TWELFTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Lakeside Inn
Mount Dora, Florida
April 11-12, 2014

WELCOME

The Animal Molecular and Cellular Biology (AMCB) Symposium Committee would like to welcome faculty and students to this year's Research Symposium held at the state's oldest continuously operated hotel. The hotel was built in 1883 and was once the vacation destination of President Calvin Coolidge and his wife in 1930.

The hotel is a great venue for scientific discussions, with a grand veranda at the front of the hotel, poolside cooking facilities (location of our barbeque), and Tremain's Tavern located in the main building of the hotel. On behalf of the faculty of the AMCB, we welcome you to our 12th symposium and wish you hearty portions of good science, exceptional fellowship and lasting memories.

Pete Hansen, Director

ACKNOWLEDGMENTS

The faculty and students of the AMCB Program thank the following for support of the 12th Annual Research Symposium

Dr. John Hayes, Dean for Research, IFAS, University of Florida

Dr. David Norton, Vice President for Research, University of Florida

L.E. “Red” Larson Endowment

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

Drs. Adegbola Adesogan, Graduate Coordinator, Animal Molecular and Cellular Biology Graduate Program, University of Florida

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

Dr. Geoffrey E. Dahl, Professor and Chair, Dept. of Animal Sciences, University of Florida

Peter Hansen and John Driver, Chair and Co-Chair of the AMCB Graduate Program

Special thanks to Jim Moss and company for preparing the Friday night meal and to Sofia Ortega for local arrangements

2014 AMCB DISTINGUISHED LECTURER

Cynthia L. Baldwin, PhD
Department of Veterinary & Animal Sciences, University of Massachusetts

Cynthia Baldwin has been an investigator in the area of immunology for over 30 years. Her research has focused on cellular responses to bacterial and protozoan pathogens of humans and livestock. She has had a particular emphasis on γδ T cells using the bovine model. She and her colleagues have demonstrated that the γδ T cell expresses a novel pattern recognition receptor known as WC1 which designates antigen reactivity in conjunction with the T cell receptor by direct binding of pathogen. Gamma delta T cells stimulated in this manner have immunological memory and appear to be an important component in the response to leptospira vaccination. These discoveries influence the way we think about γδ T cells as players in adaptive immunity and vaccine development. Her work on brucellosis has shown that, in mice, responses by B lymphocytes actually impede protective immune responses through their production of regulatory cytokines. Professor Baldwin has also put effort into reagent development which is shared with the veterinary immunology research community. She currently leads the US-Veterinary Immunology Reagent Network (VIRN), a consortium of academic researchers developing reagents and improving immunological capability in a number of species. Dr. Baldwin is a long-serving Editor-in-Chief of *Veterinary Immunology and Immunopathology*, a journal for comparative immunology. She also serves as a Jefferson Science Fellow at the US Department of State. As part of her duties, she travels to Africa in conjunction with development of the Obama administration's "Feed the Future" program to increase world food and which includes a research agenda for reducing infectious diseases in livestock. She is currently the Principal Investigator on three federally-funded grants including one from the National Institute of Food and Agriculture (NIFA) that aims to develop "High Priority Reagents for Food Security" and a second co-sponsored by NIFA and the National Institute of Health to use large animal models to investigate the role of γδ T cells in immune responses against *Mycobacteria* and *Leptospira* for benefit of humans and animals.

AMCB FACULTY

Lokenga Badinga, Department of Animal Sciences

John Bromfield, Department of Animal Sciences

Samantha Brooks, Department of Animal Sciences

Mary Brown, Department of Infectious Diseases and Pathology

Geoffrey Dahl, Department of Animal Sciences

John Driver, Department of Animal Sciences

Peter J. Hansen, Department of Animal Sciences

Kwang Cheol Jeong, Department of Animal Sciences

Maureen Keller-Wood, Department of Pharmacodynamics

Christopher Mortensen, Department of Animal Sciences

Corwin Nelson, Department of Animal Sciences

Jose Santos, Department of Animal Sciences

Stephanie Wohlgemuth, Department of Animal Sciences

Charles Wood, Department of Physiology and Functional Genomics

Emeritus Faculty

William C. Buhi, Departments of Obstetrics & Gynecology, Animal Sciences

Kenneth C. Drury, Department of Obstetrics & Gynecology

Michael J. Fields, Department of Animal Sciences

Daniel C. Sharp, Department of Animal Sciences

William W. Thatcher, Department of Animal Sciences

CURRENT AMCB STUDENTS

PhD Students

Sarah Cochran (Advisor: PJ Hansen)

Anna Denicol (Advisor: PJ Hansen)

Kyle Dobbs (Advisor: PJ Hansen)

Leandro Greco (Advisor: JEP Santos)

Chengcheng Li (Advisor: S Wohlgemuth)

Veronica Negron Perez (Advisor: PJ Hansen)

M. Sofia Ortega (Advisor: PJ Hansen)

Eduardo Ribeiro (Advisor: JEP Santos)

Leticia Del-Penho Sinedino (Advisor: JEP Santos)

Luiz Siqueira (Advisor: PJ Hansen)

Paula Tribulo (Advisor: PJ Hansen)

MS Students

Jasmine Francis (Advisor: PJ Hansen)

Gabriel Carvalho Gomes (Advisor: JEP Santos)

Kathryn Merriman (Advisor: CD Nelson)

GRADUATES 2013

Dale Kelley, PhD (Advisor: C Mortensen)
Currently, professional student in the College of Veterinary Medicine, University of Florida

Christina Vasquez, PhD (Advisor: D Julian)
Currently, Lecturer, Biological Sciences Dept., California Polytechnic University

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 Wauburg Gainesville, FL	Ina Dobrinski University of Pennsylvania
2007	Whitney Laboratory St. Augustine, FL	Doug Bannerman USDA-ARS, Beltsville, MD
2008	Cedar Cove Beach & Yacht Club Cedar Key, FL	Eckhard Wolf LMU Munich, Germany
2009	Plantation Golf Resort and Spa Crystal River, FL	Dean Betts University of Western Ontario
2010	Whitney Laboratory St. Augustine, FL	Marc-Andre Sirard Laval University
2011	Steinhatchee Landing Resort Steinhatchee, FL	Kimberly Vonnahme North Dakota State Univ.
2012	Holiday Isle Oceanfront Resort St. Augustine, FL	Rocio Rivera University of Missouri
2013	Harbor Front Hampton Inn Fernandina Beach, Florida	Martin Sheldon Swansea University
2014	Lakeside Inn Mount Dora, Florida	Cynthia Baldwin University of Massachusetts

"Matthews … we're getting another one of those strange 'aw blah es span yol' sounds."

SCHEDULE OF EVENTS

FRIDAY, APRIL 11

1:00 PM Pete Hansen
Welcome, introductory comments

Session 1: Lactating Dairy Cow
Anna Denicol, Chair

1:15 PM Eduardo Ribeiro, Animal Sciences
Plasma anti-müllerian hormone in adult dairy cows and associations with fertility

1:30 PM Sofia Ortega, Animal Sciences
Single nucleotide polymorphisms in candidate genes related to daughter pregnancy rate in Holstein cows

1:45 PM Leandro Greco, Animal Sciences
Effect of altering the dietary ratio of n-6 to n-3 fatty acids on lactational performance, timing of luteolysis, uterine production of prostaglandins, and endometrial fatty acid profile and gene expression in dairy cows

2:00 PM Rafael Bisinotto, Animal Sciences
Regimens of progesterone supplementation for lactating dairy cows according to the presence of CL at the initiation of the timed artificial insemination (AI) program

2:15 PM Luiz Siqueira, Animal Sciences
Consequences for fetal development of exposure of bovine embryos to colony stimulating factor 2 during the preimplantation period

2:30 BREAK

Session 2: 2014 AMCB Distinguished Lecturer Presentation
John Driver, Chair

3:00 PM Professor Cynthia L. Baldwin
Dept. of Veterinary & Animal Sciences, University of Massachusetts
The role of a new family of pattern recognition receptor (WC1) in activation of bovine γδ T cells & promotion of vaccine efficacy

4:00 PM BREAK AND CHECK INTO ROOMS

Session 3: Preimplantation Embryo

Kathryn Merriman, Chair

4:45 PM Anna Denicol, Animal Sciences
Regulation of WNT signaling by the antagonist Dickkopf-1 directs lineage commitment and promotes survival of the preimplantation embryo

5:00 PM Paula Tribulo, Animal Sciences
Expression of genes associated with WNT signaling in the preimplantation bovine embryo

5:15 PM Verónica M. Negrón Pérez, Animal Sciences
Possible role of CCL24 in differentiation of the inner cell mass of the bovine embryo

5:30 PM Jasmine Kannampuzha Francis, Animal Sciences
Possible involvement of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway in signaling by colony stimulating factor 2 (CSF2) in the bovine preimplantation embryo

5:45 PM BREAK

6:00 PM GROUP PICTURE, POOL DECK

6:30 PM COOKOUT, POOL DECK

SATURDAY, APRIL 12

7:30-10:15 AM Breakfast buffet, Alexander Room

Session 3: Immunology
Chengcheng Li, Chair

9:00 AM Kathryn Merriman, Animal Sciences
Evaluation of the effects of vitamin D and toll-like receptor signaling pathways on expression of antibacterial β-defensin genes in bovine monocytes, neutrophils, and mammary epithelial cells

9:15 AM Jessi Powell, Animal Sciences
Vitamin D signaling in the porcine monocyte

9:30 AM Gabriel Gomes, Animal Sciences
Effects of prepartum evaporative cooling and vitamin E supplementation on adaptive and innate immunity of Holstein cows during summer in Florida

9:45 AM Bianca Artiaga, Animal Sciences
Targeting natural killer T cells to enhance immunity: A novel swine model

10:00 AM BREAK

Session 4: AMCB Lecture
Leandro Greco, Chair

10:30 AM Eduardo Ribeiro, Animal Sciences
Cooperative effort between bovine conceptus and dam mitigates their immunological conflict during peri-implantation

11:30 AM BREAK

Session 5: Environmental and Aging Stress
Jasmine Francis, Chair

11:15 AM Ana Mesa, Animal Sciences
Effect of exercise on embryonic epigenetic patterns and uterine environment in mares

11:30 AM Chengcheng Li, Animal Sciences
Examination of age-related change in mitochondrial function in the horse

11:45 AM CLOSING (AND VERY BRIEF) REMARKS, PETE HANSEN

ABSTRACTS
(Arranged alphabetically by first author)

Targeting natural killer T cells to enhance immunity: A novel swine model

B.L. Artiaga, P.M. Mercadante, Q. Li, M.R. Di-Lernia, S.T. Lewis, Z. Siemienski, and J.P. Driver

Department of Animal Sciences, University of Florida, Gainesville

Natural killer T (NKT) cells are an immune cell subset that recognize glycolipid molecules presented by the major histocompatibility complex (MHC) class I-like molecule CD1d. NKT cells stimulated by the glycolipid-CD1d complex induce profound and multiple effects on the immune system in part by secreting large quantities of cytokines. In this way it is believed that activated NKT cells play an important immunomodulatory function that prevents a host of infections diseases. The aim of the present study was to evaluate if pigs, a species immunologically similar to humans, provide a suitable model to determine how NKT cells may be harnessed to enhance immunity against foreign antigens in humans and livestock. Animals were ranked according to the frequency of NKT cells at two weeks of age, and assigned to one of four different treatment groups after weaning. They consisted of pigs injected on day 0 and day 14 of the experiment with the neoantigen hen-egg-lysozyme (HEL) in combination with one of three superagonists or PBS as a vehicle control [α-GalCer + HEL (n=6), C-glycoside (C-gly) + HEL (n=6) and OCH + HEL (n=6) or PBS + HEL (n=6)]. Blood samples were collected at 4 h, 12 h, 24 h, 4 d, 9 d and 12 d after each injection and analyzed for the frequency of NKT cells, T cells, and myeloid cell populations. Blood samples were also analyzed at 12 and 26 days after injection to determine the frequency of T lymphocytes capable of responding to HEL re-stimulation *in vitro*. Similar analyses to those described for blood were performed using homogenized spleen, cervical and mesenteric lymph nodes collected after animals were euthanized on day 28. Plasma was collected at all time points to detect the presence of anti-HEL antibodies. Compared to control animals, T cell and antibody responses against HEL developed for pigs injected with α-GalCer+HEL or OCH+HEL. In contrast, pigs treated with C-gly+HEL developed only the antibody response. Significant variability in immune responses was detected between individuals within each treatment, which was not correlated to NKT cell frequency. Collectively, our results indicated that swine are capable of responding to NKT cell therapeutics in a way that may be harnessed to prevent and treat infectious agents that threaten the health of humans and commercial swineherds. Future studies will determine whether NKT cells can be targeted to protect swine against a live H1N1 pandemic influenza virus infection.

Regimens of progesterone supplementation for lactating dairy cows according to the presence of CL at the initiation of the timed artificial insemination (AI) program

R.S. Bisinotto, L.O. Castro, C.D. Narciso, N. Martinez, M.B. Pansani, L.D.P. Sinedino, P.E. Carneiro, N.S. Van de Burgwal, H.M. Bosman, R.D. Medina, W.W. Thatcher, and J.E.P. Santos

Department of Animal Sciences, University of Florida, Gainesville

Objectives were to evaluate the effects of supplemental progesterone on fertility in dairy cows according to the presence of CL at the initiation of the Ovsynch program (d-10 GnRH, d-3 $PGF_{2\alpha}$, h-16 GnRH, d0 AI). Cows had their ovaries evaluated on d-10 and those without CL were assigned randomly to receive 0 (**NoCL**; n=652) or 2 intravaginal inserts containing progesterone (**CIDR**) from d-10 to -3 (**2CIDR**; n=645). Cows with CL were used as positive controls (**Diestrus**; n=637). The remaining cows bearing CL were assigned randomly to receive 0 (**Control**; n=869) or 1 CIDR (**1CIDR**; n=866). Ovaries were scanned on d-3 for assessment of ovulation to the first GnRH and the presence of a new CL. Blood was sampled in a subgroup of cows (n=146) on d-10, -9, -7, -5, -3, and 0 for the evaluation of progesterone concentrations during the growth of the ovulatory follicle. Estrus was detected based on removal of tail chalk beginning on d-10. A subgroup of cows (n=365) had their ovaries scanned on d-1 and d1 for measurement of the ovulatory follicle and evaluation of the response to the second GnRH. From the cows that ovulated to the second GnRH, blood was sampled on d6, 13, 19, 27, and 30 after AI. Progesterone concentration will be measured at all time points. Leukocytes were isolated from samples collected on d19 and the expression of interferon-stimulated genes will be performed to evaluate the effects on early embryo viability. Concentration of pregnancy-specific protein B will be measured in plasma on d27 and 30 after AI to evaluate the effects of treatment on initial development pregnancy. Pregnancy was evaluated 32 and 60 d after AI. Preliminary results were analyzed using the GLIMMIX procedure of SAS. Progesterone supplementation tended to reduce the diameter of the ovulatory follicle and to improve pregnancy per AI in cows lacking CL (Table). This benefit in fertility was observed exclusively in cows not detected in estrus at AI. Pregnancy per AI on d32 after insemination was smaller for 1CIDR compared to Control. However, pregnancy loss was reduced by progesterone supplementation, which resulted in similar proportion of pregnant cows for 1CIDR and Control on d60 after AI.

	Cows without CL			Cows with CL	
	NoCL	2CIDR	Diestrus	Control	1CIDR
	-------------------- % -------------------			------------ % ------------	
New CL on d -3	71.6[a]	68.7[a]	43.2[b]	51.2	56.6
CL on d -3	71.6[b]	68.7[b]	88.0[a]	86.4	87.6
Ovulation to 2nd GnRH	86.7	86.6	94.2	91.2	89.2
Ovulatory follicle, mm	18.1 ± 0.4[A]	17.0 ± 0.4[B]	18.1 ± 0.4[A]	17.8 ± 0.4	17.5 ± 0.4
Bred before timed AI					
First AI postpartum	17.7[a]	10.9[b,B]	17.3[A]	6.6	6.2
Subsequent AI	17.5	19.0	17.1	12.3	9.8
Pregnant d 32 after AI	32.7[B]	38.4[A]	35.9	45.7[a]	38.3[b]
Pregnant d 60 after AI	28.3[B]	33.3[A]	31.4	37.4	33.4
Pregnancy loss	9.8	11.5	6.8	12.6[a]	4.0[b]

[a,b] LS means differed ($P \leq 0.05$); [A,B] LS means tended to differ ($0.05 < P \leq 0.10$).

Regulation of WNT signaling by the antagonist Dickkopf-1 directs lineage commitment and promotes survival of the preimplantation embryo

Anna C. Denicol[*], Jeremy Block[*†], Dale E. Kelley*, Ky G. Pohler[‡], Kyle B. Dobbs[*], Christopher J. Mortensen[*], M. Sofia Ortega[*], Peter J. Hansen[*]

[*]*Department of Animal Sciences, D.H. Barron Reproductive and Perinatal Biology Research Program, and Genetics Institute, University of Florida, Gainesville, Florida, USA 32611-0910;* [†] *Ovatech LLC, Gainesville FL 32608;* [‡]*Division of Animal Sciences, University of Missouri, Columbia, MO, USA 65211-5300.*

Successful embryonic development is dependent upon maternally-derived factors secreted by the endometrium. Dickkopf-1 (DKK1), an antagonist of the WNT signaling pathway, is one endometrial secretory protein potentially involved in maternal-embryo communication. Using in vitro-produced bovine embryos, we demonstrate that exposure of embryos to DKK1 during the period of morula-to-blastocyst transition (between Days 5 and 8 of development) promotes the first two cell-fate decisions leading to increased differentiation of cells towards the trophectoderm and hypoblast lineages. Moreover, treatment of embryos with DKK1 or colony-stimulating factor 2 (CSF2, an endometrial cytokine known to improve embryo development and pregnancy establishment) between Days 5 and 7 of development improves embryo survival following transfer to recipients. Pregnancy success at Day 32 of gestation was 27% (19/71) for cows receiving control embryos, 41% (35/95) for cows receiving embryos treated with DKK1 and 39% (37/94) for cows receiving embryos treated with CSF2 ($P < 0.05$). These novel findings regarding DKK1 modulation of embryonic development and survival represent the first evidence for a role for maternally-derived WNT regulators during this period, and could also lead to improvements in conditions for assisted reproductive technologies in mammalian species. Support: USDA NIFA Grant No. 2011-67015-30688.

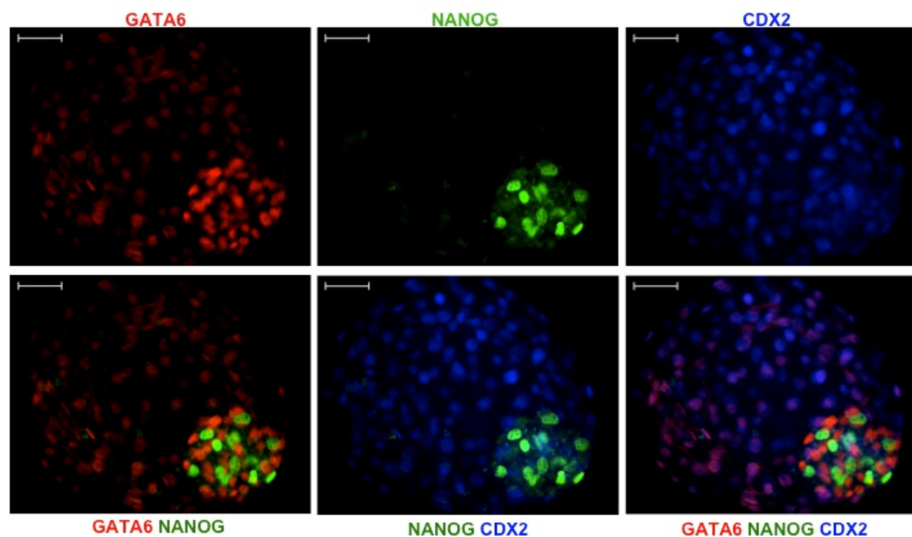

Figure 1. Immunolabeling of a day 8 blastocyst for the cell lineage markers GATA6 (hypoblast), NANOG (epiblast) and CDX2 (trophectoderm). Scale bar: 50 µm.

Possible involvement of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway in signaling by colony stimulating factor 2 (CSF2) in the bovine preimplantation embryo

Jasmine Kannampuzha Francis and Peter J. Hansen

Department of Animal Sciences, University of Florida

Pregnancy involves cross-talk between the preimplantation embryo and the reproductive tract of the mother. One molecule involved in this communication is the cytokine colony-stimulating factor 2 (CSF2) that is produced by the oviduct and endometrium. In the bovine embryo, CSF2 has anti-apoptotic actions, promotes cell numbers in the inner cell mass (ICM) of the blastocyst, decreases expression of the implantation inhibiting molecule, *MUC1*, and increases competence of the embryo to establish pregnancy after transfer to recipients. These actions occur despite the embryo expressing only one of the two subunits required for receptor mediated signaling. The CSF2 receptor (CSF2R) is comprised of two cytokine-specific α-subunits (CSF2RA) and two signal transducing β-subunits (CSF2RB). CSF2RA binds specifically to CSF2 with low affinity and recruits CSF2RB, which increases the affinity of the receptor for the ligand and is involved in signal transduction via the JAK-STAT signaling pathway. While the preimplantation embryo expresses CSF2RA, there is no detectable expression of CSF2RB in the mouse, human or cow. This result indicates that CSF2 affects cellular function in the embryo through a cell-signaling mechanism that is distinct from the CSF2RAB-JAK/STAT pathway. In two other cell types in which CSF2RB is absent, *Xenopus* oocytes and porcine trophectoderm, actions of CSF2 are mediated through the PI3K pathway. Therefore, we have hypothesized that actions of CSF2 on the bovine preimplantation embryo are mediated via the PI3K signaling pathway. If so, inhibition of the pathway with wortmannin will decrease effects of CSF2 on *MUC1* expression and inner cell mass (ICM) number. Embryos will be produced in vitro. On day 5 after fertilization, embryos will be treated with or without wortmannin (100 nM) and CSF2 (10 ng/ml) in a 2 x 2 factorial design. On day 7 post IVF, blastocyst number will be counted and blastocysts will be harvested after removing zona pellucida to measure *MUC1* mRNA via RT PCR (Experiment 1) or used to measure ICM cell numbers by immunohistochemical labeling of ICM (CDX2-) and trophectoderm (CDX2+) (Experiment 2). Another experiment, Experiment 3, will be performed to test whether CSF2 increases phosphorylation of the AKT enzyme downstream from PI3K (i.e., pAKT). At day 5 post fertilization, embryos will be treated with vehicle, CSF2 (10 ng/ml) or IGF1 (100 ng/ml; positive control) for 15 min. Embryos will be fixed in 4% (w/v) paraformaldehyde and labeled using immunofluorescence procedures with anti pAKT. Support: USDA NIFA Grant No. 2011-67015-30688.

Effects of prepartum evaporative cooling and vitamin E supplementation on adaptive and innate immunity of Holstein cows during summer in Florida

G.C. Gomes, J. Zuniga, E. Karakaya, L.F. Greco, L.D.P. Sinedino, N. Martinez, R.S. Bisinotto, E.S. Ribeiro, T.C. Bruinje, J. P. Driver, J.E.P. Santos, and C.R. Staples

Department of Animal Sciences, University of Florida, Gainesville

The objective of this study was to evaluate the effect of vitamin E (VitE) supplementation above the NRC (2001) recommendations and evaporative cooling on immune function of dairy cows under heat stress. Holstein cows (36 nulliparous and 34 parous) were blocked at 30 days before expected date of parturition by parity, milk yield, and body weight. Within each block, cows were randomly assigned to 1 of 4 treatments arranged in a 2 x 2 factorial. Cows were housed until calving in either a free-stall barn equipped with fans and sprinklers (Cooling - **C**) or in an open lot provided only with shade (No cooling - **NC**). After parturition, all cows were housed in a free-stall barn equipped with fans and sprinklers. All-rac-alpha-tocopherol (DSM, Belvidere, NJ) was top dressed daily onto the diet in individual feed bunks at 1,000 IU prepartum and 500 IU postpartum for moderate VitE (**M**) or 3,000 IU prepartum and 2,000 IU postpartum for high VitE (**H**) treatments. Blood was sampled on days -30, and -14 relative to the expected date of parturition. After calving, blood was sampled on days 3, 7, 14, 21, 28, 35 and 42. Analyses included phagocytosis and oxidative burst by neutrophils, percentage of lymphocytes positive for interleukin (IL) 10 and interferon (IFN) γ production after phorbol myristate acetate/ionomycin stimulation, percentage of total T lymphocytes, and the T lymphocyte subtypes CD4(+), CD8(+), and γδ(+), concentrations of serum IgG against ovalbumin challenge on days -30, -14, and 3, haptoglobin and acid soluble proteins. Data were analyzed by ANOVA for repeated measures with the PROC GLIMMIX of SAS, and data with residuals non-normally distributed were appropriately transformed. Results from cytokine production and neutrophil activity analyses are presented as fold increase in the percentage of positive cells or mean fluorescence intensity (MFI) fold increase relative to unstimulated control cells. Feeding **H** amounts of VitE increased ($P < 0.05$) the percentage of T lymphocytes producing IFN-γ (10.0 vs. 5.4). Prepartum cooling tended ($P < 0.10$) to increase the percentage of T lymphocytes (31.0 vs. 23.4%) relative to total lymphocytes number, CD4(+) subtype (12.4 vs. 7.4%), and serum IgG against ovalbumin (0.552 vs. 0.480, optic density). Providing **H** VitE to **NC** cows tended ($P < 0.10$) to increase phagocytic activity (MFI) by neutrophils (4.83 vs. 4.25), whereas the reverse occurred when cows were cooled prepartum (4.52 vs. 5.20). Oxidative burst activity (MFI) of neutrophils from multiparous cows was enhanced when cows were cooled (8.72 vs. 6.59) whereas the opposite occurred for primiparous cows (4.84 vs. 5.84). No effects of treatments were observed for serum concentrations of haptoglobin, acid soluble proteins, or percentage of lymphocytes positive to IL-10 production. VitE supplementation and prepartum evaporative cooling caused changes in adaptive and innate immune response patterns in dairy cattle in vivo and in vitro. Additionally, prepartum cooling provided a conditional improvement of innate immunity that was dependent on the amount of VitE supplemented and parity, which might reflect differences in metabolic and oxidative status of those animals.

Effect of altering the dietary ratio of n-6 to n-3 fatty acids on lactational performance, timing of luteolysis, uterine production of prostaglandins, and endometrial fatty acid profile and gene expression in dairy cows

L.F. Greco, J.T. Neves Neto, A. Pedrico, F.S. Lima, R.S. Bisinotto, N. Martinez, E.S. Ribeiro, W.W. Thatcher, C.R. Staples, and J.E.P. Santos

Department of Animal Sciences, University of Florida, Gainesville

Objectives were to evaluate the impacts of altering the ratio of dietary n-6 to n-3 fatty acids (FA) on lactational performance, timing of luteolysis, uterine production of prostaglandin $F_{2\alpha}$, and endometrial fatty acid profile and gene expression in dairy cows. Diets were supplemented (1.43 % DM) with a mixture of Ca salts of fish oil, safflower oil and palm oil to create different ratios of n-6 to n-3 FA; 4, 5 and 6 parts of n-6 to 1 of n-3 FA (R4; R5; R6). Cows were blocked by milk production from 6-10 DIM and then randomly assigned to one of the three dietary treatments at 15 DIM. Dry matter intake, milk yield and milk components were measured daily for 90 d. Cows had the estrous cycle synchronized starting at 40 DIM. An indwelling catheter was inserted in the tail vessel on d 15 of the estrous cycle and blood was sampled every 2 hours from estrous cycle d 16 to 23. Progesterone and 13,14,dihydro-15-keto-$PGF_{2\alpha}$ metabolite (PGFM) were measured in plasma. Cows had the estrous cycle resynchronized and endometrial tissue was collected for biopsy on d 8 of the cycle. Gene expression and FA profile were measured. Data were analyzed using MIXED procedure of SAS. Increasing the ratio of n-6 to n-3 FA from R4 to R6 resulted in a linear decrease in dry matter intake (R4 = 26.1, R5 = 24.6, and R6 = 24.7 kg/d; $P = 0.05$), with concurrent decreases in yields of 3.5% fat corrected milk (R4 = 48.0, R5 = 45.4, and R6 = 43.4 kg/d; $P < 0.01$), milk fat (R4 = 1.71, R5 = 1.60, and R6 = 1.53 kg/d; ; $P < 0.01$), milk true protein (R4 = 1.32, R5 = 1.28, and R6 = 1.24 kg/d; $P = 0.01$), and milk lactose (R4 = 2.29, R5 = 2.19, and R6 = 2.12 kg/d; $P < 0.01$). Treatment did not influence the length of the estrous cycle or concentrations of progesterone in plasma. Basal PGFM concentrations did not differ ($P = 0.66$) among treatments. The number of PGFM pulses decreased ($P = 0.05$) as the ratio n-6 to n-3 FA increase, and they averaged 5.6, 4.3, and 3.8 pulses, for cows fed R4, R5 and R6, respectively. The area under the curve of the largest PGFM pulse increased ($P = 0.02$) as the ratio n-6 to n-3 increased and was 764, 958, and 1953 pg/h per mL, for cows fed R4, R5 and R6, respectively. The concentrations of arachidonic acid increased (R4 = 8.09, R5 = 10.35, and R6 = 11.04 % of the identified FA) and of eicosapentaenoic acid decreased linearly (R4 = 2.29, R5 = 1.90, and R6 = 1.83 % of the identified FA) in the endometrium by altering the ratio of n-6 to n-3 from R4 to R6 ($P = 0.01$, $P = 0.03$, respectively). Of the genes evaluated, the expressions of estrogen receptor-α and steroidogenic acute regulatory protein increased linearly ($P < 0.01$) as the diet changed from R4 to R6. Altering the dietary ratio of n-6 to n-3 FA of lactating dairy cows influenced production, altered the pattern of prostaglandin synthesis, the FA profile, and gene expression of the endometrium but did not influenced the length of the estrous cycle.

Examination of age-related change in mitochondrial function in the horse

Chengcheng Li, Sarah H. White, Lori K. Warren and Stephanie Wohlgemuth

Department of Animal Sciences, University of Florida

As the population of old horse grows, their owners strive to use their older horses for athletic and/or recreational activities. Many horses are still actively working even at ages greater than 20 years old. Research involving rodents and human shows an age-related decrease in muscle metabolism, muscle mass and strength; and the effectiveness of appropriate exercise training to improve muscle function. Yet, few similar studies have been done in the horse. The overall goal of this project is to examine age-related changes in muscle energy metabolism, especially mitochondrial function, in equine skeletal muscle. Our specific objective is to evaluate mitochondrial changes in old compared to young horses. As a first step, we assessed mitochondrial function in skeletal muscle from young horses. Gluteal muscle samples were taken from young horses (n=24; 1.77± 0.07 yr) that were housed at the UF Equine Teaching Unit. High-resolution respirometry (HRR) on permeabilized muscle fibers was performed on the same day the biopsy was taken to measure mitochondrial oxygen consumption. Activity and content of mitochondrial enzymes such as Citrate Synthase and Cytochrome *c* Oxidase will be determined in homogenates of the skeletal muscle samples using enzymatic assays and immunoblotting, respectively. The next step in this project will be to sample skeletal muscle from older horses, and to compare mitochondrial function to the young cohort using the same measures as outlined above.

Our long term goal is to evaluate differences in skeletal muscle mitochondrial function between young and old horses. Once we will have collected baseline data of equine skeletal muscle aging, we aim to identify if and how exercise training and dietary interventions that target mitochondrial biology (such as resveratrol) affect mitochondrial function in older horses.

Evaluation of the effects of vitamin D and toll-like receptor signaling pathways on expression of antibacterial β-defensin genes in bovine monocytes, neutrophils, and mammary epithelial cells

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Toll-like receptor (TLR) signaling stimulates conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) in macrophages during mastitis. RNA sequence analysis of 1,25$(OH)_2D_3$-treated monocytes identified bovine β-defensin 3 (DEFB3) and β-defensin 6 (DEFB6) as potential targets of the activated vitamin D receptor. The genes DEFB3 and DEFB6 are located on bovine chromosome 27 along with ten other β-defensin genes. The β-defensin genes encode for small cationic peptides that have potent bactericidal and immunomodulatory activity. Neutrophils and mammary epithelial cells (MEC) are additional sources of β-defensin peptides in the udder. It was hypothesized that 1,25$(OH)_2D_3$ and TLR agonists would promote expression of the β-defensins in monocytes, neutrophils, and MEC. Therefore, the objective of this study was to determine the contribution of vitamin D and TLR signaling pathways to expression of β-defensin genes located on chromosome 27 in bovine monocytes, neutrophils, and MEC. Peripheral blood monocytes and neutrophils from cattle and primary bovine MEC cultures were treated with 0 or 1 μg/mL lipopolysaccharide (LPS) in combination with 0 or 10 nM 1,25$(OH)_2D_3$. The mRNA transcripts of *DEFB3, DEFB4, DEFB6, DEFB7,* and *DEFB10* genes were quantified by real-time PCR. The threshold cycle (Ct) for each gene was normalized to ribosomal protein S9 transcript abundance and the normalized Ct values for each treatment were analyzed with a general linear model to test for effects of 1,25$(OH)_2D_3$ and LPS treatments. In the non-stimulated monocytes, the 1,25$(OH)_2D_3$ treatment increased DEFB3, DEFB6, DEFB7, and DEFB10 gene expression (P<0.05; 10±3, 17±8, 4±1, and 5±2 fold change ± SE, respectively). Similarly, the 1,25$(OH)_2D_3$ treatment increased DEFB3, DEFB6, DEFB7, and DEFB10 gene expression in the LPS-stimulated monocytes (P<0.05; 10±3, 60±28, 7±2, and 20±6 fold change ± SE, respectively). DEFB1, DEFB4, DEFB5, DEFB13, enteric β-defensin (EBD), and lingual antimicrobial peptide (LAP) were not affected by 1,25$(OH)_2D_3$ in either resting or LPS-stimulated monocytes. LPS alone did not significantly affect expression of the DEFB3, DEFB6, DEFB7, and DEFB10 genes in this study (P>0.05). In contrast to macrophages, 1,25$(OH)_2D_3$ did not upregulate expression of the *DEFB3, DEFB6, DEFB7,* or *DEFB10* genes in either non-stimulated or LPS-stimulated neutrophils or MEC (P>0.05). However, the 1,25$(OH)_2D_3$ treatment increased *DEFB4* gene expression 5±1-fold (P<0.05) in MEC not treated with LPS, and 3±1-fold (P<0.05) in MEC treated with LPS. Furthermore, LPS combined with 1,25$(OH)_2D_3$ upregulated *DEFB4* 30-fold (P<0.05) compared to MEC cultured in the absence of LPS and 1,25$(OH)_2D_3$. The LPS treatment alone upregulated *DEFB3, DEFB4*, and *DEFB7* in MEC (P<0.05; 3±1, 11±3, and 8±3 fold change ± SE, respectively), and *DEFB3, DEFB4, DEFB6, DEFB7*, and *DEFB10* in neutrophils (P<0.05; 121±44, 10±7, 144±85, 112±51, and 56±22 fold change ± SE, respectively). In conclusion, 1,25$(OH)_2D_3$ triggers a strong increase of DEFB3, DEFB6, DEFB7, and DEFB10 gene expression in bovine monocytes. However, in bovine MEC and neutrophils, 1,25$(OH)_2D_3$ does not enhance β-defensin gene expression as it does in bovine monocytes but LPS strongly enhances several of the β-defensins in MEC and neutrophils. Therefore, activation of the TLR pathway in neutrophils and MEC, combined with activation of the vitamin D pathway in macrophages, may serve to boost the innate defense system of the udder during mastitis infections.

Effect of exercise on embryonic epigenetic patterns and uterine environment in mares

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Intense exercise has been shown to have a deleterious effect on reproductive activity in humans, rodents and equines. Our lab has demonstrated that exercised mares have abnormal ovarian follicle development and estrous cycle length, reduced embryo recovery and embryo quality. While maternal exercise during the reproductive cycle in mares has been shown to negatively affect their reproductive ability, less is known on any potential negative effects on the offspring. We hypothesize that maternal exercise during the reproductive cycle will lead to an altered uterine environment and directly influence the epigenome in early developing embryos.

The first aim of this study will be to investigate the effects of maternal exercise on equine reproduction using DNA methylation as an indicator of possible altered gene expression in recovered Day 7 embryos. Using high resolution meting analysis (MS-HRM) techniques will help detect nucleotide changes based on the temperature at which dsDNA sequence denatures. Additionally we will evaluate the expression of the de novo methylation enzymes (DNMT3 A and B). Labeling embryos with immuno-fluorescent 5-Methylcytosine, and quantify these values using image-assisted software, will allow us to compare groups and identify further differences between genders, and embryo mass cell type. Our second aim will be to evaluate changes in the activity of antioxidant enzymes and oxidation byproducts on the maternal uterus after exercise. Uterine biopsies would clarify the oxidative response to exercise *in vivo* in the mare's uterine environment.

Finally, our third aim will be to evaluate potential negative changes of the exercised uterine environment on stallion gametes. For the sperm experiment, we aim to simulate temperature and oxidative environment in vitro and analyze consequences on sperm parameters such DNA damage or lipid peroxidation.

In addition to vastly expanding our knowledge of the epigenetic patterns of early embryo development on horses, this research project will benefit of the potential use of this specie to elucidate the effects of exercise during the peri-fertile period and the resulting embryos.

Possible role of CCL24 in differentiation of the inner cell mass of the bovine embryo

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Chemokine (C-C motif) ligand 24 (CCL24) is a member of the CC family of cytokines involved in eosinophil migration, angiogenesis and proliferation. At Day 8 of development, *CCL24* is preferentially expressed in the inner cell mass (ICM) as compared to the trophectoderm (TE) (Ozawa et al., BMC Dev Biol 12:33). A role for CCL24 in the blastocyst has not been identified. However, blastocyst differentiation is likely to involve cell migration associated with the process whereby ICM cells give rise to epiblast cells, located in the inner part of the ICM, and hypoblast cells, on the edge of the ICM adjacent to the blastocoele. We hypothesized that CCL24 plays a role in formation and migration of hypoblast and epiblast at the blastocyst stage of development. The first objective was to evaluate whether *CCL24* expression is temporally related to events in the blastocyst associated with hypoblast and epiblast formation. Steady-stage amounts of mRNA for *CCL24* were evaluated for pools of 10-40 embryos collected at the oocyte, 2 cell, 3-4 cell, 5-8-cell, 9-16 cell, morula, and blastocyst (collected separately on Days 7, 8, and 9) stages using quantitative real-time PCR with *YWHAZ*, *SDHA*, and *GAPDH* as internal control genes. Amounts of *CCL24* mRNA were non-detectable for all stages before Day 7. Thereafter, *CCL24* mRNA was detectable for 4 of 4 pools of Day 7 blastocysts, 2 of 4 pools of Day 8 blastocysts and 0 of 4 pools of Day 9 blastocysts. In a second experiment, expression was determined for morulae at Day 5 and 6 and blastocysts at Day 6 and 7. Amounts of mRNA were non-detectable for morulae and present in all blastocyst samples, with 2.1-fold greater expression at Day 7 than Day 6 ($P=0.09$). Thus, *CCL24* expression peaks at a time coincident with formation of the blastocyst. The second objective was to determine whether inhibition of the CCR3 receptor (receptor for CCL24) alters the pattern of blastocyst formation. Embryos were treated with a CCR3 antagonist called SB328427 beginning at Day 6. The number and location of cells positive for NANOG (epiblast) and GATA6 (hypoblast) were determined in blastocysts at Day 8 and Day 9. Preliminary results indicate no effect on numbers of total ICM cells or numbers of NANOG+ or GATA6+ cells in the ICM. There was also no effect of inhibitor on location of GATA6+ cells in the ICM. However, the percentage of NANOG+ cells located towards the inside of the ICM was reduced ($p<0.05$) by inhibitor at Day 8 ($62.2 \pm 5.2\%$ vs $42.2 \pm 6.3\%$) but not at Day 9 ($56.5 \pm 4.8\%$ vs $60.7 \pm 4.6\%$). Future experiments are planned to further evaluate effects of SB328427 on ICM differentiation as well as the consequences of inhibition of the MAPK pathway activated by CCR3. Support: USDA NIFA Grant No. 2011-67015-30688.

Single nucleotide polymorphisms in candidate genes related to daughter pregnancy rate in Holstein cows

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Daughter pregnancy rate (DPR) is a trait widely used in the United States and elsewhere to estimate genetic merit for reproductive ability. Heritability of this trait is very low (0.04%) so that progress in selection using traditional breeding programs is slow. Moreover, genome wide association analysis has been less effective at increasing reliability of genetic estimates of DPR than for more heritable traits. Recently, SNPs in 40 genes were identified to be related to DPR in dairy bulls. We evaluated 39 of these SNPs for relationship to DPR in a separate population of Holstein cows grouped on their predicted transmitting ability for DPR: ≤ -1 (n=1266) and ≥ 1.5 (n=1071). A total of 11 farms were included in the study. Cows were genotyped by Sequenom MassARRAY®. Allele frequency was determined and linear and dominance effects of each SNP were estimated for the deregressed values of DPR. Additionally, a multiple regression analysis was performed using the 39 SNPs as explanatory variables for DPR. There were significant relationships with DPR for 23 SNPs, with 19 having a linear effect (*COQ9, HSD17B12, APBB1, FUT1, C7H19or60, DSC2, ACAT2, MS4A8B, CAST, BSP3, PCCB, OCLN, RABEP2, CACNA1D, HSD17B7, MON1B, TDRKH* and *GPLD1*), 2 having a dominance effect (*LBD3* and *TSHB*) and 2 (*C7H19or60* and *DSC2*) having linear and dominance effects. SNPs that explained the greatest proportion of variation in DPR were *COQ9* (3.0%), *HSD17B12* (1.6%), *APBB1* (1.3%), *FUT1* (1.2%), and *C7H19orf60* (1.2%). Using multiple regression analysis, the 39 SNPs explained 17.6% of the variation in DPR. Our results indicate that a large proportion of candidate gene SNPs previously related to DPR are predictive in a separate population. Further research will be conducted to determine effectiveness of incorporation of genotype information from these candidate gene SNPs into genotyping arrays for improvement of genomic estimates of DPR. Support: AFRI Grant No. 2013-68004-20365 from USDA-NIFA.

Vitamin D signaling in the porcine monocyte

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An intracrine vitamin D signaling pathway activates innate bactericidal defenses in human and bovine monocytes. In contrast to the classical endocrine vitamin D pathway, toll-like receptor signaling stimulates conversion of 25-hydroxyvitmin D_3 to1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) by the enzyme 1α-hydroxylase (CYP27B1). The $1,25(OH)_2D_3$ binds to the vitamin D receptor and triggers the expression of several antimicrobial genes. The contribution of vitamin D signaling to the function of pig monocytes has not been studied yet, but is critical because vitamin D deficiency is common in young pigs. It is hypothesized that toll-like receptor signaling induces the expression of CYP27B1 in pig monocytes, and that synthesis of $1,25(OH)_2D_3$ by CYP27B1 enhances antimicrobial defenses of the monocytes. Peripheral blood monocytes were isolated from pigs and treated with 0 or 1 µg/ml lipopolysaccharide (LPS) in combination with 0 or 10 nM $1,25(OH)_2D_3$ and 0 or 250 nM $25(OH)D_3$. The mRNA transcripts of *CYP27B1, CYP24A1, CD14*, *PR39, β-Defensin 1* (*DEFB1*), and *DEFB2* were quantified by real-time PCR. Stimulation with LPS induced *CYP27B1* expression 10-fold compared to the non-stimulated monocytes ($P<0.05$). The $1,25(OH)_2D_3$ and $25(OH)D_3$ treatments increased *CYP24A1* gene expression, a standard marker of vitamin D receptor activity, in both non-stimulated and LPS-stimulated monocytes. *DEFB2* expression was not affected by any of the treatments and *PR39* and *DEFB1* transcripts were not detected. *CD14* was enhanced by LPS treatment ($P<0.05$), but $25(OH)D_3$ and $1,25(OH)_2D_3$ did not have a significant effect on *CD14* ($P>0.05$). In conclusion, TLR signaling stimulates the vitamin D pathway in pig monocytes, but none of the antimicrobial genes evaluated so far are affected by the vitamin D pathway. Still, the vitamin D receptor is functional in pig monocytes, so RNA sequencing experiments will be performed in the future to identify genes that are affected by $1,25(OH)_2D_3$ in pig monocytes. Additional experiments will also be performed to determine if the vitamin D pathway is activated *in vivo* at sites of infection.

Plasma Anti-Müllerian Hormone in Adult Dairy Cows and Associations with Fertility

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Objectives were to identify factors associated with concentrations of AMH in plasma of dairy cows and to investigate the relationships between plasma AMH and fertility responses during a 100-d breeding season. Lactating cows, 1,237, in two seasonally calving herds had estrous cycles presynchronized and were enrolled in a timed artificial insemination (AI) protocol. All cows were inseminated on the first day of breeding season, considered study d 0. Blood was sampled on d -8 and analyzed for concentrations of AMH and progesterone. From d 19 to 35, detection of estrus was performed daily and cows detected in estrus were reinseminated. On d 36, bulls were placed with cows for 65 d of natural service breeding. Factors identified to be associated with concentrations of AMH in plasma were breed of the cow, lactation number, and body condition score (BCS) change from d 0 to d 30. Concentrations of AMH were greater for Jerseys, followed by crossbreds and then Holsteins. Cows on lactations 2 and 3 had greater concentrations of AMH than those on lactations 1 and ≥ 4. Cows that gained BCS had less AMH in plasma than those that lost or maintained BCS. The proportion of cows with low progesterone (< 1 ng/mL) at the beginning of the timed AI protocol was greater for cows classified as having high AMH than for those classified as having low and intermediate AMH. Although pregnancy per AI (P/AI) at the timed AI was not associated with concentrations of AMH, cows with low AMH had greater detection of estrus at timed AI, and the latter benefited P/AI, particularly in cows that had low progesterone at the beginning of the synchronization protocol. Pregnancy loss between gestation d 30 and 65 increased in cows with low AMH. Return to estrus in cows that failed to become pregnant from the timed AI was not associated with AMH, but pregnancy rate in cows bred on estrus (reinsemination + natural service) was associated positively with AMH. In conclusion, breed, age, and BCS were identified to be associated with concentrations of AMH in plasma. Cows with high concentration of AMH were more likely to be anovular and had less detection of estrus during the timed AI protocol. Nonetheless, concentration of AMH was associated positively with pregnancy maintenance and pregnancy rate after the first postpartum AI.

Consequences for fetal development of exposure of bovine embryos to colony stimulating factor 2 during the preimplantation period

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In vitro production (IVP) of embryos can disrupt fetal and placental development, causing early pregnancy loss, increased fetal growth, abnormal placental function, altered gene expression, and perturbations in DNA methylation and expression of imprinted genes. Together, these phenomena are referred to as abnormal offspring syndrome. Colony stimulating factor 2 (CSF2) is a cytokine present in the endometrium and oviduct of a variety of species that can improve competence of the preimplantation embryo to establish pregnancy when transferred to recipient females. In one experiment with cattle, treatment of embryos with CSF2 from Day 5-7 of development also reduced pregnancy loss after initial pregnancy diagnosis at Day 30-35 of gestation. Taken together, these results suggest that CSF2 may prevent some of the abnormalities in fetal development associated with in vitro production. Therefore, an experiment is underway to test the hypothesis that treatment of embryos with 10 ng/ml CSF2 from Day 5-7 of development reduces abnormal fetal development by altering gene expression and cellular differentiation so as to make embryos produced *in vitro* similar to their *in vivo* counterparts. Holstein cows are randomly assigned to three treatments: artificial insemination (AI, in vivo control; n=10); IVP (n=10) or IVP-CSF2 (IVP where embryos are cultured with 10 ng/mL CSF2 from Day 5 to 7 of culture; n=10). Fetuses will be collected on day 88±2 of gestation. Fetal and placental growth will be assessed by external morphometric measurements. Gene expression will be determined in maternal placenta (caruncles and intercaruncular region), extraembryonic membranes (cotyledons and intercotyledonary region), and in tissues from the three different embryonic layers: ectoderm (cerebrum), mesoderm (heart and skeletal muscle) and endoderm (liver). Because the insulin-like growth factor (IGF) system is disrupted by in vitro conditions, among the genes to be studied will be *IGF1*, *IGF2* and their receptors (*IGF1R* and *IGF2R*) in fetal liver and skeletal muscle. Expression of solute carrier family 22 genes (*SLC22A2* and *SLC22A3*) will also be examined because disruption of these genes has been associated with cardiac and renal dysfunctions, which is a commonly observed characteristic of abnormal offspring syndrome in cattle. Support: USDA NIFA Grant No. 2011-67015-30688.

Expression of genes associated with WNT signaling in the preimplantation bovine embryo

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WNT signaling participates in early embryonic development to maintain pluripotency, control cell-cell communication, and modulate cell polarization and migration. To gain an understanding of the regulation of WNT signaling during preimplantation development, expression patterns of a variety of molecules involved in WNT signal transduction were evaluated. Specific genes were *DKK1,* an endogenous inhibitor of canonical WNT signaling, the WNT co-receptors *LRP5* and *LRP6,* WNT-responsive transcription factors, *LEF1*and *TCF7*, and two repressors of WNT-regulated genes, the bovine orthologue of *Groucho* (LOC505120) and *AES1*. Embryos were produced in vitro. Groups of 30 matured oocytes or embryos at the 2-cell [28–32 h post-insemination (hpi)], 3-4 cell (44–48 hpi), 5-8 cell (50–55 hpi), 9-16 cell (72–75 hpi), morula (120–123 hpi), and blastocyst (168–171 hpi) stages were collected. Zona pellucidae were removed with proteinase, RNA purified, cDNA synthesized and real-time qPCR performed. *YWHAZ* was used as an internal control. Transcript abundance was expressed as fold-change relative to the oocyte. A total of 5 replicates were analyzed for each developmental stage. Results show variation in number of transcripts at different developmental stages ($p<0.05$). *DKK1* expression was high from the oocyte to 9-16 cell stage and then declined thereafter (fold-change for 9-16 cell stage, morula and blastocyst was 2.1, 0.4 and 0.02, respectively). Expression of *LRP6* also declined as development advanced beginning at the 5-8 cell stage (fold-change for 5-8 cell, 9-16 cell, morula and blastocyst was 0.63, 0.55, 0.11 and 0.06, respectively). In contrast, *LRP5* levels were maintained from the oocyte to the morula stage and increased at blastocyst stage (fold-change for morula and blastocyst were 1.6 and 4.1). Expression of *AES1* generally declined as development advanced and then increased slightly at the blastocyst stage (fold-change for 2-cell, 9-16 cell, morula and blastocyst was 0.36, 0.06, 0.04 and 0.16). Expression of *TCF7, Groucho* and *LEF1* followed a similar pattern except there was no increase at the blastocyst stage. Results suggest that canonical WNT signaling is reduced at the morula and blastocyst stages. Support: USDA-NIFA 2011-67015-30688.

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