2000 Endocrinology-Reproductive Physiology

Annual Symposium

University of Wisconsin Madison
Memorial Union

Friday, September 15, 2000

Symposium Schedule
Friday, September 15, 2000

8:00 Registration and Poster Set up (refreshments and pastries)

9:15 Opening Statements

9:20 Keynote Speaker – Joan S. Hunt, Ph.D.
"Immune Mechanisms in Pregnancy
Introduction by Ronald Magness

:20 minute refreshment break

11:00 Talks

Jeremy Sullivan *Differential control of eNOS by multiple agonists: $Ca^{2~}$ and non-$Ca^{2~}$ dependant eNOS activation in UA endothelial cells (UAEC).*

Jacqueline Cale *Use of micro array membranes to characterize differential gene expression in cultured and freshly isolated uterine artery endothelial cells.*

Don Neff *Effects of aspirin and tylenol on the release of luteinizing hormone-releasing hornione in cultured rat hypothalami.*

Michael Byers *Estrogen receptor expression and steroid regulation in ovine uterine arteries.*

12:00 Lunch Break

1:00 Invited Speaker – Reinhold J. Hutz, Ph.D.
"Modulation of the Female Reproductive Axis by Dioxins"
Introduction by Ted Gobs

2:00 Talks

Francisco Diaz *PGF_2a d~erentially regulates LHr mR/VA, but not cfos/cjun mRNA in porcine CL after acquisition of luteolytic capacity.*

Michael Wolfgang *Nonsurgical transfer of in vivo and in vitro produced blastocysts in the rhesus monkey.*

Jim Haughing *Effect of estradiol cypionate in early postpartum dairy cattle.*

Sheirlie Vella *Use of a novel cell per~fusion system for the study of hypothalmnic and pituitary hormone release*

3:40-4:30 Closing Statements and Poster Session

Joan S. Hunt, Ph.D.

University of Kansas Medical Center
Departments of Anatomy and Cell Biology and
Pathology and Laboratory Medicine

"IMMUNE MECHANISMS IN PREGNANCY"

In natural pregnancies, the embryo/fetus is genetically different from the mother because of carrying a complement of paternal genes. This situation comprises one of the major paradoxes of contemporary immunology, raising the question of how the genetically different embryo/fetus and mother co-exist during gestation. Human pregnancy, where cells from the implanted blastocyst migrate in massive numbers into the decidualized maternal endometrium, presents the greatest challenge.

Over the course of the past two decades, many conditions providing protection to the human fetal semiallograft have been identified. These are provided by both the mother and by the extraembryonic tissues of the embryo/fetus. They include soluble substances such as prostaglandins, progesterone and anti-inflammatory cytokines that include transforming growth factor-p and interleukin-l 0. All of these are produced in the female reproductive tract during pregnancy and are present in the uterus and placenta.

Remarkably, the placenta and extraplacental membranes themselves have evolved an array of powerful protective mechanisms. To prevent destruction by maternal complement-fixing antibodies to paternally-derived and fetal antigens, the placenta exhibits high levels of the cell surface regulatory proteins that interrupt the complement cascade. Recent experiments in knockout mice show clearly that in the absence of appropriate complement regulatory proteins, complement is activated at the implantation site and pregnancy is prevented. To protect against maternal cytotoxic immune cells directed against paternally-derived major histocompatibility antigens, known in humans as the HLA antigens, the placenta exerts strict control over their expression. Unlike other organs, placentas do not express HLA class II antigens, and the major placental HLA class I antigens, HLA-E and HLA-G, have few alleles and are unlikely to be recognized as foreign by the mother. HLA-E expressed on migrating trophoblast cells appears to prevent cytotoxic activity in natural killer cells, which are abundant in the uterus, and HLA-G may inhibit macrophage cytotoxicity. Circulating soluble HLA-G may have a profound suppressive effect on maternal cytotoxic cells.

Placental immune privilege may also be conferred by members of the tumor necrosis factor (TNF) supergene family, as is the case in other organs such as the eye and testis. All 8 of the apoptosis-inducing ligands and all but one of the 10 receptors capable of transducing death signals are expressed in human placentas. Of the 8 ligands, at least two, Fas ligand and TRAIL, seem to have roles in protecting the placenta and extraplacental membranes from attack by maternal immune cells. Receptors are also protective, with soluble TNF receptors protecting against TNFα and LTα, dysfunctional receptors protecting against FasL and decoy receptors protecting against TRAIL.

In summary, multiple mechanisms come into play during human pregnancy which, collectively, provide a safe environment for the developing fetus by circumventing normal immunological reactions in the mother that could result in recognition and rejection of foreign tissue.

Reinhold J. Hutz, Ph.D.

University of Wisconsin-Milwaukee
Department of Biological Sciences

"Modulation of the Female Reproductive Axis by Dioxins"

Environmentally relevant concentrations of tetrachlorodibenzo-p-dioxin (TcDD, dioxin) inhibit ovarian steroid production by reducing levels of rnRNAs for steroidogenic enzymes, and induce programmed cell death in granuiosa cells: TCDD has been referred to as the most potently toxic chemical known to man. It is a by-product of chlorination procedures, paper production, herbicide overuse, certain plastics production *(e.g.,* polyvinylchloride [PVC]), wood burning, and general waste incineration. TCDD has been shown to be anti-estrogenic and to affect fertility in certain model systems. In these NIEHS-sponsored studies, we show that very low concentrations of TCDD (pM and nM), similar to those present in the environment, can inhibit *in vivo* and *in vitro* the production of the female hormone estrogen by the cells that produce it in the ovary; that this effect is at last partly transcriptionally mediated, as reflected in the reduced steroidogenic enzyme mRNAs (quantified using competitive RT-PCR); and that TCDD causes programmed cell death (apoptosis) in these same cells, as detennined by augmented immunofluorescence of DNA fragmentation. These data support our contention that in addition to TCDD affecting other components of the female reproductive axis, it exerts a direct effect on the ovary and estrogen-signaling pathway. These discoveries provide insight into some of the anti-fertility effects exerted by this ubiquitous, noxious environmental contaminant.

A DIFFERENTIAL CONTROL OF eNOS BY MULTIPLE AGONISTS: Ca2+ AND NON-Ca2+ DEPENDANT eNOS ACTIVATION 114 UA ENDOTHELIAL CELLS (UAEC). JA Sullivan[1], T Di[1], RR Magness[1,2] and IM Bird[1], *Departments of Obstetrics & Gynecology, Perinatal Research and Animal Science[2], University of Wisconsin Madison, Madison WI 53715.*

Dramatic changes occur during pregnancy to promote healthy gestation and maintain optimal fetal development. A number of pregnancy related changes support increasing blood flow to the uteroplacental unit, including an upregulation of uterine artery endothelial capacity for vasodilator biosynthesis (increase in eNOS) and increases in agonist stimulated Nitric Oxide (NO) production. We have developed a UAEC model that retains pregnancy-rebated differences in agonist stimulated vasodilator production in cells from nonpregnant (NPUAEC) and pregnant (PUAEC) ewes. In PUAEC, AII, ATP, bFGF and EGF stimulate an increase in NO production, while only bFGF and EUF stimulate NO in NPUAEC. Activation of eNOS is believed to be Ca2+ sensitive, but recently, kinases such as MAPK family members and PKB/Akt have been implicated in the Ca2+ independent activation of eNOS. Comparison of agonist signaling in UAEC shows different agonists working through distinctly different pathways, strongly suggesting that changes in signaling pathways may in part underlie pregnancy-induced changes in eNOS activation by specific agonists. Only ATP (in PUAEC and NPUAEC) stimulate detectable increases in Ca2+. AII, ATP, bFGE and EGF substantially activate a prototypical member of the MAPK family, ERK 1/2 in P-UAEC. In contrast, AII, ATP and bFGF fail to retain ERK 1/2 activation in NPUAEC whereas EGF does retain ERK 1/2 activity. The inability of AII and ATP to stimulate ERK 1/2 in NPUAEC may explain the lack of All or ATP stimulated NO. However, the lack of ERK 1/2 activation by bFGE accompanied by the retention of bFGF to stimulate NO in NPUAEC leads us to believe alternate signaling pathways are involved in the bFGF activation of eNOS. All, ATP and bFGF in both P and NPUAEC give poor Akt activation. EGF, in PUAEC, also gives a weak Akt activation but in NPUAEC, EGF leads to a dramatic *(5-6* fold) increase in Akt activity. EGF, therefore, is the only agonist which in NPUAEC retains ERK 1/2 activation and stimulates Akt. We conclude that: All and ATP may acquire the ability to stimulate eNOS in PUAEC through coupling to ERK1/2. bFGF stimulates eNOS in NPUAEC through a non-ERK 1/2 or Akt pathway. EGF signaling differs substantially from that observed with other growth factors (bFGF) and more classical hormone related signals (AII,ATP). The retention of ERK 1/2 activation in NPUAEC and the novel stimulation of Akt in our model, provide two distinct or possibly one integrated pathway where EGF could activate eNOS in a Ca2+ independent fashion. Supported by USDA 9601773, NIfl-HL56702, HL49210 and HD33255.

B. Use of Micro Array Membranes to Characterize Differential Gene Expression in Cultured and Freshly Isolated B Uterine Artery Endothelial Cells. Jacqueline M. Cale*, Steven Tsoi, Ronald R. Magness[1], Ian M. Bird. Dept. Ob/Gyn, UW-Madison, 'Dept. Animal Sciences, UW-Madison

Background: During pregnancy, the uterine artery endothelium markedly increases production of the vasodilators nitric oxide (NO) and prostacyclin (PCI_2) through probable distinct intracellular signaling pathways as compared to the nonpregnant state. We have established an ovine uterine artery endothelial cell culture system (UAEC) derived from pregnant (P-UAEC) and nonpregnant (NP-UAEC) ewes (Endocrinology 141, 2000). P-UAEC have retained pregnancy-specific coupling to the MAPK pathway at the level of ERK 1/2 with an associated stimulation of NO and PGI_2 production. **Hypothesis:** Characteristic gene expression pertinent to signaling pathways of the P- and NP-UAEC is retained in our culture system. Using micro array technology, we are able to approach this investigation from a genomics standpoint. **Methods:** Total RNA was extracted from either passage 4 cells (80-90% confluent) or freshly isolated uterine artery endothelium (UAEndo). 33-P labeled cDNA was generated using poly dT primer and Superscript II RT. Following hybridization and washing, the gene filters were exposed to Hyperfilm-MP. Autoradiographs were analyzed with Research Genetics Pathways 2 software. **Results and Conclusions:** Both the UAEC and the UAEndo showed mRNAs that encode proteins essential to heptahelical, growth factor, and insulin signaling pathways functional in our model. Few striking differences were seen in mRNAs between the UAEC and UAEndo. The array experiment demonstrated a 5-fold increase (UAEC/UAEndo) in connective tissue growth factor (CTGF) mR.NA. Increased CTGF in culture may assist endothelial cell growth until contact inhibition or reflect the absence of a basement membrane for UAEC.

C EFFECTS OF ASPIRIN AND TYLENOL ON THE RELEASE OF LUTEINIZING HORMONE-RELEASING HORMONE IN CULTURED RAT **HYPOTHALAMI.**

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The research in our laboratory has been focused on the broad topic of cell-cell communication in a particular neuroendocrine network, that controlling luteinizing hormone-releasing hormone (LHRH) release in the median eminence. LHRH controls pituitary effects on reproduction in males and females, and is the central regulator of reproductive function. Given this, we are interested in regulation of pulsatile LHRH release. Prostaglandins have been implicated as one of several possible regulatory intermediaries in LHRH release. Our study used isolated rat hypothalami in a perifusion culture system to evaluate LHRH release patterns in response to treatment with aspirin and tylenol. The primary action of these conirnon medications is through inhibition of arachidonate cyclo-oxygenase, an important enzyme in pro staglandin synthesis. Infusion of aspirin resulted in a reduction in LHRFI release in 12 of 12 chambers (Preaspirin = 5.71±0.79 vs. Aspirin infusion = 0.97±0.17 pg/ml; $p<0.0)$. Later infusion of Tylenol resulted in consistent, but less dramatic reduction in LHRH release (PreTylenol = 2.51±0.39 vs. Tylenol = 1.81±0.28 pg/ml; ns). These preliminary results suggest a role for prostaglandins in LHRH regulation. Further, there may be reproductive implications to the widespread use of these medications that have not been considered.

D Estrogen Receptor Expression and Steroid Regulation in Ovine Uterine Arteries. MJ Byers*, DB Chen&, IM Bird, HL Rupnow[1], TM Phernetton&, RR Magness[12]. Perinatal Res. Labs, Dept of Ob/Gyn[1] & Animal Sciences[2], Univ. of WI-Madison.

The pregnancy-associated rise in estrogen is associated with a dramatic increase in utero-placental blood flow, 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. However, ER expression and cellular origin in uterine arteries (UAs) are currently unknown. **Objective:** To examine the expression, cellular localization, and steroid regulation of ERs in ovine UAs. **Methods**: UAs were fixed and mounted for sectioning and mRNA analysis. ER mRNA localization was determined by *in situ* hybridization (ISH) using [^{35}S]-labeled riboprobes. UA endothelial protein (UAendo) was isolated and the presence of ER protein was determined via Immunoblotting. To study steroidal regulation of UAendo ERα protein expression, ovex ewes were treated with vehicle, chronically with estrogen, progesterone, or estrogen in combination with progesterone. **Results:** We observed by Immunoblot analysis that ERα and ERβ protein were present in ovine UAendo. In addition, when compared to controls, treatment with estrogen, progesterone or their combination increased levels of ERα protein in the UAendo of ovex ewes. Also, 1511 experiments revealed the presence of ERα and ERβ mRNA in the UAendo. **Conclusion:** Taken together, we show that ER protein and mRNA are expressed in UAendo and are regulated by exogenous treatment with ovarian steroids, indicating UA endothelium is a target for estrogen actions *in vivo (Supported by NIH HD33255, HL57653, HL49210, HL57602 and NIEHS).*

E **PGF_2a differentially regulates LHr mRNA, but not cfoslcjun mRNA in porcine CL after acquisition of luteolytic capacity.**

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Porcine CL acquire luteolytic capacity (ability to regress to a single $PGF_2\alpha$ treatment) after day 12 of the estrous cycle. The mechanisms involved in this process remain undefined. $PGF_2\alpha$ regulates many physiological phenomena in CL with luteolytic capacity, including inhibition of LH receptor mRNA and induction of cfos/cjun mRNA. We hypothesized that cloprostenol (PGFZU analog) would inhibit LFr mRNA and induce cfos/cjun mRNA in d17 pseudopregnant (with luteolytic capacity), but not in d9 (without luteolytic capacity) CL. Methods: Mature gilts were randomized as follows: d9 saline *(n=5),* d9 cloprostenol *(500* μg w=4), dI7 pseudopregnant saline (n=5) and d17 pseudopregnant cloprostenol (500 μg *n=5).* Treatments were given 10 hours prior to ovariectomy. A separate group of gilts were randomized as above (n4/5 group). On d9 or d17 one ovary was removed (control). Immediately, 500 μg of cloprostenol was given and the other ovary removed *0.5* h later. **Results:** Cloprostenol had no acute *(0.5* h) effect on LHr mRNA or late (10 h) effect on cfos/cjun rnRNA. Cloprostenol acutely *(0.5* h) induced cfos mR.NA in both d9 CL (control 0.037±0.010; cloprostenol 0.889±0.203; $p<0.05$) and dll CL (control 0.063±0.043; cloprostenol 1.093±0.138; $p<0.05$). Cloprostenol also acutely induced cjun mRNA in both d9 CL (control 0.505±0.102; cloprostenol 1208±0.077; $p<0.05$) and dl 7 CL (control 0.488±0.150; cloprostenol 1.72±0.304; $p<0.05$). At 10 h, cloprostenol inhibited LHr mRNA in dl 7 CL (saline 556±85; cloprostenol 248±36; *$p<0.05$)* but not in d9 CL (saline *475±140;* cloprostenol 443±98). **Conclusion:**Acquisition of luteolytic capacity is associated with $PGF_{2\alpha}$-Mduced decrease in LHr mRNA, but not with differential induction of the early response genes cfos/cjun.

F **Effect of Estradiol Cypionate in Early Postpartum Dairy Cattle**

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Estradiol cypionate (ECP) is frequently used to treat uterine problems in early postpartum (PP) cattle; however, there are concerns that ECP may increase the incidence of follicular cysts. This study examined the effects of ECP on follicular dynamics in dairy cattle. Lactating Hoisteins received 10 mg ECP (ECP; n=17) or placebo (CON; n=16) on d7 PP. Data were collected from *d5-90* of lactation and included daily serum samples and every other day ultrasound examination of ovarian activity. After d90, all animals were bred using the Ovsynch protocol (GnRH-7d-PGF-2d-GnRH- 1 d-AI). Detection of the first PP follicle $\geq$10 mm was later in ECP (29.1±7. id) than CON (12.4±3.ld). First PP ovulation was delayed in ECP *(55.6±4.ld)* vs. CON (37.9±4.9d). Ovulation prior to 30d in milk occurred in 38% of CON but in none of the ECP cows. A total of 69% of CON cows had ovulated at least once by 50d; whereas, only *35%* of ECP cows. Nevertheless, when cows were evaluated at the time of Ovsynch (90d) regular estrous cycles were found in only 50% of CON but in 88% of ECP cows. The reasons for lack of cyclicity were no ovulation by 90d PP (3 CON, I ECP), ovulation followed by anovulatory follicular waves (3 CON), and persistent corpus luteum (>50d; 2 CON, 1 ECP). Only *3* cows developed follicular cysts (follicle$\geq$ 25mm; 2 CON, 1 ECP). Thus, although treatment with ECP on day 7 postpartum delayed the time to first ovulation, it did not induce follicuhir cysts, and it decreased cows with reproductive problems at 90 d PP.

G **Nonsurgical Transfer of *in vivo* and *in vitro* Produced Blastocysts in the Rhesus Monkey.**

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The ability to obtain viable offspring from preimplantation embryos is the first step in developing transgenic technologies. We have developed a unique nonsurgical and noninvasive technique to transfer uterine stage rhesus monkey embryos. Embryos were produced by *in vitro* fertilization from gonadotropin stimulated monkeys or were obtained by nonsurgical uterine flushing of naturally mated or artificially inseminated females. Nonsurgical transfer was accomplished by inserting a metal guide through the cervix into the uterus, after which a hollow cell sampler was inserted over the guide. The guide was removed and a catheter was inserted containing 1-5 embryos. Several pregnancies resulted from *in vitro* and *in vivo* derived blastocysts with two term pregnancies resulting in one live birth. The recipients received progesterone as a subcutaneous implant or daily injections in corn oil from the day of transfer. Bloods were taken regularly to monitor levels of plasma chorionic gonadotropin, luteinizing hormone, estrogen and progesterone. The establishment of nonsurgieal embryo transfer in the rhesus monkey will diminish problems associated with surgical transfer, enable further investigation of embryos beyond the eight-cell stage, and provide a noninvasive alternative to deliver late stage embryos. We have established several pregnancies with *in vitro* and *in vivo* produced embryos and have obtained live offspring. The results of this study provide the means to explore the transfer of transgenic and chimerie models in the monkey.

H **USE OF A NOVEL CELL PERIFUSION SYSTEM FOR THE STUDY OF HYPOTHALMIC AND PITUITARY HORMONE RELEASE**

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This project uses a flow-through cell perifusion system (Acusyst) which allows continuous sampling of Luteinizing Hormone (LH) from pituitary and hypothalamic cell culture. Rat pituitary tissue was used to determine the efficacy of this system for use with common marmoset tissue. Colleagues at the Wisconsin Regional Primate Research Center have been investigating *in vivo* release of LH and LHRH in the marmoset. The marmoset is a primate with a reproductive strategy linked to social order. Only the dominant female undergoes reproductive cycles, but determining the mechanism that suppresses cycles in subordinate females has been difficult. By performing cultures of three or more days employing *in vitro* methods already in use in our laboratory, we hope to complement *the in vivo* studies by limiting uncontrollable variables. Rat hypothalamic tissue was perifused and samples were collected for 8-10 hours in a refrigerated fraction collector and assayed for LHRH by radioimmunoassay. Rat pituitary was perifused similarly under varying culture conditions (media and serum) and samples were assayed for LH concentration. By running the culture for three days, we can extend the amount of data eollectable from each piece of marmoset tissue. Our preliminary results are encouraging but require further evaluation. Indications are that we will be successful in using this system to culture marmoset tissues for several days.

1. Angiogenic Factors Modulate Shear Stress Induced Nitric Oxide (NO) Production and eNOS Expression by Ovine Fetoplacental Artery Endothelial (OFPAE) Cells

Y. LP*, J. Zheng [1]*, I.M.Bird[1]. and R.R. Magness[1,2]. *Perinatal Res Labs, Dept Ob/Gyn[1] and Anim Sci[2], UW-Madison, WI 53715.*

Placental blood flow, endothelial NO production, endothelial nitric oxide synthase (eNOS) expression, and angiogenic factors secretion increase in placental vasculature during pregnancy. Shear stress is the frictional force exerted on endothelial cells with increasing blood flow, and it induced NO production by ovine fetoplacental artery endothelial (OFPAE) cells. *Hypothesis:* Angiogenic factors, bFGF and/or VEGF may modulate shear stress induced increases in NO production and eNOS expression by OFPAE cells. *Methods:* OFPAE cells were inoculated ($5xlO^6$) into CellMAX artificial capillary modules and were grown at a shear stress of 3 dynes/cm^2 till they reach confluence. Then they were exposed to shear stresses of 10, 15 or 25 dynes/cm^2 (physiologic range ≈12-15 dynes/cm^2) for 24 hrs. If angiogenic factors were tested, confluent OFPAE cells were pretreated with bFGF 10ng/ml) or VEGF (l0ng/mi) for 30 mm. Culture media were sampled from the cartridge for NO measurement. OFPAE cells were recovered from each cartridge by trypsin/EDTA, cell pellets were subjected for protein or RNA extraction for future Western blotting or RT-PCR. *Results:* Basal shear stress of 3 dynes/cm^2 for 24 hours did not alter either eNOS or GAPDH protein expression. However eNOS but not GAPDH protein levels were increased at 10 and *25* dyncs/cm^2 for 6 hours and continued to go up till 24 hours. And the effects of 25 dynes/cm^2 on cNOS expression were more prominent than 10 dynes/cm^2. Moreover, we found bFGF but not VEGF tended to further increase NO produced by OFPAE cells at 15 dynes/cm^2, while there were little or no further increases in eNOS expression by bFGF or VEGF after 24 hours at either 3 or 15 dynes/cm^2. *Conclusion:* Shear stress induces eNOS mRNA and protein expression by OFPAE cells. Angiogenic factors bFGF but not VEGF tends to further augment NO production by OFPAE cells, while the effects were not through elevating eNOS protein expression.

2. Endogenously Expressed Estrogen Recptor and Coactivator AIB1 Interact in MCF-7 Human Breast Cancer Cells

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Coactivators mediate estrogen induced gene responses via interaction with estrogen receptors (ER). Currently, a major challenge is to determine the importance of each coactivator in a specific cell type and promoter context in response to a particular ligand. The potential of ER to interact with a growing list of coactivators has been shown in a variety of *in vitro* and gene transfer assays, yet very few data have demonstrated the interaction of endogenous coactivators with ER in intact cells. We can release liganded ER from nuclei with low concentrations of polyanions, minimizing disruption of protein complexes formed *in situ.* We observe a ligand dependent interaction of ER and the AIB 1 coactivator in MCF-7 human breast cancer cells using immunoprecipitation and sucrose density gradient analyses. Complexes are induced by estradiol and to a much lesser extent by the partial agonist, monohydroxytamoxifen. We were unable to detect an ER-SRC-l complexes from MCF-7 cells. The *in vitro* binding affinity for ER interaction with AIB 1 was estimated to be 80 ± 40 nM. We conclude that AIB I is a major coactivator for ER in MCF-7 cells. The AIB 1 coactivator is overexpressed in 64% of primary human breast cancers (Science 277:5328). Our data are consistent with the hypothesis that AlE I :ER interaction is important in promoting estrogen-dependent breast cancer.

3. Identification of Genes Expressed During Preimplatation Bovine Embryo Development

'ESTHER OFULUE AND NEAL L. FIRST[2].
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We have used Differential Display PCR, Reverse Northern dot-blot, and RT-PCR to locate and clone transcribed sequences of thirty-two genes that are differentially expressed at in vitro fertilized preimplantation embryo stages of 2-cell, 8-cell, 16-cell, compacted morula, and blastocysts. Genbank sequence analyses revealed that pEN 14B, pEN 14C, pEN22, and pEN23 share 90%, 97%, 76% and 94% homology, respectively to the 3' ends of human thermostable phenol sulfo-transferase gene, human cytoplasmic chaperonin hTRiC5 mRNA, putative prenylated protein gene, and human mRNA for alanyl-tRNA synthetase. These genes are differentially expressed from the embryonic genome at the morula and blastocyst stages. pEN21 and pEN35 which are expressed at all embryo stages but at low levels at the 2-cell stage, share 100% homology with bovine mitochondrial geneme regions encoding NADH dehydrogenase subunits 4 & 5 and ATPase subunit 6, respectively.

Several other genes with interesting patterns of stage-specific expression (seeTable 2) which may relate to stage-specific functions share homologies with known genes as shown in Table 1. The pattern of expression of these genes in nuclear transfer cloned bovine embryos is being examined in order to provide some explanations for the abnormal or failed development of cloned animals.

4. MT3: Mammalian Cells Stably Expressing Wild-type Estrogen Receptor.

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The study of the estrogen receptor (ER) has led researchers to take many approaches to further characterize its function. Among these, transient transfection has been widely utilized. Studies in our lab, however, suggest that cells that are transiently transfected fail to regulate ER concentration in response to estrogen treatment. Historically, stable introduction of ER has proven to be difficult, however, leading to the idea that ER was lethal to ER- cells. Using a retroviral expression vector, we introduced ERa into HEK293 (human embryonic kidney) cells that normally do not contain ER. Colonies were selected for hygromycin resistance and screened by Western blot analysis for ER. Binding analysis of a selected cell line, MT3, showed the amount of receptors per cell to be 134,732 with a Kd of 0.24 nM. The ER in the MT3 cells was shown to be transcriptionally functional by measuring the induction of a transfected estrogen responsive reporter plasmid. Proliferation studies revealed that estrogen arrests MT3 cells in the Go/Gl phase, which may explain the perceived effect of toxicity. In contrast to transient systems, when treated with various amounts of estrogen, the estrogen response of down-regulation was intact in MT3. These data shows that estrogen responsiveness can be reconstituted in a cell that does not endogenously produce ER. The generation of MT3 cells demonstrates that ER is not lethal and provides a novel and practical model system for the study of ER action.

5. **Differential Control of eNOS vs cPLA2 activation via Ca2+ and ERK1/2 in UAEC.**

Tao Di, Jeremy Sullivan, Ronald Magness and Ian Bird.
Dept OB/Gyn, Perinatal Research Laboratories. & Dept Animal Science, Madison WI 53715.

We have developed a culture model for Uterine Artery Endothelial Cells (UAEC) that retains poor PGI2 synthesis and no detectable NO production in response to ATP when isolated from nonpregnant ewes (NP-UAEC), but shows enhanced PGI2 and NO production when isolated from pregnant ewes (P-UAEC). ATP can stimulate both Ca2+ mobilization and activate ERK-l/2 via MEK in P-UAEC, but shows reduced Ca2+ mobilization and poor ERK-l/2 activation in NP-UAEC. While Ca2+ responsiveness correlates well with PGI2 biosynthesis in NP vs P-UAEC, ERK-activation colTelates better with NO production. We have investigated this relationship more directly by examining the effects of Ca2+ blockade (using 10 uM BAPTA) vs MEK blockade (using U0126) on ATP stimulated NO and PGI2 production in NP vs P-UAEC. In P-UAEC, BAPTA was able to block ATP-stimulated Ca2+ mobilization but only marginally inhibit ATP-stimulated ERK-l/2 phosphorylation. UO 126 was able to fully block ATPstimulated ERK-l/2 phoshorylation to below basal levels but had no effect on ATPstimulated Ca2+ mobilization. BAPTA pretreatment of P-UAEC largely inhibited NO production while UO126 fully inhibited NO production. In contrast both BAPTA and UO 126 were fully effective in blocking ATP-stimulated PGI2 production by P-UAEC and blocking the lesser response in NP-UAEC. Thus Ca2-f- and ERK-I/2 may both be key regulators of PGI2 production in UAEC but NO production is more dependent on ERK1/2 activation than Ca2+ mobilization. *Supported by USDA 9601 773 and NIH HL56702.*

6. **Steroid hormones and paternal care in the monogamous mouse, *Peromyscus californicus***

Brian C. Trainor* & Catherine A. Mailer
Department of Psychology, University of Wisconsin-Madison, Madison, WI 53706

The importance of the steroid hormone testosterone (T) in the control of paternal behavior was investigated in the California mouse, *Peromyscus Galifornicus.* Surprisingly, castrated males provided significantly less paternal care than sham and castrated males with T-implants. This finding is contrary to experimental manipulations in other polygynous species. These results suggest that the low levels of T (mean = 0.5 ng/ml) found in male California mice play an important role in the maintenance of paternal behavior. There are at least two explanations for these results. First, and rogens in the form of T or dihydrotestosterone could directly maintain paternal behavior. A related alternative is that T is aromatized into estrogen in the brain and that estrogen maintains paternal behavior. Current research is underway to distinguish between these hypotheses.
Supported by: Sigma Xi GIA to B.C.T. & NSF IBN-9703309 to C. A. M.

7. **Follicular cysts in cattle involve an alteration in the hypothalamic action of estradiol due to lack of progesterone exposure.**

A Gumen*, MC Wiltbank; Dept Dairy Sci, Univ WI-Madison

Cattle can develop large ovarian follicular structures termed follicular cysts. The physiological mechanism(s) leading to this condition remain undefined. We hypothesized that follicular cysts develop because estradiol can induce a GnRFI/LFI surge on one occasion but progesterone exposure is required before another GnRH/LH surge can be induced by estradiol. In expt 1; 14 cows were synchronized with intravaginal progesterone implants (IFI) for 7 d and $PGF_{2\alpha}$ was given on the day of [P1 removal. Estradiol benzoate (EB, 5 mg) was given 3 d before WI removal to induce atresia of follicles. Cows were given a second ER (5 mg) treatment I d after IFI removal to induce a GnRH/LH surge in the absence of an ovulatory follicle. All cows had an LH surge following the estradiol treatment and 10 of 14 cows developed a large follicle anovulatozy condition (LFAC) that resembled follicular cysts. These LFAC cows were given a third ER *(5* mg) treatment 15 d after IPI removal and none the cows had an LII surge or ovulation. Cows were then untreated (n5 control) or treated for 7 d with an IPI (n=5) starting 7 d after third ER injection. Cows were treated for a fourth time with *5* mg EB 12 hr after WI removal. All WI but no control cows had an LH surge in response to estradiol and ovulated. In expt 2; 10 of 20 cows had LFAC after the same protocol in expt I. After the third ER treatment cows with LFAC were divided into 4 groups: l)LPI±EB, 2)IPI±GnRH, 3)control+EB, and 4)control+GnRH. EB *(5* mg) or GnRH (100 gg) were given 12 hr after WI removal. All GnRII treated cows had a similar LH surge and ovulation. In contrast, only IPI treated cows had an LH surge following ER. Thus, an initial GnRH/LH surge can be induced with high estradiol but estradiol induction of a subsequent GnR/LH surge requires prior progesterone exposure. This eftéct is mediated in the hypothalamus as evidenced by similar LII release in response to exogenous GnRH.

8. **Role of Ubiquitin and Dynamin in Prolactin Receptor Internalization**

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The proiactin receptor (PRLR) belongs to the cytokine receptor superfamily- In the cow, two isoforms, the tong (IPRLR) and the short (sPRLR), have been identified, differing only in length of their cytoplasmic domains and 11 unique amino acid tail of the sPRLR. Upon binding to ligand, the receptors form dimers and undergo internalization. By using temperature-sensitive cells (ts20) which express a thermolabile ubiquitin activating enzyme El, it has been suggested that the ubiquitin-conjugating system is required for GHR internalization. A phenylalanine in the cytoplasmic domain of the GHR has been shown to be essential for ubiquitin-dependent endocytosis. Therefore, a stable ts20 cell expressing the IPRLR was created and a mutant IPRLR (IPRLR F290A) was generated. Incubating ts20 cells at non-permissive temperature inhibited GHR internalization, but not PRLR, suggesting that ubiquitin-conjugation system may not be required for PRLR internalization.. The IPRLR-F290A mutant demonstrated a 20% decrease in intemaiization, compared to a dramatic decrease in the GHR mutant, also arguing against a role of ubiquitin-dependent endocytosis in PRLR internalization. Involvement of endocytic pathways in PRLR internalization was also examined by overexpression of a dominant-negative mutant dynamin-I, a GTP-binding protein known to mediate both clathrin-mediated uptake and the caveolar pathway. Overexpression of mutant dynamin-l (K44E-Dyn-l) inhibited PRLR internalization by 40-60%, implying involvement of either or both pathways in PRLR internalization. Further studies to distinguish the involved pathway (s) will increase our understanding of PRLR expression and function.

9. Phenotypic and Functional Characterization of Rhesus Decidual Leukocytes.

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We carried out a phenotypic and functional characterization of decidual leukocytes from the pregnant rhesus monkey. A majority (80%) of these cells were CD56bright/CD3-, with typical large granular lymphocyte/uterine NK cell morphology. Flow cytometry showed that CD56bright cells shared other phenotypic features of human uterine NK cells, including low levels of CD45RA and CD62L expression. A majority of rhesus uterine CD56bright cells expressed low levels of CD16 and were CD2-. In contrast, most rhesus CD 16+ peripheral blood cells were CD56-. A minor subset of smaller and less granular CD56intermediate decidual lymphocytes were largely CDI 6-CD2+. Decidual lymphocytes effectively lysed K562, Raji and 721.221 targets in cytotoxicity assays. Cytotoxic activity of decidual lymphocytes was significantly reduced when MHC class I-negative 721.221 cells were transfected with the rhesus monkey non-classical MHC class I molecule Mamu-AG, which is preferentially expressed in trophoblasts. These results suggest that as in the human and rodent uterus, rhesus decidual CD56 bright cells represent a distinct lymphocyte subset that belongs to the NK cell lineage. The ability of rhesus decidual NK cells to recognize cells transfected with the rhesus monkey analog of HLA-G, Mamu-AG, suggests that interaction between decidual NK cells and fetal trophoblasts through MHC class I receptors is conserved in primates and may play an important role in normal placental development or fetal survival.

10. Early Embryonic Development in High Producing Lactating Cows Contrasted with Nulliparous Heifers during Summer and Dry Cows during Winter.

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Lactating cows with high milk production have lower fertility than heifers with similar genetic. Two experiments evaluated early embryonic development in lactating Holstein cows (C; n=27; 50-130 days post-partum; >40 Kg milk/d) and nulliparous heifers (H; n=28; 11-17 months old) during the summer, and in lactating cows (L; n=27; 40-80 dpp; >40 Kg milk/d) and dry cows (D; n=27) during the winter of 1999. The animals were bred at estrus with a straw containing combined semen from 4 high fertility bulls. Five days after ovulation, embryos/ocytes were recovered and evaluated for fertilization, embryo quality (1=excellent to 5=degenerated), cell nuclei count, and number of accessory sperm. On d 6, animals received $PGF2_{\alpha}$ and were bred again at estrus. Each animal was flushed 2 or 3 times.

SUMMER: Thirty-two embryos and no unfertilized oocyte (UFO) were recovered from It and 21 embryos and 17 UFOs were recovered from C (100.0% vs. *55.3%* fertilization; pc.0l)- The quality of fertilized embryos from C was inferior to H (3.8±.4 vs. 2.3±.2; p<.0l). Embryos from C had fewer cells than from H (20.1±3.4 vs. 37.9 p<.0l). Surprisingly, The accessory sperm number in the embryos from C was greater than H (36.4-i-6.2 vs. l9.6±4.6; pc.01). Eighty per cent of the IJFOs had sperm attached (17.8±12.1 spermUFO). WINTER: Thirty-four embryos and 4 UFOs were recovered from I), and 36 embryos and 5 UFOs were recovered from L *(89.5%* vs. 87.8% fertilization; p>.10). Embryo quality from L was inferior to D (3.lt3 vs. 2.2+.3; p<.06). Embryos from L had similar cell number as D (27.2±2.7 vs. 30.6±2]; p<.01), and there was no difference in the numbers of accessory sperm in the embryos from L and D (42.0±9.4 vs.. 36.5+6.3; p>.10). Thus, it is very likely that high milk production exerts a negative effect on oocyte quality and early embryonic development, which can be potentiated by heat stress. Reductions in fertilization do not appear to be due to a sperm problem based on attached sperm to UFOs and the high accessory sperm numbers in embryos from lactating cows.

11. **Effects of posterior hypothalamic lesions on pubertal LHRH elevations and precocious menarche.**

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It has been well established that an increase in pulsatile LHRH levels triggers the onset of puberty. Further, it has been shown that posterior hypothalamic lesions advance the onset of puberty in female rhesus monkeys. However, the causal relationship between posterior hypothalamic lesions and an increase in LHRH release prior to precocious puberty has not been demonstrated. In the present study, we examined the effects of the posterior hypothalamus on pulsatile LHRH release and the timing of menarche. To induce precocious puberty, five prepubertal female rhesus monkeys (17.5±0.1 months of age) received lesions in the posterior hypothalamus. As controls, 3 prepubertal females *(17.5±0.1* months) received sham lesions and 2 monkeys did not receive lesions. A cranial pedestal for push-pull perfusion was implanted at 14 months and push-pull perfusates were collected for the measurement of LHRH release before and after the lesion surgery. All 5 monkeys with posterior hypothalamic lesions exhibited perineal sex-skin swelling starting 2 weeks after the lesion, and had menarche 1-2 months after the lesion (18.9±0.2 months). In contrast, none of the controls have yet exhibited any signs of precocious puberty. In all monkeys, LHRH levels were Tow during the prepubertal period, as is characteristic of this stage. However, after lesions, but prior to menarche, LHRH release in lesioned animals increased 200-700% over pre-lesion levels ($p<0.05$), whereas no LHRH increase was observed in controls at a comparable age. Since the menarche age of our colony monkeys is 3 0.6±0.9 months, the age of menarche in animals with posterior hypothalamic lesions was significantly early ($p<0.01$). These preliminary data suggest that posterior hypothalamic lesions result in an increase in LHRH release, which triggers precocious menarche. (Supported by NIH grants HD1 *1355,* HD15344, RR00167).

12. **Trophoblast Differentiation from Rhesus Macaque Embryonic Stem Cells *In Vivo***

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Rhesus macaque embryonic stem (ES) cells allowed to differentiate can give rise to all three embryonic germ layers (endoderm, ectoderm, and mesoderm). RT-PCR analysis of differentiated rhesus ES cells shows the expression of placenta specific mRNAs. This indicates that rhesus ES cells can differentiate into placental tissue, in contrast to murine ES cells, which cannot. To determine the expression of various placental markers, we injected rhesus ES cells into SCID mice, removed the resulting teratomas, or tumors, at various time points, and used RT-PCR to detect the expression of placental markers. RT-PCR of the tumors andlor the tissue into which the ES cells had been injected showed the expression of rhesus specific mRNAs as well as the expression of the placental markers CGα, CGβ, Mamu-AG, mGHV and GnRH. CGcz and CGI3 were expressed at d57 postinjection, Mamu-AG was expressed at d15, d22, d43 and d57, mGHV was expressed at d22, d43, and d57, and GnRH was expressed at dl5, d22, d28, d43, d57 and d98. These results suggest that trophoblasts develop within teratomas and that ES cells may provide a model for placental development.

13. **Effects of the Ovarian Cycle and Steroid Replacement Therapy on Ovine-Uterine Tissue Expression of VEGF and Fit-i KING, AG[1], JOYCE JM[1], MAGNESS, RR[1,2]**

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Uterine blood flow (UBF) is greatest during follicular (Fol) phase, when Estradiol-l 7β ($E_2β$) is the predominant-reproductive hormone and growth of endometrium (ENDO) is minimal; versus luteal (Lut) phase, when progesterone (P_4) predominates and uterine ENDO experiences maximum growth. Prolonged treatment with $E_2β$ increases UBF and cardiac output. Vascular endothelial growth factor(VEGF) regulates angiogenesis in many vascular beds and may modulate uterine ENDO vascularization. Interactions between ovarian-dependant hormonal changes, uterine structural changes, and the role of VEGF and its receptor Fit-l are unclear. **Purpose:** Evaluate the effects of endogenous and exogenous P_4 with and without $E_2β$ on expression of VEGF and Flt-1 in ENDO luminal tissue, glands and vasculature. **Method**: Uterine tissue was procured from ewes synchronized into Lut and Fol phase (Endogenous steroids) and from nonpregnant-ovariectomized ewes receiving 10 days of either V (n=5), P_4 (0.9g CIDRS vaginal implants; n=6), $E_2β$ (5μg/kg bolus followed by 6μg/kg/d; *n=5),* or P_4 +$E_2β$ (n=5)(Exogenous steroids). VEGF and FIt- l protein expression in ENDO glands, luminal tissues, and, vasculature was determined using immunohistochemistry and digital-photographic densitometry. Results: ENDO artery and gland VEUF expression was greatest in Lut phase and $E_2β$+ P_4 treatment groups ($P < 0.05$); ENDO gland staining showed the highest VEGF levels. Flt-l expression of vascular tissue was similar between Lut and Fol groups; ahhough, Flt-l predominated in the $E_2β$+P_4 over V treatment groups ($P < 0.05$). ENDO glands had the greatest increase in Fit-i with $E_2β$ over V. Conclusion: P_4 and P_4 + $E_2β$ significantly increases expression of VEGF and Flt-l in ENDO vasculature, supporting a role for VEGF in angiogenesis of uterine ENDO when tissue growth is in most need to prepare for implantation. ENDO gland expression of VEUF and Flt-l protein is increased by P_4 and decreased by $E_2β$, respectively, indicating possible protein production in ENDO glands. *Supported byHL -49210, HL-5 7653, and HD-33255.*

14. Estrogen and Pregnancy Increase Cardiac and Coronary Artery Levels of basic Fibroblast Growth Factor (bFGF) and the FGF Receptor-1. M.L. Modrick[1], J. Zheng[1], and R.R. Magness[1,2] *Perinatal Res. Labs, Dept. Ob/Gyn[1] and Ani Sci[2], U W-Madison.*

Estradiol-17B (E_2B) and bFGF enhance endothelium-dependent coronary artery dilation and coronary perfusion. E_2B and Pregnancy increase cardiac output, enlarge left ventricular chamber volume, and decrease systemic vascular resistance. Cardiac microvascular disease increases in women after menopause, but is attenuated with estrogen therapy and can be alleviated by Local cardiac FOF administration. Molecular mechanisms underlying these improved coronary effects of estrogen and pregnancy may involve endogenous bFGF or its receptor. Hypothesis: E_2B treatment and Pregnancy will increase coronary artery expression of bFGF and FGFR-1. Methods: Nonpregnant ovariectomized (OVX) ewes were treated with i.v. E_2B (5 μg/kg then 6 μg/kg/d; n=16) or Veh (10% EtOH; n8) and cardiac tissues were obtained on days 0,3,6,8, and 10 for immunohistochemistry (IHC) analysis of bFGF and FGFR- 1. Additional coronary artery cross-sections were obtained from intact Nonpregnant (NP; Luteal; n=8 /Follicular; n=6), and Pregnant (P; n6, 120-130 days'gestation) ewes. Results: IHC staining of bFGF and FGFR-l was observed in coronary artery endothelium, vascular smooth muscle (VSM), endocardium, and myocardium. OVX decreased bFGF and FGFR-l staining in all tissues vs intact NP controls (Luteal = Follicular). E_2B progressively increased bFGF and FGFR-l staining in all tissues vs OVX Veh controls (Day 0 & Veh3<6<8=l0 days of E_2B). Pregnancy increased bFGF and FOFR-l staining in coronary artery endotheliurn, VSM, endocardium, and myocardium; coronary artery staining P>> NP (Luteal=Pollicular) = OVX E_2B > OVX Veh. Conclusion: E_2B and Pregnancy increase coronary artery endothelial, VSM, endocardial, and myocardial bFGF and FGFR-l protein. Thus elevation of endogenous bFGF and FGFR-l expression during estrogen therapy and pregnancy may modulate improved cardiac function and/or increases in coronary blood flow observed during these physiologic states. *Support: NIH HL492]0, HD33255, HL57653,*

15. Evaluating the Effect of Calf Removal on Pregnancy Rates to Timed A1 or Natural Service in Suckled Nelore Cows.

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The main cause of prolonged postpartum anestrus in suckled cows is not the failure of dominant follicles (DF) to develop but rather their failure to ovulate. In a previous study we observed a greater number of cows ovulating at GnRH injection after calf removal (CR) for 48h. These data suggest that CR extends the period of DF responsiveness to an ovulatory stimulus. The present study evaluated the effect of CR before or during a timed Al (TAI) protocol on pregnancy rates at TAI and also l6-30d post Al at natural service. Suckled Nelore cows (n=167; 45-120d post-partum) were synchronized with GnRH-7d-$PGF_{2\alpha}$ -24h-Estradiol Benzoate-30h-AI. In a 2x2 factorial experiment, cows were subject to CR or no CR either 48h prior to GnRH, from $PGF_{2\alpha}$ treatment to Al or both. Pregnancy was diagnosed by manual palpation 70d after TAT. Data were analyzed using chi-square test of SAS. CR prior to GnRH and at $PGF_{2\alpha}$ improved pregnancy rates per AI when compared to no CR. Each method of CR improved pregnancy rate at natural service, increasing the total number of pregnant cows in the first 30 days. Results are presented below.

Treatments	Pregnancy Rates (%)	
	Timed AI	Natural service
No CR (n=42)	14.3(6/42)[a]	11.l(4/36)[a]
CR at $PGF_{2\alpha}$ (n=58)	172 (10/58)[a.b]	292(14/48)[b]
CR prior to GnRH (n=32)	189(6/32)[a.b]	34.6(9/26)[b]
CR prior to GnRH and at $PGF_{2\alpha}$ (n=35)	34.3(12/35)[b]	39.1(9/23)[b]

[a,b] Significantly different within column (P<0.05)

In conclusion, we propose that CR applied during a synchronization protocol improves pregnancy rate by increasing the period of DF responsiveness to GnRH/LH and by inducing cyclicity in suckled Nelore cows.

16. Effects of OvulationCalf Removal on the Diameter of the Dominant Follicle and Rate at GnRH Treatment in a Protocol for Synchronization of Ovulation in Anestrous Nelore Cows.

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Treatments to reduce postpartum anestrus in beef cows must focus on increasing the frequency of LH pulses that allow follicles to reach the final stages of maturation. Some studies have shown that calf removal (CR) increases LH pulse frequency. This study was designed to evaluate whether CR 48h prior to GnRH injection in a protocol for synchronization of ovulation results in larger dominant follicles (DF) and improves ovulation rate at GnRH treatment. Anestrous suckled Nelore cows (n=99) were used. Blood samples were collected IOd before and at the time of GnRH treatment for serum progesterone (P4) concentrations. Cows were considered in anestrus if P4 was under 1.5 ng/ml at both blood sampling. Diameter of the DF and ovulation after treatment were evaluated by ultrasonography on the day of GnRH and 7 days later, respectively. Data were analyzed by chi-square test and ANOVA in SAS. More cows with CR ovulated (P<0.0l; 41/48, 85.4%) when compared with cows without CR (26/51, *5* 1.0%). The size of the DF was influenced (P<0.05) by CR and was 10.5 1±0.3 (n=48) and 10.07±0.2 *(n=51)* in cows with and without CR, respectively. The size of the DF in the cows that ovulated at GnRH (n=67) was larger (P<.05) than the cows that did not ovulate (n=32) (10.6±0.2 vs. 9.6±0.3, respectively). These data show that CR is an important tool that may be applied to induce the ovulation in TAT protocol in anestrous Nelore cows. We would propose that CR probably increases LH pulsatility and therefore increases the size and persistence of the DF.

17. An Inducible Gene Expression System to Evaluate the Effects of Estrogen Receptor α Overexpression on Treanscriptional Activation in Response Estradiol

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One method of classifying human breast cancer is according to whether the neoplastic cell contains estrogen receptor (ER). In certain ER positive breast cancers, significantly increased levels of ERa have been observed. Elucidating the effects of ER overexpression on gene transcription and cell proliferation in response to estrogen may provide insights into whether this plays an important step in the neoplastie transformation of human breast epithelium. To determine whether overexpression affects estrogen-stimulated transcriptional activation, a tetinducible gene expression system was used to overexpress wild-type ERa tagged with the human influenza virus hemagglutinin peptide (HA) in MCF-7 cells. The HA tag did not interfere with the normal response of ER to estradiol. Inducibility of ER-HA expression by doxycycline was shown to occur in both a time- and dose-dependent manner. Transient transfection experiments using reporter genes showed that estrogen-stimulated transaetivation were not significantly different in cells overexpressing ER-HA compared to control cells. However in the absence of estradiol, increased transaetivation was observed in cells overexpressing ER-HA, suggesting an increase in ligand-independent activation. These findings imply that ER is not the limiting factor in the response of MCF-7 cells to estrogen and that overexpression may selectively favor estrogen-independent activation of ER.

18. Immunohistochemical Characterization of eNOS Express in the Ovine Adrenal.

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Nitric oxide is a potential negative regulator of steroidogenesis, and NOS activity has been found in rat zona glomerulosa (ZG) and rat and bovine zona fasciculata (ZF). eNOS expression has been reported in rat and human ZG, but has been reported to be absent in bovine ZG. The objective of this study was to analyze the pattern of endothelial nitric oxide synthase (eNOS) expression in adrenal glands and possible changes in expression during pregnancy. To investigate this, adrenal sections from pregnant (120-130 days gestation, n=6) and non-pregnant (n=7) ewes were stained using a mouse monoclonal antibody for eNOS. The staining was quantified using digital-grayscale densitometry. **Results:** The adrenal cortex from both pregnant and non-pregnant animals showed higher eNOS expression than did the capsule, which sewed as a negative control ($p<0.05$). eNOS expression in the ZF was significantly higher than in the ZG and significantly higher in the ZG than in the capsule for the pregnant animals ($p<0.05$), and non-pregnant animals showed a similar trend. The levels of eNOS expression did not vary significantly between the pregnant and non-pregnant adrenals. **Conclusions:** eNOS is present in the ovine adrenal cortex in both pregnant and non-pregnant animals, with expression levels higher in the ZF than in the ZG. Thus eNOS may regulate both aldosterone and glucoeorticoid production in the sheep. *Supported by NIH HL56702 and USDA 0002159.*

19. Liver blood flow and steroid metabolism are increased by both acute feeding and hypertrophy of digestive tract.

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Steroid metabolism primarily occurs in the liver. We hypothesized that changes in liver blood flow are due to acute feed consumption or growth of the digestive tract that could produce important alterations in steroid metabolism. To test this hypothesis, liver blood flow and steroid metabolism were evaluated in 2 experiments. Exp. I evaluated the acute (0-6 h post feeding) effect of feeding in high producing lactating cows. Exp.2 evaluated the chronic effect of high feed consumption by comparing weight-matched 'lactating (high feed consumption) and dry (low feed consumption) cows. In both experiments, liver blood flow was measured by continuous infusion of bromosulfotbalein (BSP), which is specifically metabolized in the liver. Steroid metabolism was measured by continuous infusion of progesterone and estradiol 17 β.
Steady state serum concentration of all three compounds were achieved within 0.5 b of infusion. The steady state concentration was used to calculate the clearance rate for each compound. Acute feeding increase liver blood flow (BSP clearance) from *1035* to 1637 1/h (158%) by 4 h after feeding (n=2) In contrast, liver blood flow decreases (83% of control) when cow were left unfed at the same time. In Exp.2, lactating (n=4) and dry (n=3) cows had liver blood flow and steroid metabolism measured on 3 consecutive days at 3-5 h after feeding. Although variability between cows was substantial (CV=l3.5% in lactating cows and 27.7% in dry cows) the variability between days for an individual cow was relatively low (CV=4.8% in lactating cows and *5.5%* in dry cows). Lactating cow had greater ($p<.05$) liver blood flow (1184+69 vs. 756+120 l/h)and lower serum progesterone (2.3+0.13 vs. 3.55+.25 ng/ml) and estradiol (265±2.7 vs. 352±6.8 pg/ml). Thus liver blood flow and concomitant steroid metabolism is acutely (>50% within 4 hour) and chronically (>50% higher in high feed intake cows) increased by feeding. Decreases in reproductive efficiency in high producing dairy cows may be mediated by high steroid metabolism due to high feed consumption and elevate liver blood flow.