TENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Holiday Isle Oceanfront Resort
St. Augustine, FL
March 30-31, 2012

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 Holiday Isle Oceanfront Resort in St. Augustine, FL.

We trust that this 10th Annual Retreat will be marked by good science, exceptional fellowship and lasting memories.

Alan Ealy, Director
David Julian, Co-Director

ACKNOWLEDGMENTS

The faculty and student of the AMCB Program thank the following for support of the 10th Annual Research Symposium

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

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

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

Drs. Joel Brendemuhl and Gbola Adesogan, Graduate Coordinators, Department of Animal Sciences, University of Florida

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

Special thanks to those who helped to organize this year's symposium

Ms. Rebecca Matta, Program Assistant, Department of Animal Sciences, University of Florida

Mr. Kyle Dobbs, AMCB PhD Student and Chair, Program Committee for 2012 AMCB Retreat

Mr. Leandro Greco, AMCB PhD Student and Chair, Social Activities Committee for 2012 AMCB Retreat

Special thanks to Dr. Peter Hansen for maintaining the AMCB website and Drs. Peter Hansen and Jim Moss for preparing the Friday night meal.

2012 AMCB DISTINGUISHED LECTURER

Rocio M. Rivera, PhD
Division of Animal Sciences
University of Missouri, Columbia

Dr. Rocio Rivera was born in San Juan, Puerto Rico. She began her undergraduate studies at the University of Puerto Rico, Mayaguez Campus then transferred to Iowa State University after two years. She graduated from Iowa State University in 1993 with BS degrees in Animal Science and Dairy Science. Dr. Rivera worked with Dr. Steve Ford during her undergraduate program, and she stayed at Iowa State to complete a Masters of Science with Dr. Ford, which she received in 1995. The title of her MS thesis was *A Comparison of Preimplantation Development of Embryos from Chinese Meishan and Yorkshire Pig Breeds*. Dr. Rivera then moved to Gainesville, FL and began working for Dr. Pete Hansen as a laboratory technician. In 1998 she began a PhD program with Dr. Hansen. In 2003 she completed her PhD in the AMCB Program. The title of her dissertation was *Cellular, Subcellular and Developmental Responses of Two-cell Bovine Embryos to a Physiologically Relevant Heat Shock*. Thereafter Dr. Rivera completed a Postdoctoral Fellowship with Dr. Richard Schultz at the University of Pennsylvania. In 2007 She accepted a tenure-track faculty position in the Division of Animal Sciences at the University of Missouri.

Dr. Rivera's research focuses on mammalian embryology and specifically on developmental imprinting. She currently has NIH funding to establish a phenotypic model of adverse outcomes associated with assisted reproductive technologies (ART). Her group also studies 1) epigenetic effects of ovarian hyperstimulation in mouse oocytes, 2) the effects of assisted reproductive technologies and/or fat-based hypercaloric diet on cardiovascular performance in mice, 3) the effects of aging on oocyte competence and epigenetics, and 4) determinations of the cellular and molecular mechanisms involved in ART-induced loss-of-imprinting in mouse embryos.

Dr. Rivera also is the first AMCB Alum to return as the AMCB Distinguished Lecturer.

2012 AMCB GUEST LECTURER

Dr. Tara Sabo-Attwood
Department of Environmental and Global Health
Center for Environmental and Human Toxicology
University of Florida, Gainesville, Florida.

Dr. Sabo-Attwood is an Associate Professor of Toxicology in the University of Florida's College of Public Health and Health Professions and Center for Environmental and Human Toxicology. She has a joint appointment in the University of Florida's College of Veterinary Medicine. She received her BS from the University of Connecticut in Genetics and Ph.D. from the University of Florida in Biomedical Sciences and Environmental Toxicology. Her doctoral training was in the area of aquatic toxicology and she moved into environmental pulmonary pathology as an NIEHS postdoctoral fellow at the University of Vermont. Dr. Sabo-Attwood directs a laboratory group that investigates molecular mechanisms that drive various health impacts associated with environmental exposures to agents such as mineral fibers, hormonally active agents and nanomaterials. Dr. Sabo-Attwood is currently a member of SOT, FASEB and SETAC and sits on the Editorial Board of Frontiers in Toxicogenomics. She has also chaired international nanotoxicology-related conferences and -workshops and participated in the Kavli Frontiers of Science Symposium endorsed by the National Academy of Sciences.

COMMITTEES FOR 10TH ANNUAL AMCB RETREAT

Student Program Committee
Kyle Dobbs, Chair
Anna Denicol
Ashley Grapes
Dale Kelley
Eduardo Ribeiro
Sha Tao

Student Social Activities Committee
Leandro Greco, Chair
Firdous Khan
Guilherme Marquezini
Paula Morelli-Mercadante
Maria Belen Rabaglino
Christina Vasquez

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 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

AMCB FACULTY

Jeffrey R. Abbott, Department of Infectious Diseases and Pathology

Lokenga Badinga, Department of Animal Sciences

Mary B. Brown, Department of Infectious Diseases and Pathology

Geoffrey E. Dahl, Department of Animal Sciences

Nancy Denslow, Department of Physiological Sciences

Alan D. Ealy, Department of Animal Sciences

Daniel A. Hahn, Department of Entomology and Nematology

Peter J. Hansen, Department of Animal Sciences

Kwang Cheol Jeong, Department of Animal Sciences

Sally E. Johnson, Department of Animal Sciences

David Julian, Department of Biology

Maureen Keller-Wood, Department of Pharmacodynamics

Christopher Mortensen, Department of Animal Sciences

Jose E.P. Santos, Department of Animal Sciences

Stephanie Wohlgemuth, Department of Animal Sciences

Charles E. Wood, Department of Physiology and Functional Genomics

Joel V. Yelich, Department of Animal Sciences

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

Anna Denicol (Advisor: PJ Hansen)

Kyle Dobbs (Advisor: PJ Hansen)

Sarah Fields (Advisor: PJ Hansen)

Ashley Grapes (Advisor: C Wood)

Leandro Greco (Advisor: JEP Santos)

Dale Kelley (Advisor: C Mortensen)

Firdous Khan (Advisor: PJ Hansen)

Maria Belen Rabaglino (Advisor: C Wood)

Eduardo Ribeiro (Advisor: JEP Santos)

Sha Tao (Advisor: GE Dahl)

Christina Vasquez (Advisor: D Julian)

MS Students

Paula Morelli-Mercadante (Advisor: AD Ealy)

SCHEDULE OF EVENTS

FRIDAY, MARCH 30

2:00 PM Alan Ealy
Welcome, introductory comments

Session 1: *The Environment* (Holiday Isle – Pelican North)
Kyle Dobbs, Chair

2:15 PM Dale Kelley, Animal Sciences
Pharmacokinetics of 1% oral L-arginine supplementation in mares

2:30 PM Leandro Greco, Animal Sciences
Effects of dietary fatty acids on production, reproduction, tissue composition, and hepatic gene expression in lactating dairy cows

2:45 PM Christina Vasquez, Biology
Synergistic effects of osmotic, thermal and hypoxia stress on embryo development in the horseshoe crab, Limulus polyphemus

3:00 PM Sha Tao, Animal Sciences
Effect of late gestation maternal heat stress on growth and immune function of dairy calves

3:15 PM UF Guest Lecturer
Tara Sabo-Attwood, PhD
Associate Professor, Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida

Indecent Exposure? Investigating the relationship between pathogens and emerging contaminants

4:00-6:30 PM Break, Room check-in, Free time

Session 2: Whitney Laboratory for Marine Bioscience

6:30-8:00 Poster Session

7:30-??? The Hansen/Moss BBQ

Posters:

Julia Baldrighi, Animal Sciences
The effect of dry period heat stress on oocyte development in the subsequent lactation of Holstein cows

Raquel Caserta, Horticultural Sciences
Obtention of Genetic Transgenic Model Plants - a possible tool in the study of plant pathogen interaction"

Eileen Chang, Physiology & Functional Genomics
Ketamine decreases plasma adrenocorticotropic hormone (ACTH) levels in late gestation fetuses exposed to global acute hypoxic hypoxia (HH)

Renan Di Giovanni Isola, Animal Sciences
The temporal and spatial expression of Pax7 during primary myogenesis in the bovine embryo

Miriam Garcia, Animal Sciences
Fatty acid profile and global gene expression in liver of calves supplemented with linoleic acid

Fabio Lima, Animal Sciences
Modification of the 5-d timed artificial insemination (AI) protocol to optimize fertility in dairy heifers

Paula Morelli-Mercadante, Animal Sciences
Influence of Bos indicus genetics on pregnancy-associated glycoproteins (PAGs) and their association with fetal development

Vitor Mercadante, Animal Sciences
Effects of anti-phospholipase A2 antibody (aPLA2) supplementation on DMI, feed efficiency and blood differentials of steers fed forage and grain-based diets

Manhwan Oh, Animal Sciences/Emerging Pathogens Institute
A whole genome DNA sequencing reveals genetic traits that affect survival and persistence of Escherichia coli O157:H7 in cattle

Ignacio Rodriguez-Jorquera, *Soil and Water Sciences*
What fish gene expression tells us about urban water pollution

Won-Sik Yeo, Animal Sciences/Emerging Pathogens Institute
A study of effector proteins secreted by enterohemorrhagic Escherichia coli O157:H7

SATURDAY, MARCH 31

Breakfast: Holiday Isle - Pelican South
7:30-8:30 AM Breakfast

Session 3: *Immunology and the Environment* (Pelican North)
Anna Denicol, Chair

9:00 AM Candace Lavelle, Center for Environmental & Human Toxicology
Uptake of quantum dots in fathead minnows, Pimephales promelas, ovarian explant cultures

9:15 AM Soojin Jeon, Animal Sciences/Emerging Pathogens Institute
Understanding factors that modulate prevalence of Escherichia coli O157:H7 in cattle

9:30 AM Georgia Hinkley, Center for Environmental & Human Toxicology
Oral bioavailability of 22nm gold nanoparticles in mice and the effect of PEG surface coating on gastrointestinal absorption

9:45 AM Reyna Colli-Dula, Center for Environmental & Human Toxicology
Effects in biological responses and changes in gene expression in the liver of female largemouth bass (Micropterus salmoides) by dietary exposures of EE2

10:00-10:30 AM Break – Refreshments available in Pelican South

Session 4: 2012 AMCB Distinguished Lecturer Presentation (Pelican North)
Dr. Peter J. Hansen, Chair

10:30 PM Rocio M. Rivera, PhD
Assistant Professor, Division of Animal Sciences, University of Missouri

ART and age alter the epigenetic program of oocytes, pre- and post-implantation embryos in mammals

11:30 AM-12:30 PM Lunch: Pelican South

<u>Session 5:</u> *Molecular Development* (Pelican North)
Sha Tao, Chair

1:00 PM	Kyle Dobbs, Animal Sciences *Effect of treatment of bovine blastocysts with colony stimulating factor 2 during the morula-blastocyst stages of development on growth and gene expression of trophectoderm outgrowths*
1:15 PM	Anna Denicol, Animal Sciences *Regulation of embryonic development to the blastocyst stage by canonical WNT signaling*
1:30 PM	Ashley Grapes, Physiology & Functional Genomics *The genomics of estrogen sulfoconjugation and cellular estrogen action in the late-gestation fetus*
1:45 PM	Maria Belen Rabaglino, Physiology & Functional Genomics *Microarray analysis of gene expression in the ovine fetal cerebral cortex ontogeny in late gestation suggests the development of tolerogenic myeloid-derived cells in the cortex*
2:00 PM	Firdous Khan, Animal Sciences *Are the effects of colony stimulating factor 2 on bovine preimplantation embryos mediated through the JAK-STAT pathway?*
2:15 PM	James Moss, Animal Sciences *Involvement of free cholesterol and high-density lipoprotein in development and resistance of the preimplantation bovine embryo to heat shock*
2:30 PM	Cody Smith, Center for Environment & Human Toxicology *Differential recruitment of estrogen receptor co-activators by xenoestrogens*
2:45-3:15 PM	Break: Refreshments available in Pelican South

<u>Session 6:</u> *Physiology and Fertility* (Pelican North)
Dale Kelley, Chair

3:15 Rafeal Bisinotto, Animal Sciences
Effects of follicular wave and progesterone concentration during follicle growth on conceptus global gene expression in dairy cows

3:30 Sarah Fields, Animal Sciences
Relationship between a bull's genetic merit for daughter pregnancy rate and in vitro fertilizing ability and embryonic development

3:45 Natalia Martinez, Animal Sciences
Associations among subclinical hypocalcemia, neutrophil function, and incidence of uterine disease in dairy cows of low or high risk of developing metritis

4:00 Eduardo Ribeiro, Animal Sciences
Conceptus development and global gene expression at preimplantation stages in lactating dairy cows of distinct genetic groups and estrous cyclic statuses

4:15 Closing remarks, Alan Ealy

<u>Late Afternoon and Evening Events:</u>

4:30-5:30 PM Faculty meeting: Location TBA
4:30-6:30 PM Free time for students, staff and postdocs

6:30-9:00 PM Dinner and Social: Poolside @ Holiday Isle

ABSTRACTS

(Arranged alphabetically by first author)

The effect of dry period heat stress on oocyte development in the subsequent lactation of Holstein cows

J.M. Baldrighi, K.B. Dobbs, I.M. Thompson, L. Bonilla, J. Block, P.J. Hansen, G.E. Dahl.
Department of Animal Science, University of Florida, Gainesville, Florida

Several studies indicate that the pregnancy rate of dairy cows decreases considerably in animals inseminated during the hot months of the year. There are seasonal patterns of estrus detection, day to first service, conception rate in dairy cows and lower conception rates are all observed during the summer months compared to the winter months. Negative effects of heat stress on fertility persist after summer. Cows that are no longer exposed to heat stress or cows that are dry and late pregnant during the summer, and therefore not cycling, have poor fertility in the subsequent autumn. We hypothesize that this is a carry-over effect of heat stress during the summer on the antral follicles that will develop into large dominant follicles 60 days later. Heat stress affects not only the antral follicles emerging in the follicular wave, but also the ovarian pool of small antral follicles. In-vitro and in-vivo studies support the idea that bovine oocytes are susceptible to thermal stress at different stages of follicular development. During folliculogenesis the oocyte acquires developmental competence, which is the ability to be fertilized and developed into the blastocyst stage. Alterations in the physiology of the follicle-enclosed oocyte during the lengthy period of follicular development could potentially lead to an oocyte with reduced competence for fertilization and subsequent development. The molecular changes that are involved during developmental competence are unknown. To determine if transcriptional alterations are present in the heat stressed oocyte itself, we are examining the mRNA levels of genes involved in oocyte growth and competence. Fourteen Holstein cows were exposed to heat stress or cooled (n=13) when dry, for around 46 days, during the summer of 2011. Cooled cows had shade, fans and soakers whereas the heat stressed cows had only shade. After calving all cows were assigned to the Ovsynch protocol for breeding. All cows were submitted to ovum pick-up (OPU) 3 days after the second GnRH injection, followed by another OPU section 3 days later. The range of days in milk (DIM) was from 42 to 100 days. The entire pool of oocytes among 2 to 8 mm was aspirated. Immediately after OPU, cumulus-oocyte complexes (COCs) were harvested and evaluated by their cumulus cell morphology and cytoplasmic color. The samples were stored at -80°C for subsequent Real Time RT-PCR analysis. Due to possible problems with RNA extraction, we first tested the PCR technique in bovine oocytes collected from slaughterhouse ovaries, by using the same freezing technique applied to the OPU samples. A total of 132 oocytes and their cumulus cells were frozen after OPU sections, resulting in a total recovery rate of 71.47% (223 COCs recovered/ 312 follicles). An end-point reverse transcriptase PCR (RT-PCR) was performed on slaughterhouse oocytes to test if single oocyte RNA extractions were possible or if combinations of oocytes were required to obtain the minimum necessary amount of RNA. After agarose gel visualization of the product, it was possible to verify the presence of GAPDH expression, a housekeeping gene, in a single oocyte and in pools of 2, 4 and 5 oocytes. However, using the method described, it was not possible to observe any gene expression in the cumulus cells of single oocytes or in those pools. Other experiments are underway to determine the best method for extracting and measuring gene expression in the samples from heat stressed and cooled dry cows.

Effects of follicular wave and progesterone concentration during follicle growth on conceptus global gene expression in dairy cows

R.S. Bisinotto*, E.S. Ribeiro*, L.F. Greco*, F.S. Lima*, N. Martinez*, R.L.A. Cerri¶, W.W. Thatcher*, J.E.P. Santos*
**Department of Animal Sciences, University of Florida, Gainesville, ¶University of British Columbia, Vancouver*

Previously, we reported that low concentrations of progesterone (**P4**) during development of the ovulatory follicle in cyclic and anovular cows depress fertility of lactating dairy cows[1], and that supplemental P4 reversed those effects[2]. Objectives of the current study were to evaluate the impact concentrations of P4 and wave of the ovulatory on subsequent conceptus gene expression. Non-lactating Holstein cows had their ovulation synchronized with the Ovsynch protocol (d -9 GnRH, d -2 and -1 $PGF_{2\alpha}$, d 0 GnRH, d 0 and 1 AI) starting during proestrus without supplemental P4 (**FW**, n=13), with supplemental P4 (**FWP4**, n=8), or starting on d 6 of the estrous cycle (**SW**, n=12). The rationale was to have cows ovulating at AI the dominant follicle of the first follicular wave without (FW) or with supplemental P4 (FWP4) or of the second follicular wave (SW) in which endogenous P4 is generally high. Cows in FWP4 received 3 controlled internal drug-release (**CIDR**) inserts containing P4 placed in the vagina on study d -9, -8 and -7. All inserts were removed on d-2. Cows were killed on study d 17 and uteri were flushed. Recovered concepti had mRNA extracted and global gene expression was evaluated using the Affymetrix GeneChip Bovine Genome array. A total of 20 concepti (FW = 6; FWP4 = 7; SW = 7) were selected for microarray. Data were analyzed using the MIXED procedure of JMP-Genomics/SAS. Orthogonal contrasts were performed to determine the effects of follicle wave (FW+FWP4 vs. SW) and concentration of P4 (FW vs. FWP4). Differentially expressed genes were selected if $P < 0.05$ and fold-difference > 1.5. Analyses identified 155 up-regulated and 478 down-regulated genes in response to the ovulation of a follicle from the first compared with a follicle from the second wave. Up-regulated genes are associated with 18 pathways from the Kyoto Encyclopedia of Genes and Genomes (**KEGG**). Down-regulated genes are involved with a greater variety of biological processes (50 pathways), including several of those encompassing the up-regulated transcripts. Supplementing P4 during growth of the first-wave ovulatory follicle induced up-regulation of 73 genes from 13 pathways, including MAPK (hsa04010) and Wnt signaling pathways (hsa04310), focal adhesion (hsa04510) and regulation of cytoskeleton (hsa04810). Interestingly, P4 induced up-regulation of TCF7 and EGFR in concepti, which have been associated with endometrial cell proliferation and cancer in humans; therefore, potential candidates to mediate the effects of P4 on maternal-conceptus communication. Both wave of the ovulatory follicle and concentration of P4 influenced conceptus gene expression, and these changes in gene expression might mediate the reduction in fertility of dairy cows when the ovulatory follicle originates from the first follicular wave or develops under low concentrations of P4.

[1] Bisinotto, R.S., R.C. Chebel, and J.E.P. Santos. 2010. J. Dairy Sci. 93:3578–3587.
[2] Bisinotto, R.S., F.S. Lima, E.S. Ribeiro, L.F. Greco, N. Martinez, W.W. Thatcher, and J.EP. Santos. 2012. Int. Congr. Anim. Reprod. Abstr.

Obtention of Genetic Transgenic Model Plants - a possible tool in the study of plant pathogen interaction

Caserta, R.[1, 2, 3]; Febres, Vicente.[3]; Tomas, J. P.[2]; Machado M. A.[2]; Moore, G.[3]; De Souza, A. A.[2]
1.Unicamp, Universidade Estadual de Campinas, Campinas, SP, Brazil,
2.Centro de Citricultura Sylvio Moreira/ IAC, Cordeirópolis, SP, Brazil
3.Horticultural Sciences Department, University of Florida, Gainesville, FL, USA

Model plants are widely used in plant pathogen interaction studies due to ease handling and cultivation. Genetic transformation of model plants opens possibilities for several studies that go beyond plant pathogen interaction. It can be an analysis tool for a specific gene function during this interaction. In many cases, the same defense gene can be triggered by the plant in response to different pathogens. An example is the *methyl salicylate* (*MeSA*), wich is a translocated and activator signal of the salicilic acid pathway, required for the defense against different pathogens. This gene was overexpressed in tangerins, that are resistant to the infection of *Xylella fastidiosa* (Xf), a xilem limited gram negative bacteria wich is the causal agent of Citrus Variegated Chlorosis (CVC) in Brazil. The quick triggering and expression of defense genes against a pathogen attack is the key for plant resistance. MeSA was chosed for its overexpression in *Nicotiana tabaccum*, a model plant that is host for Xf and other pathogens as *Candidatus liberibacter*, the causal agent of Greening. The gene was amplified from a cDNA library of *Citrus reticulata* previously infected with Xf, using specific primers. After purification of the target gene it was cloned between an FMV promoter and a *nos* terminator in the pUC118FMVPoly 2 plasmid. This expression cassette was designed for the overexpression of MeSA in the whole plant. The expression cassette was excised from the plasmid and cloned into the binary vector pCambia 2201, that contains the GUS reporter gene. All clonign steps were confirmed by PCR and sequencig. The strain Agl1 of *Agrobacterium tumefaciens* was transformed with the binary vector carring MeSA expression cassette and positive colonies were selected. The transformed *A. tumefaciens* was used to infect leaf disks of tobacco for genetic transformation. The disks were kept in selecion medium until shooting. Shoots were tested for the presence of the GUS gene and the positive were rooted. The test for the presence of the MeSA gene in the grown plants was done by PCR using as template DNA and cDNA of the transgenic plants. Positive plants were located in green house for flowering and colecting seeds to the next steps of the experiment. In these steps, transgenic plants overexpressing MeSA will be challenged with Xf and other pathogens of interest.

Ketamine decreases plasma adrenocorticotropic hormone (ACTH) levels in late gestation fetuses exposed to global acute hypoxic hypoxia (HH)

Eileen I. Chang and Charles E. Wood
Department of Physiology and Functional Genomics, University of Florida College of Medicine

Ketamine, a noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist, is a common pediatric anesthesia and analgesia for pre-term and neonates due to its pharmacological and physiological effects. Previously, we have shown that ketamine inhibits plasma ACTH levels in late gestation fetal sheep subjected to brachiocephalic occlusion (BCO), a potent stimulant of hypothalamic-pituitary-adrenal (HPA) axis activated by chemoreceptors responding to hypoperfusion. Fetal HPA axis is activated under stressful situations like HH, and treatment with ketamine could decrease chemoreceptor activation, consequently, reducing the HPA axis activity. We propose that treatment with ketamine will reduce fetal ACTH levels when exposed to acute HH. Fetal sheep were chronically catheterized at gestational day 125 (n=3-5/group), and tracheostomy was performed on the ewe. Ketamine (3 mg/kg) was administered intravenously to the fetus 10 min prior to maternal hypoxic scenario; fetal HH was induced by administering nitrogen gas directly to the ewe for 30 min. Maternal and fetal blood gases, and fetal heart rate and blood pressure were continuously monitored and recorded throughout the experiments. Plasma samples were collected for hormonal analysis. HH stimulation significantly increased both fetal ACTH and cortisol levels in both control and ketamine groups ($P<0.0001$, main effect of time in two-way ANOVA). Ketamine treated fetuses have significantly reduced ACTH levels versus the control ($P<0.005$, main effect of groups in two-way ANOVA). The interaction effect is also statistically significant for fetal ACTH ($P<0.001$, two-way ANOVA). Fetal cortisol levels were higher in ketamine treated group versus the control, but no significance between the two groups. Ketamine reduced fetal ACTH responses to HH, possibly due to antagonism of the NMDA receptors in the fetal brain. Interestingly, in contrast to the responses to BCO, ACTH responses to HH were only partially inhibited, suggesting that multiple neurotransmitter pathways mediate the ACTH response to HH. The lack of inhibition of cortisol, consistent with ketamine-BCO experiments, suggests that there are dynamic changes in adrenal sensitivity in response to HH that are not inhibited by ketamine. Alternatively, the increases in fetal plasma cortisol during HH could originate in the maternal HPA axis.

Effects in biological responses and changes in gene expression in the liver of female largemouth bass (*Micropterus salmoides*) by dietary exposures of EE2

Reyna Colli-Dula, Kevin Kroll, Martha E. Dowley, Marianne Kozuch, David Barber, Nancy Denslow
Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, Florida 3261

17alpha-ethinylestradiol (EE2) is a synthetic estrogen used in oral contraceptives. It is considered one of the most potent estrogens that have the ability to interfere with the endocrine system of fish. Thus, the object here was to use biological response and microarray analysis to investigate changes in gene expression caused by 60 days of dietary exposures to 0.07 mg EE2/kg and 0.2 mg EE2/kg feed in female largemouth bass (LMB) during the reproductive season. The hypothesis was that the two concentrations, 0.07 mg EE2/Kg and 0.2 mg EE2/Kg would alter physiological parameters and produce changes in the expression of sensitive genes. Body, liver and ovary weights were measured and blood was collected for measurement of plasma steroid hormones (17β-estradiol (E2), testosterone (T)) and vitellogenin (VTG) using ELISA. The 0.07 mg EE2/kg feed reduced gonadosomatic index (GSI) by approximately 30% and plasma levels of E2 and T were reduced by ~80% but this treatment did not affect VTG concentrations. The hepatosomatic index (HSI) of treated fish was higher and livers were enlarged two times greater than the control group. On the other hand, the 0.2 mg EE2/kg feed exposure reduced GSI by 75% and plasma levels of E2 and T were reduced by over 90%. Plasma VTG was increased by approximately 100% (from 4 to 8mg/ml) and HSI was reduced three times compared to the control. ANOVA analysis from microarrays revealed 1399 genes (738 induced and 661 reduced) and 1868 genes (989 induced and 879 reduced) that were significantly affected by the 0.07 mg/kg EE2 feed and 0.2 mg/kg EE2 feed, respectively. Gene ontology and pathway analysis show that the 0.07 mg EE2/kg feed exposure caused differential regulation of genes associated with metabolic effects like fatty acid biosynthesis and glucose metabolism. In contrast, the 0.2 mg EE2/kg feed exposure altered transcription of genes such as caspase and MAP kinase which is involved in the immune response and apoptosis, suggesting a toxic response at this concentration. Taken together, these results provide important insights into adverse effects caused by chronic exposures to EE2 in the diet of female LMB.

Regulation of embryonic development to the blastocyst stage by canonical WNT signaling

Anna C. Denicol, Kyle B. Dobbs, Barbara Loureiro, and Peter J. Hansen
Department of Animal Sciences, University of Florida, Gainesville

Signaling mediated by WNT proteins during embryonic development controls cell movement during gastrulation and neurulation, body axis formation and organogenesis. WNT proteins also control maintenance of pluripotency in mouse embryonic stem cells. Previous work from our laboratory demonstrated that treatment of embryos with CSF---2 at day 5 of development (day 0 being day of fertilization) improves blastocyst development and embryo survival after transfer. Microarray data of morula---stage embryos revealed expression of several WNT genes (WNT1, *WNT2B, WNT3A, WNT4, WNT5A, WNT5B, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11,* and *WNT16)* and WNT receptor genes (FZD1, *FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9,* and *FZD10).* Several of these genes are down regulated when embryos are exposed to CSF---2, suggesting that signaling by WNTs and CSF---2 may have opposite effects during early development. Embryos exposed to a canonical WNT agonist (2- Amino-4-(3,4-(methylenedioxy)benzylamino)-6-(3-methoxyphenyl)pyrimidine --- AMBMP) at day 5 of development had impaired ability to become blastocysts at day 7 (20.1 versus 7.3 ± 1.7% for control and embryos treated with 1.4 μM of AMBMP, respectively; $p < 0.01$) and also had a lower number of total cells (132.6 versus 103 ± 8 cells; $p < 0.05$) and trophectoderm cells (CDX2---positive cells) (63.6 ± 6 versus 39.2 ± 7.6 cells in control and embryos exposed to 1.4 μM of AMBMP, respectively; $p < 0.05$). Co---treatment of embryos with AMBMP and DKK1, an inhibitor of WNT co---receptors LRP5/6, revealed an interaction ($p < 0.02$) of the two that affected blastocyst development (12.6 ± 1.3% for AMBMP alone versus 16.8 ± 1.3% for AMBMP plus DKK1). DKK1 itself, however, also seems to inhibit embryo development (undergoing experiment). We hypothesize that canonical WNT signaling favors maintenance of pluripotency in blastomeres and inhibition of cell commitment into the trophectoderm lineage. Our next objectives are to determine the effect of the canonical WNT, WNT1, on blastocyst development and cell number, and the mechanism by which WNT1 exerts its effects. WNT1 was chosen based on its high level of expression in bovine morulae as determined by microarray analysis. Embryos will be treated with four different concentrations of human recombinant WNT1 at day 5 of development, and developing blastocysts will be harvested for ICM/TE cell count. Subsequent experiments will be performed to determine at what stage during the preimplantion period WNT1 exerts its effects on blastocyst development. To identify possible mechanisms by which canonical WNT signaling alters embryo development, gene expression analyses will be performed. It is hypothesized that WNT1 will increase expression of pluripotency genes. Support: USDA-AFRI No. 2009-65203- 05732

The temporal and spatial expression of Pax7 during primary myogenesis in the bovine embryo

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Skeletal muscle fiber numbers are fixed at birth in mammals. Increased size of the musculature during postnatal growth is a reflection of fiber hypertrophy. Satellite cells, a heterogeneous population of muscle stem and progenitor cells, are incorporated into the fiber thus, increasing myonuclei numbers and ensuring efficient contractile gene expression. Similar to rodents, Pax7 expression in the absence of Myf5 (Pax7+:Myf5-) defines the muscle stem cell and dual-expressing cells (Pax7+:Myf5+) denotes the muscle progenitor population in neonatal bovine satellite cells. Interestingly, a unique population defined by Myf5 only is present at birth and retained well into adulthood. To further characterize the muscle stem and progenitor populations at birth, the temporal and spatial patterns of Pax7 and Myf5 expression were examined in d28 (n=5) and d45 (n=3) bovine embryos by immunohistochemistry. Embryos were fixed with 4% paraformaldehyde, infiltrated with 30% sucrose and embedded in OCT. Ten micron serial sections were collected and immunostained with anti-Pax7, anti-Myf5, anti-desmin or anti-myosin heavy chain and the appropriate second antibody. Photomicrograph images were captured for morphometric and quantitative analysis of muscle formation. Gross anatomical features at d28 include the initial formation of a forelimb with no discernable hindlimb bud, 24-30 visible somite pairs and four branchial arches. Transverse sections through the anterior somites immediately preceding the forelimb bud reveal the presence of a multiple primary myofibers within the myotome compartment. The fibers are interspersed with Myf5-immunopositive myoblasts. Desmin-immunopositive cells are located within the myotome compartment and the dorsal aorta, a pericyte-rich structure. Midthoracic splanchnic mesoderm contains a Myf5 immunopositive population; this region is Myf5-null in rodents. By d45 of embryogenesis, both fore- and hindlimbs are present and individual somites are apparent in the anterior-most portion of the embryo. Immunohistochemistry reveals the presence of numerous primary fibers within the limbs that express both desmin and myosin. Desmin immunopositive myoblasts are not evident, a clear distinction between bovine and murine embryos. Pax7 expressing myogenic cells are located throughout the limbs as mononucleate cells in close proximity to a primary fiber. Indeed, Pax7 cells are located throughout the region extending from the initial myotome, through the trunk mesoderm and into the limb, it is likely that these muscle cells are the migratory precursors that populate the initial limb structures. Thus, the limb muscles develop from committed myogenic cells and not from unspecified mesenchyme. Results from these preliminary experiments document commonalities between bovine and mouse embryogenesis with regard to embryonic muscle formation. Importantly, these results provide the initial foundation for future efforts defining the types and numbers of muscle progenitor cells at play during fetal and neonatal myogenesis.

Effect of treatment of bovine blastocysts with colony stimulating factor 2 during the morula-blastocyst stages of development on growth and gene expression of trophectoderm outgrowths

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Colony stimulating factor 2 (CSF2) can enhance the competence of the bovine embryo for establishment and maintenance of pregnancy after transfer into recipients. In particular, treatment of in vitro produced embryos with CSF2 from day 5-7 after insemination increased conceptus survival and trophoblast elongation and interferon-tau (*IFNT*) expression at Day 15 of gestation, pregnancy rate at Day 35 of gestation and the proportion of pregnancies at Day 35 that go successfully to term. Here we used an in vitro model of trophectoderm (TE) outgrowth from Day 8 blastocysts to test the hypothesis that pretreatment with CSF2 from Day 6 to 8 after insemination would enhance outgrowth of cells from the blastocyst while maintaining commitment to the TE lineage. Bovine embryos were treated with 0 or 10 ng/ml BoCSF2 at Day 6 after insemination. Blastocysts were harvested at Day 8 and placed individually in wells of 96-well plates that had been coated in growth factor reduced Matrigel. Embryos were cultured in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum until Day 15 after insemination. Each experiment was replicated several times (r=number of replicates) with 1-12 blastocysts per treatment in each replicate. Data were recorded at Day 15 after insemination for attachment and outgrowth rate (r=15), outgrowth surface area (r=14), total cell number (r=4), steady state mRNA for *CDX2* (r=6), *GATA6* (r=6) and *IFNT* (r=4), IFNT antiviral activity (r=3), immunoreactive CDX2 (by immunofluorescence) (r=3), and percent of cells in outgrowth that were CDX2 positive (r=4). Overall, 26% (SEM=2.5) of embryos formed cellular outgrowths. Most measures of TE outgrowth were not affected by CSF2. The exception was for expression of *CDX2* , which was 1.4-fold higher for outgrowth treated with CSF2 ($P<0.04$). There was no effect of CSF2 on the percent of cells that were CDX2 positive (95.6 ± 1.16 vs. 94.2% ± 1.1) so the increase in *CDX2* expression was likely due to increased expression per cell and not to an increased proportion of cells that remained TE. Total CDX2 protein, as measured by the immunofluorescent intensity per nucleus, did not change, however (1.03 ± 0.02 vs. 1.01 ± 0.02). In conclusion, exposure to CSF2 during the morula-blastocyst stages of development can alter the developmental program of TE, as indicated by an increase in steady-state amounts of *CDX2* mRNA. The functional significance of the increase in *CDX2* expression is not clear because CSF2 did not affect growth or lineage commitment of cells in the outgrowth. Moreover, there was no significant increase in CDX2 protein, either because the change in transcript abundance did not result in increased protein synthesis or because the method for measuring CDX2 was not precise enough to detect a small increase. (Support: AFRI Grants 2009-65203-05732 and 2011-67015-30688 from USDA-NIFA).

Relationship between a bull's genetic merit for daughter pregnancy rate and *in vitro* fertilizing ability and embryonic development

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An animal's fertility is likely to depend in part on ability of sperm to fertilize oocytes (male) and on competence of zygotes to complete development to the blastocyst stage (male and female). In cattle, daughter pregnancy rate (DPR) is the most widely-measured trait related to reproduction in the United States. It is defined as the percent of cows eligible to be bred that become pregnant during each 21-day period. We hypothesize that some of the genetic variation in DPR represents variation in developmental competence of embryos and that, therefore, spermatozoa from bulls with high DPR will produce embryos with increased competence to develop to the blastocyst stage *in vitro* (measured as the proportion of cleaved embryos becoming blastocysts and termed the blastocyst development rate, BDR). Semen of bulls which have high (≥ 2.0; n = 49) or low (≤ -2.0; n = 47) DPR was obtained from various bull studs and the National Animal Germplasm Program of the USDA. Semen is being used to fertilize *in vitro* matured oocytes; the resultant embryos are being cultured until day 7 after fertilization. Each bull will be tested in four separate fertilization procedures (minimum oocytes per procedure = 100/bull) to improve the accuracy of estimates of each bull's cleavage rate (CR) and BDR. The percent of oocytes which cleave is being recorded on Day 3 (=CR), and percent of cleaved embryos which develop to the blastocyst stage (BDR) is being recorded on d 7. Bulls are randomly assigned to replicate. Once all bulls are tested, the experiment will be repeated for a total of three additional times. All 96 bulls will be used at random during each series, and each series will be completed prior to the start of the subsequent one. In addition, bulls are being genotyped for 450 single nucleotide polymorphisms in genes related to fertility. We will conduct analyses to determine if these polymorphisms are related to CR and BDR. To date, one replicate of 77 bulls has been conducted. There was no difference in CR (60.8 ± 3.7% and 64.4 ± 3.8%) between high and low DPR bulls; however, high DPR bulls had lower ($P < 0.08$) BDR (30.5 ± 3.4%) than low DPR bulls (35.4 ± 3.5%); this difference was significant ($P < 0.03$) when data were adjusted for results from an internal control. If the additional results confirm this finding, it would suggest that a bull's genetic contribution to DPR is not related to genetic contribution to *in vitro* development. Research is supported by the Southeast Milk Inc. Dairy Check-off Program.

Fatty acid profile and global gene expression in liver of calves supplemented with linoleic acid

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The aim of this study was to assess the fatty acid (FA) profile and global expression of genes in liver of calves fed increased linoleic acid (LA) during the first 30 d of life. Within 2 h of birth, bull calves (n = 31) were fed 4 L of good quality colostrum and assigned randomly to receive milk replacer (MR) with low (LLA, 0.56% LA) or high concentration of LA (HLA, 1.78% LA, DM basis) twice daily at 6.7 g of fat per kg of metabolic BW. Amounts fed were adjusted weekly. Liver biopsy was performed at 30 d of age. Total RNA was extracted using TRIzol reagent and purified according to the manufacturer's recommendation (Invitrogen, Carlsbad, CA). RNA concentrations, purity and integrity were determined (Agilent 2100 Bioanalyzer, Agilent Technologies, Inc., Santa Clara CA). Microarray analysis (n = 9 per MR) was performed using GeneChip Bovine Genome Array from Affymetrix; transcriptome expression was analyzed by one-way ANOVA. The HLA treatment up-regulated 236 genes whereas 265 genes were down-regulated ($P < 0.05$, fold change ratio ≥ 1.3). KEGG pathway analyses were employed with WebGelstat database. Main up-regulated biological pathways were focal adhesion, metabolic pathway, MAPK pathway, and PPAR alpha signaling. Some genes involved in these pathways were actinin alpha 2 (ACTN2), collagen type IV (COL4A4), acyl-CoA synthetase (ACSL6), prostaglandin D2 synthase (PTGDS), cytochrome P450 (CYP2E1), PPAR alpha (PPARA), filamin C (FLNC), and transforming growth factor B3 (TGFB3). The main down-regulated biological pathways were metabolic pathways, T cell receptor signaling, Type II diabetes mellitus, and pentose phosphate pathway. Some genes related with these pathways were pyruvate kinase (PKLR), phospholipase (PLD1), phosphofructokinase (PFKM), 3-hydroxyisobutyate dehydrogenase (HIBADH), phosphoinositide 3-kinase (PIK3R3), and protein kinase C (PRKCQ). Liver FA were analyzed by gas-liquid chromatography. Total FA concentration of liver tissue was 8.5 vs. 7.6 g FA/100 g tissue (DM basis, LLA vs. HLA, $P < 0.02$). Calves fed HLA-MR had greater ($P < 0.01$) liver concentrations (g/100 g FA) of LA (22.1 vs. 15.9), PUFA (43.1 vs. 35.5), and n-3 FA (5.1 vs. 4.2). Calves fed LLA-MR had greater ($P < 0.02$) concentrations (g/100 g FA) of SFA (45.1 vs. 40.0) and MUFA (16.3 vs. 14.3). Supplementing LA increased LA content of liver and altered the expression of key genes involved in different hepatic metabolic processes.

The genomics of estrogen sulfoconjugation and cellular estrogen action in the late-gestation fetus

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Estrogens peak dramatically prior to parturition suggesting a pivotal role in the readiness and act of birth. The placenta is the major source for sulfoconjugated estrogens, which circulate as a prohormone reservoir in fetal blood, and stimulate the fetal HPA axis and other tissues upon deconjugation. Pregnant women, pregnant sheep and their fetuses have significantly higher proportions of the sulfated forms of estrogen, which are conjugated by estrogen sulfotransferase (SULT1E1) and deconjugated by sulfatase (STS) upon timely cues. Inhibition of these enzymes could lead to endocrine disruption of pregnancy and have detrimental effects on fetal growth, development and neonatal survival. SULT1E1 inhibitors from everyday health care products have been shown to circulate in human plasma and may be contributing to preterm birth and Developmental Origins of Health and Disease (DOHAD). To date, the effects of a SULT1E1 inhibitor on estrogen bioavailability and estrogen action in the fetus have not been fully characterized. Our aims are to investigate whether the antibacterial agent and SULT1E1 inhibitor, Triclosan, increases or decreases estrogen action in the ewe fetus and whether this action could be a serious threat to normal pregnancy and fetal health. It is possible that estrogen bioavailability will be increased due to a decrease in physiological clearance of the active hormone; however, we theorize a decrease in estrogen levels because of the blockage of the active-conversion enzyme. To test this hypothesis, we will chronically-catheterize pregnant ewes and their fetuses and study them in each of the four following experimental groups: vehicle, estradiol, Triclosan, and estrogen receptor blocker, ICI182780. Infusions will be continued for 2 days. Plasma samples will be drawn daily and tissues will be collected for genomic analysis. We will use genomics to test whether the effect of Triclosan is mediated by estrogen receptors. Future studies will be conducted to test the physiological role of estrogen sulfate deconjugation and cellular uptake mechanisms during this critical peripartum period, especially with respect to fetal-maternal communication and modulation of fetal HPA activity.

Effects of dietary fatty acids on production, reproduction, tissue composition, and hepatic gene expression in lactating dairy cows

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Three studies were conducted to evaluate the effect of feeding FA to dairy cow and its effects on production, reproduction and gene expression. Objectives of the first two studies were to evaluate the impacts of supplementing diets containing low amounts of long chain fatty acids (FA, < 1.8% DM) with either mostly saturated free FA (SFA, 35% C16:0, and 52% C18:0) or with Ca salts enriched with essential FA (EFA, 27% C18:2n6 and 3.5% C18:3n3 of the FA) on production responses and hepatic FA composition and global gene expression of Holstein cows. In **Study 1**, prepartum cows were allocated randomly to 1 of 3 dietary treatments from 60 d before to 90 d after calving. Supplementation with FA (% dietary DM) consisted of 0% (**CTL**, n=26), 1.7% **SFA** (n=25, Energy Booster100), and 1.9% as Ca salts of EFA (**EFA**, n=25, Megalac-R). On d 14 postpartum, liver was biopsied and analyzed for FA and global gene expression. Feeding supplemental fat did not affect (P = 0.35) the FA content of the liver, but increased (P = 0.02) the content of n6 FA (15.4, 19.0 and 19.3 g/100 g, respectively for CTL, SFA and EFA). Feeding EFA increased (P < 0.01) the content of total CLA and C18:1 *trans*-isomers in liver. Feeding fat upregulated genes related to T cell activation, antigen receptor-mediated signaling pathway and activation of immune response. In **Study 2**, 30 cows were blocked based on milk yield in the first 12 DIM and allocated randomly to the same treatments as in study 1 (10 CTL, 10 SFA, 10 EFA). The DMI, BW, and milk yield and composition were recorded daily for 10 wk. Milk yield improved (P < 0.01) with fat feeding, and it was greater (P < 0.01) for cows fed EFA compared with those fed SFA (CTL= 37.6 vs. SFA = 40.3 vs. EFA = 43.6 kg/d). Milk composition and BW were not influenced by dietary treatments, but yields of milk components followed the same pattern as that of milk yield. In **Study 3**, 45 multiparous Holstein cows were blocked based on milk yield in the first 12 DIM and then randomly assigned to 1 of 3 dietary treatments based on the ratio of n6:n3 FA in the diet: **R3**, **R4**, and **R5**. Treatments were designed to alter the intake of n6 and n3 FA (R3 = 262 and 86 g/d; R4 = 295 and 75 g/d; and R5 = 324 and 66 g/d of n6 and n3, respectively) and evaluate their impacts on uterine secretion of $PGF_{2\alpha}$, timing of spontaneous luteolysis, and the acute phase response after an intra-mammary challenge with LPS. Dry matter intake, milk production and composition were measured daily. Ovulation was synchronized on d 48 postpartum and an indwelling catheter placed on the tail vein/artery on d 15 of the new estrous cycle. Blood was sampled every 2 h from d 16 to 22 of the estrous cycle. Plasma was harvested and frozen to later analyses of progesterone and PGFM. An intra-mammary challenge with 10 μg of LPS was conducted at 70 DIM. The solution was infused in one of the mammary glands and milk and blood were sampled every 2 h for 12 h then once daily for 5 d. Plasma concentrations of acute phase proteins and neutrophil phagocytosis and oxidative burst were evaluated. Study 3 is ongoing.

Oral bioavailability of 22nm gold nanoparticles in mice and the effect of PEG surface coating on gastrointestinal absorption

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Previous studies suggest that primary particle size is an important determinant of uptake of nanoparticles (NPs) from the gastrointestinal tract. However, the effective particle size presented to absorptive surfaces in the gut is dependent upon agglomeration behavior, which is poorly understood in the gastrointestinal environment. We used PEG-coated and uncoated gold NPs of equivalent size (22 nm) to examine the influence of agglomeration behavior on oral bioavailability. It was hypothesized that PEG-coated gold NPs would remain more dispersed, and as a result, be taken up more extensively compared to uncoated gold particles. PEG-coated gold particles remained as primary particles for over 24 hours in simulated gastric fluid, while uncoated gold NPs agglomerated within 5 minutes to sizes over 350nm and over 800nm after 2 hours. Fasted male ICR mice were given a single gavage dose of either PEG-coated or uncoated 22nm gold NPs. The presence of primary PEG-coated gold particles in the lumen of the small intestine was confirmed using transmission electron microscopy. Tissues were collected at 6 and 12 hours after dosing, and gold content was measured by ICP-MS to determine particle distribution. For both NP solutions, feces contained the highest gold levels of any tissue, indicating low oral bioavailability despite surface coating. Although oral bioavailability appears to be low, animals dosed with PEG coated gold NPs were found to have higher levels of gold in non-GI tissues compared to animals dosed with uncoated gold NPs. Further investigation is required, however these results suggest that particle agglomeration in the GI tract following oral administration may be a critical determinant of bioavailability of NPs.
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Understanding factors that modulate prevalence of *Escherichia coli* O157:H7 in cattle

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Enterohemorrhagic *Escherichia coli* O157:H7 is a significant human pathogen that can cause serious illness including hemorrhagic uremic syndrome (HUS). Cattle are the main reservoir of *E. coli* O157:H7 and the prevalence of cattle shedding this pathogen prior to processing correlates with the frequency of carcass contamination. Cattle that shed more *E. coli* O157:H7 than others are referred to as super-shedders (>10^4 CFU/g of feces). Super-shedders can cause negative effects, such as increasing the risk of food contamination and subsequent human infection. We evaluated 91 cattle to see if there are i) super-shedding animals at the farm ii) population dynamics of *E. coli* O157:H7 between steers and bulls. In 40% of animals, *E. coli* O157:H7 was detected by multiplex PCR targeting *stx1, stx2, hlyA*, and O157 after direct plating of rectal anal swab samples. We identified 16 super-shedding animals, and approximately 25% of bulls excreted at levels exceeding 10^5 CFU/swab while only 4% of steers shed at that level, implicating there are animal factors that contribute to the prevalence of *E. coli* O157:H7. Therefore, understanding the factors that play a role in super-shedding will provide insights into reducing or eliminating *E. coli* O157:H7 at the pre-harvest level, resulting in decreased contamination in the food supply. Further analyses of the correlation between genetic background of animals and prevalence of *E. coli* O157:H7 is currently under investigation.

Pharmacokinetics of 1% oral L-arginine supplementation in mares

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L-Arginine (Arg) is an essential amino acid in horses, and serves as a precursor for nitric oxide, polyamines, proline, glutamine, creatine, and agmatine. The various products from Arg can impact various physiological systems including the cardiovascular, reproductive, pulmonary, renal and immune. The objectives of this study were to evaluate changes in plasma concentrations of arginine, lysine, ornithine and citrulline in mares' diet supplemented with 1% Arg. Twelve mares were used for this study (6 American Quarter horse and 6 Thoroughbreds). Mares were blocked by breed, age (Arg: 10.2 ± 1.6 yr; Con: 9.2 ± 1.6 yr) and weight (Arg: 536.0 ± 15.2 kg; 543.5 ± 15.2 kg) and assigned to either an Arg supplemented or control group. Mares were fed 2.5% of body weight of a commercially available grain concentrate; mares were not provided any hay during this trial. Arg supplemented mares had 1% of their grain ration supplemented with Arg (136.3 ± 3.6 g; > 99% pure; Ajinomoto Aminoscience LLC., Raleigh, NC, USA). Three mares from each group were sampled on two separate days. Blood samples were drawn at 0, 0.5, 1, 2, 3, 4, and 5 h relative to feeding time. Mares' plasma was analyzed to determine Arg, lysine, citrulline and ornithine concentrations using a Hitachi L-8900® amino acid analyzer. Data were analyzed using SAS mixed procedure with a random statement to account for variability between mares and a repeated statement to account for sequential measurements. A P-value ≤ 0.05 denotes significance. Data is presented as least square means. No effect of sampling day was detected for any measurement, thus data was grouped from separate days for analysis. Arg supplemented mares had an increase in mean plasma Arg concentration (278.2 ± 25.1 μmol/L versus 103.1 ±25.1 μmol/L; P ≤ 0.01) and mean plasma ornithine concentration (112.6 ± 9.7 μmol/L versus 74.9 ± 9.7 μmol/L; P = 0.03). The Arg supplemented group had significantly higher Arg plasma concentrations at 2 through 5 h post feeding compared to control and significantly higher ornithine concentrations 3 through 5 h post feeding. No difference in mean plasma citrulline concentration were observed between Arg treated and control mares (66.0 ± 6.0 μmol/L versus 71.1 ± 6.0 μmol/L). Mean plasma lysine concentration was lower in the Arg treated group (90.6 ± 9.4 μmol/L versus 156.7 ± 9.4 μmol/L; P ≤0.01) and were significantly lower at 0.5, 1, 2, 3, 4, and 5 h post feeding. L-Arg is converted to ornithine by arginase. The elevated plasma concentrations of Arg increase substrate availability for the arginase pathway, thereby increasing plasma ornithine concentrations. Citrulline concentrations were not different between groups. This may be due to Arg concentrations present in both groups provide enough substrate for nitric oxide synthases to produce nitric oxide and citrulline, thus there is no increase when more Arg is provided. Alternatively, citrulline is recycled to produce L-arg. This recycling may account for similar cirtulline concentrations between groups. Lysine and Arg utilize the same amino acid transporter. The high concentrations of Arg result in a increase in the Arg lysine ration, resulting in a reduction in the amount of lysine absorbed by the intestines. This data demonstrates a 1% increase in Arg effectively elevates Arg concentration in the blood and impacts concentrations of other amino acids in plasma.

Are the effects of colony stimulating factor 2 on bovine preimplantation embryos mediated through the JAK-STAT pathway?

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Colony-stimulating factor-2 (CSF2) is an important cytokine regulating preimplantation embryonic development in several mammalian species. Studies on bovine *in-vitro* produced embryos have shown that addition of CSF2 to culture medium increases blastocyst development and ICM/TE ratio and, upon transfer into synchronized recipients, results in increased post-transfer survival, conceptus elongation, and interferon-τ secretion. CSF2 receptor (CSF2R) is a heterodimer consisting of an α-subunit that binds the ligand and a β-subunit that is involved in signaling downstream. However, little is known about the CSF2R and the pathways involved in its downstream signaling in bovine embryos. Therefore, we aim to study changes in expression of the α and β subunit genes throughout development and to delineate the signaling pathways by which CSF2 affects bovine embryonic development. Bovine embryos will be produced and cultured *in-vitro* and harvested at 2- to 4-cell stage, 6- to 8-cell stage, 16-cell stage, morula, and blastocyst stage. RNA will be extracted, reverse transcribed and quantified by real-time PCR using primer pairs specific for α- and β-subunits of CSF2R. Studies on other cells and tissues, for instance haematopoietic and endothelial cells, have indicated that CSF2 mainly signals through JAK-STAT pathway. We hypothesize that the effects of CSF2 on the bovine embryo are mediated through JAK-STAT signaling pathway. In a 2×2 factorial experiment, bovine embryos produced *in-vitro* will be treated on day 5 post-fertilization either with a JAK2 inhibitor (AG490 dissolved in 0.2% DMSO added at a final concentration of 20 μM) or vehicle (0.2% DMSO) followed 30 minutes later by addition of 10 ng/ml CSF2 or vehicle. After 24 h of CSF2 treatment, morulae (>16 cell embryos) will be collected, treated with 0.1% Pronase to remove zona-pellucida, and subjected to RNA isolation, DNAse treatment, RT-PCR and real-time PCR for quantification of target genes. Additional experiments will be done with blastocyst development as the endpoint.

Uptake of quantum dots in fathead minnows, *Pimephales promelas,* ovarian explant cultures

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The need for understanding the interactions of nanomaterials (NMs) with biological systems is evident, as their use in a variety of applications including cosmetics, electronics, and biomedicine is increasing. Of specific importance is the biological fate of NMs in aquatic organisms, particularly in fish, for which much is not yet understood. Previous research has shown that particle characteristics such as size and surface chemistry (including plasma protein coatings) influence biological fate and ultimately the overall effects. One class of NMs of interest are quantum dots (QD) which are structurally composed of a semiconductor core and shell that can be modified by the addition of different functional groups. The goal of this study was to determine what particle surface characteristics and physiological conditions allow for uptake of QDs into developing oocytes or their follicle cells *in vitro*. Oocytes were collected from mature female fathead minnows (FHMs) and transferred to a 24-well plate with approximately even numbers of oocytes in each well. Media was supplemented with insulin (30ug/mL), hCG (10UmL), QDs (10 nM, ± plasma containing 17ug/mL vitellogenin). Oocytes were incubated for 24 hours and some cells were removed for confocal microscopy while the rest were preserved for histology and transmission electron microscopy (TEM). Confocal images of tissues following different treatments suggest that QDs may be taken up into oocytes/follicle cells *in vitro*. TEM analysis was used to confirm the localization of the QDs within the oocytes /follicle cells. Additionally, a separate exposure to primary cultures of follicle cells was conducted to validate QD uptake into those steroidogenic cells. This assay has the potential to be a valuable tool in high-throughput screening of NMs that may cause direct toxicity to the oocyte or perturb the functions of the follicle cells.

Modification of the 5-d timed artificial insemination (AI) protocol to optimize fertility in dairy heifers

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Three experiments evaluated modifications of the 5-d timed AI protocol to optimize fertility in dairy heifers. In all studies, pregnancies per AI (P/AI) were diagnosed on d 32 and 60 after AI. In **Experiment 1**, 605 heifers were assigned randomly to receive **GnRH** on study d 0 (n = 298) or to remain as untreated **controls** (n = 307) in the 5-d timed AI with an injection of $PGF_{2\alpha}$ on d 5 only [d 0 controlled internal drug-release (**CIDR**), d 5 remove CIDR + $PGF_{2\alpha}$, d 8 GnRH + AI]. The hypothesis was that GnRH on d 0 induces a small proportion of heifers to ovulate and its benefits is not needed when $PGF_{2\alpha}$ is administered on d 5 only. Ovaries were scanned on d 0 and 5. Blood was analyzed for progesterone (**P4**) at AI. Ovulation on study d 0 and presence of a new CL were greater (P<0.01) for GnRH (35.4 and 43.1%) than control (10.6 and 20.8%). The proportion of heifers with P4<0.5 ng/mL at AI was less (P<0.01) for GnRH than controls (73.8 vs. 88.2%), which resulted in a greater (P<0.01) mean P4 at AI (GnRH=0.50 vs. control=0.28 ng/mL). P/AI did not differ between treatments (GnRH=52.5 vs. control-54.1%). When $PGF_{2\alpha}$ is administered on d 5 only, the initial GnRH is not needed to optimize P/AI in dairy heifers. In **Experiment 2**, 1,295 heifers were assigned randomly to receive a CIDR on d 0, $PGF_{2\alpha}$ and removal of the CIDR on d 5, and either GnRH 56 h after $PGF_{2\alpha}$ and AI 16 h later (**OVS56**, n=644) or GnRH concurrent with AI 72 h after $PGF_{2\alpha}$ (**COS72**; n= 651). The hypothesis was that extending the proestrus from 56 to 72 h benefits P/AI in dairy heifers. Estrus at AI was greater (P<0.01) for COS72 than for OVS56 (61.4 vs. 47.5). COS72 improved (P=0.05) P/AI on d 60 compared with OVS56 (55.6 vs. 51.6%) because of greater P/AI in cows that did not display estrus at AI (55.0 vs. 47.6%), but not in those detected in estrus (COS72 = 57.3 vs. OVS56 = 59.2%). Extending the proestrus from 56 to 72 h with GnRH administered concurrent with AI benefited fertility of dairy heifers. In **Experiment 3**, 2,118 heifers received a CIDR on d 0, $PGF_{2\alpha}$ and removal of CIDR on d 5. GnRH was given on d 8 concurrently with AI. Heifers were allocated randomly to receive no additional treatment (**NG1P**=711), a second $PGF_{2\alpha}$ on d 6 (**NG2P**=696), or an injection of GnRH on d 0 and a second $PGF_{2\alpha}$ on d 6 (**G2P**=711). Ovaries were scanned on d 0 and 5. Blood was sampled at AI to measure P4. The hypothesis was that a combination of GnRH on d 0 and a second $PGF_{2\alpha}$ on d 6 was needed to maximize P/AI in dairy heifers. Results are presented in the Table 1. Heifers with a new CL that received $PGF_{2\alpha}$ on d 5 and 6 had greater P/AI than those with a new CL that received $PGF_{2\alpha}$ on d 5 only (62.8% vs. 45.7%). Combining GnRH with 2 $PGF_{2\alpha}$ on d 5 and 6 in the 5-d timed AI protocol improved fertility because of greater ovulation associated with adequate luteolysis in dairy heifers.

Table 1. Ovarian and fertility responses of heifers in Experiment 3

	Treatment			
	NG1P	NG2P	G2P	*P*
		% (n/n)		
Ovulation d 0	13.2 (40/303)[b]	12.8 (38/296)[b]	27.6 (85/308)[a]	0.01
New CL d 5	20.5 (62/303)[b]	19.6 (58/296)[b]	33.8 (104/308)[a]	0.01
Luteolysis (P4<0.3 ng/mL)	61.2 (112/183)[b]	74.2 (135/182)[a]	71.0 (127/179)[a]	0.03
Pregnant				
d 32	52.9 (376/711)[b]	55.0 (383/696)[b]	61.7 (439/711)[a]	0.01
d 60	49.0 (348/711)[b]	51.6 (359/696)[b]	59.1 (420/711)[a]	0.01

[a,b,c] (*P*<0.05).

Associations among subclinical hypocalcemia, neutrophil function, and incidence of uterine disease in dairy cows of low or high risk of developing metritis

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Objectives were to establish relationships among subclinical hypocalcemia (**SCH**) and concentrations of energy metabolites, neutrophil (**PMN**) function and incidence of uterine diseases in dairy cows considered to be of low (**LRM**; no calving problems) or high-risk (**HRM**; dystocia, twins, stillbirth, and/or retained placenta) of developing metritis. In this prospective cohort study, 55 HRM cows were matched with 55 LRM based on parity and day of calving. Rectal temperature (**RT**) and vaginal discharge were monitored daily for the first 12 d in milk (**DIM**). Metritis was defined as fetid, watery vaginal discharge regardless of RT, and puerperal metritis was defined as metritis concurrent with RT ≥ 39.5°C. Blood was sampled at 0, 1, 2, 3, 4, 7, and 12 DIM and analyzed for concentrations of Ca, Mg, glucose, NEFA and BHBA. Neutrophil function was measured at 0, 1, and 3 DIM. Ovaries were scanned by ultrasonography at 24, 31, and 38 DIM to determine ovulation based on presence of a corpus luteum. Clinical endometritis was assessed based on vaginal mucus with pus at 31 DIM. Subclinical endometritis was diagnosed by endometrial cytology at 38 DIM. Serum Ca ≤ 8.59 mg/dL in at least one day within the first 3 DIM defined SCH based on receiver operator characteristic analysis (area under the curve = 0.77; $P < 0.01$). Cows had their estrous cycles synchronized and were inseminated after 60 DIM. Data were analyzed using PROC GLIMMIX of SAS. Cows with SCH had fewer ($P < 0.01$) circulating blood PMN (3.0±0.2 vs. 4.5±0.3x10^3 PMN/µL) in the first 3 DIM, and reduced ($P < 0.05$) proportion of PMN with oxidative burst (32.4±2.9 vs. 42.5±4.1%) and phagocytosis (61.3±3.3 vs. 73.1±4.6%) at 3 DIM compared with normocalcemic (**NC**) cows. The mean RT of cows increased (P < 0.01) when metritis was associated with SCH (39.01 °C) compared with cows with metritis and NC (38.68 °C). Among HRM cows, those with SCH had greater (P < 0.05) incidence of metritis and puerperal metritis [77.8% (35/45) and 53.5% (24/45)] compared with NC cows [(20.0% (2/10) and 10.0% (1/10)]. Similarly, among LRM cows, those with SCH had greater (P < 0.05) incidence of metritis and puerperal metritis [40.7% (11/27) and 29.6% (8/27)] compared with NC cows [(14.3% (4/28) and 0.0% (0/28)]. The population attributable risks for the impact of SCH on incidences of metritis and puerperal endometritis were 66.6 and 91.3%, respectively. Metritis did not influence concentrations of NEFA or BHBA; however, cows with SCH had greater ($P < 0.01$) NEFA (704.6±36.7 vs. 426.8±42.5 µM) and BHBA (9.9±0.4 vs. 7.7±0.5 mg/dL) concentrations compared with NC cows. In addition, cows with SCH had greater ($P < 0.04$) incidence of clinical endometritis (82.6 vs. 68.4%), and a tendency ($P < 0.07$) for greater incidence of subclinical endometritis (46.2 vs. 21.1%) compared with NC cows. Prevalence of estrous cyclic cows by 38 DIM did not differ ($P = 0.70$) between SCH and NC cows (48.6 vs. 57.9%). Pregnancy at first insemination did not differ ($P = 0.25$) between SCH and NC cows (31.3 vs. 37.1%). These findings indicate that cows with SCH (serum Ca ≤ 8.59 mg/dL in the first 3 DIM) have impaired measures of innate immunity and increased incidence of uterine diseases regardless of calving problems.

Influence of *Bos indicus* genetics on pregnancy-associated glycoproteins (PAGs) and their association with fetal development

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Cross-breeding *Bos indicus* and *Bos taurus* genotypes improves various production traits for cattle maintained in sub-tropical or tropical climates. Pregnancy-Associated Glycoprotein (PAG) concentrations are influenced by various factors during gestation and recent evidence indicates that *Bos taurus* subspecies impacts concentrations of PAG and fetal size during early gestation. We determined the correlation between maternal genotype, PAG concentrations, and fetal development during early gestation in cattle with distinct subspecies genotypes. A fixed-time AI estrous synchronization protocol with semen of multiple sires within each breed was used on multiparous Angus (n=17), Brangus (n=25) and Braford (n=9) cows. Transrectal ultrasonography was used to measure fetal size at 35 (crown-rump) and 62 d (nose-crown) of gestation. Blood was harvested to determine plasma concentrations of PAG and progesterone (P4). Orthogonal contrasts were used to compare outcomes for cattle containing *Bos indicus* genetics (Brangus and Braford) from those containing solely *Bos taurus* genetics (Angus). Concentrations of PAG tended to be greater ($p = 0.09$) in Angus (5.0 ± 0.5 ng/mL) than Brangus (4.3 ± 0.5 ng/mL) and Braford (3.4 ± 0.5 ng/mL) at d 35 of gestation. At d 62 of gestation, concentrations of PAG tended ($p = 0.09$) to be lower in Angus (2.0 ± 0.4 ng/mL) than Brangus (2.5 ± 0.4 ng/mL) and Braford (3.3 ± 0.4 ng/mL). Concentrations of P4 were not influenced by cow genotype. Concentrations of PAG were positively correlated ($p = 0.01$) with concentrations of P4 at d 35 but not at d 62 of gestation. Cow breed did not affect fetal size at d 35 of gestation(15.5 ± 0.4, 15.4 ± 0.4 and 14.6 ± 0.4 mm for Angus, Brangus, and Braford, respectively), but at d 62 of gestation Angus cows (29.6 ± 0.3 mm) contained larger fetuses ($P \leq 0.01$) than Brangus (28.0 ± 0.3 mm) and Braford (28.4 ± 0.3 mm) cows. No correlation between PAG and fetal size were observed at 35 or 62 d of gestation. In conclusion, cow genotype influenced fetal size on d 62 and tended to influence concentrations of PAG on d 35 and 62. Plasma PAG concentration had a correlation with cow breed and plasma P4 concentrations, indicating that maternal genotype may influence placental activity and early fetal development.

Effects of anti-phospholipase A2 antibody (aPLA2) supplementation on DMI, feed efficiency and blood differentials of steers fed forage and grain-based diets

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We determined whether supplementation of anti-phospholipase A2 antibody (aPLA2; BIG BEEF, Aova Technologies, Madison, WI) would alter voluntary DMI, feed efficiency (FE) and alter blood differentials (BD) due to a change in diet from a forage-based to a grain-based diet. Individual daily DMI was measured on 80 cross-bred steers during a 141 d period using a GrowSafe system (GrowSafe Systems Ltd., Alberta, Canada) at the University of Florida NFREC Feed Efficiency Facility. On d 0, steers were blocked by BW and assigned to receive a basal diet (0.97 Mcal NEg/kg of DM and 16% CP) comprised of 69% concentrate, 31 % bermudagrass hay, and a vitamins and minerals supplement containing the following treatments: 1) no additive (CON; n = 20); 2) 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM (MT; n = 20); 3) same as CON, but including aPLA2 at 0.4% of the diet DM (BB0.4%; n = 20); 4) same as CON, but including aPLA2 at 0.2% of the diet DM (BB0.2%; n = 20). On d 60 all steers were transitioned into a 90% concentrate diet (74% cracked corn; 1.32 Mcal NEg/kg of DM, 11.4% CP) over a 21 d ′step-up′ period while continuing to receive their supplement treatments. On d 0, d 60, d 81 and d 141 BW was recorded. Blood samples were collected on d 60, 63, 65, 67, 70, 72, 74, 77, 79, 81 and 84, and BD was assessed using a hematology cell counter (IDEXX ProCyte Dx Hematology Analyzer, Westbrook, ME). No differences existed for BW on d 0 (212 ± 34 kg), BW on d 141 (388 ± 46 kg), overall ADG (1.24 ± 0.16 kg/d), DMI (8.00 ± 0.94 kg/d), and residual feed intake (RFI). However, steers receiving the CON (0.34 ± 0.15 kg/d) treatment had greater ($P < 0.05$) RFI than the BB0.2% (-0.13 ± 0.15 kg/d) and BB0.4% (-0.25 ± 0.15 kg/d) treatments, whereas the MT (0.06 ± 0.15 kg/d) treatment was intermediate. During the grain-based diet period, the BB0.2% (-0.12 ± 0.21 kg/d), BB0.4% (0.38 ± 0.21 kg/d), and MT (0.09 ± 0.21) steers tended ($P = 0.07$) to have greater RFI than the CON (-0.39 ± 0.21 kg/d) steers. During the step-up period the CON (7.09 ± 0.23 k/μL) and BB0.2% (7.62 ± 0.23 k/μL) treatments had greater ($P < 0.05$) concentrations of lymphocytes than the MT (6.73 ± 0.23 k/μL) and BB0.4% (6.73 ± 0.23 k/μL) treatments, and tended ($P = 0.06$) to have greater white blood cell counts (12.87 ± 0.42; 13.61 ± 0.42; 12.16 ± 0.42; 12.37 ± 0.42 k/μL for CON, BB0.2%, BB0.4% and MT, respectively;). We conclude that aPLA2 supplementation improved FE of steers fed forage-based diets and tended to reduce blood leukocyte concentrations when exposed to a transition into grain-based diets.

Involvement of free cholesterol and high-density lipoprotein in development and resistance of the preimplantation bovine embryo to heat shock

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Development of the mammalian preimplantation embryo is susceptible to disruption by elevated temperature. The molecular and biochemical basis for developmental, genetic and other differences in embryonic resistance to heat shock is largely not known. Here we tested the hypothesis that increasing free cholesterol content could improve embryonic resistance to heat shock. In Experiment 1, embryos were cultured without treatment, with methyl-β-cyclodextrin (MBCD), or with cholesterol loaded to MBCD (0.41 mM cholesterol) for 3 h before exposure to heat shock (41.0°C for 15 h) or no stress (38.5°C continuously) beginning at 30 h after insemination (one-to-two cell stage). For all treatments, heat shock reduced ($P<0.05$) development to the blastocyst stage. For control embryos, percent of oocytes that became blastocysts was 29.9% for non-stressed embryos and 15.9% for heat-shocked embryos (SEM=2.4, n=4 replicates). Culture in methyl-β-cyclodextrin (MBCD), the carrier for cholesterol, reduced ($P<0.05$) development (19.8% for no stress and 1.8% for heat shock). Development in the presence of cholesterol-loaded MBCD (0.41 mM cholesterol) was similar to controls (34.1% for no stress and 15.0% for heat shock). For Exp. 2, embryos were not treated or received cholesterol-loaded high density lipoprotein (HDL – 0.07, 0.27 or 2.74 mM cholesterol equivalents) for 3 h before heat shock as in Exp. 1. There was a heat shock x treatment interaction ($P<0.01$) that reflected the fact that 1) the deleterious effects of heat shock were reduced by HDL and 2) high concentrations of HDL reduced development in the absence of heat shock only. Heat shock reduced development to the blastocyst stage from 39.3% to 13.5% in the absence of HDL, from 35.4% to 23.1% for 0.07 mM, 21.6% to 21.3% for 0.27 mM and from 28.3 to 26.7% for 2.74 mM cholesterol equivalents (SEM=3.5%, n=4 replicates). In Exp. 3, cholesterol assays confirmed that, as compared to untreated embryos (0.3 pmol/embryo), MBCD (0.15 pmol) and 2.74 mM cholesterol-equivalent HDL (0.23 pmol) reduced cholesterol content while cholesterol-MBCD increased cholesterol content (0.42 pmol; SEM=0.02, n=6). Results were interpreted to mean that other actions of HDL (for example, protection from free radicals) was responsible for the thermoprotective properties of this molecule. In conclusion, raising cholesterol content does not improve embryonic survival in response to heat shock. Depletion of cholesterol, in contrast, reduces competence of embryos to develop to the blastocyst stage. High density lipoprotein is thermoprotective to embryos and probably acts through a mechanism independent of its actions on embryonic content of free cholesterol.

A whole genome DNA sequencing reveals genetic traits that affect survival and persistence of *Escherichia coli* O157:H7 in cattle

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Escherichia coli O157:H7 remains a significant concern of food recalls and causes tremendous negative effect on public health. Cattle are a reservoir and considered a primary source of this foodborne pathogen. Previous studies have revealed that an O157 subtype strain (FRIK2455) was predominant on a farm while 4 other clonal variants were rarely isolated. Animal factors are presumed to contribute to the predominance of the strains. However, bacterial genetic traits responsible for the predominance of FRIK2455 have not been addressed. In this study, we have conducted whole genome DNA sequencing using one predominant and four clonal variants to identify genetic variations, which affect the persistence of *E. coli* O157:H7 in cattle. A library was prepared from chromosomal DNA using the Covaris E210, and quality of the library was verified on a Caliper LabChip GX. Sequencing (75bp paired end) was performed on the HiSeq with v3 chemistry, and cluster was generated on the cBOT. Fastq files were aligned using bwa (http://bio-bwa.sourceforge.net/bwa.shtml) to the reference *E. coli* genome O157:H7 EDL933. In addition, *de novo* assembly was performed using Abyss. Bioinformatic comparison analysis using *de novo* assembly sequences was conducted by Macrogen (Korea). Surprisingly, the predominant strain contains significantly fewer genes. It carries ~500 less genes compared to the clonal variants. Although missing genes fell into several functional categories, a majority number of genes are related to phage, suggesting phages may play critical roles in the persistence of *E. coli* O157:H7 in cattle. In addition, FRIK2455 imported ~100 new genes from other organism including a type IV secretion system, therefore the newly added genes may contribute to the survival of *E. coli* O157:H7. We are currently confirming these bioinformatic comparison data by evaluation of individual genes. Further studies will reveal insights regarding survival of *E. coli* O157:H7 in cattle.

Microarray analysis of gene expression in the ovine fetal cerebral cortex ontogeny in late gestation suggests the development of tolerogenic myeloid-derived cells in the cortex

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Remarkable physiological changes occur in the fetal brain approaching the end of gestation. In ruminants, development of the hypothalamo-pituitary function is critical for fetal survival and normal timing of parturition. However, the role of the remaining brain regions to prepare the fetus for the extra-uterine life is not completely understood. Our lab has employed the microarray technique to test the hypothesis that resulting products of genes expressed in a similar pattern during the last stage of gestation in brain regions excluding hypothalamus and pituitary are functionally related and could play an important role in the normal fetal development. Global gene expression was measured in the ovine fetal cerebral cortex, brainstem and hippocampus at 80, 100, 120, 130, 145 days of gestational life (the gestation term is around 147 days) and 1 day of extra-uterine life. The data was analyzed using the Empirical Bayes principle to rank the genes according to how well they fit an increased -or decreased- expression pattern. Gene expression was confirmed by qRT-PCR. Highly ranked genes with increased expression pattern for all the brain regions analyzed were glial fibrillary acidic protein (GFAP, marker for astrocytes) and genes encoding the antigenic myelin proteins: myelin basic protein (MBP) and alpha B-crystallin (CRYAB). In the cortex ontogeny, some of the genes with increased expression pattern were microglia markers, such as CD14, CD86 or B7.2 and CD11b. Microglia cells can be differentiated into a dendritic cell (DC) phenotype if they are exposed to colony stimulating factor 1 (CSF-1). Transcriptions of genes encoding for CSF-1, CSF-1 receptor (CSF1R), IL34 (CSF1R ligand) and DC markers such as CD1d, CD83 and CD32 were increased. The pattern of gene expression suggested increasing tolerogenic functions in late gestation. CD32, for example, is an inhibitory receptor on DC that can regulate T-cell tolerance and promotes T-regulatory cell induction by increasing IL10 production. Both IL10 and TGFb, another cytokine involved in T-regulatory cell induction, had an increased expression pattern. In contrast, a cytokine that had a decreased expression pattern was IL6. IL6 is involved in the differentiation of Th17 cells, which have been related with experimental autoimmune encephalitis (EAE). EAE can be induced in the Lewis rat by active immunization with MBP and can be prevented by tolerogenic DC. Critically important for the progress of EAE is expression of CD24, which is required for the optimal local T cell clonal expansion in the CNS. Interestingly, CD24 mRNA expression was remarkably decreased from 80 days of gestation to 1 day of extra-uterine life. Together, our results suggest that a myeloid cell type -probably DC- is differentiated from the local microglia in the fetal cortex. These cells will be destined to exert tolerogenic functions and avoid an autoimmune reaction of B- or T-cells against antigenic self-proteins highly expressed at the end of gestation, such as MBP or CRYAB.

Conceptus development and global gene expression at preimplantation stages in lactating dairy cows of distinct genetic groups and estrous cyclic statuses

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Anovular cows have impaired reproduction because of reduced establishment and maintenance of pregnancy compared with estrous cyclic cows. In addition, the genetic background of dairy cows can influence reproductive performance, and crossbreeding Holsteins with other dairy breeds has been shown to improve pregnancy per insemination (P/AI) compared with purebreds. The objectives were to compare conceptus development and global gene expression at preimplantation stages in anovular Holstein (AH), estrous cyclic Holstein (CH), and estrous cyclic Jersey/Holstein crossbred (CC) lactating dairy cows subjected to a synchronized ovulation. On postpartum d 29, cows of both breeds were randomly selected within a grazing herd and received an injection of prostaglandin. Ovaries were scanned by ultrasonography on postpartum d 29 and 39 to determine estrous cyclic status. Based on the presence or absence of CL, cows were then grouped in AH (n = 10), CH (n = 25) and CC (n = 25) and subjected to the Ovsynch protocol. The day of AI was considered study d 0. On study d 15, uteri were flushed and interferon-tau (IFN-τ) concentrations in fluid measured. Recovered conceptuses were subjected to global analysis of gene expression using Affymetrix Gene Chip® Bovine Array. Genes were considered differently expressed (DEG) when $P \leq 0.05$ and fold change ≥ 2.0.

Item	AH	CH	CC
Pregnancy d 15, % (n/n)	70.0 (7/10)	64.0 (16/25)	84.0 (21/25)
IFN-τ, ng/mL	7.27 ± 1.5[a]	0.57 ± 1.1[b]	0.85 ± 0.9[b]
Conceptus length, mm	47.8 ± 8.8[a]	9.4 ± 5.8[b]	25.3 ± 8.7[a]
Non-elongated embryos, % (n/n)	0.0 (0/7)[b]	37.5 (6/16)[a]	0.0 (0/21)[b]
DEG, all conceptuses	417	Reference	65
DEG, elongated conceptuses	284	Reference	29

[a,b,c] Values on the same row with different superscripts differ ($P < 0.05$).

Although CC ovulated a smaller follicle, they had greater concentrations of steroid hormones during the Ovsynch protocol and after AI, tended ($P = 0.11$) to have greater P/AI and presented advantages in conceptus development compared with CH. Conversely, although P/AI did not differ between the two Holstein groups, AH had more advanced conceptuses than CH, likely because of a faster rise in progesterone concentrations after AI. Moreover, estrous cyclic status within the same genetic background had a greater impact on conceptus gene expression than genetics within the same estrous cyclic status. In conclusion, expressive differences on reproductive physiology and gene expression of important biological processes were identified and might help explain the differences in fertility observed between estrous cyclic and anovular cows of distinct genetic background.

What fish gene expression tells us about urban water pollution

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Surface waters from urban areas often carry complex mixtures of toxic organic contaminants. Determining the presence of individual toxicants and/or mixtures in these waters and the potential effects exerted by these chemicals on biota is an immense task. The fact that toxicity is preceded by gene expression alteration allows the use of microarrays to determine which of thousands of genes are altered as a means to detect early signs of toxic effects on vertebrates. We use a toxicogenomic approach to link the altered liver gene expression in male fathead minnows exposed to urban waters in Gainesville and the known presence of pollutants in these waters. The collected waters included surface water downstream of a wastewater treatment plant (streamwater), a wastewater treatment plant effluent used for landscaping irrigation (wastewater), and surface water from a lake that receives urban stormwater (stormwater). These waters were used as whole effluents in a 48 hour exposure study under laboratory conditions. After LOESS normalization and ANOVA analyses, several statistically significant ($p \leq 0.05$) differences in gene expression between fish exposed to collected waters and controls were observed. The highest number of genes in the fish liver were altered in stormwater (1028), followed by streamwater (787), and wastewater (575 genes). Enrichment analysis showed that 18 biological processes were overrepresented for stormwater, 19 for streamwater, and 12 for wastewater. Exposed fish showed alteration of genes related with disruption of fatty acid metabolism, lipid and cholesterol transport, alteration of cytochrome P450 superfamily enzymes, (Stormwater and streamwater) cell death, oxidative stress and apoptosis (Wastewater). These are all biological processes commonly associated with exposure to Perfluorochemicals (PFCs), an ubiquitous class of emerging pollutants. Also, all exposed fish showed genes related with DNA damage. In particular, the strongest effects on fish exposed to stormwater and streamwater was the downregulation of several key transcripts that code for enzymes involved in cholesterol and sterol biosynthesis. The presence of PFCs in wastewaters, their environmental persistence and the similarity of our data with other known effects on transcripts, suggest that the set of genes differentially regulated in fathead minnows after 48 hours of exposure may be attributed to PFCs exposure.

Indecent Exposure? Investigating the relationship between pathogens and emerging contaminants

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Despite extraordinary advancement in manipulating materials at the nanoscale and their current use in industrial, consumer and biomedical products, we lack sound understanding of the biotoxicity associated with environmental exposures. As the use of engineered nanoparticles (ENPs) is rapidly growing there is eminent concern regarding adverse health effects. The unusual physico-chemical properties that make ENPs promising for novel product enhancement applications may also influence their ability to modulate immune defense and inflammatory responses in humans. Inhalation is a primary exposure route which underscores the critical need to comprehend how ENP impact the lungs. We have a particular interest in single-walled carbon nanotubes (SWNT) as they possess a superficially resemble asbestos which may be relevant to their long-term health consequences. Additional concerns surround the ability of ENP to influence the behavior of infectious agents thereby increasing susceptibility to infections. This can have critical consequences particularly for viruses, such as influenza A (IAV) that are notorious for causing pandemics. The objective of the present work is to characterize the mechanisms controlling the immune response of lung cells exposed to SWNT and IAV. We concentrate on toll-like receptors (TLRs) as they are an early line of defense against foreign invaders in the human body. Our hypothesis is that SWNT stimulate TLRs resulting in the production of pro-inflammatory cytokines through activation of transcription factors NF-κβ. Furthermore, combined exposures of SWNT and IAV will synergistically activate TLR-driven pathways leading to enhanced inflammation and injury. To begin to address this hypothesis, we exposed lung cells to SWNT and assessed cytotoxicity, activation and expression of TLRs and downstream immune genes using suite of molecular assays. Results show that although SWNT are not acutely cytotoxic, they activate TLR2 and NF-κβ, alter the expression of TLR7, and stimulate immune target genes. Furthermore, if we modify the SWNT surface (oxidize), activation of TLR2 does not occur. These data indicate the ability of SWNT to alter first line defense receptors and subsequent immune responses in an ENP-specific manner, highlighting the importance of surface chemistry in biologic effects. As an effort to determine how these results impact the normal immune response of pathogens through TLRs, we have begun to characterize H1N1 pandemic 2009 virus in our system. This research will not only lay a foundation for studying the health impacts of emerging contaminants and infectious disease, but will also generate a comprehensive understanding of how multiple aspects of ENP affect cell function and will provide reliable in vitro model systems to evaluate and engineer safe nanomaterials.

Differential recruitment of estrogen receptor co-activators by xenoestrogens

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Xenoestrogens are environmental contaminants that interfere with endocrine activity by modulating estrogen receptor (ER) transcriptional activity. One hypothesis that may explain contaminant-specific impacts of estrogen (E_2) signaling is that co-accessory proteins are differentially recruited to the ER. Using Time Resolved-Fluorescence Resonance Energy Transfer recruitment screening assays we revealed that E_2 weakly recruited a peptide specific to SRC-3, a known ER co-activator, to the human (h)ERα. Conversely, greater recruitment of the same peptide to the hERα was observed upon stimulation with the phytoestrogen genistein (Gen, EC_{50} 17nM). To begin to assess the functional role of SRC-3 in ER activation by these compounds, human embryonic kidney cells were transfected with an ERE-driven luciferase reporter and wildtype (w) SRC-3, or mutant (m) SRC-3, which impairs interaction with the ER through mutated phosphorylation sites. Cells were exposed to 10nM E_2, or 1uM Gen for 24 hours and luciferase activity was measured. Results showed a significant increase in hERα activation in response to Gen (16 fold) with the addition of wSRC-3 over baseline values (3 fold). Although E_2 had higher baseline activation (8 fold) addition of wSRC-3 did not significantly increase the response. In the presence of mSRC-3, ER activation was ablated by both compounds. These results suggest that although SRC-3 is essential for ERE-driven responses by both agents, the enhanced response of Gen in the presence of additional SRC-3 suggests differential recruitment likely plays a role in xenoestrogen-specific effects on ER activity. Mechanisms driving these observations are under current investigation but may involve SRC-3 site-specific modifications that are ligand dependent.

Effect of late gestation maternal heat stress on growth and immune function of dairy calves

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Heat stress during the dry period affects the cow's mammary gland development, metabolism, and immunity during the transition period. However, the impact of late gestation heat stress on calf performance and immune status is unknown. Our objective was to evaluate the effect of heat stress during the final ~45 d of gestation on growth and immune function of calves. Calves (17/treatment) were delivered from cows that were exposed to cooling (CL) or heat stress (HT) during the dry period. Heifer calves were fed 3.78 L of colostrum from their respective dams within 4 hours of birth. Additionally, only heifer calves (CL, n=12; HT, n=9) were used in the measurements of growth and immune status after birth. All the heifers were weaned at 2 month of age (MOA) and managed under identical conditions. Body weight (BW) was obtained at weaning and then monthly until 7 MOA. Withers height (WH) was measured monthly from 3 - 7 MOA. Hematocrit and plasma total protein was assessed at birth, 1, 4, 7, 11, 14, 18, 21, 25 and 28 days of age (DOA). Total serum IgG was evaluated at 1, 4, 7, 11, 14, 18, 21, 25 and 28 DOA and apparent efficiency of absorption was calculated. Peripheral blood mononuclear cells were isolated at 7, 28, 42 and 56 DOA and the proliferation rate was measured by ^{3}H-Thymidine incorporation into newly synthesized DNA. Calves from CL cows had greater BW than those from HT cows at birth (42.4 vs. 36.7 kg). Compared with CL heifers, HT heifers had lower weaning BW (78.5 vs. 65.9 kg) and similar BW (154.6 vs. 146.4 kg) and WH (104.8 vs. 103.4 cm) from 3-7 MOA. Compared with CL, heifers from HT cows had lower total plasma protein (6.25 vs. 5.89 g/dL), total serum IgG (1577.3 vs. 1057.8 mg/dL) and apparent efficiency of absorption (33.6 vs. 19.2%) and tended to have decreased hematocrit (33 vs. 30%). Additionally, CL heifers had higher peripheral blood mononuclear cell proliferation relative to HT heifers (23.8 vs. 14.1 fold). We conclude that heat stress of the dam during the dry period compromises the growth and immune function of offspring from birth through weaning.

Synergistic effects of osmotic, thermal and hypoxia stress on embryo development in the horseshoe crab, *Limulus polyphemus*

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Marine organisms in nature experience a variety of abiotic stressors, which often occur in combination and may interact synergistically, antagonistically and/or additively. Simultaneous exposure to multiple stressors may, in particular, negatively affect marine larvae and juveniles, significantly impacting development. We investigated the effects of multiple abiotic stressors on embryo development of the American horseshoe crab, *Limulus polyphemus.* Newly fertilized horseshoe crab eggs from 6 females were collected from Seahorse Key, FL in September 2011. Eggs were placed into experimental treatments consisting of fully factorial stressor combinations of temperature (25°, 30° and 35° C), salinity (5, 15 and 34 ppt), and dissolved oxygen (DO) (equilibration with 5%, 13% and 21% O_2). Eggs were incubated in the treatment conditions for 14 days after which we quantified the proportion of individuals that reached the embryo stage. Development was most successful at 30° C, 15 ppt and 13% O_2, suggesting adaptation to hyposaline and mildly hypoxic conditions. Development was significantly reduced by 27% at 35° C, 34 ppt and 13% O_2 compared to 30° C at the same salinity and DO ($p=0.03$), and it was reduced by 38% at 35° C, 5 ppt and 21% O_2 compared to 30° C at the same salinity and DO ($p<0.01$). Therefore, increased temperature limits embryo development in hyposaline conditions under normoxic and mildly hypoxic conditions. Understanding how multiple stressors affect marine organisms is essential for understanding the evolution of the stress response and for predicting vulnerability to climate change.

A study of effector proteins secreted by enterohemorrhagic *Escherichia coli* O157:H7

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Bacterial pathogens interact with host cells to disrupt a wide range of cellular processes during infection. Enterohemorrhagic *Esherichia coli* (EHEC) O157:H7 is a foodborne pathogen that can cause diseases in humans ranging from mild diarrhea to the potentially fatal hemolytic uremic syndrome (HUS). EHEC utilizes a type III secretion system (T3SS) to deliver /translocate the bacterial effector proteins into the host cell, which subvert cellular processes. The T3SS is essential for EHEC to colonize and survive inside host cells. This pathogen encodes many effectors identified by bioinformatics and biochemical analyses, which are located in the locus of enterocyte effacement (*LEE)* pathogenic island and outside the *LEE* locus. However, the roles of the majority of the effectors and/or other virulence factors encoded by the *LEE* and non-*LEE* loci remain yet to be understood. Currently, we are investigating the functionality of 72 effector-like proteins by using biochemical and cellular biology studies to ascribe cellular functions in virulence. These extensive and comprehensive approaches will provide insights into the molecular mechanisms of EHEC underlying the cellular process that produces virulence in the host cell.

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