FOURTEENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Chinsegut Hill Retreat

Brooksville, Florida
April 15-16, 2016

WELCOME

This year's AMCB symposium, the 14th in the existence of the program, will convene at Chinsegut Hill Retreat in Brooksville Florida. The location is significant for many reasons. It is the oldest house in Hernando County. Originally, built in c. 1847, the main section of the house was built between 1852 and 1854 and additions continued at various times up to 1933. The last private owner, Colonel Raymond Robins, was very active politically; guests at the house included Helen Keller, Jane Addams, William Jennings Bryan, Thomas Edison, J.C. Penney, Marjorie Kinnan Rawlings, Senator Claude Pepper, and U.S. Secretary of the Interior Harold L. Ickes. The house and surrounding land was donated to the Federal Government by Col. Robins in 1932. The Dept. of Animal Sciences at the University of Florida conducted much research here in conjunction with the USDA-ARS at the Subtropical Agricultural Research Station that was formed in large part land donated by Col. Robins.

Chinsegut Hill has also been a popular site for the AMCB. This year's symposium will mark the third time our group has held its symposium at Chinsegut Hill.

During the 2015-2016 academic year, the AMCB has continued to thrive. Faculty membership remains high at 16, the program has 13 PhD and 4 MS students, and ~60 attendees of the symposium are expected. Importantly, graduates of the program continue to do well in finding good positions. This year, two graduates of the AMCB, Eduardo Ribeiro and Kathryn Merriman, received the IFAS Award of Excellence for Graduate Research for Best PhD Dissertation and Best Master's Thesis, respectively.

As always, the AMCB Symposium is the capstone to the academic year. It is a time to share our science with each other, with our distinguished lecturer, Shawn Donkin, and with our guest from the University of South Florida, Ron Magness. It is also a great opportunity to build fellowship and have some fun!

Pete Hansen, Director
John Driver, Co-Director

ACKNOWLEDGMENTS

The faculty and students of the AMCB Program thank the following for support of the 14th Annual Research Symposium

Dr. Jacqueline K. Burns, Dean for Research and Director of the Florida Agricultural Experiment Station, IFAS, University of Florida

Dr. R. Elaine Turner, Dean, College of Agricultural & Life Sciences, University of Florida

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

L.E. "Red" Larson Endowment

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

Dr. Raluca Mateescu, Graduate Coordinator, Animal Molecular and Cellular Biology Graduate Program, University of Florida

Ms. Renee Parks-James, Program Assistant, Dept. of Animal Sciences, University of Florida

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

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

Special thanks to Jim Moss and company for preparing the Friday night meal

2016 AMCB DISTINGUISHED LECTURER

Shawn S. Donkin, PhD
Purdue University, West Lafayette, Indiana

Shawn Donkin is Professor of Animal Sciences and Assistant Dean for Agricultural Research and Graduate Education at Purdue University, West Lafayette, Indiana. He received the BSc degree with distinction from McGill University (Montreal) in 1982, an MS degree from The Pennsylvania State University in (1987), and a PhD from the University of Wisconsin-Madison (1992). Dr. Donkin has developed an internationally-recognized research program exploring the control of liver function and its importance to food animal production, animal well-being, and human health. Dr. Donkin's laboratory was among the first to describe the molecular events that control glucose metabolism in liver of dairy cattle and developing calves. Ongoing fundamental studies in food animals in Dr Donkin's lab explore the role of nutrition, physiological changes, and environmental stressors on genes critical to health and productivity. Key discoveries in the Donkin lab have exposed the role of pyruvate carboxylase in regulating gluconeogenesis and TCA cycle activity in ruminants. Collaborative research with colleagues in Nutritional Sciences has extended this role of pyruvate carboxylase to human health outcomes and identified this enzyme as a central mediator of response to vitamin D in energy partitioning and mammary cancer biology. Ongoing applied nutrition studies in ruminants evaluate the potential for alternative energy and protein feeds for transition and lactating cows and serve to link applied nutrition outcomes to hepatic metabolism. Dr. Donkin has been the primary mentor for 11 PhD students, 12 MS students, and 4 postdoctoral fellows, and has served on the advisory and examining committees for more than 40 additional graduate students. He has authored over 85 referred publications, over 125 abstracts, 8 extension publications, and 6 book chapters. On a personal note - one of Dr Donkin's favorite pastimes is riding his many cycles (9 bicycles and 2 unicycles at last count) and he usually logs about 6000 miles on the road each year.

AMCB FACULTY

John Bromfield, Department of Animal Sciences
Samantha Brooks, Department of Animal Sciences
Mary Brown, Department of Infectious Diseases and Pathology
Geoffrey Dahl, Department of Animal Sciences
John Driver, Department of Animal Sciences
Timothy Hackmann, Department of Animal Sciences
Peter Hansen, Department of Animal Sciences
Kwang Cheol Jeong, Department of Animal Sciences
Maureen Keller-Wood, Department of Pharmacodynamics
Jimena Laporta, Department of Animal Sciences
Raluca Mateescu, Department of Animal Sciences
Christopher Mortensen, Department of Animal Sciences
Corwin Nelson, Department of Animal Sciences
José Santos, Department of Animal Sciences
Stephanie Wohlgemuth, Department of Animal Sciences
Charles Wood, Department of Physiology and Functional Genomics

Emeritus Faculty

Lokenga Badinga, Department of Animal Sciences
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

Turky Omar H Asar (Advisor: G Dahl)

Leticia Del-Penho Sinedino (Advisor: J Santos)

Zaira Estrada Reyes (Advisor: R Mateescu)

Sossi Iacovides (Advisor: J Bromfield)

Mercedes Kweh (Advisor: C Nelson)

Choonghee Lee (Advisor: KC Jeong)

Chengcheng Li (Advisor: S Wohlgemuth)

Adriana Zolini (Advisor: P Hansen)

Veronica Negrón Pérez (Advisor: P Hansen)

M. Sofia Ortega (Advisor: P Hansen)

Luiz Siqueira (Advisor: P Hansen)

Paula Tribulo (Advisor: P Hansen)

Guan Yang (Advisor: J Driver)

MS Students

Laila Ibrahim (Advisor: J Bromfield)

Gulnur Jumatayeva (Advisor: P Hansen)

Michael Poindexter (Advisor: C Nelson)

Cheng Ye (Advisor: J Driver)

GRADUATES 2015

Kathryn E. Merriman, MS (Advisor: CD Nelson)
Currently dairy consultant, Standard Nutrition

Eduardo de Souza Ribeiro, PhD (Advisor: JEP Santos)
Currently assistant professor in Dept. of Animal Biosciences, University of Guelph

HISTORY OF THE AMCB RESEARCH SYMPOSIUM

YEAR	LOCATION	DISTINGUISHED LECTURER
2003	Whitney Laboratory St. Augustine, FL	Randy Prather University of Missouri
2004	Chinsegut Hill Brooksville, FL	John Dobrinsky USDA-ARS Beltsville, MD
2005	Chinsegut Hill Brooksville, FL	Doug Stocco Texas Tech University
2006	Lake Wauburg Gainesville, FL	Ina Dobrinski University of Pennsylvania
2007	Whitney Laboratory St. Augustine, FL	Doug Bannerman USDA-ARS, Beltsville, MD
2008	Cedar Cove Beach & Yacht Club Cedar Key, FL	Eckhard Wolf LMU Munich, Germany
2009	Plantation Golf Resort and Spa Crystal River, FL	Dean Betts University of Western Ontario
2010	Whitney Laboratory St. Augustine, FL	Marc-Andre Sirard Laval University
2011	Steinhatchee Landing Resort Steinhatchee, FL	Kimberly Vonnahme North Dakota State Univ.
2012	Holiday Isle Oceanfront Resort St. Augustine, FL	Rocío Rivera University of Missouri
2013	Harbor Front Hampton Inn Fernandina Beach, Florida	Martin Sheldon Swansea University
2014	Lakeside Inn Mount Dora, Florida	Cynthia Baldwin University of Massachusetts
2015	Jekyll Island Club Hotel, Jekyll Island, Georgia	Pat Lonergan & Trudee Fair University College Dublin
2016	Chinsegut Hill Retreat, Brooksville, Florida	Shawn Donkin Purdue University

SCHEDULE OF EVENTS

All events in the Learning Center unless otherwise noted

FRIDAY, APRIL 15

9:50 AM Pete Hansen
Welcome, introductory comments

Session 1: Genetics
Adriana Zolini and Choonghee Lee, Chairs

10:00 AM M. Sofia Ortega, Animal Sciences
Characteristics of single nucleotide polymorphisms in candidate genes associated with embryonic development in the cow

10:15 AM Mesfin Gobena, Animal Sciences
The effect of a single nucleotide polymorphism in the STAT6 *gene on resistance to internal parasites and production traits in small ruminants*

10:30 AM Zaira Magdalena Estrada Reyes, Animal Sciences
Genetic markers identification and genotyping for resistance to internal parasites in sheep and goat infected with Haemonchus contortus

10:45 AM Joel Leal, Animal Sciences
Association of SNPs in calpain and calpastatin genes with meat tenderness in an Angus-Brahman population

11:00 AM Samantha L Lewis, Animal Sciences
Candidate gene and marker for equine metabolic syndrome

11:15 AM Amy J. Dinerman, Animal Sciences
Polymorphism identification in candidate genes for Juvenile Idiopathic Epilepsy in the Arabian Horse

11:30 AM Laura Patterson Rosa, Animal Sciences
Inversion on STXBP5L *possible affecting intermediate speed locomotion patterns in the Mangalarga Marchador horse*

11:45 AM TOURS OF MANOR HOUSE (optional; preregistration required) and LUNCH (Dining Hall)

Session 2: Physiology and Nutrition
Guan Yang and Michael Poindexter, Chairs

1:30 PM Chengcheng Li, Animal Sciences
Age-associated attenuation of autophagy in skeletal muscle of Quarter Horses

1:45 PM Turky O. Asar, Animal Sciences
Effect of late gestation maternal heat stress on epigenetic patterns of dairy calves

1:45 PM Ana M. Mesa, Animal Sciences
Effect of exercise on ovarian function in cycling gilts

2:00 PM Camilo Lopera, Animal Sciences
Effect of level of dietary cation-anion difference (DCAD) and duration of prepartum feeding on calcium and measures of acid-base status in transition cows

2:15 PM Leticia D.P. Sinedino, Animal Sciences
Effects of feeding different types of polyunsaturated fatty acids on performance, plasma metabolites and fatty acid composition of milk in dairy cows

2:30 PM BREAK

Session 3: 2014 AMCB Distinguished Lecturer Presentation
John Driver, Chair

2:45 PM Professor Shawn Donkin
Dept. of Animal Sciences, Purdue University
On the Connectedness of Cows, Carbon Cycles, and Cancer

3:45 PM BREAK AND CHECK INTO ROOMS

Session 4: Infectious Disease and Inflammation
Chengcheng Li and Cheng Ye, Chairs

4:30 PM Choonghee Lee, Animal Sciences and Emerging Pathogens Institute
Whole genome analysis of a predominant Escherichia coli *o157:h7 reveals unique genetic features that may enable bacteria to colonize proficiently in host animals*

4:45 PM Federico Cunha, Large Animal Clinical Sciences
Droplet digital PCR quantification of uterine bacteria associated with metritis in lactating dairy cows

5:00 PM Sossi M. Iacovides, Animal Sciences
Granulosa cells do not exhibit oxidative stress in response to bacterial lipopolysaccharide

5:15 PM Rachel L. Piersanti, Animal Sciences
Tumor necrosis factor-alpha alters cumulus oocyte expansion and interleukin-6 production during bovine in vitro maturation

5:30 PM Laila A. Ibrahim, Animal Sciences
Improving calving rates in dairy cows by infusion of seminal proteins at insemination

5:45 PM BREAK

6:00 PM GROUP PICTURE, MANOR HOUSE

6:30 PM COOKOUT

SATURDAY, APRIL 16

7:30-9:00 AM BREAKFAST, Dining Hall

Session 5: Immunology
Turky Omar H Asar and Sossi Iacovides, Chairs

9:00 AM Mercedes F. Kweh, Animal Sciences
There is a missing link in the immunoregulatory mechanism of the vitamin D pathway

9:15 AM Michael Poindexter, Animal Sciences
Effects of feeding 25-hydroxy vitamin D on innate immunity and mastitis resistance in lactating dairy cows

9:30 AM Achilles Vieira-Neto, Animal Sciences
Use of 1,25-dihydroxyvitamin D3 to maintain postpartum blood calcium and improve immune function in dairy cows

9:45 AM Cheng Ye, Animal Sciences
Gene targeting the costimulatory molecule CD70 attenuates autoimmune diabetes in NOD mice

10:00 AM Guan Yang, Animal Sciences
Characterization of natural killer T cell subsets in pigs

10:15 AM Bianca L. Artiaga, Animal Sciences
Therapeutic activation of NKT cells in pigs infected with influenza improves disease outcome and reduces virus shedding

10:30 AM BREAK

Session 6: Embryology
Mercedes Kweh and Zaira Estrada Reyes, Chairs

10:45 AM Adriana Zolini, Animal Sciences
Effects of L-carnitine on development and cryotolerance of bovine embryos produced in-vitro

11:00 AM Gulnur Jumateyeva, Animal Sciences
Influence of sex on response of bovine embryos to endogenous cannabinoids

11:15 AM Verónica M. Negrón Pérez, Animal Sciences
Role for CCL24 in differentiation of the inner cell mass of the bovine embryo

11:30 AM Paula Tribulo, Animal Sciences
Developmental changes in canonical WNT signaling in the preimplantation bovine embryo

11:45 AM Luiz G.B. Siqueira, Animal Sciences and EMBRAPA Gado de Leite
Male-female differences in basal and CSF2-regulated gene expression in the bovine morula

12:00 PM Closing (and very brief) remarks, Pete Hansen

12:00 PM TOURS OF THE MANOR HOUSE (optional; preregistration required)

ABSTRACTS

(Arranged alphabetically by first author)

Therapeutic activation of NKT cells in pigs infected with influenza improves disease outcome and reduces virus shedding

Bianca L. Artiaga[1*], Guan Yang[1*], Julia C. Loeb[2, 3], Jürgen A. Richt[4], Shahram Salek-Ardakani[5], William L. Castleman[6], John A. Lednicky[2, 3], and John P. Driver[1]

[1]Dept. of Animal Sciences, University of Florida, Gainesville, FL; [2]Dept. of Environmental and Global Health, University of Florida, Gainesville, FL; [3]Emerging Pathogens Institute, University of Florida, Gainesville, FL; [4]College of Veterinary Medicine, Kansas State University, Manhattan, KS; [5]Dept. of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL; [6]Dept. of Infectious Diseases and Pathology, University of Florida, Gainesville, FL.
* These authors contributed equally to this study.

Swine influenza (SI) is an acute respiratory disease that often leads to secondary respiratory diseases and results in substantial losses for the pig industry. Currently, the most efficient strategies to control SI are preventative, including implementing strict biosecurity measures and vaccinating pigs at risk of disease. Unfortunately, due to the high mutation rate of influenza A viruses (IAVs), vaccination effectiveness is usually low as vaccine strains often do not match viruses circulating in the field. Also, there are no available treatments for pigs already infected with IAVs, which is greatly needed for limiting IAV transmission and production losses. Invariant natural killer T (NKT) cells are a lymphocyte population capable of potently stimulating innate and adaptive immune responses against numerous infectious diseases. We previously demonstrated that these cells can be activated in swine using therapeutic glycosphingolipid antigens [e.g. alpha-galactosylceramide (αGC)] to produce powerful adjuvant effects that improve vaccine responses against SI virus. In mice, activation of NKT cells by αGC at the time of influenza challenge improved disease outcome and decreased lung immune pathology. Therefore, we hypothesized that NKT cell agonists have exciting potential for antiviral therapy against infectious diseases in NKT cell-expressing livestock species, including pandemic H1N1 SI. Thus, piglets (n=3) were challenged with CA04 and immediately administered αGC (100 μg/kg) by the intranasal route (IN αGC/CA04) or by intramuscular injection (IM αGC/CA04). Another group of virus-infected pigs were administered media via the intranasal route (Mock/CA04). Two additional piglets were mock infected and mock treated (Mock/Mock). We found that intranasal but not intramuscular administration of αGC decreased viral replication in the respiratory tract. Moreover, viral titers in nasal swabs collected from IN αGC treated pigs were significantly reduced when compared to the other virus-infected groups. This was associated with a better disease course including amelioration of symptoms and restoration of body weight gain. These results suggest activation of NKT cells within the airway has potential to inhibit the replication of IAVs in swine and limit the impact of this disease for swine producers. Our results also demonstrate the potential of using NKT cell therapy to treat humans infected with influenza viruses.

Effect of late gestation maternal heat stress on epigenetic patterns of dairy calves

T. O. Asar, F. Peñagaricano and G. E. Dahl

Department of Animal Sciences, University of Florida, Gainesville FL

Epigenetics is the study of heritable changes in gene function that occur because of chemical modification to nucleic acids rather than DNA sequence changes. DNA methylation is an epigenetic event that results mostly in the silencing of gene expression and may be passed on to the next generation. Data of animals obtained from previous experiments conducted during five consecutive summers in Florida were used as a model of environmentally induced epigenetic effects. In those studies, cows were dried off 46 days before expected calving and randomly assigned to one of two treatments, heat stress (HT) and cooling (CL). CL cows were housed with sprinklers, fans and shade, whereas only shade was provided to HT cows. The cows began treatment approximately 46 d before parturition, and continued on the treatment until calving (i.e. the dry period). Birth weight, growth rate, and milk production in the first lactation from HT and CL heifers were analyzed. These analyses indicated that there are substantial differences between HT and CL of heifers in terms of body weight and height up to one year of age. More importantly, we observed significant differences in milk production in the first lactation. Compared with CL heifers, HT heifers produced less milk up to 35 weeks of the first lactation (26 ± 1.7 vs. 31.9 ± 1.7 kg/d; $p = 0.03$; Monteiro et al., 2014). To detect potential differences in DNA methylation, liver samples were collected from 10 bull calves born to cows that experienced maternal heat stress or cooling. Reduced Representation Bisulfite Sequencing (RRBS) is used to profile DNA methylation. The samples were subjected to DNA isolation, enzymatic digestion (MSP1 + TaqI), library preparation, bisulfite conversion, Bioanalyzer QC, and multiplex NGS on an Illumina HiSeq2500. After getting the sequencing data, FastQC is done to check the quality of the sequencing reads. After trimming low quality bases using the Trimmomatic tool, Fast QC will be repeated to check the results and to begin mapping the reads against the bovine reference genome. Then, methylome analysis will be done in order to determine if significant differences are apparent between CL and HT groups in terms of 5-methyl-cytosine, the most common form of DNA methylation. We hypothesize that environmental factors, such as heat stress exert the dramatic effects on the phenotype through epigenetic mechanisms. Further, phenotypic differences of the successive generations may persist due to epigenetic effects. Recent studies investigating different physiological states suggest that methylation may also play an acute, regulatory role in gene transcription. The first aim of this project will be to investigate the effect of maternal environmental (heat stress) factors and how it would modify the epigenome to develop stable alteration of the phenotype. Additionally, if this change in DNA methylation in mature animals would transfer to successive generations is unknown. Thus, a second aim is to test for similar methylation patterns in offspring of the founder calves. The third aim is to determine specific genes that may be affected by in utero heat stress and have a global effect, such as DNMT1. Collectively, these studies should provide insight to the mechanisms of in utero induced differences in mature phenotype.

Droplet digital PCR quantification of uterine bacteria associated with metritis in lactating dairy cows

Federico Cunha, Soo Jin Jeon, Anthony F. Barbet, Kwang C. Jeong, Carlos A. Risco, and Klibs N. Galvão

Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL

Metritis in lactating dairy cows is widely considered to arise from mixed bacterial infections, primarily by pathogenic gram negative facultative and obligate anaerobes. The objective of this study was to quantify the number of potentially pathogenic uterine bacteria that have been associated with metritis and fever in lactating dairy cows. Based on relative abundance data of recent metagenomic studies we hypothesized that F. necrophorum, B. heparinolyticus, B. pyogenes, P. levii, and H. ovis will be present in higher numbers in the uterus of metritic cows than in healthy cows, and that metritic cows will not have a significantly higher quantity of E. coli, P. Melaninogenica, and T. pyogenes compared to healthy cows. Droplet digital PCR, a system that works by partitioning PCR samples into ~1 nL individual reactions and estimating copy number based on the ratio of positive to negative reactions, was used for bacterial quantification. Raw data were log10 transformed before analysis. Cows in the Metritis no fever (MNoFever) and Metritis with fever (MFever) had a mean overall bacterial count one order of magnitude greater than the mean bacterial count of the Healthy group (7.8 ± 0.2 vs. 7.5 ± 0.2 vs. 6.8 ± 0.2; $P < 0.05$). This higher bacterial count is in accordance with the fact that metritis is caused by mixed bacterial infection. Higher bacterial abundance for MNoFever and MFever cows than for Healthy cows was observed for F. necrophorum (6.2 ± 0.4 vs. 6.5 ± 0.4 vs. 3.8 ± 0.4; $P < 0.001$), B. pyogenes (5.3 ± 0.4 vs. 5.9 ± 0.4 vs. 3.8 ± 0.4; $P < 0.02$), and P. levii (6.0 ± 0.4 vs. 5.8 ± 0.4 vs. 4.3 ± 0.4; $P < 0.04$). There was no difference in bacterial abundance among MNoFever, MFever and Healthy groups for E. coli (3.4 ± 0.4 vs. 3.9 ± 0.4 vs. 3.3 ± 0.4; $P = 0.46$) and T. pyogenes (4.7 ± 0.2 vs. 4.5 ± 0.2 vs. 4.3 ± 0.2; $P = 0.44$), and they were present at a relatively low abundance compared to other species. These findings suggest E. coli and T. pyogenes are not significant components in the mixed infection of the uterus at 6 ± 3 DPP. MFever cows had a significantly higher copy number of P. melaninogenica than healthy cows (4.10 ± 0.23 vs. 3.01 ± 0.23; $P = 0.006$); however, the biological significance of this finding is unclear due to the low abundance of this bacterium. F. necrophorum, B. pyogenes, and P. levii may be important factors in the etiology of metritis. Fever associated with metritis is not dependent on bacterial load but is more likely dependent on host response.

Polymorphism identification in candidate genes for Juvenile Idiopathic Epilepsy in the Arabian Horse

Amy J. Dinerman, Heather M. Holl, and Samantha A. Brooks

Department of Animal Sciences, College of Agriculture and Life Sciences, University of Florida, Gainesville, FL

As an economically important species, the health of the Arabian horse is paramount for breeders. Genetic selection for athletic ability, temperament, trainability, and above all, health is vital for the current and future success of the breed. Here we describe a candidate gene study exploring the basis of Juvenile Idiopathic Epilepsy (JIE) in the Arabian. The phenotype presents shortly after birth, from 2 days to 6 months of age. Affected foals are stricken with clusters of classic tonic-clonic seizures, in addition to blindness, lethargic behavior, and a decrease or loss of the menace response. Fatality can occur if seizures cannot be controlled pharmacologically; however, most horses outgrow seizures by 18 months of age and are healthy as adults, perpetuating this deleterious allele if they are used as breeding stock. As JIE is a rare condition in the Arabian horse, few affected individuals are available for study. Thus, utilization of a candidate gene sequence approach was used to identify mutations in a small sample set. We targeted two candidate genes, *KCNQ2* and *KCNQ3*, which encode potassium voltage gated channels within the pyramidal neurons in the brain. We identified a promising single nucleotide polymorphism (SNP) in exon 14 of *KCNQ3* that may contribute to the disease. This C/G switch when modeled in Expasy, changed the tertiary structure of the protein in the presence of the alternate allele. After analysis, the change from arginine to cysteine in the identified Ankyrin G-binding motif was predicted to be detrimental to protein function (SIFT $p = 0.03$). The alternate allele is frequent in JIE cases and parents but not all cases are homozygous ($X^2 = 0.134$). Ongoing sample collection to increase statistical power is still underway and future work will verify the association with this SNP. Additionally, full genome sequencing of an affected individual is complete and analysis is underway to identify any other novel mutations present. This approach aims to provide a genetic test to identify affected and carrier individuals to screen breeding stock and reduce the incidence of JIE affected foals.

Genetic markers identification and genotyping for resistance to internal parasites in sheep and goat infected with *Haemonchus contortus*

Zaira Magdalena Estrada Reyes*, Arthur L. Goetsch#, Terry A. Gipson#, Zaisen Wang#, Megan Rolf†, Tilahun Sahlu#, Ryszard Puchala#, Steve Zeng# and Raluca Mateescu*

*Department of Animal Sciences, University of Florida, Gainesville, FL, #American Institute for Goat Research, Langston University, Langston, OK, †Department of Animal Sciences, Oklahoma State University, Stillwater, OK

Gastrointestinal nematode infections (GNI) have a great economic impact for small ruminant production in humid areas including temperate, tropical and subtropical regions of the world. In these regions, *Haemonchus contortus* is the most important gastroenteric nematode. Unfortunately, the massive and indiscriminate use of anthelmintic drugs to control GNI has generated resistance to these chemical compounds. Among the alternative strategies proposed, the genotypic and phenotypic variability of the small ruminants have encouraged the identification of the most resistant animals. Faecal egg count (FEC) is the standard method for assessing the level of resistance in individuals infected with *H. contortus*. Research is focused on the host genome and the analysis of the genetic basis associated with parasite immunity in order to identify genetic genotypes. In order to identify genetic markers associated with the control of nematode populations within the host, the detection of single nucleotide polymorphisms (SNPs) HLA-DRA20 gene was performed in sheep and goats experimentally infected with *H. contortus*. Animals from 3 different breeds of sheep and goat were used for the study during three year of evaluation. Individuals were selected by using positive assortative matting of the most resistant individuals each year. Before the trial starts, animals were treated with levamisole (7.5 mg/kg of live weight), to eliminate any possibility of an accidental GIN infection. Individuals received a complete diet (15% Crude Protein) *ad libitum* for the duration of the trial. Each experimental animal was infected with 10,000 L_3 of *H. contortus* per kg of body weight per oral route. Fecal samples were obtained to determine fecal egg count using the modified Mc Master technique. Blood samples were collected from the jugular vein with sterile vacuum tubes with sodium heparin to evaluate blood package cell volume (PCV) and levels of IgA, IgM and IgG. DNA were purified from blood samples using DNeasy Blood & Tissue Kit (Qiagen). One SNP in the HLA-DRA20 segregating in this population was analysed using High Resolution Melting assays and three genotypes were observed (AA, GA, GG). A GLM was fitted with MPCV, DMI, ADG, RFI, IgM, IgG, IgA levels and genotype as predictors and a mean of FEC as the response variable. According the results, the best significant predictors to fit the model were Genotype, Breed (Species), MPCV, DMI, ADG and genotype ($p < 0.05$). In conclusion, the polymorphism in the HLA-DRA20 gene could have an important role in the immune mechanisms against *H. contortus* infections in sheep and goats. Indeed, these results provided evidence that there is a significant difference among FEC and production traits between breeds within species.

The effect of a single nucleotide polymorphism in the *STAT6* gene on resistance to internal parasites and production traits in small ruminants

M. Gobena[†] , Y. Tsukahara[#], A. L. Goetsch[#], T. A. Gipson[#], S. P. Hart[#], L. J. Dawson[#], Z. Wang[#], R. Puchala[#], T. Sahlu[#], and R. Mateescu[†]

[†]Department of Animal Sciences, University of Florida, Gainesville, FL and [#]American Institute for Goat Research, Langston University, Langston, OK

The main objective of this study is to develop genetic markers for resistance to internal parasites in order to facilitate efforts to selectively breed animals for parasite resistance. Among the candidate genes being considered in this study is *STAT6* which codes for a transcription factor for interleukin-4. A total of 130 sheep (3 breeds) and 141 goats (3 breeds) were dewormed before being challenged by infection with *Haemonchus contortus* larvae. Fecal egg count (FEC), average daily gain (ADG), dry matter intake (DMI), residual feed intake (RFI), IgA, IgG and IgM were measured post infection. The animals were genotyped for a synonymous single nucleotide polymorphism that is located in exon 7 of the *STAT6* using PCR-RFLP and High Resolution Melt, and three genotypes (AA, GG, and AG) were observed. Linear regression models were fit with genotype, breed and year as predictors and FEC (square root transformed), DMI (log transformed), ADG, RFI and immunoglobulin levels as response variables. There was marginally significant difference in FEC between different breeds (P=0.077) but not between genotypes (P=0.108) and years (P=0.386) among goats. In sheep, FEC was significantly different between breeds (P=0.025) and years (P=0.006) but not between genotypes (P=0.942). IgA level was significantly different between years (P<0.001) marginally different between genotypes (P=0.075) and not different between breeds (P=0.306) among goats. Within sheep, IgA level was significantly different between breeds (P<0.001) and years (P<0.001) but not genotypes (P=0.46). IgG level showed significant difference between years (P<0.001) and breeds (p<0.001) but not genotypes (P=0.53) among goats. Similarly, in sheep, IgG level was significantly different between breeds (P<0.001) and years (P<0.001) but not between genotypes (P=0.69). IgM level was significantly different between years (P<0.001) and breeds (P<0.001) but not genotypes (P=0.301) among goats. IgM level was significantly associated with year (P<0.001) but not breed (P<0.111) and genotype (P=0.775) within sheep. ADG showed significant difference between breeds (P<0.001) but not genotypes (P=0.762) and years (P=0.984) among goats. Within sheep, there were significant differences between breeds (P<0.001) and years (P=0.036) but not genotypes (P=0.191) in ADG. There were significant differences in DMI between years (P<0.001) and breeds (P=0.002) but not genotypes (P=0.398) among goats. In sheep, DMI was significantly associated with breed (P<0.001) and year (P<0.001) but not with genotype (P=0.971). There was significant difference between genotypes (P=0.019) but not between years (P=0.986) and breeds (0.61) in RFI among goats whereas there was no significant difference between years (P=0.977), breeds (P=0.841) and genotypes (P=0.493) in sheep. Although polymorphism in STAT6 was not associated to resistance to internal parasites, it showed strong association to RFI in goats. The results also showed that there is considerable difference between breeds within both sheep and goats in terms of resistance to internal parasites and production traits. These results provide further evidence that selection can improve resistance to internal parasites in small ruminants.

Granulosa cells do not exhibit oxidative stress in response to bacterial lipopolysaccharide

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Bacterial infections of the uterus occur in approximately 40% of postpartum dairy cows. Following uterine infection cows have reduced fertility. It is currently unknown how uterine infection results in infertility of cows after the resolution of infection. Following the clearance of uterine infection cows display retarded follicle growth, reduced cyclicity and extended luteal phases, suggesting the ovary as a potential secondary target of uterine infection. Bacterial factors such as the cell wall component lipopolysaccharide have been shown to stimulate an innate immune response by non-hematopoietic cells of the endometrium and ovarian follicle through the Toll-like receptors. Elevated reactive oxygen species are indicative of cellular stress and are further elevated in phagocytic immune cells to kill pathogenic bacteria. We hypothesize that lipopolysaccharide will increase oxidative stress in granulosa cells. Bovine ovaries were collected from healthy cows at slaughter and transported to the laboratory. Growing follicles (4-8 mm) were aspirated to collect granulosa cells which were then cultured for 48 hours before treatment. Blood was collected from cows and leukocytes concentrated by centrifugation to be used as positive controls. Leukocytes or granulosa cells were treated with the antioxidant n-acetyl cysteine, the oxidative agent menadione or one of five concentrations of ultra-pure LPS (1 ng/ml – 10 μg/ml). Following treatment for 1 or 24 hours cells were stained using CellROX to quantify oxidative stress using flow cytometry. CellROX staining was increased in leukocytes exposed to menadione or LPS for 1 hour compared to vehicle treated controls (7.9-fold and 15.6-fold, respectively). The addition of the antioxidant n-acetyl cysteine reduced both menadione and LPS induced CellROX staining to basal levels in leukocytes. Treatment of granulosa cells with the oxidative agent menadione for 1 hour increased CellROX staining 4.4-fold compared to vehicle treated controls. However, treatment of granulosa cells with LPS for 1 hour did not induce any detectable increase in CellROX staining. Treatment of either leukocytes or granulosa cells with LPS for 24 hours did not increase CellROX staining. Next we will quantify expression of genes involved in reactive oxygen defense: superoxide dismutase 1 (*SOD1*), superoxide dismutase 2 (*SOD2*), glutathione reductase (*GSR*), and glutathione disulfide (GSSG). We do not expect an upregulation of genes involved in reactive oxygen defense in granulosa cells exposed to lipopolysaccharide. Granulosa cells have been demonstrated to exhibit phagocytic properties comparable to leukocytes, here our data suggests granulosa cells do not induce reactive oxygen when stimulated by pathogen associated molecules.

Improving calving rates in dairy cows by infusion of seminal proteins at insemination

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For decades, seminal plasma has been known as a transport, survival and nourishment medium for mammalian sperm. However, a new role for seminal plasma is emerging to optimize reproductive outcomes by targeting the female reproductive tract. Briefly, seminal plasma acts as a key regulator of the female tract environment providing optimal support for the developing embryo. The cellular and molecular environment of the uterus is critical for implantation success and optimal foetal and placental development. In humans, rodents and swine seminal plasma contains immuno-regulatory molecules which induce strong changes within the lining of the uterus (endometrium). It is unknown if seminal plasma from the bovine contains such immuno-regulatory molecules or has the ability to modulate the uterine environment following insemination. Here, we hypothesize that infusion of seminal plasma during bovine artificial insemination will increase pregnancy rates and decrease pregnancy loss in dairy cows by altering the molecular and cellular environment of the early pregnant uterus. The objective of our current project is to identify active signaling factors in bovine seminal fluid and examine their contribution to modulating the cellular and molecular environment of the early pregnant reproductive tract. Firstly, we will evaluate bovine seminal plasma for the presence and concentration of significant seminal proteins identified in other species, namely TGFβ and PGE_2. Using a developed endometrial explant model we will then evaluate cellular and molecular changes occurring within the female endometrium following exposure to seminal plasma. This in vitro model will also allow us to identify unique bovine seminal moieties responsible for inducing changes to the endometrial environment. Finally, we will evaluate the responsiveness of the endometrium in cows following seminal plasma exposure at the time of artificial insemination. Evaluation of the endometrium following seminal plasma exposure (or seminal proteins) will provide us novel information on the endometrial environment which is potentially lacking after traditional artificial insemination. Ultimately we aim to develop a new protocol whereby targeted seminal proteins can be added back to semen at the time of artificial insemination to optimize reproductive outcomes in commercial dairy herds.

Influence of sex on response of bovine embryos to endogenous cannabinoids

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Development of the mammalian embryo is dependent on molecules produced by the female reproductive tract called embryokines that regulate embryonic growth, differentiation and other aspects of cellular function. One putative embryokines is anandamide (N-arachidonoylethanolamine, AEA), an endogenous cannabinoid derived from arachidonic acid, which has been found to be produced in the uterus of several species. Cannabinoids signal through two G protein-coupled receptors called CNR1 and CNR2. In mice, low concentrations of AEA (7-14 nM) stimulate development of embryos to the blastocyst stage while higher concentrations AEA (28 nM) inhibit development. Recently, we have shown that the bovine morula expresses *CNR2* and that expression is greater for female embryos than male embryos. Thus, it is possible that female embryos are affected by AEA differently than for male embryos. Two series of experiments are planned to address the role of endogenous cannabinoids in embryonic development in cattle. The first will be to quantify amounts of AEA in uterine flushings of cows from Days 0-7 of the estrous cycle. Amounts of AEA will be quantified by liquid chromatography-mass spectrometry. The second will be to test the hypothesis that AEA regulates competence of embryos to develop to the blastocyst stage and that female embryos are more sensitive to AEA than males. Embryos will be produced using X- or Y-sorted sperm and treated with concentrations of AEA ranging from 7-28 nM. The number of blastocysts on day 7 after development will be determined. Also, number of inner cell mass and trophectoderm cells will be measured to test whether AEA affects differentiation of the blastocyst.

There is a missing link in the immunoregulatory mechanism of the vitamin D pathway

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Vitamin D insufficiency has been associated with elevated risk of bacterial and viral infections. Previous studies have shown that in cattle the underlying basis for the association between vitamin D and disease risk is vitamin D-mediated activation of the antibacterial peptide response, such as regulation of the inducible nitric oxide synthase (iNOS) and β-defensin genes. We have shown that this protective action against bacterial pathogens is in part mediated through crosstalk between the vitamin D, toll-like receptor and interferon-γ (IFN-γ) pathways, yet the mechanism by which vitamin D regulates these responses remains unclear. In order to shed further light on the role of vitamin D in regulation of antibacterial genes, we investigated the effects of the protein synthesis inhibitor, cycloheximide (CHX) on vitamin D-mediated gene expression in bovine monocytes. Bovine monocytes were treated with either 1,25-dihydroxyvitamin D_3 (1,25D; 4 ng/mL) or cycloheximide (10 μg/ml), or a combination of both at zero and four hours. Cells were cultured for 12 h and RNA was extracted and used for RT-PCR and qPCR. Suprisingly, there was a >3000 fold increase in expression of *CYP24A1*, the gene encoding the vitamin D catabolic enzyme, with treatment of both 1,25D and CHX when compared to 1,25D alone. Although treatment of cells with CHX partially inhibited 1,25D-induced iNOS expression (>350 fold), 1,25D upregulated iNOS (>350 fold) in the presence of CHX compared to CHX alone. In contrast, CHX treatment in the presence of 1,25D completely suppressed the expression of β-defensin genes *BNBD3* (>150 fold), *BNBD4* (>100 fold), *BNBD6* (>150 fold), *BNBD7* (>250 fold), and *BNBD10* (about 100 fold), compared to 1,25D alone. Interestingly, expression of *BNBD5,* which is normally hindered by 1,25D treatment, was recovered by treatment with 1,25D and CHX. In addition, CHX in the presence of 1,25D decreased (>200 fold) the expressions of *S100A12,* a gene involved in the calcium-dependent signal transduction pathway and *IL1β,* the lymphocyte activating factor, compared to 1,25D alone. Consequently, we hypothesize an intermediate factor directly regulated by the vitamin D receptor/retinoid X receptor complex is responsible for several of the vitamin D-mediated responses (e.g. β-defensin genes) of bovine monocytes. Further studies are needed to identify direct vitamin D target genes which may be central in elucidating the antibacterial actions of vitamin D.

Association of SNPs in calpain and calpastatin genes with meat tenderness in an Angus-Brahman population

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Meat quality traits are important in customer purchasing decision and eating satisfaction. Ability to deliver a consistently superior quality product is important if beef industry is to maintain and expand its share of the market. Meat quality could be described by several sensorial features, like tenderness, juiciness and flavor. Tenderness is the most important attribute and an objective measure of tenderness is the Warner-Bratzler Shear Force (WBSF) which measures the force required to shear a cooked steak after post-mortem ageing. The challenge with these quality traits is that they are complex traits, controlled by many genes and by the environment. Many of these traits are measured after the animal is slaughtered, are difficult and costly to measure and have relatively low heritability. Genomic selection provides the best strategy to implement a genetic improvement program for these traits. The Calpain/Calpastatin enzymatic system has been extensively evaluated in tenderness association studies of beef quality and several polymorphisms in these genes have been reported previously as associated with phenotypic variation in bovine populations. This project examined the association of three single nucleotide polymorphisms (SNPs) in calpain and calpastatin genes with tenderness measured by WBSF. One 2.54-cm steak from 173 steers from a multibreed Angus-Brahman population was removed, vacuum packaged, aged for 14 d from the harvest date at 2°C and frozen at -20°C. Steaks were cooked to an internal temperature of 68ºC Six cores, 1.27-cm in diameter, were removed parallel to muscle fiber orientation and sheared once, using a Warner-Bratzler head attached to an Instron Universal Testing Machine. The Warner- Bratzler head moved at a crosshead speed of 200 mm/min. Peak load (kg) of each core was recorded and mean peak load (kg) was analyzed for each sample. Three SNPs: Capn-316, Cast1 and Cast5 were genotyped by RFLP procedure. Capn-316 is a missense C/G SNP in the exon 9 of μ-Calpain, Cast1 is a C/G polymorphism in intron 9 of the Calpastatin gene, and Cast5 is an A/G change in exon 35 located in the 3'UTR of Calpastatin gene. The allelic and genotypic frequencies were calculated using Proc Frec procedure of SAS. A regression model was fitted using WBSF as response variable in a General Linear Model procedure in SAS. Live weight at slaughter, age at slaughter, degree of marbling in the ribeye, subcutaneous fat, ribeye area, cooking loss, breed group and Capn-316, Cast1 and Cast5 genotypes were used as possible predictors. All three SNPs were polymorphic in this population. The genotypic frequencies for Capn-316 were: 4.17% CC, 43.45% GC, 52.38% GG; for Cast1 were 59.04% CC, 40.96%GC; and for Cast5 were 60.23% AA, 36.26% GA, 3.51% GG. Among predictors, weight at slaughter, cooking loss and marbling score were significant. The association of each SNP with WBSF was analyzed by including cooking loss and marbling score as covariates, and the effect of Capn-316 and Cast1 were found to be significant. Meat from animals with genotype GG and GC in Capn-316 were more tender (5.03 ± 1.24 kg and 4.67 ± 1.39 kg) than CC animals (6.25 ± 0.88 kg). For Cast1, steers with GC genotype had more tender meat (4.59 ± 1.40 kg) than CC animals (5.20 ± 1.23 kg). Marker Cast5 had no significant effect on WBSF.

Whole genome analysis of a predominant *Escherichia coli* o157:h7 reveals unique genetic features that may enable bacteria to colonize proficiently in host animals

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Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important foodborne pathogen causing outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS). Cattle are a major asymptomatic reservoir of STEC O157 and this pathogen colonizes primarily in the terminal recto-anal junction (RAJ) of cattle. Cattle shedding O157 of more than 10^4 CFU/g of feces are regarded as super-shedders. Since STEC O157 is able to survive in animals, drinking water, and feces, it can be transmitted easily to other hosts. Predominant STEC O157 strains, which are well adapted to hosts and environments, are responsible for a large part of O157 outbreaks. Previous studies have revealed that an *E. coli* O157:H7 subtype strain (FRIK2455) was predominant on the farm R while other clonal variants were rarely isolated (FRIK2069 and FRIK2533). However; genetic factors of these predominant isolates that may explain their dominance in cattle are not well understood. In this study, we conducted whole genome sequencing to identify genetic factors that may confer predominance in cattle. By conducting comparative genome analysis of those genomes, we found that these strains share similar genetic composition and structure, but distinct features in plasmids. Only the predominant strain FRIK2455 carries a plasmid, p35K that encodes a type IV secretion system (T4SS) that may provide an advantage for survival in hosts and environments. Further analysis will focus on understanding molecular mechanisms of T4SS in the predominant strains in hosts.

Candidate gene and marker for equine metabolic syndrome

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Increasing equine obesity rates, comparable to human and other companion animal populations, give rise to loss of use and life-threatening secondary chronic disease. High insulin levels often characterize the primary disease associated with equine obesity, Equine Metabolic Syndrome (EMS). Due to clinical similarities with other diseases, Equine Cushing's disease (PPID) and Hypothyroidism, diagnosis of EMS proves challenging. Other than diet and exercise, few treatments are available and effective for EMS; emphasizing the need for genetic testing to identify at risk individuals to implement preventative measures. A previous genome-wide association study, using horses with EMS and/or PPID and exhibiting severe lameness, revealed statistically significant markers for the condition near a single candidate gene, *FAM174A*. A single study describing the function of this gene suggests it may play a role in cholesterol homeostasis. Sequencing of the *FAM174A* gene in EMS affected Arabian horses identified at least five polymorphic haplotypes. In this study, additional samples from a larger population of horses diagnosed with EMS disease were genotyped by Sanger sequencing for polymorphisms in the *FAM174A* gene and the results assessed for association with the EMS condition. Additionally, we genotyped the most significant marker SNP from previous GWAS, BIEC2-263524, by High Resolution Melt (HRM). An 11-guanine homo-polymer allele in a 3' UTR of *FAM174A* correlated with both elevated insulin values (p=0.0082) and BCS >6.5 (p=0.0116). An A→G substitution at the BIEC2-263524 marker SNP displayed similar associations to elevated insulin values (p=0.0060) and BCS>6.5 (p=0.0049). The G allele at BIEC-263524 presented in 11-G allele horses at a 95% frequency, indicating strong LD across this haplotype. Further analyses with larger samples sizes and Genotype by Sequencing is currently underway. Confirmation of the association between these markers and the EMS condition will enable genetic tests for the horse as a helpful tool in diagnosing and preventing EMS, as well as improve our understanding of the etiology of this troubling condition. The *FAM174A* locus may also prove an interesting candidate gene for genetic causes of human obesity.

Age-associated attenuation of autophagy in skeletal muscle of Quarter Horses

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In our previous study, we found that mitochondrial function decreased in both *gluteus medius* and *triceps brachii* muscle from old Quarter Horses, and that the mitochondrial density, indicated by citrate synthase activity, decreased only in *triceps* muscle from aged Quarter Horses. One underlying cause for the age-associated decrement of mitochondrial density and function could be an impairment of mitochondrial quality control mechanisms. Mitochondrial biogenesis and selective degradation of damaged mitochondria (autophagy) are two of the most prominent quality control mechanisms that have been described. The balance between those two processes is necessary to maintain a healthy population of mitochondria. The objective of the present study was to investigate the underlying mechanisms that caused the age-associated decline in mitochondrial density and function in equine skeletal muscle by analyzing the expression of proteins involved in mitochondrial biogenesis and autophagy pathways. Muscle biopsies of the *gluteus* and *triceps* muscles were collected from young (1.8±0.1 y; n=24) and aged (20±5 y; n=12) Quarter Horses, subsequently frozen in liquid nitrogen and stored at -80°C until further biochemical analysis. Frozen muscle samples were homogenized and prepared for protein analysis using Western Blot. Statistical differences between ages and muscle groups were analyzed using Two-Way ANOVA and Holm-Sidak post hoc analysis (Sigmaplot 12.0). Consistent with previously assessed enzyme activity data, we found that the protein expression of citrate synthase was decreased in aged *triceps* muscle *(*$P<0.05$), but not in aged *gluteus* muscle. Interestingly, the expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a master regulator of mitochondrial biogenesis, was not affected by age in either muscle. Age had no effect on expression of autophagy protein 7 (Atg7) in either muscle, but significantly decreased protein expression of autophagy protein 5 (Atg5; $P<0.05$) and the autophagosome-bound form of microtubule-associated protein 1 light chain 3 (MAP-LC3-II; $P<0.05$) in *triceps* muscle; and increased protein expression of ubiquitin-binding protein p62 in both *gluteus* and *triceps* muscle ($P<0.05$ for both). The decrease of LC3II and the accumulation of the autophagosome cargo protein p62 are often used as indicators of impaired autophagic flux. Taken together, our data suggest that mitochondrial density and autophagic activity but not mitochondrial biogenesis were impacted by age in equine *triceps* muscle.

Role for CCL24 in differentiation of the inner cell mass of the bovine embryo

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During blastocyst formation in the cow embryo, the chemokine (C-C motif) ligand 24 (*CCL24*) is more highly expressed in the inner cell mass (ICM) than the trophectoderm (TE) (BMC Dev Biol 12:33). No function has yet been identified for CC cytokines during early embryo development. We hypothesize that CCL24 is involved cell migration events needed for establishment of the hypoblast adjacent to the blastocoele on the outer edge of the ICM. The first objective was to evaluate temporal expression of *CCL24* throughout early development. Steady-stage amounts of mRNA were evaluated for pools of 10-40 embryos collected at the oocyte, 2 cell, 3-4 cell, 5-8 cell, 9-16 cell, morula (collected separately on Days 5 and 6), and blastocyst (collected separately on Days 6, 7, 8 and 9) stages using qPCR with *YWHAZ* as the internal control gene. Expression of *CCL24* was non-detectable until Day 6 of development, at the morula stage, peaked in the Day 7 blastocyst, and declined thereafter and became non-detectable by Day 9. Thus, *CCL24* expression is maximal at a time coincident with formation of the blastocyst. The second objective was to determine whether inhibition of CCR3 (receptor for CCL24) alters the pattern of blastocyst formation. Embryos were treated with two CCR3 antagonists (SB328437 or SB297006) or vehicle at beginning of Day 6. Embryos were collected at Day 8 and subjected to immunolabeling for GATA6 (hypoblast) and NANOG (epiblast). The number and location of cells positive for NANOG and GATA6 was determined using epifluorescence or confocal microscopy. SB328437 (n=25-26 per group) decreased the percent of GATA6+ cells that were in the outer part of the ICM (61±1.7%) as compared to controls (66±1.6%; P=0.057). The experiment was repeated with additional embryos (n=8-9 per group) using confocal microscopy. Again, treatment with SB328437 decreased the percent of GATA6+ cells that were on the periphery of the ICM (77±2.6% for treated vs 86±2.9% for control; P<0.04). SB297006 (n=68 per group) also decreased the percent of GATA6+ cells that were on the periphery of the ICM (65±1.41% for treated vs 69±1.49% for control; P<0.04). Although the CCR antagonist altered localization of GATA6+ cells, it is likely that the antagonist affects an alternative receptor because *CCR3* mRNA was non-detectable in embryos at all stages of development through the blastocyst stage. The final objective was to knockdown the *CCL24* mRNA to confirm previous results. A subset of embryos was randomly selected and microinjected at the zygote stage with a specific morpholino antisense oligomer against *CCL24* or a standard negative control morpholino. A separate group of embryos were uninjected but exposed to all other manipulations. Blastocysts were collected at Day 7 for qPCR or fixed at Day 8 for immunolabeling. Results from two pools of 9-15 blastocysts indicated that the antisense morpholino decreased *CCL24* mRNA by 40%. There was a tendency for the antisense to decrease the percent of GATA6+ that were on the periphery of the ICM (68.4±3.4% for antisense morpholino vs 76.0±3.2% for control morpholino; P=0.08). In summary, the bovine embryo expresses a C-C chemokine, *CCL24,* at a time coincident with blastocyst formation and first differentiation of the ICM. Moreover, inhibition of a C-C receptor disrupted localization of hypoblast cells in the ICM. We hypothesize that CCL24 acts through a SB328437- or SB297006- sensitive mechanism to regulate position of hypoblast cells in the ICM. USDA AFRI Grant No. 2011-67015-30688.

Effect of level of dietary cation-anion difference (DCAD) and duration of prepartum feeding on calcium and measures of acid-base status in transition cows

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Objectives were to determine the effects of extending the feeding of acidogenic salts prepartum at two levels of negative DCAD on mineral metabolism and acid-base status in dairy cows. One-hundred and twelve Holstein cows at 230 d of gestation were blocked by lactation (1 vs. > 1) and 305-d milk yield and, within each block randomly assigned to one of four treatments arranged as 2 x 2 factorial with two levels of DCAD (-60 vs. -160 mEq/kg) and two durations (**DUR**) of feeding the negative DCAD, short (**S**; 21 d) or long (**L**; 42 d). Cows in S received an isonitrogenous and isocaloric diet with a DCAD of +130 mEq/kg from 233 to 254 d of gestation. Therefore, during the first 21 d of the experiment cows were fed one of three DCAD diets, +130, -60, or -160 mEq/kg, whereas during the last 21 d of gestation they were fed either -60 or -160 mEq/kg. Urine was collected twice weekly and pH measured. Cows were weighed and body condition scored once weekly prepartum. Intake of dry matter (**DMI**) was measured daily. Blood was sampled from the jugular vein at 250, 269 and 272 d of gestation and on d 0, 1, 2, 3, and 4 postpartum and analyzed for concentrations of ionized Ca **(iCa)**, blood gases, pH, base excess, and bicarbonate **(HCO_3^-)**. Data were analyzed by ANOVA with repeated measures using the MIXED procedure of SAS. Intake of DM in the first 21 d in the experiment decreased (P<0.01) by reducing the DCAD, and averaged 11.8, 10.9 and 10.4 kg/d for cows fed +130, -60, and -160 mEq/kg, respectively. Similarly, urinary pH decreased (P<0.01) with a reduction in DCAD, and averaged 8.11, 6.59, and 5.67 for cows fed +130, -60, and -160 mEq/kg, respectively. Results for the last 21 d of gestation and first 4 d postpartum are depicted in Table 1. Reducing the level of negative DCAD from -60 to -160 mEq/kg reduced DMI, induced a more exacerbated metabolic acidosis prepartum, and increased the concentration of iCa in blood. Extending the duration of negative DCAD had minor impacts on blood iCa and measures of acid-base status.

Table 1. Effect of level of prepartum DCAD and duration (Dur) of feeding on intake and blood measures

	Treatment[1]					P value		
Prepartum	S -60	S -160	L -60	L -160	SEM	Dur	DCAD	Dur x DCAD
DMI (-21 to -1), kg/d	12.1	9.9	11.4	10.0	0.6	0.45	<0.01	0.29
Urinary pH	6.19	5.38	6.41	5.47	0.09	0.10	<0.01	0.47
Blood pH	7.419	7.382	7.413	7.384	0.007	0.80	<0.01	0.58
Blood HCO_3^-, m*M*	26.2	22.6	25.7	23.8	0.5	0.49	<0.01	0.13
Base excess, m*M*	1.62	-2.40	1.04	-1.43	0.63	0.75	<0.01	0.21
Blood iCa, m*M*	1.26	1.29	1.25	1.28	0.01	0.44	<0.01	0.93
Postpartum								
Blood pH	7.448	7.452	7.444	7.454	0.003	0.74	0.05	0.47
Blood pCO_2, mm Hg	41.9	41.7	41.6	42.5	0.5	0.73	0.49	0.34
Blood HCO_3^-, m*M*	29.3	29.1	28.6	30.1	0.4	0.59	0.07	0.02
Base excess, m*M*	5.17	5.51	4.56	6.11	0.37	0.99	0.01	0.10
Blood iCa, m*M*	1.12	1.13	1.13	1.13	0.02	0.81	0.66	0.70

Effect of exercise on ovarian function in cycling gilts

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Exercise can alter reproductive function in the mare. To identify this phenomenon in other species, the effects of daily exercise on ovarian function in cycling gilts was evaluated. A total of 18 gilts (mean age = 225±8.3 d) were treated orally with a synthetic progestin, and subsequently injected with a gonadotropin to synchronize estrous cycles. Gilts were then trained to follow a target and run voluntarily along an 80 m track. Thereafter, gilts were randomly assigned to either an exercise or control group. Exercised pigs were worked twice daily for 6 min each period, during the last 10 d of the estrous cycle. Each exercise period consisted of travel of and average distance of 0.25 km at an average speed of 6.0 km/h. Rectal temperatures increased from values at rest of 38.5±0.3°C to a mean of 38.8±0.4°C immediately after exercise ($P < 0.05$). Respiration rates also increased from 30.8±3.5 to 59.7±12.6 breaths/min ($P < 0.05$). Cortisol was measured in saliva the day before the exercise protocol started and 5 and 9 d later. Cortisol concentrations were higher ($P < 0.05$) in exercised pigs at 5 and 9 d compared to controls. Gilts were slaughtered 2 d after the onset of estrus and reproductive organs were collected. No differences were found amongst treatments in the total number of follicles, corpus hemorragica, or corpora lutea. Exercised gilts had more medium (18.5 vs. 7.6; $P < 0.01$) and small (24.6 vs. 20.1; $P < 0.05$) sized follicles compared to control gilts. Cumulus-oocyte complexes were aspirated from small follicles (<2 mm in diameter), medium follicles (3–6 mm) and large follicles (>6 mm) and classified by quality based on a qualitative scale considering the number of layers of compact cumulus cells and ooplasm homogeneity. There was no effect of treatment on oocyte complex quality. Data indicate that exercise of pigs is associated with a stress response and can influence ovarian follicle development.

Characteristics of single nucleotide polymorphisms in candidate genes associated with embryonic development in the cow

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The goal was to understand mechanisms by which SNP in 12 genes associated with development of an embryo to the blastocyst stage (*BRINP3, C1QB*, *HSPA1L*, *IRF9*, *MON1B*, *PARM1*, *PCCB*, *PMM2*, *SLC18A2*, *TBC1D24*, *TTLL3* and *WBP1*) could affect preimplantation development. We evaluated temporal changes in gene expression to understand the period during development when the gene is active, modelled how changes in amino acid sequence changes tertiary structure of the proteins and performed phylogenetic analysis to determine whether the SNP associated with poor development arose coincident with divergence of cattle from ancestral species. Gene expression was evaluated in matured oocytes and in embryos from the 2-cell to blastocyst stages. *BRINP3* and *C1QB* were not detected at any stage. For most of the other genes, transcript abundance declined as the embryo developed to the blastocyst stage. The exception was for *PARM1* and *WBP1,* where steady-state mRNA increased at the 9-16 cell stage. There were large differences in the predicted three-dimensional structure of WBP1 and smaller changes in PARM1, SLC18A2, and TTLL3 caused by the mutation. Moreover, the mutation in WBP1 is located close to a P-P-X-Y motif involved in protein-protein interactions. There were three genes in which the SNP associated with embryonic development appeared to arise in cattle – *MON1B, PCCB,* and *PMM2*. Moreover, *PARM1* is in a selection signal associated with breed development in cattle. In conclusion, results indicate that SNPs in *PARM1* and *WBP1* affect competence of an embryo to develop to the blastocyst by changing protein structure and altering important developmental processes occurring after genome activation at the 8-cell stage. *BRINP3* and *C1QB* are not directly involved in embryonic development and results for the other mutations are equivocal.

Inversion on *STXBP5L* possible affecting intermediate speed locomotion patterns in the Mangalarga Marchador horse

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Locomotion patterns in vertebrates are controlled by the central pattern generators (CPGs) a group of neurons present in the spinal cord. All types of locomotion, quadrupedal, bipedal, flying or swimming, have reflexive features in common. Central pattern generators are not isolated entities but are interconnected in terms of circuitry and overlap in the behaviors that they generate across diverse species. The functional properties of the CPG can be altered by neuromodulators, neurotransmitter-like substances, delivered by the bloodstream or more rapidly via synaptic terminals that enhance or diminish the effect of the primary neurotransmitters with which they coexist in nerve terminals. These substances alter the functional properties of neuronal circuits by facilitating, depressing, or initiating motor activity as well as by modifying the cellular and synaptic characteristics of neurons. The modulating effects of supraspinal input, sensory afferents, and, neuromodulators make it abundantly clear that CPGs do not produce immutable, stereotyped motor patterns but rather flexible, adaptive patterns that are sculpted by plastic mechanisms. *Tomosyn-2* or, as currently known, the *sintaxyn-binding protein 5-like* (*STXBP5L*) gene, is implicated in locomotion in mice and humans , although its main function in mammals is not yet well understood. *STXBP5L* in the murine model is expressed in the adult central nervous system (CNS) as well as at high levels in embryonic CNS structures. It may participate in several different functional pathways, as enzyme regulator, cellular component in plasma membrane and/or cytoplasmic vesicles. A previous study conducted in our lab documented a 200kb inversion in the re-sequenced genome of a Mangalarga Marchador (MM) horse, the national horse breed of Brazil. This computationally detected structural variation divides the *STXBP5L* gene after the second intron and should eliminate normal transcription of the gene product. Notably, the MM horse breed is prized for a smooth and unique gait. MMs must pass rigid standards for conformation, gait, performance and endurance, as defined by a regulation book and supervised by the Associação Brasileira dos Criadores do Cavalo Mangalarga Marchador (ABCCMM) and the Brazilian Department of Agriculture (MAPA). The quality of gait in the MM is certified by a system of official registration inspections and organized competitions that have been in place for more than three decades in Brazil. To be registered, the MM is inspected twice by a technician (veterinary or animal science professional) trained and authorized by the ABCCMM and MAPA. These regulations do not permit the trot or pace; two-beat intermediate gaits exhibited by many other breeds of horse. Our hypothesis is that the inversion disrupting the *STXBP5L* disrupts transcription, and that lack of this gene product results in the unique intermediate-speed locomotor patterns observed in this breed of horses. RNA-seq is an extremely useful method for exploring the expression of sequence variants and might help elucidate the role and function of *STXBP5L* in future studies of these horses.

Tumor necrosis factor-alpha alters cumulus oocyte expansion and interleukin-6 production during bovine in vitro maturation

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The collaborative communication between the oocyte and the somatic cells of the ovarian follicle is essential for proper follicular development and for the oocyte to acquire developmental competence. Postpartum bacterial uterine infection affects 40% of dairy cattle and results in infertility even after the resolution of disease. Cows with uterine infection show retarded follicle growth, decreased estradiol secretion and aromatase expression. The pathogen associated molecule lipopolysaccharide is present in the follicular fluid of the dominant follicle of cows affected by uterine infection, causing granulosa cells to exhibit an innate immune response and express proinflammatory cytokines including tumor necrosis factor-alpha (TNFα). Granulosa cells cultured in the presence of TNFα reduce estradiol secretion. We hypothesize that exposure of cumulus oocyte complexes to the proinflammatory cytokine TNFα during maturation will reduce oocyte competence by disrupting the collaborative communication with the cumulus-granulosa cells. Ovaries were acquired from a local abattoir and oocytes obtained from follicles less than 8mm in diameter. Cumulus oocyte complexes underwent in vitro maturation for 24 hours in the presence of 0, 1, 10 or 100 ng/ml of recombinant bovine TNFα and gonadotropins. Following 24 hours of maturation cumulus oocyte complex expansion was recorded and supernatants collected for analysis of IL-6 accumulation by ELISA. Rates of cumulus oocyte expansion were reduced in the presence of increasing concentrations of TNF-α when compared with the vehicle treated controls (24%, 26% and 14% reductions in expansion, respectively). While not statistically significant a 42% increase in IL-6 accumulation was observed in supernatants following maturation of cumulus oocyte complexes in the presence of 1 ng/ml of TNFα compared to vehicle treated controls. The presence of appropriate IL-6 during oocyte maturation is critical to oocyte competence. This observed increase in IL-6 may be indicative of changes to other important factors related to oocyte development and as such, have negative biological impact on the oocyte. While TNFα is involved in the physiological balance between cell survival and apoptosis, inappropriate accumulation following exposure to pathogen associated molecules may perturb this balance. We propose that lipopolysaccharide induces inappropriate granulosa cell TNFα secretion, disrupting the delicate communication between somatic cells and the oocyte, resulting in reduced fertility following postpartum bacterial uterine infection.

Effects of feeding 25-hydroxy vitamin D on innate immunity and mastitis resistance in lactating dairy cows

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Vitamin D contributes to calcium homeostasis and immunity of dairy cattle. The majority of vitamin D in an animal circulates as 25-hydroxyvitamin D in blood. Activation of vitamin D from 25-hydroxyvitamin D (25(OH)D) to 1,25-dihydroxyvitamin D initiates an antimicrobial response in bovine macrophages. Hypothetically, greater availability of 25(OH)D benefits the immune system, but excessive amounts can lead to calcification. Dairy cattle are routinely supplemented with vitamin D and typically have 25(OH)D concentrations ranging from 60 to 80 ng /mL of serum. The National Research Council nutrient requirements for dairy cattle recommend 0.5 mg (20,000 IU) of supplemental vitamin D, whereas, dairy producers often supplement 0.75 – 1.25 mg of vitamin D per day. Recent data, however, has indicated that increasing supplemental vitamin D up to 3 mg/day does not lead to greater serum 25(OH)D concentrations. Rather, feeding 25(OH)D directly has been shown to effectively increase serum 25(OH)D concentrations of dairy cattle. Whether increased serum 25(OH)D concentrations as a result of feeding 25(OH)D, however, was still in question. The objective of this study is to evaluate the effects of feeding two levels (1 mg and 3 mg per day) of vitamin D and 25(OH)D on vitamin D metabolite concentrations, innate immunity and mastitis resistance of dairy cattle. In total, sixty pregnant, lactating Holstein cows will be enrolled in the study 5 enrollment blocks with 12 cows per block. Within each enrollment block cows will be blocked by milk yield and somatic cell count and randomly assigned to 1 of the 4 dietary treatments. The treatments will be given as a top-dress to a standard lactating cow ration for 28 d with blood and milk samples collected weekly. On day 21 of the trial the cows in the 1 mg vitamin D and 3 mg 25(OH)D groups will receive an intramammary *Streptococcus uberis* challenge**.** Presented here are preliminary data of the serum 25(OH)D concentrations of the cows in response to the treatments. Cows fed the 3mg 25(OH)D treatment ranged from 260 to 410 ng/mL after 4 weeks. Cows fed the 1 mg 25(OH)D ranged from 130 ng/mL to 188 ng/mL. Cows fed either 1mg or 3 mg vitamin D treatments both had similar serum 25(OH)D ranging from 55 ng/mL to 80 ng/mL after 4 weeks of their respective treatments. On the basis of the present data, feeding 25(OH)D increased serum 25(OH)D concentrations leading to more available substrate for 1α-hydroxylase while feeding additional vitamin D (1 mg vs 3 mg) did not increase serum 25(OH)D. Data on blood and milk leukocyte immune status and mastitis severity are being collected to determine if immunity of the cows receiving the 25(OH)D treatments benefit from the greater serum 25(OHD concentrations. Toxicity can occur if concentrations exceed 200 ng/mL for prolonged periods of time, so caution may be needed when supplementing 25(OH)D in the diets of lactating cattle.

Effects of feeding different types of polyunsaturated fatty acids on performance, plasma metabolites and fatty acid composition of milk in dairy cows

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Objectives were to determine the effects of feeding different types of polyunsaturated FA (**PUFA**) on performance and yield of fatty acids (**FA**) in Holstein cows. Eight ruminally cannulated primiparous cows were randomly assigned to a replicated 4 x 4 Latin square design (28-d, 19-d adaptation, 7-d collection, 2-d rumen evacuation). Diets were identical except for the type of FA supplements that were incorporated at 2.1% of dietary DM. Supplements were: Ca salts (**CaS**) of palm oil (**CaSP**); Oil (**O**; a blend of 45% palm and 55% soybean oils); CaS of O in a granular form (**CaSOG**); and CaS of O in a pelleted form (**CaSOP**). Intake, and yield and composition of milk were averaged from d 20 to 26. The FA profile of milk fat was analyzed in samples collected from d 24 to 26. Blood was sampled at 0, 3, 6, 9 and 12 h relative to feeding on d 26 and analyzed for hormones and metabolites. Ruminal pH was measured for 72 h in each period. Data were analyzed by ANOVA with the MIXED procedure of SAS for a replicated 4x4 Latin square. Results of cow performance are presented in Table 1. Results in the text are presented in the following sequence: CaSP, O, CaSOG, and CaSOP. Feeding O increased ($P \leq 0.05$) rumen fluid pH compared with CaSP or CaSOG (6.22, 6.31, 6.20, 6.26±0.07). Plasma concentration of glucose increased ($P \leq 0.05$) in O compared with CaSP and CaSOG, and tended (P=0.08) to be greater than CaSOP (64.8, 66.3, 64.7, 64.9±0.55 mg/dL). Glucagon concentration in plasma tended ($P=0.06$) to be greater in CaSP compared with CaSOP (156.4, 152.6, 149.4, 144.8±8.0 pg/mL). There were no treatment effects on plasma insulin, NEFA, and urea N concentrations. Milk linoleic (3.29, 3.22, 4.88, 4.71±0.16 g/100g of FA) and linolenic acids (0.43, 0.46, 0.61, 0.53±0.02), total n-3 (0.46, 0.48, 0.64, 0.56±0.02) and n-6 FA yields (3.78, 3.64, 5.37, 5.19±0.17) increased ($P<0.01$) in cows fed CaSOG and CaSOP compared with CaSP or O. Conjugated linoleic acids *trans*-10 *cis*-12 and *trans*-9 *cis*-11 were reduced ($P<0.01$) in cows fed CaSP compared with other treatments (0.004, 0.013, 0.019, 0.019±0.003). Source of FA did not affect DMI or milk yield, but feeding O as CaS in a pelleted form improved milk fat content. Feeding CaS of O either as granular or pelleted increased content of PUFA in milk fat.

Table 1. Performance of dairy cows fed different forms of FA

	Treatment[1]					
Item	CaSP	O	CaSOG	CaSOP	SEM	P
DM intake, kg/d	20.5	20.6	20.5	20.0	0.68	0.46
Milk yield, kg/d	28.7	28.6	28.7	28.2	0.91	0.96
Milk fat, %	3.47[a]	3.28[b]	3.25[b]	3.44[a]	0.14	0.05
Milk fat yield, kg/d	0.99[a,A]	0.93[B]	0.92[b]	0.96	0.04	0.13
Milk NE_L, Mcal/kg	0.67[a]	0.65[b,B]	0.65[c]	0.67[a,b,A]	0.01	0.03
Water intake, L/d	103.4[b]	109.5[a]	102.1[b]	100.0[b]	4.59	0.03

[a,b,c] Different superscripts differ ($P < 0.05$). [A,B] Different superscripts tend to differ ($P < 0.10$).

[1] CaSP = CaS of palm oil; O = blend of 45% of palm and 55% soybean oils; CaSOG = CaS of O in granular form; CaSOP = CaS of O in pelleted form.

Male-female differences in basal and CSF2-regulated gene expression in the bovine morula

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Development of the preimplantation embryo is modulated by sex. In the cow, gene expression differs between female and males at the morula and blastocyst stages. Moreover, response to the embryokine colony-stimulating factor 2 (CSF2) is sex-dependent. In females, CSF2 improved development to the blastocyst stage and tended to increase the ratio of trophectoderm to inner cell mass. There was no effect of CSF2 on these endpoints for males. Here, we tested whether gene expression of the bovine morula is modified by CSF2 in a sex-dependent manner. Embryos were produced in vitro using X- or Y-sorted sexed semen and treated, beginning at day 5 of culture, with 10 ng/ml bovine CSF2 or vehicle. Morulae were collected at day 6 and, after removal of the zona pellucida, stored in liquid nitrogen. Biological replicates (n=8) were pools of 50 morulae. Transcript abundance was determined by RT-qPCR using the Fluidigm® Delta Gene™ assay system. Transcripts quantified included those for 48 genes reported to be regulated by CSF2 in the bovine morula, 13 genes regulated by sex in the bovine morula, 31 other genes important for development, cellular differentiation or apoptosis, and four housekeeping genes. ΔCt values were calculated relative to the geometric mean of the housekeeping genes and fold-changes were calculated as $2^{-\Delta Ct}$. Data represent least-squares means ± SEM of fold-change relative to the housekeeping genes. Expression of three genes was downregulated by CSF2: *DDX3Y,* downregulated 1.07 fold, (0.77±0.02 vs 0.72±0.02, control vs CSF2, respectively; P=0.063), *MYF6,* downregulated 1.25 fold (0.001±0.0002 vs 0.0008±0.0002; P=0.0123) and *NANOG,* downregulated 1.33 fold (0.08±0.006 vs 0.06±0.006; P=0.0779). Expression of one gene, *PPP2R3A*, was upregulated 1.33 fold by CSF2 (0.003±0.0006 vs 0.004±0.0006; P=0.0599). A treatment by sex effect was observed for four genes. In each case, CSF2 increased expression in females but decreased expression in males. Genes affected were *POU5F1* (0.73±0.02 vs 0.76±0.02 in females and 0.73±0.02 vs 0.67±0.02 in males, control vs CSF2, respectively; P=0.0301), *DAPK1* (0.05±0.004 vs 0.06±0.004 and 0.05±0.004 vs 0.04±0.004; P=0.0587), *HOXA5* (0.002±0.0006 vs 0.003±0.0006 and 0.004±0.0006 vs 0.003±0.0006; P=0.0627), and *TNFSF8* (0.007±0.0008 vs 0.009±0.0008 and 0.005±0.0008 vs 0.004±0.0008; P=0.0568). Expression of 32 genes were affected by sex (P<0.05), with 22 genes being more expressed in females and 10 being more expressed in males. Among the sex-regulated genes were seven involved in embryonic development (*AMOT*, *BMP15*, *CDX2*, *FGF4*, *H2AFZ*, *POLR2D*, *UBE2A*), the anti-apoptotic genes *BAK1, RNF7*, and *TNFSF8,* and the pro-apoptotic genes *CREM* and *DAPK1*. In conclusion, sex plays an important role in regulation of gene expression at the morula stage. CSF2 affected expression of only a small subset of genes examined and the magnitude of change was small. Thus, actions of CSF2 on blastocyst development likely either involve changes in gene expression later in development or in post-transcriptional regulation of cell function. Of the 8 genes whose expression was modified by CSF2, response to CSF2 was affected by sex in half the cases (n=4). Such a result is consistent with sexually-dimorphic responses of the preimplantation embryo to this embryokine. (Support: NIH HD080855).

Developmental changes in canonical WNT signaling in the preimplantation bovine embryo

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A series of experiments was conducted to determine changes in WNT signaling during preimplantation development in the bovine. Expression of WNT co-receptors (*LRP5* and *LRP6*), antagonist (*DKK1*), transcription factors (*TCF7* and *LEF1*) and transcription factor antagonists [*AES* and *LOC505120* (a *GROUCHO*-like gene] declined to a nadir at the morula or blastocyst stage. An RNA-Seq database was used to determine expression of 91 genes associated with WNT signaling in the morula and inner cell mass (ICM) and trophectoderm (TE) of the blastocyst. Many genes associated with WNT signaling were characterized by low transcript abundance. Expression of only six genes increased from the morula to blastocyst stage (most notably *WNT6*) while expression of seven genes decreased. Ten genes were overexpressed in TE as compared to ICM, including *WNT6, FZD1, FZD7,* and *LRP6.* Immunoreactive β-catenin, assessed by immunolabeling, was relatively unchanged from the 2-cell stage to the morula stage and then increased slightly to the blastocyst stage. However, immunoreactive non-phospho (active) β-catenin in the nucleus exhibited a decrease from the 2-cell stage onward. Strikingly, β-catenin was not observable in the nucleus of blastomeres at any stage of development even after activation of canonical WNT signaling. Results indicate that canonical WNT signaling is partially silenced at the morula stage in the bovine embryo. Based on gene expression, WNT signaling may be more active in TE than ICM. Lack of observable nuclear β-catenin implies that WNT acts on the embryo largely through signal transduction pathways distinct from the canonical signaling pathway. (Support NIH HD080855).

Use of 1,25-dihydroxyvitamin D3 to maintain postpartum blood calcium and improve immune function in dairy cows

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Objectives were to determine the effects of a slow-release injectable formulation of 1,25-dihydroxyvitamin D_3 (calcitriol) on mineral metabolism and measures of immune function in recently calved Holstein cows. Cows were blocked by parity (2 vs. >2) and calving sequence and, within each block, randomly assigned to receive subcutaneously 300 μg of calcitriol (**DHVD**, n=25) or vehicle (**CON**, n=25) within 6 h of calving. Blood and urine were sampled before treatment application, 12 h later, and on d 1, 2, 3, 5, 7, 9, 12, and 15 postpartum. Samples were analyzed for total (**tCa**) and ionized Ca (**iCa**), magnesium (**Mg**), phosphorus (**P**), calcitriol, nonesterified fatty acids (**NEFA**), beta hydroxybutyrate (**BHBA**), glucose, serotonin (**5-HT**) and crosslaps (**CTX-1**). Neutrophil function was evaluated in the first week postpartum. Intake of DM and production performance were evaluated for the first 42 d postpartum. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. DHVD increased (P<0.01) concentrations of calcitriol within 4 h of application from 24 to 420 pg/mL, which returned to baseline within 3 d. Blood iCa and tCa took 12 and 24 h, respectively, to increase after treatment with VitD compared with CON. Concentrations of iCa (CON=1.05 vs. DHVD=1.18 m*M*), tCa (CON=2.11 vs. DHVD=2.35 m*M*), and P (CON=1.51 vs. DHVD=2.06 m*M*) remained elevated (P<0.01) in DHVD until 3, 5 and 7 d postpartum, respectively. Concentration of Mg (CON=0.76 vs. DHVD=0.67 m*M*) was less (P<0.01) in DHVD cows until 5 d postpartum. DHVD cows excreted more urinary Ca (CON=0.6 vs. DHVD=1.7 g/d; P<0.01) and Mg (CON=3.6 vs. DHVD=5.5 g/d; P=0.02) in the first 5 and 1 d postpartum, respectively. Concentrations of glucose, NEFA, BHBA, 5-HT and CTX-1 in plasma did not differ between treatments. DHVD improved neutrophil function compared with CON. Relative to a reference cow, the percentage of neutrophils with oxidative burst activity (CON=80.0 vs. DHVD=101.0%; P=0.03), the mean fluorescence intensity (**MFI**) for oxidative burst (CON=96.0 vs. DHVD=105.0%; P=0.09), and the MFI for phagocytosis (CON=94.0 vs. DHVD=110.0%; P=0.03) were all greater for DHVD than CON cows. Intake of DM and yields of milk and milk components did not differ between treatments. Administration of 300 μg of calcitriol at calving was safe and effective in increasing plasma concentrations of calcitriol, iCa, tCa, and P for the first few days after treatment, and improved measures of innate immune function in early lactation Holstein cows.

Characterization of natural killer T cell subsets in pigs

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Invariant natural killer T (NKT) cells are an unusual T cell subset that recognize glycolipid antigens presented by the MHC class I-like molecule CD1d. Murine and human NKT cells have been classified into three functional subsets, NKT1, NKT2 and NKT17, based on their expression of cytokines usually produced by T helper 1 (Th1) (IFN-γ), Th2 (IL-4, IL-5, IL-10, IL-13) and Th17 (IL-17) CD4 T cells, respectively. Their ability to produce many diverse cytokines enables NKT cells to play a role regulating both pro-inflammatory responses that can be targeted against cancer and infectious disease and anti-inflammatory responses useful for suppressing autoimmune diseases. We previously showed that pigs are similar to humans for NKT cell frequency and tissue localization. However, as yet little is known about the function and localization of NKT cell subsets in swine. For the first time we have extensively characterized the porcine NKT cell subsets by flow cytometry and our results show that these lymphocytes can be divided into $CD8^+$ and $CD8^-$ cells and that respectively secrete IFN-γ and IL-4 and IL-4 alone after *in vitro* stimulation. Interestingly, unlike murine and human NKT cells, pig NKT cells did not express IL-17. Instead a subset of natural killer (NK) cells was mostly responsible for producing IL-17 under the stimulation conditions tested. Collectively, these findings demonstrated that swine poses distinctive NKT cell subsets that in future might be differentially targeted to treat and prevent a range of diseases of veterinary importance.

Gene targeting the costimulatory molecule CD70 attenuates autoimmune diabetes in NOD mice

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Type-1 diabetes (T1D) is an autoimmune disease caused by the destruction of β-cells by autoreactive CD4 and CD8 T lymphocytes. The events that pathogenically activate autoreactive T cells are not yet well understood, but it appears that this process involves signaling via a range of different activating and inhibiting costimulatory molecules that control T cell activation, including autoreactive T cell clones. Here we report that costimulatory molecule CD70 that interacts with CD27, a tumor necrosis factor receptor (TNFR) family member on T cells, plays an inhibitory role for the development of T1D. We found that T1D is accelerated in our newly generated CD70 deficient NOD mice. Accelerated disease was associated with a lower frequency of circulating immunosuppressive regulatory T cells (Tregs) that expressed less of the transcription factor FoxP3, which mediates Treg effector function, compared to intact NOD mice. These results indicate that the CD27-CD70 signaling pathway has important effects on immune responses that contribute to T1D. Understanding the mechanism through which CD27-CD70 pathway modulates T1D progression may lead to promising therapeutic intervention to suppress disease.

Effects of L-carnitine on development and cryotolerance of bovine embryos produced *in-vitro*

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The aim was to determine effect of supplementation of oocyte maturation and embryo culture media with L-carnitine (LC) on embryo development and cryotolerance. Bovine embryos were produced *in-vitro* using abattoir-derived cumulus-oocyte complexes. Cleavage, blastocyst and advanced blastocyst (expanded, hatching and hatched) rate were assessed on Day 3 and 7 after insemination (day 0), respectively. Blastocyst and expanded blastocyst stage embryos were harvested on Day 7 and subjected to controlled-rate freezing following equilibration in 1.5 M ethylene glycol. After thawing, embryos were cultured for 72 h in SOF-BE1 supplemented with 10% (v/v) FBS and 50 mM dithiothreitol at 38.5^{o} C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Post-thaw re-expansion and hatching rates were determined at 24, 48 and 72 h. Data were analyzed by logistic regression. In experiment 1, presumptive zygotes (n=2,768) were collected after fertilization and randomly assigned in a 2 x 4 factorial design to *in vitro* culture (IVC) in SOF-BE1 supplemented with 0% or 5% fetal bovine serum (FBS) and concentrations of either 0.0, 0.75, 1.5 or3.03 mM LC (T1-T4, respectively) at 38.5^{o} C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2. There was no effect of FBS on cleavage rate, but the presence of FBS during IVC increased ($P<0.05$) the blastocyst (19.7 ± 1.1% vs. 25.3 ± 1.4%) and advanced blastocyst (9.1 ± 0.8% vs. 12.4 ± 1.2%) rates. Addition of LC during IVC did not affect cleavage rate or blastocyst rate. However, treatment of embryos with 3.03 mM LC reduced ($P<0.05$) advanced blastocyst development (10.9 ± 1.2% - T1 , 12.2 ± 1.4% - T2, 13.5 ± 1.5% - T3, vs. 7.0 ± 1.0% - T4). Addition of FBS during IVC reduced ($P<0.05$) rates of re-expansion (78.1 ± 3.4% vs. 65.5 ± 3.1%, 81.0 ± 3.0% vs. 68.4 ± 2.7%, 78.4 ± 3.4% vs. 65.8 ± 3.1%, for 24, 48 and 72 h, respectively) and hatching (52.0 ± 4.0% vs. 39.8 ± 3.6%, 45.4 ± 3.8% vs. 61.2 ± 4.1%, 45.4 ± 3.8% vs. 61.2 ± 4.1%, for 24, 48 and 72 h, respectively) at all time points. In contrast, LC increased post-thawing re-expansion rates ($P<0.05$) at 24 and 48 h and tended to increase hatching rate ($P<0.08$) at 48 h (Table 1). There was no interaction between FBS and LC treatment on embryo development or cryotolerance. In experiment 2, immature bovine cumulus-oocyte complexes (n=1,796) were randomly assigned to be matured in maturation medium supplemented with or without 3.03 mM LC for 22 to 24 h at 38.5 °C in a humidified atmosphere of 5% CO_2. Addition of LC during maturation had no effect on cleavage rate, blastocyst development and post-thaw re-expansion or hatching rates. In conclusion, post-thaw viability of bovine embryos produced in vitro can be improved by the addition of LC during IVC.

Table 1. Effect of L-carnitine supplementation on cryotolerance of bovine embryos produced in vitro.

L-carnitine (mM)	Re-expansion			Hatching		
	24 h	48 h	72 h	24 h	48 h	72 h
0	63.1 ± 4.4[a]	67.2 ± 3.4[a]	68.5 ± 4.4	24.7 ± 4.7	38.0 ± 5.1	47.2 ± 5.3
0.75	77.4 ± 4.6[b]	80.0 ± 4.0[b]	76.2 ± 4.6	26.3 ± 5.0	47.1 ± 5.3	56.5 ± 5.6
1.5	72.1 ± 4.5 [b]	74.6± 3.9[b]	71.3 ± 4.4	31.5 ± 4.8	50.4 ± 5.2	55.6 ± 5.4
3.03	74.7 ± 4.7 [b]	77.3 ± 4.1[b]	72.4 ± 4.7	23.8 ± 5.1	48.1 ± 5.5	54.1 ± 5.8

[ab]Values with different superscripts within columns differ significantly