenBank accession # AF016050). The predicted amino acid sequence of ovine NP-1 had 83%, 81%, and 87% identity to human (GenBank accession # AAC12921.1), mouse (GenBank accession # NP_032763), and rat (GenBank accession # AAC53345.1), respectively. Two CU-rich motifs were identified in the 3' UTR of ovine NP-1. These CU-rich sequences are the potential binding site for mRNA-binding proteins which may regulate the stability of NP-1 mRNA. Two destabilizing elements 5'-U(U/A)(U/A)AUUUAA-3' also existed in the 3' UTR of ovine NP-1. Expression of VEGF and NP-1 in OFPAE cells and fetal placentas was confirmed by Northern and Western blotanalyses as well as immunohistochemistry. These results indicate, for the first time, that VEGF is expressed in endothelial cells using multiple approaches. Moreover, we also identified, for the first time, a complete 3' UTR of NP-1 in any species. Together with expression of NP-1 and other VEGF receptors in endothelial cells, we proposed that there is an autocrine mechanism by which VEGF regulates angiogenesis and other functions of endothelial cells.

Endocrinology-Reproductive Physiology Program Faculty Members				
Name	**Department**	**Address**	**Phone #**	**E-Mail and Fax #**
Abbott, David **Professor**	Primate Center; Obstetrics & Gynecology	203 Primate Center 1220 Capitol Court	3-3583	abbott@primate.wisc.edu Fax: 3-4031
Alarid, Elaine **Assistant Professor**	Physiology	120 Service Memorial Institute 1300 University Avenue	5-9319	alarid@physiology.wisc.edu Fax: 5-5512
Bertics, Paul **Professor**	Biomolecular Chemistry	571A Medical Sciences Center 1215 Linden Drive	2-8667	pbertics@macc.wisc.edu Fax: 2-5253
Bird, Ian **Associate Professor**	Obstetrics & Gynecology	7E Meriter Hospital/Park 202 South Park Street	267-6314 **267-6240**	imbird@facstaff.wisc.edu Fax: 257-1304
Bosu, William **Professor**	Animal Health & Biomedical Science; Sch. of Vet. Med	2031 Veterinary Medicine Building 2015 Linden Drive	2-8106	bosuw@svm.vetmed.wisc.edu Fax: 5-6748
Downs, Karen **Assistant Professor**	Anatomy	263 Bardeen Medical Labs 1300 University Avenue	5-5411	kdowns@facstaff.wisc.edu Fax: 2-7306
Duello, Theresa **Associate Professor**	Obstetrics & Gynecology	5240 Medical Sciences Center 1300 University Avenue	2-7456	tmduello@facstaff.wisc.edu Fax: 2-7454
First, Neal **Professor**	Animal Science	752 Animal Sciences Building 1675 Observatory Drive	3-4307	nlf@calshp.cals.wisc.edu Fax: 2-5157
Fricke, Paul **Assistant Professor**	Dairy Science Cooperative Extension	278 Animal Sciences Building 1675 Observatory Drive	3-4596	fricke@calshp.cals.wisc.edu Fax: 3-9412
Ginther, Oliver **Professor**	Animal Health & Biomedical Sciences	B1 Animal Health & Biomedical Sc. 1656 Linden Drive	2-1037	ojg@ahabs.wisc.edu Fax: 2-7420
Golos, Thaddeus **Associate Professor**	Obstetrics & Gynecology; Primate Center	115 Primate Center 1220 Capitol Court	3-3567	golos@primate.wisc.edu Fax: 3-3524
Jefcoate, Colin **Professor**	Pharmacology;	3630 Medical Science Center 1300 University Avenue	3-3975	jefcoate@facstaff.wisc.edu Fax: 2-5245
Magness, Ronald **Professor**	Obstetrics & Gynecology	7E Meriter Hospital/Park 202 South Park Street	267-6314 **267-6498**	rmagness@facstaff.wisc.edu Fax: 257-1304
Martin, Thomas **Professor**	Biochemistry	5519 Biochemistry 420 Henry Mall	3-2427	tfmartin@macc.wisc.edu Fax: 2-3453
Ntambi, James **Associate Professor**	Biochemistry & Nutritional Sciences	180A Biochemistry Bldg. 420 Henry Mall	5-3700	ntambi@biochem.wisc.edu Fax 2-3453

Endocrinology-Reproductive Physiology Program Faculty Members (Continued)				
Name	**Department**	**Address**	**Phone #**	**E-Mail and Fax #**
Parrish, John **Professor**	Animal Science	714 Animal Sciences Building 1675 Observatory Drive	3-4324	parrish@calshp.cals.wisc.edu Fax: 2-5157
Peterson, Richard **Professor**	Pharmacology	5203 Chamberlin Hall 425 N. Charter Street	3-5453	rep@pharmacy.wisc.edu Fax: 5-3316
Schuler, Linda **Professor**	Comparative Biosciences; Sch. of Vet. Med.	4354/B Veterinary Medicine Bldg. 2015 Linden Drive	3-9825	schulerl@svm.vetmed.wisc.edu Fax: 3-3926
Shapiro, Sander **Professor**	Obstetrics & Gynecology	H4/630 Clinical Science Center 600 Highland Avenue	3-1217	ss.shapiro@hosp.wisc.edu Fax: 3-0191
Sheffield, Lewis **Professor**	Dairy Science	864 Animal Sciences Building 1675 Observatory Drive	3-9867	lgsheffi@facstaff.wisc.edu Fax: 3-9412
Smith, Susan **Associate Professor**	Nutritional Sciences	340A Nutritional Sciences 1415 Linden Drive	3-4316	suesmith@nutrisci.wisc.edu Fax: 2-5860
Terasawa, Ei **Professor**	Dept. of Pediatrics; Primate Center	217 Primate Center 1223 Capitol Court	3-3579	terasawa@primate.wisc.edu Fax: 3-3524
Thomson, James **Associate Professor**	Department of Anatomy: Primate Center	Primate Center 1220 Capitol Court	3-3585	thomson@primate.wisc.edu Fax: 3-3517
Wentworth, Bernard **Professor**	Animal Science	562 Animal Sciences Building 1675 Observatory Drive	2-8945	wentwort@calshp.cals.wisc.edu Fax: 2-5157
Wiltbank, Milo **Associate Professor**	Dairy Science	856 Animal Sciences Building 1675 Observatory Drive	3-9413	wiltbank@calshp.cals.wisc.edu Fax: 3-9412

All telephone numbers have a 608 area code plus the numbers 26 before each given telephone number.
University of Wisconsin-Madison postal zip code is 53706

Endocrinology-Reproductive Physiology Program Office Staff				
Name	**Department**	**Address**	**Phone #**	**E-Mail and Fax #**
Bachmann, Tiffany	Endocrinology-Reproductive Physiology Program	260 Animal Science Bldg. 1675 Observatory Dr.	2-3222	tabachmann@facstaff.wisc.edu Fax: 2-5157

Endocrinology-Reproductive Physiology Program Student Directory

Student	Email	Degree Objective	Major Professor
Lori Anderson	leanders@students.wisc.edu	Ph.D.	Wiltbank
Zeki Beyhan	zbeyhan@students.wisc.edu	Ph.D.	First
Michael Byers	mjbyers@facstaff.wisc.edu	Ph.D.	Magness
Jacqueline Cale	jmcale@students.wisc.edu	MD/Ph.D.	Bird
Gabriella Cezar	gcezar@infigen.com	Ph.D.	First
Tien-cheng (Arthur) Chang	tchang2@students.wisc.edu	Ph.D.	Golos
Francisco Diaz	fjdiaz@students.wisc.edu	Ph.D.	Wiltbank
Xavier Donadeu	donadeu@ahabs.wisc.edu	Ph.D.	Ginther
Joel Eisner	jeisner@students.wisc.edu	Ph.D.	Abbott
Lefric Enwall	leenwall@students.wisc.edu	Ph.D.	Parrish
Xuezhu Feng	xfeng@primate.wisc.edu	Ph.D.	Thomson
Jennifer Gavinski	jjgavins@students.wisc.edu	Ph.D.	Sheffield
Behzad Gerami	bgeraminaini@students.wisc.edu	Ph.D.	Golos
Tiffini Gibson	tgibson@students.wisc.edu	MS	Magness
Shannon Gifford	sgifford@students.wisc.edu	Ph.D.	Bird
Jim Haughian	haughian@students.wisc.edu	MS	Wiltbank
Jeffery Joyce	jmjoyce@students.wisc.edu	MS	Magness
Adam King	agking@students.wisc.edu	MS	Magness
Kinarm Ko	kinarmko@students.wisc.edu	Ph.D.	Peterson
Lindsey LaPlante	lnlaplan@students.wisc.edu	MS	Parrish
Yun Li	yunli@students.wisc.edu	Ph.D.	Magness
Juu-Chin Lu	jlu@students.wisc.edu	Ph.D.	Schuler
Rasterifi Medley	rdmedley@students.wisc.edu	Ph.D.	Abbott
Pavla Misica	pmisica@infigen.com	MS	First
Christina Pattison	jcpattison@students.wisc.edu	Ph.D.	Bird
Dinesh Rao	drao@students.wisc.edu	MS	Golos
Jacqueline Smith	jlsmith23@students.wisc.edu	Ph.D.	Sheffield
Jeremy Sullivan	jasullivan@students.wisc.edu	Ph.D.	Bird
Michael Wolfgang	wolfgang@primate.wisc.edu	Ph.D.	Golos
Rao Zhou	raozhou@primate.wisc.edu	Ph.D.	Abbott

Beckman Coulter Ad

Thank You
Fryer Company Inc.

Thank You
Department of Animal Sciences

UW
Animal Sciences
University of Wisconsin-Madison

Dr. Daniel Schaefer, Chair