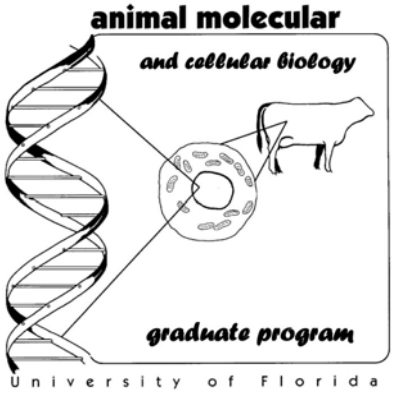
FIFTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Whitney Laboratory for Marine Bioscience
St. Augustine, Florida
April 13-14, 2007

UF | UNIVERSITY of FLORIDA



Animal Molecular and Cellular Biology Graduate Program

Fifth Annual Research Symposium

WELCOME

It is our great pleasure to welcome you to the Fifth Annual Research Symposium of the Animal Molecular and Cellular Biology Graduate Program of the University of Florida. The fifth anniversary of our symposium brings us back to the site where our first symposium was held. The Whitney Laboratory is one of the world's foremost laboratories of marine biology and is at a locale that is most conducive to science and fellowship.

As we hold our 5th symposium, we will also be entering a new chapter in the AMCB's history. The AMCB was founded in 1993 as an Interdisciplinary Graduate Concentration. In 2007, the AMCB was approved as a formal graduate program by the Board of Governors of the State University System. This change will bring new resources into the program, raise its visibility and will mean that student diplomas will read "Animal Molecular and Cell Biology". Special thanks are due to Karen Moore, Jimmy Cheek, Jane Luzar, Kirby Barrick, and Elaine Turner for their efforts on our behalf.

We wish you good science, good fellowship, good food and drink, and good memories of the symposium.

Bill Buhi, AMCB Director
Pete Hansen, AMCB Co-director

ACKNOWLEDGEMENTS

The faculty and students of the Animal Molecular and Cellular Biology Program thank the following for support of the 5th Annual Research Symposium

Dr. Mark McLellan, Dean of Research, IFAS, University of Florida

Dr. George J. Hochmuth II, Associate Dean for Research, IFAS, University of Florida

Dr. Winfred Phillips, Vice-President, Research and Graduate Programs, University of Florida

Dr. Geoff Dahl, Dept. of Animal Sciences, University of Florida

Dr. Peter A.V. Anderson, Director, Whitney Laboratory for Marine Bioscience, University of Florida

Appreciation is also expressed to those who have supported the AMCB throughout the year

Dr. Kirby Barrick, Dean for Academic Programs, IFAS, University of Florida

Ms. Joann Fischer, Program Assistant, Dept. of Animal Sciences, University of Florida

Dr. Joel Brendemuhl, Professor and Graduate Coordinator, Dept. of Animal Sciences, University of Florida

2007 Animal Molecular and Cellular Biology Distinguished Lecturer

Dr. Douglas Bannerman

Dr. Douglas Bannerman is a recognized authority in the areas of bacterial sepsis-mediated vascular inflammation and bovine mammary gland innate immunity. He received his Ph.D. in Pathology from the University of Maryland-Baltimore in 1999 and went on to complete a post-doctoral research fellowship in the Department of Surgery at the University of Washington-Seattle. His graduate and post-doctoral research focused on elucidating the mechanisms of inflammatory-mediated vascular permeability and injury. Dr. Bannerman joined the USDA's Agricultural Research Service (ARS) in 2002, where he established and maintains an active research program. He has developed a systems-based approach to investigations into the pathogenesis of bovine mastitis by employing molecular, immunological, and genomic techniques to studies at the cell and whole animal levels. His research has focused on characterizing the innate immune response to intramammary infection in dairy cows and developing immunomodulators for the prevention and/or treatment of mastitis.

Dr. Bannerman serves on the editorial board of the *Journal of Dairy Science* and has served on grant review panels for USDA-NRI and the United States - Israel Binational Agricultural Research and Development (BARD) Fund programs. During his research career, Dr. Bannerman has co-authored over 40 peer-reviewed articles, 2 book chapters, 2 patent applications, and 9 germplasm (GenBank) releases. Dr. Bannerman has been awarded several competitive grant awards and has been successful in developing cooperative research partnerships with pharmaceutical companies targeting vaccines and novel therapeutics to control mastitis in dairy cows. Dr. Bannerman has received the Northeast Branch of the American Dairy Science Association's Young Scientist Award in 2005 and the USDA-ARS Herbert L. Rothbart Outstanding Early Career Scientist of the Year Award in 2006.

Guest Lecturer

Dr. Leonid Moroz

Dr. Leonid Moroz is Professor of Neuorscience, Zoology, and Chemistry at the University of Florida. Dr. Moroz's laboratory works to characterize basic mechanisms underlying the design of nervous systems and evolution of neuronal signaling mechanisms. The major questions are: (1) why are individual neurons so different from each other, (2) how do they maintain such precise connections between each other, (3) how does this fixed wiring result in such enormous neuronal plasticity and (4) how does this contribute to learning and memory mechanisms? By taking advantage of relatively simpler nervous systems of invertebrate animals as models, Moroz conbines neuroscience, genomics, bioinformatics, evolutionary theory, zoology, molecular biology, microanalytical chemistry and nanoscience to understand how neurons operate, remember and learn.

As part of the NIH Center of Excellence in Genomic Sciences, Moroz investigates the genomic basis of neuronal identity and plasticity. Due to the tremendous difficulties in mapping single cells and processes in the mammalian brain, his group studies the giant neurons of the sea slug *Aplysia californica*, a well-established model organism for cellular neuroscience. The objective is to investigate nearly all messenger RNA (mRNA) involved in simple feeding and defensive networks.

The origins of the Whitney Laboratory stem from the 1930's, when Cornelius Vanderbilt Whitney's long-standing interest in the natural history of marine animals provided the basis for his founding of Marineland, the world's first oceanarium. This enterprise, launched in 1938, included a small research laboratory that immediately attracted the attention of many academic biologists. When the University of Florida College of Medicine opened in Gainesville in 1956, researchers from that institution came to the modest facility at Marineland to study physiological adaptations of marine animals - both fish and invertebrates. In the early 1970's, prompted by the possibility that experimental studies of marine animals could bring about medical advances, C.V. Whitney donated to the University of Florida over three acres of land adjacent to Marineland, as the site for a new marine biological research facility. Whitney provided about half of the construction costs. The Laboratory opened its doors on January 30, 1974. Two years later a second building, Whitney Hall, containing dormitory rooms, apartments, and a conference center, was constructed with funds provided by Cornelius and Marylou Whitney.

Today, the Whitney Laboratory is a marine biomedical research center of the University of Florida. It is particularly well equipped for cell and molecular biology, and provides a highly interactive research and training environment. The main laboratory building includes about 20,500 square feet of space and houses the research units of the individual faculty members. Research interests of the faculty include chemical senses (Barry W. Ache), structure and function of ion channels (Peter A. V. Anderson), biochemistry of vision (Barbara-Anne Battelle), molecular physiology and evolution of membrane transport systems (Dmitri Boudko), plasticity and homeostasis in motor systems (Dirk M. Bucher), integrative membrane physiology and functional genomics (William R. Harvey), molecular and cell biology of complex systems (Paul J. Linser), neurogenomics, nanotechnology and neuronal evolution (Leonid L. Moroz and Andrea B. Kohn), synaptogenesis and synaptic physiology (Fumihito Ono), ecology and productivity of coastal marine ecosystems (Edward J. Phlips and Shirley M. Baker), structure and function of neuropeptide receptors (David A. Price), and cellular neurobiology and signal transduction (David A. Zacharias).

SCHEDULE

Friday AM, April 13, 2007
Room C/G 101 - Cancer & Genetics Research Complex Auditorium
AMCB Distinguished Lecturer Series

8:00 – 9:00 AM Douglas Bannerman, USDA-ARS, Beltsville, MD. FLICE-Like Inhibitory protein (FLIP) protects Against bacterial lipopolysaccharide-induced apoptosis

Friday PM, April 13, 2007
Auditorium, Whitney Laboratory

12:00 – 1:00 PM LUNCH

Session 1. Research Reports (James Moss, chair)

1:00 – 1:15 PM Kramer, J.M., K. Drury and K. Moore, Dept. of Animal Sciences, University of Florida. Characterization of aneuploidy in bovine oocytes and in vitro produced embryos

1:15-1:30 PM Loureiro, B., G. Entrican and P.J. Hansen. Dept. of Animal Sciences, University of Florida and Moredun Research Institute, Ednurgh, UK. Granulocyte-macrophage colony-stimulating factor improves the proportion of bovine embryos that develop to the blastocyst stage.

1:30-1:45 PM Bonilla, A.Q.S., E. Wroclawska, and K. Moore. Dept. of Animal Sciences, University of Florida. Characterization of CPG islands in bovine cloned and in vitro produced embryos using bisulfite mutagenesis.

1;45 – 2:00 PM Cooke, F.N.T. and A.D. Ealy. Dept. of Animal Sciences, University of Florida, The expression of fibroblast growth factor receptors and their ligands in pre- and peri-attachment bovine conceptuses.

BREAK 2:00 - 2:30 PM

Session 2 – Guest Lecture (Peter Hansen, Chair)

2:30 – 3:30 PM Leonid L. Moroz, Dept of Neuroscience & The Whitney Laboratory for Marine Bioscience, University of Florida. Genomics of single cells and cell compartments and evolutionary aspects of animal genomics

3:30 – 4:00 PM GROUP PICTURE

Session 3 – Research Reports (Katherine Hendricks, Chair)

4:00 – 4:15 PM Esterman, R.D., and J.V. Yelich. Dept. of Animal Sciences, University of Florida. Follicle developemnt in *Bos taurus* and *Bos indicus x bos taurus* cattle treated with GnRH and a progesterone insert

4:15 – 4:30 PM Eroh, M.L., A. D. Ealy and D.C. Sharp. Dept. of Animal Sciences, University of Florida. Pregnancy status and steroid exposure impact the abundance of endometrial cyclooxygenase-2 mRNA in mares.

4:30 - 4:45 PM Oliveira, L. and P.J. Hansen. Dept. of Animal Sciences, University of Florida. Differences in peripheral blood mononuclear cell populations between periparturient and nonpregnant cows.

4:45 – 5:00 PM Silva, L.A., D. Sharp and O.J. Ginther. Dept. of Animal Sciences, University of Florida and Eutheria Foundation, Cross Plains, WI. Endometrial vascular perfusion during early pregnancy in heifers assessed by color-doppler ultrasonography.

Check into Rooms

Volleyball

Cookout

Saturday PM, April 14, 2007
Auditorium, Whitney Laboratory

8:00 – 8:45 AM Breakfast

Session 4 – Distinguished Lecture (William Buhi, Chair)

8::45 - 9:45 AM Douglas Bannerman, USDA-ARS, Beltsville, MD Pathogen-dependent innate immune responses to intramammary infection in dairy cows.

9:45 – 10:00 AM BREAK

Session 5 – Research Reports (Kathleen Pennington, Chair)

10:00 – 10:15 AM Doty, A.L., M. Varest, S. Benson, M.A. Pozor, M.L. Macpherson, W.C. Buhi, and M.H.T. Troedsson. Depts of Animal Sciences, Large Animal Clinical Sciences, and Obstetrics & Gynecology, University of Florida. Heat shocked spermatozoa induce functional and structural damage to PMNs.

10:15 – 10:30 AM Hendricks, K.E.M. and P.J. Hansen. Dept. of Animal Sciences, University of Florida. Effects of heat shock of frozen/thawed ejaculated spermatozoa on fertilization and subsequent embryonic development

10:30 – 10:45 AM de Castro e Paula, L.A., and P. J. Hansen. Dept. of Animal Sciences, University of Florida. Protective effects of the antioxidant dithiothreitol (DTT) on preimplantation bovine embryos exposed to heat shock.

10:45 – 11:00 AM BREAK

Session 6 – Research Reports (Katherine Hendricks, Chair)

11:00 – 11:15 AM Padua, M. B., and P. J. Hansen. Dept. of Animal Sciences, University of Florida. Evolutionary relationship between epitheliochorial placentation and presence of uterine serpins

11:15 – 11:30 AM Pennington, K.A., and A.D. Ealy. Department of Animal Sciences, University of Florida, Gainesville Florida. Several fibroblast growth factors stimulate interferon-tau production in bovine trophectoderm.

11:30 – 11:45 AM Reed, S.A and Sally E. Johnson, Dept. of Animal Sciences, University of Florida. Characterization of equine umbilical cord blood-derived stem cells.

11:45 – 12:00 PM Yang, Q. and A.D. Ealy. Dept. of Animal Sciences, University of Florida. Potential involvement of PKC and MEK in mediating fibroblast growth factor 2 activity in trophectoderm.

ADJOURNMENT

ABSTRACTS

CHARACTERIZATION OF ANEUPLOIDY IN BOVINE OOCYTES AND IN VITRO PRODUCED EMBRYOS

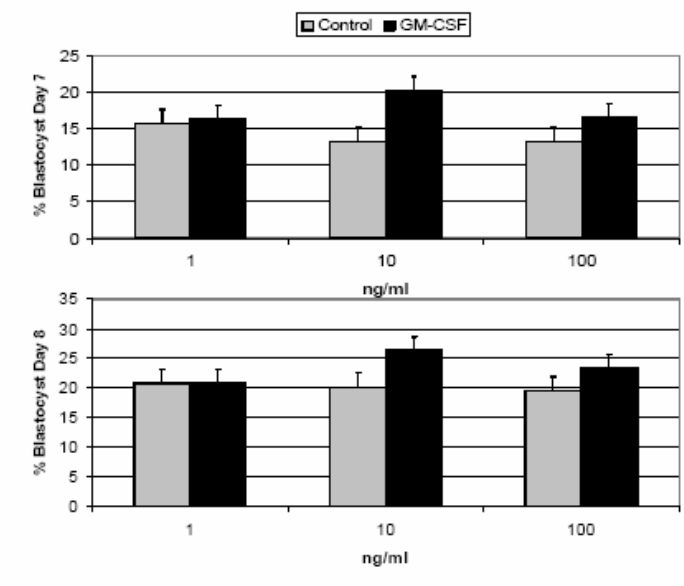
Joseph M. Kramer, Ken Drury and Karen Moore
Department of Animal Sciences, University of Florida, Gainesville, Florida

ABSTRACT: A substantial portion of bovine in vitro produced (IVP) embryos arrest during in vitro culture. Even after transfer to the uterus, more than half of all transferred IVP embryos fail to achieve pregnancy. It is most likely that oocyte quality, as well as in vitro culture conditions are the major sources for these losses. Several studies have shown that chromosomal aberrations, such as polyploidy and mixoploidy, occur at greater frequency in IVP embryos compared to in vivo controls. The most common chromosomal aberration is mixoploidy, which occurs when individual blastomeres within an embryo have different ploidy for particular chromosomes. The origin of aneuploidy, which is a non-diploid number for one or more chromosomes, is unknown in bovine. In humans, aneuploidy has been investigated in sperm, oocytes, primary and secondary polar bodies, as well as cleavage stage embryos up to the blastocyst stage. These detailed studies in humans suggest aneuploidy primarily arises through non-disjunction during meiosis I and II, and to a lesser extent during mitotic divisions in early cleavage stage embryos. In bovine, however, studies have only focused on matured oocytes, early cleavage stage embryos, or blastocysts. To date, no single study has analyzed bovine embryos from gametes to blastocysts. Furthermore, chromosomal aberrations in primary and secondary polar bodies have yet to be studied in the bovine. The purpose of this research is to characterize aneuploidy within bovine oocytes and in vitro produced embryos using fluorescence in situ hybridization (FISH). Our hypothesis is that bovine aneuploidy primarily arises because of non-disjunction occurring during meiosis I and II and less frequently during cleavage divisions. This study will investigate aneuploidy within oocytes and polar bodies and within embryos at pronuclear (PN), two-cell, four-cell, eight-cell, and blastocyst stages of development. Oocytes and whole embryos will be fixed using methanol:glacial acetic acid (3:1). For blastocysts, trophectoderm will be dissected from inner cell mass and each tissue will be independently fixed. Bovine BAC DNA specific for chromosomes 22, 27, X, and Y will be used for nick translation to produce a four color FISH probe panel. Oocytes will be deemed normal haploid if one signal for each chromosome is detected. Multiple signals for a particular chromosome suggest non-disjunction occurred during meiosis I and will be confirmed by analysis of the associated primary polar body. PN stage embryos will be deemed normal diploid if two signals can be detected for each chromosome. Observation of more than two signals for a particular chromosome in a PN stage embryo suggests non-disjunction occurred during meiosis II and will be confirmed by examining associated primary and secondary polar bodies. Mitotic non-disjunction will be determined by examining the population of cells within cleavage stage embryos. Embryos containing cells with different levels of ploidy will be considered mixoploid. Inner cell mass and trophectoderm will be examined separately to determine if aneuploid cells are preferentially routed to the trophectoderm. Data obtained from this study will help elucidate the origin of chromosomal aberrations in bovine IVP embryos and allow future studies to investigate the influence of the in vitro culture environment on chromosomal aberrations. Ultimately, the optimization of the in vitro culture environment will likely lead to fewer aneuploidies and therefore improvement in embryo development and embryonic potential.

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IMPROVES THE PROPORTION OF BOVINE EMBRYOS THAT DEVELOP TO THE BLASTOCYST STAGE

Bárbara Loureiro[1], Gary Entrican[2] and Peter J. Hansen[1]
[1]Department of Animal Sciences, University of Florida, Gainesville, Florida; [2]Moredun Research Institute, Edinburgh, United Kingdom

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein originally recognized as a regulator of cell proliferation and differentiation. GM-CSF has also been implicated in preimplantation embryo development. In mice, GM-CSF increases embryo cell number by facilitating glucose uptake and inhibiting apoptosis (Robertson et al., Biol Reprod 64:1206–1215, 2001). In cattle, bovine GM-CSF has been reported to improve the proportion of embryos that become blastocysts in vitro (Moraes and Hansen, Biol Reprod 57:1060-1065, 1997). The long-term goal of the present research is to evaluate effects of GM-CSF on post-transfer survival of bovine embryos. As a first step, conditions for enhancement of embryonic development in vitro by GM-CSF are being established. A preliminary study is underway to determine effects of various concentrations of GM-CSF under culture conditions of 5% or atmospheric oxygen conditions and when added at either Day 1 or 5 after fertilization. Embryos were cultured in the presence of 1, 10 or 100 ng/ml of recombinant ovine GM-CSF produced in transfected CHO cells or an equivalent volume of cytokine-free CHO cell supernatant (control). Cleavage rate was accessed on day 3 after insemination. Stage of development was recorded at days 7 and 8 after insemination. Results obtained to date involved 2200 oocytes. There was no effect of GM-CSF on cleavage rate. When added at 10 ng/ml, GM-CSF increased the percent of oocytes that became blastocysts at Day 7 ($P<0.02$) and Day 8 ($P<0.08$). This increase tended to be seen when added at Day 1 or 5 and for both oxygen environments. There was no significant effect of 1 or 100 ng/ml GM-CSF on blastocyst In conclusion, GM-CSF can affect competence for embryos to become blastocysts in a concentration-dependent manner (BL is supported by a CAPES (Brazil)/Fulbright Grant).



CHARACTERIZATION OF CpG ISLANDS IN BOVINE CLONED AND IN VITRO PRODUCED EMBRYOS USING BISULFITE MUTAGENESIS

A.Q.S. Bonilla*, E. Wroclawska, K. Moore
University of Florida, Dept. of Animal Sciences, University of Florida, Gainesville, Florida

Somatic cell nuclear transfer (NT) is quite inefficient with several associated problems, such as low in vitro embryo development, as well as early embryonic, fetal and postnatal losses. These problems are thought to be due to abnormal gene expression which is regulated epigenetically. Aberrant DNA hypomethylation or hypermethylation can contribute to inappropriate gene expression or gene silencing, respectively, leading to problems seen in cloned and in vitro produced embryos. In cloned embryos the processes of erasure and reestablishment of DNA methylation during early embryonic development do not follow what normally happens with in vivo produced embryos. Usually the embryonic genome undergoes rapid demethylation after fertilization, and becomes remethylated between the 8-cell and the blastocyst stages. However, in nuclear transfer embryos the methylation process is altered and embryos are not adequately demethylated. We hypothesize that the introduction of a somatic form of DNA methyltransferase, Dnmt1s, into the nuclear transfer embryo may be related with the abnormally high methylation found in clones, and leads to abnormal gene expression. Normally only the oocyte specific form, Dnmt1o, is found at the preimplantation stage of development and is not found in the nucleus, thus allowing DNA demethylation. However, Dnmt1s is found in the nucleus and cytoplasm of the donor cell, and when the cell is transferred to the enucleated oocyte the early cloned embryo will have Dnmt1s both in the nucleus and cytoplasm, increasing DNA methylation. To test this hypothesis a donor cell line with low Dnmt1 expression has been selected to produce cloned embryos, and compared with clones produced from a cell line with high Dnmt1 expression, and with in vitro produced controls to evaluate global methylation using immunocytochemistry. As a second step we will determine DNA methylation at specific regions of euchromatin and heterochromatin using bisulfite sequencing. The bisulfite reaction converts non-methylated cytosines to uracils, whereas 5-methylcytosines stay unchanged. DNA from pools of clones produced from high or low Dnmt1 cell lines and in vitro produced embryos at the 2-cell, 8-cell, and blastocyst stages will be harvested and used in a bisulfite conversion assay. Bisulfite converted DNA will be purified and used in PCR reactions using bisulfite primers specific on regions with known CpG islands in IGF2, Oct4, Satellite I, and Bov-B. Following PCR, products will be gel purified and subcloned into pGEM-T easy (Promega). Subclones will be sequenced and aligned to assess methylation status across each treatment, to determine if donor cell selection can be utilized to reduce DNA methylation in cloned embryos. Clones prepared from donor cells with low Dnmt1 expression would be expected to have lower methylation at these loci, and thus be more like the in vitro produced embryos. However clones prepared from donor cells with high Dnmt1 expression will be expected to have high methylation at these loci, and be more prone to abnormalities. We believe that reducing DNA methylation similar to what occurs in vivo should improve development of cloned offspring to term.

THE EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTORS AND THEIR LIGANDS IN PRE- AND PERI-ATTACHMENT BOVINE CONCEPTUSES

F.N.T. Cooke and A.D. Ealy
Dept. of Animal Sciences, University of Florida, Gainesville, Florida

A series of developmental events must occur in the bovine conceptus in order for pregnancy to succeed to term. During early pregnancy the outermost layer of the primitive placenta, termed the trophectoderm, must proliferate extensively and secrete interferon-tau (IFNT), the signal for maintenance of pregnancy in ruminants. The bovine and ovine endometrium expresses fibroblast growth factor 2 (FGF2) and secretes it into the uterine lumen during early pregnancy where it is suspected to play a role in the establishment of pregnancy by increasing IFNT secretion. We hypothesize that other members of the FGF family, which currently contains at least 23 distinct factors, also modulate conceptus development and maternal recognition of pregnancy in ruminants. The FGFs exert their actions through binding to specific FGF receptors (FGFR), which have different subtypes that exhibit ligand specificity to FGFs. The objectives of this study were 1) to identify the FGFR expressed by d8 and d17 bovine conceptuses, and 2) to identify candidate FGFs expressed by conceptuses that may interact with these FGFR. The total cellular RNA was extracted from cow trophectoderm cells (CT1) and from three pools of d17 bovine conceptuses. RT-PCR was used to study the expression of FGFR1, FGFR2, FGFR3 and FGFR4 mRNA. The amplified products were cloned and their DNA was sequenced to identify each FGFR. The same procedures were applied to pools of *in vitro* produced (IVP) bovine blastocysts collected at day 8 post-IVF. These analyses indicated that FGFR1IIIb, -2IIIb, -3IIIc and -4 mRNA are expressed by both day 8 and 17 conceptuses and CT1 cells. In follow-up studies, the total cellular RNA was extracted from d17 conceptuses, CT1 cells and bovine endometrium. RT-PCR was performed to verify the expression of mRNA for FGF1, FGF2, FGF7 and FGF10. These analyses revealed that FGF1, FGF2 and FGF10 mRNA are expressed by d17 conceptuses and endometrium whereas FGF7 mRNA is expressed only by the endometrium. CT-1 cells contain FGF1 and 2 but not FGF10 mRNA. We are still investigating the expression of these ligands in day 8 conceptuses. In summary, there are at least four distinct FGFRs in cultured bovine trophectoderm as well as within the bovine conceptus by the blastocyst stage when IFNT production is first evident. Moreover, at least four candidate FGFs are expressed either by the endometrium or conceptus during early pregnancy. Further studies are required to examine how some or all of these ligands interact with their receptors to regulate conceptus development in cattle and other ruminants.

FOLLICLE DEVELOPEMNT IN *BOS TAURUS* AND *BOS INDICUS* × *BOS TAURUS* CATTLE TREATED WITH GNRH AND A PROGESTERONE INSERT

R.D. Esterman and J.V. Yelich
Department of Animal Sciences, University of Florida, Gainesville

Bos indicus × *Bos taurus* cattle are commonly raised in subtropical regions like Florida because of their superior tolerance to heat, disease, and ability to utilize low quality forages compared to *Bos taurus* cattle. Slight differences exist in the reproductive physiology between *Bos indicus* compared to *Bos taurus* cattle, which create challenges in developing effective estrous synchronization protocols for cattle of *Bos indicus* breeding. In lactating *Bos indicus* × *Bos taurus* cows synchronized with GnRH concomitant with a progesterone insert (CIDR) and prostaglandin $F_{2\alpha}$ (PG) 7 d later (GCIDR + PG), conception rates decreased significantly when estrus was expressed greater than 72 h after PG. The reason for this decrease is unclear but it could be due to differences in stage of follicle development at CIDR removal, which is probably related to the ability of GnRH to initiate ovulation at CIDR insertion, resulting in follicle turnover. Stage of the estrous cycle at GnRH administration effects ovulation rates in *Bos taurus* cattle but the extent of this effect is unclear in *Bos indicus* cattle. Cattle of *Bos indicus* breeding have more three and four follicle wave patterns than *Bos taurus* cattle. Therefore, cows with more three and four follicular wave patterns could have fewer opportunities for ovulation to GnRH, which could result in longer durations of follicle dominance during treatment with a 7 d CIDR. An ongoing study in our lab will evaluate the ability of GnRH to initiate ovulation in estrous cycling cows undergoing GCIDR + PG protocol in suckled Angus (*Bos taurus*) and Brangus (*Bos indicus* × *Bos taurus*) cows. Estrous cycling cows will be pre-synchronized to generate cows to start the synchronization protocol on either day 2, 6, 10, 14, or 18 of their estrous cycle (n = 5 Angus and n = 5 Brangus in each group). Cows will be evaluated by daily ultrasonography and blood sampling from initiation of GCIDR + PG treatment until 3 d after PG to evaluate follicle growth patterns and estradiol and progesterone concentrations. Cows not exhibiting estrus will be timed-AI 73 to 80 h after PG concurrent with GnRH. Results of this study should provide a better understanding of the effectiveness of GnRH to initiate ovulation at the initiation of a GCIDR + PG treatment in Angus and Brangus cows across several stages of the estrous cycle. Furthermore, evaluating follicle growth patterns during the CIDR treatment should lead to a better understanding of follicle dynamics between Angus and Brangus cows and cows that ovulated or failed to ovulate to GnRH at the initiation of a CIDR treatment. Subsequent research will investigate what effect stage of follicular growth has on follicle development and fertility in Angus and Brangus cows. Cows will be pre-synchronized to be either day 2 or 6 of the estrous cycle at the initiation of a GCIDR + PG treatment. Follicle development will be evaluated daily until CIDR removal. Following CIDR removal and an observed estrus, dominant follicles will be aspirated using ovum pickup technique (OPU), collecting both the follicular fluid and oocyte. Measurements of estradiol, progesterone, estradiol:progesterone ratio, adrosteindione, androsteindione:progesterone ratio, inhibin, and activin follicular fluid will be evaluated to characterize follicle quality. Evaluation of IGF1 and IGF binding proteins in the follicular fluid will characterize follicle growth and development potential. Quality of follicular fluid plays an essential role in growth and maturation of oocytes and dictates future oocyte competence. Oocytes collected will be matured, fertilized, and developed in culture to evaluate cleavage rates on day 3 and blastocyst development on day 8 post-fertilization. Data generated from these experiments should lead to a better understanding of follicle development between *Bos taurus* and *Bos indicus* × *Bos taurus* cattle, which can be used to design more effective estrous synchronization protocols in *Bos indicus* × *Bos taurus* and *Bos indicus* cattle.

PREGNANCY STATUS AND STEROID EXPOSURE IMPACT THE ABUNDANCE OF ENDOMETRIAL CYCLOOXYGENASE-2 MRNA IN MARES

M.L. Eroh, A. D. Ealy and D.C. Sharp
Department of Animal Sciences, University of Florida, Gainesville, Florida

The equine embryo must signal its presence to the uterus in order for pregnancy to continue to term. This concept of maternal recognition of pregnancy remains unresolved in horses. It is understood that locomotion of the conceptus throughout the uterus is crucial for its survival, and this action presumably permits the embryo to transmit its luteostatic signal to the endometrium, thereby preventing endometrial prostaglandin production. Synthesis of the luteolysin prostaglandin F2α (PGF2α), is controlled primarily by cyclooxygenase-2 (COX-2) in the endometrium. Relative expression of COX-2 and production of PGF2α reaches a maximum concentration within the uterus 14 days after ovulation in non-pregnant mares. However during pregnancy, endometrial production of PGF2α is greatly diminished. Our hypothesis is that the equine conceptus inhibits production of PGF2α by inhibiting the expression of COX-2 in the equine endometrium. To investigate this hypothesis, our first aim was to determine equine COX-2 mRNA abundance in endometrium derived from cycling verses pregnant mares using Quantitative Real Time Polymerase Chain Reaction (real time-PCR). Equine COX-2 and 18s cDNAs were cloned to serve as positive controls. Endometrial biopsies were taken on day 14 post ovulation (day 0) from cycling (n = 10) and pregnant mares (n = 9) using alligator-type uterine biopsy forceps. Total RNA was extracted, reverse transcribed, and quantitative real-time PCR was completed with equine COX-2 or 18S specific primers and SybrGreen. COX-2 mRNA abundance was 19.6 fold less in endometrial biopsies from pregnant mares than from non-pregnant mares (P=.02). In a second study, use of long term ovariectomized (OVX), steroid-treated mares was tested as a model to study endometrial COX-2 mRNA expression. OVX mares (n=4) were treated with estradiol (10mg in 3mL sterile sesame oil daily) for seven days followed by progesterone (200 mg in 3 mL sterile sesame oil daily) for 14 days or sesame oil only (negative control). On the 21st day of treatment, endometrial biopsies were obtained, and the relative expression of COX-2 mRNA was evaluated. Endometria obtained from OVX mares without steroid treatment did not yield COX-2 mRNA values reliably different from background cycles whereas the steroid treatment regiment provided reliable detection of COX-2 mRNA. In summary, a clear reduction in COX-2 mRNA was observed in endometria of pregnant mares compared with endometria of non-pregnant mares. Moreover, COX-2 abundance in the steroid treated OVX mares was similar to mares on day 14 of the estrous cycle. These data support the hypothesis that the reduction in PGF2α in pregnant mares reflects a reduction in COX-2 mRNA expression. These findings support our idea of targeting the changes in expression of COX-2 mRNA to determine the conceptus derived factor responsible for COX-2 mRNA reduction during early pregnancy using steroid treated OVX mares as the model. (Supported by a grant from the Florida Pari-mutuel Wagering Trust to DCS).

DIFFERENCES IN PERIPHERAL BLOOD MONONUCLEAR CELL POPULATIONS BETWEEN PERIPARTURIENT AND NONPREGNANT COWS

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Pregnancy involves maternal and fetal mechanisms acting together to prevent harmful immune responses against the fetus. Recent studies have shown that specific subsets of immune cells are involved in maternal tolerance to the conceptus. For example, the frequency of $CD4^+CD25^+$ T cells in the circulation was higher in women during early pregnancy compared to nonpregnant women. The γδ T-cell has been shown to increase in frequency in the peripheral mononuclear cell (PBMC) population of women during the first trimester. In sheep, pregnancy is associated with accumulation of γδ T-cells and $CD68^+$ macrophages in the uterine endometrium. The purpose of the present study was to use flow cytometry to determine differences in selected subpopulations of PBMC ($CD4^+$, $CD4^+CD25^+$, $\gamma\delta^+$ T and $CD68^+$) associated with the periparturient period in cattle. Blood was collected from 8 nonpregnant Holstein cows and 8 periparturient Holstein cows (5.9 ± 1.8 days of pregnancy). The PBMC were stained for single color analysis with anti-γδ and anti-CD68 and for dual-color analysis with anti-CD4 and anti-CD25. Cells were gated by forward and side scatter for lymphocyte (G1) and monocyte (G2) regions. The percentage of $CD4^+$, $CD25^+$ and $CD4^+CD25^+$ cells in the lymphocyte gate were higher ($p<0.05$) in periparturient cows compared to nonpregnant cows (25.0 vs 19.4; 8.2 vs 5.3 and 4.0 vs 2.2%, respectively). The difference in frequency of $CD4^+CD25^+$ cells probably represents changes in frequency of $CD4^+$ cells because the percent of $CD4^+$ cells that were $CD25^+$ did not change with pregnancy status. An increase of γδ T-cells ($p<0.01$) was observed in the periparturient group in the lymphocyte region. There was also a tendency ($p<0.1$) for the proportion of cells positive for γδ T-cells in the monocyte region to be greater for periparturient cows (G1: 4.0 vs 2.2; G2: 6.6 vs 4.9; respectively). The percentage of $CD68^+$ cells tended to be lower ($p<0.5$) for periparturient cows for both gates (G1: 2.7 vs 1.3; G2: 10.2 vs 2.3%, respectively). It was concluded that, as compared to nonpregnant cows, there is an increase in the proportion of PMBC that are $CD4^+$, $CD4^+CD25^+$,and $\gamma\delta\ T^+$ in periparturient cows and a decrease in the proportion that are $CD68^+$. The increase in γδ T-cells may represent recirculation of these cells from the uterus to the periphery. The decrease in $CD68^+$ cells may occur because of recruitment of macrophages to the uterus at this time of pregnancy.

ENDOMETRIAL VASCULAR PERFUSION DURING EARLY PREGNANCY IN HEIFERS ASSESSED BY COLOR-DOPPLER ULTRASONOGRAPHY

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Conceptus-mediated endometrial vascular changes in mares are transiently higher in the horn containing the embryo during the embryo mobility phase and remain higher in the embryo-containing horn than in the opposite horn after fixation. It was hypothesized in this study that endometrial vascular perfusion during early gestation in heifers is also conceptus-mediated. Heifers were inseminated and data collected until Day 60 (Day 0 = ovulation) in pregnant heifers or until the second ovulation in nonpregnant heifers. Endometrial perfusion was subjectively evaluated by color-Doppler scanning of each uterine horn (scored 1-4; none to maximal, respectively). Blood samples were collected daily for progesterone (P4) and estradiol (E2) assay. Heifers were retrospectively assigned to pregnant (n = 11) or nonpregnant (n = 6) groups after pregnancy diagnosis on Day 25, and horns ipsilateral and contralateral to the corpus luteum were compared. The data were partitioned for Days 0 to 18 so retrospective comparisons could be made between pregnant and nonpregnant status. Endometrial perfusion scores were not different ($P>0.05$) between uterine horns within each group until Day 18, so data were combined for the two horns for group comparisons. There was an increase ($P>0.001$) in endometrial perfusion scores in nonpregnant heifers between Days 14 to 18 that was temporally associated with the preovulatory rise in estradiol. When data from the nonpregnant group were normalized to the day of second ovulation, the increase in endometrial perfusion scores was first detected between Days -4 to -1. Plasma P4 decreased sharply between Day -4 and -3 and plasma E2 increased sharply between Day -5 and -4. There was no evidence for a similar increase in endometrial perfusion scores in pregnant heifers at this time. In the pregnant heifers, endometrial perfusion scores were compared between ipsilateral and contralateral horns from Days 0 to 60. Increased endometrial perfusion scores in the ipsilateral horn were detected between Days 18 and 20, and continued to increase until Day 40. Perfusion scores of the contralateral horn remained low until Day 32, when they began to rise, reaching perfusion scores approximately similar to the ipsilateral horn around Day 40, then declining until Day 60. In summary, an increase in endometrial vascular perfusion in nonpregnant heifers was temporally associated with decreased P4 concentrations and increased E2 concentrations. In contrast, increased endometrial perfusion scores were detected later, around Day 20, in pregnant heifers only in the horn ipsilateral to the corpus luteum and conceptus, and without temporal association with increased estradiol or decreased P4. The hypothesis that an increase in endometrial vascular perfusion is conceptus-mediated in heifers was supported. These findings suggest different pathways of endometrial vascular perfusion stimulation in pregnant and nonpregnant heifers. (LAS: Supported by CAPES – Brazil scholarship)

HEAT SHOCKED SPERMATOZOA INDUCE FUNCTIONAL AND STRUCTURAL DAMAGE TO PMNS

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Phagocytosis by polymorphonuclear neutrophils (PMNs) is an important mechanism of sperm elimination from the mare's reproductive tract. While viable spermatozoa need to be protected from PMN-phagocytosis, dead and damaged spermatozoa should be eliminated from the uterus soon after ejaculation or AI. We have recently identified a specific seminal plasma protein (SPPα) that selectively protects viable spermatozoa from PMN-binding and phagocytosis. We also found that heat shocked spermatozoa were readily bound and phagocytosed by PMNs in the presence of seminal plasma. However, heat shocked spermatozoa appeared resistant to PMN-binding and phagocytosis in the absence of seminal plasma. We hypothesized that spermatozoa release substances that cause functional and/or structural changes to PMNs in the absence of seminal plasma. Three stallions were collected three times each, and spermatozoa were washed and reconstituted in a commercial semen extender (EquiPro, Minitube of America, Verona, WI). Samples were stored at room temperature for 1 hr (viable) or heat shocked at 45^{o}C (non-viable) for one hour. This heat shock protocol has been validated to induce changes to equine spermatozoa consistent with cell death. Aliquots were divided into six equal fractions, washed three times each, centrifuged to separate spermatozoa from supernatant, and reconstituted into one of the following: 1) viable sperm plus supernatant from non-viable sperm 2) viable sperm plus supernatant from viable sperm, 3) viable sperm plus extender 4) non-viable sperm plus supernatant from non-viable sperm, 5) non-viable sperm plus supernatant from viable sperm, 6) non-viable sperm plus extender. PMN's were isolated from peripheral blood from a healthy mare, and samples were subjected to *in vitro* assays for PMN binding, stained and examined for PMN-sperm binding and viability of PMN's. PMN-sperm binding was expressed as the percentage of PMN's (mean±SEM) that bound at least one sperm cell. PMN viability was expressed as the percentage of PMN's (mean±SEM) that were ruptured or degranulated. Differences between treatment groups were determined by randomized complete block ANOVA and Bonferronis comparison tests. Viable sperm + extender (66.2±2.1), viable sperm + supernatant from viable sperm (64.9±2.1), and non-viable sperm + supernatant from viable sperm (61.0±2.1), showed superior PMN-binding compared to viable sperm + supernatant from non-viable sperm (43.5±2.1), non-viable sperm + supernatant from non-viable sperm (39.1±2.1) and non-viable sperm + extender (34.3±2.1; $P<0.05$). PMN-damage was less common in viable sperm + supernatant from viable sperm (17.2±2.9), viable sperm + extender (19.6±2.9) and non-viable sperm + supernatant from viable sperm (21.4±2.9) compared to non-viable sperm + extender (64.8±2.9), non-viable sperm + supernatant from non-viable sperm (70.6±2.9) and live sperm + supernatant from non-viable sperm (80.6±2.9; $P<0.05$). These results demonstrate that dead spermatozoa secrete substances that induce damage to PMNs. Previous studies have shown that seminal plasma protects PMNs from this effect, maintaining their capacity to phagocytose dead spermatozoa in the uterus.

EFFECTS OF HEAT SHOCK OF FROZEN/THAWED EJACULATED SPERMATOZOA ON FERTILIZATION AND SUBSEQUENT EMBRYONIC DEVELOPMENT

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Heat shock can compromise the function of spermatagonia, spermatocytes, oocytes and preimplantation embryos. It is not known, however, whether heat shock can damage ejaculated sperm in a manner that would affect fertilization and development of embryos formed from heat-shocked sperm. Here, it was hypothesized that heat shock (41°C for 4 h) of frozen/thawed ejaculated spermatozoa would lead to a reduction in fertilization (measured as a reduction in cleavage rate and percent of oocytes exposed to sperm that became blastocysts) and embryo development (measured as a reduction in percent of cleaved embryos that became blastocysts). Extended semen was thawed, subjected to Percoll gradient purification to obtain motile spermatozoa, diluted in Sp-TALP medium to 20 x 10^6 spermatozoa/ml, and incubated at 38.5°C or 41°C in air for 4 h using a water bath. Additional semen was prepared for an unincubated spermatozoa control. In this case, semen was thawed, subjected to Percoll purification, and diluted to 20 x 10^6 spermatozoa/ml at a time coincident with the end of the incubation period for heat shock. Following treatment, sperm was used to fertilize oocytes that had been matured for 22 h. Treatments were fertilized with non-incubated sperm, sperm incubated at 38.5°C, sperm incubated at 41°C, and a parthenogenesis control (incubation of oocytes in fertilization medium without sperm). Oocytes were fertilized in groups of 30 with 500,000 spermatozoa/well. Following fertilization, presumptive zygotes and oocytes were placed in groups of 30 in 50 μL microdrops of KSOM-BE2 and cultured at 38.5°C in an humidified atmosphere of 5% CO_2, 5% O_2 and the balance nitrogen in Experiment 1 (low O_2) or at 38.5°C in 5% CO_2 and humidified air in Experiment 2 (high O_2). Cleavage was assessed on Day 3 after insemination and blastocyst development on Day 8 after insemination. In Experiment 1 (n=9 replicates with seven bulls), exposure to 41°C reduced cleavage rate (P=0.05) as compared to 38.5°C (75.7$\pm$ 5.4 vs 59.0 $\pm$ 5.7% for 38.5 vs 41°C). Temperature also tended to affect the proportion of oocytes that became blastocysts (P=0.08; 32.2$\pm$ 4.5 vs 19.8 $\pm$ 4.8%) and the proportion of cleaved embryos that became blastocysts (P=0.09; 42.3$\pm$ 5.2 vs 28.8 $\pm$ 5.5%). In Experiment 2 (n=5 bulls), exposure to 41°C reduced cleavage rate (P=0.06; 73.5$\pm$ 3.8 vs 61.7 $\pm$ 3.8% for 38.5 vs 41°C) but did not reduce the proportion of oocytes that became blastocysts (15.4$\pm$ 4.1 vs 12.7 $\pm$ 4.1%) or the proportion of cleaved embryos that became blastocysts (20.3$\pm$ 5.3 vs 19.4 $\pm$ 5.3%). In conclusion, exposure of sperm to heat shock prior to fertilization had a detrimental effect on cleavage rate in high and low oxygen environments and subsequent embryonic development in a low oxygen environment. The mechanism of action by which heat shock of sperm affects developmental competence of embryos formed from that sperm is unclear. The proportion of oocytes that cleaved under parthenogenetic conditions was 35.1% under low oxygen and 13.3% under high oxygen. One possibility is that a large proportion of embryos formed in the 41°C group were parthenotes and this reduced development of cleaved embryos. Further experiments are required to resolve this issue.

PROTECTIVE EFFECTS OF THE ANTIOXIDANT DITHIOTHREITOL (DTT) ON PREIMPLANTATION BOVINE EMBRYOS EXPOSED TO HEAT SHOCK

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Effects of heat shock (HS) on bovine embryos are greater in culture under high oxygen (20.95%) when compared to low oxygen (5%). It was hypothesized that HS effects involve reactive oxygen species (ROS) and DTT reduces these effects. For Experiment (Exp) 1 (culture in high oxygen), two-cell embryos were cultured at 38.5°C (control) or 41°C (HS) for 15 h with 0, 50 or 500 µM DTT. Embryos were then cultured at 38.5°C for 9 h in the same DTT treatment (trt) and then at 38.5°C without DTT until day 8. DTT increased the percent of control embryos becoming blastocysts ($P<0.05$) and heat shock reduced blastocyst development ($P<0.05$). This reduction was less for embryos treated with 500 µM DTT ($P<0.05$). For Exp 2, two-cell embryos were cultured at 38.5°C or 41°C for 15 h in high or low oxygen and with 0 or 500 µM DTT. Embryos were then cultured at 38.5°C for 9 h in either high or low oxygen in the same DTT trt and then cultured in low oxygen at 38.5°C without DTT until day 8. HS decreased blastocyst development in all trts except for the 0 µM DTT group cultured in low oxygen (temp x DTT; $P<0.05$). For Exp 3 (culture in high oxygen), embryos ≥16 cells were cultured at 38.5°C or 41°C for 15 h in the presence of 0, 50 or 500 µM DTT. Embryos were then cultured at 38.5°C for 9 h in their same DTT trts and then at 38.5°C without DTT until day 8. DTT increased the percent of control embryos becoming blastocysts ($P<0.05$). HS reduced blastocyst development ($P<0.05$) but the reduction was less for embryos treated with DTT ($P<0.05$). Exp 4 was conducted as for Exp 3 except that embryos were fixed 24 h after start of HS and analyzed by TUNEL assay. The percent of TUNEL-positive cells was increased by HS in the absence of DTT ($P<0.05$) but not in the presence of 50 or 500 µM DTT ($P>0.1$). In summary, DTT improved development of embryos cultured in high oxygen and conferred partial protection from HS. Protection was incomplete and it is likely that there are ROS-independent actions of HS. Since DTT was detrimental to HS embryos in low oxygen, there may be a ROS-dependent thermoprotective mechanism deployed by the embryo in low oxygen.

EVOLUTIONARY RELATIONSHIP BETWEEN EPITHELIOCHORIAL PLACENTATION AND PRESENCE OF UTERINE SERPINS

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The superfamily of serine proteinase inhibitors, also known as serpins, are single chain proteins containing a conserved domain of 370-390 aminoacids. The structure of these proteins is formed by 6 antiparallel β-sheet strands, at least 7 α helices and a reactive center loop (RCL) which contains the proteinase recognition site. Serpins inhibit serine proteinases by an irreversible suicide substrate mechanism. However, some serpins are non-inhibitory, but are structurally similar to the inhibitor type including corticosteroid binding globulin (CBG) thyroxine binding globulin (TBG). A group of related serpins called uterine serpins are expressed in the uterine endometrium under the influence of progesterone. These serpins have been found in the pregnant uterus of sheep, cow, goat, sow and water buffalo. All of these species are of the Superorder Laurasiatheria and have a placental structure characterized by a non-invasive epitheliochorial placenta. The best studied of this group is ovine uterine serpin (OvUS) which has been proposed to regulate the immune response against the fetus during pregnancy and to inhibit proliferation of certain other cells. The homology of the aminoacid sequence between OvUS and the caprine (CaUS), bovine (BoUS) and porcine uterine serpins 1 and 2 (PoUS-1 and PoUS-2) is 96%, 82%, 55% and 56% respectively. The objective for the present study is to evaluate the hypothesis that uterine serpins arose as uterine-expressed genes during evolution of Laurasiatheria in conjunction with the development of an epitheliochorial placenta. A blast search in the NCBI (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) using the nucleotide sequence of OvUS showed that there are no homolog genes in the completed genomic sequences of Canis familiaris (dog), Homo sapiens (human), Mus musculus (mouse), Macaca mulata (rhesus monkey), Rattus norvegicus (rat). All of these species have a placenta that is either endotheliochorial or haemochorial. One of these, the dog, is also a member of Laurasiatheria. There are also no homolog genes of OvUS in the completed genome sequences of Danio rerio (zebra fish) and Gallus gallus (red jungle fowl). This analysis is consistent with the idea that the gene for uterine serpins evolved with the development of epitheliochorial placantation in Laurasiatheria. However, further experiments to localize the uterine serpin gene in other species within Laurasiatheria that have different types of placentation, as well as for species with epitheliochorial placentation in other Superorders are needed to corroborate these findings.

SEVERAL FIBROBLAST GROWTH FACTORS STIMULATE INTERFERON-TAU PRODUCTION IN BOVINE TROPHECTODERM

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Maintenance of early pregnancy in bovids depends on interferon-tau (IFNT), a protein produced by the first differentiated placental cell line, termed the trophectoderm, prior to placental attachment to the uterine lining. IFNT prevents maternal rejection of the conceptus by blocking oxytocin receptor expression, thereby preventing pulsatile secretions of prostaglandin F2 alpha and luteloysis. It also induces several uterine proteins implicated in various pregnancy-regulatory functions. Several uterine-derived factors may regulate IFNT gene expression. One factor produced by the endometrium, fibroblast growth factor 2 (FGF2), stimulates IFNT production in bovine blastocysts and a bovine trophectoderm cell line (CT1). Recently, the laboratory determined that other FGFs, notably FGF1, 7 and 10, are produced by the elongating bovine conceptus and/or endometrium. Several FGF receptors (FGFR) exist in the bovine conceptus, and the aforementioned ligands share a common receptor, termed FGFR2b, which mediates placental formation and function in other mammals. The overall goal of this work is to determine if these uterine and conceptus-derived FGFs increase IFNT production in bovine trophectoderm. The following study determined if FGF1, 7 and 10 increase IFNT mRNA abundance in CT1 cells. Cultures were incubated in medium lacking serum for 24 h then replaced with medium containing 0, 0.05, 0.5, 5, 50 and 500 ng/ml of recombinant bovine FGF1, human FGF7, or human FGF10. RNA was extracted 24 hours later and the abundance of IFNT mRNA relative to an internal control (18S RNA) was determined using real-time RT-PCR. Two to three replicate dose response experiments were completed for each FGF. Treatment of CT1 cells with $\geq$ 50 ng/ml FGF1 increased IFNT mRNA concentrations ($P<.05$) compared with controls. This laboratory identified FGF1 mRNA in elongating bovine conceptuses. Others localized FGF1 protein to the trophectoderm and endometrial epithelium during mid pregnancy in cows. CT-1 exposure to 500 ng/ml FGF10 increased ($P<.05$) IFNT mRNA abundance compared with lower doses. In the ewe, FGF10 is expressed by the stromal endometrium and the developing embryonic mesoderm, a tissue layer juxtaposed to trophectoderm during conceptus elongation. Treatment with $\geq$ 5 ng/ml FGF7 increased ($P<.05$) IFNT concentration compared with controls. Current evidence indicates that FGF7 is not secreted into the uterine lumen in ruminants. However, FGF7 acts exclusively through FGFR2b, thereby implicating this receptor subtype in transducing the effects of this and potentially other FGFs on trophectoderm. To conclude, we have yet to identify sites of FGF1 and 10 expression in the pre-attachment phase conceptus and endometrium, but current findings implicate both as putative mediators of IFNT production during early pregnancy. Additional replicates of these studies as well as evaluation of IFNT protein secretion, CT-1 cell proliferation, and localization of specific FGFs and receptors within reproductive tissues must be completed before the importance of these FGFs during early bovine conceptus development can be firmly established.

CHARACTERIZATION OF EQUINE UMBILICAL CORD BLOOD-DERIVED STEM CELLS

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Equine athletes have a high risk of musculo-skeletal injury. In every aspect of the performance horse industry, injury and break-down represent a significant loss to owners, trainers, and breeders. Human umbilical cord blood (UCB) contains progenitor cells that can differentiate into osteocytes and chondrocytes, among others. In this study, we show that stem cells can be isolated from equine UCB. UCB was collected from mares at foaling and within 12 hours were isolated by RosetteSep negative selection and Ficoll density gradient centrifugation. A standard marker of stem cell pluripotency, Oct4 was detectable in eUBC by both immunofluorescence and western blot. eUBC also expressed STAT3 and Twist mRNA, inhibitors of differentiation found in embryonic and adult stem cells. Using tissue-specific defined media, eUBC were capable of differentiation into osteocytes and chondrocytes. Osteogenic cells formed nodules that stained positive with Alizarin Red and Von Kossa, indicating the presence of calcium deposits. eUBC were pelleted to a micromass, promoting chondrogenic differentiation in a three-dimensional environment. Micromass cryosections stained positive for glycosaminoglycans and proteoglycans via Alcian Blue and Safranin O. Additionally, after 14 days of culture in hepatogenic medium, cells were densely packed and had acquired large vacuoles, characteristic of hepatic cells. RT-PCR confirmed the presence of albumin mRNA, a protein produced by hepatocytes. eUBC hold immense potential as therapeutic aids. Human UCB stem cells aid regeneration of the central nervous, muscular, and skeletal systems of injured mice. In other species, autologous transplantation of MSC increased the efficiency of recovery from tendon injuries, ligament injuries, and bone fractures. Equine UCB is a source of stems cells that are easily obtainable and can differentiate into multiple cell lineages. Collection of UCB poses no danger to either mare or foal, requiring a much less invasive collection procedure than bone marrow aspiration. Equine UCB presents a unique source of stem cells that may provide an invaluable therapy for equine musculo-skeletal injury and disease.

POTENTIAL INVOLVEMENT OF PKC AND MEK IN MEDIATING FIBROBLAST GROWTH FACTOR 2 ACTIVITY IN TROPHECTODERM

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The critical maternal recognition signal of pregnancy in ruminants, interferon-tau (IFNT), is regulated by uterine-derived cytokines and growth factors. Fibroblast growth factor-2 (FGF-2) is a uterine-derived factor released into the uterine lumen that stimulates IFNT production in a bovine trophectoderm cell line (CT-1), bovine blastocysts, and human choriocarcinoma cells (JEG-3) transfected with IFNT promoter/enhancer reporters. The overall objective of the present work was to investigate expression of FGF receptors and intracellular signaling events involved in mediating IFNT expression in trophectoderm. In the first study, end-point RT-PCR was used to screen for FGF receptor (FGFR) mRNA populations in CT-1 and JEG-3 cells. Amplified products encoding FGFR1c, FGFR2b, FGFR3c, and FGFR4 were detected in both cell lines. In the second study, CT-1 cells were treated with either a pan-inhibitor of PKC isoforms (0.5 μM calphostin C) or an inhibitor of ERK1/2 (50 μM PD98059) prior to FGF2 supplementation to determine if either PKC or MAPK signaling modules mediate the FGF2 action on IFNT expression. After 24 h of FGF2 treatment, real time RT-PCR quantified the abundance of IFNT mRNA relative to an internal RNA control (18S RNA). Preliminary observations suggest that both the pan-PKC and ERK1/2 inhibitors attenuate FGF2-induced IFNT expression. Similar studies focusing on whether PKC- and ERK1/2-mediated pathways impact IFNT promoter/enhancer activity in JEG-3 cells are underway. In summary, bovine and human trophectoderm cells express several FGFR isotypes, and FGF2 binding may recruit MEK and/or PKC signaling molecules to mediate IFNT expression. Continued effort is needed to confirm that PKC and ERK1/2 are inactivated by their respective inhibitors. Once we are convinced that either PKC or ERK1/2 is involved with mediating FGF2 responses on CT-1 and JEG-3 cells, subsequent work will focus on identifying the PKC isoforms responsible for this effect, determining the FGFR isoforms used to mediate this response, and pinpointing the promoter response elements used to regulate IFNT expression.

Current Students in the AMCB

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Sarah Reed (Advisor: Sally Johnson)
Luciano Silva (Advisor: Dan Sharp)
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Qien Yang (Advisor: Alan Ealy)

M.S. Students
Flavia Cooke (Advisor: Alan Ealy)
Michelle Eroh (Advisor: Dan Sharp)

"OK, you know what to do, get in there and make my post docs fall in love with their research."

History of the AMCB Research Symposium

Year	Location	Distinguished Lecturer
2003	Whitney Laboratory, St. Augustine, FL	Randy Prather, University of Missouri
2004	Chinsegut Hill, Brooksville, FL	John Dobrinsky, USDA-ARS, Beltsville, MD
2005	Chinsegut Hill, Brooksville FL	Doug Stocco, Texas Tech University
2006	Lake Wauberg & Animal Science Bldg, UF, Gainesville, FL	Ida Dobrinski, Univ. Pennsylvania
2007	Whitney Laboratory, St. Augustine, FL	Douglas Bannerman, USDA-ARS, Beltsville, MD