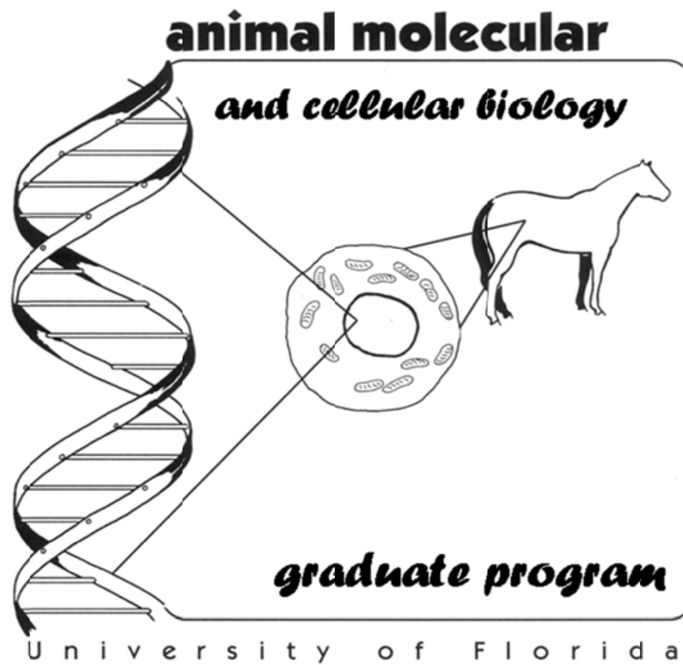
NINTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Steinhatchee Landing Resort
Steinhatchee, Florida
April 1-2, 2011

UF | UNIVERSITY of FLORIDA



Animal Molecular and Cellular Biology Graduate Program

Ninth Annual Research Symposium

WELCOME

The AMCB symposium committee would like to welcome all faculty and students to this year's Research Symposium which is held at the Steinhatchee Landing Resort on Florida's gulf coast.

We wish all the best to Rafael Bisinotto, Aline Bonilla, Regina Esterman, Justin Fear, Joseph Kramer, Barbara Loureiro, Kathleen Pennington, and Qi En Yang, who completed their degrees in 2010.

We welcome all new students and faculty to the premier scientific event in the AMCB calendar. As for the veteran students and faculty, we trust the Ninth Annual Symposium will be marked by good science, good fellowship, and good memories.

Lokenga Badinga, AMCB Director

Alan Ealy, AMCB Co-Director

ACKNOWLEDGEMENTS

The faculty and students of the Animal Molecular and Cellular Biology Program thank the following for support of the 9th Annual Research Symposium

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. Mark Reiger, Acting 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 for help with the symposium

2011 ANIMAL MOLECULAR AND CELLULAR BIOLOGY DISTINGUISHED LECTURER

Dr. Kimberly Vonnahme

Kimberly Vonnahme grew up on a livestock and grain farm near Breda, Iowa (West-Central Iowa). Upon graduation from high school, Kim attended Iowa State University majoring in Animal Science and worked for three years in a reproductive biology laboratory. Her interest in reproductive biology was sparked, and after graduation in 1996, pursued her Master of Science degree at Oklahoma State University under the guidance of Dr. Rodney Geisert working on embryo-uterine interaction during early pregnancy in the pig with a thesis entitled "*Detection of Glandular Kallikrein and Low Molecular Weight Kininogen in the Porcine Uterus during the Estrous Cycle and Early Pregnancy*". She returned to Iowa State University in 1998 to begin her PhD program with Dr. Steve Ford, and moved with Ford to the University of Wyoming in 2000, where she completed her PhD degree in 2003. While her dissertation title was "*The Impacts of Placental Size and Vascularity on Litter Size in the Pig*", Kim also helped with the early studies in the Center for the Study of Fetal Programming developing a nutritional model using pregnant sheep. In the fall of 2003, Kim moved to North Dakota State University with interests in learning measurements of vascularity in sheep and cow placenta from Dr. Larry Reynolds. In April, 2004, Kim accepted an assistant professor position in the Department of Animal Sciences to teach and conduct research. She was promoted to associate professor in 2010. She also is currently the co-director for the Center for Nutrition and Pregnancy.

Dr. Vonnahme's research programs focuses on the impacts of maternal nutrition on fetal and placental development in sheep, cattle, and pigs. More specifically, Kim is interested in how the maternal nutrition impacts uteroplacental blood flow, development of the placenta, and nutrient transfer. She has 58 peer reviewed journal articles, over 130 abstracts, 1 book chapter, and over $1.8 million in grant funding.

Kim also enjoys instructing the Physiology of Reproduction course. She also has established a Research in Reproduction course where undergraduate students undertake an independent research project, which 14 students have participated. She has mentored 13 MS and PhD students and 1 post doctorate scholar.

Kim is married to Michael Kangas and they have one daughter, Katie.

GUEST LECTURER

Dr. Maria Belen Rabaglino

Maria Belen Rabaglino is a faculty member of the Animal Reproduction Department at the National University of Rio Cuarto (UNRC) Cordoba, Argentina since 2004. She obtained the Veterinary and Laboratory Technician degrees at the (UNRC) in 2003, and the Specialist in Bovine Reproduction degree at the National University of Cordoba (UNC) in 2006. Maria Belen came to UF in 2007, under a special Fulbright scholarship (Faculty Development program) to pursue a Master of Sciences program in the Large Animal Clinical Sciences Department, College of Veterinary Medicine with Dr. Carlos Risco as advisor. She conducted her MS thesis research in the area of reproductive management of dairy heifers and earned her Master in Veterinary Sciences in 2009. In the same year, Maria Belen joined the AMCB program as a PhD student of Dr. Charles E. Wood. Her current research is based on the analysis of the global gene expression in the ovine fetal brain close to parturition.

Committees of the AMCB, 2010-2011

Director: Lokenga Badinga
Co-Director: Alan D. Ealy
Graduate Coordinator: Joel H. Brendemuhl
Program Assistant: Joann Fischer

Research Symposium
Dr. Lokenga Badinga
Dr. Alan Ealy
Dr. Peter Hansen
Ms. Sarah Fields
Mr. Sha Tao
Mr. Kun Zhang

Scholarship
Chris Mortensen
Sally Johnson
Stephanie Wohlgemuth

Social Events
Dr. Peter Hansen
Dr. Alan Ealy
Dr. Jose Santos
Mr. Rafael Bisinotto

Web Site
Dr. Peter Hansen

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
2010	Whitney Laboratory for Marine Bioscience, St. Augustine, FL	Marc-André Sirard, Laval University
2011	Steinhatchee Landing Resort, Steinhatchee, FL	Kimberly Vonnahme, North Dakota State University

AMCB Faculty

Jeffrey R. Abbott, Ph.D.
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, Ph.D.
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, Ph.D.
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 Biology

Maureen Keller-Wood, Ph.D.
Department of Pharmacodynamics

Christopher Mortensen, Ph.D.
Department of Animal Sciences

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

Stephanie Wohlgemuth, Ph.D.
Department of Animal Sciences

Charles E. Wood, Ph.D.
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
Anna Denicol (Advisor: Pete. Hansen)
Kyle Dobbs (Advisor: Pete Hansen)
Sarah D. Fields (Advisor: Pete Hansen)
Leandro Greco (Advisor: Jose Santos)
Maria Belen Rabaglino (Advisor: Charles Wood)
Sha Tao (Advisor: Geoff Dahl)
Maria Christina Vasquez (Advisor: David Julian)
Kun Zhang (Advisor: Alan Ealy)

M.S. Students
Alice Chow (Adisor: Mary Brown)
Paula Morelli (Advisor: Alan Ealy)

SCHEDULE

Friday PM, April 1, 2011
Conference Center

Session 1, Distinguished Lecture

1:00-1:15 PM	Lokenga Badinga, Welcoming remarks.
1:15-2:00 PM	Kimberly Vonnahme, Department of Animal Sciences, North Dakota State University. Life before birth: how the maternal environment "programs" offspring growth and development.
2:00-2:30 PM	BREAK

Session 2. Research Reports – Fertility and Embryonic Development (Sarah Fields, chair)

2:30 - 2:45 PM	Bisinotto, R.S., Ayres, H., Carvalho, H.R., Ribeiro, E.S., Cerri, R.L.A., Greco, L.F., Lima, F.S., Favoreto, M.G., Monteiro, A.P., Perdomo, M.C., Thatcher, W.W., and Santos, J.E.P. Dept. of Animal Sciences, University of Florida, Gainesville and Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada. Effect of follicular wave and progesterone concentration during follicle growth on fertility of dairy cows.
2:45 – 3:00 PM	Zhang, K., and Ealy, A.D. Dept. of Animal Sciences, University of Florida, Gainesville, Florida. Disruption of fibroblast growth factor receptor signaling in bovine cumulus-oocyte complexes during in vitro maturation impacts subsequent embryonic development.
3:00 – 3:15 PM	Dobbs, K.B., and Hansen, P.J. Dept. of Animal Sciences, University of Florida, Gainesville. Regulation of expression of genes involved in epigenetic programming in the bovine morula by colony stimulating factor 2, bone morphogenic protein 4 and activin A .
3:15 – 3:30 PM	Denicol, A.C., Dobbs, K.B., and Hansen, P.J. Dept. of Animal Sciences, University of Florida, Gainesville. Wnt signaling regulates development of bovine embryos to the blastocyst stage.
3:30 – 4:00 PM	GROUP PICTURE

Session 3 – Research Reports – Environment (Leandro Greco, Chair)

4:00 – 4:15 PM	Vasquez, M.C., Crombie, T.A., and Julian, D. Department of Animal Science and Department of Biology, University of Florida, Gainesville.

	Lysosome number and size do not vary during a tidal cycle in erythrocytes of the bloodworm *Glycera dibranchiata.*
4:15 - 4:30 PM	Sakatani, M., and Hansen, P.J. Dept. of Animal Sciences, University of Florida, Gainesville and National Agricultural Research Center for Kyushu Okinawa Region, Kumamoto, Japan. Effect of physiological heat shock on development and expression of stress response genes in bovine preimplantation embryos.
4:30 – 4:45 PM	Tao, S., Thompson, I. M., Monteiro, A.P., Hayen, M.J., and Dahl, G.E. Dept. of Animal Sciences, University of Florida, Gainesville. Effect of heat stress during the dry period on insulin sensitivity of multiparous dairy cows.
5:00 – 6:00 PM	FACULTY MEETING
6:00 PM - ????	COOKOUT

Saturday AM, April 2, 2011
Conference Center

7:00 AM - 8:30 AM Breakfast, Steinhatchee Landing Welcome Center

Session 4 – Guest Lecture (Alan Ealy, Chair)

8:30 - 9:15 AM Maria Belen Raboglino, Dept. of Physiology & Functional Genomics, University of Florida. Ontogeny of global gene expression in the ovine fetal hypothalamus in late gestation.

9:15 – 9:30 AM BREAK

Session 5 – Research Reports - Infectious Disease and Imune function (Sha Tao, Chair)

9:30 – 9:45 AM Chow, A. and Brown, M.B., Dept of Infectious Diseases & Pathology, University of Florida, Gainesville. Mechanosensitive channel protein (MscL) in *Mycoplasma bovis.*

9:45 – 10:00 AM Perdomo, M.C., and Badinga, L. Dept. of Animal Sciences, University of Florida, Gainesville. Conjugated linoleic acid and rosiglitazone attenuate lipopolysaccharide-induced TNF-α production by bovine blood cells.

10:00 – 10:15 AM Lima, F.S., Bisinotto, R.S., Ribeiro, E.S., Greco, L.F., Ayres, H., Favoreto, M.G., Carvalho, M.R., Sevaroli, A.L., Galvão, K.N., Risco, C.A., Thatcher, W.W. and Santos, J.E.P. Depts. of Animal Sciences and Large Animal Clinical Sciences, University of Florida, Gainesville. Therapy and underlying mechanisms associated with uterine disease of dairy cows.

10:15 – 10:30 AM Martinez, N., Risco, C.A., Maunsell, F., Galvão, K.N., and Santos, J.E.P. Dept. of Animal Sciences and Dept. of Large Animal Clinical Sciences, University of Florida, Gainesville. Evaluation of peripartal mineral and energetic statuses and neutrophil function of dairy cows of low or high risk of developing uterine diseases.

10:30 – 11:00 AM BREAK

Session 6 – Research Reports – Genetics/Nutrition (Kun Zhang, Chair)

11:00 – 11:15 AM Fields, S.D., Cole, J.B., and Hansen, P.J. Dept. of Animal Sciences, University of Florida, Gainesville and USDA Animal Improvement Programs Laboratory, Beltsville, MD. Identifying genetic markers for fertility in dairy cattle.

11:15 – 11:30 AM Ribeiro, E.S., Monteiro, A.P.A., Bisinotto, R.S., Lima, F.S., Greco, L.F., Thatcher, W.W., and Santos, J.E.P. Dept. of Animal Sciences, University of Florida, Gainesville. Effects of anovulation and breed on conceptus development at preimplantation stages in lactating dairy cows subjected to synchronized ovulation.

11:30 – 11:45 AM Mercadante, P.M., Rae, D.O., Johnson, S.E., Ealy, A.D. Depts. of Animal Sciences and Large Animal Clinical Sciences, University of Florida, Gainesville. Effects of *Bos indicus* breeding on plasma pregnancy-associated glycoprotein (PAG) concentrations and fetus size in early gestation.

11:45 – 12:00 PM Greco, L.F., Garcia, M., Bisinotto, R.S., Ribeiro, E.S., Favoreto, M.G., Marsola, R.S., Martins, L.T., Loureiro, B., Lima, F.S., Thatcher, W.W., Staples, C.R., and Santos, J.E.P. Dept. of Animal Sciences, University of Florida, Gainesville. Effect of fatty acid supplementation to dairy cows fed diets containing low concentrations of fatty acids on follicular fluid composition and embryo quality.

ADJOURNMENT

ABSTRACTS

EFFECT OF FOLLICULAR WAVE AND PROGESTERONE CONCENTRATION DURING FOLLICLE GROWTH ON FERTILITY OF DAIRY COWS

R.S. Bisinotto[1,*], H. Ayres[1], M.R. Carvalho[1], E.S. Ribeiro[1], R.L.A. Cerri[2], L.F. Greco[1], F.S. Lima[1], M.G. Favoreto[1], A.P. Monteiro[1], M.C. Perdomo[1], W.W. Thatcher[1], and J.E.P. Santos[1]

[1]University of Florida, Gainesville, USA, and [2]University of British Columbia, Vancouver, Canada

Effects of wave of the ovulatory follicle and P4 concentration during follicle growth on corpora lutea (**CL**) function and conceptus development were evaluated in dairy cows. Non-lactating Holstein cows had their estrous cycles synchronized with GnRH and a controlled internal drug release (**CIDR**) device containing progesterone (**P4**), followed 7 d later by CIDR removal and 2 injections of $PGF_{2\alpha}$ 24 h apart. All cows received GnRH 1 d after the 2nd $PGF_{2\alpha}$ which, for cows induced to ovulate a first wave follicle (**FW**, n=13) or a FW follicle supplemented with P4 (**FWP4**, n=8), was the 1st GnRH of the timed artificial insemination (**AI**) protocol (d-9 GnRH, d-2 and d-1 $PGF_{2\alpha}$, d0 GnRH and AI, d1 AI). Cows induced to ovulate a second wave follicle (**SW**, n=12) received the timed AI protocol beginning 6 d after the previous GnRH. Cows in FWP4 received 3 CIDR, one at 12, 24 and 48 h after the GnRH (d-9), that were removed at the $PGF_{2\alpha}$ (d-2). Blood was sampled from d-9 to 17 for P4 and estradiol (**E2**) analyses. Cows were slaughtered on d17 and uteri were flushed. Interferon-tau (**INF-τ**) on uterine flush was quantified. Concepti IFN-τ mRNA expression was accessed by RT-PCR. Orthogonal comparisons were performed to determine the effects of P4 (FW vs. FWP4 + SW) and follicle wave (SW vs. FWP4).

	Treatment			P	
	FW	FWP4	SW	Wave	P4
P4, ng/mL					
d-9 to -2	1.4 ± 0.2	3.8 ± 0.3	5.4 ± 0.3	<0.01	<0.01
d4 to 16	6.3 ± 0.2	5.0 ± 0.3	5.0 ± 0.3	0.84	<0.01
Ovulatory follicle (d0), mm	17.9 ± 0.6	15.3 ± 0.7	14.7 ± 0.6	0.47	<0.01
E2 peak before AI (d-1), %	58.3	12.5	0.0	0.77	0.01
E2 at peak, pg/mL	8.0 ± 0.6	7.0 ± 0.7	5.9 ± 0.6	0.25	0.05
CL on d7, mm^3	5.2 ± 0.5	3.6 ± 0.6	4.2 ± 0.5	0.39	0.03
Pregnant, %	50.0	87.5	72.7	0.45	0.10
Conceptus length, cm	17.5 ± 2.8	13.7 ± 2.6	11.2 ± 2.6	0.52	0.15
INF-τ on uterine flush, ng/mL	300 ± 92	211 ± 86	60 ± 92	0.06	0.22
INF-τ mRNA, dCt ratio	1.5 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	0.13	0.78

Ovulation of a FW follicle reduced pregnancy and this effect was mediated by low P4 concentration during their development. Luteal function during early gestation, concepti elongation and their ability to produce IFN-τ were not compromised by ovulation of follicles developing under low concentrations of progesterone.

DISRUPTION OF FIBROBLAST GROWTH FACTORRECEPTOR SIGNALING IN BOVINE CUMULUS-OOCYTE COMPLEXES DURING *IN VITRO* MATURATION IMPACTS SUBSEQUENT EMBRYONIC DEVELOPMENT

Kun Zhang and Alan D. Ealy

Department of Animal Sciences, University of Florida, Gainesville, Florida

Folliculogenesis and oogenesis is regulated by several autocrine, paracrine and endocrine factors. Members of the fibroblast growth factor (FGF) family have been implicated in this regard. Several FGFs and their receptors (FGFRs) are expressed in the follicle and oocyte throughout folliculogenesis and supplementing cumulus oocyte complexes (COCs) with selective FGFs during *in vitro*maturation (IVM) improves subsequent embryo development. The overall objectives of this work were to examine changes in FGFR transcript abundance in cumulus and oocytes during IVM and determine if FGFR activity during IVM is required for subsequent embryo development. In the first study, COCs were collected either before culture (time 0) or after 6 or 22 h of culture in the presence or absence of 25µg/ml FSH in serum-free maturation medium. At each time point cumulus cells were separated from oocytes by repeat pipetting and RNA was extracted (n=10 COCs/sample). Quantitative RT-PCR was completed to determine time- and FSH-dependent changes in *FGFR1b*, *R1c*, *R2b* and *R2c* transcript abundance. In cumulus cells, increases ($P<0.05$) in *R1b* and *R2c* mRNA abundance were observed in the absence of FSH after 6 and 22 h. Supplementation with FSH produced substantial increases ($P<0.001$) in the abundance of each *FGFR* after 6 h of culture. This effect was transient and the relative abundance of all *FGFR* transcripts decreased to non-FSH treated levels by 22 h in culture. No effects of FSH supplementation and time in culture were detected in oocytes. In the second study, COCs were cultured in the presence of FSH with one of two FGFR kinase inhibitors (25 µM SU5402 [SU] or 1 µM PD173074 [PD]) or the carrier only (DMSO). *In vitro* fertilization was performed by co-culture of matured COCs with a pool of frozen-thawed semen from three bulls. After 8-10 h, putative zygotes were cultured with modified synthetic oviductal fluid. Fertilization rates were not affected by exposure to inhibitors but subsequent embryo production was influenced by COC exposure to FGFR inhibitors. Specifically, adding SU or PD decreased ($P<0.05$) the percentage of 8-16 cell stage embryos at day 3 (66.4±2.8 %[control] vs. 47.1±4.2% [SU]; 74.3±4.6% [control] vs. 50.1±4.2% [PD]). Also,blastocyst formation at day 7 post-fertilization was decreased ($P<0.05$) by FGFR inhibitor treatment during COC maturation (23.2±2.0% (control) vs. 8.2±2.6% [SU]; 24.7±1.9% [control] vs. 12.2±2.6% [PD)]. These observations indicate that FSH regulates *FGFR1* and *R2* mRNA abundance in cumulus during final oocyte maturation, and activation of these receptors during IVM appears important for subsequent embryo development. This project was supported by National Research Initiative Competitive Grant no. 2008-35203-19106 from the USDA National Institute of Food and Agriculture.

REGULATION OF EXPRESSION OF GENES INVOLVED IN EPIGENETIC PROGRAMMING IN THE BOVINE MORULA BY COLONY STIMULATING FACTOR 2, BONE MORPHOGENIC PROTEIN 4 AND ACTIVIN A

K. B. Dobbs and P. J. Hansen

Dept. of Animal Sciences, University of Florida

Development of the preimplantation embryo is under autocrine and paracrine control by cytokines and growth factors. Among these are colony-stimulating factor (CSF2), which increases inner cell mass number and embryo survival, bone morphogenic factor 4 (BMP4), which promotes mesoderm formation and inhibits neural tube development, and activin A, which promotes maintenance of pluripotentcy. Here we tested whether actions of these molecules involve regulation of genes involved in epigenetic regulation. Genes examined participate in gene silencing through trimethylation of H3K27me3 (*EZH2, EED, SUZ12, SUV39H1, HDAC1*) or DNA methylation (*DNMT3a*). Bovine embryos were treated with 0 or 10 ng/ml BoCSF2, HuBMP4 or HuActivin A at Day 5 after in vitro fertilization. Embryos $\geq$ 16 cells were harvested at either 12 h (all genes) or 24 h (EZH2 only) after treatment. Pools of 10 embryos were processed for qPCR. The experiment for the 12 h time point was replicated 8 times for *EZH2*, 6 times for *EED*, *SUZ12*, *SUV39H1* and *HDAC1* and 2 times for *DNMT3a*. The experiment for the 24 h time point was replicated 2 times. There were no significant effects of treatment on expression of *EZH2* (at 12h or 24h), *SUZ12*, *HDAC1, EED, or SUV39H1*. BMP4 and activin A increased *DNMT3a* transcript abundance ($P<0.04$ and $P<0.06$ respectively). As compared to control, the fold change after 12 h was 1.76, 2.57 and 2.39 for CSF2, BMP4 and activin A, respectively. *DNMT3a* is one of two *de novo* methyltransferases responsible for re-establishing methylation marks on DNA following global demethylation. In conclusion, the addition of BMP4 and activin A at day 5 for 12 h caused an increase in *DNMT3a*, suggesting these growth factors can change *de novo* methylation. More experiments are needed to confirm changes in *DNMT3a* transcript abundance and to determine effects on expression of other genes involved in epigenetic modification. (Supported by USDA AFRI Grant 2009-65203-05732)

WNT SIGNALING REGULATES DEVELOPMENT OF BOVINE EMBRYOS TO THE BLASTOCYST STAGE

Anna C. Denicol, Kyle B. Dobbs, and Peter J. Hansen

Dept. of Animal Sciences, University of Florida, Gainesville

The Wnt signaling pathway regulates cell proliferation and differentiation. Studies in mice have demonstrated that this signaling system is a critical regulator of embryonic development during the peri- and post-implantation period. Recently, it has been demonstrated that treatment of bovine embryos with CSF2 improved embryo development and post-transfer survival while also affecting expression of genes that would downregulate Wnt signaling. Here the hypothesis was tested that activation of Wnt signaling at d 5 after fertilization reduces development to the blastocyst stage. Experiment 1 was performed in 6 replicates using 1103 embryos produced in vitro. At day 5 after insemination, embryos received either vehicle (0.1% DMSO) or 0.35, 0.7, 1.4 or 2.8 μM of Wnt agonist 2-Amino-4-(3,4-(methylenedioxy)benzylamino)-6-(3-methoxyphenyl)pyrimidine (AMBMP). Embryos were evaluated for blastocyst development at d 7, 8 and 9, and harvested for staining of total cells (Hoechst) and trophectoderm cells (Cdx2-labeled antibody). Addition of AMBMP at 0.7 μM or higher decreased percent of blastocysts at d 7, 8 and 9 ($P < 0.05$) compared to controls. The percent of oocytes that developed to the blastocyst stage at d 7 was 20.3, 19.8, 12.4, 7.3, and 2.9 for 0, 0.35, 0.7, 1.4 and 2.8 μM AMBMP, respectively. In addition, AMBMP reduced blastocyst total cell number, inner cell mass number (2.8 μM only) and trophectoderm number ($P < 0.05$). Experiment 2 was conducted to verify if the effect of AMBMP was mediated by Wnt signaling. In particular, it was tested whether an inhibitor of Wnt signaling, human recombinant DKK1, blocked inhibitory effects of AMBMP. This experiment was performed in 5 replicates with 1691 embryos. On d 5, embryos received vehicle (DMSO 0.1%), AMBMP (0.7 μM), DKK1 (100 ng/ml), or AMBMP and DKK1. Development of embryos to blastocyst stage was evaluated at d 7 and 8. Addition of AMBMP decreased blastocyst development at d 7 and d 8 ($P < 0.05$). There were agonist x antagonist interactions at d 7 and 8 ($P < 0.05$). Effects of AMBMP were blocked by DKK1 but, in the absence of AMBMP, DKK1 reduced development. The percent of oocytes that developed to the blastocyst stage at d 7 was 29.9, 15.9, 21.1 and 22.2 for control, AMBPM, DKK1, and AMBPM + DKK1, respectively (SEM=2.1). In conclusion, addition of AMBMP at d 5 post-insemination reduces development of embryos to the blastocyst stage and blastocyst cell number through a process dependent upon activation of Wnt signaling. In addition, however, DKK1 can inhibit development, suggesting endogenous signaling through Wnt receptors that facilitates blastocyst development. Support: USDA-AFRI No. 2009-65203-05732

LYSOSOME NUMBER AND SIZE DO NOT VARY DURING A TIDAL CYCLE IN ERYTHROCYTES OF THE BLOODWORM *GLYCERA DIBRANCHIATA*

M. Christina Vasquez[1], Timothy A. Crombie[2] and David Julian[2]
[1]Department of Animal Science, [2] Department of Biology, University of Florida, Gainesville, FL 32611

Intertidal marine organisms experience a variety of abiotic stressors during tidal emersion, including exposure to hypoxia, anoxia and hydrogen sulfide. However, the return of oxygenated water during tidal immersion may also be a stressor. Biomedical research on mammalian systems has shown that a rapid return to normoxia from hypoxia or anoxia (termed ischemia-reperfusion) may cause cellular damage from increased reactive oxygen species (ROS) production. To remove the cellular damage, cells utilize lysosome-mediated autophagy, in which specialized vacuoles isolate the damaged proteins and organelles and then bind to lysosomes allowing for the degradation and recycling of the damaged components. If immersion after low tide increases ROS production, similar to reperfusion after ischemia, then tidal immersion may activate lysosome-mediated autophagy. We investigated whether cells from the intertidal bloodworm *Glycera dibranchiata* showed changes during a tidal cycle that are characteristic of increased autophagy. We collected erythrocytes from bloodworms at a single location over a tidal cycle. Collected erythrocytes were stained for lysosomes using a lysosome specific fluorescent marker. We determined the number of lysosomes within each cell, the cross-sectional area of each lysosome and the total lysosome area per cell. We found statistically significant effects of tidal cycle on the number of lysosomes present per cell and on the cross-sectional area of the lysosomes per cell. Overall, the number of lysosomes and the lysosome cross-sectional area decreased during the tidal cycle but the magnitudes of these changes were very small. We found no significant differences between time points for the total lysosome area. Based on these data, we failed to find support for the hypothesis that re-immersion during a tidal cycle is associated with increased lysosome-mediated autophagy.

EFFECT OF PHYSIOLOGICAL HEAT SHOCK ON DEVELOPMENT AND EXPRESSION OF STRESS RESPONSE GENES IN BOVINE PREIMPLANTATION EMBRYOS

Miki Sakatani and Peter J Hansen

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Heat shock can decrease development of preimplantation bovine embryos. Embryos become more resistant to heat shock as they advance in development; by the morula stage, embryos are resistant to heat shock. Conclusions about heat shock are based on experiments using temperatures of 41-43°C that are greater than those commonly experienced by embryos in utero during maternal hyperthermia. In the present study, we evaluated whether a temperature more commonly experienced by heat-stressed cows (40°C) can inhibit embryonic development and induce expression of genes that stabilize cellular function in response to heat shock. Two experiments were performed with *in vitro* produced embryos of various genotypes that were cultured in microdrops of SOF-BE1 medium (30 zygotes/50 µL drop). Unless otherwise stated, culture was at 38.5°C in 5% CO_2 in air. In Experiment 1, embryos were exposed to 38.5 or 40°C for 24 h beginning at 8 h post insemination (hpi) (zygote stage), or 116 hpi (morula stage). After heat shock, embryos were returned to 38.5°C in 5% CO_2 in air and cultured through day 8. Cleavage and development were assessed o Day 3 and 8 after insemination Control embryos were cultured at 38.5°C in 5% CO_2 from day1 through day 8. Cleavage rates were not affected by treatment but the percent of embryos that were > 5-cell stage on Day 3 and the percent of embryos that were blastocysts at Day 8 was significantly lower for embryos heat shocked beginning at 8 hpi than for control embryos. In contrast, heat shock beginning at 116 hpi had no effect on percent blastocyst at Day 8. Experiment 2 was conducted to determine whether resistance of more advanced embryos was related to expression of genes involved in thermoprotection. At 116 hpi, embryos were exposed to 40°C and 5% CO_2 in air for 2, 4, and 8 h. After heat shock, 20 morula-stage embryos were collected for gene expression analysis. Control embryos were cultured at 38.5°C and 5% CO_2 in air and collected at the same times (118, 120 and 124 hpi). Transcript abundance was determined for *HSPA1A, HSP90AA1, SOD1* and *CAT* using quantitative real time PCR. Results were normalized to *GAPDH* expression. Transcript abundance for all genes increased in response to as little as 2 h of heat shock. The magnitude of response to heat shock for *HSPA1A* varied between replicates. Results indicate that physiological heat shock affects embryo competence at the zygote stage but not at the morula stage of development. Increased expression of specific genes involved in thermoprotection occurs in response to heat shock and may explain, in part, the increased resistance of morula to heat shock (Support: Southeast Milk Dairy Checkoff Program and NARO Study Abroad Program).

EFFECT OF HEAT STRESS DURING THE DRY PERIOD ON INSULIN SENSITIVITY OF MULTIPAROUS DAIRY COWS

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Heat stress during the dry period affects hepatic gene expression and adipose tissue mobilization during the transition period. One of the possible outcomes may be altered insulin action on peripheral tissues. Our objective was to evaluate the effect of heat stress during the dry period on insulin sensitivity in the transition period. Cows were dried off 46 d before expected calving and assigned to one of two treatments: heat stress (HT, n=16) or cooling (CL, n=16). During the dry period, the average THI was 78, but CL cows were cooled with sprinklers and fans and HT cows were not. After calving, all the cows were housed together in the same barn and cooled. Rectal temperatures (RT) were measured twice daily (0730 and 1430h) and respiration rate (RR) recorded thrice weekly during the dry period. DMI was recorded daily from dry-off to 42 d relative to calving (RTC). BW and BCS were measured weekly from dry-off to 42 DIM. Milk yield and composition were recorded daily to 126 DIM. Glucose and insulin tolerance tests were performed at dry-off, -14, 7 and 28 d RTC from a subset of cows (HT, n=8; CL, n=8). Relative to HT, CL cows had lower RT in the afternoon (39.3 vs. 39.0 ^{0}C; $P < 0.01$) and lower RR (69 vs. 48 breaths/min; $P < 0.01$). CL cows consumed more feed than HT cows prepartum (11.4 vs. 10.2 kg/d; $P = 0.05$), but not postpartum ($P = 0.25$). Compared with HT, CL cows gained more weight before calving ($P = 0.01$) but lost more weight in the early lactation ($P = 0.02$). Treatment did not affect BCS. CL cows produced more milk than HT cows (40.4 vs. 32.7 kg/d; $P < 0.01$), but prepartum cooling did not affect milk composition. Preliminary data from the glucose tolerance test indicate that CL cows had similar glucose disposal rates ($P = 0.3$) relative to HT cows 2 weeks before calving. Regardless of treatment, cows had increased glucose disposal rate at -14 d RTC compared with dry-off. We conclude that heat stress during the dry period compromises lactation performance but does not affect insulin sensitivity late in the dry period.

ONTOGENY OF GLOBAL GENE EXPRESSION IN THE OVINE FETAL HYPOTHALAMUS IN LATE GESTATION

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Introduction: Parturition is a highly regulated neuroendocrine process that requires a complete maturation of the fetal hypothalamus-pituitary-adrenal (HPA) axis in order to activate the endocrinal signal cascade that concludes gestation. The activation of the HPA axis by the end of gestation occurs together with a gradual increased transcription of specific recognized genes. However, many of the genes expressed on the ovine fetal brain close to parturition remain unknown. The resulting products of those genes could play an important role in the normal fetal development and expected time of parturition. The objective of this study was to determine the genes (and corresponding biological processes) showing a pattern of increased expression toward the end of gestation in the hypothalamus of ovine fetuses.

Materials and methods: mRNA was extracted from the hypothalamus of ovine fetuses at 80, 100, 120, 130, 145 days of gestation and 1 day of extra uterine life, taking 4 biological replicates per gestational age (n=24). RNA samples were purified according the RNA STAT-60 Protocol and analyzed with the Agilent 2100 Bioanalyzer to determine RNA integrity. Microarray was performed following the Agilent protocol for 1-color 8x15 microarrays. Each sample was hybridized with the 15744 probes contained in each array, and the intensity of the resulting fluorescence was quantified with an Agilent Scanner. Microarray data analysis was performed following the Empirical Bayes model proposed by Tai and Speed (Biometrics 65, 40, 2009). The list of genes obtained from this analysis was subjected to a functional enrichment analysis employing WebGestalt (WEB-based GEne SeT AnaLysis Toolkit). The statistical method used was the hypergeometric test and the p-value was adjusted by Bonferroni method. Some genes were seleceted for qRT-PCR validation using SYBR-Green. Expression of each gene was normalized to β-actin expression. Relative quantification was obtained by the difference in cycle times (Ct), calculating fold changes in gene expression for each sample.

Results: A total of 604 genes were selected by the Empirical Bayes analysis after duplicates removal. From them, 486 genes showed an increased expression pattern after 120 days of gestation, while the 118 genes remaining had a decreased pattern. From the functional enrichment analysis of the genes with increased expression pattern, the main significant biological processes ($p<0.05$) obtained and number of involved genes, were: response to external stimulus (57 genes); response to stress (83 genes); defense response (41 genes) and inflammatory response (30 genes). The genes related with defense/inflammatory response selected for qRT-PCR validation were: Interleukin 10 (IL 10); Interleukin 18 (IL18); Toll-like receptor 2 (TLR2) and Toll-like receptor 3 (TLR3). The fold change in mRNA relative to 80 days was significantly increased at 145 days and 1 day of life for all the genes ($p<0.05$).

Conclusion: These results suggest that could be a pathway related with inflammatory/immune response that is turning on in the fetal brain parallel with the HPA activation close to the time of parturition. The activation of anti or pro inflammatory cytokines and immune related genes in the fetal hypothalamus could be part of the global gene activation in the placental-fetal unit or, it could be a critical pathway in the fetal neuronal development leading to parturition. Further studies are proposed to elucidate this issue.

MECHANOSENSITIVE CHANNEL PROTEIN (MSCL) IN *MYCOPLASMA BOVIS*

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Mollicutes represent the smallest free-living microorganisms, have minimal genomes, and have arisen by reductive evolution. Because the cell wall has been lost, mycoplasmas are susceptible to desiccation and osmotic damage. Based on *in silico* analysis of the available complete genome sequences of Mollicutes, a homolog of *MscL*, the mechanosensitive channel protein that forms pores within bacterial membranes and regulates solute transport and water flow during osmotic challenge, was found in selected avian, fish, reptilian, ruminant, and plant pathogens. The species that have acquired *MscL* share unique characteristics of persistence in the environment and the ability to colonize sites with wide osmotic ranges. *Mycoplasma bovis* was among the pathogens with a gene encoding MscL. We hypothesize that the acquisition of the *MscL* gene has provided an evolutionary advantage and confers resistance to osmotic lysis. The specific aims of this project are (1) to determine the degree of diversifying and stabilizing selection of the known *MscL* genes among the Mollicutes; (2) to determine the degree of diversifying and stabilizing selection of the *MscL* gene in different clinical isolates of *M. bovis*, and (3) to determine if a *MscL* mutant of *M. bovis* has altered resistance to osmotic shock. The following experimental approaches will be used. In order to determine the degree of diversifying and stabilizing selection of the known MscL genes, sequences from GenBank will be analyzed using Bayesian models of sequence evolution available in the Selecton v2.4 software suite. This will identify highly conserved as well as variable regions of the protein among the Mollicutes. The *MscL* gene was identified in >70 clinical isolates of *M. bovis* in a preliminary screening. From this group, I will select 10 strains and sequence the gene. These sequences will be analyzed using the Selecton software. We predict there may be substitutions or deletions within the *MscL* coding sequence among the different species of mycoplasmas, but that the strains of *M. bovis* will have more limited substitutions or deletions. In order to determine if MscL confers resistance to osmotic shock in *M. bovis*, a wild type strain and a mutant (gift of R. Rosenbusch, Iowa State University) will be grown to midlogarithmic phase of growth and subjected to hypotonic shock. After 5, 10, 15 and 30 min exposure, the CFU will be determined. We expect that at least a two-fold log difference will be observed between the wild type and mutant.

CONJUGATED LINOLEIC ACID AND ROSIGLITAZONE ATTENUATE LIPOPOLYSACCHARIDE-INDUCED TNF-α PRODUCTION BY BOVINE BLOOD CELLS

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Lipopolysaccharide (LPS) modulates innate immunity through alteration of cytokine production by immune cells. The objective of this study was to examine the effect of exogenous conjugated linoleic acid (CLA) and peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist, rosiglitazone, on LPS-induced tumor necrosis factor alpha (TNF-α) production by cultured blood from prepubertal Holstein heifers (mean age = 5.5 months). Compared to unstimulated cells, addition of LPS (10 µg/mL) to the culture medium increased peripheral blood mononuclear cell (PBMC) proliferation up to 2.5-fold. Co-incubation with interferon gamma (IFN-γ, 5 ng/mL) further stimulated ($P < 0.01$) the proliferative response to LPS. Lipopolysaccharide increased ($P < 0.01$) TNF-α concentration in cultured whole blood in a dose- and time -dependent manner. The greatest TNF-α stimulation occurred after 12 h of exposure to 1 µg/mL of LPS. Co-incubation with *trans*-10, *cis*-12 (*t*10,*c*12) CLA isomer (100 µM) or rosiglitazone (10 µM), a PPAR-γ agonist, decreased LPS-induced TNF-α production by 13 and 29%, respectively. Linoleic acid (LA) and *cis*-9, *trans*-11 (*c*9,*t*11) CLA isomer had no detectable effects on LPS-induced TNF-α production. The PPAR-γ agonist-induced TNF-α attenuation was reversed when blood was treated with both rosiglitazone and GW9662, a selective PPAR-γ antagonist. Addition of rosiglitazone to the culture medium tended to reduce NF-κBp65 concentration in nuclear extracts isolated from cultured PBMC. Results demonstrate that LPS is a potent inducer of TNF-α production in bovine immune cells, and that *t*10,*c*12 CLA and PPAR-γ agonists decrease TNF-α response to LPS in cultured bovine blood. Additional studies are needed to fully characterize the involvement of NF-κB in LPS-signaling in bovine blood cells.

THERAPY AND UNDERLYING MECHANISMS ASSOCIATED WITH UTERINE DISEASE OF DAIRY COWS

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Contamination of the female reproductive tract following parturition is common in dairy cows (> 90%) leading to high prevalence of uterine diseases (> 20%), which ultimately impacts herd profitability (decreased milk yield and fertility) and animal welfare. Objectives were to investigate the efficacy of prostaglandin (**PG**) $F_{2\alpha}$ early postpartum as a therapy to reduce the incidence of subclinical endometritis (**SCE**) and improve pregnancy per insemination (**P/AI**; study 1); and to investigate mechanism by which *Arcanobacterium pyogenes* alters the length of the estrous cycle and impair fertility (study 2). In study 1, we hypothesized that PGF2a improves P/AI of dairy cows by reducing the risk of subclinical endometritis. A total of 1,342 lactating Holstein dairy cows on 25±3 day in milk (**DIM**) were randomly allocated to 3 treatments: (Control= No treatment; 1PGF= 1 dose of $PGF_{2\alpha}$ at 39±3 DIM; and 2PGF = 2 doses of $PGF_{2\alpha}$ at 25±3 and 39±3 DIM). A subset of 357 cows had uterine cytology sample collected at 25±3, 32±3 and 46±3 DIM to determine percentage of polymorphnuclear cells (**PMN**). Subclinical endometritis was defined based on the percentage of PMN in the cytology as two thresholds, ≥5% and ≥10%. All cows were enrolled in the double Ovsynch program for 1st AI. Pregnancy was diagnosed 32 d after AI and reconfirmed 28 d later. Data were analyzed by the GLIMMIX procedure of SAS fitting the proper distribution type. Subclinical endometritis as determined by a threshold of ≥5% of PMN decreased P/AI at d 32 (36.7% vs. 44.3%; P=0.018) and d 60 (27.3% vs. 39.1%, P<0.001). Threshold of ≥10% of PMN also decreased P/AI at d 32 (36.0% vs. 43.6%; P=0.04) and 60 (26.0% vs. 38.1%, P=0.002). Nonetheless, 1PGF or 2PGF treatments did not affect incidence of SCE neither P/AI at d 32 nor 60 post AI. Therefore, incidence of subclinical endometritis and P/AI were not affected by early postpartum treatment with $PGF_{2\alpha}$. The aim of study 2 is to elucidate the mechanism of CL lysis as well as blockade of the luteolytic cascade when cows are challenged with A. pyogenes live uterine infusion. *A. pyogenes* is considered one of the most relevant pathogens involved in uterine diseases because of severity of endometrial lesions and chronicity of infections. Intrauterine inoculations of live *A. pyogenes* lead to abnormal estrous cycle length, including shortening of the luteal phase as well as extension of the luteal phase. The mechanisms by which *A. pyogenes* can induce luteolysis in some cows and promote extension of the luteal phase in other cows have not been completely elucidated. In vitro studies have shown that *A. pyogenes* can increase the secretion of $PGF_{2\alpha}$ as well as decrease the expression of oxytocin receptors by the endometrium. The luteotropic prostaglandin E2 (PGE_2) has also been found to be increased when endometrial cells were exposed to *A. pyogenes*. Therefore, the initial response to infection might be to induce the release of $PGF_{2\alpha}$ leading to the lysis of the CL in some cows and later to decrease oxytocin receptors leading to extension of luteal life span. Increase of PGE_2 might also help to maintain the CL later on. Increase of $PGF_{2\alpha}$ secretion as well as decrease in oxytocin receptor gene expression have been observed when uterine epithelial cells have been exposed to pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β); therefore, we hypothesized that inoculation of live *A. pyogenes* would initially lead to increased secretion of $PGF_{2\alpha}$ as a result of increased TNFα and IL-1β, but later would have decreased secretion of $PGF_{2\alpha}$ because of down regulation of oxytocin receptors.

EVALUATION OF PERIPARTAL MINERAL AND ENERGETIC STATUSES AND NEUTROPHIL FUNCTION OF DAIRY COWS OF LOW OR HIGH RISK OF DEVELOPING UTERINE DISEASES

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Objectives were to establish the relationships among postpartum Ca status, neutrophil function and incidence of uterine diseases in dairy cows considered to be of low or high risk of developing metritis. Our hypothesis was that subclinical hypocalcemia reduces in vitro neutrophil function and increases the risk of metritis in early lactation. In this prospective cohort study, 55 Holstein cows considered to be of high risk to develop metritis based on calving problems (HRM; dystocia, twins, stillbirth, and/or retained placenta) were matched with 55 low risk (LRM) herdmates based on day of calving and parity. Cows were monitored daily for rectal temperature, vaginal discharge, and attitude score for the first 12 d in milk (DIM). Blood was sampled and neutrophils analyzed for their phagocytic and oxidative burst activities at 0, 1 and 3 DIM using a dual flow cytometric assay. Blood was also sampled at 0, 1, 2, 3, 4, 7 and 12 DIM and analyzed for serum concentrations of Ca, Mg, and K, nonesterified fatty acids and 3-βOH-butyrate. Ovaries were scanned by ultrasonography at 24, 31, and 38 DIM to determine ovulation based on presence of a corpus luteum (CL). Clinical endometritis (CE) was assessed based on vaginal mucus with pus at 31 DIM. Subclinical endometritis (SE) was assessed based on the prevalence of neutrophils on endometrial cytology performed at 38 DIM. Data were analyzed by the PROC GLIMMIX of SAS (2001) fitting appropriate distribution functions. Receiver operator characteristic (ROC) curves were used to determine the ideal cut point for serum Ca to predict metritis. Serum Ca concentrations below 8.59 mg/dL in the first 4 d postpartum predicted metritis with 88.5% sensitivity and 55.2% specificity (area under the curve = 0.77, 95% CI=0.68-0.84; $P<0.001$). Changes in serum Ca concentrations after calving were associated ($P=0.03$) with an increased risk of metritis. For every 1 mg/dL decrease in serum Ca in the first 4 d postpartum, the odds of having metritis increased 2.6 fold (AOR=2.6; 95% CI=1.1-6.2). Serum Ca concentrations in the first 4 d postpartum were less ($P<0.01$) for cows of HRM than LRM (8.60±0.07 vs. 8.91±0.07 mg/dL). Cows that developed metritis had reduced ($P<0.01$) oxidative burst in the first 3 d postpartum compared with healthy cows (36.3±2.7 vs. 46.4±2.3% of neutrophils). Neutrophil function and incidence of metritis are depicted in the Table below according to subclinical hypocalcemia and LMR and HRM.

	Subclinical hypocalcemia[1]		Normocalcemia	
	LMR	HMR	LMR	HMR
Neutrophil function				
Oxidative burst	38.9±3.2	39.8±2.7	45.3±3.4	44.7±5.2
MFI[2,§]	42.6±12.2	67.8±10.2	70.8±13.2	33.8±19.8
Metritis, % [*,¶]	40.7 (11/27)	77.8 (35/45)	14.3 (4/28)	20.0 (2/10)
Puerperal metritis, % [*,¶]	29.6 (8/27)	53.5 (24/45)	0 (0/28)	10.0 (1/10)

* Effect of hypocalcemia ($P < 0.05$); ¶ Effect of metritis risk ($P < 0.05$); § Interaction between hypocalcemia and metritis risk ($P < 0.05$).
[1] Serum Ca < 8.59 mg/dL in the first 3 d postpartum; [2] Mean fluorescence intensity for oxidative burst.

In conclusion, subclinical hypocalcemia and a reduction in serum Ca concentrations after calving are important risk factors for uterine diseases, and these were observed in cows considered to be of low and high risks for metritis at calving.

IDENTIFYING GENETIC MARKERS FOR FERTILITY IN DAIRY CATTLE

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Daughter Pregnancy Rate (DPR) is an estimate of a bull's daughter fertility and is defined as the percent of cows eligible to be bred that become pregnant during each 21-day period. The term is calculated from days open; every 1% increase in DPR equals a decrease of 4 days open. The heritability of DPR is only 0.04 so identifying genetic markers for this trait would be useful for enhancing rate of genetic progress in selection for the trait. We hypothesize that allelic variants of genes involved in reproductive processes will have significant effects on DPR. Semen of bulls which have high (≥ 2.5; $n = 240$) or low (≤ -2.5; $n = 240$) DPR was obtained from the Cooperative Dairy DNA Repository of the USDA. The DNA will be extracted from semen using the DNeasy Blood and Tissue kit (Qiagen) and quantified by the Quant-it PicoGreen Assay (Invitrogen). A custom Golden Gate SNP array (Illumina) will be designed that contains SNPs in genes related to fertility or regulated in the embryo by factors such as IGF1 and CSF2 which have been shown to increase embryo survival. A total of 384 SNPs will be incorporated in the array, and each DNA sample will be genotyped using the array. Effects of each SNP on DPR will be tested using the MIXED procedure of the Statistical Analysis System. Contrasts will be performed to determine additive effects of the SNP (by comparing the two homozygotes), and dominance effects (by comparing the heterozygotes with the average of the two homozygotes). We will also test for Hardy-Weinberg equilibrium on all the SNPs. SNPs which have a significant effect on DPR could be used as biomarkers for fertility. This project is supported by the Southeast Milk Inc. Dairy Check-off Program.

EFFECTS OF ANOVULATION AND BREED ON CONCEPTUS DEVELOPMENT AT PREIMPLANTATION STAGES IN LACTATING DAIRY COWS SUBJECTED TO SYNCHRONIZED OVULATION

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Objectives were to compare the steroid hormone concentrations and conceptus development to preimplantation stages of anovular Holstein (HA), cyclic Holstein (HC), and cyclic Jersey/Holstein crossbreed (CC) lactating dairy cows subjected to a synchronized ovulation. On postpartum d 29±3, cows of both breeds were randomly selected within a seasonal grazing herd and received an injection of prostaglandin (PG) $F_{2\alpha}$. Cows had their ovaries scanned by ultrasonography on postpartum d 29±3 and 39±3 to determine cyclic status. Cows were then grouped in CC (n=25; presence of a CL on both examinations), HC (n=25; presence of CL on both examinations) and HA (n=10; lack of CL in both examinations) and subjected to the Ovsynch protocol (d 39 GnRH, d 46 PGF2a, d 48 GnRH, d 49 AI). Blood was sampled and analyzed for concentrations of progesterone and estradiol during the Ovsynch protocol, at AI, d 7 and 15 after AI. On d 15 after AI, uteri were flushed and endometrium biopsied. The IFN-tau concentration in the uterine flush was measured. Recovered conceptuses and endometria were subjected to global analysis of gene expression using microarray. Size and morphology of the conceptuses were recorded. Data were analyzed using PROC GLIMMIX of SAS.

Parameter	HA	HC	CC	*P*
Diameter of dominant follicle, mm				
at $PGF_{2\alpha}$ of Ovsynch	16.5 ± 1.0 ^{a}	13.2 ± 0.7 ^{b}	13.2 ± 0.7 ^{b}	0.02
at 2nd GnRH of Ovsynch	23.5 ± 0.9 ^{a}	20.7 ± 0.6 ^{b}	19.1 ± 0.6 ^{c}	<0.01
Estradiol concentration, pg/mL				
at $PGF_{2\alpha}$ of Ovsynch	3.0 ± 0.4 ^{a}	0.8 ± 0.3 ^{b}	0.9 ± 0.3 ^{b}	<0.01
at 2nd GnRH of Ovsynch	4.8 ± 0.9 ^{b}	5.4 ± 0.6 ^{ab}	7.1 ± 0.6 ^{a}	0.05
Diameter of CL, mm				
at d 7 after AI	31.8 ± 1.8 ^{a}	26.6 ± 1.2^{b}	28.1 ± 1.2 ^{ab}	0.07
at d 15 after AI	31.4 ± 1.4 ^{a}	28.0 ± 0.9 ^{b}	30.0 ± 0.9 ^{ab}	0.10
Progesterone concentration, ng/mL				
at 1st GnRH of Ovsynch	0.1 ± 0.7 ^{b}	3.7 ± 0.4 ^{a}	3.9 ± 0.4 ^{a}	<0.01
at PGF2a of Ovsynch	2.5 ± 0.7 ^{c}	8.0 ± 0.5 ^{b}	9.4 ± 0.5 ^{a}	<0.01
at 2nd GnRH of Ovsynch	0.1 ± 0.4	0.3 ± 0.3	0.3 ± 0.3	0.73
at d 7 after AI	4.1 ± 0.3 ^{a}	3.0 ± 0.3 ^{b}	4.0 ± 0.3 ^{a}	<0.01
at d 15 after AI	7.6 ± 0.4 ^{a}	6.7 ± 0.3 ^{b}	7.7 ± 0.3 ^{a}	<0.05
Pregnancy based on IFN-tau, %	70.0 (7/10)	64.0 (16/25)	84.0 (21/25)	0.28
Ovoid embryos, %	0.0 (0/0) ^{b}	27.3 (3/16) ^{a}	0.0 (0/0) ^{b}	0.05
Conceptus length, mm	47.8 ± 8.8 ^{a}	9.4 ± 5.8 ^{b}	25.3 ± 8.7 ^{b}	<0.01
IFN-tau concentration in pregnant, ng/mL	7.3 ± 1.5 ^{a}	0.6 ± 1.1 ^{b}	0.9 ± 0.9 ^{b}	<0.01

^{a,b,c} Different superscripts in the same row differ (P < 0.05)

Although the proportion of pregnant cows was similar, HA cows had more advanced conceptuses likely because of a faster rise in progesterone concentrations after AI resulting from ovulation of a larger follicle and formation of a larger CL compared with HC. Although CC ovulated a smaller follicle, they had greater progesterone concentrations after AI and less incidence of ovoid conceptuses than HC on d 15

after AI. These preliminary data suggest that postovulatory rise in progesterone is more critical for d 15 conceptus development than pre-ovulatory concentrations.

EFFECTS OF BOS INDICUS BREEDING ON PLASMA PREGNANCY-ASSOCIATED GLYCOPROTEIN (PAG) CONCENTRATIONS AND FETUS SIZE IN EARLY GESTATION

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Cross-breeding *Bos indicus* and *Bos taurus* breeds improves various production traits for cattle maintained in hot climates. Limited information exists describing pregnancy specific events that are influenced by these cross-breeding strategies. In this study, transrectal ultrasonography was used to measure fetal size at 52 to 55 d of gestation in cows composed of Angus (>80% Angus; n=17), Brangus (n=15) and Brahman (≥25% Brahman; n=58) genetics. Blood was collected for the measure of plasma pregnancy associated glycoprotein (PAG) content by ELISA. Multiparous cows were used in a timed AI protocol for this study. Multiples sires were used to generate fetuses with varying degrees of Angus/Brahman cross-breeding. Blood was harvested once between day 52 and 55 post-TAI. Day of blood collection and ultrasonography was used as a covariate. PAG concentrations were greater ($P \leq 0.05$) in Brangus and Brahman cows than Angus cows (9.8, 10.4 and 5.9 ng/ml, respectively; SE=1.93). Fetus size was smaller ($P \leq 0.05$) in Brangus and Brahman cows than Angus cows (27.7, 28.8 and 34.8 mm, respectively; SE=3.24). No differences in PAG concentrations and fetus size were observed based on the amount of Angus, Brangus and Brahman genetics in the fetus. In summary, both PAG concentrations and fetus size differed based on the degree of Angus/Brahman cross-breeding of the cow but not the fetus. This suggests that the maternal system plays an active role in controlling placental activity and early fetal development, and Brahman-based cows control these events differently than Angus cows during early pregnancy.

EFFECT OF FATTY ACID SUPPLEMENTATION TO DAIRY COWS FED DIETS CONTAINING LOW CONCENTRATIONS OF FATTY ACIDS ON FOLLICULAR FLUID COMPOSITION AND EMBRYO QUALITY

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Objectives were to evaluate the impacts of supplementing diets containing low amounts of long chain fatty acids (FA, < 1.8%) with either mostly saturated free FA (SFA) or with Ca salts enriched with polyunsaturated FA (PUFA, 27% C18:2n6 and 3.5% C18:3n3 of the total FA) on follicular fluid composition and embryo quality of Holstein cows. At 60 d from the expected calving date cows were allocated randomly to 1 of 3 dietary treatments. Supplementation with FA (% dietary DM) consisted of 0% (**Control**, n=26), 1.7% **SFA** (n=25), and 1.7% as Ca salts of PUFA (**EFA**, n=25). At 40 DIM the estrous cycle of cows was synchronized with an injection of 25 mg of prostaglandin $F_{2\alpha}$ followed 3 days later by an injection of 100 µg of GnRH, and the ovaries were mapped by ultrasonography. The first wave dominant follicle was aspirated on d 6 after GnRH. Concentrations of progesterone, estradiol and total and free IGF-1 were measured in the fluid (Table 1). Embryo collection was performed twice in each cow. A standard superovulation protocol started after follicle aspiration, and cows were inseminated and then flushed on d 7 after AI. Embryos-ova were evaluated for fertilization and grade quality according to IETS (1998). After the first embryo collection, cows had their estrous cycle synchronized and were inseminated. The uteri were flushed on d 15 after AI, and interferon-tau quantified in the flushes. Data were analyzed by the Proc Glimmix of SAS (2001). Orthogonal comparisons were performed to determine the effect of fat feeding and source of fatty acid.

	Treatment			P[1]		
Parameter[2]	Ctrl	SFA	EFA	TRT	Fat	FA
Follicle						
Follicle, mm	14.1 ± 1.2	14.0 ± 0.8	16.4 ± 0.8	0.08	0.42	0.03
Estradiol, ng/mL	459 ± 373	232 ± 212	349 ± 192	0.87	0.66	0.69
P4, ng/mL	97.9±148.5	67.3±128.5	152.5±89.8	0.92	0.99	0.73
Total IGF-1, ng/mL	37.2 ± 8.9	42.4 ± 6.5	44.8 ± 6.2	0.80	0.55	0.79
Free IGF-1, ng/mL	0.59 ± 0.08	0.64 ± 0.05	0.54 ± 0.05	0.44	0.98	0.20
E2:P4 ratio	6.6 ± 4.8	3.9 ± 3.3	2.8 ± 5.4	0.85	0.59	0.88
Embryo day 7, %						
Fertilization	59.8 ± 15.4	75.3 ± 11.2	74.5 ± 11.3	0.68	0.38	0.96
Grades 1 and 2	72.9 ± 22.8	56.1 ± 14.5	70.2 ± 13.9	0.73	0.70	0.49
Transferable	76.4 ± 18.9	74.5 ± 12.0	76.7 ± 11.5	0.99	0.97	0.89
Blastocyst	10.0 ± 9.8	16.4 ± 6.2	6.3 ± 6.0	0.51	0.89	0.25
Embryo day 15						
Elongated, %(n/n)	71.4 (5/7)	50.0 (2/4)	66.7 (4/6)	0.86	0.77	0.63
Length, mm	7.1 ± 3.6	11.1 ± 4.2	5.2 ± 5.5	0.68	0.93	0.51
IFN-tau, ng/mL	0.14 ± 0.16	0.55 ± 0.18	0.11 ± 0.25	0.45	0.72	0.49

[1] Fat = CTRL (no fat) vs. fat (EFA + SFA); FA = EFA vs. SFA;
[2] P4 = progesterone E2:P4 ratio = Estradiol to progesterone ration;.

DIRECTIONS

Steinhatchee Landing Resort
203 Ryland Circle
(GPS Address: 228 US Highway 51 North)
Steenhatchee, FL 32359
☎ (352) 498-7046

From Gainesville – approximately 76 miles
Take SR 26 West (University Avenue) through Newberry and Trenton
Before Fanning Springs, turn slight right onto US-19/US-27
At Tennille, turn left (South) on SR 51
Go 7.9 miles to destination in Steinhatchee