THIRTEENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Jekyll Island Club Hotel

Jekyll Island, Georgia
April 17-18, 2015

WELCOME

This year's AMCB symposium is the 13th in the existence of the program and the first time we meet outside the borders of Florida. Jekyll Island was the playground of titans of industry in the late 19th Century and first half of the 20th Century. Members of the Jekyll Island Club included Morgans, Vanderbilts, Fields, Goulds, Pulitzers, Goodyears and others. The hotel where we are staying was built in 1888 to house guests of the club. Our Friday night cookout will be on the porch and lawn of Indian Mound, the cottage built by oil executive William Rockefeller, brother of John D. Rockefeller who founded Standard Oil. Presentations will be in a conference center that was once an indoor tennis court of the club.

It was on Jekyll Island that the meeting to draft the legislation to create the US Federal Reserve was held (1910) and where the first transatlantic telephone call was made in 1915 (involving Alexander Graham Bell and President Woodrow Wilson).

The island has miles and miles of seashore, golfing, tennis, several historic sites, and over 20 miles of bike trails. Bikes can be rented at the hotel.

It is appropriate that we convene in such grand surroundings since 2014 has been a great year for the AMCB. Faculty membership has grown to an all-time high of 17 and over 60 attendees of the symposium are expected. This will be a great occasion to share our science with each other and with our distinguished lecturers, Pat Lonergan and Trudee Fair. We trust that this will be memorable symposium not only in terms of scientific exchange but also in terms of fellowship and fun!

Pete Hansen, Director
John Driver, Co-Director

ACKNOWLEDGMENTS

The faculty and students of the AMCB Program thank the following for support of the 13th Annual Research Symposium

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

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

L.E. "Red" Larson Endowment

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

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

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

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

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

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

2015 AMCB DISTINGUISHED LECTURER

Patrick Lonergan
School of Agriculture and Food Science, University College Dublin

Pat Lonergan is Professor of Animal Reproduction at University College Dublin, Ireland. He graduated from UCD with a degree in Animal Science in 1989. He then did a Masters and PhD under Prof Ian Gordon in the area of in vitro embryo production in cattle. After his PhD he spent a one-year Post- doctoral Fellow in the Norwegian College of Veterinary Medicine, Oslo and 4 years at INRA, Nouzilly in Tours, France working in the area of bovine embryo development. He subsequently returned to UCD as a Post-doctoral Fellow and joined the academic staff in 2001. His main areas of interest are early embryo development in vivo and in vitro and understanding embryo mortality in cattle. He has a large volume of research publications, an associated h-factor of about 50, and was awarded a D.Sc. on Published Work by UCD in 2005. He was elected Member of the Royal Irish Academy in 2012. He has supervised numerous Masters and PhD students, has served on the Boards of the International Embryo Transfer Society (IETS; www.iets.org) and European Embryo Transfer Association (www.aete.eu) and was elected President of IETS in 2009. He currently serves as Associate Editor of *Biology of Reproduction* and *Reproduction, Fertility and Development*.

2015 AMCB DISTINGUISHED LECTURER

Trudee Fair
School of Agriculture and Food Science, University College Dublin

Trudee Fair is a Senior Lecturer in the School of Agriculture & Food Sciences at University College Dublin (UCD), Ireland. She studied Animal Science in UCD, completing a Masters Degree under Prof Ian Gordon in the area of in vitro embryo production in cattle. She subsequently carried out the studies for her PhD under the supervision of Prof Torben Greve and Prof Poul Hyttel, in University of Copenhagen, Denmark. She has been an investigator in the area of cow fertility for almost 20 years. Her research has focused on bovine oocyte growth and maturation and the role of the maternal immune system in cow fertility. Dr Fair was among the first to identify the precise size and morphology at which the bovine oocyte growth phase is completed, transcription ceases and developmental competence is acquired. Her group published the first details of the timing of the establishment of the maternal imprints in bovine oocytes and showed that this process can be disrupted by the cow's metabolic status. She published the initial evidence of the impact of assisted reproductive technology on bovine embryo morphology, cryotolerance, gene expression and more recently, the methylome. Her group has characterized the expression profile of the MHC-I in bovine embryos and demonstrated that the initial bovine endometrial immune response to the presence of an embryo is an increase in macrophages and dendritic cells. Dr. Fair has published extensively and her Web of Science h-factor is 30. She has supervised numerous Masters and PhD students and Postdoctoral Fellows. She is a Management Committee Member and Work Package Leader of FP7 funded EU Cost Action 'Epiconcept'. Dr Fair is an Editorial Board Member of *Reproduction* and a sub-Editor of *Biology of Reproduction*.

AMCB FACULTY

Lokenga Badinga, Department of Animal Sciences
John Bromfield, Department of Animal Sciences
Samantha Brooks, Department of Animal Sciences
Mary Brown, Department of Infectious Diseases and Pathology
Geoffrey Dahl, Department of Animal Sciences
John Driver, Department of Animal Sciences
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
Jose Santos, Department of Animal Sciences
Stephanie Wohlgemuth, Department of Animal Sciences
Charles Wood, Department of Physiology and Functional Genomics

Emeritus Faculty

William C. Buhi, Departments of Obstetrics & Gynecology, Animal Sciences
Kenneth C. Drury, Department of Obstetrics & Gynecology
Michael J. Fields, Department of Animal Sciences
Daniel C. Sharp, Department of Animal Sciences
William W. Thatcher, Department of Animal Sciences

CURRENT AMCB STUDENTS

PhD Students

Turkey Omar H Asar (Advisor: G Dahl)

Leticia Del-Penho Sinedino (Advisor: J Santos)

Sossi Iacovides (Advisor: J Bromfield)

Mercedes Kweh (Advisor: C Nelson)

Chengcheng Li (Advisor: S Wohlgemuth)

Veronica Negron Perez (Advisor: P Hansen)

M. Sofia Ortega (Advisor: P Hansen)

Eduardo Ribeiro (Advisor: J Santos)

Luiz Siqueira (Advisor: P Hansen)

Paula Tribulo (Advisor: P Hansen)

Guan Yang (Advisor: J Driver)

MS Students

Jasmine Kannampuzha Francis (Advisor: PJ Hansen)

Kathryn Merriman (Advisor: CD Nelson)

Cheng Ye (Advisor: J Driver)

GRADUATES 2014

Anna Denicol, PhD (Advisor: PJ Hansen)
Currently postdoctoral scientist in the laboratory of Jonathan Tilly, Northeastern University

Kyle Dobbs, PhD (Advisor: PJ Hansen)
Currently postdoctoral scientist in the laboratory of Jonathan Tilly, Northeastern University

Gabriel Carvalho Gomes, MS (Advisor: JEP Santos)
Currently resident, Large Animal Clinical Sciences, University of Florida College of Veterinary Medicine

Ashley Grapes, MS (Advisor: CE Wood)
Currently teacher, Duval Country School District

Leandro Ferreira Greco, PhD (Advisor: JEP Santos)
Currently, assistant professor, Faculdade Anhanguer, Anápolis, Goiás, Brazil

HISTORY OF THE AMCB RESEARCH SYMPOSIUM

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

SCHEDULE OF EVENTS

All events in Morgan Center unless otherwise noted

FRIDAY, APRIL 17

9:50 AM Pete Hansen
Welcome, introductory comments

Session 1: Immunology and Microbiology
Leticia Del-Pinho Sinedino and Mercedes Kweh, Chairs

10:00 AM Cheng Ye, Animal Sciences
Unmasking genes controlling how CD4+ T-cells pathogenically activate type-1 diabetes inducing CD8+ T-cells

10:15 AM Kathryn Merriman, Animal Sciences
Intramammary infusion of vitamin D metabolites stimulates host defense genes in the bovine mammary gland

10:30 AM Mercedes Kweh, Animal Sciences
Unraveling the mechanisms that regulate activation of β-defensin antimicrobial peptide responses in cattle

10:45 AM Guan Yang, Animal Sciences
CD1d-knockout pigs: a novel model to study the role of natural killer T cells in immunity

11:00 AM Bianca Artiaga, Animal Sciences
Adjuvant effects of therapeutic glycolipids administered to a cohort of NKT cell-diverse pigs challenged with influenza H1N1 virus

11:15 AM Zhengxin Ma, Animal Sciences and Emerging Pathogens Institute
Localization and characterization of enterohemorrhagic Escherichia coli *O157:H7 T3SS effectors EspR, EspX, and EspY in host cells*

11:30 AM Soon Jin Jeon, Large Animal Clinical Sciences
Uterine microbiota from calving until establishment of metritis in dairy cows

11:45 AM Junyi Tao, Animal Sciences
Transport of a fluorescent analog of glucose (2-NBDG) by rumen bacteria

12:00 PM LUNCH – New York Deli

Session 2: Reproduction

Luiz Siqueira, Chair

1:30 PM Rodolfo Daetz, Large Animal Clinical Sciences
Use of chitosan microparticles to prevent metritis in lactating dairy cows

1:45 PM Sossi Iacovides, Animal Sciences
Do metritis associated bacterial components alter the follicular environment and perturb oocyte competence?

1:45 PM Leticia Sinedino, Animal Sciences
Effects of feeding algae rich in docosahexaenoic acid (DHA) on lactation and reproductive performance of dairy cows

2:00 PM A. Vieira-Neto, Large Animal Clinical Sciences
Association between gestation length with health, reproduction, and production in Holstein cows

2:15 PM Ana Mesa, Animal Sciences
Effect of hydrogen peroxide and heat stress on stallion sperm function

2:30 PM Paula Tribulo, Animal Sciences
Endometrial expression of genes involved in growth factor, cytokine, hormone, and WNT signaling during the early estrous cycle of the cow

2:45 PM BREAK

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

3:00 PM Professor Patrick Lonergan
School of Agriculture and Food Sciences, University College Dublin
Embryo-maternal interactions leading to establishment of pregnancy in cattle

4:00 PM BREAK AND CHECK INTO ROOMS

Session 4: Pregnancy
Sofia Ortega, Chair

4:45 PM Verónica M. Negrón Pérez Animal Sciences
Role for CC cytokines in differentiation of the inner cell mass of the bovine embryo

5:00 PM Luiz G. B. Siqueira, Animal Sciences and EMBRAPA
Colony-stimulating factor 2 affects development of the bovine preimplantation embryo differently for females than males

5:15 PM Jasmine Kannampuzha-Francis, Animal Sciences
Exposure to colony stimulating factor 2 during preimplantation development increases postnatal growth in cattle

5:30 PM Eduardo S. Ribeiro, Animal Sciences
Patterns of expression of genes involved in lipid metabolism in bovine preimplantation conceptuses at the onset of elongation

5:45 PM BREAK

6:00 PM GROUP PICTURE, INDIAN MOUND

6:30 PM COOKOUT, INDIAN MOUND

SATURDAY, APRIL 12

7:30-9:30 AM CONTINENTAL BREAKFAST

Session 5: Physiology and Nutrition
Guan Yang, Chair

9:00 AM Jessi Powell, Animal Sciences
Vitamin D status of dairy calves fed pasteurized whole-milk

9:15 AM Turky O. Asar, Animal Sciences
Effect of late gestation maternal heat stress on epigenetic patterns of dairy calves

9:30 AM Chengcheng Li, Animal Sciences
Effects of aging on mitochondrial function in skeletal muscle of Quarter Horses

9:45 AM BREAK

Session 6: 2014 AMCB Distinguished Lecturer Presentation
Peter Hansen, Chair

10:00 AM Trudee Fair
School of Agriculture and Food Sciences, University College Dublin
Immunological aspects of early pregnancy in cattle

11:00 AM BREAK

Session 7: Genetics and Genomics
Cheng Ye, Chair

11:15 AM	Sofia Ortega, Animal Sciences *Effect of a single nucleotide polymorphism in* COQ9 *on cellular energy metabolism, fertility and milk production in Holstein cows*
11:30 AM	Mesfin Gobena, Animal Sciences *Genomics of resilience in sheep to climatic stressors*
11:45 AM	Amy Dinerman, Animal Sciences *Polymorphism identification in candidate genes for juvenile idiopathic epilepsy in the Arabian Horse*
12:00 PM	CLOSING (AND VERY BRIEF) REMARKS, PETE HANSEN

ABSTRACTS

(Arranged alphabetically by first author)

Adjuvant effects of therapeutic glycolipids administered to a cohort of NKT cell-diverse pigs challenged with influenza H1N1 virus

Bianca L. Artiaga*, Guan Yang*, Robert Whitener†, Charles R. Staples*, and John P. Driver*

*Dept. of Animal Science, University of Florida, Gainesville FL
†Dept. of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville FL

Natural killer T (NKT) cells are a lymphocyte population capable of potently stimulating innate and adaptive immune responses. They can be activated using therapeutic glycolipid antigens for multiple purposes, including as powerful adjuvants to improve vaccine responses against various infectious diseases. We hypothesize that NKT cell antigens have exciting potential for preventing infectious diseases in NKT cell-expressing livestock species, including swine. Thus, our objective was to survey NKT cell frequencies and function amongst commercial pigs and to determine if porcine NKT cells can be therapeutically activated to enhance immunity against foreign antigens. For this, immune tissues from 85 pigs of mixed genetics were compared for NKT cell frequency, cytokine secretion and subset ratios. Pigs were also injected with the model antigen hen-egg lysozyme (HEL) in conjunction with one of three therapeutic glycosphingolipids, alpha-galactosylceramide (αGC), OCH, and C-glycoside, which selectively activate NKT cells, to assess the adjuvant potential of each. Significant variation was found between individual pigs for all NKT cell characteristics measured. However, piglets within litters contained similar frequencies of NKT cells compared to piglets between litters, suggesting that the variation is partially due to genetic effects. All three agonists elicited HEL-specific cellular and humoral immune responses of varying quality, but only αGC increased the systemic concentration of NKT cells. A similar study was conducted to test whether αGC could be used to enhance vaccination against a strain of the viral pathogen pandemic H1N1 swine influenza virus A/California/04/2009 (CA04). Sixteen piglets were vaccinated twice, 16 days apart, with a mock solution, UV-killed CA04 (kCA04) or a combination of αGC and kCA04. Following the vaccination period twelve piglets were challenged with live CA04, NKT cell activation with αGC induced a dramatic increase in the frequency of virus-specific cells in blood, lung and spleen, as measured by interferon-γ (IFNγ ELISPOT) assay. αGC administered with vaccine also significantly reduced viral titers in nasal swabs and airway tissues compared to pigs treated with vaccine alone. For the first time, important information was generated about pig adjuvant responses to different varieties and dosage levels of NKT cell antigens. Additionally, we demonstrated that NKT cells could be harnessed to protect swine from a dangerous pathogen.

Supported by University of Florida's Research Opportunity Seed Fund and IFAS Early Career Seed Fund awards. NIH Tetramer Core Facility provided OCH, C-glycoside and CD1d tetramers.

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

T.O. Asar 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 in the silencing of gene expression and may be passed on to the next generation. DNA is methylated by at least two de novo methyltransferases – DNA methyltransferase 3a (Dnmt3a) and DNA methyltransferase 3b (Dnmt3b). DNA methyltransferase 1 (Dnmt 1), through its action on hemi – methylated DNA ensures complete methylation, and hence is responsible for maintaining the methylation pattern in specific coding and noncoding regions. 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). All calves born to these dams were managed in the same manner and weaned at 49 d of age. Birth weight, growth rate, and milk production in the first lactation from HT and CL heifers were analyzed. Analysis 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, but weight differences were not apparent at calving. More importantly, we observed that the 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; $p = 0.03$). We hypothesize that environmental factors, such as heat stress exert these dramatic effects on the phenotype through epigenetic mechanisms because there are no genotypic differences between the groups. 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. Epigenetic shifts in DNA methylation have an important role in development but can also arise stochastically as animals mature. So, in aim 3, the samples will be collected from multiple tissues across different ages of F1 and F2 animals, to determine if tissue specific patterns of methylation occur. Collectively, these studies should provide insight to the mechanisms of in utero induced differences in mature phenotype.

Use of chitosan microparticles to prevent metritis in lactating dairy cows

R. Daetz[1], F. Cunha[1], Y. Maeda[4], F. Magalhaes[1], C.A. Risco[1], K.C. Jeong[2], J.E.P. Santos[3], and K.N. Galvão[1]

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[2]Department of Animal Sciences and Emerging Pathogens Institute, University of Florida, Gainesville, FL
[3]Department of Animal Sciences, University of Florida, Gainesville, FL
[4]School of Veterinary Medicine, Kitasato University, Towada, Japan

The objective was to determine the efficacy of chitosan microparticles (CM) in preventing metritis in dairy cows. Holstein cows (n=101) from a 4,500-cow commercial herd that had risk factors for metritis (dystocia, twins, stillbirth, retained placenta) were randomly assigned to one of two treatments 1 d (24 h) postpartum (DPP): CM (n = 51) = intrauterine (i.u.) infusion of 8 g of CM dissolved in 40 mL of sterile water for 5 days; Control (n = 50) = i.u. infusion of 40 mL of sterile saline solution for 5 days. Metritis prevalence was analyzed by logistic regression using the LOGISTIC procedure of SAS using a one-side test in accordance with sample size calculation for reduction in metritis prevalence using CM. Continuous outcomes were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. Models included the effects of treatment, parity, specific risk factor, body condition score at enrollment and interaction between treatment and other covariates. The effect of time and interaction between treatment and time was also included in repeated measures analyses. Treatment with CM resulted in decreased incidence of metritis at 7 DPP compared with Control (45.1 vs. 64.0%; $P = 0.03$); however, there were only numerical differences at 4 (11.8 vs. 18%; $P = 0.23$), 10 (60.1 vs. 72%; $P = 0.12$), and 14 (62.7 vs. 72.0%; $P = 0.16$). Treatment with CM resulted in decreased NEFA plasma concentrations at 10 DPP (464.2 ± 57.2 vs. 639.5 ± 57.2 μEq/L; $P = 0.04$); however, there were no differences at 4 (813.8 ± 56.7 vs. 780.4 ± 56.7 μEq/L; $P = 0.67$), 7 (669.9 ± 56.7 vs. 692.9 ± 56.7 μEq/L; $P = 0.77$), and 14 (527.6 ± 57.7 vs. 420.7 ± 57.7 μEq/L; $P = 0.18$). The uterine discharge pH was lower in Control than in CM cows (6.84 ± 0.03 vs. 6.93 ± 0.03; $P = 0.02$). BHBA (647.4 ± 30.0 vs. 589.3 ± 30.0 μmol/L; $P = 0.36$), temperature (39.2 ± 0.04 vs. 39.1 ± 0.04 ºC; $P = 0.62$) and milk production (29.3 ± 1.0 vs. 28.8 ± 1.0 L/day; P = 0.69) were not different between CM and Control groups. In conclusion, CM may be a viable alternative for treatment of metritis; however, the duration of treatment may have to be extended in order to maintain differences in the incidence of metritis.

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 to achieve a profit. 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 Horse. The phenotype of this condition typically presents shortly after birth, at 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 complete loss of the menace response (Aleman et al, 2006). However, foals may not display all of these signs, and these signs may vary widely in severity from one individual to the next (Aleman et al, 2006). JIE can be fatal, if seizures cannot be controlled pharmacologically (Aleman et al, 2006); however, some horses will outgrow JIE seizures by 18 months of age and can be healthy as adults, perpetuating this deleterious allele if they are used as breeding stock.

JIE is a fairly rare condition in the Arabian horse and few affected individuals are available for study. Thus a candidate gene sequence approach will be utilized to identify possible mutations in a small sample set. Thus far, we have targeted a promising candidate gene, *KCNQ2*, which encodes a potassium voltage gated channel within the neurons in the brain. KCNQ2 mutations cause Benign Familial Neonatal Seizures (BFNS) in man, which is characterized by classic tonic –clonic seizures beginning by day 7 of the child's life. Dysfunction of potassium channels in nerve cells of the frontal lobe, cerebellum, and to a lesser extend the cerebral cortex, triggers seizures by reducing the M current used to regulate firing action in the neurons (Volkers et al, 2009). Like JIE horses, BFNS affected humans outgrow seizures by 12 months of age. Additional candidate genes responsible for homologous phenotypes in other species may also be investigated. Future work will include whole genome sequencing in affected individuals using Illumina Hi-Seq 2500 technology to identify novel and unique polymorphisms. Newly identified polymorphisms may provide a genetic test to identify affected, and carrier individuals in hopes of screening breeding stock and reduce the incidence of JIE affected foals.

Aleman, M. *et al,* 2006. Juvenile idiopathic epilepsy in Egyptian Arabian foals: 22 cases (1985-2005). *Journal of Internal Veterinary Medicine*, 6, 1443-9.

Volkers, L. *et al,* 2009 Functional analysis of novel KCNQ2 mutations found in patients with Benign Familial Neonatal Convulsions. *Neuroscience Letters*, 462, 24-29.

Exposure to colony stimulating factor 2 during preimplantation development increases postnatal growth in cattle

Jasmine Kannampuzha-Francis,[1] Anna C. Denicol,[1] Barbara Loureiro,[2] and Peter J Hansen[1]

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The microenvironment of the preimplantation embryo can cause changes in development that affect postnatal phenotypes. Potential mediators of this effect are embryokines secreted by the maternal reproductive tract that regulate embryonic function. One of these, colony stimulating factor-2 (CSF2) can increase competence of mouse, cattle, and human embryos to establish pregnancy after transfer into recipients. Moreover, culture of mouse embryos in CSF2 alleviated some adverse effects of embryo culture on postnatal growth in adult mice (Endocrinology 146:2142). Accordingly, we hypothesized that treatment of in vitro produced embryos with CSF2 in culture would alter birth weight and postnatal growth of the resultant calf. Embryos were produced in vitro from Holstein oocytes inseminated with X-sorted Holstein semen. Embryos were cultured with or without 10 ng/ml recombinant bovine CSF2 from day 5-7 after insemination and transferred at day 7 to lactating Holstein dairy cows. Calves were weighed at birth and at intervals thereafter until 13 mo of age. Birth weights were not affected by treatment but CSF2 calves grew faster than controls (treatment x month of age, P=0.0029) (Figure 1). Results indicate that exposure to CSF2 during the preimplantation period can alter the trajectory of growth in the postnatal period. Perhaps the embryo can be programmed in culture to improve postnatal functions important for health and productivity.

Support: (USDA-AFRI-2011-67015-30688).

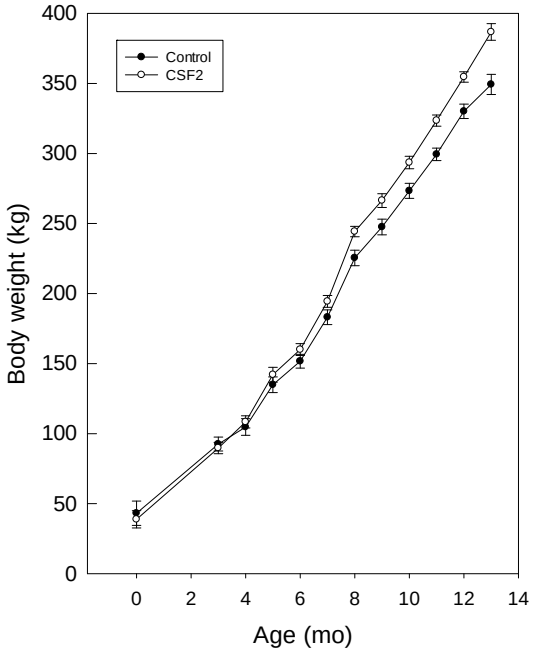

Figure 1. Body weights in the first 13 mo of life.

Genomics of resilience in sheep to climatic stressors

Mesfin Gobena*, Arthur L. Goetsch[#], Terry A. Gipson[#], Zaisen Wang[#], Megan Rolf[†], Pascal Oltenacu[*], Tilahun Sahlu[#], Ryszard Puchala[#], Steve Zeng[#] and Raluca Mateescu[*]

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[#]American Institute for Goat Research, Langston University, Langston, OK
[†]Department of Animal Sciences, Oklahoma State University, Stillwater, OK

According to projections by the United Nations Intergovernmental Panel on Climate Change, climatic changes projected to happen in the foreseeable future will include more intense and frequent heat waves, severe droughts and floods. These changes are likely to have a substantial effect on ruminant livestock production. One sustainable approach to prepare for these changes is to select and breed livestock that are able to maintain the level of production under environmental conditions projected to happen in the future. The long term goal of this project is to improve the resilience of sheep to environmental elements. This will be accomplished by providing the knowledge and tools necessary to enable selection of animals that can cope up with the rapidly changing climate. It is hypothesized that enough variability of the genetic background for adaptation to climatic elements/stressors exists between and within different sheep breeds and ecotypes to enable selection of animals better suited to tomorrow's climate. Four sheep breeds (i.e., Katahdin, St. Croix, Dorper, and Rambouillet) will be selected from each of the 4 locations in the US identified based on landscape environmental data to provide a wide gradient of conditions. Whole-genome genotyping using the 60K SNP chip will be performed for 10 randomly selected mature ewes and the 5 oldest ewes per breed and location/ecotype (160 randomly selected and 80 oldest in total). The randomly selected ewes will be exposed to environmental conditions that are expected to be more common in the future such as limited nutritional plane, restricted water availability and high heat load index, and they will be evaluated for their ability to minimize of energy, conserve water and tolerate heat under the respective conditions. Climate resilience phenotypes associated with specific stressors will be identified by characterizing relationships between resilience and environmental data. Genomic loci with adaptive significance will be identified by determining relationships between SNP markers and environmental variables in a landscape genomic analysis. Association mapping analysis will be used to assess the association of SNP markers with resilience phenotypes in order to identify quantitative trait loci controlling adaptation to environmental stressors. Genomic breeding value for resilience traits will be estimated and the accuracy of breeding values will be evaluated using a four-fold cross-validation method. A targeted SNP panel will be developed to be used in selection programs to improve genetic resilience to climatic stressors.

Do metritis associated bacterial components alter the follicular environment and perturb oocyte competence?

Sossi Iacovides and John J. Bromfield
Department of Animal Sciences, University of Florida

Bacterial uterine infection (metritis) is highly prevalent in dairy cows. Cows with metritis not only suffer pain and discomfort due to infection, but also display reduced fertility resulting in increased culling for failure to calf. Bacterial factors, such as the cell wall component lipopolysaccharide (LPS), have been shown to stimulate an innate immune response by non-hematopoietic cells of the endometrium and granulosa cells of the ovarian follicle via the Toll-like receptor 4 (TLR4) complex. Interestingly, the ovary and ovarian function appear to be potential secondary targets of bacterial infection. *In vitro* studies show that LPS exposure causes changes in granulosa cell gene expression and reduces meiotic competence of oocytes. These LPS derived changes may result in infertility of cows suffering uterine infection.
Elevated reactive oxygen species (ROS) are indicative of cellular stress and drive additional cell damage and apoptosis. However, ROS produced by cellular metabolism also mediates normal cellular homeostasis. Hematopoietic immune cells increase ROS in response to LPS, yet this has not been validated in the cumulus oocyte complex (COC) or granulosa cells. *We hypothesize that biochemical and mechanical cellular stress caused by LPS mediated inflammation, could promote changes to the follicular environment and perturb oocyte competence.* We intend to examine the role of ROS in altering the follicular environment and subsequent oocyte competence with 3 aims:
1. To examine ROS production in granulosa cells following LPS exposure. We will determine if LPS (and other bacterial components) induces elevated ROS production in granulosa cells, which may alter the follicular environment. We hypothesize that LPS exposure will increase ROS production by granulosa cells, thereby compromising the developmental environment of the oocyte.
2. To explore the role of ROS in oocyte maturation and developmental potential. We will determine if the presence of elevated ROS (via LPS exposure) in the maturation environment has a negative impact on meiotic progression of oocytes. We hypothesize that elevated ROS during oocyte maturation will have a negative effect on meiotic progression and the developmental competence of oocytes.
3. To assess the impact of ROS and LPS on cytoskeletal structure of granulosa cells.
Cytoskeleton is vital for the regulation of cell shape changes and the creation of mechanical forces. Granulosa cell cytoskeletal dynamics contribute to the regulation of ovarian steroidogenesis, while ROS impacts the dynamics of the cytoskeleton. We hypothesize increased ROS in response to LPS exposure has negative effects on granulosa cell steroidogenesis.
Understanding the mechanisms by which metritis effects fertility is critical. The studies outlined here will increase our knowledge of how innate immunity of the ovarian follicle results in reduced oocyte quality and infertility.

Uterine microbiota from calving until establishment of metritis in dairy cows

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Objective was to characterize the progression of uterine miocrobiota from calving until establishment of metritis. Uterine swabs were collected at 0 (20 min from calving), 2, 4, 6, and 8 d postpartum (DPP) from 92 cows. Twelve cows were diagnosed with metritis at 4, 6, or 8 DPP (6 ± 2 DPP), and 12 healthy cows were selected for comparison. Swabs (n=72) collected at 0, 2, and 6 ± 2 DPP were used for metagenomic sequencing of 16S rRNA gene on the Illumina MiSeq platform. A heat map showed that uterine microbiota was established in cows shortly after calving. The microbiota changed rapidly from 0 to 6 ± 2 DPP by decreasing the abundance of Proteobacteria and increasing the abundance of Bacteroidetes and Fusobacteria. At 6 ± 2 DPP, abundance of Bacteroidetes was significantly higher in metritic cows than healthy cows ($P < 0.01$). Although most genera were shared, healthy and metritic cows could be discriminated based on relative abundance at 0, 2 and 6 ± 2 DPP using discriminant analysis ($P < 0.01$). Also, discriminant analysis showed that Bacteroides, Filifactor, Porphyromonas, Fusobacterium, and Arcanobacterium/Trueperella were important predictors of metritis. Furthermore, Bacteroides and Fusobacterium were significantly correlated with uterine discharge score ($rs = 0.51$ and $rs = 0.49$, respectively; $P = 0.02$). Likewise, at species level, Bacteroides heparinolyticus and Fusobacterium necrophorum were the main bacteria for the development of metritis because they were both prevalent (16.8% and 20.2% in metritic cows; 7.0% and 15.8% in healthy cows) and correlated with uterine discharge score ($rs = 0.42$ and $rs = 0.42$, respectively; $P \leq 0.05$). In addition, there was a second (Fusobacterium gonidiaformans, Helcoccocus ovis, and Filifactor villosus) and third (Bacteroides pyogenes, Porphyromonas levii and others) line of bacteria that acted synergistically with the main bacteria causing metritis.

Unraveling the mechanisms that regulate activation of β-defensin antimicrobial peptide responses in cattle

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β-defensins are multifunctional peptides that not only function as antimicrobial peptides, but also act as signal molecules for cells of the immune system such as T-lymphocytes and monocytes. Bovine β-defensins exhibit antimicrobial properties against bacterial species specifically associated with mastitis, and expression of several β-defensin genes has been reported in the udder during mastitis. In cattle, toll-like receptor and vitamin D signaling pathways induce expression of multiple β-defensin genes; however, β-defensin expression in mammary epithelial cells is only activated via the TLR pathway and is much lower compared to neutrophils and macrophages. The promoter regions of the bovine chromosome 27 β-defensin gene cluster contain multiple potential DNA methylation sites, suggesting the hypothesis that epigenetic mechanisms contribute to vitamin D-mediated induction of the β-defensin response in cattle. The objective of this study was to investigate the effects of DNA methylation and histone deacetylation inhibitors, 5-aza-2'-deoxycytidine (5-Aza) and Trichostatin A (TSA), respectively, on TLR and 1,25D-induced expression of β-defensins in primary bovine mammary epithelial cells (bMEC). Primary bMEC cultures obtained via mammary biopsy were treated with 5-Aza, TSA or control in combination with lipopolysaccharide (LPS; 100 ng/mL; 16 h) and 1,25-dihydroxyvitamin D3 (1,25D; 10 nM, 16 h). Expression of β-defensin 3 (*BNBD3*), *BNBD4, BNBD7, BNBD10,* and lingual antimicrobial peptide (*LAP*) genes was determined using qPCR. The 5-Aza treatment resulted in a greater than 10-fold increased expression of the *BNBD3* (P=0.0006), *BNBD4* (P<0.0001), *BNBD7* (P=0.001), *BNBD10* (P<0.0001), and *LAP* (P<0.0001) genes. There was also an interaction seen between LPS and 5-Aza resulting in an increased expression of the *BNBD3* (P=0.0001), *BNBD10* (P=0.0008) and *LAP* (P<0.0001) genes. TSA treatment also increased expression of the *BNBD7* (P<0.0001), *BNBD10* (P<0.0001), and *LAP* (P=0.0023) genes approximately 5-fold compared to control. There was no 1,25D and 5-Aza or TSA interaction affecting these genes. These data suggest that DNA methylation and histone acetylation both contribute to regulation of the β-defensin response, and methylation of the *BNBD3, BNBD10,* and *LAP* promoters in particular may affect TLR activation of the β-defensin antimicrobial response of bMEC.

Effects of aging on mitochondrial function in skeletal muscle of Quarter Horses

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Abstract. Research in human and rodents has shown an age-associated decline in physical function, aerobic capacity and skeletal muscle mitochondrial function, which in humans begins around the age of 50. On the other hand, many horses can still actively work or compete beyond 20 years of age, an age equivalent to a 65-year old human. The purpose of the present study was to determine the age-related changes in fiber type composition and mitochondrial function in equine skeletal muscle. Muscle biopsies of right *gluteus medius* and *triceps brachii* from young (1.8±0.1 y; n=24) and aged (20±5 y; n=10) Quarter Horses were compared. High-Resolution Respirometry was performed on freshly sampled and subsequently permeabilized muscle fibers. Remaining tissue was frozen in liquid nitrogen and stored at -80°C for measurement of fiber type composition and enzyme activities. Statistical differences were analyzed using One-Way ANOVA and Holm-Sidak post hoc analysis (Sigmaplot 12.0). We found that aged horses had a higher percentage of oxidative type I myosin heavy chain (MHC) isoform in both *gluteus* (P<0.001) and *triceps* (P=0.024) compared to young horses. The proportion of glycolytic type IIX MHC isoform tended to decrease with advancing age, particularly in *triceps* (P=0.061). The proportion of intermediate fiber type IIA MHC isoform was not affected by age. Age had no effect on mitochondrial respiration in *gluteus;* but *triceps* from aged compared to young horses had greater leak respiration (P=0.038), electron transport system capacity (P=0.032), and a tendency for a lower respiratory control ratio (P=0.076). Cytochrome *c* oxidase activity in both *triceps* (P<0.001) and *gluteus* (P<0.001) was lower in aged compared to young horses. Using citrate synthase activity as a marker, mitochondrial density increased by 26.8% in the *gluteus* of aged horses (P=0.034), but was unaffected by age in the *triceps* (P=0.183). Our data suggest that aging resulted in an increased percentage of oxidative type I fibers, increased mitochondrial density, and impaired mitochondrial function in Quarter Horse skeletal muscle.

Localization and characterization of Enterohemorrhagic *Escherichia coli* O157:H7 T3SS effectors EspR, EspX, and EspY in host cells

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 subverts host signaling pathways by injecting multiple effectors into host cells via a type III secretion system (T3SS) encoded at the locus of enterocyte effacement (LEE). Although many of the T3SS effectors are expressed from LEE, EHEC encodes a number of effectors from genetic loci distinct from LEE and their roles during infection remain largely uncharacterized. Determining their function has been complicated as many of the effectors contain multiple paralogs including members of the EspR, EspX, and EspY families. Interestingly, despite their homology, inactivation of individual family members results in specific phenotypic differences including changes in cytotoxicity, decreased adherence to epithelial cells and defects in actin cytoskeleton formation. Consistent with these differences, individual family members were targeted to different cellular compartments when ectopically expressed within mammalian cells. For examples, the paralogous effectors EspR3 and EspR4 were localized to the endoplasmic reticulum and the nucleus, respectively, and nuclear localization of EspR4 was dependent on a c-terminal leucine-rich motif. These findings provide an example whereby homologous effectors have evolved to manipulate host cell signaling in diverse ways, thereby contributing to the exquisite ability of EHEC to cause disease.

Intramammary infusion of vitamin D metabolites stimulates host defense genes in the bovine mammary gland

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In bovine peripheral blood monocytes, the active form of vitamin D, 1,25-dihydroxyvitamin D_3 (1,25D), enhances the expression of multiple β-defensin antimicrobial genes, inducible nitric oxide synthase (iNOS), and the chemokine RANTES/CCL5 (Merriman et al., submitted). In the mammary gland, CYP27B1, the enzyme that catalyzes 1,25D production from 25-hydroxyvitamin D_3 (25D), is triggered in response to pathogens, and intramammary 25D treatment inhibited experimental *Streptococcus uberis* infection (Lippolis et al., 2011). The intramammary 25D treatment was thought to inhibit infection via 1,25D-mediated upregulation of iNOS, RANTES, and β-defensin antimicrobial genes. The objective of this study was to determine the effects intramammary 25D and 1,25D treatments on mammary host-defense gene expression. The objective was performed two separate experiments. In the first experiment, contralateral quarters of seven healthy Holstein cows (SCC ≤ 200,000 cells/mL) were treated with an intramammary infusion of 10 ug of 1,25D or placebo (10 mL of PBS with 10% FBS) at the completion of milking. Milk samples were collected at 0, 2, 4, 8, and 12 h relative to treatment, and somatic cells were collected for analysis of host-defense gene expression. At 2, 4, 8, and 12 h post 1,25D infusion CYP24A1, a positive control gene for 1,25D activity, was increased in the infused quarters relative to the control quarters ($P < 0.0001$). The 1,25D treatment increased iNOS expression 2-fold in milk somatic cells at 8 h and 12 h post infusion relative to the control quarters ($P < 0.05$), and β-defensin 3 (DEFB3) and DEFB7 expression 2-fold at 4 h post infusion relative to the control quarters ($P < 0.05$). In addition, macrophages isolated from milk somatic cells using fluorescence-activated cell sorting (FACS) had 2-fold greater DEFB4 and DEFB7 expression in response to 1,25D treatment ($P < 0.05$). In the second experiment, all four quarters of five cows were treated with either 5 ug of LPS, 100 ug of 25D, a combination of 100 ug 25D and 5 ug LPS (LPS+25D), or placebo (10 mL of PBS with 10% FBS). The 25D, LPS, and LPS+25D treated quarters had increased CYP24 expression at 4 h post infusion relative to control quarters ($P < 0.01$), indicating that vitamin D signaling is present in activated somatic cells. At 8 h post infusion, somatic cells from LPS and LPS+25D treated quarters had increased CYP27B1, iNOS, DEFB3, DEFB4, DEFB7, DEFB10, and lingual antimicrobial peptide gene expression relative to control quarters and pre-challenge ($P < 0.05$). Treatment with 25D either alone or in combination did not affect host-defense gene expression. Macrophages and neutrophil populations also were isolated from milk using FACS. The neutrophils from the LPS and LPS+25D treated quarters had increased CYP27B1, iNOS, RANTES, DEFB3, DEFB7, and DEFB10 expression relative to the control quarters at 8 hours post treatment ($P < 0.05$). In contrast, only iNOS and CYP27 were increased in macrophages from LPS and LPS+25D treated quarters compared to macrophages from the control quarters ($P < 0.05$). In conclusion, 1,25D enhances expression of critical host-defense genes in mammary macrophages, however, intramammary 25D treatment did not enhance host-defense gene responses to LPS challenge even though vitamin D pathway signaling was activated.

Effect of hydrogen peroxide and heat stress on stallion sperm function

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Spermatozoa are highly specialized cells, sensitive to environmental factors such as temperature and redox state. Increased temperature and reactive oxygen species can result from exercise, and when applied *in vitro* are associated with a decline in sperm function. The aim of this study was to evaluate the effect of H_2O_2 and heat stress on DNA damage, membrane integrity and motility of stallion spermatozoa. We hypothesized that *in vitro* applied stressors would deteriorate sperm competence and would be reflected in a decline of membrane integrity, decreased motility and induce a higher percentage of DNA damaged sperm cells. Spermatozoa from three stallions were organized using a 2x2 factorial design and subjected to control (38 °C, 1 h), heat stress (41°C, 1 h), oxidative stress (50 μM H_2O_2, 1 h) or a combination of both (41°C + 50 μM H_2O_2, 1 h). Horses often can reach 41 °C with exercise and was chosen as our heat stress temperature. The experiments were replicated twice per stallion. All initial parameters were considered normal and semen was extended prior to processing. Upon arrival to the laboratory, semen was purified using a 40 % and 80 % discontinuous Percoll gradient to remove dead sperm and extender. After treatment, motility parameters were measured using a computer-assisted sperm analysis (CASA) system and DNA damage was assessed with the terminal dUTP nick end labelling (TUNEL) assay. To evaluate membrane integrity, an amine reactive dye was utilized. DNA and membrane integrity were simultaneously assessed using flow cytometry. Compared to control, heat-treated sperm were observed to have decreased total motility (76.2 % vs. 66.6 %, respectively; $P = 0.048$) but did not have an effect on progressive motility (39.2 % vs. 33.4 %) or membrane integrity (30.8 % vs. 37.4 %). In addition, heat did not induce DNA damage (19.7 % vs. 17.3 %). Oxidative stress treatments did not affect total motility (76.2 % vs. 78.6 %), progressive motility (39. 2% vs. 46.4%), or membrane integrity (30.8 % vs. 25.9 %). Interestingly when compared to control, sperm cells subjected to oxidative stress were observed to have decreased DNA damage in both H_2O_2 treatments (19.7 % vs. 7.1 %, respectively; $P < 0.001$) and combination treatments (17.3 % vs. 6.5 %; $P < 0.001$). There were no interactions between heat and oxidative stress treatments in any of the parameters analyzed. It is interesting from our results a favorable effect of 50 μM H_2O_2 on limiting DNA damage in stallion spermatozoa, whereas this concentration has induced DNA damage in other species. Further studies need to be conducted to confirm these findings, and to investigate a potential signaling mechanism that H_2O_2 may be exerting to decrease DNA damage.

Role for CC cytokines in differentiation of the inner cell mass of the bovine embryo

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Previous research indicates that the chemokine (C-C motif) ligand *CCL24* is overexpressed in the inner cell mass (ICM) of the blastocyst as compared to the trophectoderm (TE) (BMC Dev Biol 12:33). Given the potential importance of cell migration for establishment of the hypoblast adjacent to the blastocoele on the outer edge of the ICM, we hypothesize that CCL24 or another embryo-derived chemokine plays a role in differentiation 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 quantitative real-time PCR with *YWHAZ*, *SDHA*, and *GAPDH* as internal control genes. 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 a CCR3 antagonist (SB328437) beginning at Day 6. Embryos (n=25-26 per group) were collected at Day 8, fixed with 4% paraformaldehyde 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 microscopy. SB328437 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). 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. In summary, the bovine embryo expresses a C-C chemokine, *CCL24,* at a time coincident with blastocyst formation and the 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-sensitive mechanism to regulate position of hypoblast cells in the ICM. USDA AFRI Grant No. 2011-67015-30688.

Effect of a single nucleotide polymorphism in *COQ9* on cellular energy metabolism, fertility and milk production in Holstein cows

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Previously, a single nucleotide polymorphism (SNP) for *COQ9* was found to be significantly related to genetic merit for fertility in two separate populations of Holstein cattle, with the minor allele (G) being associated with higher fertility. COQ9 is necessary for the synthesis of coenzyme Q_{10} which is a component of the mitochondrial (mt) electron transport system (ETS). Here, we evaluated the effect of *COQ9* genotype on the ETS, and phenotypic measurements of fertility and production in Holstein cows. The SNP in *COQ9* was genotyped using a Sequenom MassARRAY® assay. In the first study, cows ≥ 200 days in milk were selected for analysis of mitochondrial oxygen consumption [*COQ9* genotype: AA (n=10), AG (n=10) and GG (n=11)]. Peripheral blood mononuclear cells (PBMC) were isolated and mt respiration assessed using the Oroboros O2k high resolution respirometer to evaluate routine respiration, *R*, leak respiration, *L*, and ETS capacity, *E* (expressed as the uncoupling control ration *UCR=E/R*)). There were additive effects of genotype on mt respiratory function ($P<0.05$): *R* was 4.7±0.4, 3.8±0.4 and 3.6±0.3 pmol $O_2/s/10^{-6}$ cells, *L* was 2.7±0.3, 1.5±0.3 and 1.9±0.3 pmol $O_2/s/10^{-6}$ cells and *UCR* was 2.0±0.3, 2.8±0.3 and 3.0±0.3 for AA, AG, and GG, respectively. In a second study, we evaluated days open (DO), services per conception (SPC) and 305-day milk yield (MY) for the first lactation in a population of 2273 Holstein cows grouped based on predicted transmitting ability for daughter pregnancy rate (DPR): ≤ -1 (n=1220) and ≥ 1.5 (n=1053), and located on 11 farms in Florida and California. Farm records were retrieved from on-farm computers and combined with records from the national genetic evaluation system. The association of the genetic variants was evaluated using SAS V9.4 (SAS Institute, Inc., Cary, NC). Continuous data were analyzed by analysis of variance using the MIXED procedure while categorical data were analyzed by logistic regression using the GLIMMIX procedure. The model included farm, number of copies of the minor allele, and the numerator relationship matrix to account for (co)variances among animals. Genotype was considered as a categorical variable to estimate additive and dominance effects. Genotype affected each trait ($P<0.05$). Values for AA, AG and GG were as follows: DO, 139.4±3.5, 134.3±2.8, and 123.6±3.5 d; SPC, 2.7±0.1, 2.5±0.1 and 2.4±0.1 services; and MY, 9776±57, 9727.±44.4 and 9610±57 kg. Results indicate that *COQ9* genotype affects mt respiratory function with the genotype previously associated with genetic merit for fertility (GG) displaying lower routine and leak respiration, , and higher ETS capacity. Moreover, genotype associated with favorable phenotypic measurements of fertility was less favorable for MY. Relationships between *COQ9* genotype and phenotypic characteristics mirror the relationships with genetic estimates observed earlier. Further research will be required to elucidate the role of *COQ9* variants on Q_{10} production and mt function (USDA AFRI 2013-68004-20365).

Vitamin D status of dairy calves fed pasteurized whole-milk

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Calves need vitamin D to support bone growth and immunity. Feeding dairy calves pasteurized whole-milk is a common practice, but the vitamin D status of milk-fed calves has not been widely appreciated. The objectives of this study were to determine serum 25-hydroxyvitamin D (25D) concentrations of dairy calves fed pasteurized whole-milk and the effects of subcutaneous vitamin D injections on serum 25D concentrations of milk-fed dairy calves. Two experiments were conducted on two separate farms and serum 25D concentrations were measured using a 25D ELISA. In the first experiment, 23 Holstein calves received a vitamin AD&E (1200 IU D-α-tocopherol, 400,000 IU retinyl-palmitate, and 40,000 IU vitamin D3; n= 11) or saline injection (n=12) at birth, and fed pasteurized whole milk and housed indoors. Serum 25D concentrations of the control calves were 8.1±1.0, 11.2±2.6, 13.0±3.2, and 13.3±1.6 ng/mL (mean±SE) on days 0, 7, 22, and 37, respectively, past birth. In contrast, serum 25D concentrations of the vitamin AD&E treated calves were the same as control calves at birth (10.0±1.0 ng/mL), greater at days 7 and 22 (25.4±2.8 and 24.9±3.4 ng/mL, repectively; $P<.05$), and not different from controls at day 37 (14.3 ± 1.6 ng/mL; treatment x time interaction, $P<0.05$). In the second experiment, 13 Holstein bull calves received either 80,000 IU of vitamin D3 via subcutaneous injection at birth and once weekly for 3 weeks (n=5) or no injection (control, n=8), and fed pasteurized whole milk and housed under shaded structures. Serum 25D concentrations of the non-treated calves were 13.4±3.3, 5.1±3.4, 8.0±4.1, and 8.7±4.9 ng/mL (mean± SE) on days 0, 7, 14, and 21, respectively, past birth. In the vitamin D-treated calves, serum 25D was the same as control calves at birth, but was increased to 25.6±4.4, 38.1±5.2, and 41.2±6.2 ng/mL (mean±SE) at 7, 14 and 21 days of age, respectively (treatment x time interaction, $P<.01$). $CD14^+$ cells were isolated from peripheral blood and treated with either 0 or 100 ng/ml lipopolysaccharide (LPS) in the second experiment. The mRNA transcripts of CYP27B1, CYP24A1, inducible nitric oxide synthase (INOS), β-defensin 3 (DEFB3), and β-defensin 7 (DEFB7) were quantified by real-time PCR. There was an overall effect of LPS on expression of CYP 24A1, CYP27B1, and INOS ($P<.0001$). There was an overall effect of time on expression of CYP27B1 ($P<.001$). Expression of BD3 was higher at day 7 for calves that received vitamin D3 injection ($P<.05$). In conclusion, milk-fed dairy calves, particularly if housed indoors or under shade, are at risk for vitamin D deficiency (serum 25D <10 ng/mL) if they do not receive supplemental vitamin D, and continuous vitamin D supplementation is needed to maintain vitamin D status of calves.

Patterns of expression of genes involved in lipid metabolism in bovine preimplantation conceptuses at the onset of elongation

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Objectives were to investigate changes in transcriptome related to lipid metabolism in preimplantation conceptus cells at the onset of elongation. Lactating dairy cows (n = 160) had estrous cycles and ovulation synchronized for timed artificial insemination (AI). The day of AI was considered study d 0. On d 15, uteri were flushed and recovered conceptuses were classified based on morphology/length as ovoid (OV; 1-4 mm), tubular (TUB; 5-19 mm) and filamentous (FIL; 20-85 mm). A subsample of conceptuses from each morphology group had mRNA extracted and subjected to transcriptome analysis using the Affymetrix Gene Chip® Bovine Array (8 OV, 17 TUB, and 17 FIL). The experimental design was a prospective cohort study with 3 independent groups. Microarray data were analyzed using the Bioconductor software within R statistical computing programming. Data were pre-processed using Gene Chip Robust Multi-Array function. The Limma package was used to fit a linear model and adjust variances by empirical Bayes method. Moderate t-test was performed for all pairwise comparisons and *P* values were adjusted for multiple testing using the Benjamini and Hochberg false discovery rate. Adjusted $P < 0.05$ and fold change > 1.5 characterized significant differences. Functional analyses were performed using IPA. Results revealed substantial differences in transcriptome of cells from OV, TUB, and FIL conceptus. A total of 1,610 transcripts were differently expressed in one or more pairwise comparisons. From those transcripts, at least 132 annotated genes are known to be associated with lipid metabolism. Functional analysis revealed the transcription factor PPARγ as a potential upstream regulator of some of the changes observed in transcriptome. Corroborating with this prediction, expression of *PPARG* increased 16-fold from OV to FIL and was highly correlated with the expression of other genes also involved in lipid metabolism. Among them were genes associated with lipid uptake (*SCARB1, SCL27A6, SCL27A2*), lipid droplet formation (*PLIN2*), activation (*ACSL3, ACSL6, ACSS2, CRAT*) and oxidation (*ABCD3, ACOT8, ACOT4, ACOX1, ALDH3A2*) of fatty acids, peroxisomes biogenesis (*CAT, PEX11A*), transport of lipids metabolites (*ABCG2, SLC10A1*), mobilization, biosynthesis and transport of fatty acids (*FADS2, FADS1, PLA2G7, PLA2G12A, TECR*), phospholipids (*AGPAT9, AGPAT3, DGKD, GPAM, LPCAT3, LPCAT1, MOGAT1, PLCL2, SLC37A1, STARD10*), and prostaglandins (*PTGES, PTGIS, PTGS2, SLCO2A1*). In conclusion, lipid metabolism seems to be essential for preimplantation conceptus elongation and is mainly coordinated by PPARγ control of transcription. As the bovine conceptus elongates, the cellular machinery for metabolism of lipids is reinforced by increased expression of genes involved in oxidation of long chain fatty acids and synthesis of lipid derivatives such as prostaglandins and phosphatidic acid. Oxidation generates ATP, whereas lipid derivatives are likely important for downstream cell signaling and coordination of additional changes required in cell biology of conceptus and endometrium cells during preimplantation development.

Effects of feeding algae rich in docosahexaenoic acid (DHA) on lactation and reproductive performance of dairy cows

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Objectives were to determine the effects of supplementing DHA by feeding algae on lactation and reproductive performance of Holstein cows. Weekly cohorts of cows were blocked by parity and randomly assigned at 27 DIM to a control [**CON**; n=372, 115 primiparous and 258 multiparous] or an algae supplemented (**ALG**; n=366, 105 primiparous and 261 multiparous) diet. Cows were fed the same TMR; however, ALG received 100 g/cow/d of a 17% DHA supplement that was top-dressed and hand-mixed in the TMR for 120 d. Milk yield was recorded daily and sampled every 3 weeks and analyzed for fat, protein, lactose, and somatic cells. Cows were subjected to the Presynch-Ovsynch protocol starting at 44±3 DIM and those in estrus after 58±3 DIM were inseminated on the same day, otherwise, they received timed AI at 80±3 DIM. Pregnancy was diagnosed on d 60 after AI. Open cows were resynchronized with Ovsynch. Data were analyzed by ANOVA, logistic regression or the Cox's proportional hazard model using the GLIMMIX and PHREG procedures of SAS. Intake of DM did not differ between treatments (ALG = 24.8 vs. CON = 25.5±0.6 kg/d). Milk yield was greater (P=0.01) in ALG than CON (43.6 vs. 42.5±0.3), but yield of energy-corrected milk was similar between treatments and averaged 39.2±0.2 kg/d. Feeding ALG reduced (P≤0.03) content and yield of milk fat (3.08 vs. 3.23±0.03% and 1.30 vs. 1.34±0.01 kg/d); however, ALG increased (P<0.01) yields of protein (1.23 vs. 1.20±0.01 kg/d) and lactose (2.07 vs. 2.02±0.01 kg/d). Feeding ALG increased (P=0.04) the proportion of primiparous cows that resumed estrous cyclicity by 58 DIM (77.6 vs. 65.9%) and pregnancy at first AI (47.6 vs. 32.8%). Feeding ALG increased (P<0.01) pregnancy per AI at all AI for primiparous and multiparous cows (41.4 vs. 30.8%). Cows fed ALG became pregnant 21 d (103 vs. 124 d) sooner (P< 0.01) than those fed Control (adjusted HR = 1.38; 95% CI = 1.13 to 1.69). Supplementing DHA by feeding ALG did not affect intake of DM, increased yields of milk and protein, but reduced milk fat content and yield. Cows fed ALG had improved estrous cyclicity, pregnancy per AI, and reduced interval to pregnancy.

Colony-stimulating factor 2 affects development of the bovine preimplantation embryo differently for females than males

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Colony-stimulating factor 2 (CSF2) regulates early embryonic development by modifying the epigenome, reducing apoptosis, and altering ratio of cells in the trophectoderm (TE) and inner cell mass (ICM) of the blastocyst. Previously, CSF2 reduced trophoblast elongation in female embryos but increased elongation in males. Here it was tested whether sexual dimorphism in response to CSF2 can be observed as early as the blastocyst stage. Embryos were produced in vitro using X- or Y-sorted sexed semen (n=1612 putative zygotes). On day 5 of culture, droplets were supplemented with 5 μL vehicle (control) or 10 ng/ml bovine CSF2. Blastocysts (n=210) were collected at Day 7 and labeled with a nuclear dye (Hoescht 33342; total cells) and a TE cell marker (CDX2). Number of ICM cells was calculated by subtraction. Statistical analysis was performed using the Proc Mixed procedure of SAS; data represent least-squares means ± SEM. Treatment of female embryos with CSF2 increased the proportion of zygotes (P=0.0213) and cleaved embryos (P=0.0252) to become a blastocyst but there were no effects in males (P>0.10). The percent of zygotes becoming blastocysts on Day 7 was 14.7±2.1 vs 21.5±2.1% for control and CSF2 in females and 16.2±2.0 vs 16.3±2.0% in males. There was no effect of CSF2 treatment, sex, or the interaction on the total cell number or number of TE (P>0.10). There was a tendency (P=0.0934) for ICM number to be less in females (56.2±3.1 vs 61.0±2.9) and the TE:ICM ratio was greater (P=0.0217) for females (1.64±0.91) compared to males (1.45±0.09). Numerically (but not significantly), CSF2 tended to decrease ICM in females (53.9±3.6 vs 58.6±3.7) but not in males (60.4±3.5 vs 61.5±3.4). There was a tendency for a CSF2 by sex interaction (P=0.0955) for TE:ICM ratio. In females CSF2 increased ratio (1.73±0.11 vs 1.55±0.11), but no effect was observed in males (1.41±0.10 vs 1.50±0.10). In conclusion, CSF2 exerts different responses on development of female and male preimplantation embryos. Consequences of actions of CSF2 on ICM and TE cell differentiation require further investigation (Support: NIH HD080855).

Transport of a fluorescent analog of glucose (2-NBDG) by rumen bacteria

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Our objective was to determine if cultured strains of rumen bacteria would transport a fluorescent analog of glucose (2-NBDG) with the same specificity and kinetics as glucose. Our rationale was that 2-NBDG could be used to identify uncultured, glucose-utilizing bacteria if it were transported similarly to glucose. Pure cultures of bacteria were harvested in the mid-to-late log phase, washed, and dosed with 2-NBDG or radiolabeled sugar (0 to 100 μM). Transport was halted by adding -5°C stop buffer and filtering through a membrane. The membrane was taken for fluorometry or liquid scintillation counting. Transport of 2-NBDG could be detected within 2 s of 2-NBDG addition for *Streptococcus bovis* and *Selenomonas ruminantium* (2 strains each), but it was not detected at any time for 6 other glucose-fermenting species. Genomes of *S. bovis* and *S. ruminantium* strains were found to possess genes for the mannose phosphotransferase system, whereas the other species had genes for other glucose transporters. For *S. bovis* JB1, the Michaelis constant (K_m) for 2-NBDG transport was 10.6-fold lower than that for [^{14}C]-glucose transport (P = 0.006). The maximum velocity (V_{max}) was 2.9-fold lower than that for [^{14}C]-glucose, but this difference was not significant (P = 0.100). In another set of experiments, transport of 2-NBDG at a single concentration (100 μM) was compared to that of [^{14}C]-glucose, [^{3}H]-mannose, [^{14}C]-deoxy-2-glucose. For *S. bovis* JB1, transport of 2-NBDG was 3.2-fold lower than that of [^{14}C]-glucose (P = 0.002) but similar to that for [^{3}H]-mannose (P = 0.992) and [^{14}C]-deoxyglucose (P = 0.955). 2-NBDG could identify uncultured, glucose-utilizing bacteria, but only those with a mannose phosphotransferase system (not other glucose transporters). Its transport may more closely reflect that of mannose and deoxy-2-glucose than glucose.

Endometrial expression of genes involved in growth factor, cytokine, hormone, and WNT signaling during the early estrous cycle of the cow

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Knowledge of the molecules used by the maternal reproductive tract to regulate development of the preimplantation embryo is largely incomplete. To identify possible candidates for this function, an experiment was conducted to assess expression patterns during the first seven days after ovulation for 93 genes that could potentially be involved in control of development. Included were genes for 31 growth factors, 10 cytokines, 21 interleukins, 3 hormones, 19 WNT ligands and 9 WNT regulatory molecules. Ovulation was synchronized in 15 cows and cows were slaughtered at days 0, 3, 5 and 7 relative to predicted ovulation. Reproductive tracts were obtained and endometrial tissue was harvested for gene expression analysis from uterine horns. Purified RNA was analyzed by the NanoString nCounter analysis system to determine abundance of specific mRNA molecules. Data were normalized against 6 housekeeping genes (*ACTB, ERK1, GAPDH, RPL19, SLC30A6, SUZ12*) and internal positive controls. Genes were considered expressed if the number of reads was greater than two standard deviations above the mean of negative controls. Data were analyzed by analysis of variance using the GLM procedure of SAS with day as fixed effects and cow as a random effect. All genes were detected at some point of the evaluated period. Eight genes were significantly affected by day with values highest at estrus *(BMP7*), Day 5 (*TDGF1*) or Day 7 (*CXCL16, FGF13, NGF, VEGFB, WNT11, WNT7A*). There was a wide variation in the magnitude of gene expression. Among the 20 most expressed genes, the reads varied from 150 to over 36000. For day 5, the ten most highly expressed genes in descending magnitude were *CXCL3, WNT5A, TDGF1, CTGF, SFRP1, GRO1, HDGF, IK, VEGFA, IGF1.* For day 7, the ten most highly expressed genes were *TDGF1, CTGF*, *CXCL12, VEGFB, WNT5A, IK, CXCL10, HDGF, IGF2, SFRP1*. This experiment points to potential maternal regulators of embryonic development. Further studies are needed to determine the effect of these maternally secreted molecules on embryonic development (Support NIH HD080855).

Association between gestation length with health, reproduction, and production in Holstein cows

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Objectives were to evaluate the association between gestation length (**GL**) and incidence of diseases, reproduction, and milk production. The data were screened to eliminate cows with GL longer or shorter than 3 SD from the population mean, resulting in 104 cows excluded from the analyses. Holstein cows (n=6,254) on a farm using only artificial insemination (**AI**) were evaluated. Responses measured included the incidences of stillbirth, retained placenta (**RP**), metritis, mastitis, and other diseases within 90 d in milk (**DIM**). Pregnancy at first AI and interval to pregnancy were evaluated. Milk yield and removal from the herd by death or culling were recorded for the first 300 DIM. Gestation length was categorized as short (**S**; at least 1 SD below the population mean; group mean = 266, range 256 to 269), normal (**N**; population mean ± 1 SD; group mean = 276, range 270 to 282 d), and long (**L**; > 1 SD above the population mean; group mean = 285, range 283 to 296 d). Data were analyzed by ANOVA, logistic regression, and the Cox's proportional hazard model using the GLIMMIX and PHREG procedures of SAS. Models included the fixed effects of GL category (S, N, L), gender of calf (female, male, twin), parity (1 or > 1), season of calving (cool or hot), and all two-way interactions. Gestation length affected (P<0.01) the incidences of stillbirth (S=11.4, N=7.3, L=6.7%), RP (S=32.7, N=10.3, L=9.4%), metritis (S=51.1, N=36.5, L=33.9%), but not (P=0.85) that of mastitis (S=4.7, N=4.0, L=5.2%). The rate of removal from the herd by culling or death was faster (P<0.01) for S than N (adjusted HR=1.32; 95% CI=1.10-1.58) and tended (P=0.10) to be faster for L than N (adjusted HR=1.12; 95% CI=0.98-1.29). Pregnancy at first AI did not differ (P=0.94) among groups (S=33.2, N=34.3, L=33.5%). The rate of pregnancy was greater (P<0.05) for N than L (adjusted HR=1.11; 95% CI=1.02-1.21), but it did not differ between N and S or L and S. Daily milk yield was greater (P<0.01) for N than S or L (S=35.2, N=38.1, L=35.8±0.4 kg/d). Cows with GL within 1 SD of the population mean (range 270 to 282 d) had improved health, reproduction, and production.

CD1d-knockout pigs: a novel model to study the role of natural killer T cells in immunity

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Studies in mice genetically lacking invariant natural killer T (iNKT) cells show that these lymphocytes make important contributions to both innate and adaptive immune responses. However, the usefulness of murine models to study human iNKT cells is limited by the many differences between mice and humans, including that their iNKT cell frequencies, subsets and distribution are dissimilar. A more suitable model may be swine that share many metabolic, physiological and growth characteristics with humans and are also similar for iNKT cells. Thus, we analyzed genetically modified pigs made deficient for CD1d that is required for the development of iNKT cells. Peripheral blood analyzed by flow cytometry and interferon-γ (IFNγ) enzyme-linked immuno spot (ELISPOT) assays demonstrated that CD1d-knockout pigs completely lack iNKT cells while other leukocyte populations remain intact. CD1d and iNKT cells have been shown to be involved in shaping the composition of the commensal microbiota in mice. Therefore, we also compared the fecal microbiota profile between pigs expressing and lacking iNKT cells. However, no differences were found between pigs lacking or expressing CD1d. Our results are the first to show that knocking-out CD1d prevents the development of iNKT cells in a non-rodent species. CD1d-deficient pigs should offer a useful model to more accurately determine the contribution of iNKT cells for human immune responses. They also have potential for understanding how iNKT cells impact the health of commercial swine.

Unmasking genes controlling how $CD4^+$ T-cells pathogenically activate type-1 diabetes inducing $CD8^+$ T-cells

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Most beta-cell destruction in type-1 diabetes (T1D) is due to the cytotoxic effects of autoreactive $CD8^+$ T-cells that rely on $CD4^+$ T-cell-help for their activation. Here we report that this process is in part controlled by our previously described T1D susceptibility locus on distal chromosome 11 (Idd32), discovered through a first-backcross between diabetes-susceptible NOD mice and diabetes-resistant C57BL/6 (B6) mice congenic for the NOD-derived $H2^{g7}$ MHC haplotype ($B6.H2^{g7}$). In adoptive transfer studies we found that lymphopenic recipients injected with $CD8^+$ T-cells transgenically expressing the diabetogenic AI4 TCR were completely resistant and susceptible to T1D when co-injected with $CD4^+$ T-cells from donors respectively expressing heterozygous ($Idd32^{NOD/B6}$) or homozygous ($Idd32^{B6/B6}$) B6 alleles at Idd32. Diabetogenic $Idd32^{B6/B6}$ $CD4^+$ T-cells required CD40-CD40L interactions to pathogenically activate AI4 T-cells. Surprisingly, protective $Idd32^{NOD/B6}$ $CD4^+$ T-cells inhibited AI4 T-cell activation through a mechanism that requires interferon-γ (IFNγ), generally considered a pro-inflammatory cytokine. In support that our findings are clinically significant, pre-activation with IFNγ reduced the cytotoxicity of human diabetogenic $CD8^+$ T-cells used in a cell-mediated lympholysis assay. Our results are important for addressing how $CD4^+$ T-cells control when tissue-specific $CD8^+$ T-cells, which harmlessly circulate in most individuals, sometimes become pathogenically activated. They are also important for interventions to block this disease-critical process.

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