FIFTEENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Safety Harbor Resort and Spa

Safety Harbor, Florida
April 7-8, 2017

WELCOME

For the first time, the AMCB will meet along the shores of Tampa Bay. We will be joined by some participants from the nearby University of South Florida. We welcome the participation of our colleagues at USF and look forward to increased collaborations in the future.

The AMCB is all about connections - connections between areas of research, between technical approaches to science, and between scientists themselves. The program has 16 faculty in four colleges and work in diverse areas ranging from genetics of the horse to bacterial physiology. This year's distinguished lecturer, Kieran Meade of Teagasc, can serve as an example of how thinking broadly about biology can help us develop greater understanding. His research on β-defensins involves immunology, reproduction, genetics and microbiology and should be of broad interest to the group.

The AMCB remains effective at educating scientists with an understanding of molecular and cellular biology in a large-animal context. The program has 14 PhD and 3 MS students, and ~40 attendees of the symposium are expected. Graduates of the program have been successful in finding good positions. Last year, for example, Anna Denicol, who received her PhD in 2014, obtained a faculty position at the University of California-Davis, Eduardo Ribeiro, who received the PhD in 2015, is now on the faculty at the University of Guelph.

On behalf of all the faculty of the AMCB, welcome to the 15th Annual Research Symposium of the AMCB. Have some fun, build some connections, and learn!

Pete Hansen, Director
John Driver, Co-Director

ACKNOWLEDGMENTS

The faculty and students of the AMCB Program thank the following for support of the 15th 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

2017 AMCB DISTINGUISHED LECTURER

Kieran G. Meade, PhD
Teagasc, the Agriculture and Food Development Authority of Ireland

Kieran Meade is a senior Animal Health Bioscientist in Teagasc and adjunct Assistant Professor of Immunology at Trinity College Dublin, Ireland. He received his bachelor's degree in Animal Science from University College Dublin in 2000 and subsequently a PhD in molecular genetics from the same institution. As an undergraduate, Kieran received a prestigious bursary in Agriculture award from the National University of Ireland. This award funded training in functional genomics including in the laboratory of Professor Paul Coussens in Michigan State University. During his PhD studies, Kieran also spent time in the International Livestock Research Institute (ILRI) in Kenya applying these techniques to understand bovine Trypanosomiasis. Subsequently as a postdoctoral research scientist, Kieran spent periods of research in the Vaccine and Infectious Disease Organisation (VIDO) in Canada, working on avian *Campylobacter* and *Salmonella* infections. Kieran's research was the first to apply functional genomics techniques to gain a deeper understanding of the immune response to multiple infectious agents, most particularly in relation to TB in cattle. The suppression of innate immune gene expression detected by his work led to the first analysis of the role of methylation in controlling gene expression in TB infected cattle. Amongst his other achievements was the discovery and functional characterisation of natural antibiotics, β-defensins, in the genomes of multiple livestock species including horses, chickens and cattle. His lab was the first to show the expression of these newly discovered β-defensins on bull sperm and document their association with fertility. Current funded work centers on exploitation of β-defensins as novel therapeutics as well as the identification of immune phenotypes to breed cattle with superior resistance to disease. Kieran is also interested in immunometabolism and has been shortlisted for a Fulbright award to perform research at Harvard investigating energetic costs associated with enhanced immunity in cattle. Dr. Meade has graduated 4 PhD students and 2 MSc students and has authored over 50 peer-reviewed publications. He is currently the primary mentor for 5 additional students and 2 postdoctoral fellows. On a personal note - one of Dr Meade's favourite pastimes is perfecting his coop building skills to house his rare-breed poultry.

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

Eliab Estrada Cortes (Advisor: P Hansen)

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)

Paula Tribulo (Advisor: P Hansen)

Guan Yang (Advisor: J Driver)

Cheng Ye (Advisor: J Driver)

Achilles Vieira Neto (Advisor: J Santos)

Roney Zimpel (Advisor: J Santos)

MS Students

Bethany Margaret Dado (Advisor: J Laporta)

Laila Ibrahim (Advisor: J Bromfield)

Michael Poindexter (Advisor: C Nelson)

Jason Rizo (Advisor: J Bromfield)

GRADUATES 2016

Jasmine Kannampuzha-Francis, MS (Advisor: PJ Hansen)
Currently scientist, Cornell University

GRADUATES 2017 (to date)

M Sofia Ortega Obando, PhD (Advisor: P Hansen)
Currently postdoctoral scientist, University of Missouri

Leticia Del-Penho Sinedino Pinheiro, PhD (Advisor: J Santos)
Currently postdoctoral scientist, Colorado State University

Luiz GB Siqueira, PhD (Advisor: PJ Hansen)
Currently, scientist, EMBRAPA Gado do Leite, Juiz de Fora, MG, 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	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
2017	Safety Harbor Resort and Spa, Safety Harbor, Florida	Kieran Meade Teagasc

SCHEDULE OF EVENTS

All events in the Athena Room unless otherwise noted

FRIDAY, APRIL 7

1:05 PM Pete Hansen
Welcome, introductory comments

Session 1: Embryo and Pregnancy
Achilles Vieira Neto and Laila Ibrahim, Chairs

1:15 PM Veronica Negrón-Pérez, Animal Sciences
Analysis of single-cell gene expression of the epiblast, hypoblast and trophectoderm of the late bovine blastocyst

1:30 PM Paula Tribulo, Animal Sciences
Consequences of exposure of embryos produced in vitro in a serum-containing medium to dickkopf-related protein 1 and colony stimulating factor 2 on blastocyst yield, pregnancy rate and birthweight

1:45 PM Adriana Zolini, Animal Sciences
Effect of addition of L-carnitine during culture on pregnancy rate obtained after transfer of cryopreserved bovine embryos produced in vitro

2:00 PM Gulnur Jumatayeva, Animal Sciences
Effects of sex on response of bovine preimplantation embryos to activin A and WNT7A

2:15 PM Vladimir Vargas, University of South Florida
Leptin receptor expression and leptin-induced MAPK signaling regulation of uterine artery angiogenesis in ovine endothelial cells of the ovarian cycle and pregnancy

2:30 PM BREAK

Session 2: 2014 AMCB Distinguished Lecturer Presentation
Corwin Nelson, Chair

3:00 PM Kieran Meade
Teagasc, Grange, Co. Meath, Ireland
Bovine β-defensins - at the interface of reproduction and immunity

4:00 PM BREAK AND CHECK INTO ROOMS

Session 3: Uterus/Muscle/Postpartum Cow
Roney Zimpel and Cheng Ye, Chairs

5:00 PM Laila Ibrahim, Animal Sciences
The effect of seminal fluid on modulating gene expression in endometrial tissue

5:15 PM Jason Rizo, Animal Sciences
Improving pregnancy outcomes in cattle: Does seminal plasma induces endometrial inflammation and alter ovarian function?

5:30 PM Chengcheng Li, Animal Sciences
Examination of age-related changes in equine skeletal muscle satellite cell function

5:45 PM Roney Zimpel, Animal Sciences
Effect of dietary cation-anion difference (DCAD) on acid-base status and dry matter intake in pregnant-nonlactating cows

6:00 PM Break

6:15 PM GROUP PICTURE

7:00 PM SOCIAL and DINNER (8:00 PM), BRADY'S BACKYARD GRILL, 340 Main St.

SATURDAY, APRIL 8

7:30-8:30 AM BREAKFAST, East Room

Session 4: Immunology/Bacteriology
Mercedes Kweh and Zaira Estrada Reyes, Chairs

9:00 AM Mercedes F. Kweh, Animal Sciences
Are epigenetic regulations governing immunostimulatory cell specific responses to 1,25-dihydroxyvitamin D3 in cattle?

9:15 AM Michael Poindexter, Animal Sciences
Feeding 25-hydroxyvitamin D3 increases mineral concentrations and decreases severity of mastitis in lactating dairy cows

9:30 AM Guan Yang, Animal Sciences
Characterizing porcine invariant natural killer T cells: a comparative study with NK cells and T cells

9:45 AM Cheng Ye, Animal Sciences
Unmasking a killer: targeting a novel gene that precipitates autoimmune diabetes

10:00 AM Choonghee Lee, Animal Sciences and Emerging Pathogens Institute
Identification of novel mechanism of non-LEE effector protein EspR1 in EHEC pathogenicity

10:15 AM Junyi Tao, Animal Sciences
Uptake of a fluorescent glucose analog (2-NBDG) by mixed rumen bacteria

10:30 AM BREAK

Session 5: Genetics/Nutrition
Adriana Zolini and Michael Poindexter, Chairs

10:45 AM Laura Patterson Rosa, Animal Sciences
Investigation of computationally predicted structural polymorphisms in gaited horses

11:00 AM Katelyn Palermo, Animal Sciences
Comparison and annotation of a structural polymorphism in the LATH *gene of equine and related species*

11:15 AM Zaira Estrada Reyes, Animal Sciences
Effect of MHC DRB1 variation on the resistance of sheep and goats to Haemonchus contortus *infection*

11:30 AM Eliab Estrada Cortes, Animal Sciences
Potential effect of choline chloride on the development of bovine embryos to the blastocyst stage of development

11:45 AM Achilles Vieira Neto, Animal Sciences
Use of calcitriol to reduce subclinical hypocalcemia and improve postpartum health in dairy cows

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

ABSTRACTS

(Arranged alphabetically by first author)

Effect of heat stress during the dry period on milk and colostrum yield and quality and mammary gland tight junction formation in the subsequent lactation

B. Dado Senn, A. L. Skibiel, T. F. Fabris, G. E. Dahl, and J. Laporta

Department of Animal Sciences, University of Florida, Gainesville

Heat stress during the dry period (**DP**) impairs milk yield in the subsequent lactation. Our objective was to examine if exposure to heat stress during the DP alters milk and colostrum quality and tight junction (**TJ**) permeability during the subsequent lactation. Holstein cows, selected based on parity and mature equivalent milk production, were enrolled into two groups: heat stress (**HT**, access to shade, n=12) or cooled (**CL**, access to shade, fans and soakers, n=12) during the entire DP (~46 d, temperature-humidity index ≥ 68). After calving, all cows were managed together and had access to shade, fans and soakers. Colostrum and milk volume and mastitis events were recorded daily until 84 d in milk (**DIM**, AfiFarm System). Milk samples were collected at 0, 1, 2, 7, 14, 21, 49, and 84 DIM and analyzed for somatic cell count (**SCC**) by flow cytometry. Blood was collected at these time points and at -7, -2, -1 d relative to calving and assayed for lactose concentrations. Mammary gland biopsies (n=6 per treatment) were collected at 14, 42, and 84 DIM. Total RNA was extracted to analyze the expression of genes related to TJ formation (ZO1, 2, 3, and OCLN) by real-time PCR. Data were analyzed by general linear mixed models with DIM as a repeated measure. Cooled cows had greater colostrum and milk yield compared with HT cows (7.79 vs. 3.72 ± 0.79 kg; 39.93 vs. 34.27 ± 0.80 kg/d; for colostrum and milk respectively; P<0.01). Colostrum SCC was lower for CL compared to HT cows (839 vs. 2729 ± 518 x 1000 cells/mL, respectively; P=0.01), and CL cows tended to have lower milk SCC (230 vs. 441 ± 95 x 1000 cells/mL; P=0.11). Cooled cows had fewer cases of mastitis relative to HT cows (1 vs. 4 cases, respectively). There was no difference in blood lactose concentrations. Expression of most TJ genes in the mammary gland did not differ between CL and HT cows, however, ZO3 expression was upregulated at 14 DIM in HT cows (P=0.04). Cows exposed to heat stress during the DP produced less colostrum and milk with higher SCC. However, blood lactose and genes related to TJ formation were not substantially impacted by DP heat stress.

Potential effect of choline chloride on the development of bovine embryos to the blastocyst stage of development

Eliab Estrada-Cortes, Charles R. Staples, and Peter J. Hansen

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

Choline is a nutrient required for synthesis of phospholipids and acetylcholine and which serves as a methyl donor for DNA methylation. Feeding rumen-protected choline from 17 d prepartum to 21 d postpartum was reported to increase first service pregnancy rate. Genes encoding enzymes involved in the pathways to synthesize choline-containing phospholipids and to process methyl groups from choline are expressed in the preimplantation bovine embryo. The objective of this study was to determine whether choline plays a role in developmental competence of the bovine embryo. It was hypothesized that addition of choline chloride to culture would increase the proportion of embryos that developed to the blastocyst stage. Embryos were produced in vitro. Cumulus oocyte complexes (COCs) obtained from bovine ovaries collected in a slaughterhouse were incubated in maturation medium for 24 h at 38.5°C and 5% (v/v) CO_2 in a humidified atmosphere. Matured COCs were fertilized with a pool of spermatozoa from three bulls for 12 h at 38.5°C, 5% (v/v) CO_2 in a humidified atmosphere; a different pool of bulls was used for each replicate. In Experiment 1, putative zygotes were placed randomly in 50 µl microdrops of SOF-BE2 culture medium supplemented with 0.0, 1.3 or 13.0 mM choline chloride. Concentrations were chosen to approximate the total concentrations of choline in plasma of lactating cows at week 1 (1.30 mM) and 34 postpartum (13.0 mM) (Artegoitia *et al.,* Plos One 9:e103412). All treatments were adjusted with NaCl to be isotonic. Embryos were incubated at 38.5°C in a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2 (v/v). The proportion of embryos becoming blastocysts was determined at Day 7 after fertilization. The experiment was replicated nine times; the total number of COCs per treatment ranged from 633-661. Data were analyzed by the GLIMMIX procedure of SAS. Treatment affected the percent of oocytes becoming blastocysts (P=0.032). Moreover, orthogonal contrasts indicated that development was increased by 1.3 mM (P=0.003) and decreased by 13 mM (P=0.063). Least-squares means $\pm$ SEM were 13.4, 20.2 and 13.1% for 0, 1.30, and 13.0 mM, respectively. Similar results were found for the percent of cleaved embryos becoming blastocysts (P=0.0046); least-squares means $\pm$ SEM were 24.3, 32.6, and 21.9% for 0, 1.30, and 13.0 mM, respectively (0 vs 1.3 mM, P=0.019; 13 vs 0 +1.3 mM, P=0.029). Experiment 2 was conducted as for Experiment 1 except the culture medium was BBH7 and concentrations of choline were chosen to approximate total choline concentration in plasma during week 1 postpartum (1.30), at week 1 in cows fed rumen-protected choline (1.80), and at day 70 postpartum in non-supplemented cows (6.37; day 70 is around the time when cows are typically inseminated). The experiment was replicated three times; the total number of COCs per treatment ranged from 181-209. Treatment did not affect cleavage rate but the percent of oocytes becoming blastocysts was least for the greatest concentration of choline (P=0.032). Least-squares means $\pm$ SEM were 35.9, 36.8, 38.9 and 27.0% for 0, 1.30, 1.80, and 6.37 mM, respectively. Similar results were found for the percent of cleaved embryos becoming blastocysts (6.37 vs others; P=0.088) with values being 46.0, 48.1, 47.9, and 37.9% for 0, 1.30, 1.80, and 6.37 mM, respectively. In conclusion, high concentrations of choline chloride inhibited development to the blastocyst stage. Beneficial effects of low concentrations of choline appeared to depend on culture medium. Support: Larson Endowment.

Effect of MHC DRB1 variation on the resistance of sheep and goats to *Haemonchus contortus* infection

Zaira Magdalena Estrada Reyes*, Y. Tsukahara†, Arthur L. Goetsch#, Terry A. Gipson#, Tilahun Sahlu#, Ryszard Puchala#, 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) represent a challenge for livestock producers worldwide. Moreover, the emergence of anthelmintic resistance in different GIN species have stimulated interest in the development of alternative strategies to control GINs. Besides phenotypic markers such as fecal egg count (FEC), genetic markers could be included in sheep/goat breeding programs. Important chromosomal regions containing QTL associated with low FEC have been identified in chromosome 20, a region close to the major histocompatibility complex (MHC). Most of the allelic polymorphisms within the MHC class II B genes are located in the second exon, which contributes directly to the diversity of bound peptides presented to T cells. In order to evaluate the effect of different loci within the MHC DRB1 gene, in the present study we identified two single nucleotide polymorphisms (SNPs) that could be associated with resistant sheep and goats infected with *H. contortus*. Animals from 3 different breeds of sheep and goat were used for the study. Individuals were mated each year as follow: Resistant X Resistant and Moderate Resistant X Moderate Resistant. Before the trial started, animals were treated with levamisole (7.5 mg/kg of live weight). Animal were infected with 10,000 L3 of *H. contortus* per kg of body weight per oral route. Fecal samples were obtained to determine FEC. Blood samples were collected from the jugular vein with vacutainer tubes to evaluate blood package cell volume (PCV) and levels of IgA, IgM and IgG at 21 days post-infection. DNA was extracted from blood samples using DNeasy Blood & Tissue Kit (Qiagen). Respective primers were designed to amplify exon 2 of the MHC DRB1. Two SNPs in the MHC DRB1 gene segregating in this population were selected from the sequencing results and new primers were designed to evaluate the SNPs targeting the 3'end of the forward primer respectively. The SNPs were analyzed using High Resolution Melting and conventional PCR assays. The PCR products were 113 bp for SNP1 and 135 and 147 bp for SNP2, respectively. Three different genotypes were identified from each SNP as follow: TT, AT and AA for SNP1, and AA, AG and GG for SNP2. General Linear Models were fitted with ADG, specie, year, breed and genotype as predictors and the square root of the mean of FEC as the response variable. According to the results, the best significant predictors to fit the model were Genotype, Breed and Year ($p < 0.05$). The results from the Least Square Means (LSM) showed that the AA genotype for SNP1 had the lowest FEC and the TA genotype had the highest FEC. Statistical differences were identified between AA and TA genotypes. In conclusion, the results demonstrate that genetic differences at the MHC DRB1 gene exist and the AA genotype could be associated to low FEC in our population.

The effect of seminal fluid on modulating gene expression in endometrial tissue

Laila A. Ibrahim[1], Matthew D. Utt[2] and John J. Bromfield[1]

[1] Department of Animal Sciences, University of Florida, Gainesville FL 32611
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Seminal fluid is conventionally thought to have a single purpose which is carriage of spermatozoa to the oocyte to achieve conception and commence pregnancy. However, its role is now recognized to extend beyond this to targeting female tissues. The cellular and molecular environment of the uterus during the pre- and peri-implantation period of early pregnancy is critical for implantation success and optimal foetal and placental development. Recent studies in rodents, swine and humans show that seminal fluid contains active moieties that interact with epithelial cells lining the female reproductive tract to influence the uterine environment. This is proposed to occur by activating a cascade of cytokine and leukocyte-mediated events that appear to contribute to endometrial receptivity for embryo implantation. Epithelial cytokines activated by seminal fluid exert embryotrophic actions on the developing pre-implantation embryo, modulate maternal immunity toward the conceptus and potentially improve pregnancy success. It is unknown if a similar communication network exists in the bovine to modulate the uterine environment following insemination. Evaluation of the endometrium following seminal fluid exposure in the bovine provides us novel information on the endometrial environment which is potentially lacking after the utilization of artificial insemination in the cow. We hypothesize that exposure of endometrial tissue to seminal fluid alters the production of cytokines important in early pregnancy success. Endometrial explants from slaughterhouse material were cultured for 24h with either seminal fluid, whole semen, semen cell pellet or culture medium alone. Total mRNA was extracted from explants and gene expression quantified by qPCR. A dose response to seminal plasma for 24 hours demonstrated an increased endometrial expression of several genes including the embryokine *CSF2* (12.8-fold) and prostaglandin synthase *COX2* (2.2-fold). This response was absent when explants were exposed to semen cell pellet. Interestingly, the expression of *IL6* and *IL8* was decreased following seminal fluid treatment compared to vehicle treated controls. A time course experiment revealed that increased expression of *CSF2* and *COX2* was maximal after a 30 minute exposure to 5% seminal fluid. The data presented here describe a role for seminal fluid in communicating between sire and dam in the bovine, a communication network that is not present due to the ubiquitous use of artificial insemination in the cow. Ultimately, we aim 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 and 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.

Effects of sex on response of bovine preimplantation embryos to activin A and WNT7A

G. Jumatayeva[1], K. Lehloenya[2], P. Tribulo[1], and P.J. Hansen[1]

[1]Dept. of Animal Sciences, University of Florida;
[2]Department of Animal & Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa

Maternal regulation of preimplantation development involves secretion of cell signaling molecules called embryokines. The objective of this study was to determine whether preimplantation embryos respond to embryokines in a sex-dependent manner. Two experiments were designed to independently test the effect of activin A and WNT7A on development of male and female bovine embryos. Embryos were produced in vitro from oocytes obtained from ovaries collected at a local abattoir. Following oocyte maturation, fertilization was performed with X- or Y- sorted semen from a pool of semen from two bulls. Within a replicate, the same bulls were used for X and Y-sorted semen. Cleavage rate was assessed on day 3 of culture. On day 5 of culture embryos were treated with 1 nM human recombinant Activin A or vehicle (Experiment 1); or 66 ng/ml human recombinant WNT7A or vehicle (Experiment 2). Blastocyst development was assessed on day 7 of culture. Experiment 1 was replicated 7 times using a total of 6 bulls, and Experiment 2 was replicated 8 times using a total of 6 bulls. Blastocyst development was evaluated as the proportion of oocytes or cleaved embryos becoming a blastocyst (Bl/COC and Bl/cleaved, respectively). Results are presented as least-square means ± SEM in the following order: vehicle female, vehicle male, treatment female, and treatment male. Activin A improved development as revealed by higher Bl/COC (7.2 ± 1.5, 10.8 ± 1.8, 12.6 ± 2.1, 17.0 ± 2.4; P= 0.0004) and Bl/cleaved (13.3 ± 3.2, 21.2 ± 4.2, 23.8 ± 4.5, 33.3 ± 5.4; P=0.003). Similarly, WNT7A was beneficial for Bl/COC (13.4 ± 2.0, 15.0 ± 2.1, 16.9 ± 2.3, 20.2 ± 2.5; P= 0.01) and Bl/cleaved (21.6 ± 2.9, 24.0 ± 3.1, 28.2 ± 3.4, 31.0 ± 3.5; P=0.005). Effect of sex on development was only observed in Experiment 1 (P=0.01 and P=0.003 for Bl/COCe and Bl/cleaved, respectively). There was no interaction of treatment by sex in either experiment. Results indicate that male and female embryos respond similarly to Activin A and WNT7A. (Support USDA-AFRI 2011-67015-30688 and NIH R03 HD080855).

Are epigenetic regulations governing immunostimulatory cell specific responses to 1,25-dihydroxyvitamin D_3 in cattle?

Mercedes F. Kweh, Michael Poindexter and Corwin D. Nelson

Animal Molecular and Cellular Biology Graduate Program, Department of Animal Sciences, University of Florida

The innate immune response to microorganisms includes induction of antimicrobial and inflammatory mechanisms. Recent studies have given evidence for epigenetic control of the bovine innate response to microorganisms. We have previously shown that transcription of several β-defensin genes located in the chromosome 27 β-defensin gene cluster can be induced by toll-like receptor (TLR) and vitamin D signaling pathways in bovine monocytes, neutrophils and mammary epithelial cells; however, the induction pathway was cell-type specific. We also showed that TLR-induced β-defensin, but not pro-inflammatory cytokine, gene expression of mammary epithelial cells was enhanced by inhibition of DNA methylation. Dermal fibroblasts also play a role in initiating antimicrobial and inflammatory responses when invading microorganisms are present. 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 vitamin D-induced expression of immunostimulatory genes in bovine dermal fibroblasts. Primary cultures of dermal fibroblasts (a gift from Dr. David Kerr, University of Vermont) were cultured with 5-Aza, TSA or media alone in combination with lipopolysaccharide (LPS; 100 ng/mL; 16 h) and 1,25-dihydroxyvitamin D3 (1,25D; 10 nM, 16 h). Expression of vitamin D pathway, β-defensin, and cytokine genes was determined using qPCR. We did not observe interactions between 1,25D and 5-Aza or TSA treatments on any of the immune genes measured. The vitamin D catabolism gene, *CYP24A1,* was increased by 1,25D treatment as expected, and TSA treatment enhanced 1,25D-induced *CYP24A1* expression. To our surprise, the β-defensin genes tested (*BNBD3, BNBD4, BNBD6, BNBD7, BNBD10, LAP* and *TAP*) were not detected for any of the treatment conditions. Treatment with TSA enhanced LPS-induced expression of the *IL1β*, *IL8* and *CCL5* genes. TSA treatment also increased expression of *CYP27B1*, which encodes the enzyme that synthesizes 1,25D. These data have shown that that epigenetic modifications contribute to not only the regulation of the immune response genes assessed in bovine dermal fibroblast, but also contribute to the cell specific expressions we have observed in previous studies using other cell types. In addition, acetylation of the promoters in particular affects TLR activation of these genes in bovine dermal fibroblast.

Identification of novel mechanism of non-LEE effector protein EspR1 in EHEC pathogenicity

Choonghee Lee[1,2], Won-Sik Yeo[1,2], and K. C. Jeong[1,2]

[1]Emerging Pathogens Institute and [2]Department of Animal Sciences, University of Florida, Gainesville, Florida

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a particular type of *E. coli* which causes life-threatening disease such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis. During EHEC infection, host cells are damaged and their signaling pathways are disrupted by bacterial effector proteins secreted through a type III secretion system (TTSS). In the early studies, many of the effector proteins were found at a specific region, called the Locus of Enterocyte Effacement (LEE) and their function have been extensively investigated. However, other effector proteins encoded outside of the LEE (non-LEE effectors) have been recently discovered and their functions remain to be fully characterized. Among numerous non-LEE effectors, our previous study focused on EspR, EspX, and EspY that contain multiple homologs especially on the *E. coli* O157:H7 genome. To identify their roles, deletion mutants of individual effectors were constructed and following changes in their pathogenicity were observed. Despite their homology, deletion of individual family members caused different effects on their adherence ability, host cell cytotoxicity, and actin polymerization ability. Consistent with these results, individual effector families exhibited distinct localization in host cells. After these experiments, EspR1 was chosen to be investigated further since it is involved in the entire pathogenic process and localized to the host cell nucleus. Amino acid analysis of EspR1 revealed that there are five leucine-rich repeat (LRR) domains and two leucine zipper domains that can function as nuclear localization signal and bind to DNA and regulate host gene expression, respectively. Remarkably, we found that C-terminal truncated EspR1 failed in nuclear localization and resulted in decreased adherence ability and host cell cytotoxicity. Further study will be focused on identifying target molecule(s) of EspR1 and its molecular mechanism in EHEC pathogenicity.

Examination of age-related changes in equine skeletal muscle satellite cell function

Chengcheng Li and Stephanie E. Wohlgemuth

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Myogenic stem cells, commonly referred to as satellite cells (SC), are responsible for muscle repair and hypertrophic growth in adults. In response to injury or a growth stimulus such as strength exercise, satellite cells become activated, and their progeny, myoblasts, proliferate and subsequently differentiate and fuse to form mature muscle fibers. As subtle myofiber injuries can occur routinely during daily muscle activity, continuous muscle repair is essential for muscle maintenance throughout life. However, the ability to regenerate muscle fibers declines with age. This poor regeneration capacity of aged muscle is reportedly due to environmental (SC niche) impairment of aged satellite cell function. However, the intrinsic changes within aged satellite cell may also underlie this decline. Primary culture of satellite cells under standard conditions *in vitro* enables us to gain insight into the intrinsic changes of satellite cells with age. Therefore, the overall goal of this project is to examine differences in the intrinsic myogenic capacity of satellite cells isolated from young and aged horses. Muscle biopsies were taken in a sterile manner from the *Gluteus medius* and the *Triceps brachii* of young (2-4 y; n=4) and aged (20-27 y; n=4) American Quarter Horses. In brief, cells were freshly isolated from biopsied muscle samples by pronase digestion, and pre-plated for 30 min on uncoated, sterile culture dishes to remove fibroblasts. The non-attached cells (satellite cells) were subsequently seeded onto sterile, gelatin-coated culture dishes, and proliferated for 3 days in growth medium. Cells were passaged three times before storage in liquid nitrogen, with pre-plating at each passage to remove fibroblasts and enrich the satellite cells population. For proliferation and differentiation experiments, frozen satellite cells were thawed and grown in growth medium. The replacement of growth medium by differentiation medium containing less horse serum induced rapid fusion of myoblasts to form large multi-nucleated myotubes *in vitro*. Using this culture system, we will compare the capacity to proliferate, differentiate and fuse *in vitro* between satellite cells isolated from young and aged horses. The cells' developmental and differentiation capacity will be determined through expression of myogenic genes and proteins. The quantitative analysis of the number of myonuclei in terminally differentiated myotubes will enable us to calculate a fusion index to evaluate the fusion capacity of satellite cells *in vitro*. Finally, we will assess mitochondrial density and functional quality in isolated satellite cells from young and aged horses. The overall aim of this study is to gain a deeper understanding of the effects of aging on myogenic and metabolic capacity of equine satellite cells, which will help to identify interventional targets to combat age-related impairment of physical function and regenerative ability.

Analysis of single-cell gene expression of the epiblast, hypoblast and trophectoderm of the late bovine blastocyst

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The first two differentiation events in the bovine blastocyst result in three cell types – pluripotent epiblast, trophectoderm (TE), which gives rise to the placenta, and hypoblast, which forms the yolk sac. The purpose of the present experiment was to identify molecular markers for each cell type. The resultant information could be used for cell identification purposes and to understand processes controlling pluripotency and differentiation in the bovine embryo. Embryos were produced *in vitro*. Blastocysts were collected at 208±1 h (day 8.75) after fertilization and dissociated into single cells using TrypLE Select Enzyme 10X. Individual cells were captured in wells of the C1 Single-cell Auto Prep IFC device (Fluidigm, South San Francisco, CA) and cDNA obtained following manufacturer guidelines. A total of 67 cells were captured in three replicates. Transcript abundance for 96 genes was determined using the Fluidigm® qPCR microfluidic device Biomark^{TM} HD system. Included in the PCR analysis were genes representing 10 potential markers of TE (i.e., identified in other studies with embryos from the cow or other species), 9 potential epiblast markers, 11 potential hypoblast markers, as well as genes involved in chemokine signaling (n=16), the Hippo signaling pathway (n=9), epigenetic modulation (n=13), tight junctions (n=4), cell polarity (n=7), axon guidance (n=3), steroidogenesis (n=2), other genes of interest (n=10) and housekeeping genes (n=2). Genes with a Ct value greater than 27 were considered to be non-detected. Data were normalized to the geometric mean of the two housekeeping genes (*ACTB* and *GAPDH*) to obtain the delta Ct; fold changes were calculated as 2^{-dCt} relative to housekeeping genes. The Gene Cluster 3.0 clustering software was used to identify cell populations through hierarchical clustering. The GLM procedure of SAS was utilized to compare gene expression of the 94 genes in the identified populations. The hierarchical cluster output indicated that the 67 cells belonged to two major cell populations (A and B) with two subpopulations of clade A and four subpopulations of clade B. Use of lineage-specific markers indicated that the two subpopulations of clade A represented epiblast and hypoblast while the four subpopulations of clade B were TE. Among the genes significantly upregulated were: *AJAP1*, *FGF4*, *H2AFZ* and *HDAC8* in epiblast; *ALPL*, *FGFR2*, *FN1*, *GJA1*, *HDAC1*, *PDGFRA* and *SOX17* in hypoblast; *CDH2*, *DNMT3A*, *HNF4A*, *KDM2B*, *POU5F1* and *RUNX1* in epiblast and hypoblast; *GATA6* and *HPRT1* in hypoblast and all four TE; *CCL26*, *LATS2* and *TET2* in TE1; *ACKR4*, *CCL11*, *CCR2*, *CCR3*, *CRB2*, *DNMT1*, *EPHA4*, *INADL*, *MST1*, *PPBP*, *TAZ* and *TJAP1* in TE2; *ELF5* and *PECAM1* in TE3; *ASGR1*, *CCL24*, *CCR5*, *IL4*, *ITK*, *KLF2*, *PLCB1*, *SOX30* and *TCF23* in TE4; *CDX2*, *RAC1* and *KRT8* in all four TE subpopulations. The subpopulations of TE varied amongst each other in amount of expression of prototypical TE markers, *IFNT* and *EOMES*, suggesting that cells were at different degrees of differentiation. In summary, new candidate genes for markers for each cell type in the bovine blastocyst have been identified. Results also indicate heterogeneity in gene expression among TE cells. Further studies are needed to confirm whether subpopulations of TE cells represent different stages in development of a committed TE phenotype. (USDA AFRI Grant No. 2011-67015-30688 and Larson Endowment Fund)

Comparison and annotation of a structural polymorphism in the *LATH* gene of equine and related species

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Cooling mechanisms are vital to thermoregulation and survival of all mammals. Equids and higher primate species have uniquely evolved to produce sweat as a primary means of cooling. Unlike humans, equine sweat is high in protein, specifically Latherin, a surfactant protein which increases pelt wetting and aids in evaporation. Encoded by the gene *LATH*, the locus is homologous to human Bactericidal Permeability-Increasing Protein Family A Member 4 (*BPIFA4*). A previous study observed a polymorphic copy number variant (CNV) encompassing the *LATH* gene region of the domesticated horse and varying in copy number among horses of diverse breeds. Although underappreciated as a source of phenotypic variation, CNVs are repeated sections of DNA that occur naturally during recombination and replication and may account for much of the structural polymorphism observed in mammals. We hypothesize that *LATH* CNVs among the extant living equid species may be a result of adaptive evolution for survival. To fully understand the impact of this structural polymorphism, we propose a precise re-assembly and annotation of the *LATH* gene region of multiple equid species and members of the perissodactyl family such as rhinoceros, zebras, and tapirs. Through evaluation of homologous regions in the horses' closest relatives, we will better understand the impact of domestication on the evolution of copy number variants for this gene. Future work regarding the *LATH* gene region will include identification of subjects with extreme CNV gains or losses and quantification of the amount of *LATH* protein produced in sweat samples, which will be performed by western blot. Through our experimentation, we will bring novel information to both the equine and genetic communities regarding the importance of CNVs to phenotypic traits; our research will elucidate new insight into equine thermoregulation, as it is a vital part of homeostasis.

Investigation of computationally predicted structural polymorphisms in gaited horses

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Improved understanding of variation in genome structure is crucial to provide insight into genomic influence on phenotypic traits. In a previously conducted analysis, six re-sequenced horse genomes were inspected for single nucleotide polymorphisms (SNPs), insertions and deletions (INDELs), copy number variations (CNVs) and structural variations (SVs). SVs, specifically inversions, are usually hard to detect but have been linked to major phenotypical variations in the horse. For example, the Tobiano white spotting pattern is caused by a paracentric inversion on the *Equus caballus* chromosome 3, likely disrupting a promoter region of the *KIT* gene. INDELs are the second most common form of genomic variation. An example of an INDEL is the deletion in *MYO5A* gene which causes Lavender Foal Syndrome.

Further analysis of the data provided by the previous study demonstrated that regions of the genome under selection in the three gaited horse breeds possessed unique genes ontology (GO) terms for biological processes in locomotion. Notably one of the hits - a computationally predicted insertion fragment - is similar between two of these horses, but is not present in the other four horses nor in Twilight, the horse reference genome.

With about 14,075 annotated SVs and an average of 357,000 homozygous alternative INDELs amongst the six re-sequenced horses, such diversity has the capacity to be a major source of phenotypic variation in the horse. Further investigations will assess gene disrupting SVs and INDELs in the equine genome and association with phenotypic effects.

Feeding 25-hydroxyvitamin D_3 increases mineral concentrations and decreases severity of mastitis in lactating dairy cows

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The objectives of this experiment were to determine the effects of feeding supplemental 25-hydroxyvitamin D_3(25D) on concentrations of 25D and minerals in serum, lactation performance, and mastitis resistance in dairy cows. Sixty Holstein cows (multiparous, pregnant, lactating, SCC < 165,000/mL) were blocked by milk yield and, within each block, randomly assigned to receive a daily dietary supplement containing 1 mg vitamin D (**1mgD**), 1 mg 25D (**1mg25D**), 3 mg vitamin D (**3mgD**), or 3 mg 25D (**3mg25D**) for 28 days (n = 15/group). Blood and milk were sampled at 0, 7, 14, and 21 d for measurement of vitamin D metabolites, minerals, and energy metabolites in serum. At 21 d, cows fed 1mgD and 3mg25D received an intramammary *Streptococcus uberis* challenge. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Significance was declared at $P < 0.05$. The 1mg25D and 3mg25D cows had greater serum 25D concentrations at 7, 14 and 21 d, greater serum 24,25-hydroxyvitamin D (24,25D) at 21 d, and lower vitamin D at 21 d compared with cows fed 1mgD and 3mgD (25D = 62 ± 7, 66 ± 8 ng/mL, 135 ± 15, and 232 ± 26 ng/mL; 24,25D = 4.9, 4.0, 11.8, and 30.6 ± 2.8 ng/mL; vitamin D = 7.6, 15.7, 1.9, and 3.1 ± 1.6 ng/mL for 1mgD, 3mgD, 1mg25D and 3mg25D, respectively, at 21d). The 3mg25D cows had greater concentrations of Ca and P at 21 d compared with other treatments, which did not differ (Ca = 2.38, 2.4, 2.37, 2.48 ± 0.02 m*M*; P = 1.69, 1.87, 1.88 and 2.10 ± 0.08 m*M* for 1mgD, 3mgD, 1mg25D and 3mg25D, respectively). Milk yield and components, DMI, BW, NEFA, BHBA, glucose, 1,25-dihydroxyvitamin D and Mg did not differ between treatments. The 3mg25D cows had less severe mastitis at 60 and 72 h after challenge with *S. uberis* compared with 1mgD cows. The 3mg25D cows also had slightly lower ($P = 0.06$) rectal temperature compared with 1mgD cows during the challenge period (38.9 vs. 39.1 ^{o}C). Feeding 25D increases serum 25D more effectively than supplemental vitamin D, resulting in increased serum mineral concentrations and less severe mastitis in lactating dairy cows.

Improving pregnancy outcomes in cattle: Does seminal plasma induces endometrial inflammation and alter ovarian function?

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Seminal plasma (SP) is not just a transport medium for spermatozoa. It can also interact with the uterus as a complex communication pathway to improve pregnancy outcomes. Seminal plasma induces an acute inflammatory response in the endometrium as a result of the recruitment of phagocytic immune cells including neutrophils, macrophages and dendritic cells. It is thought that this inflammation aids in the establishment of immune tolerance to the paternal antigens expressed on the conceptus, potentially optimizing the reproductive performance of females. The role of SP in preparing an optimal uterine environment for early embryo development has been investigated in rodents, swine and humans. Studies in mice show that the absence of SP reduces fecundity by affecting embryo formation, implantation, placentation, and even subsequent offspring health. In swine, an increase in the average number of viable embryos and plasma progesterone has been reported after SP infusion at insemination. Post-insemination inflammation of the endometrium or cervix is absent following coitus with seminal vesicle deplete mice or condom protect men, establishing a causal link between SP and cellular inflammation. Does seminal plasma improve reproductive performance in cattle? In cattle, SP is diluted or removed during semen processing prior to artificial insemination; potentially reducing sire to dam communication via SP. We hypothesize that uterine exposure to SP at AI drives acute endometrial inflammation and alters ovarian function in cows. Long-term, we aim to evaluate the potential of SP on improving the efficiency of artificial insemination by optimizing pregnancy outcomes. We infused 54 synchronized beef cows with one of 4 randomly assigned treatments (saline, SP, commercial AI semen, or SP + semen). Endometrial biopsies in both ipsilateral and contralateral uterine horns were collected 24 hours after treatment infusion. Gene expression of inflammatory mediators (*IL6*, *IL8*) and embryokines (*CSF2*) will be measured using qPCR. We predict seminal plasma infusion to cause acute inflammation in the endometrium greater than saline or AI semen. Using immunohistochemistry we will evaluate the recruitment of leukocytes into the endometrium to assess cellular inflammation. In addition, we will evaluate the effect of seminal plasma on blood progesterone content during the early luteal phase to assess effects of SP on ovarian function. These data will help us explain how SP may improve pregnancy outcomes by modulating the uterine environment and changing ovarian function, potentially improving pregnancy outcomes.

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

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Most rumen bacteria are uncultured, making their niche hard to identify. Fluorescent substrates could potentially identify the substrate preferences and the niche of these uncultured bacteria, but uptake of these substrates has not been tested with mixed rumen bacteria. Our objective was to determine if a fluorescent analog of glucose (2-NBDG) would be taken up by mixed bacteria from the rumen. A second objective was to determine if we could separate cells taking up 2-NBDG by using fluorescence-activated cell sorting. We prepared mixed bacteria from rumen fluid by centrifugation, incubated them in 2-NBDG (0 to 100 μM) for up to 60 s, and monitored uptake of 2-NBDG with fluorimetry. We found mixed bacteria took up 2-NBDG, and they did so with a maximum velocity (V_{max}) of 0.180 (0.05 SEM) nmol mg protein^{-1} min^{-1} and Michaelis constant (K_m) of 6.37 (4.86 SEM) μM (n = 3). We confirmed that cells took up 2-NBDG by using flow cytometry, which revealed that up to 18.5% cells were positive after incubating them in 100 μM 2-NBDG for 5 min. Positive cells could be separated with fluorescence-activated cell sorting, and we obtained 94% purity during post-sort analysis. Work is ongoing to 1) optimize cell sorting, 2) sequence sorted cells for identification, and 3) synthesize and test uptake of other glucose analogs. Our work supports that 2-NBDG, in combination with other analogs, could be used to identify which bacteria consume which substrates, helping define what role uncultured bacteria play in the host.

Consequences of exposure of embryos produced in vitro in a serum-containing medium to dickkopf-related protein 1 and colony stimulating factor 2 on blastocyst yield, pregnancy rate and birthweight

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Embryokines are molecules secreted by the mother that regulate embryonic development. Among the embryokines in cattle are colony stimulating factor 2 (CSF2) and dickkopf-related protein 1 (DKK1). Here we evaluated actions of CSF2 and DKK1 alone or in combination on characteristics of embryos produced in vitro in the presence of serum. A total of 70 beef cows from four farms were subjected oocyte retrieval on 1 to 4 occasions. Within each farm, donors were randomly allocated to one of four treatment groups (vehicle, CSF2, DKK1, CSF2 + DKK1). Embryos from a given donor were always exposed to the same treatment. Treatments were added to the culture medium on day 5 after insemination and blastocyst stage embryos were transferred to recipient females 2 days later. Treatment did not affect the percent of oocytes or cleaved embryos that developed to the blastocyst stage or the percent of recipients that became pregnant after embryo transfer. However, calves derived from embryos treated with DKK1 were smaller at birth, regardless of CSF2 treatment [42.6 ± 3.2, 40.3 ± 2.8, 35.9 ± 4.0, and 34.3 ± 2.9 (LSMean (kg) ± SEM for vehicle, CSF2, DKK1, CSF2 + DKK1, respectively)]. Results indicate no beneficial effects of addition of CSF2 or DKK1 to cultures of embryos produced in vitro with serum-containing medium on development to the blastocyst stage or embryo competence to establish pregnancy after transfer to recipients. The fact that embryos cultured with DKK1 resulted in calves with altered postnatal weights illustrates the ability of this embryokine to alter development to affect postnatal phenotype. Results support the concept that properties of the offspring can be programmed as early as the preimplantation period. Thus, culture conditions of embryos produced in vitro could affect animal performance after birth.

Leptin receptor expression and leptin-induced MAPK signaling regulation of uterine artery angiogenesis in ovine endothelial cells of the ovarian cycle and pregnancy

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Leptin regulates reproductive processes, vascular function, and angiogenesis. The follicular phase and pregnancy are physiological states of elevated estrogen, angiogenesis, and uterine blood flow. However, little is known concerning leptin and its leptin receptor (LEPR) in regulating uterine artery (UA) angiogenesis. We hypothesized: 1) *Ex vivo* expression of the LEPR in UA endothelium (UAendo) and UA vascular smooth muscle (UAvsm) is elevated in pregnant vs. nonpregnant (Luteal and Follicular) sheep; 2) *In vitro* leptin treatments differentially modulate cell proliferation in uterine artery endothelial cells from pregnant (P-UAECs) greater than nonpregnant (NP-UAECs) ewes; 3) *In vitro* LEPRs are also upregulated in P-UAECs vs. NP-UAECs in association with leptin activation of intracellular p-STAT3 and; 4) Leptin binding to the LEPR recruits the MAPK signaling pathway to stimulate angiogenesis in UAECs. For *ex vivo* expression studies, we utilized a unilateral pregnant (120-130d, term=147d) sheep model where pre-breeding uterine horn isolation (nongravid) restricted pregnancy to one horn (gravid). *Ex vivo* LEPR protein expression was determined on UAendo/UAvsm by immunohistochemistry (IHC), and Western analysis; *in vitro* LEPRa, LEPRb, phospho STAT3, ERK1/2, p38, and JNK, were also evaluated in NP-UAECs and P-UAECs. To evaluate angiogenesis, UAECs obtained from NP-UAECs (n=4/group) and P-UAECs (n=4) sheep were treated with vehicle (control), or 7 doses of leptin (0.001-1000 ng/ml; 24 hours). NP-UAECs, and P-UAECs were pretreated for 1 hour with or without 5 μmol/L of the pure antagonists PD98059 (ERK1/2), SB203580 (p38), or SP600125 (JNK) inhibitors, followed by 1 ng/ml of leptin for 0.25, 1, 12, and 24 hours to analyze activation of MAPKs. UAECs were collected separately at each time point. Untreated (0 minutes) cells were collected at the start of each experiment for basal controls. An additional set of NP-UAECs and P-UAECs were pretreated for 1 hour with PD98059, SB203580, or SP600125 followed by 1 ng/ml of leptin treatment for 24 hours to determine the involvement of individual MAPKs in leptin-induced NP-UAECs and P-UAEC proliferation. The effect of leptin on UAEC proliferation was evaluated using the 5-ethynyl-2-deoxyuridine (EdU-labeled) assay technique. IHC revealed expression of the LEPR in UAendo/UAvsm from nonpregnant and pregnant sheep. Western analysis revealed that compared to follicular UAendo/UAvsm, LEPR were reduced ($P<0.05$) in UAendo/UAvsm from luteal, nongravid, gravid, and control pregnant groups. Leptin treatment significantly increased *in vitro* cell proliferation in NP-UAECs from follicular (1.70±0.18-fold;$P<0.05$), and P-UAECs (1.50±0.12-fold;$P<0.05$), but not luteal (0.80±0.20-fold) phase. Although leptin receptors in UAECs were expressed at similar levels between all groups, leptin treatment activated phospho STAT3, ERK1/2, p38, but not JNK in the follicular phase and P-UAECs more than luteal NP-UAECs. MAPK antagonists inhibited the time dependent expression changes of phospho-ERK1/2, p38, and JNK. Inhibition of ERK1/2, and p38, but not JNK blocked the proliferation response of follicular NP-UAECs and P-UAECs; luteal UAECs did not respond significantly to either inhibitor or leptin treatment. Thus leptin may play an angiogenic role particularly in preparation for the increase UBF during the periovulatory period and subsequently in pregnancy to meet the demands of the growing fetus. (NIH-HL49210, HD38843, HL87144, HL117341, NIH-NHLBI Post-doctoral award).

Use of calcitriol to reduce subclinical hypocalcemia and improve postpartum health in dairy cows

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Objectives were to determine effects of an injectable formulation of calcitriol on Ca concentration, risk of subclinical hypocalcemia, and health in dairy cows. Cows were blocked by lactation number (1 vs. ≥2) and calving sequence, and within each block, randomly assigned to receive, within 6 h of calving, subcutaneously vehicle only (**Control**, n=450), 200 μg of calcitriol (**Cal200**, n=450), or 300 μg (**Cal300**, n=450). Blood samples were collected before treatment administration, and on d 1, 2, 3, and 5. Samples were analyzed for blood ionized Ca, and total plasma Ca and Mg. Vaginal discharge (**VD**) was evaluated at 4, 6, and 8 DIM, and cows with VD reddish/brownish foul smell were diagnosed with metritis. Morbidity was evaluated until 60 DIM, and responses measured included metritis, mastitis, displaced abomasum, digestive and respiratory disorders. At 35 DIM, VD was scored for diagnosis of purulent vaginal discharge (**PVD**, VD>2, mucopurulent discharge). Cyclicity was evaluated by presence of a corpus luteum (>20mm) in at least one of two ovarian ultrasound scans performed at 35 and 49 DIM. Data were analyzed using PROC MIXED and PROC GLIMMIX of SAS. Cows receiving calcitriol resulted in greater concentration of blood ionized Ca and plasma total Ca during the first 5 and 3 DIM, respectively, whereas concentration of plasma Mg were reduced during the first 3 DIM (Table). Treatment with calcitriol did not affect the incidence of metritis, puerperal metritis, morbidity by 60 DIM, PVD, and cyclicity (Table). Calcitriol treatment was effective to improve Ca concentrations during the first 3 DIM, but was unable to improve health performance.

	Treatment				
Parameter	Control	Cal200	Cal300	SEM	P value
Ionized Ca, mM	1.12	1.22	1.27	0.02	< 0.01
Total Ca, mM	2.31	2.65	2.70	0.03	< 0.01
Total Mg, mM	0.65	0.54	0.52	0.02	< 0.01
Metritis, %	37.5	38.1	34.1	3.0	0.51
Puerperal metritis, %	9.9	8.3	9.6	1.5	0.78
Morbidity by 60 DIM, %	60.2	62.6	63.4	2.9	0.60
Purulent vaginal discharge, %	26.7	32.8	30.2	2.5	0.23
Cyclicity, %	68.3	74.7	73.5	2.9	0.15

Characterizing porcine invariant natural killer T cells: a comparative study with NK cells and T cells

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CD1d-restricted invariant natural killer T (iNKT) cells are innate-like T cells that share phenotypic characteristics of both NK and conventional T cells (Tconv). Although iNKT cells have been well characterized in mice and humans, functional CD1d and CD1d-restricted iNKT cells are not universally expressed in mammals. Swine express iNKT cells that can be detected using α-galactosylceramide (α-GalCer)-loaded CD1d tetramers. In the present study, we characterized iNKT cells from the blood, spleen, lymph node, lung and liver of commercial mixed-breed pigs, and compared their phenotype to NK cells and Tconv. The principal findings are that pig iNKT cells are CD8α and CD44 positive and CD11b and Nkp46 negative. Most are also negative for the CD4 co-receptor, which is used to distinguish functionally distinct mouse and human iNKT cells subsets. CD8α^{bright} iNKT cells produced 3-4 fold more IFN-γ but similar levels of IL-4 than CD8α^{dull} iNKT cells, suggesting that CD8α expression identifies iNKT cells with unique functional roles in immune responses. IFN-γ production by NK cells did not correspond to CD8α expression in the same way. Instead, NK cell subsets that differentially produce IFN-γ and IL-17 could be distinguished according to CD11b and NKp46. CD11b also marked a population of Tconv capable of producing high levels of IFN-γ. Finally, large variability was detected among pigs in interactions between iNKT cells and monocytes when iNKT cells were activated with α-GalCer, which raises a cautionary note about manipulating iNKT cells for immunotherapy. Collectively, our study provides important phenotypic and functional information about porcine iNKT cells that will be useful for understanding how iNKT cells contribute to immune responses in swine, with potential implications for human health.

Unmasking a killer: targeting a novel gene that precipitates autoimmune diabetes

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CD4 T cells provide vital help for autoreactive CD8 T cells activation that causes most beta-cell destruction in type-1 diabetes (T1D). Previously, we identified a T1D susceptibility locus on distal chromosome 11 (Idd32) controlling how $CD4^+$ T-cells suppress or support T1D induced by the diabetogenic $CD8^+$ T-cells clone AI4 when adoptively transferred into lymphopenic hosts. AI4-reciepients co-injected with CD4 T cells from donors expressing NOD ($Idd32^{NOD/NOD}$) or B6 alleles ($Idd32^{B6/B6}$) were respectively completely protected and susceptible to diabetes. To reduce the number of Idd32 candidate genes we backcrossed B6.$H2^{g7}$ Idd32 congenic mice to B6.$H2^{g7}$, a diabetes resistant B6 stock congenically express NOD-derived $H2^{g7}$ MHC haplotype, to generate subcongenic stocks carrying different NOD-derived segments of the original Idd32 interval. Our approach shortened the original Idd32 support interval to a < 520 kb region that contains 13 genes and 4 pseudogenes with polymorphisms between NOD and B6. Diabetes resistance in B6.$H2^{g7}$. $Idd32^{NOD/NOD}$ mice was associated with much reduced circulating AI4 T cells in peripheral blood. Instead, AI4 T cells accumulated in the spleen and lymph nodes where they remained in a naïve state. In contrast, AI4 T cells from T1D susceptible mice B6.$H2^{g7}$ $Idd32^{B6/B6}$ mice were found at high concentrations in the blood that proliferated and became highly activated in the spleen. Together, our results indicate that a gene within Idd32 controls the migration and activation of autoreactive CD8 T cells in T1D. One candidate is *sphk1* that encodes sphingosine kinase 1, an enzyme that phosphorylates sphingosine to sphingosine-1-phosphate (S1P). S1P plays an important role in lymphocytes circulation and FTY720, a receptor agonist for S1P, has been shown to prevent onset of T1D. Identifying and understanding the gene underlying Idd32 could shed new light on how $CD4^+$ T-cells control tissue-specific $CD8^+$ T-cells in T1D and may provide a way to block this cornerstone of disease.

Effect of dietary cation-anion difference (DCAD) on acid-base status and dry matter intake in pregnant-nonlactating cows

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Objectives were to determine if the mechanisms that mediate decreases in dry matter intake **(DMI)** in cows fed acidogenic diets. Our hypothesis was that acidogenic diets suppress appetite because of metabolic acidosis and not because of addition of salts of containing strong anions. Ten nulliparous pregnant Holstein cows at 130 to 150 days of gestation were utilized in a duplicated 5 x 5 Latin Square design. Acid-base status was manipulated by adding strong anions (Cl and S) or by buffering the strong anions with strong cation sources (Na and K). Treatments were formulated to manipulate the dietary cation-anion difference (**DCAD**). Treatments were: control (**TRT1**), a base diet containing (dry matter basis) 55% corn silage, 10% grass hay, and 35% concentrate mixture (soybean hulls, citrus pulp, soybean meal) without the addition of acidogenic salts (**AS**) or other extra mineral sources to result in a DCAD of +200 mEq/kg. Treatment 2 (**TRT2)**: control diet with 2% added mixture of 1:1 NaCl and KCl to result in the same DCAD of +200 mEq/kg, but with additional Cl and K. Treatment 3 (**TRT3**), the control diet with added AS and a mixture of K_2CO_3 and $NaHCO_3$ to result in the same DCAD of + 200 mEq/kg, but with added AS. Treatment 4 (**TRT4**), the control diet with added AS to reduce the DCAD to -120 mEq/g. Treatment 5 (**TRT5**), the control diet with added AS, KCl, and NaCl to result in a diet with a DCAD of -120 mEq/kg, but additional K and Cl contents. Periods lasted 14 days, with 7 days of adaptation to the diets and 7 days of data collection. DMI was measured daily. Body weight was recorded on days 10 and 12 of each period. Spot urine samples were collected twice daily from days 8 to 12 of each period and evaluated for pH, Ca, Mg, and creatinine. Measures of blood acid-base status (pCO_2, HCO_3^-, PO_2, sO_2, base excess, and pH), mineral concentrations (Na, K, Cl, total and ionized Ca), metabolites (glucose and NEFA) and hematocrit were evaluated on days 10 and 12 of each period. Respiration rate was measured twice daily on days 8 to 12 of each period. Feeding behavior was evaluated on days 13 and 14 of each period, and hourly feed intake was recorded. Data from individual cows were either averaged per period before analyses or analyzed as repeated measures. The statistical model of analyses was: *Response =μ + TRT + Period + Square + TRT x Square + TRT x Period + Cow (Square) + e*. Table 1 depicts the expected differences among treatments.

Table 1. Dietary cation-anion difference, ash content, additional mineral intake, and expected acid-base measures

Item	Treatment				
	Control **(TRT1)**	Control+ KCl+NaCl (**TRT2)**	AS[1]+KCO_3 + $NaHCO_3$ **(TRT3)**	AS **(TRT4)**	AS+KCl +NaCl (**TRT5)**
DCAD,[2] mEq/Kg	+196	+194	+192	-114	-132
Dietary ash, %	5.30	7.00	7.80	5.70	7.80
Added mineral,[3] g/d	0	170	250	40	250
Blood and urine pH	High	High	High	Low	Low
Acid base status	Alkalosis	Alkalosis	Alkalosis	Acidosis	Acidosis

[1]AS = acidogenic salts.
[2]The DCAD is calculated as (mEq of K + mEq of Na) – (mEq of Cl + mEq of S). Results from chemical analyses.
[3]Includes the sum of Cl and S in AS, and the additional NaCl, KCl, $NaHCO_3$ and K_2CO_3. Based on 10 kg of DM intake.

Effect of addition of L-carnitine during culture on pregnancy rate obtained after transfer of cryopreserved bovine embryos produced *in vitro*

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The susceptibility of in vitro produced embryos to cryopreservation is related to sub-optimal cultivation conditions that lead to cytoplasm lipid accumulation in embryos. The use of L-carnitine, a component of lipid metabolism, has been reported to reduce lipid contend in bovine embryos and can be an alternative to solve this problem. The aim of this study was to determine the effect of supplementation of culture medium with L-carnitine on competence of in vitro produced embryos to develop to the blastocyst stage and establish pregnancy after cryopreservation. Embryos were produced invitro using cumulus-oocyte complexes collected by ovum pick-up (OPU) from pregnant Holstein heifers (n=24) following superstimulation. Superstimulation was induced 48 h after dominant follicle removal with two intramuscular injections of 90 mg of follicle-stimulating hormone (FSH; Folltropin-V ®) diluted in hyaluronic acid (MAP^{TM}-5) given 48 h apart. OPU was performed 32 h after the second FSH injection. After fertilization with X-sorted semen, presumptive zygotes (n=417) were randomly assigned in a crossover design to culture in SOF-BE1 supplemented with 0 or 0.75 mM L-carnitine at 38.5^{o}C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2 (v/v). The proportion of oocytes that cleaved was assessed at d 3 after insemination and the proportion of oocytes that developed to the blastocyst stage was determined at d 7. Grade 1 and 2 morula and blastocyst stage (early, blastocyst, expanded and hatched) embryos were harvested at d 7 and subjected to controlled-rate freezing following equilibration in 1.5 M ethylene glycol. Lactating Holstein cows were synchronized for timed embryo transfer using the OvSynch-56 protocol (Carvalho et al. 2014). At d 7 after presumptive ovulation, a single embryo (n = 102) was randomly thawed and transferred into cows having a corpus luteum confirmed by ultrasonography. Pregnancy was diagnosed at d 33, 45 and 72 of gestation. Data were analyzed using the GLIMMIX procedure of SAS ($P<0.05$). There was no effect of L-carnitine supplementation on cleavage rate, blastocyst rate or on the proportion of embryos selected for freezing. The proportion of cows pregnant at d 33, 45 and 72 of gestation was not effected by L-carnitine (33.3% ± 0.06 vs. 27.7% ± 0.06, 31.2% ± 0.06 vs. 27.7% ± 0.06, 22.9% ± 0.06 vs. 22.2% ± 0.06 respectively). L-carnitine also had no effect on pregnancy loss between d 33 and 45 and d 45 and 72 (6.0% ± 0.1 vs. 0.0% and 26.6 ± 0.1 vs. 20.0% ± 0.1, respectively). In conclusion, supplementation of embryo culture media with L-carnitine had no effect on embryo development or pregnancy rate after cryopreservation.

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