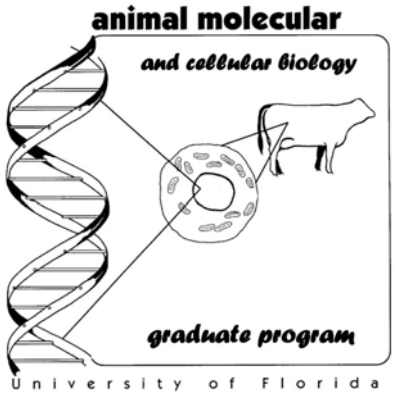
SEVENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Plantation Golf Resort and Spa
Crystal River, Florida
April 17-18, 2009

UF | UNIVERSITY of FLORIDA



Animal Molecular and Cellular Biology Graduate Program

Seventh Annual Research Symposium

WELCOME

Once again, we gather for another Research Symposium of the Animal Molecular and Cellular Biology Graduate Program of the University of Florida. We meet in Crystal River, famous for manatees and golf, and soon to be memorialized as the location for an outstanding scientific event.

The AMCB has undergone significant growth since our last symposium. Our emphasis has changed from livestock to comparative biology. As part of this refocusing, seven new faculty have joined the program. They are Jeff Abbott and Mary Brown from the Dept. of Infectious Diseases & Pathology, Nancy Denslow, from the Dept. of Physiological Sciences, Dan Hahn, from Entomology & Nematology, Maureen Keller-Wood, from the Dept. of Pharmacodynamics, Jose Santos, from the Dept. of Animal Sciences, and Charlie Wood from the Dept. of Physiology & Functional Genomics. The program will be richer because of their involvement.

One faculty member retired in 2008, Mike Fields, and one, Mats Troedsson, left for a new position at the University of Kentucky. Good luck to both of them. We are glad to know Mike Fields will continue to be involved with the AMCB in retirement.

In 2008, two students, Katherine Hendricks and Flavio Silvestre, completed their Ph.D. degree. Flavia Cooke obtained a MS in the AMCB program, which is the first awarded since the AMCB was approved as a formal graduate program in 2007.

The symposium will mark the end of Dr. Hansen's term as Director. Dr. Badinga will begin a two-year stint as Director after the meeting and Dr. Alan Ealy will assume the duties of Co-Director.

To new students and faculty, we welcome you to the premier scientific event in the AMCB calendar. To new and veteran students and faculty, we trust the Seventh Symposium will be marked by good science, good fellowship, and good memories.

Pete Hansen, AMCB Director Lokenga Badinga, AMCB Co-Director

ACKNOWLEDGEMENTS

The faculty and students of the Animal Molecular and Cellular Biology Program thank the following for support of the 7th Annual Research Symposium

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

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

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

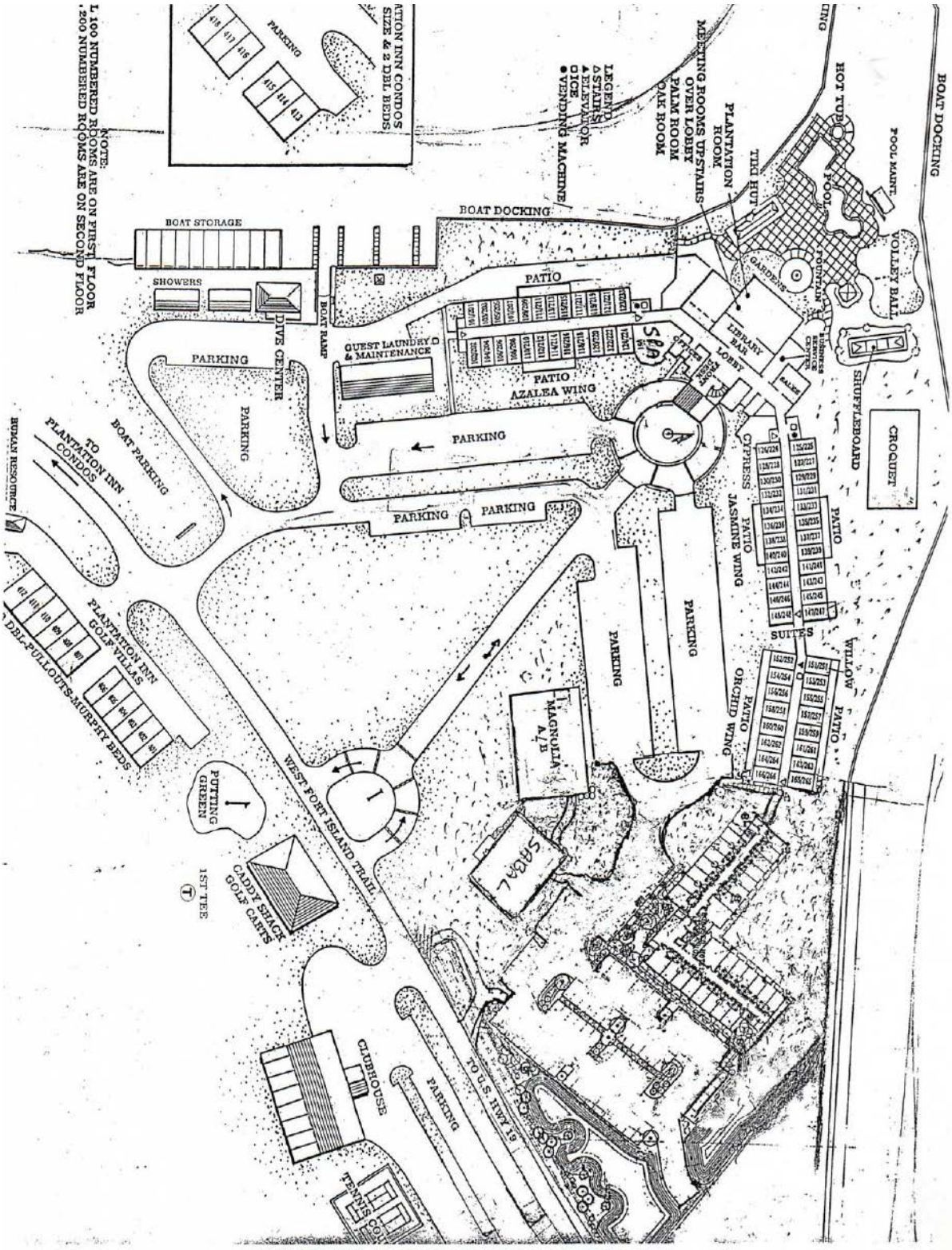
Appreciation is also expressed to those who have supported the AMCB throughout the year

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 H. Brendemuhl, Professor, Dept. of Animal Sciences, University of Florida; Graduate Coordinator, AMCB

Special thanks to Sarah Fields and Kathleen Pennington for on-site research

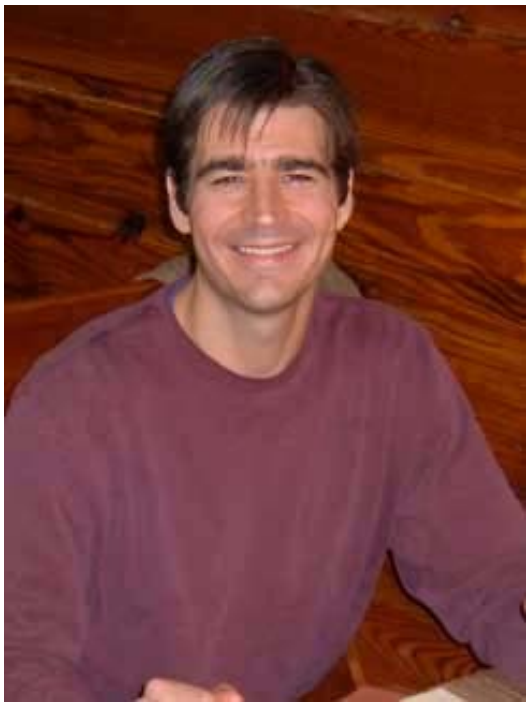

BOAT DOCKING
HOT TUB
POOL
POOL MAINT.
VOLLEY BALL
TIKI HUT
PLANTATION ROOM
PALM ROOM
OAK ROOM
MEETING ROOMS UPSTAIRS OVER LOBBY
LEGEND
STAIRS
ELEVATOR
ICE
VENDING MACHINE
BOAT STORAGE
SHOWERS
BOAT DOCKING
PATIO
GARDENS
FOUNTAIN
LIBRARY BAR
LOBBY
BUSINESS CENTER
SHUFFLEBOARD
CROQUET
DIVE CENTER
BOAT RAMP
GUEST LAUNDRY & MAINTENANCE
PARKING
PATIO
AZALEA WING
CYPRESS
JASMINE WING
ORCHID WING
WILLOW
SUITES
TO PLANTATION INN CONDOS
BOAT PARKING
HUMAN RESOURCE
PLANTATION INN GOLF VILLAS
DBL-PULLOUTS-MURPHY BEDS
MAGNOLIA A/B
WEST FORT ISLAND TRAIL
PUTTING GREEN
1ST TEE
CADDY SHACK GOLF CARTS
CLUBHOUSE
TO U.S. HWY 19
TENNIS
NOTE:
L 100 NUMBERED ROOMS ARE ON FIRST FLOOR
200 NUMBERED ROOMS ARE ON SECOND FLOOR

2009 Animal Molecular and Cellular Biology Distinguished Lecturer

Dr. Dean H. Betts

Dr. Dean Betts is currently an Associate Professor in the Departments of Physiology & Pharmacology and Obstetrics & Gynecology at the University of Western Ontario, London, Canada. He received degrees from the University of Western Ontario (BSc, MSc), and the University of Guelph (PhD). Following a post-doctoral fellowship at Case Western Reserve University, Dr. Betts joined the faculty at the Ontario Veterinary College in 2001. Dr. Betts' research, which has resulted in 37 peer-reviewed publications, has focused on characterizing and understanding the molecular and cellular mechanisms of early mammalian development using cattle embryos as his main experimental model. Notably, his studies on telomere length regulation presented evidence of a telomere-lengthening event during the first week of embryogenesis and that some healthy and fertile cloned animals and their offspring exhibit variant telomere lengths compared to their reproductively bred counterparts suggesting that epigenetic alterations could be passed through the germ line. Dr. Betts was one of the first to apply RNA interference technology to study gene function in bovine embryos. Using live cell imaging and embryo microinjections his lab revealed that the stress adaptor protein p66Shc is mechanistically involved in the ROS signaling pathway of permanent embryo arrest. Applying global gene expression analyses to stably transfected bovine cell lines, his research has discovered alternative function(s) for the catalytic subunit of telomerase (TERT) that changes the cell state towards a progenitor stem cell-like condition. This information explains the utility of ectopically expressed TERT as a potent reprogramming factor in the production of induced pluripotent stem (iPS) cells. Just prior to Dr. Betts' move to the University of Western Ontario in 2008, his lab was one of the first groups to generate and characterize numerous canine embryonic stem cell lines and the first to isolate a mesenchymal stem cell population from umbilical cord blood of foals. These recent discoveries have led Dr. Betts to focus his research activities in developing clinically relevant animal models for stem cell – based transplantation therapies.

Guest Lecturer



Dr. Daniel A. Hahn

Daniel A. Hahn is an Assistant Professor in the Department of Entomology and Nematology at the University of Florida. Born and raised in south Florida, Dan spent much time in the ocean and thought he wanted to grow up to be Jacques Cousteau. A love of animal diversity coupled with a habit of taking things apart to try and understand how they worked lead Dan to Florida State University (BS), where he discovered insects and was hooked by the fact that physiologists and cell biologists were also obsessed with taking things apart to understand how they worked. Dan followed with graduate work at the University of Arizona (MS, Ph.D.) and a postdoctoral fellowship at Ohio State University before taking a position at UF four years ago. Most of the current work in Dan's group focuses on physiology and evolutionary ecology of insect diapause (hibernation). Surviving without eating is a challenging task faced by most diapausing insects. Diapause offers a tremendous adaptive advantage allowing survival in seasonal environments that could otherwise not be tolerated and permits precise synchronization of the life cycle with periods suitable for growth, development and reproduction. However, successfully completing diapause implies an impressive capacity for managing energy reserves. Diapauses of 9-10 months are common and in a few cases may stretch to several years. Insects use two strategies to mitigate the energetic costs of diapause: accumulation of greater reserves and metabolic depression. Dan's laboratory uses a variety of insect model systems to study both the proximate, physiological and genetic, mechanisms underlying metabolic depression and nutrient storage in diapause and the ultimate, ecological and behavioral, costs and benefits of diapause-induced nutrient storage and metabolic depression.

Committees of the AMCB, 2009-2010

Director: Peter J. Hansen
Co-Director: Lokenga Badinga
Graduate Coordinator: Joel H. Brendemuhl
Program Assistant: Joann Fischer

Bylaws
Ken Drury (chair)
Pete Hansen

Symposium Committee
Pete Hansen (chair)
Lokenga Badinga
Alan Ealy
Sarah Fields
Lilian Oliveira
Kathleen Pennington
Sarah Reed

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
2008	Cedar Cove Beach and Yacht Club, Cedar Key, FL	Eckhard Wolf, Genzentrum der LMU-München
2009	Plantation Golf Resort and Spa, Crystal River, FL	Dean H Betts, University of Western Ontario

AMCB Faculty

Jeffrey R. Abbott
Department of Infectious Diseases and Pathology

Lokenga Badinga, Ph.D.
Department of Animal Sciences

Mary B. Brown, Ph.D.
Department of Infectious Diseases and Pathology

Geoffrey E. Dahl, Ph.D.
Department of Animal Sciences

Nancy Denslow
Department of Physiological Sciences

Kenneth C. Drury, Ph.D., HCLD
Department of Obstetrics & Gynecology

Alan D. Ealy, Ph.D.
Department of Animal Sciences

Daniel A. Hahn
Department of Entomology and Nematology

Peter J. Hansen, Ph.D.
Department of Animal Sciences

Sally E. Johnson, Ph.D.
Department of Animal Sciences

David Julian, Ph.D.
Department of Zoology

Maureen Keller-Wood
Department of Pharmacodynamics

Karen Moore, Ph.D.
Department of Animal Sciences

Jose E. P. Santos, D.V.M., Ph.D.
Department of Animal Sciences

Charles E. Wood
Department of Physiology and Functional Genomics

Joel V. Yelich, Ph.D.
Department of Animal Sciences

Emeritus Faculty

William C. Buhi, Ph.D.
Departments of Obstetrics & Gynecology, Biochemistry & Molecular Biology, and Animal Sciences

Michael J. Fields, Ph.D.
Department of Animal Sciences

Daniel C. Sharp, Ph.D.
Department of Animal Sciences

William W. Thatcher, Ph.D.
Department of Animal Sciences

Current Students in the AMCB

Ph.D. Students
Aline Bonilla (Advisor: Pete. Hansen)
Andria Doty (Advisor: Mats Troedsson)
Regina Esterman (Advisor: Joel Yelich)
Sarah D. Fields (Advisors: Pete Hansen and Alan Ealy)
Leandro Greco (Advisor: Jose Santos)
Joe Kramer (Advisors: Ken Drury)
Barbara Loureiro (Advisor: Pete Hansen)
Lilian Oliveira (Advisor: Pete Hansen)
Maria Beatriz Padua (Advisor: Pete Hansen)
Kathleen Pennington (Advisor: Alan Ealy)
Sarah Reed (Advisor: Sally Johnson)
Luciano Silva (Advisor: Dan Sharp)
Sha Tao (Advisor: Geoff Dahl)
Qien Yang (Advisor: Alan Ealy)
Kun Zhang (Advisor: Alan Ealy)

M.S. Students
Rafael Bisinotto (Advisor: Jose Santos)
Justin Fear (Advisor: Pete Hansen)
Izabella Thompson (Advisor: Bill Thatcher)

"My old lab was cluttered with test tubes, bottles, flasks, paper...Lord, how I miss it!"

SCHEDULE

Friday PM, April 17, 2009
Plantation Golf Resort & Spa
Sable Room

Session 1, Distinguished Lecture

1:00-1:15 PM	Peter J. Hansen, Welcoming remarks.
1:15-2:00 PM	Dean H. Betts, AMCB Distinguished Lecturer, Depts of Physiology & Pharmacology and Obstetrics & Gynecology, University of Western Ontario, London. Equine cord blood stem cells - from farm to point of care.
2:00-2:30 PM	BREAK

Session 2. Research Reports (Sarah Fields, chair)

2:30 – 2:45 PM	Yang, Q., Johnson, S.E., and Ealy, A.D. Dept. of Animal Sciences, University of Florida, Gainesville. Protein kinase C mediates fibroblast growth factor 2-induced expression of interferon tau in bovine trophectoderm.
2:45 - 3:00 PM	Pennington, K.A., and Ealy, A.D. Dept. of Animal Sciences, University of Florida, Gainesville.Identification of selective bone morphogenetic proteins (BMPs) and their receptors in peri-attachment bovine conceptuses and the trophectoderm cell line, CT1.
3:00 - 3:15 PM	Cerri, R.L.A. Thompson, I.M., Kim, I.H. Ealy, A.D., Hansen, P.J., Staples, C.R., Li, J.L. and Thatcher, W.W. Dept. of Animal Sciences, University of Florida, Gainesville and Chungbuk National University, South Korea Effects of lactation and pregnancy on endometrial gene expression in dairy cattle.
3:15 – 3:30 PM	Thompson, I.M., Cerri, R.L.A., Kim, I.H., Green, J.A., and Thatcher, W.W. Dept. of Animal Sciences, University of Florida, Gainesville and Chungbuk National University, South Korea. Bovine pregnancy associated glycoproteins (PAGs): conceptus and endometrial expression and subsequent dynamics in plasma during pregnancy.
3:30 – 4:00 PM	GROUP PICTURE

Session 3 – Research Reports (Kathleen Pennington, Chair)

4:00 – 4:15 PM	Kramer, J.M., Ali, L., and Drury, K.C. Depts. of Animal Sciences and Obstetrics & Gynecology, University of Florida . Serum substitute supplement (SSS) improves control-rate cryopreservation and post thaw re-expansion of murine blastocysts.
4:15 – 4:30 PM	Fear, J. M. Bonilla, L., Kennedy, J.W., Talbot, N.C., Stodieck, L., and Hansen, P.J. Dept. Animal Sciences, University of Florida, Zero Gravity, Inc., Stevensville, MD, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, and BioServe Space Technologies, University of Colorado, Boulder, CO. Cows in space: A preliminary investigation to determine effects of space flight on preimplantation development of bovine embryos.
4:30 - 4:45 PM	Zhang, K., and Ealy, A.D. Dept. Animal Sciences, University of Florida. Global assessment of the conceptus transcriptome in response to fibroblast growth factor 2 and 10 supplementation in vitro.
4:45 – 5:00 PM	Fields, S.D., Hansen, P.J., and Ealy, A.D. Dept. of Animal Sciences, University of Florida. Actions of fibroblast growth factor 2 on development of bovine embryos to the blastocyst stage.
4:45 – 5:00 PM	Loureiro, B., Bonilla, L., Block, J., Fear, J.M., Bonilla, A.Q.S. and Hansen, P.J. Dept. of Animal Sciences, University of Florida. Granulocyte-macrophage colony stimulating factor improves development and post-transfer survival of bovine embryos produced in vitro.
5:00 – 5:15 PM	Bonilla, A.Q.S., and Hansen, P.J. Dept. of Animal Sciences, University of Florida. Developmental changes in actions of insulin-like growth factor-I to protect the preimplantation bovine embryo from heat shock.
5:15 - 5:30 PM	Carambula, S., Oliveira, L., and Hansen, P.J. Dept. of Animal Sciences, University of Florida. Represssion of the intrinsic pathway to apoptosis in the two-cell bovine embryo involves histone deacetylation and DNA methylation.

CHECK INTO ROOMS

COOKOUT – AT THE VILLAS

Saturday AM, April 18, 2009
Plantation Golf Resort & Spa
Sable Room

7:30 – 8:45 AM Breakfast (on your own)

Session 4 – Guest Lecture (Lokenga Badinga, Chair)

8:45 - 9:30 AM Daniel A. Hahn, Dept. of Entomology & Nematology, University of Florida, Metabolic Mechanisms Mediate the Miserable Months: a journey through fly hibernation physiology.

9:30 – 10:00 AM BREAK

Session 5 – Research Reports (Barbara Loureiro, Chair)

10:00 – 10:15 AM Esterman, R.D., Austin, B.R., McKinniss, E.M., and Yelich, J.V. Dept. of Animal Sciences, University of Florida. Comparison of a Select Synch/CIDR + timed-AI (TAI) to a Co-Synch/CIDR synchronization protocol in suckled *Bos indicus x Bos taurus* cows.

10:15 – 10:30 AM Bisinotto, R.S., Chebel, R., C., and Santos, J.E.P. Dept. of Animal Sciences, University of Florida and School of Veterinary Medicine, University of California Davis. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows.

10:30 – 10:45 AM Greco, L.F., Garcia, M., Favoreto, M., Thatcher, W.W., Staples, C.R., and Santos, J.E.P. Dept. of Animal Sciences, University of Florida. Fatty acid supplementation to periparturient dairy cows receiving diets containing low amounts of essential fatty acids.

10:45 – 11:00 AM BREAK

Session 6 – Research Reports (Regina Esterman, Chair)

11:00 – 11:15 AM Reed, S.A., Li, J., and Johnson, S.E. Dept. of Animal Sciences, University of Florida. Effects of three dimensional culture and fibroblast growth factor supplementation on equine umbilical cord blood derived stem cells.

11:15 – 11:30 AM Oliveira, L., and Hansen, P.J. Dept. of Animal Sciences, University of Florida. Phenotypic characterization of macrophages in the endometrium of the pregnant cow.

11:30 – 11:45 AM Bubolz, J.W., Tao, S., do Amaral, B.C., Hayen, M.J., Olson, T.A., and Dahl, G.E. Dept. of Animal Sciences, University of Florida. Heat stress

does not alter immune status of Holstein calves but slick genotype confers reduced immune function.

11:45 – 12:00 PM Tao, S., and Dahl, G.E. Dept. of Animal Sciences, University of Florida. Environmental effects during dry period on the mammary gland development of dairy cows.

ADJOURNMENT

ABSTRACTS

PROTEIN KINASE C MEDIATES FIBROBLAST GROWTH FACTOR 2-INDUCED EXPRESSION OF INTERFERON TAU IN BOVINE TROPHECTODERM

Qien Yang, Sally E. Johnson, Alan D. Ealy
Department of Animal Sciences, University of Florida, Gainesville, FL, 32611

Several uterine- and conceptus-derived factors are recognized as essential components of peri-implantation conceptus development in placental animals. In cattle and sheep, several uterine- and conceptus-derived fibroblast growth factors (FGFs) are implicated as regulators of interferon tau (IFNT) expression, the trophectoderm-derived factor responsible for maternal recognition of pregnancy signal in cattle, sheep and likely other ruminants. An FGF of central interest is FGF2. It is produced by both the uterine epithelium and the conceptus during peri-attachment development in sheep and cattle, and FGF2 supplementation stimulates IFNT mRNA and protein concentrations in bovine blastocysts and a bovine trophectoderm cell line (CT1). The goal of this project was to build on previous observations and determine whether protein kinase C-mediated systems are required for the induction of IFNT expression by FGF2 in bovine trophectoderm. To inhibit or activate PKC signaling pathway, the CT1 cells were treated with PKC inhibitors or phorbol 12-myristate 13-acetate (PMA), respectively. The impact of treatments on IFNT mRNA abundance was determined after 24h of FGF2 supplementation by using quantitative RT-PCR. The result revealed a 10.70 ± 1.93 fold increase in IFNT mRNA abundance in response to FGF2 ($P<0.001$). Exposure to the pan-PKC inhibitor (0.5μM Calphostin C) limited ($P<0.05$) the ability of FGF2 to increase in IFNT mRNA abundance (2.24±1.12). In a subsequent study, supplementation 100nM PMA stimulated IFNT mRNA levels to the same extent as FGF2 treatment. Also, the combined exposure to PMA and FGF2 did not provide any added effect on IFNT mRNA levels, suggestive of PMA and FGF2 acting on the same cellular cascade. Identification of the specific PKC isoform impacted by FGF2 was examined by exposing CT1 cells to chemical antagonists of classical PKCs (5 μM Gö6976) or PKC-delta (5 μM rottlerin), a novel PKC (PRKCD). Both FGF2 and PMA increased IFNT mRNA abundance in CT1 cells exposed to the classical PKC inhibitor. By contrast, inhibition of PKC-delta attenuated the PMA (0.29±0.06) and FGF2 (1.11± 0.75) responses ($P<0.01$). PRKCD mRNA was detected in CT1 cells and day 14, 17 bovine conceptus using end-point RT-PCR. Both PMA and FGF2 activated PKC-delta via phosphorylation of Tyr311 loop. To summarize, these observations support the contention that regulation of PKC-delta activity is controlled by FGF2 and PMA in trophectoderm and this signaling module controls FGF2-mediated IFNT expression. This Project was supported by the National Research Initiative Competitive Grant no.2008-35203-19106 from the USDA Cooperative State Research, Education, and Extension Service.

IDENTIFICATION OF SELECTIVE BONE MORPHOGENETIC PROTEINS (BMPS) AND THEIR RECEPTORS IN PERI-ATTACHMENT BOVINE CONCEPTUSES AND THE TROPHECTODERM CELL LINE, CT1

Kathleen A. Pennington and Alan D. Ealy
Dept. Animal Sciences, University of Florida, Gainesville Florida

The TGF beta super family is a collection of autocrine, paracrine and endocrine factors involved in multiple developmental and physiological functions in various species. BMPs are members of this gene super family. BMPs were first described for their roles in bone formation, but they now also are known to possess additional activities, including those relating to gametogenesis and embryogenesis. BMPs are best know in farm animals for their ability to control oogenesis and follicle development, but it also is possible that they may control specific features of embryogenesis in farm animals. In human embryonic stem cells, BMP 2 and 4 induce differentiation of embryonic stem cells to trophoblast cells. In the mouse, BMP 4 plays a key role in gastrulation and mesoderm formation. Recent work by this group discovered BMP 2 and 4 mRNA in elongating day 17 bovine conceptuses and diestrus-phase pregnant bovine endometrium when using a custom-designed bovine DNA Nylon Array. The goal of this work of was to determine if elongated bovine conceptuses and CT1 cells contain transcripts for BMP2 and 4 and whether the receptors associated with BMP2/4 activity (Type I and II serine/threonine receptors known as Alk3, 6, BMPRII) and their innate inhibitor (noggin) are expressed in bovine conceptuses and CT1 cells. Primers were designed based on predicted transcript sequences available on the Genbank Database. RNA from elongated bovine conceptuses and CT1 cells was reverse transcribed and 30-40 cycles of polymerase chain reaction(RT-PCR) was completed. The presence or absence of amplified products was determined by gel electrophoresis and ethidium bromide staining. Amplified products were cloned into the pCR4-ZeroBluntTOPO plasmid and sequenced to verify the specificity of amplification. Both BMP2 and 4 mRNA were amplified in elongating day 17 bovine conceptuses and CT-1 cells. Interestingly, several additional PCR cycles were required before BMP2 and 4 could be visualized in CT1 RNA preparations, suggesting that perhaps elongating conceptuses contain greater amounts of BMP2 and 4 mRNA than CT1 cells. Quantitative approaches have not been competed to verify this supposition. Alk3, Alk6, and BMPR-II mRNA also were detected in day 17 conceptuses and CT-1 cells. Transcripts for noggin were identified in day 17conceptus preparations but not in CT1 cell preparations. Further studies are needed to elucidate the gene expression profiles and localization pattern of these ligands during pre- and peri-attachment conceptus development and discover the functional significance of these factors on bovine conceptus development and maternal-fetal communication.

EFFECTS OF LACTATION AND PREGNANCY ON ENDOMETRIAL GENE EXPRESSION IN DAIRY CATTLE

R.L.A. Cerri[1], I.M. Thompson[1], I.H. Kim[2], A.D. Ealy[1], P.J. Hansen[1], C.R. Staples[1], J.L. Li[1], and W.W. Thatcher[1]
[1]*University of Florida, Gainesville,* [2]*Chungbuk National University, South Korea*

Lactation is associated with decreased fertility in dairy cows. Objectives were to determine the effects of lactation and pregnancy on endometrial gene expression on day 17 of the estrous cycle and pregnancy in Holstein first calf heifers. Heifers (n=33) were assigned randomly after parturition to a lactating (L, n=17) or non-lactating group (NL, n=16). All cows were subjected to an ovulation synchronization program to allow for a timed artificial insemination; 10 cows in L and 12 in NL were inseminated. All cows were slaughtered 17 days after the day of timed artificial insemination, and conceptus and intercaruncular endometrial tissues collected. Only pregnant (L, n=8; NL, n=6) and non-inseminated cyclic (L, n=7; NL, n=4) cows were included in analyses. Total RNA was extracted from endometrium from the uterine horn ispilateral to the corpus luteum, and microarray analysis performed using the bovine Affymetrix GeneChip platform (23,000 *Bos taurus* transcripts). Data were analyzed using Bioconductor GCRMA and Limma methods. Differentially expressed genes were selected when P-value < 0.01. Approximately 4861 expressed sequence tags were absent in all arrays. Analysis detected 16 genes expressed differentially because of the main effect of lactation (P< 0.01; 7 down-regulated and 9 up-regulated) and 674 genes expressed differentially because of a main effect of pregnancy (P < 0.01; 389 down-regulated and 285 up-regulated). In addition, 27 genes were expressed differentially in association with both lactation and pregnancy (P<0.01; i.e., concurrent main effects of both pregnancy and lactation, or lactation by pregnancy interactions). Gene ontology (GO) analyses of down-regulated genes during pregnancy revealed 90 GO terms being differentially expressed. Examples include cell adhesion (GO:0007155) and multicellular organismal development (GO:0007160). Genes up-regulated in pregnant cows were associated with 91 GO terms like defense response (GO:0006952), response to virus (GO:0009615) and cell death (GO:0008219), among others. Differentially expressed genes due to lactation were overrepresented for cell differentiation (GO:0030154) and cellular protein metabolic process (GO:0044267). Differentially expressed genes, related to concurrent effects of lactation and pregnancy, were associated with regulation of signal transduction (GO:0009966). In summary, endometrial gene expression on day 17 post-ovulation was affected by presence of the conceptus. In addition to the expected interferon-regulated genes, genes related to cell adhesion, glucose homeostasis and lipid metabolism were also affected by pregnancy. The effect of lactation and the interaction of lactation and pregnancy revealed a smaller number of differentially expressed genes than initially anticipated but this set includes genes whose expression may contribute to sub-fertility often observed in lactating dairy cows.

BOVINE PREGNANCY ASSOCIATED GLYCOPROTEINS (PAGS): CONCEPTUS AND ENDOMETRIAL EXPRESSION AND SUBSEQUENT DYNAMICS IN PLASMA DURING PREGNANCY

I.M. Thompson*[1], R.L.A. Cerri[1], I.H. Kim[2], J.A. Green[3] and W.W. Thatcher[1]
[1]University of Florida, Gainesville, [2]Chungbuk National University, South Korea, [3]University of Missouri,Columbia

The pregnancy associated glycoproteins (PAGs) constitute a large gene family expressed in trophoblast cells of ruminant ungulates. PAGs are sub-divided into two distinct groups: ancient PAGs, expressed in all trophoblast cells; recently evolved PAGs, expressed only in binucleate cells. The detection of bovine PAGs in maternal serum is used for early pregnancy diagnosis and as an indicator of fetal development and pregnancy loss. The expression of PAG family members in early gestation is associated with trophoblast cell differentiation and may indicate that these genes are involved in implantation. Pregnant Holstein dairy heifers (n=34) were randomly assigned after parturition to either Lactating (n=17) or Non-Lactating (n=17) Groups. All cows were placed on a Presynch/CIDRSynch program (~77days postpartum) and either inseminated (Pregnant; n=14) or not inseminated (Cyclic; n=12) and were slaughtered for tissue collection on day 17 after GnRH injection (day 0 is day of GnRH injection comparable to day of estrus). The Bovine Affymetrix GeneChip was used to assess conceptus and endometrial gene expression. Conceptuses (n=14) expressed PAG1 (n=1), PAG2 (n=13), PAG7 (n=3), PAG8 (n=13), PAG9 (n=1), PAG10 (n=1), PAG11 (n=13), PAG12 (n=13), PAG17 (n=2), PAG18 (n=1), PAG20 (n=1) and PAG21 (n=5). In contrast, PAG2 (n=4), PAG8 (n=1), PAG11 (n=7) and PAG12 (n=3) were expressed in the endometrium of pregnant cows. Correlation and multiple regression analyses detected associations of PAG11 expression with the following genes: HPGD (hydroxyprostaglandin dehydrogenase 15-[NAD]), PGRMC2 (progesterone receptor membrane component 2), PIBF1 (progesterone immunomodulatory binding factor 1), and PTGDS (prostaglandin D2 synthase 21kDa). A direct association was detected between PAG11 and HPGD ($r = 0.47$; $P < 0.01$). A further assessment of HPGD expression within this gene cluster independent of PAG11 (i.e., prolifically expressed endometrial genes of pregnant/cyclic and lactation/dry cows) detected an association of HPGD with PGRMC2 and PIBF1, whereas PTGDS was negatively associated with PIBF1. In a second experiment, primiparous and multiparous (n=1578) cows were assigned randomly to two treatment groups: Resynch and Control. Blood samples for analyses of PAGs were taken every 2 days from day 18-30 (n=100) and continued weekly to day 60 in pregnant cows (n=43). Plasma concentrations of PAGs in pregnant cows were lower ($P < 0.01$) for Resynch on days 39 ($2.8 < 4.1$ ng/ml) and 46 ($1.34 < 3$ ng/ml). Cows pregnant at day 32, that had pregnancy loss by day 60 (n=7), had lower ($P < 0.05$) plasma concentrations of PAGs at day 30 than cows (n=36) that maintained pregnancy until day 60 ($2.9 < 5$ ng/ml). PAGs at day 30 (> 0.33ng/ml) were predictive of a day 32 pregnancy (Sensitivity 100%, Specificity 90.6%). A second subset of blood samples (n=101) were collected at the day of parturition to relate PAG plasma concentrations with difficulty at parturition, calf gender and occurrence of peripartum health disorders. However, no significant relationships were detected. Early expressions of PAGs within the endometrium of pregnant cows and in the conceptus infer a possible role of PAGs in regulating prostaglandin metabolism that may alter vascularization, steroidogenesis and immune function of the conceptus-maternal unit. Dynamics of PAGs in plasma were indicative of pregnancy status and pregnancy loss.

SERUM SUBSTITUTE SUPPLEMENT (SSS) IMPROVES CONTROL-RATE CRYOPRESERVATION AND POST THAW RE-EXPANSION OF MURINE BLASTOCYSTS

Joseph M. Kramer[1], Larissa Ali[2] and Kenneth C. Drury[2]
[1]*Department of Animal Sciences, University of Florida, Gainesville, FL*
[2]*Department of Obstetrics and Gynecology, University of Florida, Gainesville, FL*

Improvements in the technology of in vitro fertilization (IVF) have led to increased rates of conversion of embryos to the blastocyst stage such that there are usually surplus embryos remaining in culture post embryo transfer. Among the options for remaining embryo disposition, cryopreservation of surplus embryos poses the greatest benefit to the patient, but only if the IVF center has established a successful freezing program. Put simply, a successful cryopreservation program can be characterized as having high rates of thaw recovery and survival followed by successful implantation and pregnancy. To date, there are a limited number of publications from various IVF centers that demonstrate these high standards. Interestingly, these successful programs commonly supplement α- and β-globulins as a part of a complex protein source in both freeze and thaw solutions. In addition, several of the reports incorporated the use of cryo-vials instead of traditional freezing straws. Our main objective was to test whether the supplementation of cryo solutions with a complex protein source containing globulins would improve blastocyst cryopreservation. In addition, we also set out to determine if cryo-vials offered any advantages over the use of freezing straws. Frozen two-cell murine embryos were thawed and cultured in vitro for 72 hr to the blastocyst stage. Only cavitating blastocysts with discernable inner cell mass (ICM) and well structured trophectoderm were considered for cryopreservation. Standard protocols were used for freezing and thaw procedures. Specimens were stored in LN2 for at least 24 hr, but not more than 1 wk. Following thaw, embryos were cultured for 24 hr in G2v5 PLUS medium (Vitrolife) containing human serum albumin (HSA, 5mg/ml) and then assessed for blastoceol re-expansion as a measure of survival. Data were analyzed using chi-square test, with significance deemed $P<0.05$. Experiment 1 was a 2x2 design comparing cryopreservation of blastocysts using freeze and thaw solutions (Vitrolife) supplemented with SSS (Irvine Scientific, 10mg/ml HSA + 2mg/ml globulins) or HSA (Vitrolife, 10mg/ml). Fewer blastocysts re-expanded if freeze→thaw solutions contained HSA→HSA or SSS→HSA compared to SSS→SSS ($P<0.05$). Experiment 2 was similar to the first experiment except that post-thaw culture medium was supplemented with SSS (5mg/ml HSA + 1mg/ml globulin). No differences were observed across treatments with all demonstrating high rates of re-expansion. Experiment 3 compared control-rate cryopreservation using either High Security CBS straws (Conception Technologies) or cryo-vials (Nunc). No differences between freezing apparatuses were observed for either rates of recovery or blastocyst re-expansion. In conclusion, our data demonstrate that the inclusion of a complex protein source containing globulins in cryo solutions improves post-thaw survival and that supplementation of post-thaw culture medium with SSS is able to restore the potential for blastocyst re-expansion even if previously frozen and/or thawed in solutions devoid of globulins. In addition, usage of cryo-vials was shown to perform equally as well as freezing straws. Future studies will need to verify whether these results can be transferred to a clinical IVF setting for the establishment of a successful cryopreservation program.

COWS IN SPACE: A PRELIMINARY INVESTIGATION TO DETERMINE EFFECTS OF SPACE FLIGHT ON PREIMPLANTATION DEVELOPMENT OF BOVINE EMBRYOS

J. M. Fear[1], L. Bonilla[1], J. W. Kennedy[2], N. C. Talbot[3], L. Stodieck[4] and P.J. Hansen[1]
[1]*Department of Animal Sciences, University of Florida, Gainesville, Florida*
[2]*Zero Gravity, Inc., Stevensville, MD*
[3]*Agricultural Research Service, United States Department of Agriculture, Beltsville, MD*
[4]*BioServe Space Technologies, University of Colorado, Boulder, CO*

Organisms in space are exposed to a variety of stressors including microgravity and ionizing radiation. Microgravity can affect cytoskeletal strcuture (Uva et al 2002, Brain Res 934:155) and rates of apoptosis (Sarkar et al. 2000, J Gravit Physiol 7:P71). The objective of this study was to evaluate effects of space flight on development of bovine preimplantation embryos. We hypothesized that space flight would inhibit development, possibly because of interference with tight junction formation, blastocoele formation or induction of apoptosis. Oocytes were collected, matured in vitro and fertilized with sperm pooled from three bulls. Procedures were carried out at the Space Life Sciences Laboratory at Kennedy Space Center. At 16 h post insemination (hpi), embryos were washed and placed into well culture inserts (BioServe Space Technologies, Boulder CO) with 45 µL pre-equlibrated modified BBH7 medium. Inserts were loaded into Fluids Processing Apparatuses (FPA) (BioServe Space Technologies) containing a volume of 0.7 mL modified BBH7. FPAs were then placed for 1 h at 38.5^{o}C in low oxygen (5% CO_2, 5% O_2, 90% N_2) to allow for equilibration. A fixative [3.4% (w/v) gluteraldehyde or 6.8% (w/v) paraformaldehyde] was loaded into the fixative chamber of the FPAs. FPAs were then loaded into the plunger device which was then gassed with low oxygen, sealed and placed into one of two Group Activation Packs (GAPs) (BioServe Space Technologies) and held at 38.5^{o}C. After FPAs and GAPs were examined for leaks, they were placed into either a flight or ground incubator at 37^{o}C. The flight incubator was then turned over to Kennedy Space Center staff for loading onto the *Endeavour* approximately 24 h pre-launch. The *Endeavour*, mission STS-126, launched at approximately 52 hpi (i.e., while embryos were at the 4-8 cell stage). While in orbit, embryos were either fixed on Day 9 or Day 16 after insemination by activating the plunger device. Embryos were maintained at 37^{o}C until recovery at landing on Day 20. Ground control embryos were treated the same. Upon recovery, development was assessed for both flight and ground controls. Of the 1200 embryos loaded (600 flight and 600 ground), 1067 were recovered (547 flight and 520 ground). No blastocysts were obtained from either flight or ground control embryos. Cleavage rate was not significantly different between flight embryos (9%) and ground controls (12%). Of those embryos that cleaved, the majority were at the 2-4 cell stage (78% flight and 89% ground). The percent of cleaved embryos that were ≥6 cells (i.e., embryos that developed at a time typically coincident with space flight) tended to be greater for space flight embryos (22% vs 11% ground) but differences were not significant (P=0.12). In conclusion, our initial attempt to achieve development of bovine embryos in space was limited by inadequate culture conditions. Future experiments to improve these conditions are planned.

GLOBAL ASSESSMENT OF THE CONCEPTUS TRANSCRIPTOME IN RESPONSE TO FIBROBLAST GROWTH FACTOR 2 AND 10 SUPPLEMENTATION IN VITRO

Kun Zhang and Alan D. Ealy
Animal Molecular & Cellular Biology Program, University of Florida, Gainesville, Florida 32611, USA

Pregnancy failures are prevalent in dairy and beef cattle, and one period of notable losses occurs as tissues with the developing conceptus (*i.e.* a fetal-placental unit) expand and differentiate in preparation for uterine attachment. During this period ruminant conceptuses must secrete ample amounts of interferon-tau (IFNT), the maternal recognition of pregnancy factor for these species. It is well known that IFNT limits the pulsatile release of prostaglandin $F_{2\alpha}$, thereby preventing CL regression. It also regulates uterine-derived factors that support pregnancy. Our laboratory determined that fibroblast growth factors (FGFs) promote IFNT expression in bovine trophectoderm. FGF2 and 10 are of particular interest. FGF2 is produced by the endometrial epithelium and detected in uterine luminal fluid throughout early pregnancy in cattle and sheep. Also, specific FGF2 polymorphisms are associated with bovine blastocyst formation in vitro. FGF10 is a key mesoderm-derived factor found in the uterus and conceptus. Its role in bovine conceptus development remains unexplored, but FGF10 transcript abundance increases dramatically during conceptus elongation coincident with the rapid surge in IFNT transcription, trophectoderm morphogenesis, and gastrulation. We propose that FGF2 and 10 impact key developmental events during bovine conceptus development in addition to their pre-described effects on IFNT expression. The first step in describing FGF2 & 10 actions on bovine conceptuses is to determine how these factors modify the conceptus transcriptome. Day 8 in vitro produced bovine blastocysts will be used for this analysis. Groups of blastocysts (n=10-15) will be placed into medium drops (50 µl/drop) and treated with 0 (control), 100 ng/ml of recombinant bovine FGF2 or 100 ng/ml human FGF10. After 8 hours, total cellular (tc) RNA will be extracted. TcRNA will be collected from five replicate pools of blastocysts exposed to each treatment. cRNA will be purified after amplification and Cy3/Cy5 labeling. Labeled cRNA will be hybridized to the Agilent Bovine DNA Microarray (4x44K) at the UF-ICBR Gene Expression Core Facility. After washing and detection, expression profiles will be examined to provide an initial assessment of FGF2- and 10-dependent expression profiles. Based on what is known about FGF actions in other cells, we anticipate the expression of various genes will be impacted in embryos exposed to FGF2 or 10, and these genes likely will include those related to cell fate, differentiation, adhesion, invasion and regulation of IFNT expression. Quantitative RT-PCR will be used to validate the expression profiles of selected genes. To summarize, this work will provide a holistic view of how FGF2 and 10 impacts the conceptus transcriptome. This effort will provide valuable insight into events of conceptus development that may be controlled by FGFs.

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ACTIONS OF FIBROBLAST GROWTH FACTOR 2 ON DEVELOPMENT OF BOVINE EMBRYOS TO THE BLASTOCYST STAGE

S. D. Fields, P. J. Hansen, and A. D. Ealy
Dept. of Animal Sciences, University of Florida, Gainesville, Florida

The maternal environment secretes several factors that regulate embryonic development during the early stages of pregnancy. Fibroblast growth factor-2 (FGF2) may be one of these. FGF2 mRNA is present in the endometrium throughout the estrous cycle of the cow, and embryos produce FGF2 during the preimplantation period. Furthermore, polymorphisms in *FGF2* have been linked to embryo mortality. Therefore, we theorize that FGF2 is a key factor in regulating embryo development. The objective of this work was to determine the effect of varying concentrations of FGF2 on the competency of cleaved embryos produced in vitro to become blastocysts. The hypothesis was that FGF2 would increase the percent of embryos that become blastocysts and alter the characteristics of those blastocysts in terms of increased total cell number, reduced trophectoderm (TE) to inner cell mass (ICM) ratio, and increased competence for blastocoele expansion and hatching from the zona pellucida. In experiment 1, bovine embryos were produced in vitro and cultured in modified synthetic oviductal fluid (mSOF) containing one of three treatments: 1) 0 ng/mL (control), 2) 5 ng/mL FGF2, or 3) 100 ng/mL FGF2. Cleavage rates were recorded on Day 3 after insemination and blastocyst development was determined on Days 7 and 8. There was no effect of FGF2 on cleavage (67.5 ± 4.1, 66.4 ± 4.1, and 70.1 ± 4.1 for 0, 5 and 100 ng/mL, respectively). The percent of oocytes that were blastocysts on Day 7 tended to be lower (P = 0.06) for embryos cultured with 5 ng/mL FGF2 than for embryos cultured with 100 ng/mL; neither was different from the control (18.4 ± 2.8, 14.0 ± 2.8, and 21.9 ± 2.8). At Day 8, there were no differences between groups (26.6 ± 3.2, 22.7 ± 3.2, and 27.5 ± 3.2). Treatment with 100 ng/mL FGF2 increased the percent of oocytes that were hatched blastocysts on Day 7 (0.4 ± 0.5, 0.4 ± 0.5, and 2.4 ± 0.5; P = 0.007) and the percent of blastocysts that were either expanded, hatched, or hatching (advanced blastocysts) on Day 7 (66.8 ± 5.6, 71.9 ± 6.0, and 77.1 ± 5.6; P = 0.05). At Day 8, however, there were no treatment effects, suggesting FGF2 hastened development of blastocysts to advanced stages rather than increased the proportion of embryos that could become advanced blastocysts. The objective of Experiment 2 was to determine effects of FGF2 added at Day 5 after insemination. The rationale was that FGF2 added at Day 0 may be degraded before later stages of development. Embryos were produced in vitro and cultured in mSOF. On Day 5, embryos were washed and placed in fresh mSOF containing 0, 5, or 100 ng/mL FGF2. Cleavage rates were recorded on Day 3 and blastocyst development was determined on Days 7 and 8. Blastocysts were harvested at Day 8 and numbers of ICM and TE cells were determined. There was no effect of either concentration of FGF2 on number of oocytes that were blastocysts on Day 7 (23.3 ± 6.3, 20.7 ± 6.3, and 26.8 ± 6.3) or 8 (44.1 ± 5.3, 40.9 ± 5.3, and 42.7 ± 5.3), TE number (89.1 ± 7.4, 89.8 ± 6.6, and 94.9 ± 7.8), ICM number (38.3 ± 2.0, 37.3 ± 1.8, and 37.6 ± 2.1), or TE:ICM (2.5 ± 0.3, 2.5 ± 0.3, and 2.6 ± 0.3). In summary, there was no effect of addition of FGF2 on competence of embryos to develop to the blastocyst stage although addition at Day 0 hastened the onset of hatching. The lack of effect of FGF2 could reflect non-involvement of this growth factor in development to the blastocyst stage, the need for a higher concentration to be effective, or presence of FGF2 produced endogenously by embryos negating actions of FGF2 added to culture medium.

GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR IMPROVES DEVELOPMENT AND POST-TRANSFER SURVIVAL OF BOVINE EMBRYOS PRODUCED IN VITRO

B. Loureiro, L. Bonilla, J. Block, J.M. Fear, A.Q.S. Bonilla and P.J. Hansen
Department of Animal Sciences, University of Florida, Gainesville, Florida

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multifunctional cytokine that has been implicated in the regulation of preimplantation embryonic development in several species. The objective of this study was to verify the effects of GM-CSF on blastocyst yield and post-transfer survival and to evaluate the properties of the blastocyst formed after GM-CSF treatment. In vitro, treatment with 10ng/ml of recombinant GM-CSF significant increased (P=0.05) the percentage of oocytes that became blastocysts at day 8 after fertilization comparing to control embryos. Blastocyst development was better for embryos that received GM-CSF at day 5 rather that day 0 after fertilization (P=0.06). For the transfer experiments Holstein cumulus-oocyte-complexes were fertilized with X-chromosome selected sperm from Holstein bulls. Morulae and blastocysts were collected at Day 7 after insemination and transferred at day 7 after ovulation to lactating dairy cows subjected to a modified OvSynch protocol. In the first transfer experiment, conducted during heat stress, embryos were cultured in KSOM-BE2 alone, KSOM-BE2 with 100 ng/ml Arg^3-IGF-1 or KSOM-BE2 with 10 ng/ml GM-CSF. IGF-1 was used as a positive control, because to date it is the only growth factor shown to improve post-transfer survival of bovine embryos. Treatments were added at day 1 after insemination. As compared to control embryos (17% $\pm$ 2%), the percent of cleaved embryos that became transferable morulae or blastocysts at Day 7 was increased (P<0.05) by GM-CSF (25 $\pm$ 2%) but not by Arg^3-IGF-1 (18 $\pm$ 2%). There was no significant effect of treatment on pregnancy rate at Day 30-35 [34% (n=52), 35% (n=51), and 43% (n=55) for control, GM-CSF, and IGF-1, respectively] or calving rate (27%, 35%, and 40%) although values were numerically greater for cows receiving IGF-1 treated embryos. In the second experiment, conducted mostly in cool weather, embryos were cultured in KSOM-BE2 alone, KSOM-BE2 with 100 ng/ml Arg^3-IGF-1 added at Day 1 after insemination, or KSOM-BE2 with 10 ng/ml GM-CSF added at day 5 after insemination. GM-CSF, but not IGF-1, increased the percent of oocytes (P<0.03) and the percent of cleaved embryos (P=0.05) that became transferable morulae or blastocysts at Day 7 (14 $\pm$ 1%, 14 $\pm$ 2% and 10 $\pm$ 1% for controls, GM-CSF and Arg^3-IGF-1, respectively). Treatment with GM-CSF increased (P=0.056) the percent of cows pregnant at day 30-35 [34% (n=79), 43% (n=107) and 27% (n=44) for control, GM-CSF, and IGF-1]. When the two experiments were pooled, pregnancy loss was significant higher (P<0.025) for cows that received a control embryo. When GM-CSF treated embryos were analyzed at day 7 the number of ICM cells (P=0.07) and the ratio of ICM/TE (P=0.02) was greater for GM-CSF treated embryos. However, GM-CSF had no effect on TUNEL-positive cells. Results indicate that treatment with GM-CSF at day 5 after fertilization can affect embryonic development and enhance its competence for post-transfer survival. Research supported by USDA Grant 2006-55203-17390, BARD Grant US-3986-07 and the Southeast Milk Dairy Checkoff Program. BL was supported by a CAPES (Brazil)/Fulbright Fellowship.

DEVELOPMENTAL CHANGES IN ACTIONS OF INSULIN-LIKE GROWTH FACTOR-I TO PROTECT THE PREIMPLANTATION BOVINE EMBRYO FROM HEAT SHOCK

A.Q.S. Bonilla, P.J. Hansen
University of Florida, Department of Animal Sciences, Gainesville, Florida

Insulin-like growth factor-I (IGF-I) is a survival factor for the bovine preimplantation embryo exposed to heat stress. It is not known, however, whether actions of IGF-I on preimplantation embryos are developmentally regulated. The first experiment was designed to test the hypothesis that IGF-I will protect day 5 embryos from effects of heat shock on embryonic development but not two-cell embryos. Two-cell embryos (selected at 28-30 h post-insemination) and day 5 embryos (≥ 16-cells) were selected and received or not (control) 100 ng/mL IGF-I. After 1 h of preincubation, embryos received thermal treatments as follow: a) KSOM-BE2 ± IGF-I with incubation at 38.5°C for 24 h; b) KSOM-BE2 ± IGF-I with incubation at 41°C for 15 h and 38.5°C for 9 h. Embryos were then washed 3 times in KSOM-BE2 drops to remove IGF-I and cultured at 38.5°C until day 8 pi when the percentage of embryos that became blastocysts was evaluated. The experiment for day 5 embryos was replicated 15 times using 193 to 201 embryos per group, and the experiment with two-cell embryos was replicated 11 times using 169 to 174 embryos per group. There was no effect of temperature or IGF-I treatment when day 5 embryos were heat-shocked at 41°C. The least-squares means for percent blastocyst at day 8 pi was 57.7% (control) vs 47.6% (heat shock) for embryos without IGF-I and 54.4% (control) vs 50.0% (heat shock) for embryos cultured with IGF-I (SEM=4.5%). For two-cell embryos, the percent that became blastocysts at day 8 was reduced by heat shock (P<0.005) but was not affected by IGF-I or IGF-I x heat shock. The least-squares means for percent blastocyst was 39.5% (control) vs 20.9% (heat shock) for embryos without IGF-I and 37.1% (control) vs 20.1% (heat shock) for embryos cultured with IGF-I (SEM=3.2%). The lack of a heat shock effect at day 5 precluded a test of whether IGF-I blocks negative effects of heat shock. Therefore, a second experiment was performed where day 5 embryos were exposed to a more severe heat shock of 42°C. The experiment was conducted the same way as the one above, except that the heat-shock temperature was 42°C. The experiment was repeated 4 times using 59-60 embryos per group. The percent of embryos that became blastocysts was reduced by heat shock (P<0.001) and increased by IGF-I (P=0.05). The least-squares means for percent blastocyst at day 8 pi was 86.9% (control) vs 47.7% (heat shock) for embryos without IGF-I and 88.7% (control) vs 66.3% (heat shock) for embryos cultured with IGF-I (SEM=5.6%). The third experiment was conducted to evaluate whether the lack of thermoprotective response of two-cell embryos to IGF-I was due to absence of IGF-IR. Two-cell embryos (selected at 28-30 h pi), day 5 embryos (≥ 16-cells) and day 7 blastocysts were selected. Zona pellucida was removed from embryos with pronase, total cellular RNA was extracted from groups of 30 embryos and RT-PCR was performed for IGF-IR mRNA. There was expression of IGF-IR transcripts at the two-cell, day 5 and blastocyst stages (Figure 1). It is concluded that IGF-I induces thermotolerance in day 5 bovine embryos but not in two-cell embryos despite the presence of IGF-IR.

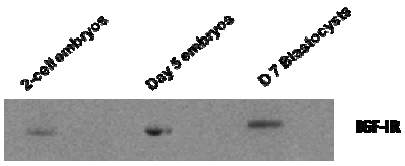

Figure 1. RT-PCR showing the presence of IGF-IR in two-cell, day 5 and blastocyst-stage embryos.

REPRESSSION OF THE INTRINSIC PATHWAY TO APOPTOSIS IN THE TWO-CELL BOVINE EMBRYO INVOLVES HISTONE DEACETYLATION AND DNA METHYLATION

Carambula, SF; Oliveira, LJ; Hansen, PJ
Dept. of Animal Sciences, University of Florida, Gainesville, Florida

Apoptosis in the bovine embryo cannot be induced by activators of the intrinsic or extrinsic apoptosis pathways until the 8-16 cell stage. Depolarization of mitochondria with the decoupling agent carbonyl cyanide 3-chlorophenylhydrazone (CCCP) can activate caspase-3 in two-cell embryos but DNA fragmentation does not occur. Here we hypothesized that the repression of apoptosis is caused by hyperacetylation of histones and methylation of DNA. To test this hypothesis, we evaluated whether reducing DNA methylation or histone deacetylation would make two-cell embryos susceptible to DNA fragmentation caused by CCCP. Bovine oocytes were maturated and fertilized in vitro for 18 h. Putative zygotes were then treated for 12 h with vehicle, 100 μM 5-aza 2' deoxycytidine (AZA), to cause DNA demethylation, or 100 nM Trichostatin A (TSA), to inhibit histone deacethylase. Embryos were then were treated $\pm$ 100 μM CCCP. Fragmentation of DNA fragmentation was determined at 24 h after CCCP using the TUNEL reaction. The experiment was replicated 6 times using a total of 458 embryos. The percent of blastomeres positive for TUNEL (% TUNEL positive cells) was affected by a treatment x CCCP interaction ($P<0.0001$). CCCP did not cause an large increase in % TUNEL positive cells in control embryos (2.0 $\pm$3.4% vs 7.7 $\pm$ 5.5%) but caused a large increase in % TUNEL positive cells for embryos treated with AZA (5.4 $\pm$ 2.9% vs 42.3 $\pm$ 3.2%). Results for TSA indicated an increase in %TUNEL positive cells in embryos without CCCP and only a slight increase with CCCP treatment (17.1 $\pm$ 2.8% vs 24.9 $\pm$ 4.2%). Further analysis of TSA treated embryos focused on the subset of embryos that were < 8 cells (i.e., those that are ordinarily not susceptible to apoptosis). In this subset, which included 68% of the TSA-treated embryos, there was a clear increase in % TUNEL positive nucleus after CCCP treatment (10.0 $\pm$ 4.2% vs 24 $\pm$ 4.5%, $P<0.025$). In conclusion, disruption of DNA methylation and histone deacetylation can remove the block to apoptosis in bovine two-cell embryos. Moreover, inhibition of histone acetylation leads to some spontaneous apoptosis in the absence of a proapoptotic signal. It is likely that interactions between methylated DNA and histones masks DNA at early stages of development and prevents access of caspase-activated DNAses to DNA.
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COMPARISON OF A SELECT SYNCH/CIDR + TIMED-AI (TAI) TO A CO-SYNCH/CIDR SYNCHRONIZATION PROTOCOL IN SUCKLED *BOS INDICUS* X *BOS TAURUS* COWS

R.D. Esterman, B.R. Austin, E.M. McKinniss and J.V. Yelich
Dept. of Animal Sciences, University of Florida, Gainesville

Development of a synchronization protocol that achieves acceptable pregnancy rates in cattle of *Bos indicus* breeding is particularly important to producers in subtropical regions such as Florida. One of the most effective synchronization protocols in both *Bos taurus* and *Bos taurus* x Bos *indicus* cattle is GnRH and a CIDR on d 0 with $PGF_{2\alpha}$ at CIDR removal on d 7, followed by estrous detection for 3 d and a clean-up TAI + GnRH of cows failing to exhibit estrus. This protocol is commonly termed the Select Synch/CIDR + TAI (SSC) protocol. Pregnancy rates of 40 to 55% can be achieved in *Bos indicus* × *Bos taurus* cattle using this protocol; however, the protocol is labor intensive due to the estrus detection required. Many producers do not want to detect estrus, which is also difficult to detect in cattle of *Bos indicus* breeding. When a timed-AI + GnRH was performed 48 hours after CIDR removal (Co-Synch/CIDR – COS) previous work in *Bos indicus* type cattle demonstrated pregnancy rates of approximately 30% compared to 50% in *Bos taurus* cattle. Previous research from our lab has indicated that peak estrous response after CIDR removal in the Select Synch/CIDR protocol in cattle of *Bos indicus* breeding is 60 to 72 hours. Therefore, the objective of this experiment was to evaluate the effectiveness of the SSC protocol compared to a COS with the timed-AI at 66 to 70 hours in postpartum lactating *Bos indicus* × *Bos taurus* cows. Multiparous suckled *Bos indicus* × *Bos taurus* cows were used to compare the SSC and COS protocols. Five groups of suckled *Bos indicus* × *Bos taurus* cows were utilized (n = 659) and allotted to treatments based on days postpartum (DPP) and body condition (BCS) on day 0 of the experiment. Blood samples were also collected 10 days before the start of the experiment and on day 0 to evaluate progesterone concentrations and determine estrous cycling status. Cows in both treatments received GnRH (100 μg; Cystorelin) and a CIDR (Eazi-Breed CIDR) on day 0. On day 7, SSC cows had CIDR removed and received $PGF_{2\alpha}$ (25 mg; Lutalyse Sterile Solution); whereas, COS cows had CIDR removed and received $PGF_{2\alpha}$ on day 7.5. For SSC cows, estrus was detected for 3 days following $PGF_{2\alpha}$ and cows were inseminated 8 to 12 hours after observed in estrus. Additionally, cows not exhibiting estrus by 72 hours after $PGF_{2\alpha}$ received GnRH and were timed-AI at 76 to 80 hours. The COS cows received GnRH and were timed-AI at 66 to 70 hours after $PGF_{2\alpha}$. The SSC cows had a 3 day estrous response of 50.6% (168/332), conception rate of 66.1% (111/168), and timed-AI pregnancy rate for non-responders of 32.3% (53/164). Synchronized pregnancy rates were similar ($P > 0.05$) for SSC (49.4%; 164/332) and COS (47.1%; 154/327). Estrous cycling status did not ($P > 0.05$) influence synchronized pregnancy rates between treatments. Synchronized pregnancy rates differed ($P < 0.05$) for cows ≤ 55 (38.5%; 37/96), 56 to 65 (53.1%; 121/228), 66 to 75 (52.0%; 77/148), and ≥ 76 (44.4%; 83/187) DPP, but there was no ($P > 0.05$) effect of treatment × group. Although there were group differences ($P < 0.05$) for synchronized pregnancy rate, there were no ($P > 0.05$) treatment x group effects. In summary, the COS TAI protocol yielded similar synchronized pregnancy rates compared to the SSC protocol and the COS appears to be an effective TAI synchronization protocol in suckled *Bos indicus* × *Bos taurus* cows when the timed-AI is performed at 66 to 70 hours after CIDR removal.

FOLLICULAR WAVE OF THE OVULATORY FOLLICLE AND NOT CYCLIC STATUS INFLUENCES FERTILITY OF DAIRY COWS

Rafael S. Bisinotto[1], R.C. Chebel[2], and J.E.P. Santos[1]

[1]*Department of Animal Sciences, University of Florida*

[2]*School of Veterinary Medicine, University of California Davis*

Two studies evaluated the influence of follicular wave at AI on fertility of dairy cows. In EXP1, data from 5,630 lactating cows enrolled in a presynchronized timed AI protocol were analyzed. Cows had blood analyzed for progesterone (P4) 12 to 14 d apart, on the days of second PGF of the presynchronization and the first GnRH (GnRH1) of the timed AI protocol (Ovsynch or Cosynch72). Cows were classified as cyclic if P4≥1 ng/mL and anovular if both samples had P4<1 ng/mL. Cyclic cows were categorized as low (CLow: <1 ng/mL) or high (CHigh: ≥1 ng/mL) P4 on the day of GnRH1, which would result in ovulation of the dominant follicle of the first (FW) and second (SW) follicular waves, respectively, at AI. Pregnancy per AI (P/AI) was determined 31 and 66 d after AI. In EXP2, 220 Holstein received 2 PGF given 14 d apart. The Ovsynch protocol (GnRH, 7 d PGF, 48 h GnRH, 12 h timed AI) initiated either 3 or 10 d after the second PGF to result in insemination to the FW or SW dominant follicles. Blood was analyzed for P4 and ovaries were scanned on the days of the second PGF of the presynchronization, GnRH1 and PGF of the Ovsynch. Only cyclic cows were enrolled. Pregnancy was determined on days 32 and 66 after AI. In EXP1, P/AI on d 31 was greater (P<0.001) for CHigh than anovular and CLow cows (43.0 vs. 29.7 vs. 31.3%, respectively). Short-cycling differed (P<0.001) among groups and were 7.1, 11.9, and 15.7% for CHigh, anovular and CLow, respectively. Pregnancy loss differed (P<0.05) only between anovular and CLow (10.0 vs. 15.0%), and it was intermediate for CHigh (13.5%). In EXP2, 9.8 and 97.2% of the FW and SW cows, respectively, had P4>1ng/mL at the GnRH1. Concentrations of P4 at GnRH1 (0.4±0.1 vs. 2.6±0.1 ng/mL) and PGF (2.1±0.2 vs. 2.9±0.2 ng/mL) were greater (P<0.002) for SW than FW cows. P/AI was greater (P=0.04) for SW than FW cows (41.7 vs. 30.4%), despite less (P=0.05) ovulation to GnRH1 in SW than FW cows (78.7 vs. 88.3%). These data indicate that follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. Synchronization programs that induce ovulation of the FW follicle reduced P/AI of cows. Ovulation of a first-wave follicle is one of the mechanisms by which anovular cows have reduced fertility.

FATTY ACID SUPPLEMENTATION TO PERIPARTURIENT DAIRY COWS RECEIVING DIETS CONTAINING LOW AMOUNTS OF ESSENTIAL FATTY ACIDS

Leandro F. Greco, M. Garcia, M. Favoreto, W.W. Thatcher, C.R. Staples, and J.E.P. Santos
Department of Animal Sciences, University of Florida

Dietary supplementation with lipid usually influences lactation and reproduction of dairy cows. Effects on reproduction are thought to be independent on provision of calories. Fatty acids can also modulate immune responses in dairy cows. The objectives of this study are to determine the impact of supplementing a diet containing low amounts of essential fatty acids with either mostly saturated free fatty acids or with essential fatty acids linoleic (LA,C18:2 n6) and α-linolenic (LNA, C18:3 n3) on A) Dynamics of EFA composition of different tissues; B) Metabolic status; C) Uterine involution and health; D) Resumption of ovulatory cycles; E) Embryo quality; F) Immune competence early postpartum; G) Lactation performance; H) Global gene expression of hepatic and embryonic tissues. The hypotheses are: Feeding diets with minimum concentrations of essential FA will lower the cow's tissues of LA and LNA, which will compromise lactation, immune responses, health, and reproduction; and supplementing cows with saturated FA will not reverse the negative effects of a diet marginal in EFA. Cows and diets: 80 prepartum cows were blocked by parity and body condition and, within each block, randomly allocated to 1 of 3 dietary treatments at 60 d before the expected date of calving and continue until 90 d postpartum. Treatments consist of 0% of the dietary DM as supplemental fat (negative control, n = 26), 1.7% of the dietary DM as saturated free fatty acids (positive control, n = 27), and 2% of the dietary DM as calcium salts rich in LA (n = 27). Milk production, reproduction and health will be evaluated. Measures of immune responses will be characterized by neutrophil function [phagocytic and oxidative burst capacity of neutrophils; expression of the adhesion molecules (β-Integrin and L-selectin], peripheral blood mononuclear cell isolation and cytokine production (IL-1, IL-4, TNF-α, and INF-γ), and antibody titers against ovalbumin. Liver tissue will be collected by percutaneous biopsy from all cows at 14 d postpartum. Samples will be analyzed for its chemical composition. An aliquot of liver tissue will be preserved for RNA extraction and global gene expression by microarray. Embryos/oocytes collected on days 7 and 14 after insemination will be used for evaluation of fertilization, grade quality, and tissue will be preserved for global gene expression by microarray. Plasma samples collected 3 to 5 times weekly will be analyzed for concentrations of metabolites, progesterone, estradiol, acute phase proteins. Follicular fluid collected from the dominant follicle on day 6 of the estrous cycle will be analyzed for fatty acid composition, steroid concentrations, free and total IGF-1, and insulin. Expected results: Feeding a diet with marginal FA content will significantly reduce the maternal stores of essential FA, which will alter blood, tissue and milk lipid composition, compromise measures of postpartum health and immune function, uterine health and involution, resumption of postpartum ovulatory cycles. These changes are expected to influence global gene expression in embryos and hepatic tissue. The experiment is currently underway and the animal portion of the study will be completed on June 30, 2009.

EFFECTS OF THREE DIMENSIONAL CULTURE AND FIBROBLAST GROWTH FACTOR SUPPLEMENTATION ON EQUINE UMBILICAL CORD BLOOD DERIVED STEM CELLS

Sarah A. Reed, Ju Li, and Sally E. Johnson
Dept. of Animal Sciences, University of Florida

Mesenchymal stem cells are a current therapeutic aid for a wide variety of musculoskeletal diseases and injuries. In the horse, tendon injury is a severe injury that has lifelong consequences on the usability of the animal. This translates into thousands of dollars lost every year in the racing and performance horse industries. Current therapies include rest, physical rehabilitation, and stem cell treatments from adipose and bone marrow. The objective of this study was to evaluate the potential of equine umbilical cord blood derived stem cells (eUCB) and equine adipose derived stem cells (AdMSC) for use in tendon injury.

Not only is growth in a three dimensional environment crucial for engraftment and survival in a bodily structure, but use of a scaffold to support the maintenance of the stem cells at the site of injury is also likely to be required for successful treatment. As such, eUCB were cultured on collagen beads and embedded into 30% Matrigel. Compared to control monolayers on gelatin, eUCB cultured on collagen beads maintained a more compact, less flattened morphology. Those embedded in Matrigel formed colonies of cells with filopodia that protruded into the surrounding matrix. Despite differences in morphology, eUCB cultured on gelatin, collagen beads and Matrigel maintained similar levels of *scleraxis* expression, a marker of early tenocyte differentiation.

Fibroblast growth factors (FGF) elicit differing effects on naïve cells. FGF2 inhibits differentiation while FGF4 and FGF5 can promote the tenocytic lineage. As such, the effects of FGF2, FGF4, and FGF5 on eUCB and AdMSC were examined. eUCB treated with FGF5 increased proliferation after 48 hours of treatment while AdMSC exhibited growth inhibition. Conversely, FGF2 stimulated proliferation in AdMSC while inhibiting BrdU incorporation in eUCB. FGF4 had no effect on growth of either cell type. Changes in proliferation were blocked by inclusion of the MEK inhibitor PD98059 in the FGF treatment media. No difference in *scleraxis* expression was detected when AdMSC or eUCB were treated with FGF2, FGF4 or FGF5. Both eUCB and AdMSC are capable of transcriptional responses to the FGFs measured by changes in AP-1 activity.

While stimulation of eUCB and AdMSC with FGFs did not increase *scleraxis* transcription, it is capable of eliciting signaling and transcriptional responses. Further investigation into the effects of these growth factors on adult stem cell identity is warranted. Furthermore, sequential signaling and/or the stresses of a three dimensional environment may be required for tenocytic differentiation and *scleraxis* expression.

PHENOTYPIC CHARACTERIZATION OF MACROPHAGES IN THE ENDOMETRIUM OF THE PREGNANT COW

Lilian Oliveira and P.J. Hansen
Department of Animal Sciences, University of Florida, Gainesville, Florida, USA

There is large-scale recruitment of macrophages to the lamina propria (LP) and submucosa (SM) of the intercaruncular endometrium during pregnancy in the cow. In this study, we evaluated whether macrophages are also located in the placentomes and whether macrophages are regionally differentiated based on expression of cell surface markers. Intercaruncular endometrium and placentomes from uteri of pregnant cows (n=10; estimated fetal ages of 133-247 d) were subjected to dual-color immunofluorescence using CD68 as a pan-macrophage marker in combination with CD14 (macrophage marker), CD11b or MHC class II. CD68+ cells were abundant in the LP and SM of intercaruncular endometrium and in the caruncular septa (maternal tissue) of the placentomes. The CD68+ cells were not present in fetal villi of the placentomes. Regardless of location, the majority of CD68+ cells also expressed CD14. In intercaruncular endometrium, cells dual labeled for CD68 and CD11b were present in SM but not in LP. In contrast, cells dual labeled for CD68 and MHC class II were present in LP but not in SM. There were also some MHC class II+ cells in the SM around endometrial glands that were not positive for CD68. In caruncular septa, most CD68+ cells were negative for MHC class II and all were negative for CD11b. These data indicate that 1) CD68+ macrophages are present in the stroma of the ICE and caruncular septa of the placentome during pregnancy of the cow and 2) these macrophages are regionally differentiated. Cells located deep in the stroma express CD11b, cells closer to the epithelium express MHC class II, and cells in the placentome being largely negative for CD11b and MHC class II.

HEAT STRESS DOES NOT ALTER IMMUNE STATUS OF HOLSTEIN CALVES BUT SLICK GENOTYPE CONFERS REDUCED IMMUNE FUNCTION

J. W. Bubolz, S. Tao, B. C. do Amaral, M. J. Hayen, T. A. Olson, G. E. Dahl
Dept. of Animal Sciences, University of Florida, Gainesville, FL, USA

Environmental factors such as photoperiod and heat stress influence health and hormone secretion in dairy cattle. Genetic background may also modulate immune status in cattle. The objective of the present study was to test the hypothesis that heat stress depressed immune function in cattle. A second objective was to examine the affect of the Slick hair gene on immune status. Calves defined as slick-haired possess a dominant gene of Senepol origin that when expressed produces a very short, sleek coat. Slick (n=4) and wild-type (n=4) calves were kept in controlled-temperature chambers for a period of 9 weeks. Calves were exposed to heat stress and neutral thermal conditions with a 1 week pretreatment acclimation and 2 week washout period between temperature treatments in a 2x2 cross-over design. Dry matter intake (DMI), water intake and infrared (IR) skin temperature were measured daily. Jugular blood samples were collected weekly and evaluated for lymphocyte proliferation, neutrophil phagocytosis and neutrophil oxidative burst activity. Relative to thermoneutral condtions, heat stress increased AM (35.0 vs. 30.6 °C; $P < 0.001$) and PM skin temperatures (36.8 vs. 31.6 °C; $P < 0.001$). Calves under heat stress increased daily water consumption (29.2 vs. 17.8 L; $P < 0.04$) and decreased DMI as percentage of body weight (2.29 vs. 3.83%; $P < 0.001$) compared with the thermoneutral period. Relative to thermoneutral treatment, no difference in any immune variable was observed during heat stress. However, neutrophils from wild type calves had greater phagocytic ($P < 0.01$) and oxidative burst ($P < 0.07$) activity compared with slick-haired calves. In addition, lymphocytes from wild type calves had greater proliferation relative to slick calves ($P < 0.05$). Results indicate that wild type calves had improved immune status compared to slick-haired calves. Immune status of slick-haired calves was depressed relative to wild type, but heat stress did not influence immune status of Holstein calves in controlled-temperature chambers.

ENVIRONMENTAL EFFECTS DURING DRY PERIOD ON THE MAMMARY GLAND DEVELOPMENT OF DAIRY COWS

S. Tao and G. E. Dahl
Dept. of Animal Sciences, University of Florida

The dry period, a phase in late pregnancy when cows cease lactating before parturition, is important to achieve maximal milk production during the next lactation. And the mammary gland is the key regulator. Mammary glands are skin glands comprised of extraparenchymal fat pad and parenchymal tissue which includes epithelial structure and associated stromal tissue that are derived from the ectodermal cells in the embryo. Alveoli are the secretory units made up of secretory epithelial cells, myoepithelial cells, basement membrane and a luminal space.

Morphologically, alveolar structure is apparent during the entire dry period. During a standard 60 d dry period, the number of secretory epithelial cells reaches a minimum at -35 d relative to calving and increases thereafter to the peak at -7 d relative to calving. Total mammary parenchymal mass and DNA increase during the entire dry period while at the same time the mammary cells undergo extensive cell turnover by increasing high proliferation and apoptotic rate throughout the dry period.

Any factors that increase the number of mammary epithelial cells or secretory activity of epithelial cells may improve milk production. Manipulation of photoperiod during the dry period, such as exposure to short day photoperiod (SDPP), enhances milk production during the subsequent lactation. There is evidence that the increase in milk production is mediated through enhanced prolactin signaling. Compared with cows exposed to long day photoperiod (LDPP), cows exposed to SDPP have greater mammary development through increased mammary cell proliferation and decreased apoptosis during the dry period. It appears that the increased mammary remodeling of SDPP cows occurs at a specific time during the dry period (3 – 6 wk before calving).

Cows exposed to heat stress during dry period have impaired performance in the next lactation, whereas heat stress abatement during the dry period reverses the negative effect on subsequent yield. Based on previous studies, we hypothesize that heat stress abatement during the dry period increases mammary development and mammary cell turnover relative to heat stress. Further, we postulate that prolactin signaling is depressed in the mammary gland of heat stressed cows relative to cooled cows. These cellular responses would depress subsequent milk yield regardless of management interventions after parturition.

DIRECTIONS

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From Gainesville – approximately 65 miles
Southwest on SR 121 to US19
South on US19 thru SR 44 Intersection in Crystal River
Continue 19 south to 2nd traffic light West Ft. Island Trail,
Turn right go 0.8 mile, located on the right side.