SEVENTEENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

International Palms Resort & Conference Center
Cocoa Beach, Florida

April 12-13, 2019

WELCOME

Our location this year is notable not only because we get to enjoy the beauty of the Atlantic Ocean but because we are meeting at the home of the Kennedy Space Center, one of the most consequential locations for science in the United States. It was from here that humans first went to the Moon. It is often overlooked but the National Aeronautics and Space Administration (NASA) is also involved in biological research. The University of Florida is a partner in that research and maintains a laboratory building at the Kennedy Space Center called the Space Life Sciences Lab. The photo on the cover is of Space Shuttle *Endeavour* on mission STS-126 as it prepared to dock with the International Space Station on November 16, 2007. On board were hundreds of bovine embryos sent up by researchers from the UF Dept. of Animal Sciences.

This year, for the first time, the presentations have not been organized by topic but instead to maintain intellectual diversity in each session. In recognition of our location, the sessions are named after important NASA space programs.

We are pleased to have Dr. Brian O'Neill as the AMCB Distinguished Lecturer. We are also grateful for those who support the AMCB during the year. We receive significant and sustained funding from the College of Agricultural and Life Sciences and the L.E. "Red" Larson Endowment. The symposium itself also received support from the University of Florida Office of Research and, for the first time, Balchem Corporation.

During the year, the daily operations of the AMCB are maintained by Renee Parks-James. Thank you Renee. In addition, the symposium would not have been possible without the tireless efforts of Liz Jannaman of the Dept. of Animal Sciences. Almost every aspect of the local arrangements was organized by Liz. Please thank her when you see her.

As always, the AMCB is all about connections – and it is hoped that discussions during the breaks and other informal occasions during the symposium will lead to some new insights and new collaborations.

On behalf of all the faculty of the AMCB, welcome to the 17th 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 17th Annual Research Symposium

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

Balchem Corporation

BALCHEM
Real People. Real Science. Real Results.

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

Elizabeth Jannaman, Dept. of Animal Sciences

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

2019 AMCB DISTINGUISHED LECTURER

Brian T. O'Neill, MD, PhD
University of Iowa Carver College of Medicine

Brian T. O'Neill is an assistant professor and endocrinologist at the University of Iowa, Carver College of Medicine. He received his MD/PhD from the University of Utah, where he completed his thesis work with Dr. E. Dale Abel on the regulation of cardiac metabolism by PI3-Kinase and Akt. Dr. O'Neill then did endocrinology fellowship and post-graduate training at Beth Israel Deaconess Medical Center and Joslin Diabetes Center, affiliates of Harvard Medical School. Under the mentorship of Dr. C. Ronald Kahn, Dr. O'Neill discovered that insulin receptors and IGF-1 receptors coordinate muscle growth and suppress atrophy by regulating FoxO transcription factors.

Muscle atrophy and decreased muscle strength are under-appreciated complications of uncontrolled diabetes in older populations, which can lead to disability or even death from surgery or severe illness. Dr. O'Neill's lab uses mouse and cell models to investigate the roles of insulin signaling and FoxO regulation in the control of muscle strength and mitochondrial metabolism, in hopes of identifying the intracellular signaling defects that occur in the diabetic state. The ultimate goal of Dr. O'Neill's research is to provide therapeutic targets that benefit muscle energy production in diabetes, speed recovery after severe illness or surgery, and prevent disability.

AMCB FACULTY

Mario Binelli. Department of Animal Sciences
John J. Bromfield, Department of Animal Sciences
Samantha A. Brooks, Department of Animal Sciences
Mary B. Brown, Department of Infectious Diseases and Pathology
Geoffrey E. Dahl, Department of Animal Sciences
John Driver, Department of Animal Sciences
Timothy J. Hackmann, Department of Animal Sciences
Peter J. Hansen, Department of Animal Sciences
Kwang Cheol Jeong, Department of Animal Sciences
Qiu-Xing Jiang, Department of Microbiology & Cell Science
Maureen Keller-Wood, Department of Pharmacodynamics
Jimena Laporta, Department of Animal Sciences
Raluca Mateescu, Department of Animal Sciences
Corwin Nelson, Department of Animal Sciences
José E.P. Santos, Department of Animal Sciences
Charles E. 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

Thiago F. Amaral (Advisor: Hansen)
Usman Arshad (Advisor: Santos)
Mackenzie J Dickson (Advisor: Bromfield)
Eliab Estrada Cortes (Advisor: Hansen)
Zaira Estrada Reyes (Advisor: Mateescu)
Weihong Gu (Advisor: Driver)
Ali Husnain (Advisor: Santos)
Melany D Darling Madrid (Advisor: Driver)
Paula Cardoso Molinari (Advisor: Bromfield)
Michael B. Poindexter (Advisor: Nelson and Santos)
Froylan Sosa Hernandez (Advisor: Hansen)
Achilles Vieira Neto (Advisor: Santos)
Cheng Ye (Advisor: Driver)
Bo Zhang (Advisor: Hackmann)
Roney Zimpel (Advisor: Santos)

MS Students

Samantha Bohm (Advisor: Nelson)
Sena Field (Advisor: Laporta)
Courtney McCourt (Advisor: Hackmann)

GRADUATES OF THE AMCB, 2018-2019

Bethany Dado Senn, MS (Advisor J Laporta)
Currently an Animal Sciences doctoral student in the Laporta laboratory, University of Florida

Laila A. Ibrahim, MS (Advisor: JJ Bromfield)
Currently a faculty member of University of Benghazi; will return to UF in the fall of 2019

Jason A. Rizo, MS (Advisor: JJ Bromfield)
Currently a doctoral student, University of Missouri

Mercedes Kweh, PhD (advisor: C, Nelson)
Currently a postdoctoral fellow at University of Miami Cancer Epigenetic Training Program

Guan Yang, PhD (Advisor J Driver)
Currently a postdoctoral fellow in the Dept of Pathology, Microbiology and Immunology, Vanderbilt University with Luc Van Kaer

Adriana Zolini, PhD (Advisor: PJ Hansen)
Currently, Dairy Business, Zoetis, Sioux Falls, SD

HISTORY OF THE AMCB RESEARCH SYMPOSIUM

YEAR	LOCATION	DISTINGUISHED LECTURER
2003	Whitney Laboratory St. Augustine, Florida	Randy Prather University of Missouri
2004	Chinsegut Hill Brooksville, Florida	John Dobrinsky USDA-ARS Beltsville, MD
2005	Chinsegut Hill Brooksville, Florida	Doug Stocco Texas Tech University
2006	Lake Wauburg Gainesville, Florida	Ina Dobrinski University of Pennsylvania
2007	Whitney Laboratory St. Augustine, Florida	Doug Bannerman USDA-ARS, Beltsville, MD
2008	Cedar Cove Beach & Yacht Club Cedar Key, Florida	Eckhard Wolf LMU Munich, Germany
2009	Plantation Golf Resort and Spa Crystal River, Florida	Dean Betts University of Western Ontario
2010	Whitney Laboratory St. Augustine, Florida	Marc-Andre Sirard Laval University
2011	Steinhatchee Landing Resort Steinhatchee, Florida	Kimberly Vonnahme North Dakota State Univ.
2012	Holiday Isle Oceanfront Resort St. Augustine, Florida	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, Co. Meath, Ireland
2018	Plantation on Crystal River Crystal River Florida	Christopher Geyer East Carolina University
2019	International Palms Resort & Conf. Center, Cocoa Beach, Florida	Brian O'Neill University of Iowa

SCHEDULE OF EVENTS

All events in the Manatee Room unless otherwise noted

FRIDAY, APRIL 12

1:05 PM Pete Hansen
Welcome, introductory comments

Session 1: Mercury
Roney Zimpel and Cheng Ye, Chairs

1:15 PM Weihong Gu, Animal Sciences
iNKT cells: Russian roulette or a silver bullet in influenza

1:30 PM Jason Rizo, Animal Sciences
Seminal plasma or TGFβ modulates expression of proinflammatory cytokines in bovine endometrial cells

1:45 PM Bo Zhang, Animal Sciences
Determining how much ATP is yielded from fermentation by rumen microbes

2:00 PM Darling Melany de C Madrid, Animal Sciences
Targeting NKT cells to block influenza A infections in swine

2:15 PM Eliab Estrada-Cortes, Animal Sciences
Developmental programming of bovine preimplantation embryos by choline chloride

2:30 PM BREAK

Session 2: 2019 AMCB Distinguished Lecturer Presentation
José E.P. Santos, Chair

3:00 PM Brian T. O'Neill
University of Iowa Carver College of Medicine
Insulin signaling in the regulation of muscle strength and mitochondrial metabolism

4:00 PM BREAK AND CHECK INTO ROOMS

Session 3: Gemini
Ali Husnain and Thiago Amaral, Chairs

4:30 PM Roney Zimpel, Animal Sciences
Effects of dietary vitamin D3 or 25-hydroxyvitamin D3 on mineral metabolism in growing calves

4:45 PM Cheng Ye, Animal Sciences
Unmasking a killer: discovery of a gene that controls the pathogenic activation of CD8 T cells in Type 1 diabetes

5:00 PM Zaira Magdalena Estrada Reyes, Animal Sciences
Association analysis identifies Th1/ Th2/ Threg loci associated with resistance to natural Haemonchus contortus *infections in Florida Native sheep*

5:15 PM Sena L. Field, Animal Sciences
Supplementation of 5-HTP or fluoxetine impacts bioenergetics in dairy calves

5:30 PM Break

6:00 PM Group photo on the beach (entrance to beach next to Mambo's Grill)

7:00 PM SOCIAL and DINNER, Mambo's Grill (restaurant on site)

SATURDAY, APRIL 13

7:00-8:30 AM BREAKFAST, The Breakfast Spot (restaurant on site)

Session 4: Apollo
Melany Darling Madrid and and Froylan Sosa Hernandez, Chairs

8:45 AM Yao Xiao, Animal Sciences
Dickkopf WNT signaling pathway inhibitor 1 is not able to maintain pluripotency during derivation of bovine embryonic stem cells

9:00 AM Peixin Fan, Animal Sciences and Emerging Pathogens Inst.
Understanding the role of host genetics on gut microbiota through life by using the Angus-Brahman multibreed herd

9:15 AM Usman Arshad, Animal Sciences
Meta-analysis of the effects of supplemental rumen-protected choline during the transition period on performance and health of dairy cows

9:30 AM Samantha R. Bohm, Animal Sciences
Effects of dietary 25-hydroxyvitamin D3 on vitamin D status and puberty of dairy heifers

9:45 AM Michael B. Poindexter, Animal Sciences
Effects of dose and source of vitamin D on mineral homeostasis and performance in transition dairy cows

10:00 AM Paula C.C. Molinari, Animal Sciences
Dairy cows have an increased incidence of uterine disease during the hot season with no difference in vaginal bacteria load

10:15 AM BREAK

Session 5: Space Shuttle
Usman Arshad and Sena Field, Chairs

10:30 AM Achilles Vieira-Neto, Animal Sciences
Effect of duration of exposure to diets differing in DCAD on Ca metabolism after a parathyroid hormone (PTH) challenge in dairy cows

10:45 AM Froylan Sosa Hernandez, Animal Sciences
Effect of colony stimulating factor 2 on competence of bovine blastocysts to survive vitrification

11:00 AM Rachel L. Piersanti, Animal Sciences
Short and long term effects of uterine disease on oocyte transcriptome in dairy cows

11:15 AM Ali Husnain, Animal Sciences
Impact of induced uterine inflammation on conceptus development in dairy cows

11:30 AM BREAK

Session 6: International Space Station
Michael Poindexter and Weihong Gu, Chairs

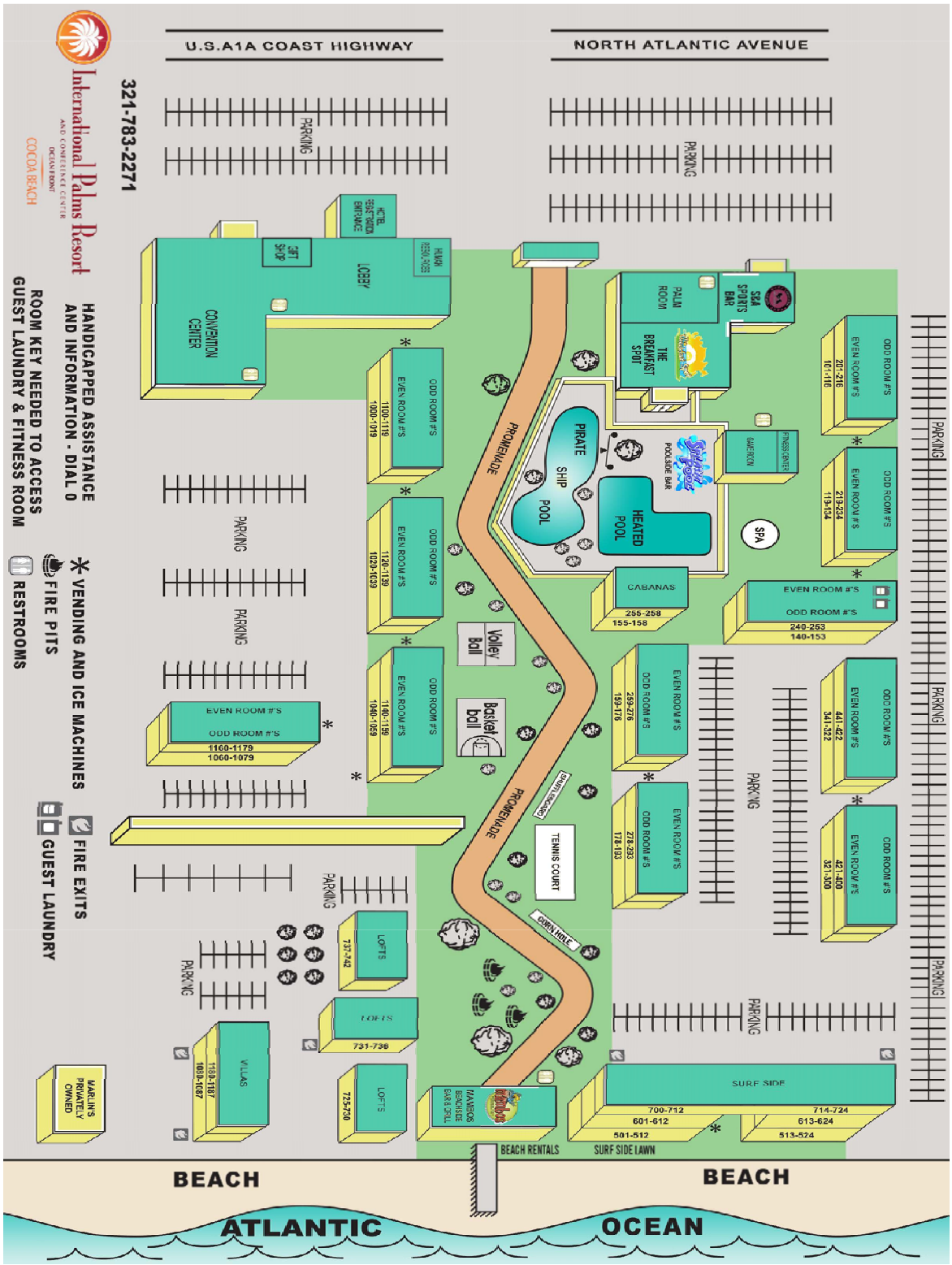
11:45 AM Courtney McCourt, Animal Sciences
Catalytic characterization of the enzyme involved in the finil step of acetate formation: in Selenomonas ruminantium *HD4*

12:00 PM Mackenzie Dickson, Animal Sciences
Uterine disease impairs bovine oocyte quality and embryo development in vitro

12:15 PM Thiago Amaral, Animal Sciences
Expression of KREMEN1 *in the bovine preimplantation embryo*

12:30 PM Closing (and very brief) remarks, Pete Hansen

Sitemap of International Palms Cocoa Beach Hotel & Report



ABSTRACTS

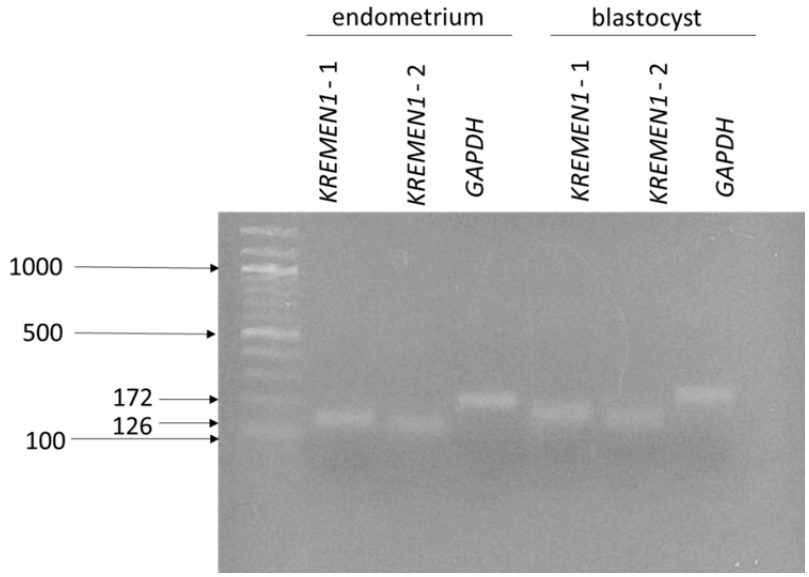
(Arranged alphabetically by first author)

Expression of *KREMEN1* in the bovine preimplantation embryo

Thiago F. Amaral and Peter J. Hansen

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

Embryonic development is regulated by signaling molecules produced by the reproductive tract and embryo. Among these molecules, dickkopf-1 (DKK1) is a secreted inhibitor of canonical WNT signaling expressed in endometrium that can increase the competence of bovine blastocysts to establish pregnancy after transfer into recipients. The mechanism by which DKK1 increases embryo competence is not known. The long-term goal of this research is to identify cell signaling pathways through which DKK1 affects embryo function. Actions of DKK1 on other cells involve either inhibition of canonical WNT signaling or activation of the planar cell polarity (PCP) pathway. Inhibition of the canonical WNT pathway by DKK1 involves endocytosis of LRP5/6 WNT co-receptor by formation of a ternary complex of DKK1/KREMEN/LRP. The current hypothesis being examined is that DKK1 regulates embryo function through actions involving KREMEN-mediated internalization of LRP5/6. As a preliminary experiment, we evaluated expression of the two KREMEN genes (*KREMEN1* and *KREMEN2*) in bovine embryos. First, data mining of an existing transcriptome dataset of morula, blastocyst inner cell mass and blastocyst trophectoderm (BMC Dev Biol 12:33) was performed. Both *KREMEN1* and *KREMEN2* were expressed in all cell types examined, with transcript abundance of *KREMEN1* being 5 to 14-fold greater than for *KREMEN2*. Highest expression of *KREMEN1* was in morula (50 reads for morula, 11 reads for inner cell mass and 29 reads for trophectoderm). Subsequently, reverse transcription-polymerase chain reaction (RT-PCR) was performed to confirm expression of *KREMEN1* by in vitro produced blastocysts using two sets of primers. Endometrium was used as a positive control. Results confirmed expression of *KREMEN1* in both blastocysts and endometrium (Figure 1). Future experiments will be conducted to determine whether knocking down mRNA for KREMEN1 blocks effects of DKK1 to alter blastocyst gene expression and reduce accumulation of β-catenin.



Meta-analysis of the effects of supplemental rumen-protected choline during the transition period on performance and health of parous dairy cows

U. Arshad, M. G. Zenobi, C. R. Staples, and J.E.P. Santos

Department of Animal Sciences, University of Florida, Gainesville 32611

The objectives were to use meta-analytic methods to determine the effects of supplementing rumen-protected choline starting prepartum on production and health of parous dairy cows. The literature was systematically reviewed and 21 experiments with 66 treatment means, with a range of choline ion fed prepartum, from 3.2 to 25.2 g/d of diet, reported data for 1,309 parous cows. Duration of prepartum feeding of diets reported in the experiments averaged (± SD) 22.0 ± 5.9 days. All 21 experiments had a non-supplemented treatment (0 g choline/d). Data collected included number of cows, days on treatment pre- and postpartum, choline ion fed (g/d), ingredients and nutrient content of prepartum diets, number of days of measurements postpartum, and LSM and respective SEM for productive and health responses. The contents of diet NE_L (Mcal/kg), metabolizable protein (MP) as % of DM intake (MPDMI), and metabolizable methionine as % of MP (METMP) were estimated for each prepartum diet using NRC (2001). Mixed models were fitted using the MIXED and GLIMMIX procedures of SAS. Models contained the random effect of experiment and data were weighted by the inverse of the SEM squared to account for the precision of each experiment. Models included the linear and quadratic effects of choline ion, diet NE_L, MPDMI, and METMP, days on treatment prepartum, milking frequency, and interactions between choline and diet NE_L, and choline and METMP in parous cows. Additional meta-analytical statistics were used to estimate the effect size using the METAN and METAREG procedures of STATA. The estimated effect of supplementing 0 vs. 12.9 g/d of choline ion during the transition period is presented in Table 1. Increasing prepartum intake of choline ion linearly ($P<0.001$) increased pre- ($\beta = 0.018 \pm 0.004$; weighted mean difference [WMD] = 0.27 kg/d) and postpartum DM intake ($\beta = 0.045 \pm 0.012$; WMD = 0.59 kg/d), energy-corrected milk ($\beta = 0.174 \pm 0.021$; WMD = 1.90 kg/d), fat yield ($\beta = 0.006 \pm 0.001$; WMD = 0.08 kg/d), and protein yield ($\beta = 0.005 \pm 0.001$; WMD = 0.06 kg/d) in parous dairy cows. However, supplementing prepartum choline ion had no effect on retained placenta, mastitis, displaced abomasum, ketosis, or metritis in parous cows. Although, responses were linear up to 25 g/d of choline ion, yet, the dose of choline ion fed did not allow for the detection of an optimum value for postpartum performance.

Table 1. Effect of prepartum supplementation of 0 vs. 12.9 g of choline ion on performance (LSM ± SEM) in parous transition dairy cows

Item	Control	Choline[1]	*P*-value
Prepartum			
DM intake, kg/d	12.0 ± 0.5	12.3 ± 0.5	< 0.001
Postpartum			
DM intake, kg/d	20.5 ± 0.8	21.0 ± 0.8	< 0.001
ECM, kg/d	35.5 ± 1.6	37.7 ± 1.6	< 0.001
Fat yield, kg/d	1.30 ± 0.07	1.38 ± 0.07	< 0.001
Protein yield, kg/d	1.05 ± 0.04	1.12 ± 0.04	< 0.001

[1] Choline = Linear effect of supplementation of choline ion during transition period

Effects of dietary 25-hydroxyvitamin D_3 on vitamin D status and puberty of dairy heifers

S. R. Bohm, M. B. Poindexter, A. C. M. Silva, T. L. Williams, and C. D. Nelson

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

Vitamin D plays a role in maintaining calcium and phosphorous homeostasis and is required for normal growth and development of heifers. We hypothesized that supplementing dairy heifers with 25-hydroxyvitamin D_3 [25(OH)D_3] would improve growth and development of dairy heifers. The objective of this experiment was to test the effects of dietary 25(OH)D_3 on growth and the onset of puberty in dairy heifers. One hundred forty-three Holstein dairy heifers were assigned to one of two treatments, control (CON) and 25(OH)D_3 (25D) starting at approximately 14 d of age. Control diets were formulated to provide vitamin D_3 at a rate of 1.5 μg vitamin D_3/kg BW and 25D heifers were supplemented with an additional 1.7 μg 25(OH)D_3/kg BW. Treatments were applied three times weekly to individual heifers. Analysis of growth data from birth to 3 m of age indicated 25D calves had greater BW gain through 3 m of age compared to the CON. For the present report serum 25(OH)D_3 and BW were analyzed from 3 to 9 m of age. Age at onset of puberty was analyzed by concentrations of progesterone in biweekly serum samples collected from heifers when they were between 200 to 300 kg BW. Serum 25(OH)D_3 and BW data were analyzed with the mixed procedure of SAS that included fixed effects of treatment and age and heifer within treatment as a random effect. Age and BW at onset of puberty was defined as serum progesterone greater than 1 ng/mL. Serum 25(OH)D_3 concentrations of 25D heifers were greater ($P < 0.001$) compared with CON (118 vs. 59 ± 4 ng/mL). Effect of treatment ($P = 0.03$) and interaction between treatment and age ($P = 0.05$) were significant such that heifers receiving 25D were heavier at 3 m of age compared with CON heifers (109 vs. 113 kg) but BW of CON heifers increased over time such that at 9 m of age, BWs were similar between 25D and CON heifers (252 vs. 254 kg). Age and BW at puberty did not differ between treatment groups with BWs of (267 ± 5 kg) and age being (283 ± 6 d) at onset of puberty. In conclusion, these data suggest that the supplementation of 25(OH)D_3 improves serum 25(OH)D_3 levels. Although 25(OH)D_3 supplementation increases BW up to 3 m of age, the difference in BW was not maintained and it had no effect at age at onset of puberty.

Uterine disease impairs bovine oocyte quality and embryo development in vitro

Mackenzie J. Dickson[1], Jeremy C. Block[2], José E. P. Santos[1], I. Martin Sheldon[3], and John J. Bromfield[1]

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[2]OvaTech, LLC., FL 32609.
[3]Institute of Life Science, Swansea University Medical School, Swansea, SA2 8PP, UK.

Uterine disease is common in postpartum cows and is associated with subsequent subfertility even after resolution of disease. We used an experimental infection model of uterine disease to characterize the impact of bacterial endometritis on the oocyte and its ability to develop into an embryo in culture. Estrous cycles were synchronized in 23 non-lactating, primiparous Holstein cows, and cows were blocked by days postpartum then assigned randomly to receive an intrauterine infusion of vehicle (Luria-Bertani broth; Control, *n*=11), or endometrial pathogenic bacteria (*Escherichia coli* and *Trueperella pyogenes*; Bacteria, *n*=12) on day 2 of the estrous cycle. Data were analyzed by ANOVA for continuous data and logistic regression for binomial data. Increased endometrial expression of *CXCL8*, *IL1B*, *IL6*, *PTGS2* and *TNF* on day 6 after infusion, and the presence of vaginal pus confirmed acute endometrial inflammation in bacteria infused cows compared with controls. Oocytes were collected 2, 24, 45, and 66 days after intrauterine infusion by transvaginal ultrasound guided oocyte pick-up and used for *in vitro* fertilization. The number of oocytes collected did not differ between treatments, but there was a tendency ($P = 0.08$) for a greater proportion of oocytes to undergo cleavage when collected from bacteria infused cows compared with controls. Interestingly, the proportion of cleaved zygotes to reach the morula stage of development was reduced ($P = 0.04$) when oocytes were collected from bacteria infused cows (33.9%) compared with controls (42.2%). Morulae derived from bacteria infused cows also had increased ($P = 0.02$) expression of heat shock 70 kDa protein 1A (*HSPA1A*) compared with controls. These data provide evidence that uterine disease reduces the capacity of oocytes to progress to the morula stage of development following in vitro fertilization. This work is supported by NICHD R01HD084316.

Developmental programming of bovine preimplantation embryos by choline chloride

Eliab Estrada-Cortes[1], William Ortiz[1], Elizabeth Jannaman[1], Charles R. Staples[1], Jeremy Block[2], and Peter J. Hansen[1]

[1]Department of Animal Sciences, University of Florida, Gainesville, Florida, USA.
[2]Ovatech, Gainesville, Florida, USA.

The process of global DNA demethylation and re-methylation occur during the pre-implantation period in bovine embryos; therefore, this is a critical period to establish the epigenome. Choline is a precursor of betaine, a methyl donor for DNA methylation and supplementation with rumen-protected choline has improved reproductive performance in dairy cows. The objective was to determine whether addition of choline chloride to culture medium improves the competence of in vitro-produced embryos to become blastocysts, to establish pregnancy after embryo transfer (ET) and alter characteristics of resultant offspring. Oocytes were obtained by ovum pick up from Brahman cows (n=11). Oocytes from each donor were fertilized using conventional (n=4) or sexed semen (n=1) from five Brahman sires. Half of the presumptive embryos from each donor were incubated in BBH7 culture medium (control; n=387 oocytes) and half with culture medium containing 1.8 mM choline chloride (n=332 oocytes). Treatments were isotonic. At day 7, blastocysts were transferred into Angus x Brangus recipients. Pregnancy diagnosis was performed at day 28 after ET and resultant offspring were evaluated at birth. There was no effect of treatment (P>0.1) on the percent of cleaved embryos becoming blastocyst (15.9±3.4% control vs 15.8±3.3% choline) or pregnancy per ET [50.0% (31/62) vs 40.0% (20/50)] for control or choline, respectively. Birth weight was affected by the treatment x sex of calf interaction (P=0.0022). For females, choline calves were heavier than control calves [49.4±2.5 (n=4) vs 34.3±2.1kg (n=7)] whereas the opposite was observed for male calves [27.5±3.2 (n=3) vs 36.2±3.2kg (n=4)]. In conclusion, addition of 1.8 mM choline chloride to culture medium did not affect competence of embryos to become blastocysts but programmed development to alter fetal growth in a sex-dependent manner (Support: Larson Endowment).

Association analysis identifies Th1/ Th2/ Threg loci associated with resistance to natural *Haemonchus contortus* infections in Florida Native sheep

Zaira Magdalena Estrada Reyes, Owen Rae, Carol Postley, Myriam Berenice Jiménez Medrano, Joel Leal Gutiérrez, and Raluca Mateescu

*Department of Animal Sciences, University of Florida, Gainesville, FL

The aim of this study was to identify single nucleotide polymorphisms (SNPs) associated to internal parasite resistance in Florida Native sheep, using a targeted sequencing approach. One hundred and fifty three lambs (5 months and three months old) were evaluated in this study. At the start of the trial, phenotypic records for fecal egg count (FEC), FAMACHA score, body condition score (BCS), and weight were recorded and deworming of sheep with levamisole (18 mg per kg of body weight) was performed. Ten and 28 days post deworming, a full hematogram of each sheep was obtained and FEC, FAMACHA score, BCS and weight were assessed. Average daily gain was calculated at the end of the trial. Out of 153 animals, 100 sheep were selected for genotyping using a targeted sequencing approach. Targeted sequencing panel included 100 candidate genes for immune response against *H. contortus*. SNPs were discarded if call rate < 95% and minor allele frequency ≤0.05. A mixed model was used to analyze the response variables and included the identity by state matrix to control for population structure. A contemporary group (age, group and sex) was included as fixed effect. Bonferroni correction was used to control for multiple testing. Eighteen SNPs on chromosomes 1, 2, 3, 4, 6, 7, 11, 15, 18, 20 and 26 were significant for different traits. For FEC, significant SNPs were located within *ITGA4*, *STAT3*, *MUC15*, *IL2RB* and *CFI* genes. Two SNPs within *IL12RB2* and *GPX2* genes were significantly associated with FAMACHA score. One SNP in *IL16* gene was associated with both red blood cell count (RBC) and hemoglobin level (HGB). Significant SNPs in *STAT6*, *PCDH7* and *MUC15* genes were identified for white blood cell count (WBC). *CXCL10*, *TNF*, *CCL26*, *ITGA4* and *TLR3* genes contained significant SNPs associated with neutrophil, basophil and eosinophil counts. These results suggest that loci related to Th1/Th2 and Treg responses play an important role in the expression of resistant phenotypes. In conclusion, potential immune like loci could be used as genetic markers for resistance in sheep exposed to natural *H. contortus* infections.

Understanding the role of host genetics on gut microbiota through life by using the Angus-Brahman multibreed herd

Peixin Fan[1, 2], C. D. Nelson[1], J. D. Driver[1], M. A. Elzo[1], and F. Peñagaricano[1], K . C. Jeong[1, 2*]

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

Commensal bacteria in the intestinal tract provide energy for the host and inhibit pathogen colonization. However, the role of host genetics on the gut microbiota structure and its consequences on animal growth are largely unknown. In this study, we investigated the effects of breed composition on the gut microbiota of Angus-Brahman multibreed (MAB) calves at three different growing stages. Fecal samples were collected from a cohort of MAB calves at preweaning, postweaning and fattening stages, and gut microbiota structure was analyzed by using 16S rRNA gene sequencing. We found that breed composition influenced the gut microbiota structure at all three stages, but the variation in the relative abundance of bacteria driven by breed composition was greater in the preweaning and fattening stages compared to the postweaning stage. The relative abundance of *Clostridium* and unclassified Ruminococcaceae as well as microbial genes involved in nitrogen metabolism showed consistent positive correlation with Angus proportion through life, while the relative abundance of *Sutterella* was positively correlated with Brahman proportion. Interestingly, weight gain of the MAB calves was higher in calves with more Angus proportion in the preweaning and fattening stages, but did not show significant difference in the postweaning stage. Strong positive correlations were detected between weight gain and the relative abundance of butyrate producing bacteria *Faecalibacterium*, *Blautia*, *Oscillospira*, and *Prevotella* only at the preweaning stage, suggesting profound effect of preweaning gut microbiota on animal growth. Moreover, SNP genotyping showed high agreement between breed composition estimated from pedigree and genetic distance, and 87,807 SNP markers showed correlation with breed composition. Further studies will identify the relationship between host SNPs and abundance of bacteria to understand the role of genetic effects on the gut microbiota. In conclusion, breed composition influences the gut microbiota of MAB calves greatly depending on growing stages that contributes to animal growth.

Supplementation of 5-HTP or fluoxetine impacts bioenergetics in dairy calves

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Serotonin (**5-HT**) is a monoamine that regulates energy balance through the modulation of insulin and lipid metabolism. Here, we examined if manipulating 5-HT pathway by administering Fluoxetine (**FLX**, a 5-HT reuptake inhibitor) or 5-hydroxytryptophan (**5-HTP**, a 5-HT precursor), would impact energy metabolism in pre-weaned dairy calves. Bull Holstein calves (21±2 d) were fed milk replacer (8 L/d) with saline (**CON**, 8 mL/d *n*=8), FLX (40 mg/d, *n*=8) or 5-HTP (90 mg/d, *n*=8) for 10 consecutive d in a complete randomized block design. Blood samples were collected before (d-1), during treatment (d1-10) and during withdrawal (d2, 3, 4, 7, 14) periods to measure insulin and NEFA concentrations. Calves were euthanized after treatment or withdrawal period to harvest pancreas and adipose tissue to measure gene expression of 5-HT receptors (*5-HTR*), 5-HT transporter (*SERT*) and tryptophan hydroxylase (*TPH1*) by real-time PCR. Data was analyzed by period using one and two-way ANOVAs in R. Insulin had a treatment by day interaction ($P<0.01$), where 5-HTP group had higher circulating concentrations compared to the CON, but had only a day effect during withdrawal period ($P<0.001$). Circulating NEFA concentrations were not different during treatment or withdrawal period ($P>0.10$). In the adipose and pancreas, for both treatment and withdrawal periods, *SERT*, *TPH1* and *5-HTR* were expressed with the exception of *5-HT1D, -2C, -3A, -3B* and *-4*, whereas *5-HT5* was only expressed in pancreatic tissue and *5-HT6* in adipose tissue. After treatment period, adipose gene expression of *5-HT1A* tended to be downregulated in the 5-HTP group ($P<0.10$), but after withdrawal period *5-HT1F* was upregulated by 5-HTP and FLX ($P<0.01$) when compared to CON. After treatment period, there was a tendency for downregulation of pancreatic gene expression of *SERT* ($P<0.08$) and *5-HT1F* ($P<0.10$), in FLX and 5-HTP group, respectively, when compared to CON. After withdrawal, serotonin pancreatic receptors were not differentially expressed. Manipulating serotonin bioavailability of dairy calves increases circulating insulin and modifies the expression of serotonin receptors in the pancreas and adipose tissue.

iNKT cells: Russian roulette or a silver bullet in influenza A virus immunity

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CD1d-restricted invariant natural killer T (iNKT) cells are a distinct subset of αβ T cells that express NK cell receptors. iNKT cells are called "bridging cells" because they have characteristics of both the innate and adaptive immune system. Not all mammals express iNKT-CD1d system, but pigs do. We have found that pigs are more similar to humans than mice for the frequency, distribution, NKT cell T cell receptor structure and antigen recognition. Along with their similar size to humans, these qualities make swine an attractive preclinical model to study the physiological role of iNKT cells for humans. There is also value in understanding how iNKT cells contribute to immunity against important pig pathogens. However, current understanding of porcine iNKT cells is rudimentary, and in need of study. Because single-cell RNA sequencing is a powerful tool to distinguish rare immune cell subsets and dynamic cellular changes at transcriptomic level, we are using this technology to establish the developmental pathway of iNKT cells in pig thymus and determine functional iNKT subsets. Here, we report a magnetic bead-based purification technique that we developed to enrich thymic iNKT cells for sequencing. Also described is a recent study to study the contribution of porcine iNKT cells to immunity against influenza A virus (IAV), a pathogen of great importance for humans and swine. In contrast to what has been reported for mice, it was found that iNKT cell-deficient pigs have reduced and delayed virus shedding compared to iNKT cell intact pigs, indicating that iNKT cells may induce inflammatory immune responses that actually hinder virus clearance. This implies that pigs and humans with high iNKT cell levels could be more at risk for spreading influenza during an outbreak.

Impact of induced uterine inflammation on conceptus development in dairy cows

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Epidemiological data in dairy cows demonstrate that postpartum uterine diseases, which affect 20 to 40% of postpartum cows, are associated with reduced pregnancy per artificial insemination, and increased risk of pregnancy loss. Despite the extensive body of work, the underlying mechanisms resulting in failure of establishment and maintenance of pregnancy remain mostly unclear. We hypothesize that inflammation of the uterus alters the uterine environment that impairs conceptus development in dairy cows. The aim of the current experiment is to utilize an animal model of induced endometritis to investigate the underlying molecular mechanisms of how uterine inflammation affects conceptus development during the peri-implantation period. Postpartum lactating dairy cows with no history of retained placenta, metritis, or clinical endometritis and having metricheck score < 2 and endometrial cytology with less than 10% of polymorphonuclear neutrophils on 23 ± 3 days in milk (DIM), will be enrolled. Cows will have their estrous cycle synchronized using the double Ovsynch protocol starting at 25 DIM. Cows will be assigned randomly to receive no intrauterine treatment (negative control, NC; n = 18) or to be treated (n =18) at 40 DIM with an intrauterine inoculation of 5 x 10^8 CFU of *Escherichia coli* and 5 x 10^8 CFU *Trueperella pyogenes*, a model already validated to induce inflammation and signs compatible with clinical endometritis. The uterus of cows will then be evaluated for discharge, presence of endometrial polymorphonuclear neutrophils and expression of pro-inflammatory cytokines (IL-1, IL-6, IL-12, TNF and IFNγ). Single fresh in vitro produced Holstein embryo will be transferred on day 7 of the estrous cycle (56 DIM) and the uteri will be flushed 9 days later, on day 16 of the presumptive pregnancy. The uterine flush will be collected and frozen for later quantification of interferon-tau, pro-inflammatory markers, and characterization of the lipidome and metabolome through mass-spectrometry. After flushing, endometrial tissue from the horn ipsilateral to the corpus luteum will be collected for biopsy for fatty acid analysis and quantification of mRNA expression for genes of interest. Immune cell infiltration into the endometrial tissue will be characterized through immunohistochemistry to identify uterine natural killer cells, macrophages and T- lymphocytes. The length of recovered concepti will be measured and the presence of the embryonic disc identified. Conceptus mRNA expression will be investigated to characterize genes involved in fatty acid metabolism, cell signaling, growth and development, inflammation, immune function, cell division, and apoptosis through real time PCR in fluidigm platform (Biomark HD). Blood will be collected throughout the experiment for measurements of concentrations of progesterone, haptoglobin, serum amyloid A, and total and differential white blood cell count. Concentrations of estradiol will be measured during proestrus immediately before induction of ovulation. It is anticipated that inducing endometritis will increase plasma and endometrial markers of inflammatory response. It is expected that elevated pro-inflammatory cytokine would upregulate the expression of non-classic major histocompatibility complex class I (MHC-1) on trophoblast and activate the genes associated with cell death and/or apoptosis in conceptus. Infiltration of immune reactive cells into the uterus and their interactions with the upregulated MHC-1 on trophoblast would initiate the immune response that potentially result in death of the embryo. In addition, it is speculated that altered histotroph composition most likely affects the metabolism of fatty acids and glucose, mediated through peroxisome proliferator activated receptor gamma (PPARγ), resulting in reduced embryo elongation that eventually will lead to less production of IFN-tau.

Targeting NKT cells to block influenza A infections in swine

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Antiviral therapy options for treatment of influenza A(IAV) infection are limited for humans and animals. The leading therapy currently used to treat influenza in humans, oseltamivir (Tamiflu®), has to be administered at early onset of symptoms to affect the course of disease, which requires quick diagnosis of symptoms. In addition, cases of viral resistance against this drug have been reported because the therapy targets the virus. Thus, there is a need for novel antiviral therapies that can overcome these limitations by targeting the immune system. Swine are natural hosts for influenza A virus and are also capable of being infected by virus of human and avian origin, which makes them a potential "mixing vessel" for reassortant virus. Currently, there is no antiviral available to control disease pathology and transmission for commercial swine. It has been reported in mice that the activation of natural killer T cells (NKT), a special subtype of T cell, can improve the outcome of diseases caused by a plethora of pathogens, including influenza A virus. Studies show that NKT cell activation reduces mortality and viral shedding in IAV-infected mice. Pigs and humans also possess this special population of cells that can be activated by a synthetic marine-sponge-derived agonist, α-Galactosylceramide (α-GalCer). We describe how intranasal delivery of α-GalCer to pigs infected with IAV inhibited viral replication in airway tissues, decreased virus shedding and also reduced lung pathology without causing any disadvantageous immune reaction in newly-weaned pigs. We also show that NKT cell agonists therapy is comparable to oseltamivir for controlling lung pathology and virus shedding in young piglets infected with pandemic H1N1influenza virus A/CA/04/09. Our results demonstrate the potential to use NKT cell therapy to block influenza transmission in swine and possibly humans, which is needed and deserves further study.

Catalytic characterization of the enzyme involved in the finil step of acetate formation: in *Selenomonas ruminantium* HD4

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The majority of bacteria in the ruminant are unculturable, and their substrate consumption as well as product formation must be predicted from reconstructed genomes. The prediction method most commonly used only includes the classic pathways and their respective enzymes. *Selenomonas ruminantium* HD4 a well characterized rumen bacterium was used to validate this method of pathway prediction. The results of which reveal that *S. ruminantum* HD4 is not predicted to produce acetate, yet *S. ruminantum* HD4 has been experimentally validated to produced acetate through Acetate Thiokinase. Our hypothesis is to validate an alternative pathway predicted by a less exclusive method to which Succinyl:Acetyl-CoA Transferase is used in the conversion of acetyl-coa to acetate and not Acetate Thiokinase, in *S. ruminantium* HD4. Extracts from *S. ruminantum* HD4 were tested for the enzymatic activity of Acetate Thiokinase, and Succinyl:Acetyl-CoA Transferase. Additionally, extracts from *S. ruminantum* HD4 were dosed with cofactors predicted in the enzymatic reaction to secondarily validate results of enzymatic activity. Methods where completed aerobically as well as anaerobically. We detected no enzymatic activity for Acetate Thiokinase, and Succinyl:Acetyl-CoA Transferase in *S. ruminantum* HD4.We did detect that phosphate significantly increased ($P<0.05$) the ability of the unknown enzyme to catalyze the reaction from acetyl-coa to acetate. The addition other cofactors had no effect: pyrophosphate, AMP and pyrophosphate, ADP and phosphate, GDP and phosphate, Succinate and phosphate, Succinate ADP and phosphate, or Succinate GDP and phosphate. We further determine that the unknown enzyme was not aerotolerant, requiring anaerobic conditions. We reject our hypothesis that Succinyl:Acetyl-CoA Transferase is used in the conversion of acetyl-coa to acetate, as well we further provide evidence against Acetate Thiokinase. We conclude by hypothesizing that a hydrolase is the unknown enzyme responsible for the conversion of acetyl-coA to acetate.

Dairy cows have an increased incidence of uterine disease during the hot season with no difference in vaginal bacteria load

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Bacterial infections cause metritis about 7 days after calving in approximately 30% of dairy cows, and uterine disease persists as endometritis in some animals beyond 21 days postpartum. Although heat stress reduces milk production and impairs immune function, little is known about its effect on uterine infection and disease. Our aim was to evaluate uterine disease severity and quantify the bacterial load in vaginal mucus collected from dairy cows on the same farm, during a cool (n=51, average maximum temperature: 22.5°C) season and a hot season when animals are subjected to heat stress (n=51, 31°C). We hypothesize that the incidence and severity of uterine disease is increased during the hot season, accompanied by an elevated bacterial load in the reproductive tract. All animals received heat abatement with fans and sprinklers after calving. Vaginal mucus was scored according to the abundance of purulent discharge and odor on days 7 and 21 postpartum, at the time of diagnosis of metritis and endometritis, respectively. Bacterial 16S rRNA was quantified by qPCR in vaginal mucus samples. During the hot season a higher proportion of cows had persistent uterine disease at both day 7 and day 21 (58.8% vs. 29.4%) and increased incidence of endometritis (64.7% vs. 43.1%). The concentration of bacterial 16S rRNA in vaginal mucus was higher in animals with endometritis compared with healthy cows at day 21 (2.35 vs. 0.04 ng/mg mucus) but did not differ between the hot and cool seasons (2.12 vs. 2.68 ng/mg mucus). In conclusion, our data indicate that despite a similar vaginal bacteria load, heat stress increased the incidence of endometritis. This work is supported by NICHD R01HD084316.

Short and long term effects of uterine disease on oocyte transcriptome in dairy cows.

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Postpartum uterine disease is a common cause of reduced fertility in dairy cows. The effect of uterine disease on subsequent fertility may be related to the accumulation of pathogen-associated molecules and inflammatory mediators in follicular fluid, and their impact on oocyte development. In order to better understand the influence of uterine disease on the oocyte, we used an experimental model of induced uterine disease in virgin heifers. Animals received an intrauterine infusion of sterile medium (control, n=5) or bacteria (pathogenic *Escherichia coli* and *Trueperella pyogenes*, n=4). Uterine disease was confirmed in bacteria infused animals by the presence of clinical endometritis symptoms. Ovum pick-up was performed on day 4 (during disease) and day 60 (after resolution of clinical disease) relative to infusion, and zona-free oocytes were subjected to RNAseq analysis. Using a *P*-value < 0.05 cut-off, 474 genes were differentially expressed in oocytes from bacteria infused animals on day 4, and 929 genes on day 60. Only 56 genes were differentially expressed in oocytes of bacteria infused animals on both day 4 and day 60. Further analysis of the differentially expressed genes identified canonical pathways impacted at day 4 including, interferon signaling, TGFβ signaling, TNFR2 signaling, IL-6 signaling and BMP signaling; while at day 60 glycolysis, bile acid biosynthesis neutral pathway, gluconeogenesis, ILK signaling, interferon signaling and chondroitin sulfate degradation pathways were affected. Predicted upstream regulators of differentially expressed genes in oocytes of bacteria infused animals on day 4 included SOX2, LPS and NFκB; while upstream regulators at day 60 included IL-1β, IL-6, and LH. These data provide evidence that uterine disease alters the oocyte transcriptome, and that unique effects are also apparent in the oocyte after the resolution of disease. This work is supported by NICHD R01HD084316.

Effects of dose and source of vitamin D on mineral homeostasis and performance in transition dairy cows

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Objectives of the experiment were to determine the effects of supplementing two doses of either vitamin D_3 (VitD) or 25-hydroxyvitamin D_3 (25D) on peripartum mineral metabolism and lactation performance. One hundred and thirty-three parous and forty-four nulliparous pregnant Holstein cows were enrolled in the experiment. Cows were blocked by parity and previous lactation milk yield or genetic merit and then assigned randomly to receive a daily dietary top-dressed supplement containing 1 mg VitD (**1mgD**), 1 mg 25D (**1mg25D**), 3 mg VitD (**3mgD**), or 3 mg 25D (**3mg25D**) from 250 days in gestation until parturition. The prepartum diet was approximately -130 mEq/kg in dietary cation anion difference. Data were analyzed by ANOVA with mixed models using the Mixed procedure of SAS. Cows receiving the 25D treatments had greater ($P = 0.01$) concentrations of P in serum prepartum and a tendency for greater ($P = 0.07$) concentrations of P postpartum. Cows receiving the 25D treatments had greater ($P = 0.04$) concentrations of total Ca in serum postpartum. Cows receiving the 3mg25D treatment produced more milk ($P < 0.01$) in the first 42 d in milk compared with other treatments. Prepartum DM intake, postpartum disease incidence, colostrum production, and concentrations of Ca and Mg in colostrum and milk did not differ among treatments. Cows receiving 25D were in a better mineral status postpartum and performed better compared with cows receiving just VitD.

Item	1mgD	3mgD	1mg25D	3mg25D	SE
Prepartum					
Ca, m*M*	2.34	2.32	2.34	2.35	0.02
Mg, m*M*	0.85	0.84	0.83	0.83	0.02
P, m*M*[*]	1.89	1.87	1.96	2.05	0.03
Postpartum					
Ca, m*M*[*]	2.13	2.11	2.15	2.18	0.02
Mg, m*M*	0.88	0.85	0.87	0.84	0.02
P, m*M*	1.72	1.7	1.75	1.8	0.04
Colostrum yield, kg	4.3	3.9	5.4	4.5	0.55
Milk yield, kg/d[¥]	35.3	32.4	33.4	36.6	1.3

*Source (P<0.05), [§]Level (P<0.05), [¥]Level x Source (P<0.05)

Seminal plasma or TGFβ modulates expression of proinflammatory cytokines in bovine endometrial cells

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Seminal plasma is the cell-free fraction of semen that induces endometrial inflammation following coitus in rodents and swine. Seminal plasma-derived TGFβ is the active molecule that modulates inflammation in female reproductive tissues. In rodents and swine, endometrial responses to seminal plasma promote embryo development and improve pregnancy outcomes. However, the endometrial response to seminal plasma is not well-characterized in cattle, where diluted semen is routinely used for artificial insemination. We hypothesize that seminal plasma or TGFβ increases expression of proinflammatory cytokines in bovine endometrial cells. Bovine endometrial (BEND) cells were treated for 24 hours with increasing concentrations of pooled seminal plasma (0.001% to 20%), rhTGFβ-1 (1 to 100 ng/mL) or rhTGFβ-2 (0.1 to 10 ng/mL). Cell viability was measured by MTT assay and gene expression was quantified by qPCR. Viability of BEND cells was reduced following exposure to 1% seminal plasma and higher concentrations. Heat-treatment of seminal plasma reduced cytotoxic effects of seminal plasma. Exposure of BEND cells to 1% seminal plasma (non-cytotoxic) increased expression of *IL1B* and *IL6* compared to controls (6.7-fold and 36.7-fold), while expression of *TNF* and *CSF2* was unchanged. Exposure of BEND cells to rhTGFβ-1 or rhTGFβ-2 increased *TNF* expression in the absence of estrogen, while *IL6* expression was increased only after estrogen supplementation. Exposure to TGFβ did not affect *CSF2* or *IL1B* expression. These data suggest that seminal plasma is cytotoxic to BEND cells and alters expression of proinflammatory cytokines. Similar expression of cytokines were observed in BEND cells following TGFβ exposure. Further investigation is required to determine the extent by which semen components elicit endometrial inflammation in the bovine which may be useful to optimize reproductive efficiency. This research was supported by Select Sires and the Southeast Milk checkoff.

Effect of colony stimulating factor 2 on competence of bovine blastocysts to survive vitrification

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Colony stimulating factor 2 (CSF2) is an important regulator of embryonic development. Treatment of in vitro produced embryos with CSF2 from days 5 to 7 after insemination can increase competence of embryos to establish pregnancy after transfer. One action of CSF2 is to protect embryos from stress. Treatment with CSF2 reduced heat-shocked induced apoptosis of blastomeres and increased the percent of embryos developing to the blastocyst stage after heat shock. Here we tested whether CSF2 would promote survival of embryos to another stress: cryodamage caused by vitrification. Ovaries were recovered from an abattoir and used to harvest cumulus-oocyte complexes (COC). Groups of 10 COC were matured for 20-22 h. After maturation, COC were fertilized with~1x10^6/mL Isolate®-purified spermatozoa from a pool of frozen-thawed semen from 3 bulls. Fertilization proceeded for 20-22 h. Cumulus cells were removed from putative zygotes using 1000 U/ml hyaluronidase by agitation and putative zygotes were cultured in groups of 30 in 50-µL drops of synthetic oviductal fluid – bovine embryo 2 (SOF-BE2) medium until day 7 after insemination. Drops of cultured embryos were randomly assigned to receive either 10 ng/ml CSF2 or vehicle [90% (v/v) SOF-BE2 and 10% (v/v) Dulbecco's phosphate buffered saline containing 1 mg/ml bovine serum albumin) on day 5. Blastocyst stage embryos were selected for vitrification at Day 7. Blastocysts were vitrified and rewarmed according to procedures of (Vajta *et al*. Theriogenology 45: 683-689, 1996). After warming, embryos cultured for 72 h in groups of 5-12 in SOF-BE2 containing 10% fetal bovine serum and 50 µM dithiothreitol (DTT) were assessed for re-expansion (defined as restoration of a reconstituted blastocoele) and hatching (defined as partial or completion exit from the zona pellucida). To date, a total of eight replicates have been performed using 170 blastocysts treated with CSF2 and 185 blastocysts treated with vehicle. Data on percent re-expansion and hatching were calculated for each replicate and analyzed by analysis of variance using the GLM procedure of SAS. There were no significant differences due to CSF2 treatment (Table 1). Preliminary conclusions are that CSF2 does not increase blastocyst survival to vitrification. More replicates will be performed. In addition, vitrified blastocysts will be transferred to recipients to determine survival in vivo.

	Re-expansion (%)			Hatching (%)		
Treatment	24 h	48 h	72 h	24 h	48 h	72 h
Vehicle	70 ± 5	66 ± 5	66 ± 5	19 ± 4	32 ± 5	38 ± 4
CSF2	73 ± 5	71 ± 5	71 ± 5	25 ± 4	32 ± 5	40 ± 4

Effect of duration of exposure to diets differing in DCAD on Ca metabolism after a parathyroid hormone (PTH) challenge in dairy cows

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Objectives were to determine the length of exposure to an acidogenic diet (ACD) to elicit increased response to PTH-induced changes in blood Ca and vitamin D_3 in prepartum cows. The hypothesis was that cows have increased PTH responsiveness within 3 d of feeding ACD. Ten parous Holstein cows at 242±7 d of gestation were blocked by lactation and pretreatment DMI and, within block, they were assigned randomly to an alkalogenic (ALKD; DCAD = +209 mEq/kg DM; n=5) or an ACD (DCAD = -168 mEq/kg DM; n=5) on experiment d 0. Water and DMI were measured, and urine and blood were sampled. The PTH challenges were performed on d 3, 8, and 13. Cows received 0.05 μg PTH/kg BW i.v. every 20 min for 9 h to mimic the pulsatile release of endogenous PTH. Jugular blood was sampled at 0 h, and hourly thereafter until 10 h, and at 12, 18, 24, 36, and 48 h relative to the challenge. Blood acid-base measures and concentrations of ionized Ca (iCa) were evaluated, and vitamin D metabolites will be assayed. Results were available for the first challenge on d 3 and data were analyzed by ANOVA with mixed models with SAS. Cows fed ACD had smaller (P<0.01) blood pH (7.429 vs. 7.382 ± 0.005), base excess (4.3 vs. -2.4 ± 0.5 m*M*), and bicarbonate (28.5 vs. 22.8 ± 0.4 m*M*) within 24 h of the experiment compared with cows fed ALKD. Urine pH declined and differed (P<0.01) by 15 h of feeding the ACD (8.18 vs. 7.32 ± 0.17), and differences increased by 24 h (8.10 vs. 6.46 ± 0.17). Blood iCa increased (P<0.01) in ACD compared with ALKD by d 3 (1.216 vs. 1.281 ± 0.013 m*M*). During the PTH challenge on d 3, cows fed ACD had greater (P<0.01) concentration of blood iCa than cows fed ALKD (1.331 vs. 1.423 ± 0.013 m*M*). Nevertheless, the increment in iCa in the first 36 h after the challenge, relative to baseline at 0 h, did not differ between treatments (ALKD = 0.15 vs. ACD = 0.16 ± 0.01 m*M*). Diet-induced metabolic acidosis occurred within 24 h of treatment; however, an increase in blood iCa concentration was observed after 3 d of metabolic acidosis. Blood iCa response to a PTH challenge did not differ between treatments on experiment d 3.

Dickkopf WNT signaling pathway inhibitor 1 is not able to maintain pluripotency during derivation of bovine embryonic stem cells

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Pluripotent bovine embryonic stem cells (ESC) were recently derived from blastocysts by employing fibroblast growth factor 2 and a canonical WNT/β-catenin pathway inhibitor (IWR1) in a serum-free culture system. It suggested that modulation of the canonical WNT pathway played a critical role in establishment of pluripotency in bovine cells. Dickkopf WNT signaling pathway inhibitor 1 (DKK1) is a canonical WNT pathway inhibitor that controls lineage differentiation during embryogenesis. We hypothesis that IWR1 can be replaced by DKK1 during the derivation of bovine ESC. In vitro produced blastocysts (non-expanded, expanded or hatching) were treated to remove the zona pellucida, randomly divided into three groups and plated onto mouse embryonic fibroblast feeders with the standard ESC medium containing IWR1, DKK1 or vehicle (n=12 per group) in 4-well plates. Outgrowths were passaged 4-5 times and evaluated for immunolabeling with the pluripotency marker SOX2 at passages 3-5. A total of five embryo-derived cell lines were obtained from IWR1-treated cultures and all cell lines were positive for SOX2 at passage 4. Four cell lines were obtained from DKK1-treated cultures. Only one of these cell lines was positive for SOX2 at passage 3 and expression was gradually lost during subsequent passages. Four cell lines were derived from vehicle-treated cells and all were negative for SOX2. At passage 4, membrane-associated β-catenin (downstream signaling molecule for WNT signaling) was abundantly distributed on the cell membrane of IWR1-treated cells but was less abundant for both DKK1- and vehicle-treated cells. These data suggest that DKK1 was not as effective as IWR1 in maintaining pluripotency of embryo-derived cells. Perhaps, membrane-associated β-catenin has an important role in maintenance of pluripotency of cells derived from the bovine epiblast during culture. Future experiments will focusing on determining the lineage characteristics of DKK1-treated cells and testing whether IWR1 maintains pluripotency via stabilizing β-catenin and E-cadherin complexes in the plasma membrane to regulate cytoskeleton and cell adhesion.

Unmasking a killer: discovery of a gene that controls the pathogenic activation of CD8 T cells in Type 1 diabetes

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CD4 T cell help is critical for the activation of autoreactive CD8 T cells that cause most β-cell destruction in type-1 diabetes (T1D). We previously identified a 10 Mb C57BL/6 (B6)-origin recessive *Idd* susceptibility locus on chromosome 11, designated *Idd32* that controls whether CD4 T cells cause pathogenic CD8 T cells to undergo activation or become tolerized. To identify the underlying gene, we adoptively transferred CD8 T cells transgenically expressing the diabetogenic AI4 T cell receptor into B6 mice expressing the NOD-derived $H2^{g7}$ MHC haplotype (B6.$H2^{g7}$) and one of 9 different NOD-derived segments of the *Idd32* interval. This reduced the interval to a <520 kb region containing 18 polymorphic genes. To test these candidate genes (Δ) we used the CRISPR/Cas9 system to generate two sets of F1 strains: (B6.$H2^{g7}$.$Idd32^{NOD/NOD}$.$\Delta^{-/-}$ x B6.$H2^{g7}$)F1 and (B6.$H2^{g7}$.$Idd32^{NOD/NOD}$ x B6.$H2^{g7}$)F1 that were monitored for T1D development after infusing them with AI4 T cells. We found that F1mice lacking the NOD allele of the sialic acid transferase gene *St6galnac1* (B6.$H2^{g7}$.$Idd32^{NOD/NOD}$.$St6galnac1^{-/-}$ x B6.$H2^{g7}$)F1, but not other candidates, developed T1D. Furthermore, CD4 T cells from these mice strongly promoted diabetogenic AI4 T cell activation when co-transferred into *NOD.Rag1*$^{-/-}$ mice. In contrast, CD4 T cells from *St6galnac1*-intact (B6.$H2^{g7}$.$Idd32^{NOD/NOD}$ x B6.$H2^{g7}$)F1 mice suppressed AI4 activation and T1D development. In summary, we have identified a novel, recessively-acting T1D susceptibility gene hidden in the normally T1D-resistant B6 mouse strain, with striking effects on pathogenic CD8 T cell activation. Our results may aid the screening of humans at future risk for T1D as well as provide a target for possible disease interventions.

Determining how much ATP is yielded from fermentation by rumen microbes

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Rumen microbes represent the largest and cheapest source of protein for cattle, making it a perennial goal of animal nutrition to increase microbial protein production. However, both total yield and efficiency of microbial yield are low, and it is uncertain how inefficient microbes are. We must know the ATP yield of fermentation in order to determine the microbial growth efficiency accurately. The ATP yield from fermentation is thought to be well known, but recent genomic evidence suggests fermentation pathways yield more ATP than expected. Some bacteria encode two ion pumps (Ech, Rnf) that, in conjunction with ATP synthase, raise ATP yield by 50% in the pathway of fermentation of glucose to butyrate. Some bacteria that encode Rnf pump raise ATP yield by 12.5% in the pathway that forming acetate and propionate. Additionally, the existence of an unconventional glycolytic enzyme Pfp raises ATP yield by sparing ATP because this enzyme uses pyrophosphate rather than ATP when transferring fructose-6P to fructose-1,6P_2. Here, we will determine if this genomic evidence is supported experimentally for bacteria and protozoa fermenting glucose. Specifically, we will show our current finding about a new pathway for acetate formation in bacteria, and discuss how to verify and determine the activities of Ech, Rnf, and Pfp in bacteria and protozoa isolated from the rumen. By determining the activity of these enzymes (Ech, Rnf, Pfp) in rumen bacteria and protozoa, we can determine the accurate ATP yield from major fermentation pathway of glucose and reveal how inefficient microbes are.

Effects of dietary vitamin D_3 or 25-hydroxyvitamin D_3 on mineral metabolism in growing calves

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Objectives were to determine the effects of dose and source of vitamin D (vitamin D_3 or 25-hydroxyvitamin D_3 [25OHD_3]) on mineral metabolism and bone mineral density (BMD) in calves. The hypothesis was that dietary 25OHD_3 would enhance Ca accretion and BMD. Forty-five Holstein male calves were enrolled within the first week of age in a randomized complete block design. Calves were assigned to 1 of 5 treatments: **T1** (0.25 μg of vitamin D_3/kg BW), **T2** (0.25 μg of vitamin D_3 + 1.5 μg of 25OHD_3/kg BW), **T3** (0.25 μg of vitamin D_3 + 3.0 μg of 25OHD_3/kg BW), **T4** (1.75 μg of vitamin D_3/kg BW) and **T5** (3.25 μg of vitamin D_3/kg BW). Treatments were supplied from birth to 131 ± 9 d of age when calves were euthanized, and tissues collected. Calves were fed milk replacer until 49 d of age and had ad libitum access to starter grain and water. Blood was sampled on d -15, -14, -13, -12, -11, -9, -7, -5, -3 and -1 relative to slaughter. Intake of DM was evaluated in the last 15 d before slaughter. Total fecal collection and spot urine was sampled on d -4 to -2 relative to slaughter. The BMD was determined in the right metacarpus. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Contrasts evaluated included the effects of supplementing vitamin D (Sup: T1 vs. T2+T3+T4+T5), source of vitamin D (Source: T2+T3 vs. T4+T5), dose of vitamin D (Dose: T2+T4 vs. T3+T5), or the interaction between Source and Dose (Int: T2 + T5 vs. T3 + T4). The weight of liver, pancreas and thyroid gland, and weight and dimensions of kidneys and spleen did not differ among treatments**.** Intake of DM or Ca did not differ among treatments. Supplementing vitamin D reduced blood Ca; however, supplemental 25OHD_3 increased blood Ca compared with vitamin D_3. Treatment did not affect digestibility or retention of Ca, but calves fed 25OHD_3 tended to have increased BMD.

Item	T1	T2	T3	T4	T5	SE
Body weight, kg	146.4	148.5	143.3	145.9	150.1	8.7
BW gain, kg/d	1.32	1.20	1.19	1.25	1.34	0.12
Intake						
DM, kg/d	4.3	4.0	4.0	4.0	4.3	0.25
Ca, g/d	61.9	55.1	58.6	56.7	62.2	4.0
Blood						
tCa, m*M* [*,†]	2.868	2.858	2.840	2.788	2.808	0.024
iCa, m*M* [*]	1.473	1.454	1.437	1.446	1.429	0.014
Calcitriol, pg/mL	51.4	53.8	55.2	53.4	57.0	3.2
BMD, g/cm^2 [†,‡]	0.986	1.033	1.054	0.967	1.038	0.024
Ca digestibility, %	50.5	53.2	51.6	52.1	52.9	2.5
Ca retention, g/d	31.1	29.0	30.3	29.4	33.1	2.9

[*] Sup ($P<0.09$); [†] Source ($P<0.10$); [‡] Dose ($P<0.07$).

Bone density, calcium, vitamin D

NOTES

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