**10th Annual Meeting**
**Eccles Science Learning Center**
**Tuesday, August 10, 2021**

Student Research Symposium
*Experience • Research • Networking*

## MEETING SCHEDULE

| | | |
|---|---|---|
| 8:00 – 8:15 AM | Welcome/Opening Remarks | ESLC 130 |
| 8:15 – 10:10 AM | Sessions 1A & 1B: Oral/Flash Talk Presentations | ESLC 130 |
| 10:10 – 11:00 AM | Session 2: Break/Regular & Flash Talk Posters | ESLC Hallway |
| 11:00 – 12:00 AM | Guest Panel | ESLC 130 |
| 12:00 – 1:15 PM | Lunch | ESLC Basement/Patio |
| 1:15 – 2:55 PM | Sessions 3A & 3B: Oral/Flash Talk Presentations | ESLC 130 |
| 2:55 – 3:45 PM | Session 4: Break/Regular & Flash Talk Posters | ESLC Hallway |
| 3:45 – 4:30 PM | Ice Cream Social | ESLC Hallway |
| Virtual Only | Pre-Recorded Mini Presentations | SRS Canvas Page |

## MEETING DETAILS

**Welcome** **Opening Remarks** (Dirk Vanderwall & Kara Thornton-Kurth) **8:00 AM – 8:15 AM**

**Session 1A** **Oral Presentations** (Moderator: Shawn Zimmerman) **8:15 AM – 9:30 AM**

| Start – End | Student | Advisor(s) | Degree |
|---|---|---|---|
| 8:15 – 8:30 | Michael Clayton | Van Wettere/Stegelmeier | PhD |
| 8:30 – 8:45 | Laura Smith | Thornton-Kurth | PhD |
| 8:45 – 9:00 | Laura Adams | Polejaeva | PhD |
| 9:00 – 9:15 | Jiyoun Kim | Lee | PhD |
| 9:15 – 9:30 | Daphne Rodriguez | Benninghoff | PhD |

**Session 1B** **Poster Flash Talks (**Moderator: Shawn Zimmerman) **9:30 AM – 10:10 AM**

| Poster # | Student | Advisor(s) | Degree |
|---|---|---|---|
| 12 | Katherine Trepanier | Rood | DVM |
| 8 | Tyeisha Watters | Olsen | DVM |
| 16 | Joseph Goldhardt | Wang | DVM |
| 19 | Sierra Lopez | Meyer-Ficca | DVM |
| 4 | Sarah Bayles | Thornton-Kurth | DVM |
| 6 | Andy Nguyen | Coulombe | MS |
| 14 | Sawyer Fonnesbeck | Isom | MS |
| 2 | Tyler Elgiar | Lyman | MS |
| 25 | Ashlee Buist | Mason | MS |
| 29 | Irene Helper | Rood | MS |

**Session 2** **Regular Posters & Session 1B Flash Talk Posters** **10:10 AM – 11:00 AM**

| Poster # | Student | Advisor(s) | Degree |
|---|---|---|---|
| 1 | Audrey Ligard | Meyer-Ficca | Undergrad |
| 9 | Carlyan Spackman | Rutigliano | Undergrad |
| 13 | Cassie Wilker | Thornton-Kurth | Undergrad |
| 22 | Brodie Taylor | Mason | Undergrad |
| 26 | Aubrey Ukena | Wang | Undergrad |

**Guest Panel:** ***The Intersection of Science and Politics*** **11:00 AM – 12:00 PM**
Moderator: Kara Thornton-Kurth

**Dr. Angela Dunn (MD, MPH)**, Executive Director at Salt Lake County Health Department
**Br. Bart Tarbet (PhD, MS),** Research Associate Professor, Institute for Antiviral Research
**Dr. Damon Cann (PhD, MA),** Professor, Political Science, Mayor of North Logan

**Session 3A** **Oral Presentations** (Moderator: Fernanda Batistel) **1:15 PM – 2:15 PM**

| Start – End | Student | Advisor(s) | Degree |
|---|---|---|---|
| 1:15 – 1:30 | Jacob Keim | Polejaeva | PhD |
| 1:30 – 1:45 | Scott Gibson | Hurst | MS |
| 1:45 – 2:00 | Madi Lindsey | Isom | MS |
| 2:00 – 2:15 | Lexie Padilla | Batistel | MS |

**Session 3B** **Poster Flash Talks** (Moderator: Fernanda Batistel) **2:15 PM – 2:55 PM**

| Poster # | Student | Advisor(s) | Degree |
|---|---|---|---|
| 11 | Micah Forbush | Meyer-Ficca | PhD |
| 7 | Renata Hoskova | Meyer | PhD |
| 15 | Anthony Alberto | Thornton-Kurth | PhD |
| 3 | Caleb Reichhardt | Thornton-Kurth | PhD |
| 10 | Chase Goodey | Isom | MS |
| 18 | Rebecca Strong | Tarbet | MS |
| 21 | Ashley Sheesley | Tarbet | MS |
| 23 | Morgan Eggleston | Coulombe | MS |
| 27 | Ethan Gilliam | Mason | MS |
| 30 | Braden Abercrombie | White | MS |

**Session 4** **Regular Posters & Session 3B Flash Talk Posters** **2:55 PM – 3:45 PM**

| Poster # | Student | Advisor(s) | Degree |
|---|---|---|---|
| 5 | Alexie Zwerdling | Meyer | Undergrad |
| 17 | Matthew Brothers | Davies | Undergrad |
| 20 | Lillian Okamoto | Thornton-Kurth | Undergrad |
| 24 | Kaden Underwood | Mason | Undergrad |
| 28 | Tristin King | Mason | Undergrad |

**Mixer** **Ice Cream Social** **3:45 PM – 4:30 PM**

**Virtual** **Pre-Recorded Mini Presentations** **SRS Canvas Page**

| Student | Advisor(s) | Degree |
|---|---|---|
| Amber Thornton | Rutigliano | MS |
| Camila Castro Veloz | Batistel | MS |
| Elaine Dawson | Rood | MS |
| Youssef Harraq | Thornton-Kurth | Undergrad |

**Angela Dunn (MD, MPH)**

Dr. Angela C. Dunn is the Executive Director for the Salt Lake County Health Department. Prior to this position, she served as the State Epidemiologist for the Utah Department of Health. Dr. Dunn received her medical degree from the University of Miami Miller School of Medicine and completed her residency training in General Preventive Medicine and Public Health at the University of California San Diego. Following residency, she trained with the Centers for Disease Control and Prevention as an Epidemic Intelligence Service Officer where she responded to the 2014-2016 Ebola Epidemic in West Africa. Dr. Dunn lives in Salt Lake City with her husband and two sons. They love exploring the outdoors together year-round.

**E. Bart Tarbet (PhD, MS)**

I started my training in medical microbiology (infectious diseases) at the Louisiana State University (LSU) Medical Center in 1992. After completing my degree at LSU, I worked for 12 years in the veterinary vaccine industry, where I developed vaccines for emerging infectious diseases in animals, including West Nile Virus, equine influenza virus, and canine influenza virus. In the Institute for Antiviral Research at USU, my research includes developing animal models of human infectious disease for evaluation of experimental therapeutics and vaccines. Emerging viruses studied in my lab have included the 2009 pandemic influenza H1N1 virus, enteroviruses (EV-D 68, EV-71, and Echovirus), and SARS-CoV-2. In addition, from 2010 to 2015, we had funding from BARDA to train personnel from vaccine manufacturers in developing countries as part of the World Health Organization's vaccine production capacity building program. We hosted visiting scientists from 12 countries for 3-week training courses at USU, and also completed on-site training at vaccine manufacturers in Indonesia and Vietnam. My research in both human and animal infectious diseases led to development of a course in *One Health* for the Master of Public Health program through ADVS.

**Damon Cann (PhD, MA)**

Damon Cann is an award-winning teacher and researcher in the field of Political Science. He began his career at the University of Georgia in 2004, then came to Utah State University in 2008 where he is now a Full Professor. He previously served as interim head of the Political Science Department and the Journalism & Communication Department. His book on congressional campaign finance, *Sharing the Wealth*, won the 2009 Fenno Prize for the best book on Legislative Studies and his more recent book on judicial selection, *Voters' Verdicts*, won the 2016 Virginia Gray Book Award for the best book on state politics & policy. His research work focuses on elections, representation, political communication and public policy.

# 2021 ADVS Student Research Symposium Abstracts

Abstracts are in alphabetical order by first author's last name.
Tip: Search the abstracts using last names. Some authors have abbreviated their first names.

# DNA methylation profiles of developmentally important genes in Bovine SCNT and IVF embryos

Braden Abercrombie, Ying Liu, Tayler Patrick, Misha Regouski, Abby Benninghoff, Irina Polejaeva, Ken White

Department of Animal, Dairy and Veterinary Sciences. Utah State University, Logan UT.

Epigenetic reprogramming of the early embryo involves mass *de novo* DNA methylation, which can subsequently affect the ability of regulatory elements involved in gene transcription and cell differentiation. DNA Methylation largely occurs on CpG dinucleotides, a high cluster of which is a termed a CpG island. CpG islands are commonly found in the genome near genes and can occur in coding and non-coding gene regions. The importance of classifying DNA methylation patterns in somatic cell nuclear transfer (SCNT) embryos can provide insight into the mechanisms in which a donor fibroblast is reconstructed and the ability to differentiate leading to further embryo development. One aspect of our study is to compare the methylome of developmentally important genes: NANOG, SOX2, LIN28, cMYC, KLF4, and OCT4 (POU5F1) in SCNT and in vitro fertilized (IVF) embryos as well as 3 fibroblast cell lines used for SCNT. We used a high throughput bisulfite sequencing workflow to successfully classify DNA methylation profiles of each gene region in 2cell, 8cell, and blastocyst stage embryos as well as fibroblasts and oocyte samples. We found differences in DNA methylation patterns on a per base resolution between SCNT embryos and IVF embryos across the 6 genes of interest. Interestingly, we found major differences between the 3 fibroblast cell lines including most of the CpG sites on the NANOG gene region. Replicates will be further generated for robust statistical analysis.

**Use of handmade cloning protocol to reduce oocyte mitochondria**

Laura Adams[1], Ying Liu[1], Barbara Durrant[2], Elena Ruggeri[2], Carly Young[2], Irina A. Polejaeva[1]

[1] Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah, USA
[2] Reproductive Sciences, Beckman Center for Conservation Research, San Diego Zoo Wildlife Alliance, Escondido, California, USA

A significant issue in interspecies somatic cell nuclear transfer is mitochondrial heteroplasmy: two distinct mitochondrial DNA populations existing within the reconstructed embryo. This condition can lead to developmental issues. This study aims to significantly reduce mtDNA copy numbers via oocyte bisection, therefore also reducing mitochondrial heteroplasmy in the reconstructed embryo. Denuded mature bovine oocytes were centrifuged to produce a visible mitochondria-rich fraction at one end of the oocyte, and zonae pellucidae were removed to allow for bisection, which removed the mitochondria fraction. DNA was extracted from somatic cells, oocytes, and ooplasts. A standard curve ($R^2$=0.97) was created using the quantitative PCR (qPCR) results of DNA extracted from bovine somatic cell dilutions. Subsequent qPCR runs included DNA extracted from whole oocytes and bisected ooplasts, which were either mitochondria-depleted or mitochondria-rich, providing a comparison of mtDNA copy numbers before and after bisection. The average mtDNA copy number (± standard deviation) in one bovine oocyte was 45,565 ± 37,169. The average mtDNA copy number in a mitochondria-depleted ooplast was 8,396 ± 13,287. A mitochondria-rich ooplast had an average of 74,653 ± 58,877 mtDNA copies. These results indicate that the bisection of a bovine oocyte following centrifugation can significantly decrease the mtDNA copy numbers present in the ooplast when compared to the original oocyte ($P<0.0001$) and to the mitochondria-rich ooplast ($P<0.0001$). This modification would decrease the incidence of mitochondrial heteroplasmy in the reconstructed embryo, promoting proper embryonic and fetal development.

**Understanding the role of zinc and manganese in proliferation and protein synthesis of primary bovine satellite cells.**

A. Alberto[1], L. Smith[1], C.C. Reichhardt[1], S. L. Hansen[2], K. J. Thornton[1]

[1]Animal Dairy and Veterinary Sciences, Utah State University, Logan, UT, US
[2]Department of Animal Science, Iowa State University, Ames, IA, US

Trace minerals are vital for the health and growth of livestock, supporting multiple biochemical processes in the body. There are several different signaling pathways that may be affected by trace minerals, ultimately altering growth of skeletal muscle. However, it is currently unknown how trace minerals specifically impact growth of skeletal muscle. As such, the objective of this study was to determine how zinc (Zn) and manganese (Mn) affect proliferation and protein synthesis of primary bovine satellite cell (BSC) cultures. Cultures were grown to 80% confluency and treated in 1% fetal bovine serum (control), 0.05, 0.10 or 0.25 µM of Mn, or 10, 20 or 40 µM of Zn to assess proliferation. Additionally, the above treatments were applied to fused BSC cultures in serum free media (control) to measure protein synthesis. The trace mineral concentrations chosen were based off known ranges of circulating concentrations of Zn or Mn. A series of contrasts were constructed to determine whether growth of BSC cultures was different between the treated and control cultures. Treatment with 10 µM Zn increased ($P = 0.03$) proliferation when compared to control cultures. However, treatment with Mn at the tested concentration did not ($P > 0.12$) result in proliferation rates that were different than the control cultures. Treatment with 10 µM Zn, 20 µM Zn, or 0.5 µM Mn increased ($P < 0.05$) protein synthesis compared to control cultures. These results indicate Zn is capable of increasing proliferation and both Zn and Mn increase protein synthesis of BSC cultures. Additional research is needed to couple trace mineral nutrition with knowledge of BSC biology to elucidate the molecular mechanisms by which trace minerals may function to support bovine skeletal muscle growth**.**

# Examining the relationship between breed type and anabolic implant protocols on feed intake and feeding behavior of beef steers

S.A. Bayles[1,2], C.C. Reichhardt[1], B.W. Roholt[1], M. Garcia[1], K.J. Thornton[1]

[1]Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322
[2]School of Veterinary Medicine, Utah State University, Logan, UT 84322

Emerging research suggests that different cattle breed types respond differently to anabolic implants. The purpose of this research was to determine the effects of different anabolic implant protocols on feed intake and feeding behavior in feedlot steers of two different breed types. Sixty steers were stratified by weight and breed in a 2x3 factorial design examining two different breeds: Angus (AN; n=38) or Santa Gertrudis influenced (SG; n=22), and three implant strategies: no implant (CON; n=20), a medium-intensity (MI) implant protocol (d0 implant: Revalor-G, d56 implant: Revalor-IS, d112 implant: Revalor-S; MI; n=20), or a high-intensity (HI) implant protocol (d0 implant: Revalor-IS, d56 implant: Revalor-S, d112 implant: Revalor-200; HI; n=20). Steers were randomly placed into pens equipped with GrowSafe® bunks and fed the same diet. Feeding behavior was analyzed based off bunk visits (BV) and feed bouts (FB) calculated by the GrowSafe® bunks. High-intensity steers had greater (*P<0.01*) dry matter intake compared to CON and MI steers. Angus steers consumed less (*P<0.01*) dry matter than SG steers. Bunk visit and FB events decreased (*P<0.01*) over time, regardless of implant treatment. A treatment by breed interaction was observed (*P<0.01*) for the average time spent with head down for BV and FB. High-intensity steers consumed more (*P<0.01*) than MI steers for the average amount per feed bout consumed (AveFBCon) and average amount per bunk visit consumed (AveBVCon). Further, AveFBCon and AveBVCon increased (*P<0.1*) with time for all steers. Average bunk visit duration and average feed bout duration decreased (*P<0.01*) with time for all steers. Overall, both breed type and anabolic implant protocol influence feed intake and feeding behavior of beef steers.

# The interferon lambda system and its role in the survival of interferon type I receptor knockout sheep

Matthew J. Brothers[1,3], Evan K. Peterson[1,3], Heloisa M. Rutigliano[1,2], Aaron J. Thomas[1,3], Young-Min Lee[1,2], Irina A. Polejaeva[1] and Christopher J. Davies[1,2,3],

[1]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT; [2]School of Veterinary Medicine, Utah State University, Logan, UT; [3]Center for Integrated BioSystems, Utah State University, Logan, UT

Our group has developed a line of type I interferon (IFN) receptor (IFNAR) knockout sheep. These sheep fail to respond to type I IFN including IFN-alpha (IFNA), IFN-beta (IFNB) and IFN-tau (IFNT). Consequently, they are more susceptible to viral infections, and biallelic knockout ewes are likely infertile because IFNT is the signal for pregnancy recognition in ruminants. Nevertheless, some IFNAR knockout sheep survive under farm conditions. We hypothesize that in these sheep the IFN-lambda (IFNL) system helps provide protection against viruses. Our goals were: (1) to understand the gene expression of the IFNL (*IFNL3* and *IFNL4*) and IFNL receptor (*IL10RB* and *IFNLR1*) genes; and (2) to assess the rate of clearance of a long-acting progesterone (P4; BioRelease P4 LA, BET Pharm) we hope to use to maintain pregnancy in biallelic IFNAR knockout ewes. Expression of the IFNL genes was studied in peripheral blood mononuclear cells (PBMC) and an ovine kidney epithelial cell line (OA4.K/S1, ATCC). Gene expression was compared in unstimulated cells and cells stimulated with Poly(I:C), which activates an anti-viral immune response. There was a problem with genomic DNA contamination in some RNA samples. Nevertheless, the data showed that both PBMC and kidney epithelial cells constitutively expressed *IL10RB*, upregulated expression of *IFNLR1* in response to Poly(I:C), and did not express *IFNL3* or *IFNL4* even after being stimulated. Four, three-year-old wethers were used for the P4 clearance study. The wethers received two doses of 600 mg (6.6-7.8 mg/kg) long-acting P4 one week apart. Blood samples were collected every other day for measurement of P4. Progesterone levels were quite variable, while some wethers maintained high P4 levels, in other animals P4 levels dropped relatively quickly.

**Dissecting the ovarian somatic cell influence on health**

Ashlee N. Buist, Tracy L. Habermehl, Kaden B. Underwood, Kate C. Parkinson, Jeffrey B. Mason

*Department of Animal, Dairy, and Veterinary Sciences, School of Veterinary Medicine, Utah State University, Logan, Utah.*

Menopause is a natural process occurring in every adult woman's life, typically around age 51. During this time the menstrual cycle ceases, ovarian follicles are exhausted, and numerous disease symptoms increase, such as osteoporosis, cognitive decline, muscle atrophy, and among others. Previous studies in primitive species show that germ cell-depleted ovaries increase longevity, which suggests the ovarian somatic components are directly involved in extending lifespan. This was first documented in *C. elegans*, a species of nematode. Populations of *C. elegans* with germ cell-depleted ovaries lived 60% longer, when compared to no intervention. Similar results were seen in a study of *D. melanogaster* where the lifespan increased by 50% in germ cell-depleted female flies. Our lab demonstrates that when young mice ovaries are transplanted into old mice, both health and lifespan increase. This was refined by depleting the germ cells in the ovaries prior to transplantation, which again demonstrated an increase in health and a larger increase in lifespan when compared to whole ovary transplants. Going forward ovaries from young C57BL/6 mice will be collected and the somatic cells isolated. Cells tagged with antibody markers will undergo flowcytometry to separate the cells into three populations: epithelial, mesenchymal, and stem cell. Each population will be transplanted separately into groups of ovariectomized 18-month-old mice. The mice will be allowed to age then a series of health assays will be performed testing physical attributes such as grip strength, neurological status, lifespan, etc. This study hopes to compare different somatic cell interactions in the ovary that ultimately link to the increases seen in health and lifespan.

**Impact of increasing dietary cottonseed on rumen fermentation, nutrient digestibility, and microbial community composition in continuous culture fermenters**

Camila Castro, Naghme Bagheri, Fernanda Batistel

Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT

In this study we determined the impact of increasing dietary cottonseed (**CS**) on rumen fermentation, nutrient digestibility, and microbial community composition. The study was conducted as a replicated 4×4 Latin square. Treatments were a control diet (50:50 orchardgrass hay:concentrate) without CS, and the control diet plus 5, 10, or 15% of CS. Prokaryotic community was determined by sequencing of the 16S rRNA gene. Protozoa were counted. Data were analyzed using a mixed model including the fixed effect of treatment and the random effects of period and fermenter. Linear, quadratic and cubic contrasts were tested. No treatment effect was observed for NDF and starch digestibility or acetate concentration ($P \geq 0.23$). Butyrate and ammoniacal N concentrations increased (Quadratic, $P \leq 0.05$), while propionate concentration tended to decrease (Quadratic, $P = 0.08$) as CS was added. Increasing CS decreased protozoa count and relative abundance of archaea (Linear, $P = 0.01$). Analysis of the phyla abundance showed that 6 were affected by treatments, most abundant ones being *Firmicutes* and *Bacteriodetes*. *Firmicutes* abundance increased whereas *Bacteriodetes* decreased with the addition of CS (Linear, $P \leq 0.05$). *Lachnospiraceae* and *Prevotellaceae* were the most abundant bacteria families and no treatment effect was observed for *Lachnospiraceae*, while *Prevotellaceae* tended to decrease as CS was added (Linear, $P = 0.07$). The two most abundant genera across samples were *Prevotella* and *Pseudobutyribrio.* Addition of CS decreased the abundance of both (Linear, $P \leq 0.05$). Preliminary results indicate that increasing levels of CS up to 15% DM diet do not negatively impact fiber or starch digestion. Although, inclusion of CS affected the microbial community composition.

# Relative toxicity of two dehydropyrrolizidine alkaloids and two dehydropyrrolizidine N-oxides

Michael J. Clayton[1,2], Bryan L Stegelmeier[2], Arnaud J Van Wettere[1]

[1]Utah State University, College of Agriculture and Applied Sciences, Department of Animal, Dairy and Veterinary Sciences, Logan, Utah
[2]United States Department of Agriculture, Agricultural Research Service, Poisonous Plant Research Laboratory, Logan, Utah

Introduction: Dehydropyrrolizidine alkaloids (DHPAs) are plant derived toxins estimated to be present in 3% of all flowering plants. Over 600 individual DHPAs have been discovered. DHPAs can be found in the chemical forms of free bases, or dehydropyrrolizidine alkaloid N-oxides (PANOs). DHPAs have a common base structure, and variable side chains. PANO's have been characterized as being much less toxic than their corresponding DHPAs based on previous studies.
Materials and methods: Riddelliine, riddelliine N-oxide, senecionine N-oxide and heliotrine were dosed by oral gavage to mice. There were three mice in each dosing group, six different doses and six control animals for each compound. Mice were dosed for ten days, then euthanized on day eleven. Serum was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Freeze dried liver was analyzed for the presence of tissue bound pyrroles by HPLC-MSMS, and complete necropsy was performed.
Results: ALT, AST, ALP and liver pyrrole concentration increased with dose for riddelliine, riddelliine N-oxide, and senecionine N-oxide. Mice that were dosed with heliotrine had very little increase in hepatic enzymes or pyrrole concentrations. Hepatocellular necrosis was observed in mice dosed with riddelliine, riddelliine N-oxide, and senecionine N-oxide. Mice dosed with heliotrine had no lesions.
Discussion: Based on serum biochemistry values, hepatic pyrrole concentrations, and histopathology, riddelliine, riddelliine N-oxide and senecionine N-oxide appear be similarly toxic. Heliotrine appears to have little to no toxic potential based on the same parameters. These results will be used to select suitable doses for carcinogenicity studies.

# Educating 4-H students about the important zoonotic diseases in the animals that they are working with

Elaine Dawson[1], Irene Hepler[1], Jessie Hadfield[1,3], Kerry Rood[1,2]

[1]Department of Animal, Dairy, and Veterinary Sciences at Utah State University, [2]School of Veterinary Medicine at Utah State University, [3]4H and Youth Extension program at Utah State University

Many high school students join extracurricular 4-H programs to learn and work with animals but are not aware of the zoonotic diseases that can be transmitted to them while in close contact with these animals. Infectious disease control and prevention relies on understanding the different factors that determine transmission. This project aims to teach students ages 13 to 18 about common zoonotic diseases in Utah, the different disease-causing pathogens that cause them, and how they are transmitted. By understanding the risks associated with working with animals and knowing the simple yet effective safety precautions they should take, this project can help keep these students and their families safer from zoonotic diseases. This information is presented in a series of PowerPoint modules so that it is easily accessible to all students. The first module is an overview of the most common disease-causing agents, and the different routes of transmission, as well as general safety precautions that should be followed to reduce the chances of transmission and disease occurrence. The first module is required to be taken by all 4-H students in Utah. The following modules are separated by the species of animal that the students may be working with, horses, cattle, sheep and goats, pigs, and companion animals. Each of these modules explains the specific zoonotic diseases associated with these species and goes into greater detail about transmission, and safety precautions. These modules are not required but the students are recommended to take the ones that are most applicable to the animals they are working with. By separating the information into different modules, -comprehension should be higher, and less likely to overwhelm the students.

# Detection of atmospheric oxidized mercury at a mountain top site in Colorado, U.S.A: Understanding origins and oxidation mechanisms

Tyler Elgiar[1], Seth Lyman[1], Liji David[1], Lynne Gratz[2], Zoe Zwecker[2], Anna Gannet Hallar[3], Noah Hirshorn[3], Rainer Volkamer[4], Henning Finkenzeller[4], Christopher Lee[4]

[1]Utah State University, Bingham Research Center
[2]Colorado College
[3]University of Utah
[4]University of Colorado Boulder

Mercury is an environmental toxin that is emitted into the atmosphere via natural and anthropogenic sources, as either elemental or oxidized mercury. Once in the atmosphere, mercury readily undergoes oxidation and reduction, and is eventually deposited into ecosystems around the world via wet and dry deposition. This can have detrimental effects on wildlife and human health. Uncertainty surrounds the mechanisms by which mercury is oxidized in the atmosphere, and it has been thought that reactive bromine may be the globally-dominant mercury oxidant. It has been shown that reactive bromine dominates in costal and marine air masses, however, its role in the continental free troposphere and planetary boundary layer remains unclear. There may in fact be multiple different oxidation pathways that occur in different regions of the atmosphere at different times. Previous measurements of oxidized atmospheric mercury have been made with systems that utilize a KCl coated denuder. These measurements have been proven to be biased low due to conversion of oxidized mercury into elemental mercury on the denuder. New systems are needed to measure oxidized mercury with a greater degree of certainty, and to be able to understand the mechanisms by which mercury is oxidized in the atmosphere. We have developed a cation-exchange membrane-based dual-channel oxidized mercury measurement system that avoids the bias created by the KCl coated denuder. We have deployed our system at Storm Peak Laboratory in Colorado, U.S.A. (a mountain top site where high levels of oxidized mercury have been previously observed in the free troposphere) in a multi-year study. The dual channel system is deployed alongside a suite of chemical and meteorological instrumentation, including a MAX-DOAS instrument for detecting halogens and other reactive gasses. This study will elucidate the origins of oxidized mercury at Storm Peak Laboratory and the oxidation pathways that lead to its formation in the continental atmosphere.

# The Influence of Dairy Breed and Forage Type on Organic Dairy Heifer Performance

Sawyer Fonnesbeck[1], Blair Waldron[2], Kara Thornton-Kurth[1], Michael Greenland[3] Rusty Stott[1], Alexis Sweat[1], Earl Creech[3], Kerry Rood[1], Allen Young[1], S. Clay Isom[1]

[1]Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT
[2] Forage and Range Research Laboratory, USDA-ARS, Logan, UT
[3]Department of Plant, Soils, and Climate, Utah State University, Logan, UT

Many breeds of dairy cattle exist, but questions remain about their performance within exclusively forage-based systems in the United States. This paucity of data is especially apparent in relation to heifer development, which is the second largest expense on most dairy farms. We evaluated the performance of pre-pubertal heifers from four different genetic backgrounds ("breeds") within a rotational-grazing system in the Intermountain West. For each of two 105-day grazing seasons, 24 dairy heifers from each of four different breeds [Holstein (HO), Jersey (JE), Holstein/Jersey crossbred (HJ), and Viking Red/Holstein/Montbeliarde crossbred (PRO)] were randomly assigned to one of two pasture treatments that consisted of either grass only (MONO) or grass interseeded with the legume birdsfoot trefoil (BFT; MIX). Main effects of treatment and breed (and potential interactions) on average daily gain (ADG), body condition score (BCS), change in percent mature body weight (CPMBW), and blood urea nitrogen (BUN) levels were evaluated. There was a significant effect of pasture treatment on ADG, with MIX heifers averaging 0.48 kg of gain/day over the full grazing period, whereas MONO heifers averaged 0.28 kg/day ($P<0.0001$). Change in BCS was significantly affected by treatment ($P=0.0012$) and breed ($P<0.0001$). Significant effects of treatment ($P<0.0001$) and breed ($P=0.0005$) on CPMBW were also found. Overall, we found that Jersey heifers were able to gain a higher percentage of their MBW and lose less BCS while on pasture than other breeds in this study. We also found that grazing BFT can have significant positive effects on ADG, BCS, and CPMBW. Further research is needed to more thoroughly evaluate the influence of heifer genetics (breed) on heifer development in pasture settings.

# NAD Requirements for DNA repair during spermatogenesis in mice

Micah Forbush, Miles Wandersee, Renata Hoskova, [A]lexie Zwerding, Audrey Lidgard, Ralph Meyer, and Mirella Meyer-Ficca

Department of Animal, Dairy and Veterinary Science

As humans age, their fertility decreases. This phenomenon has been widely studied in females, but the potential link between increased age and decreased fertility in males has not been as well established. NAD (Nicotinamide Adenine Dinucleotide) is an essential cofactor in most biochemical reactions, including DNA repair. During the aging process, NAD availability declines. Lower NAD levels are expected to diminish the body's ability to repair DNA breaks, and potentially contribute to lower germ cell quality in aged males.

Controlled transient DNA strand breaks are required for normal spermatogenesis, and adequate NAD levels are critical in repairing those breaks. We expect NAD decline to lead to a decreased ability to repair DNA in germ cells, which could contribute to an age-associated decrease in fertility. To test this hypothesis, we quantified DNA strand breaks using comet assays on sperm from chronologically young mice with varying NAD levels due to different amounts of dietary niacin. The comet assay is an electrophoresis method to quantify DNA strand breaks on a single cell level.

An unexpected hurdle has presented itself during early analysis in the fact that niacin-rich control diets induce significant obesity in the control animals, leading to inconclusive preliminary DNA damage results. Increasing scientific evidence indicates that obesity in itself causes poor sperm integrity, which limits our ability to compare niacin-deficient sperm to healthy control sperm. To avoid unhealthy control animals, we are pursuing several alternative strategies: We will use healthy, normal-weight control animals kept on standard rodent diets, and we are currently evaluating alternative defined control diets to identify an optimal diet that does not induce obesity.

**Bone changes in osteoarthritic sheep treated with gene therapy via TIMP-3**

Ethan S. Gilliam, Crystal Collier, Jeffery B. Mason

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Current treatments of osteoarthritis (OA) include arthroscopy, pain medications, and physical therapy. None of these are curative and only prolong the disease and subsequently allow the associated pain to return. The current study included fifteen ewes surgically manipulated to induce OA. Treatment groups included seven ewes that underwent ovariectomy, and eight ewes that underwent a crucial cranial ligament discectomy. Three ewes from each group received the gene therapy injection in the left stifle joint via an rAAV-*TIMP-3* viral vector. *TIMP-3* was selected for its binding affinity in the ECM and has also been shown to decrease the breakdown of aggrecan (a proteoglycan of cartilage) and inhibit TNF-α activation. After treatment, ewes were routinely exercised on a treadmill at an oblique angle to hasten the onset of OA. Stifle radiographs were collected at three time-points. Femoral and tibial width was increased at the end of the study in controls, but not in TIMP-3-treated sheep. Digital radiographs are effective in assessing initial subjective clinical signs of OA, but inferior to quantitative micro-CT (computed tomography) analysis. Sheep stifles were taken to The University of Utah, to assess bone changes with a micro-CT scanner. We hypothesize that we will evaluate and confirm the findings of the previous radiographs and quantitatively confirm the gene therapy treatment efficacy by evaluating parameters such as Bone Mineral Density (BMD), Trabecular Spacing (TbSp) and Bone Volume as a fraction of Total Volume (BV/TV). Based on our initial analysis, *TIMP-3* gene therapy did slow the progression of OA. Upcoming work will include completion of the micro-CT scan analysis and potential clinical trials to further confirm our findings.

# The role of cholesteryl ester transfer protein in endotoxemia pathogenesis

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Sepsis is serious inflammatory condition that leads to 11 million deaths annually. Endotoxemia is a common experimental model of sepsis and is defined as the presence of lipopolysaccharide (LPS) in systemic circulation. Despite decades of research, sepsis pathogenesis is still being unraveled. Human epidemiological studies have revealed that certain gain-of-function variants of cholesteryl ester transfer protein (CETP) are correlated with decreased sepsis survival. Unfortunately, there have been contradictory results using transgenic mice, which naturally lack the *CETP* gene, to explain the relationship between CETP and sepsis. We believe that by using golden Syrian hamsters, which do have natural CETP activity, we will obtain a clearer picture on the immunological role of CETP during this life-threatening condition. Our hypothesis is that the absence of CETP activity will lead to increased endotoxemia survival, reduced LPS-associated histopathological changes, increased HDL values, and a decreased pro-inflammatory cytokine profile. To test this hypothesis, we will use our genetically engineered golden Syrian hamster *CETP* $^{(-\backslash -)}$ model and wild-type animals to experimentally induce endotoxemia through intraperitoneal (i.p.) injection of LPS. Histopathological changes in the liver, spleen, kidney, gastrointestinal, respiratory, and cardiovascular system will be recorded along with alterations in the lipid profile and D-dimer levels in the blood. Utilizing real-time PCR, we will analyze tissue-specific cytokine expression while the impact of CETP on endotoxemia survival will be assessed through the development of a Kaplan-Meier survival curve. Data from this project will be used to continue the investigation into the role of CETP in sepsis pathogenesis via other sepsis induction methods and to explore the protein's influence in other conditions such as inflammatory bowel disease (IBD).

# Measures of rumen health and function in organic pasture-raised dairy heifers

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Previous work by our research team has demonstrated that forage quality accounts for 50% of the variation of the differences in grazing heifer performance. We hypothesize that the rumen microbiome is part of the unexplained variation in the performance of grazing heifers. To test this hypothesis, we have collected rumen fluid samples from 240 dairy heifers from four distinct genetic backgrounds ("breeds"; Holstein, Jersey, Holstein/Jersey crossbred, and ProCROSS) over the course of two 105-day grazing seasons. Heifers were fed a total mixed ration (TMR; control), or were grazed on one of two treatment types: monoculture grass (MONO), or grass interseeded with the legume birdsfoot trefoil (MIX). Rumen fluid was collected via esophageal tubing on the first day of the study and at the end of each of three 35-day rotations thereafter. Rumen microbes will be harvested from rumen fluid by centrifugation. Microbiome profiling by ultra-deep 16S rRNA sequencing and bioinformatic analysis of sequencing data will be accomplished as described elsewhere (see Wallace, et al *Sci Adv* 2019, e.g.). The primary endpoints will be family- and species-level population lists with relative abundances, as well as alpha and beta diversity scores, which have been tightly associated with feed efficiency in several recent reports. By characterizing the microbiome from each of the samples collected we will be able to describe the main effects of breed and diet and time on microbiome composition. We anticipate that this research will provide clarification of the complementary relationships between animal genetic background and rumen health and function, and their effect on livestock intake and performance.

**Effects of trenbolone acetate, estradiol, polyamines, and polyamine precursors on protein synthesis rates of murine myoblasts**

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Anabolic hormones, such as testosterone and estrogen, increase growth of skeletal muscle. However, the mechanisms through which these hormones increase skeletal muscle growth remains unknown. Previous research has shown that anabolic hormones may interact with the polyamine biosynthetic pathway. Polyamines are naturally occurring amino acid derivatives found in a variety of different whole foods that are potent stimulators of cell proliferation. However, the exact effect of polyamines on skeletal muscle growth has not been well characterized. As such, the objective of this study was to investigate the effect that trenbolone acetate (a testosterone analog, TBA), estradiol (E2), polyamines (putrescine, spermine, and spermidine), and polyamine precursors (methionine and ornithine) have on protein synthesis of Sol8 and C2C12 murine myoblasts. Cultures were treated with serum-free media and 10 nM TBA, 10 nM E2, 10 nM TBA and 10 nM E2, 10 mM methionine, 8 mM ornithine, 3 mM putrescine, 1.5 mM spermidine, or 0.1 mM spermine and protein synthesis rates were compared to the control (serum-free media). A series of contrasts were constructed to determine whether protein synthesis of murine myoblast cultures was different between the treatment and control cultures. Treatment of Sol8 and C2C12 cells with TBA, E2, TBA and E2, methionine, ornithine, putrescine, spermidine, or spermine did not ($P > 0.05$) result in increased protein synthesis compared to control cultures. These results indicate that additional research is needed to understand the role that anabolic hormones and polyamines have on protein synthesis of murine myoblasts.

# The development and implementation of an agritourism education module

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After an isolated outbreak of Shiga-Toxin producing *Escherichia coli* (STEC) in Utah, state-wide agritourism venues were surveyed about their disease prevention practices and what type of materials they wished they had more of. This feedback, which primary consisted of requests for an online education platform, is what prompted the creation of our module. The module consists of a pre and post module quiz in order to measure how much was learned over the course of the module. This module was distributed to previous survey participants who wished to be contacted, along with distribution to agritourism venues who had information posted on either Utah's Own agritourism page or Agritourism World's Utah page. Results of the pre and post quizzes will be compared to see how much knowledge the participant gained from module.

**Impact of NAD+ deficiency on the histone-to-protamine transition in a niacin-dependent mouse model (ANDY)**

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Advanced paternal age is positively associated with poor sperm chromatin quality and lower rates of successful and healthy development of progeny. While underlying causes are not well understood, a phenomenon observed during aging is a depletion of body-wide $NAD^+$ levels. To test the hypothesis that low $NAD^+$ levels in aging males are causally linked to defective sperm chromatin development, biologically young, but $NAD^+$-deficient, transgenic mice with acquired niacin-dependency (ANDY) were generated. In this mouse model, niacin (a vitamin B3 compound) is the main source for $NAD^+$ synthesis, like it is in humans. By feeding male ANDY mice niacin-deficient (ND1, ND2), or niacin-complete (CD1, CD2) defined diets, various blood $NAD^+$ levels were created. Sperm chromatin quality was then analyzed using immunoblotting of testis-protein extracts and quantitative chromomycin A3 (CMA3) staining. Immunoblotting results indicate that hyperacetylation of histone H4 at residues K5, K8, and K12, which is essential during spermiogenesis for proper histone-to-protamine exchange, was incomplete in $NAD^+$-deficient mice on ND1 diet. CMA3 assay results were inconclusive due to obesity in the control mice. Currently, ANDY males are on new control diets we developed to limit obesity in future investigations. Future steps include immunoblot analyses of sperm for identification of retained histone types and their modifications and investigation of potential mechanisms of insufficient histone acetylation.

# Effect of cytokine supplemented maturation medium on bovine cloned embryo development

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*In vitro* maturation (IVM) is an important process in the *in vitro* production of embryos. It has been recently shown that 3 cytokines: fibroblast growth factor 2 (FGF2), leukemia inhibitory factor (LIF), and insulin-like growth factor 1 (IGF1) have increased the efficiency of IVM, blastocyst production, and *in vivo* development following somatic cell nuclear transfer (SCNT) in pigs (Yuan *et al.* 2017 PNAS **114**, E5796-E5804). This study was designed to assess the effect of these cytokines on IVM in bovine oocytes, their development to blastocyst and pregnancy rate when used in SCNT. Cleavage and blastocyst rates were assessed at Day 2 and Day 7, respectively. Blastocysts were transferred to estrus synchronized recipients. Pregnancy rates assessed at day 40, 90 and 180 after embryo transfer. Statistical analysis was performed using one-way ANOVA or chi-square test. Data are presented as mean ± SEM. The MII rate was significantly higher in FLI medium compared with control medium (709/885; 80.2 ± 2.33% *v.* 549/822; 66.8 ± 1.82%; $P < 0.05$,). A significant increase in blastocyst rate was observed in the treatment group compared with the control group (181/446; 40.6 ± 5.1% *v.* 156/606; 25.7 ± 2.0%; $P < 0.05$). Embryos produced from FLI treated oocytes resulted in a significant increase in initial pregnancy rates (50.3±20.9% vs. 29.0±20.6%, P<0.05), 90-day pregnancy rates (43.1±7.12% vs. 15.4±6.30%, P<0.05), and 180-day pregnancy rates (23.0±6.75% vs. 7.58±2.93%, P<0.05), but full-term rates of the FLI group was slightly higher than control, but not significant (14.6±6.7% vs. 5.4±3.1%, P=0.063). In conclusion, FLI maturation medium improves overall SCNT efficiency by increasing IVM and SCNT blastocyst rates while having no negative impacts on full-term pregnancy rates.

**Cloning of porcine alveolar macrophage-derived cell lines suitable for genetic screens for host factors involved in PRRSV infection**

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of PRRS, an economically devastating disease of swine that is characterized by reproductive failure in pregnant sows and respiratory problems and growth retardation in piglets. The goal of this study is to understand the initial process of PRRSV to gaining access to the interior of host cells. To accomplish this goal, we first cloned eight porcine alveolar macrophage-derived single-cell clones (PAM sc1 to sc8) that differ in susceptibility to PRRSV infection for our gain- and loss-of-function screens. We further engineered one of the single-cell clones (PAM sc4) displaying higher susceptibility to PRRSV infection, in order to stably express the Cas9 protein (PAM sc4/Cas9) for our loss-of-function screens. This work is an ongoing project involving the use of two complementary, technologically advanced genome-scale genetic screens for gain- and loss-of-function of PRRSV entry. For the gain-of-function screen, we are using a cyclical packaging rescue strategy with a retroviral cDNA library, derived from the PRRSV-susceptible porcine macrophage cell line PAM sc4, to identify one or more cellular genes that confer susceptibility to PRRSV infection on the PRRSV-nonsusceptible porcine kidney cell line PK-15. For the loss-of-function screen, we are using a multiplexed CRISPR screen strategy with a lentiviral porcine sgRNA library to identify cellular genes that play an important role in PRRSV entry into the PRRSV-susceptible porcine macrophage cell line PAM sc4/Cas9. The outcomes of this study will provide a framework for a complete understanding of how PRRSV-host cell interactions occur at the level of PRRSV entry and offer multiple new targets for the prevention of PRRSV infection.

**The role of ovarian FOXO signaling in oxidative stress and health**

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As an individual ages, the risk for developing diseases also increases for both males and females. However, in comparison to males, females are generally as healthy or healthier until they reach menopause which occurs on average between the ages of 42 and 51. If a female experiences ovarian failure, they are more likely to develop diseases earlier in their life, usually before they are 40 years old. If a female experiences ovarian failure their life span is also likely to be shortened. Studies previously conducted with mice have shown that once a mouse is ovariectomized, its life span decreases by about 35 days from the control mice. As an individual ages, there is an increase in oxidative stress and reactive oxygen species (ROS), which in turn activates FOXO transcription factors to defend against oxidative stress. However, if there is an excessive amount of oxidative stress or ROS, there is an increased risk of developing diseases, ovarian failure, and premature aging. The results of this study were conducted by looking at the proteins in young versus old livers from mice. Livers from young and old mice were collected and preserved by freezing and pulverizing the samples in liquid nitrogen. At a later date, samples were analyzed using Ripa buffer to determine the proteins in the sample. With over 200 proteins to look at, a preliminary analysis for SOD2 and GSTM1 was conducted. Based on the data for SOD2 and GSTM1, it shows that FOXO primarily regulates SODs, which occurs when antioxidant effects are at their highest. Continuation of this study would be conducted by looking at all other proteins in the liver samples.

# Optimal Concentration of Doxycycline to induce ACMSD Expression in ANDY Mice

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Niacin is a dietary precursor for nicotinamide adenine diculeotide (NAD), an essential cofactor in most metabolic processes. Mice that are niacin deficient, and thus NAD deficient, are useful to study the roles of NAD in aging and fertility. Since wild-type mice can produce NAD from dietary tryptophan more efficiently than humans, certain gene-technological methods are necessary to induce niacin deficiency in mice through dietary restriction. Our lab has developed a transgenic "acquired niacin dependency" (ANDY) mouse with inducible overexpression of Aminocarboxymuconate Semialdehyde Decarboxylase (ACMSD). ACMSD is an enzymatic gatekeeper that prevents the tryptophan intermediates from becoming NAD+, and thereby makes these mice effectively dependent on dietary niacin, like humans. In ANDY mice, ACMSD overexpression is induced by doxycycline (dox), an antibiotic, which acts as an activator to the Tetracycline (tet)-on system, then allowing the ACMSD transgene to be expressed. Dox is typically administered at a 2 mg/mL concentration in drinking water to induce ACMSD expression in the liver. The aim of this project was to determine if the concentration of dox could be lowered to minimize potential side effects of the antibiotic in our mice while maintaining suitable ACMSD expression. We placed 7 groups of mice each on varying dox concentrations ranging from 2 mg/mL down to 20 mic/mL. To determine the efficiency of those concentrations for transgene expression, we quantified ACMSD transgene expression in liver lysates of those mice using a Western blot. Our preliminary data suggest that lower dox concentrations are not sufficient to maintain suitable expression of ACMSD.

**Using cytoplasmic biopsies to determine bovine oocyte quality**

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Embryos resulting from assisted reproductive technologies develop with much lower efficiencies than embryos resulting from their in vivo counterparts. The reasons behind the developmental discrepancies remain largely unknown. Because the oocyte is the primary determinant of embryo developmental success, it is reasonable to consider inherent oocyte quality as a possible cause. The hypothesis for this project is that there are distinct mRNA transcript patterns that distinguish high- and low-quality oocytes developing within the same environment. In this study, a small cytoplasmic biopsy was removed from 40 Metaphase II bovine oocytes via micromanipulation and stored for later use. The oocytes were parthenogenetically activated and cultured in vitro. Following an eight-day development period, embryos that reached the blastocyst stage and embryos that failed to develop were identified. This was repeated five times, for a total of six experimental replicates. Next, the relative transcript levels of 48 gene will be evaluated in biopsies from successful versus failed oocytes using the BioMark dPCR system from Fluidigm. The functional categories of the 48 genes include apoptosis, oocyte-specific, epigenetic, metabolism, housekeeping, redox, and RNA processing. We expect to see transcript level patterns appear within these categories that correlate with either successful or failed blastocyst development. If distinct transcript patterns can be differentiated between high- and low- quality oocytes, modifications in culture medium composition or other, more invasive, measures of altering gene expression may allow for optimization of these transcript levels, leading to increased quality of oocytes and, ideally, increased developmental success in assisted reproductive technologies.

# Possible mechanisms behind impaired glucose metabolism in niacin-deficient mice

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Niacin, a component of vitamin B3, is necessary for the synthesis of nicotinamide adenine dinucleotide (NAD). NAD is an essential coenzyme in many biological functions. Humans obtain dietary niacin from meats, nuts, and certain vegetables. Severe niacin deficiency has become uncommon in the United States, but milder forms of niacin deficiency resulting in low NAD levels have been correlated with body-wide decline, particularly in the elderly. In this study, we are investigating possible mechanisms of NAD-dependent dysregulation of energy metabolism in transgenic niacin-deficient mice with Acquired Niacin-Dependency (ANDY). Mice were either fed control diets with adequate dietary niacin or diets that lack dietary niacin (ND1 or ND2) and cause NAD deficiency. Compared to mice with adequate niacin intake, niacin-deficient mice experienced a significant loss of total body weight and body fat, lower energy levels, and impaired blood glucose regulation. In glucose and pyruvate challenge tests, ND1- and ND2-fed mice had significantly lower blood glucose and higher lactate levels than controls. We hypothesize that inadequate levels of NAD prevent the efficient conversion of pyruvate to glucose during gluconeogenesis and cause a buildup of lactate. In addition, we quantified lactate dehydrogenase enzyme using immunoblotting analyses and found its expression to be significantly higher in ND1-fed mice compared to control-fed mice. Future experiments will further investigate the mechanisms that cause the metabolic dysregulation in niacin-deficient mice.

# Cache Valley $PM_{2.5}$ Activates Akt, Inflammatory Pathways, and Induces Genetic Damage in Human Lung Cells

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Northern Utah's Cache Valley frequently has some of the nation's highest concentrations of $PM_{2.5}$ particulate pollution. Exposure to $PM_{2.5}$ is associated with increased all-cause mortality, cardiovascular and cardiopulmonary diseases, heart attack, stroke, COPD, Alzheimer's disease, and lung cancer. The purpose of this study is to determine the cellular responses of human lung cells (BEAS-2B) exposed to $PM_{2.5}$ particulate pollution collected in Cache Valley (CVPM; (1 and 12 µg/ml; 24 hr). Parallel experiments were conducted using diesel exhaust particles (DEP) as a positive control. Exposure to CVPM resulted in genetic damage as assessed by the Comet Assay and a significant increase in the number of actively-dividing cells compared to control cells by flow cytometry ($p < 0.05$), with similar potency to that of DEP exposed cells. Whole-genome microarray (Affymetrix Human 2.0) identified affected genes principally related to the inflammatory and immune pathways, as well as activated serine/threonine Akt (*aka* protein kinase B or PKB)-dependent pathways. RNA sequencing with gene set enrichment pathway and clustering analysis confirmed that differentially expressed genes involved the immune response, Akt activation pathways, and MAPK activation. Treatment-related changes in expression of most genes were similar between particle types. Immunoblotting confirmed activation of Akt by phosphorylation of Thr308 in both CVPM and DEP exposed cells. Given the oncogenic nature and centrality of Akt and related pathways in cell division and proliferation, our data is consistent with the hypothesis that CVPM induces carcinogenesis with potency similar to that of DEP. This research is supported by the Marriner S. Eccles Charitable Foundation and by Utah State University.

# The effects of omega-3 supplementation on markers of inflammation and myogenesis in pig skeletal muscle

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Inflammation, caused by clinical and subclinical infection, can impact livestock production, including pigs. Skeletal muscle, the tissue responsible for producing meat, is susceptible to decreased growth during inflammation. Given the global push to decrease antibiotic use, it is important that other avenues are found to combat inflammation. Supplementation of omega-3 via fish oil is one option. The objective of this study was to determine the effects of fish oil supplementation on markers of inflammation and myogenesis in pig skeletal muscle. Forty weaned piglets (8.21 +/- 0.83 kg) were utilized in a randomized complete block design with two treatments: a basal diet or a basal diet with 3% fish oil. Treatments were delivered for 35 consecutive days and on day 34, an LPS challenge was administered to 14 piglets. On day 35, all piglets were euthanized and skeletal muscle samples were taken from the longissimus lumborum (LL) and biceps femoris (BF). Total mRNA was isolated and abundance of myogenic regulatory factors, markers of inflammation, and markers of adipose growth were analyzed. Fish oil supplementation increased mRNA abundance of cyclophilin A ($p=0.03$), a marker of inflammation, and decreased abundance of myogenic factor 5 ($p=0.10$), a myogenic regulatory factor, in skeletal muscle of fish oil supplemented piglets. Muscle location influenced mRNA abundance such that there was increased leptin ($p=0.006$) and adiponectin ($p=0.01$) in the BF when compared to the LL. At the time point investigated, results suggest a potential for decreased growth and increased skeletal muscle inflammation when piglets are supplemented with fish oil. Further research is warranted to better examine the relationship between inflammation, skeletal muscle growth, and fish oil.

**Impact of pH and palmitic acid on ruminal fermentation and microbial community composition**

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Supplemented fats are often used to increase energy density of a feed for increased efficiency with unknown effects to the microbiome. The objective of this study was to evaluate the effects of dietary palmitic acid and pH on rumen fermentation and bacteria community composition (BCC). Eight continuous culture fermenters were used in a 2×2 factorial design. The two factors in the experiment are palmitic acid treatment and pH treatment. Palmitic acid treatment (PAT) contained 1.5% palmitic acid compared to a control diet (CON). pH level treatments compared normal pH (6.6-7.0) to low pH (6.0-6.4). Digestibility, ammoniacal nitrogen ($NH_3$-N), VFA concentration, and BCC were measured to assess rumen fermentation. Data were analyzed using a mixed model that included the fixed effects of palmitic acid treatment, pH and their interaction. No interaction between PAT and pH were observed for measures of rumen fermentation ($P>0.05$) including fiber digestion, total VFAs, $NH_3$-N or individual VFAs: acetate and propionate. Butyrate showed an interaction effect ($P=0.02$). PAT increased NDF digestibility ($P=0.04$) but did not affect $NH_3$-N nor total VFAs ($P>0.05$). Low pH decreased NDF digestibility 8.2% ($P<0.01$). Low pH also decreased $NH_3$-N, total VFAs and individual VFAs: acetate and butyrate ($P\leq0.01$). No interaction effect was observed for BCC at kingdom level ($P>0.05$). Few interactions were observed in the family and phylum level. PAT did not affect alpha diversity or phylum abundance. PAT had variable effects to few families. Low pH decreased archaea and increased bacterial abundance. Low pH had variable effects to families and phyla. In conclusion, palmitic acid and pH independently affected rumen fermentation and prokaryotic community composition.

# Examining the relationship between breed type and anabolic implant protocols in performance in the feedlot, health, and temperament of beef steers

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The purpose of this research was to compare anabolic implant protocols in feedlot steers of two different breed types. Sixty steers were stratified by weight and breed examining two different breeds: Angus (AN; n=38) or Santa Gertrudis influenced (SG; n=22), and three implant strategies: no implant (CON; n=20), a moderate-intensity implant protocol (d0 implant: Revalor-G, d56 implant: Revalor-IS, d112 implant: Revalor-S; MI; n=20), or a high-intensity implant protocol (d0 implant: Revalor-IS, d56 implant: Revalor-S, d112 implant: Revalor-200; HI; n=20). Steers were randomly placed into pens equipped with GrowSafe® bunks and fed the same ration. Weight, chute score (CS), exit velocity, blood, temperature, hip height and 12$^{th}$ rib fat thickness were collected approximately every 28d over a 112d period. Over the 112 d, SG steers tended (*P=0.10*) to gain more hip height than AN steers. Anabolic implant protocol influenced total gain with both HI and MI steers gaining more (*P<0.01*) than CON. On d 0, SG steers had a higher (*P<0.01*) CS compared to AN steers, with this being maintained through the course of the trial. There was also as a tendency for there to be a breed*treatment effect (*P=0.06*) on d112, with SG-MI having a higher (*P=0.04*) CS than AN-HI, and a tendency (*P=0.08*) for SG-HI to have a higher CS than AN-HI. Moderate-intensity and HI implant protocols may be a useful tool to increase performance in feedlot steers. However, this research did find that SG influenced steers may have a more excitable temperament, but implant protocol did not influence (*P>0.05*) temperament.

# Black raspberry supplementation alters the gut microbiome and improves alpha diversity in a mouse model of inflammation-associated colorectal cancer

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Anti-inflammatory bioactives in black raspberries (BRB) have been shown to have protective effects on the colon epithelium and may influence gut microbiome. The goal of this study was to determine the effects of dietary intervention with BRB on the dynamic composition of the gut microbiome composition in mice. Using a 2x2 factorial design, C57BL/6J male mice were fed the standard AIN93G diet or the total Western diet (TWD) for 16 weeks with or without 10% (w/w) whole, freeze-dried BRB powder. The azoxymethane + dextran sodium sulfate model of inflammation-associated colorectal cancer was employed to assess the dynamic response of the gut microbiome to basal diet and BRB treatment prior to, during, and after active colitis and at the study end. Microbiome composition was determined using 16s rRNA sequencing followed by diversity analyses (alpha and beta) and identification of discriminating taxa by with linear discriminant analyses by effect size (lefse). Alpha diversity was markedly reduced during colitis for mice consuming either AIN93G or TWD, with some improvement noted by the recovery phase. Of note, consumption of BRB for two weeks significantly increased alpha diversity measures, and BRB improved alpha diversity in mice fed the AIN93G diet during colitis. Alternatively, BRB appeared less effective in mice fed TWD. Beta diversity was also significantly affected with notable clustering of microbiomes by BRB treatment during and after colitis. Consumption of BRB affected the relative abundance of several key taxa over the course of colitis and recovery from gut injury, including Erysipelotrichaceae, Bifidobacteriaceae, Streptococcaceae, Rikenellaceae, Ruminococcaceae and Akkermansiaceae, among others. Dietary supplementation with BRB shifted the composition of the gut microbiome during colitis and recovery from gut injury, though the effects were inconsistent with respect to the basal diet consumed.

# Development of a lethal hACE2-hamster model of COVID-19 for evaluation of experimental therapeutics

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Coronavirus disease of 2019 (COVID-19) caused by the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an international public health crisis. SARS-CoV-2 infection leads primarily to a respiratory disease, with a wide spectrum of severity ranging from asymptomatic infections to an acute respiratory distress syndrome and death. Viral entry into human cells is mediated by the ACE2 receptor. To establish a lethal infection model, we developed human ACE2 (hACE2) transgenic hamsters via a piggyBac-mediated transgenesis strategy in which the epithelial-specific expression of the hACE2 gene is achieved through the regulatory elements of the human cytokeratin 18 gene. When hamsters are inoculated with $10^{0.3}$ $CCID_{50}$ of SARS-CoV-2, clinical signs of infection are observed (hunched posture, lethargy, dyspnea, weight loss, and mortality). Histopathological examination of lung tissue revealed a bronchointerstitial pneumonia by day 5 post-infection. In addition, virus replication was detected in lungs, brain, heart, and kidney tissues. The highest titers ($10^6$ logs) were observed in lung tissue on day 4 post-infection. Oropharyngeal swabs collected daily for virus titrations demonstrated virus shedding from day 1-5 post-infection.
Poly (I:C), a TLR3 agonist was evaluated as a positive control. A protective effect was observed at low dose virus challenge when administered 24 hours pre-infection, but not post-infection. This severe SARS-CoV-2 infection model will be used to evaluate potential antiviral therapies and vaccines for COVID-19.
*[Supported by Contract HHSN272201700041I from the Respiratory Diseases Branch, DMID, NIAID, NIH]*

**Comparison of SARS-CoV-2 variants in a transgenic hACE2-mouse model**

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Variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may have increased transmissibility, disease severity, and/or be less susceptible to public health control measures such as vaccines, diagnostic tests, or therapeutics. The goal of this study is to evaluate five variants of SARS-CoV-2 against the original pandemic strain to identify their differences in a transgenic hACE2-mouse model. To evaluate the variants, we completed a challenge dose titration to determine the 90% lethal dose ($LD_{90}$), or in other words, the virus dilution that causes mortality in 90% of the animals for the original pandemic strain, Alpha, Gamma, and Epsilon variants. The $LD_{90}$ results were: $10^{3.6}$, $<10^{1.8}$, $10^{0.9}$, $10^{1.5}$ virus/dose, respectively. The $LD_{90}$ evaluation studies for the Beta and Delta variants are ongoing. Based on the $LD_{90}$ challenge dose results, future studies will involve a comparison of all virus variants at their optimum dose to evaluate differences in clinical presentation (*e.g.* weight loss, mortality, viral load, inflammatory cytokine responses, and histological changes).

# Effects of feeding a novel alfalfa leaf pellet product (ProLEAF MAX™) and alfalfa stems (ProFiber Plus™) on production of lactating dairy cows

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Alfalfa is a commonly grown forage in the Intermountain west and is often included in lactating dairy cow rations. This study investigated the effects of including novel alfalfa products: ProLEAF MAX™ (PLM), an alfalfa leaf pellet; and ProFiber Plus™ (PFP), alfalfa stems, in the diet of lactating dairy cows on dry matter intake, milk yield, and milk components. Holstein cows were housed in a freestall barn and milked in a free-flow automatic milking system. All cows were fed each treatment for 21 days, then switched to the next treatment utilizing a crossover experimental design. The five treatments were: control (CON, typical diet including alfalfa hay; n=65); low-quality alfalfa hay (LQ+PLM, a diet that replaced alfalfa hay with low-quality alfalfa hay and PLM; n=62); PLM+PFP (a diet that replaced alfalfa hay with PLM and PFP; n=65); PLM (a diet that replaced alfalfa hay with PLM; n=62); and PFP (a diet that replaced alfalfa hay with PFP; n=60). Cows were group fed a partial mixed ration balanced for 40.8 kg milk, 3.9% milk fat and 3.3% milk protein. Individual milk yield and milk components were recorded daily by the automatic milking system. Dry matter intake was also recorded daily. When fed the PFP diet, cows had decreased ($P < 0.01$) dry matter intake compared to the other diets. Milk yield was increased ($P < 0.01$) when cows received the PLM diet when compared to the other diets. When fed the PFP and PLM+PFP diets, milk fat was increased ($P < 0.01$) when compared to the other diets. Milk protein was decreased ($P < 0.01$) when cows were fed the PFP diet when compared to the other diets. These data indicate that inclusion of fractionated alfalfa products in the diet of lactating dairy cows has the potential to increase milk yield and milk components.

# Placental PD-L1 mRNA expression during bovine gestation

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Reproductive efficiency plays a significant role in the economic profitability of dairy cattle operations. Embryonic loss results in approximately 73% of failed pregnancies in dairy cattle. This embryonic loss often occurs during implantation due to abnormal communication between the embryo and the maternal endometrium. Modulation of T lymphocytes in the maternal endometrium is required for acceptance of the fetus. Programmed death ligand 1 (PD-L1) can regulate immune responses by preventing T cells from being activated. For this study, we hypothesize that PD-L1 mRNA is expressed in the bovine placenta and its abundance varies between gestational semesters in cattle. Placentas from first, second, and third trimesters were collected at a local abattoir. Fetal/embryonic age was estimated by crown-to-rump length. Cotyledonary tissue was collected for mRNA analysis from the conceptuses. The TRIzol Plus RNA Purification Kit (ThermoFisher Scientific) was used to extract RNA. Equal amounts of RNA (100ng) were used for real-time quantitative PCR (RT-qPCR) with the GoTaq 1-Step RT-qPCR System (Promega) to characterize PD-L1 mRNA. We observed that PD-L1 mRNA is expressed in all stages of gestation. By expressing PD-L1 the bovine placenta can evade the attack of maternal T cells.

**Vaccine production and the cross-antigenicity of five human coronaviruses**

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Much is unknown about the antigenic similarities of human coronaviruses. Antibodies to viruses that are antigenically similar have the potential to be cross-protective or cross-reactive. While cross-antigenicity alone does not elucidate the extent of cross protection or the type(s) of cross-reactivity, it does clarify how similar the immune responses created against these viruses are. The purpose of this research is to determine the cross-antigenicity of the following five human coronaviruses: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus 1, Middle East respiratory syndrome coronavirus, human coronavirus OC43 (HCoV-OC43), and human coronavirus 229E. One liter of each virus stock was grown and inactivated to produce vaccine. Mice were vaccinated, and sera collected before and after vaccination. Virus neutralization assays were completed using the collected sera to identify the cross-antigenicity between the viruses and the difference in immunogenicity over time. Our data suggest that HCoV-OC43 has no cross-antigenicity with the other four coronaviruses, as sera from mice vaccinated with heterologous vaccines had no neutralization against HCoV-OC43, while sera from mice vaccinated with the homologous vaccine did. Additionally, there was more than a two-fold increase in immunogenicity between the first and second vaccination of HCoV-OC43. The evaluation of the cross-antigenicity of the other four coronaviruses is ongoing. Future studies include evaluating the effects of time on immune responses, the cross-antigenicity of SARS-CoV-2 variants, and the evaluation of cross-reactivity through the identification of neutrophil activity and vaccine efficacy studies *in vivo*.

**Selective induction of apoptosis in senescent cells**

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Systemic senescent cell load increases with age and leads to increased rates of disease. This is particularly evident in post-menopausal/post-reproductive women. Senescent cells have ceased to divide and can produce detrimental levels of various cytokines. Senescent cells can be induced toward apoptosis using senolytic drugs such as Dasatinib and Quercetin (D+Q). Data from previous experiments demonstrated that young ovaries transplanted to post-reproductive mice increase health and lifespan. Therefore, we can conclude that the age of the ovaries affects and influences the all-around health of the mouse. Our preliminary data demonstrates that older ovaries have higher concentrations of senescent cells, compared with young ovaries. Based on these observations, we hypothesized that the senescent cell load, specifically in the ovary, is responsible for increased disease rates at the time of reproductive failure. To test this, we in vitro cultured MCF-7 breast cancer cells in control media until 75% confluence was achieved and (initially) treated culture with a 0.25 μM doxorubicin (doxo) for 24 hours after which doxo-media was replaced with control media for 2 weeks. We included variations to our concentrations/timing of doxorubicin treatments. We also included a senolytic treatment (ABT-263 @ 1 μM, targeted specifically to kill senescent cells while preserving control cells). Cells were collected and analyzed by flow cytometry to quantify the percent control, senescent and apoptotic cells in each culture/treatment**.** In conclusion, detection of apoptosis in the current cultures produced suboptimal results, while the response to doxo produced favorable and reliable cellular senescence. In future experiments we will use ovarian somatic tissue/cells as well as alternate senolytics and compare their apoptotic responses.

# The role of extracellular vesicles in bovine pregnancy immunomodulation

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During pregnancy there is a shift in the maternal immune system to allow for tolerance of the fetal allograft. Our previous studies show that there is a strong inflammatory response in the uterus of cows carrying somatic cell nuclear transfer (SCNT) pregnancies due to a dysregulation in the proteins expressed by trophoblast cells. Our long-term goal is to understand the immune tolerance mechanisms that take place to establish a successful pregnancy in cattle.
The aims of this study are to determine the role of trophoblast-derived extracellular vesicles (EVs) in healthy pregnancies established by artificial insemination (AI) and abortion-prone cattle pregnancies established by SCNT. We hypothesize that EVs from SCNT pregnancies will stimulate T-cells to express pro-inflammatory mediators and to proliferate when stimulated, while EVs from pregnancies produced by AI will induce T-cells to express a more anti-inflammatory phenotype. Pregnancies will be established by AI and SCNT (n = 6/group) and will be collected at 42+/-3 days. Placental tissue will be digested and cultured. Cell culture supernatant will be collected and EVs isolated by size exclusion chromatography. Peripheral blood mononuclear cells (PBMCs) will be collected from day 42+/-3 pregnant cows and isolated by density gradient centrifugation. PBMC populations will be sorted using flow cytometry, and reverse transcription quantitative polymerase chain reactions using primers for pro- and anti-inflammatory genes will be performed. It is expected that EVs from SCNT pregnancies will stimulate T-cells to express pro-inflammatory mediators, while EVs isolated from AI pregnancies will induce a more anti-inflammatory response.

Katherine Nicole Trepanier

Shiga toxin-producing *Escherichia coli* (STEC) is a variety of the ubiquitous gram-negative bacteria *E. coli* that infects the intestinal tract of humans and animals. Shiga toxin can cause fatal hemorrhagic colitis and hemolytic uremic syndrome in humans, with sporadic outbreaks occurring annually. In the United States, human infection with STEC results in a loss of $400 million annually in health care costs. STEC and other varieties of *E. coli* are a part of the normal flora of the cattle intestinal tract. Humans are infected with STEC through fecal contaminated meat products, milk, water, vegetables, or through direct contact with animal feces. Research in STEC reducing best management practices has focused on pre- (shedding) and post- (contamination) harvest processes. Plant tannins have been shown to reduce *E. coli* growth and shedding. Our research investigates STEC shedding of dairy heifers in a summer pasture development model that uses a high-tannin containing legume (Birdsfoot Trefoil, *Lotus corniculatus*) as a treatment.

# Genotyping *KCNQ1* knockout in Golden Syrian Hamsters

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Golden Syrian hamsters are great animal models when it comes to human research, due to the similarities in physiology, metabolism, and disease symptoms they exhibit. The goal of the project was to generate a *KCNQ1* knockout animal model using CRISPR/Cas9 to model human diseases caused by loss of function mutations in the *KCNQ1* gene. Hamsters with the *KCNQ1* gene knockout experience similar effects to those of humans with the inherited genetic mutation. The *KCNQ1* gene encodes for a potassium voltage-gated channel that is used during the action potential of the myocardium. When a mutation occurs, affected patients have increased chances of cardiac arrhythmia and cardiac arrest. This channel also plays a role in the inner ear, so affected individuals often have issues with balance which causes an abnormal gait. A F0 founder male, carrying three different INDELs (insertion and deletions) of the *KCNQ1* gene, a 68 bp deletion, a 9 bp deletion and, a 1 bp insertion, was generated by CRISPR/Cas9-mediated gene targeting experiments and was bred with wild type females. Through DNA extraction from tissues isolated from F1 spring, PCR-RFLP, TA cloning, and sequencing, four F1 offspring were determined to carry one of the INDELs: two males have the 68 bp deletion, one female has the 9 bp deletion and, one female has the 1 bp insertion. As a result, the two F1 males were selected to be bred due to the large deletion of the gene which results in premature stop codons in the *KCNQ1* gene and the ability to identify the genotype with PCR alone. In conclusion, the F1, heterozygous offspring were identified and will be bred until homozygous offspring are identified. The hamsters will then be used to further understand the *KCNQ1* gene and develop possible treatments.

# DNA Methylation and Age-related Disease

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Replacing senescent ovaries in mice with young ovaries can induce benefits such as increased lifespan and restoration of renal function. These treatments have proven beneficial, but the specific mechanisms of these changes remain unknown and may be found in DNA changes resulting from exposure to young ovarian tissue. DNA methylation, which is the addition of a methyl group to Cytosine, which may silence the gene, has been the subject of past aging studies. DNA methylation changes in mammals as they age. Manipulation of this process has been demonstrated in dietary restriction studies, which used DNA methylation to construct a 'clock' to determine the biological age of a mouse, based on hyper- and hypo-methylated regions of the DNA. Dietary restriction in female mice improves health and decreases age-related hyper- and hypo-methylation, compared with ad libitum-fed mice. We expect similar results in slowing age-related DNA methylation changes with the replacement of senescent ovaries with young ovarian tissue. Preliminary data demonstrating the health benefits of ovarian transplants, along with the current use of DNA methylation sequencing will pave the way for future results concerning ovarian transplant, which we hypothesize will show methylation patterns that are similar between transplanted aged mice and young control mice.

# The effects of propionate and butyrate on platelet aggregation measured through optical density.

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Preliminary research has shown that volatile fatty acids (VFAs) may have some anticoagulation properties. We hypothesized that VFAs propionate and butyrate may have similar inhibitory effects on platelet aggregation. Concentrated platelets were collected from Goat (*Caprus hircus*) blood through centrifugation and then resuspended into HEPES solution. To measure platelet aggregation, optical density was evaluated within individual 96 well plates over 30 minutes, interpreting a decrease in optical density as platelet aggregation. In assay development, we titrated arachidonic acid and platelet concentration throughout the plates to find the concentration that caused the greatest change in optical density. Following titration of these factors, the optimal experimental conditions were determined to be $3\times10^7$platelets/ml activated with arachidonic acid at a concentration of 15μM. For our test articles, we used propionate and butyrate at varying concentrations to determine if they inhibited platelet aggregation. Heparin was also evaluated as a putative anti-aggregation positive control agent. Results showed there was a statistically significant increase in platelet aggregation in a 2.5U/mL heparin solution as compared to normal activation of platelets by 15uM arachidonic acid (P=0.05). No other concentration of heparin or VFAs tested resulted in a statistically significant change when compared with the activated platelet control. Through these efforts, we have established a repeatable platelet aggregation multi-well assay. Unlike their observed effects on clot formation in plasma, VFAs did not decrease platelet aggregation. Future efforts will be required to determine a more appropriate positive control for inhibiting platelet aggregation.

**Comparing various breed effects on feed consumption, growth, health, and development of grazing dairy heifers on different pasture types.**

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Recent changes in the dairy industry, such as incorporation of new breeds and more dairies becoming organic, have necessitated the need for research to determine differences between dairy breeds. This research aimed to better understand how dairy heifers of different breeds perform on different organic pastures. This study included 96 heifers of four different breeds of dairy heifers: Holstein (H; n=30), Jersey (J; n=30), Holstein/Jersey (HJ; n=30), and Montbeliarde/Holstein/Swedish Red (PRO; n=30). Heifers were then placed into one of three dietary treatments: grazing grass monoculture pastures (n=48), grazing grass pastures interseeded with BFT (n=48), or fed a conventional total mixed ration in a confined setting (n=24) for 70 days. Serum and feces were collected after fasting on days 0, 35, and 70. Blood urea nitrogen (BUN) was analyzed in the serum and fecal egg counts to determine parasite load were assessed in feces. Heifers fed the feedlot diet tended (P = 0.08) to have increased BUN concentrations over time. However, breed did not affect (P = 0.12) BUN. Diet did not affect (P = 0.10) fecal egg count. However, FEC differed (P =0.02) between breeds at day 0 such that H heifers had increased (P = 0.02) FEC compared to HJ. These data suggest that the different heifer breeds analyzed in this study have similar BUN and parasite load when grazed on grass or grass + BFT pastures.

# Quantifying the effect of NAD deficiency on DNA damage in spermatozoa

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Like in women, the quality of male gametes (sperm) deteriorates with paternal age. While the causes remain unknown, aging is characterized by a decline of body-wide levels of nicotinamide adenine dinucleotide (NAD). Decreased levels of NAD may be linked to poor sperm quality and DNA integrity in men as they age. Low NAD levels may increase the amount of oxidative stress causing DNA strands breaks in the nuclei of sperm, because synthesis of the cellular antioxidant glutathione is dependent on the availability of NAD. Therefore, we hypothesized that lower levels of NAD cause increased DNA damage in the sperm of aging males. To test the hypothesis, a transgenic mouse model (ANDY mouse) was generated to create low levels of NAD, seen as men age, in chronologically young mice. TUNEL assays were performed to quantify DNA strand breaks in sperm from NAD-deficient mice. In the TUNEL assay, DNA double and single strand breaks are labeled with fluorescent dUTPs and elongated using the enzyme terminal deoxynucleotidyl transferase (TDT) at the 3’ end of the DNA cleavage site, allowing for the DNA damage to be quantified. Digital image analyses was used for fluorescent signal quantification of TUNEL-stained sperm. Due to a need for higher animal numbers, results remained inconclusive at this point. More data will be needed to determine whether sperm from NAD-deficient ANDY mice have elevated DNA strand break frequencies compared to age-matched control animals, which was a result of a previous preliminary study.