9th Annual ADVS STUDENT RESEARCH SYMPOSIUM
August 12, 2020 – Utah State University
8:30am – 12:15 pm

MEETING SCHEDULE

8:30 – 8:45 AM	Opening Remarks
8:45 – 10:00 AM	Session 1: Oral Presentations (5 presentations)
10:00 – 10:15 AM	Break
10:15 – 10:45 AM	Guest Speaker – "The Writing Process: Tips for Manuscript Writing" by Kendall Becker
10:45 – 11:00 AM	Break
11:00 – 12:15 PM	Session 2: Oral Presentations (5 presentations)

SESSION 1
Moderator: Kara Thornton-Kurth

Presentation #	Time	Student	Advisor	Degree
1	8:45 – 9:00	Caleb Reichardt	Thornton-Kurth	PhD
2	9:00 – 9:15	Elizabeth Park	Meyer-Ficca	DVM
3	9:15 – 9:30	Madi Lindsey	Isom	MS
4	9:30 – 9:45	Mike Clayton	Van Wettere	PhD
5	9:45 – 10:00	Morgan Eggleston	Coulombe	MS

Guest Speaker

10:15 – 10:45 "The Writing Process: Tips for Manuscript Writing" by Kendall Becker
Moderator: Heloisa Rutigliano

SESSION 2
Moderator: Fernanda Batistel

Presentation #	Time	Student	Advisor	Degree
6	11:00 – 11:15	Sawyer Fonnesbeck	Isom	MS
7	11:15 – 11:30	Scott Gibson	Hurst	MS
8	11:30 – 11:45	Reganne Briggs	Thornton-Kurth	MS
9	11:45 – 12:00	Heather Gaudette	Lee	BS
10	12:00 – 12:15	Jacob Keim	Polejaeva	PhD

RECORDED MINI-PRESENTATIONS

Presentation #	Student	Advisor	Degree
1	Andre Tu Nguyen	Rutigliano	BS
2	Kaden Bunch	Polejaeva	BS
3	Kaden Underwood	J Mason	BS
4	Lillian Okamoto	Thornton-Kurth	BS
5	Makenna Osborne	Hoopes	BS
6	Mason Miles	Lee	BS
7	Porter Green	Benninghoff	BS
8	Raquel Larsen	White	BS
9	Shailyn Parrish	J Mason	BS
10	Sierra Lopez	Thornton-Kurth	BS
11	Brittney Swapp	Meyer-Ficca	BS
12	Tanner Edgington	Davies	BS
13	Anthony Alberto	Batistel	MS
14	Braden Abercrombie	Ken White	MS
15	Camila Castro Veloz	Batistel	MS
16	Irene Knorr	Rood	MS
17	Lexie Padilla	Batistel	MS
18	Maren Haroldsen	Kelly	MS
19	Rebecca Echols	Kelly	MS
20	Sarah Andersen	H Mason, Pate, Smith	MS
21	Daphne Rodriguez	Benninghoff	PhD
22	Kira Morgado	Rutigliano	PhD
23	Laura Adams	Polejaeva	PhD
24	Laura Smith	Thornton-Kurth	PhD
25	Naghme Bagheri	Batistel	PhD
26	Zachary Hoopes	J Mason	PhD (withdrawn)
27	Beth Crandall	Thornton-Kurth	DVM
28	Joseph Goldhardt	Wang	DVM
29	McKenna Walters	J Mason	DVM
30	Victoria Whitworth	Kelly	MPH

ADVS SRS 2020

Abstracts

Comparing the effectiveness of anabolic implants in Santa Gertrudis sired steers versus Angus steers.

C.C. Reichhardt, T.J. Brady, R.K. Briggs, L.A. Smith, B.R. Bowman, M. Garcia, A.J. Thomas, K.J. Thornton

Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT 84322

Implants are commonly used in beef in the United States to improve production. One method to increase environmental sustainability of the beef industry even further is by increasing *Bos indicus* genetics. This research compared the use of implants in *Bos indicus* influenced animals versus *Bos taurus* in a feedlot setting. Twenty steers were blocked by weight and breed in a 2 x 2 factorial design examining two different breeds: angus (AN; n=10) or Santa Gertrudis influenced
(SGI; n=10), and two implant strategies: no implant (CON; n=10) or a combined implant (CI; n=10). Steers were randomly placed into pens equipped with GrowSafe® bunks, fed the same ration, and weighed and ultrasounded on days 0, 28, 56, 84, and 112. Blood was collected on days 0, 2, 10, 28, 56, 84, and 112. Backfat measurements (BF), weights, and blood urea nitrogen (BUN) were determined. When analyzed with repeated measures, the main effects of breed, implant, and breed*implant demonstrate that AN gained more ($P<0.05$) weight than SGI, CI gained more ($P<0.05$) weight than CON, and the AN that received a CI had increased weight gain when compared to the AN CON, SGI CON and SGI CI, with there being no statistical difference between breed, implant or implant*breed differences in weight at day 0 ($P>0.05$). There was no difference ($P<0.05$) in weight gain between the SGI that received a CI vs CON, nor was there a difference between the CON SGI and AN. There was a treatment*breed interaction on BF ($P < 0.05$), with AN tending to put on more BF than SGI CON ($P<0.08$). A breed*treatment interaction was also observed when analyzing BUN ($P<0.05$). This research provides preliminary evidence suggesting that anabolic implants are not as effective in *Bos indicus* influenced animals when compared to *Bos taurus* animals.

Assessment of NAD+ Deficiency on glutathione and DNA damage in niacin dependent mouse models (ANDY)

Elizabeth Park[1], Sierra Lopez[2], Miles Wandersee[2], Ralph Meyer[1,2] and Mirella Meyer-Ficca[1,2]

[1]School of Veterinary Medicine, [2]Department of Animal, Dairy, and Veterinary Sciences

One of the hallmarks of aging is the depletion of NAD levels in the body. A decrease in NAD^+/NADH and the phosphorylated derivatives $NADP^+$/NADPH can lead to an accumulation of oxidative stress and inflammation resulting in DNA damage. Reduced glutathione is an important molecular defense system that requires sufficient NADPH to detoxify reactive oxygen species and protect the body from DNA damage. NADPH is a cofactor that glutathione reductase needs to regenerate "used" oxidized glutathione (GSSG) to its "active" reduced state (GSH) that functions properly as the body's defense system. To determine consequences of low NAD^+ (and $NADP^+$) availability on glutathione concentrations and the amount of ROS, we used the transgenic Acquired Niacin Dependency (ANDY) mouse model. We hypothesize that restricted dietary niacin intake that results in lowered NAD^+ and $NADP^+$ concentrations will lead to decreased levels of reduced glutathione and an increase in ROS and DNA damage. To test this hypothesis, we analyzed liver tissues with defined NAD levels determined by niacin in the diet. We quantified liver GSH content and reduction status using a modified Tietze recycling assay. To measure ROS, we performed dot blot assays for 8-oxo-guanine which is commonly used as a biomarker for oxidative DNA damage. Our results indicate that liver tissue from NAD-deficient mice have significantly lower reduced glutathione levels compared to tissues from mice on control diets.

Using cytoplasmic biopsies to determine bovine oocyte quality

Madi Lindsey, Clay Isom

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

Embryos resulting from assisted reproductive technologies such as in vitro fertilization of intracytoplasmic sperm injection develop with much lower efficiencies than embryos resulting from in vivo oocyte fertilization. The reasons behind these 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. Our hypothesis for this project is that there are distinct mRNA transcript patterns that distinguish high-quality oocytes from poor quality oocytes developing within the same environment. In this study, we will remove a small cytoplasmic biopsy from each of 200 bovine oocytes via micromanipulation and store them for later use. We will then parthenogenetically activate the biopsied oocytes to stimulate development and culture the incipient embryos in vitro. Following a seven-day development period, embryos that reach blastocyst and embryos that fail to develop will be selected, and their corresponding biopsies will then be compared for transcript levels of 48 genes via the BioMark single-cell qPCR system from Fluidigm. The functional categories of the 48 genes that will be analyzed 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.

Characterization and comparison of subacute dehydropyrrolizidine alkaloid toxicosis in C57BL mice gavaged with riddelline, senecionine, seneciphylline or lasiocarpine

Michael J Clayton[1,2], Edward L Knoppel[1], Arnaud J Van Wettere[2], Jeffery O Hall[1,2], Bryan L Stegelmeier[1]

[1]USDA Poisonous Plant Research Laboratory, Logan, UT; [2]Department of Animal Dairy and Veterinary Sciences Utah State University, Logan, UT

Dehydropyrrolizidine alkaloids (DHPAs) are a globally important group of plant derived toxins, estimated to be found in 3% of flowering plants. Over 600 individual DHPAs have been discovered and many of these are hepatotoxic, pneumotoxic, nephrotoxic, genotoxic and under some circumstances, carcinogenic. There are numerous reports of poisonings in domestic animals, wildlife and humans. Animal exposure occurs via contaminated hay or grain, or less commonly from grazing. Human exposure occurs from contaminated grain, herbal supplements or teas, or animal products such as milk, honey, or eggs. Mice are less sensitive than other species to DHPA toxicosis, but they are a useful animal model due to their size, availability, degree of genomic characterization and the ability to create genetically altered models. The objectives of this experiment are to characterize microscopic lesions and compare lesion severity following subacute exposure to select DHPAs in mice to obtain suitable dose(s) for use in future chronic studies assessing genotoxic and carcinogenic potentials. Different doses of four purified DHPAs (riddelline, senecionine, seneciphylline and lasiocarpine) were gavaged to C57BL mice for ten days. The mice were necropsied on day eleven. Microscopic lesions included hepatocellular hypertrophy in zone 2 and 3, and multifocal random, hepatocellular necrosis. The severity of hepatocellular hypertrophy and necrosis increased with dose. The order of hepatotoxicity, based on severity of necrosis as correlated with dose, from most hepatotoxic to the least was senecionine, seneciphylline, riddelliine, and lastly lasiocarpine. The order based on the lowest dose that produced microscopic lesions was lasiocarpine, riddelliine, seneciphylline, senecionine.

Cache Valley $PM_{2.5}$ activates the unfolded protein response in human lung cells

Morgan Eggleston[1], Andy Nguyen[1], Nicholas Grooms[1], Rachel Sagers[1], Randy S. Martin[2], Kimberly Hageman[3], Roger A. Coulombe Jr[1]

[1]Department of Veterinary Sciences, [2]Department of Civil and Environmental Engineering[1], [3]Department of Chemistry and Biochemistry, Utah State University, Logan UT, USA

Worldwide, exposure to ambient particulate $PM_{2.5}$ air pollution is associated with increases in all-cause mortality, cardiopulmonary and cardiovascular disease, stroke, diabetes, cancer, and Alzheimer's disease. The normally picturesque Cache Valley of Northern Utah frequently experiences some of the highest $PM_{2.5}$ concentrations in the United States. However, the exact mechanism(s) of Cache Valley $PM_{2.5}$ (CVPM) toxicity are incompletely understood. We recently demonstrated that CVPM exposure is associated with endoplasmic reticulum (ER) stress, which triggers the unfolded protein response (UPR), a highly conserved stress-response mechanism common to many disease states. The purpose of this study was to focus on the dynamics of CVPM-induced ER stress and UPR in cultured human lung (BEAS-2B) cells exposed to CVPM (1 and 12 μg/mL; 24 h). All experiments were conducted in parallel with diesel exhaust particles (DEP) as a positive control. RNA sequencing with gene set enrichment pathway analysis confirmed significant upregulation (FDR adjusted p=0.05) in genes strongly associated with UPR activation, such as *BiP/GRP78*, *PERK*, *IRE1*, and *ATF6*. Significant cellular effects related to UPR activation were also observed, including reductions in mitochondrial membrane potential and alterations in intracellular Ca^{2+} homeostasis, as evidenced by a significant influx of Ca^{2+} in the cytosol and mitochondria, likely from the ER network. CVPM treatment also led to the release of cytochrome c oxidase from the mitochondria to the cytosol, a preliminary indicator of apoptosis. Biomarkers related production of reactive oxygen species (ROS), such as malondialdehyde and 4-hydroxynonenal, were also identified in CVPM treated lung cells, indicating ROS is likely contributing to the source of ER stress and activation of the UPR. Across most experiments, 1μg/mL DEP elicited similar results to CVPM at 12μg/mL, suggesting that CVPM is less potent than DEP. Taken together, these results support our hypothesis that a principal toxic mechanism of CVPM pollution involves ER stress and the UPR.

Influence of cattle breed and forage type on organic dairy heifer performance.

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

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Recent renewed interest in pasture-based farming has reopened debate about a breed's suitability in such a system. Many different breeds of dairy cattle exist, but questions remain about their relative performance within an exclusively forage-based system. This is especially true in a pre-pubertal heifer grazing system. In our trial, we evaluated the performance of four different breeds [Holstein (HO), Jersey (JE), Holstein/Jersey crossbred (HO/JE), and Swedish Red/Montbeliarde/Holstein crossbreds (SR/MO/HO)] in a rotational grazing system. Treatments consisted of either a monoculture grass paddock (MONO) or a grass paddock interseeded with the legume Birdsfoot Trefoil (MIX). Our hypothesis is that MIX treatments have a positive influence on the weight, height, and overall growth of the animal. It was also hypothesized that the crossbred animals would perform better due to hybrid vigor. We looked at physical properties on a percentage basis to equally compare inherently different sized breeds. When looking at the increases as a current percentage of a mature body weight (%MBW) of that specific breed, the Jerseys on the mixed treatment increased by 12.06 percent while the other three breeds only increased 9.95, 8.58, and 8.19 percent (Holstein/Jersey, Swedish Red/Montbeliarde/Holstein, and Holstein respectively). Increases were notably smaller in the monoculture treatment with the Jerseys at 9.03 % increase followed by the HO/JE, HO, and lastly the SR/MO/HO (6.06%, 4.53%, and 4.49% respectively). Overall average daily gains (ADG) showed a tendency to be higher in the MIX treatments ($P=0.06$). Breed differences also showed significance ($P<0.0001$) among each other for raw weight, %MBW, percent total gains based on starting weight, and hip height.

Development of a transgenic hamster model for evaluation of antiviral drugs & vaccines against SARS-CoV-2

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The emergence of the severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) presented a severe global health crisis. Antiviral drugs and vaccines against SARS-CoV-2 will be needed to treat infected patients and prevent the spread of disease. It is vital to screen these drugs and vaccines for antiviral efficacy. Animal models for infectious diseases provide an avenue to evaluate efficacy and help focus human trials on drugs that have shown promise in animals. A transgenic mouse is commercially available which expresses the human angiotensin-converting enzyme 2 receptor (hACE2), which is used by SARS-CoV-2 for cellular entry. Available published data in hACE2 mice show ~5% weight loss, histopathology in lung tissue, and viral replication in the lungs[1]. Partial mortality (50%) has been observed in another study[2].

Our goal is to establish a hamster model of SARS-CoV-2 infection for the evaluation of antiviral therapies and vaccines. Collaborating with Dr. Zhongde Wang, hamsters expressing hACE2 receptors were infected with SARS-CoV-2. Hamsters were observed for weight loss, body temperature, and clinical disease signs for 14 days. We observed mortality in 3 of 9 animals and ~15% weight loss in infected animals. In addition, an increase in body temperature was observed shortly after infection. Clinical signs of disease varied but common signs included general sickness behavior (rough fur, hunched posture), lethargy, and dyspnea. Histopathology and viral replication were observed in the lungs. We have observed clinical signs in the hACE2 hamsters that have not been observed in wild-type hamsters or hACE2 mice. *[Supported by Contract HHSN272201700041I from the Respiratory Diseases Branch, DMID, NIAID, NIH]*

Reference:

1. Bao, L. *et al.* The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* **583**, 830–833 (2020).
2. Jiang, R.-D. *et al.* Pathogenesis of SARS-CoV-2 in Transgenic Mice Expressing Human Angiotensin-Converting Enzyme 2. *Cell* **182**, 50-58.e8 (2020).

Effects of pre-mortem stress on protein expression, steak color, and myofibrillar fragmentation index in the *longissimus lumborum* following harvest

R. K. Briggs[1], J. F. Legako[2], P. R. Broadway[3], J. A. Carroll[3], N. C. Burdick Sanchez[3], Z. K. Smith[4], R. Ramanathan[5], K. J. Thornton[1]

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Undesirable variation in beef tenderness and stability of flavor and color may be associated with the abundance of heat shock proteins (HSP). This study aimed to determine whether pre-mortem stress impacts HSP expression in the skeletal muscle following harvest. Forty Holstein steers were administered an i.v. bolus dose of adrenocorticotropic hormone (ACTH; 0.1 IU/Kg BW) to mimic an acute pre-mortem stress. *Longissimus lumborum* (LD) biopsy samples were taken prior to the ACTH challenge. Serum cortisol was measured every 0.5 h from -2 to 6 h relative to the ACTH challenge. Skeletal muscle and blood samples from 10 steers were collected at each harvest timepoint at (2, 12, 24 and 48 h post-challenge). Samples were collected from the LD immediately after harvest and after 14 d of aging. Protein expression of HSPβ1, P-HSPβ1, HSPβ5, and DJ-1 was analyzed in muscle samples taken prior to the ACTH challenge, at harvest, and after 14 d of post-mortem wet aging. In addition, steak color and myofibrillar fragmentation index (MFI) was analyzed in 14 d aged samples. Harvest time point following the ACTH challenge affected ($P < 0.05$) protein expression of HSPβ1 and P-HSPβ1. Protein expression of DJ-1 prior to the ACTH challenge was different ($P < 0.05$) among steers harvested at different timepoints. In addition, time of harvest had no effect on HSPβ5 expression ($P > 0.05$). Regarding steak color, time of harvest had an effect ($P < 0.01$) on a*, b*, hue, chroma, and ratio, but no effect ($P > 0.05$) on L*. Lastly, time of harvest had an effect ($P < 0.05$) on MFI. These data indicate that HSP expression, steak color, and MFI in the LD after harvest may be related to time of harvest following a stressful event pre-mortem.

Role of the viral E glycoprotein in Japanese encephalitis virus replication

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Japanese encephalitis virus (JEV) is an emerging mosquito-borne flavivirus (family *Flaviviridae*) that has become a global concern, due to its continuous spread in Asia and Australia and recent emergence in Europe (Italy) and Africa (Angola). JEV is the leading cause of viral encephalitis in most Asian countries with its annual incidence of 50,000-175,000 JE cases. About 20-30% of JE cases are fatal, and up to 50% of JE survivors experience long-term neuropsychiatric sequalae. There is a cell culture-derived live-attenuated JEV vaccine SA14-14-2, which is derived from its wild-type virulent strain SA14. In our previous studies, we found that the viral E protein is a key determinant for the attenuation of SA14-14-2. To genetically map the amino acid residues that are critical for SA14-14-2 attenuation, we generated six SA14 E mutants based on the E protein crystal structure and showed that three amino acid residues located in the domain II of SA14 E protein promote viral replication and affect the ratio of prM to M proteins accumulated in infected cells. Our data warrant further investigation to elucidate the precise role of E DII in viral replication in cell culture and viral virulence in mice, a small animal model for JEV pathogenesis that mimics the manifestations of JEV disease in humans.

Effect of cytokine supplemented maturation medium on bovine somatic cell nuclear transfer 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 consecutive 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. Initial pregnancy rates assessed at Day 40 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 maturation medium supplemented with cytokines compared with control medium (709/885; 80.2 ± 2.33% *v.* 549/822; 66.8 ± 1.82%; $P < 0.05$, 7 replicates, one-way ANOVA). A significant increase in blastocyst rate was observed in the treatment group compared with the control group (181/446;40.6 ± 5.1% *v.* 73/300 24.3 ± 2.9%; $P < 0.05$, 8 replicates, one-way ANOVA). SCNT embryos derived from treatment group also resulted in a significant increase in initial pregnancy rates (25/48; 50.3 ± 20.9% *v.* 9/31; 29.0 ± 20.6%, $P < 0.05$, 4 replicates, chi-square). Full-term pregnancy rates are pending. In conclusion, addition of FGF2, LIF, and IGF1 to maturation medium improves bovine IVM and SCNT blastocyst development and initial pregnancy rates. The effect on full-term pregnancy success is yet to be determined.

Identification and characterization of PD-L1 in bovine placentas

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[2]School of Veterinary Medicine, Utah State University, Logan, UT

Successful bovine pregnancy requires that the maternal immune system modulate T lymphocytes. Program death-ligand 1 (PD-L1) is an inhibitory protein that is associated with immune tolerance and modulation of T cells. Previous studies have linked PD-L1 to suppressing T cell activity and modulating cytokine production, therefore, inducing maternal tolerance and acceptance of the fetus. PD-L1 may be a possible mechanism involved in establishing successful bovine pregnancies. To this day, no evidence of PD-L1 RNA or protein was found in bovine placentas. We hypothesize that PD-L1 is present in the bovine placenta and its expression differs between trimesters. To analyze the presence of PD-L1, we have developed a quantitative Real-Time PCR and Western Blotting protocol to detect and characterize PD-L1 RNA and protein, respectively. For PD-L1 RNA, recombinant plasmids were used as a positive control. The cDNA was diluted at 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 to generate a standard curve. Anti PD-L1 was used as the primary antibody for Western Blot detection. It is expected that PD-L1 is present in bovine placentas and differs through gestation periods with higher expression of PD-L1 during the first trimester of gestation. In this study, PD-L1 mRNA and protein expression were observed in bovine placentas. PD-L1 is also expected to potentially modulate immune cell expression indicating that PD-L1 RNA and proteins can be used to induce a receptive maternal immune system to fetal antigens.

In vitro correction of F508del and G542X mutations in sheep fibroblasts of cystic fibrosis models

Kaden Bunch,[1] Iuri Viotti Perisse,[2] Zhiqiang Fan,[2] Kenneth L. White,[2] Irina A. Polejaeva[2]

[1]Department of Biology, Utah State University, Logan, Utah, USA.[2]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, USA.

Cystic Fibrosis (CF) is a human genetic disease caused by mutations in the CF Transmembrane Conductance Regulator (*CFTR)* gene. Among the ~2000 known CF mutations, F508del mutation is found in 84% and G542X in 4.6% of the CF patients in US, respectively. The F508del mutation is characterized by the deletion of the 'CTT' nucleotides that leads to the misfolding of the CFTR protein. The G542X is a nonsense mutation that results in absence of protein production. We hypothesized that gene editing may be an effective tool to correct these mutations and permanently cure this genetic disease. In this study, we evaluated the efficiency of CRISPR/Cas9-meditated gene knock-in to correct the F508del and G542X mutations in sheep fibroblasts *in vitro*. We designed sgRNAs and approximate 100bp of single-stranded oligodeoxynucleotides (ssODNs) targeting the mutation sites at exon 11 and 12 to introduce either 'CTT' or replace 'T' to 'G' nucleotide in genome of F508del or G542X CF sheep cells, respectively. Each of Cas9/sgRNA ribonucleoproteins was transfected into sheep fibroblast cells along with ssODNs for the Homology-Directed Repair. The transfected cells were subsequently cultured, incubated at 38.5ºC, and DNA was extracted 48 h post-transfection for mutation efficiency validation. PCR products of the exons 11 and 12 were ligated into T-vector and bacterial colonies were selected based on blue/white screening. In total, we isolated 32 single cell bacterial colonies for each of the mutant. Sequencing results indicate that 'CTT' was introduced in 4/26 (15.3%) plasmid colonies, and 'T to G' replaced in 13/31 (41.9%) colonies. Therefore, our results indicate that the F508del and G542X mutations can be effectively corrected in CF sheep fibroblasts *in vitro* using CRISPR/Cas9 approach.

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Manipulation of ovarian influence on aging-associated DNA methylation changes

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It is well-established that as an individual continues to age, their health deteriorates. When looking at the differences in health and aging between women and men, women are generally healthier until they go through menopause around the age of 50. After menopause, their health is no longer better than their male counterparts. There is a direct correlation between the health of women, and their ovarian function. In past studies using mice, there has been evidence that shows health benefits from ovarian manipulation, such as restoration of cardioprotective influence and, renal function, and increased lifespan. In this study, we have obtained preliminary data that shows changes in miRNA and gene transcription which are associated with the health benefits of ovarian manipulation. Our results on miRNA expression are analyzed from ovarian tissue, and our results on RNA sequencing and methylation are analyzed from homogenized liver. By using multiple groups of mice, we expect to see changes between miRNA expression and RNA sequencing that will show similarities between our young mice and our mice that underwent some form of ovarian manipulation. We are looking at multiple gene groups, such as the Foxo genes. These changes and differences seen in the miRNA, RNA, and DNA methylation from ovarian manipulation are hoped to be further applied to future assays in order to dissect which specific up or down regulations in genes are related to aging associated health.

Examining the effects of estradiol, trenbolone acetate, or polyamines on bovine satellite cell differentiation

L.L. Okamoto[a], C.C. Reichhardt[a], B.P. Griffin[a], L.A. Smith[a], G.K. Murdoch[b], K.J. Thornton[a]

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The mechanism through which anabolic implants improve skeletal muscle growth of beef animals is incompletely understood. Polyamines (PA) are bioactive amino acid derivatives that act as potent growth stimulants. The objective of this study was to determine effects of anabolic implants, PA and their precursors on bovine satellite cell (BSC) differentiation. Primary BSC were cultured to approximately 80% confluency, at which time they were induced to differentiate in the presence of 3% horse serum (Con) and treated with 10nM TBA, 10 nM E_2, or 10nM TBA and 10 nM E_2 (ETBA), 10 mM methionine (Met), 8 mM ornithine (Orn), 2 mM putrescine (Put), 1.5 mM spermidine (Spd) or 0.5 mM spermine (Spe). Total mRNA was isolated 0, 2, 4, 8, 12, 24, or 48 h post-treatment and abundance of paired box transcription factor 7 (*Pax7*) and myogenic differentiation factor (*MyoD*) were analyzed. Treatment with the hormones (TBA, E_2, or ETBA) and PA (Orn, Put, Spd, and Spe) increased ($P < 0.05$) abundance of *MyoD* 4 h post-treatment when compared to Con cultures. However, 24 h post-treatment, *MyoD* abundance was decreased in the presence of hormone treatments when compared to the Con, while the PA treatments increased ($P < 0.05$) abundance of *MyoD* when compared to the Con cultures. Treatment with either the hormones or PA had no effect ($P > 0.05$) on *Pax7* abundance at 2, 4, 8, 12, 24, or 48 h post-treatment when compared to Con cultures ($P > 0.05$). These results indicate that treatment with PA or hormones increases abundance of *MyoD*, though temporally different indicating that these two classes of growth promoters impact differentiation via alternate physiological pathways. Additional research is underway in order to determine the effects of both PA and hormones on differentiation of primary BSC.

Importance of N-glycosylation of the Zika virus E glycoprotein in a live chimeric virus vaccine candidate

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Zika virus (ZIKV) is an enveloped RNA virus belonging to the genus *Flavivirus* within the family *Flaviviridae*. Within the genus, ZIKV is a mosquito-borne zoonotic pathogen closely related to other clinically important flaviviruses, such as Japanese encephalitis (JEV), West Nile, dengue, and yellow fever viruses. Originated in Africa, ZIKV has spread eastward from Asia to the Americas over the past half century. ZIKV can cause a variety of neurological illnesses from mild to life-threatening diseases, of which the two of the greatest clinical importance are microcephaly and Guillain-Barre syndrome. However, no clinically proven vaccines or drugs are currently available for the control of ZIKV infection. In our previous work, we created a live chimeric virus vaccine candidate against ZIKV, based on the clinically proven live-attenuated JEV vaccine SA14-14-2, by replacing the two viral surface glycoproteins, prM and E, of JEV SA14-14-2 with the corresponding genes of ZIKV P6-740. In the present study, we aimed to understand the importance of E protein glycosylation in replication of the chimeric JEV/ZIKV in vitro. Specifically, we analyzed the effects of the N-linked glycosylation at position E-153 in Vero cells. We found that the chimeric virus with the glycosylation consensus motif increased its plaque size with no significant effect on viral RNA replication. Our results promote further research in order to understand the role of E protein glycosylation in attenuation of the live chimeric virus vaccine candidate against ZIKV in mice.

Overview of the underlying molecular mechanisms of inflammation leading to colorectal cancer and shifts in microbiome.

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Colorectal cancer (CRC) is the third most common cancer diagnosed in men and women in the US. CRC is characterized by frequent epigenetic and genetic modifications like high microsatellite instability, chromosomal instability, and CpG island methylation. In addition, mutations of tumor suppressor genes (e.g., p53) and oncogenes (e.g., RAS genes) occurs in 55-60% of CRC patients resulting in changes in cell propagation, apoptosis, cell cycle and DNA repair mechanism. CRC has a variety of risk factors including environment, diet, genetics, health status, lifestyle choices, among others. Inflammatory bowel disease (IBD), more specifically ulcerative colitis, is one of the major risks factor for developing colitis-associated colorectal cancer (CAC). IBD is an idiopathic disorder caused by chronic and excessive inflammation. Chronic excess inflammation leads to an increase in cellular production of reactive oxygen species (ROS), which in turn increases rates of DNA mutation of tumor suppressor genes and oncogenes ultimately leading to colon tumorigenesis. IBD has several pathogenic factors such as immune dysregulation, environmental changes leading to epigenetic change and abnormal gut microbiota.
The typical human gastrointestinal tract is inhabited by approximately a thousand different bacteria species. A healthy gut microbiome is a very diverse and complex community in which the relationship between bacteria and host is usually commensal and/or symbiotic in nature. A disruption in this host-bacteria relationship leads to dysbiosis of the gut microbiome. Dysbiosis coupled with an excessive inflammatory response can lead to an acceleration of CRC initiation and progression via mucosa dysplasia and tumorigenesis in the colon and rectum. Invading commensal bacteria and their metabolites can activate Toll-like receptors which mediate the inflammatory cytokine production consequently promotes CRC development via activation of other cytokines. Thus, dysbiosis of the gut microbiome associated with IBD is connected to sustained inflammation of the gut epithelium, predisposing to development of inflammation-associated CRC.

Effect of Timed Cumulus Cell Removal in Bovine Embryo Development

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The purpose of this study was to examine the importance of the relationship between cumulus oocyte complexes, (COC's) and the specifics between embryo quality and timing of cumulus cell (CC) removal. CC's are a specialized granulosa cell that are attached to the oocyte to promote healthy oocyte function and quality. *In-vitro* methods allow for the cc to be denuded approximately eight hours post *In-vitro Fertilization* (IVF), and co-culturing is used by adding a bed of denuded cells onto the culture dish. This increased embryo quality. Our hypothesis manipulated timing of *In-Vitro culture* (IVC) methods and cc removal. Four groups were tested, including a control. CC's were denuded eight hours post IVF in the control group and incubated to culture using IVC methods. Co-Culture methods were tested as well, cc's were denuded eight hours post IVF and denuded cells were added back to the dish before culturing. In the two last groups, cc's were kept on embryos 24- and 48-hours post IVF. After, the 24- and 48-hr groups cc's were denuded and then cultured. Once embryos had reached the 8-day blastocyst stage, embryos were fixed and stained to examine blastocyst rate and embryonic quality. By comparing cleavage rates to blastocysts rates, no significance was shown ($P > .05$) between groups.Staining embryos with antibody stains, embryo quality was compared by counting Trophectoderm (TE) cells and Inner Cell Mass (ICM) in a ratio TE:ICM. Ratios were slightly higher in 24 and 48hr groups, but no correlation ($P > .05$) was found. However, ICM numbers were higher in the 24 and 48hr groups, which could suggest a biological significance, as appearance and size was substantial compared to other groups. Further studies may need to be done to find any statistical significance between the groups.

Analyzing Glucose Tolerance Testing in CBA/J mice

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Studies have shown throughout time that women have a better health span than men up until they go through menopause. CBA/J mice decline in ovarian function early in life, similar to the changes in ovarian function with menopause. To assess metabolic health, we chose to use a glucose tolerance test (GTT). The GTT was performed to see how younger mice would handle the dextrose compared to older treated mice. Mice that had no procedures done on their ovaries were compared to each other and surgery mice; 28 months (n=5), 19 months (n=6), 8 months (n=6), and 6 months (n=19). Treated mice included; germ cell-containing mice of 18 months of age (n=9), germ cell depleted mice of 18 months of age (n=10), and the ovarian somatic cell injected mice of 18 months of age (n=5). The purpose of this research is to broaden comprehension of the effect of ovarian signaling in our health as we age. Results show that older untreated mice have a greater intolerance in glucose metabolism, compared to mice with transplanted young ovaries. All treated mice show higher tolerance to glucose compared to all control groups and the ovarian somatic cell injected mice showed reduced glucose levels compared to the germ cell depleted mice. Performing a GTT in a different strain of mice would narrow down any changes in metabolism to reinforce and validate data from this study and previous studies. Using a strain such as the C57BL/6 strain will provide more comparable data to other research data since they are a more common strain used in several different fields of research, not just aging research. The goal is to determine the specific mechanisms that the young ovaries are using to positively influence aging health including glucose metabolism.

Effects of dietary omega-3 fatty acids on factors of inflammation and growth in piglets

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Historically, antibiotics have been used as routine feed additives in livestock production to prevent and treat disease, promote growth, and improve feed efficiency. Increased federal regulations on antibiotics have prompted swine producers and researchers to search for alternative feed additives that can promote growth in their animals. Omega-3 polyunsaturated fatty acids are among many of the proposed feed additives to take the place of antibiotics in swine production. Omega-3 is well documented in promoting regeneration of diseased or aged muscle due to its anti-inflammatory properties, but little research exists on its viability as a growth promoter in young, healthy animals. The purpose of this study was to determine the effects of omega-3 supplementation on inflammatory cytokines and skeletal muscle growth in piglets. Our hypothesis was that provision of dietary omega-3 fatty acids would result in decreased inflammation leading to enhanced myogenesis. Fourteen 35-day old weaned piglets were used for the study. They were divided evenly between a control group, which received only normal pig feed, and a treatment group, which received feed with an omega-3 supplement (dosage/day ~3% of their body weight). They were euthanized after 30 days. Relative abundance of mRNA related to inflammation (Ppary, Nf-kB, TNF-a, and IL-6) and myogenesis (Myf5, MyoD, MyoG and Pax7) was measured in muscle. Our results showed no difference ($P > 0.05$) between treatment groups in abundance of PPARy, Nf-KB, TNF-a, IL-6, MyoD, MyoG, or Pax7. Abundance of Myf5 was decreased ($P < 0.05$) by omega-3 supplementation. This study is useful for directing future studies examining the interaction between inflammation and skeletal muscle growth in swine production.

The Effects of Lacking Dietary Niacin on Sperm Count and Chromatin Quality

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Dietary niacin is essential for nicotinamide adenine dinucleotide (NAD) synthesis in humans. Rodents, in contrast, can satisfy their NAD requirements using tryptophan in dietary protein. Fish, vegetables and fortified wheat flour are sources of dietary niacin (vitamin B3). Insufficient dietary niacin results in reduced body-wide NAD levels in humans. Because NAD is important for cell proliferation and for DNA repair, we hypothesized that NAD will be necessary for proper sperm formation, and low NAD should result in a lower quantity and quality of sperm. To test this hypothesis, groups of transgenic ANDY mice, which resemble human NAD niacin requirements, were fed different diets without niacin and varying protein contents. This resulted in blood NAD levels ranging from very low (niacin deficient ND1 diet), low (ND2 diet) to normal (control diets CD1, CD2). Sperm obtained from these mice were counted and sperm DNA quality was measured using an alkaline Sperm Comet Assay procedure. This assay is based on single-cell electrophoresis of sperm, where DNA fragments due to strand breaks move out of the nucleus resulting in a DNA "tail" that is quantified as "tail moment" using the CASP software.
In males with extremely low blood NAD levels sperm production stopped all together. In contrast, males on ND2 niacin-deficient diet had sperm with increased tail moments and normal sperm count, indicating that these males might be fertile, but had poor quality sperm with DNA damage. Such low-quality sperm can have negative health consequences for the offspring, such as a higher risk of autism, diabetes, or metabolic disorders. Low-niacin diets are common in the western world, and the data suggest that ensuring adequate niacin in diets will promote the birth of healthy offspring.

Characterization of the ovine interferon lambda (IFNL) system and creation of an IFNL receptor knockout fetal fibroblast cell line

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Knocking out certain genes in the DNA of sheep allows us to gain a better understanding of the functions of those genes as the sheep are studied over their lifespan. In this experiment we worked to knock out the interferon lambda receptor 1 (IFNLR1) gene in fetal fibroblast cells. This was achieved through transfection of the fibroblast cells with CRISPR-Cas9 and sgRNA. To get to this point, we designed and successfully tested sets of primers for the interferon lambda (IFNL) system. Primers for the IFNLR1 gene, along with the restriction enzyme AjiI (BmgBI), have been used in PCR-RFLP to indicate the success of the transfection. Mutation efficiency from the transfection is estimated to be 86%. From this point, the goal is to take transfected cells and create single cell colonies that will replicate to the point that they can be used in a process of in vitro fertilization. Embryos containing the knockout will then be implanted into the uterus of a ewe. As lambs are born with the IFNLR1 knockout, how they respond to viral infections will give us a look at the functions of the IFNL system.

Impact of Fish Oil on Animal Performance and Intestinal Permeability in Swine

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The objective of this study was to evaluate the effects of fish oil on animal performance and nutrient digestibility in growing piglets as well as intestinal permeability and volatile fatty acid concentration after an LPS challenge. Forty male piglets (Landrace x New Hampshire) with an average body weight of 8.21 ± 0.83 kg and 30 days of age were used in a complete randomized block design and assigned to one of the two treatments: 1) a basal diet (Control) or 2) the control diet plus 3% of fish oil (Fish Oil). Animals received the treatments for 35 days. At day 34, seven animals per treatment received an LPS challenge, and 24 h after intestinal tissue and digesta were collected. The statistical model included the random effect of block, and the fixed effect of treatment, day, and their interactions. There was no treatment effect on body weight (9.56 vs 9.33 kg; $P<0.10$). Piglets that received fish oil had greater feed intake (1049 vs. 729 g; $P<0.01$) and total fatty acids digestion (78.4 vs.71.2%; $P<0.01$) compared with control. No treatment effect was observed for the cytokine TNFα (121 vs 127 pg/mL, $P=0.63$) as well as in vivo intestinal permeability (D-xylose; 1.13 vs 1.15 mg/ml, $P=0.69$). The concentration of volatile fatty acids (acetate, propionate, and butyrate) in the gastrointestinal tract after the LPS challenge was not affected by treatments ($P\geq0.27$). However, fish oil tended to decrease intestinal permeability (6.97 vs 6.63 pg/mL, $P=0.08$) in an ex-vivo assay after the LPS challenge. Although fish oil did not improve body weight, it had a positive effect on feed intake and intestinal permeability after the LPS challenge.

Key words: fatty acids, intestinal barrier, weaning.

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

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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. 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 the 3 fibroblast cell lines used. We used a 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 cell lines including most of the CpG sites on the NANOG gene region. We will use SAS logistic regression for statistical analysis to determine differential methylation between cell and embryo types for each gene region sequenced.

Effect of increasing levels of whole cottonseed on rumen fermentation, fiber digestibility and biohydrogenation extent in continuous culture fermenters

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Whole cottonseed is a byproduct largely used in dairy rations because of its nutritional value; however, there is a lack of information about how much cottonseed can be added into the diets without negatively affecting rumen fermentation. The objective of this study was to measure the effect of increasing levels of whole cottonseed in the diet on rumen fermentation parameters, digestibility and biohydrogenation of fatty acids. The study was conducted as a replicated 4×4 Latin square using continuous culture fermenters (n=8). Treatments were a control diet without cottonseed, and the control diet plus 5, 10, or 15% of whole cottonseed. The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate fed twice daily. The experiment consisted of 4 periods of 10 days each (6 days adaptation, 4 days of sampling). 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 acetate and propionate concentrations. A quadratic effect of the levels of cottonseed on butyrate concentration was observed; the addition of 5 and 10% of cottonseed increased butyrate concentration. The lack of treatment effect on acetate indicates that increasing levels of cottonseed does not negatively impact fiber digestion in the rumen. The completion of the analyses (NDF digestibility, biohydrogenation, microbial growth and microbial community) are needed to validate our preliminary conclusions.

Key Words: cottonseed, gossypol, rumen

Agritourism raw products and the potential risks to tourists

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After an isolated outbreak of Shiga-Toxin producing *Escherichia coli* (STEC) in Utah, state-wide agritourism venues were surveyed and characterized for zoonotic disease prevention best practices. The sale of raw products, direct to consumers, at agritourism venues is a known risk for STEC. Of the twelve venues reporting selling raw food products direct to consumer, 83% (n=10) allowed animal contact. Of those allowing animal contact, 7 (70%) provided education to their guests about zoonotic diseases and potential risks. This presentation will discuss best practices for venues in preventing zoonotic diseases at agritourism events.

Impact of palmitic acid and pH on ruminal NDF digestibility and fermentation in a continuous culture system

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Non-rumen bacteria incorporate exogenous long-chain saturated fatty acids to change membrane fluidity under low pH conditions. We hypothesized that rumen bacteria use a similar mechanism, thus, providing long-chain saturated fatty acids in the diet could support bacterial metabolism and growth and consequently enhance fiber digestibility. The objective of this study was to evaluate the effects of dietary palmitic acid and pH on ruminal NDF digestibility and fermentation. The study was conducted as a 2 × 2 factorial treatment arrangement in a replicated 4×4 Latin square using continuous culture fermenters (n=8). Treatments were a control diet without supplemental fatty acids or the control diet plus 1.5% of palmitic acid factorialized with normal pH (range: 6.6 - 7.0) or low pH (range: 6.0 - 6.4). The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate fed twice daily. Data were analyzed using a mixed model including the fixed effect of pH, fatty acid, and its interaction, and the random effects of period and fermenter. No interaction between fatty acids and pH were observed for the variables measured. Compared with control, palmitic acid increased NDF digestibility (45.2 vs. 39.34%, P=0.03). The lower pH decreased NDF digestibility in 8.2 percentage units compared with normal pH (46.4 vs. 38.16%, P<0.01). Furthermore, low pH decreased ammonia (7.30 vs. 5.64 mg/dL, P=0.01) and total VFA concentration (168 vs. 138 mmol/d, P=0.02) compared with normal pH; palmitic acid did not (P>0.10) affect ammonia nor total VFA concentration. Our preliminary data indicate that rumen pH and palmitic acid independently affect NDF digestibility and rumen fermentation. Palmitic acid supplementation increased ruminal fiber digestibility under low and normal pH conditions.

Key Words: fatty acids, rumen, pH

Brucellosis testing in cattle to prevent zoonotic disease spread to humans

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Brucellosis is a zoonotic disease that can infect deer, cattle, humans and many other species of animals. It causes sudden abortions and retention of fetal membranes in cattle, sheep and other ruminant populations. Brucellosis is known to be present in the bison and white tailed deer herds of Yellowstone National Park. Because of the disease presence in these wild populations, it is possible for brucellosis to infect cattle herds when they graze in the mountains near Yellowstone National Park during the summer months. In the winter months, these cattle are kept close to home for feeding. During this time, brucellosis can be transferred from the cattle to humans. Brucellosis transfers to humans through a variety of methods including: consumption of unpasteurized milk or meat from a sick cow, or any sort of close contact with infected animals. Due to the possibility of transfer, it is required by law that all cattle must be vaccinated against brucellosis when they are big enough to receive the vaccine. Generally, a calf must weigh 500 lbs. before being administered the vaccine. However, testing for the disease is still required when selling a cow to a new farm even if a vaccine has been administered. The testing and identification of brucellosis must all be documented and shown to the government as proof that the testing is being completed and approved by a veterinarian.

Planning for the Utah One Health Symposium-An MPH Practicum experience

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The MPH practicum is an opportunity for MPH students to get real-world experience in the field of public health. Serving on the planning committee for the Utah One Health Symposium was an opportunity to be engaged in One Health education for professionals and to learn the basics of planning a symposium. As a discipline, One Health explores the rich connections between human, animal and environmental health and uses multidisciplinary approaches for complex problems like emerging infectious diseases. One Health symposia help raise awareness of the One Health approach by developing strong interdisciplinary scientific programs and providing forums for One Health ideas and professional connections. The Utah planning committee avoids impediments to interdisciplinary work by modeling One Health principles in its membership and planning process. The committee is diverse, represents many organizations and disciplines. The multidisciplinary approach to planning ensures that the committee has ample resources and benefits from the advantages of the multidisciplinary environment. These include: varied expertise, experiences and perspectives, and enhanced creativity. The committee's work also benefits from collaborative leadership that engenders trust and participation. Planning is well-organized around three elements – evaluating the previous year, organizing the logistics, and designing the scientific program. A participant survey informs all aspects of planning. So it should be no surprise that the symposium garners a 97.8 percent approval rating from its audience. One Health approaches are critical to many complex challenges at the interfaces of human, animal and environmental health. The Utah One Health Symposium opens the doors for those collaborative partnerships.

Development and validation of an evaluation rubric for equine assisted interventions

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Equine assisted interventions (EAI) is a growing field that incorporates the interaction of humans who face mental, physical, emotional, or social challenges and equines for therapeutic purposes. Recreational, physical, mental, social, and/or emotional goals are met through various EAI such as adaptive riding and equine-assisted psychotherapy. As recommended by those in the equine industry seeking to reduce equine-related human injuries, equines should be evaluated prior to participation in EAI. The Professional Association of Therapeutic Horsemanship International (PATH) recommends the use of an unbiased equine assessment tool to conduct this evaluation; unfortunately, there are no validated methods that exist to meet this criterion. Therefore, our group has developed the Equine Basic Ground Skills Evaluation Rubric (EBGSER) to meet this recommendation and will be determining the reliability and validity of this tool. Surveys that incorporate the videos of a horse going through the evaluation criteria will be sent to EAI industry professionals. The collected data will be analyzed to determine intra- and inter-rater reliability. Rubric scores will be correlated with physiological parameters to validate the assessment tool as an unbiased predictor of equine stress. The results of the EBGSER evaluation could lead to a standardized evaluation tool for EAI professionals. An implication of this research is the potential to decrease the occurrence of non-mounted human injuries caused related to equine stress related behaviors.

Consumption of the Total Western Diet promotes Colitis and Inflammation-Associated Colorectal Cancer in Mice

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Consumption of a Western type diet is a known risk factor for colorectal cancer. Our group previously developed the total Western diet (TWD) for rodents with energy and nutrient profiles that emulate a typical Western diet. In prior studies, consumption of the TWD in C57BL6/J mice markedly enhanced gut inflammation and colon tumorigenesis. In this study, our objective was to assess the dynamics of immune and cancer pathway gene expression during the onset and resolution of DSS-induced colitis. Male C57BL/6J mice were fed the standard AIN93G diet or the TWD diet for 45 days; on day 21, mice were provided 1% (w/v) DSS in the drinking water for 10 days. On days 33 and 45, the colitis disease activity index was determined. Also, days 21, 33, and 45, colon mucosa was collected and total mRNA was isolated for gene expression analysis using the NanoString nCounter Mouse PanCancer Immune Profiling Panel, which targets 750 cancer- and immune-related genes. Results of these targeted gene expression analyses point to striking up-regulation of hundreds of genes associated with interferon response, inflammation, innate immunity, adaptive immunity, and chemokines and receptor pathways in mice fed TWD as compared to the standard AIN diet during active colitis. In a pattern that mirrored the persistent elevation in inflammation and mucosal injury observed in our prior longitudinal studies with TWD and DSS exposure, dysregulation of many of these genes persisted through recovery from gut injury, in addition to the stimulation of other pathways, such as B-cell activation and antigen processing. This study is the first to assess the dynamics of immune and cancer pathway gene expression during the onset and resolution of DSS-induced colitis and the first to employ highly multiplexed, direct digital detection NanoString technology for analysis of a colitis transcriptome in mouse colon mucosa. Our observations indicate that consumption of the TWD markedly enhanced colitis, delayed recovery from gut injury, and enhanced colon tumorigenesis likely via broad changes in expression of immune-related genes in the colon mucosa.

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 goals are to understand the immune tolerance mechanisms that take place to establish a successful pregnancy in cattle. In this study, we hypothesize that extracellular vesicles (EVs) modulate the cytokine expression by maternal immune cells. Our previous studies suggest a dysregulation of immune mechanisms during somatic cell nuclear transfer (SCNT) pregnancies. We will use this to model an abortion-prone gestation. The aims of this study are to determine the role of trophoblast EVs in healthy pregnancies established by artificial insemination (AI) and abortion-prone cattle pregnancies established by SCNT. Pregnancies will be collected at 42±3 days. Placental tissue will be digested and cultured. Cell culture supernatant will be collected and EVs isolated with size exclusion chromatography columns. 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 RT-PCR reactions using pro-inflammatory 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 have a more anti-inflammatory phenotype.

Effects of mitochondria on embryonic development following interspecies somatic cell nuclear transfer

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There are only two northern white rhinoceroses living on earth. One current effort to revive the species, researched by reproductive scientists at the San Diego Zoo, involves interspecies cloning: fusing an iPSC from a northern white rhino an with an oocyte from a secondary species in order to produce a northern white rhino embryo, to be developed within a southern white rhino (a sub-species of the white rhino). A more cost effective and risk averse model will be created at USU by the fusion of a bovine oocyte with a caprine donor cell. Bovine oocytes are more easily obtained, and there is a significantly greater number of caprine somatic cells available than have been obtained from previously living northern white rhinos. The embryo will be grown and developed in the uterus of the doe. The primary issue being investigated within this model is mitochondrial heteroplasmy. Xenogeneic mitochondria being present in a single cell – in this case, the embryo – has been shown to cause failure of embryonic growth, cell apoptosis, lack of intracellular protein production, and/or the presence of mitochondrial-based syndromes in the individual created via the SCNT process. To reduce the occurrence of mitochondrial heteroplasmy in the embryo, the oocyte's mitochondria will be at least partially removed, through the use of physical or chemical manipulation, or through editing the oocyte's mtDNA. Supplementation of mitochondria in the somatic cell will assist in ensuring that the embryo has adequate amounts of mtDNA to perform necessary cellular functions, and that the embryo has a greater number of mitochondria from the donor species than from the oocyte. Mitochondrial quantification, via RT-PCR, will assist in determining optimal mtDNA quantities for embryonic growth.

Effects of feeding a novel alfalfa leaf pellet (ProLEAF MAX™) and an alfalfa stem byproduct (ProFiber Plus™) on growth and conception rates of developing dairy heifers

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Alfalfa is a commonly grown forage in the Intermountain west and is often included in rations for dairy animals. 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 ration of dairy heifers on growth, feed efficiency, and conception. Heifers were stratified by weight and farm origin, and randomly allocated to one of three treatment groups (n=8/treatment): control (CON, typical ration that included alfalfa hay), PLM+PFP (a ration that replaced alfalfa hay with PLM and PFP), or PFP (a ration that replaced alfalfa hay with PFP). Heifers were fed for 84 d in individual pens and feed intake and refusals were recorded daily. Weight, hip height, and wither height were recorded every 14 d and blood was collected every 28 d. Additionally, blood urea nitrogen (BUN) and conception rates were measured. Data were analyzed with day as a repeated measure and treatment as a fixed effect. Heifers fed the CON ration had increased ($P < 0.05$) weight gain, hip height, and dry matter intake over time compared to the treatment groups. There was a treatment*time ($P < 0.05$) effect on feed efficiency where heifers fed the PFP had more variable feed efficiency than CON or PLM+PFP. Treatment had an effect ($P > 0.03$) on BUN where CON heifers had increased BUN compared to those fed PFP. No differences ($P < 0.05$) were observed in conception. These data provide insight into how performance of growing heifers is affected when novel alfalfa products are included in the ration and indicate that inclusion of PFP might decrease growth of heifers by decreasing DMI. More research needs to be done to determine whether there is an optimal amount of these products to include in heifer rations.

Maternal Methionine Supply During Late Gestation Alters the mTOR Pathway in Ovine Placenta

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Maternal nutrition and intrauterine environment have substantial effects on fetal development and postnatal health of offspring. We hypothesized that maternal rumen-protected methionine supplementation during late-gestation could affect placenta amino acid transporters via mTOR (Mammalian Target of Rapamycin) pathway. This study aimed to determine the effect of rumen-protected methionine supplementation during the last 30 days of pregnancy on placenta amino acid transporters and the mTOR pathway in the placenta tissue of sheep. Twenty ewes were used in a randomized complete block design experiment. During the last 30 days of pregnancy, ewes were fed a control diet or the control diet plus rumen-protected methionine (0.1% g/kg dry matter intake). After the natural delivery of the placenta, 4 cotyledons from the central area of the placenta were dissected, rinsed and stored at -80°C. Samples were used to assess protein expression for the following proteins: mTOR, SAMTOR, and sodium-coupled natural amino acid transporters (SNATs) 1 and 10. The data were analyzed using a mixed model including the fixed effect of treatment and the random effect of block. Protein expression of mTOR was greater in the methionine group compared with control (0.02 vs. 0.006 C.U., P=0.02). Methionine supply had no effect on SAMTOR protein. Maternal methionine supplementation decreased protein expression of SNAT10 (0.0001 vs. 0.006 C.U., P=0.04) in ewes while there was no treatment effect on SNAT1. Our preliminary data indicated that maternal methionine supply during late gestation affected mTOR but the effect on amino acid transporters was not consistent.

Key words: amino acids, mTOR pathway

Influence of breed on feed intake, health, and growth of dairy heifers in a feedlot setting.

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Recently, a wider variety of breeds of dairy cattle have been incorporated into dairy herds. However, there is little research available detailing how heifers of these different breeds perform in a drylot setting. As such, the objective of this research was to identify differences in feed intake, health, and growth between different breeds of dairy heifers. This study analyzed four different breeds (Holstein (H), Jersey (J), Holstein/Jersey (HJ), and Montbeliarde/Holstein/Swedish Red (PRO); n=6). All heifers were fed the same ration in GrowSafe bunks for 70 days and individual intakes were measured daily. Weight, hip height, and serum were collected in a fasted state on days 0, 35, and 70. Blood urea nitrogen (BUN) was measured in the serum. Rumen fluid was also collected in a non-fasted state one week after the prior collection to analyze volatile fatty acids (VFA). Data was analyzed using the Proc Mixed procedure of SAS with breed and collection day as fixed effects. The H and PRO breeds had increased ($P < 0.05$) weight gain and hip height measurements over the 70 day trial when compared to J and HJ breeds. H and PRO also had increased ($P < 0.05$) ADG and feed efficiency compared to J and HJ breeds. Two of the VFA's, Acetate and Propionate, were also increased ($P < 0.05$) in the H when compared to the J and HJ, which may be at least partially responsible for the increased growth observed in these breeds. Taken together, these data indicate that in a drylot setting, H and PRO breeds of dairy heifers have improved performance compared to J and JH breeds.

Development of a genetically engineered golden Syrian hamster model of beta-thalassemia

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Beta Thalassemia (BT) is the result of dysfunctional or absent hemoglobin subunit beta and is one of the most common hemoglobinopathies in the world with 60,000 affected individuals born annually. Currently, there is no cure and limited treatment options. Symptoms often include anemia, low hemoglobin, and systemic inflammation that directly correlates with changes in the lipid profile of affected individuals. Mice are the predominant model for studying BT but do not share a similar lipid metabolism to humans in addition to being shown as poor models for studying systemic inflammation. Due to golden Syrian hamsters (GSH) having a very similar lipid profile to humans, we hypothesize that a GSH-BT model will more accurately represent the pathophysiology of humans affected by the disease. Using a CRISPR-Cas9 system, we successfully inserted a 17 base pair deletion within the first exon of the predicted GSH hemoglobin beta gene. Using colorimetric analysis, we then measured the total cholesterol and triglyceride levels of wildtype, heterozygous, and homozygous animals. In addition to the lipid profile, we analyzed the red blood cell count and total hemoglobin through the Utah Veterinary Diagnostic Laboratory. Our study revealed hamsters homozygous for the hemoglobin beta deletion mirrored the human disease phenotype for total cholesterol and hemoglobin levels compared to heterozygous and wildtype animals. However, there was no correlation between genotype and triglyceride levels or red blood cell count. These results indicate that further investigation with a larger sample size and age-matched wildtype animals is required to determine if the GSH is a viable model for studying BT.

Young germ cell depleted ovaries in post-reproductive mice and its effect on immune function

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It has previously been shown that young, cycling ovarian transplantation in female mice increased the general health and life span in regard to their post-reproductive health. It has further been hypothesized that this enhancement of health is directly influenced by the somatic cell. To address this hypothesis, transplants of young germ cell depleted and germ cell containing ovaries were performed on female mice of varying age groups. The purpose of this study is to continue to discern the reproductive influence on aging health, specifically in the area of immunological well-being. Control group mice were separated by age and treatment mice were subsequently age matched to receive either germ cell depleted or germ cell containing ovary transplantations. All groups underwent various health span assays including immunoassays using flow cytometric analysis to determine T-cell subset alterations. Data collected from the immunoassays were analyzed with two-factor ANOVA and a Tukey-Kramer post-hoc test to determine any difference between groups. Ratio differences and trends were analyzed between the groups and between central naïve and central memory T-cells. Results showed that the group having received germ cell depleted ovaries displayed a shift in the central naïve to central memory ratio to an increased central naïve population, thus indicating an improvement of immunological function as compared to the other test groups. This may indicate that the ovarian somatic cell participates heavily in the regulation of age-associated health.

Keywords: germ cell depleted (GD); germ cell containing (GC); ovarian somatic cells

Testing Antibiotic Susceptibility of Facultative Veterinary Anaerobes Under Aerobic and Anaerobic Conditions Using Thermo-Scientific Anaerobic Sensititre™ Plates and Thermo-Scientific AnaeroGen™ Technology.

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Anaerobic bacteria continue to threaten both humans and animals, causing infections in various areas of the body due to their opportunistic nature. Discovering more accurate diagnostic tests and treatments for these anaerobes could help to prevent worsening infection and antibiotic resistance in the future. In this study we examined facultative veterinary anaerobes both aerobic and anaerobic conditions and compared their susceptibility using Thermo-Scientific Anaerobic Sensititre™ Plates. After noting growth on the Thermo-Scientific Anaerobic Sensititre™ Plates in aerobic conditions, we grew the anaerobes in anaerobic conditions using Thermo-Scientific AnaeroGen™ and an anaerobic jar. We successfully grew facultative anaerobes in both conditions and saw similar results in sensitivity and resistance when compared across methods. Increased repetition is still needed from this study to elicit more conclusive results, but the preliminary data showed good results when controlling for human error, increased inoculum concentrations, and different antibiotic mechanisms of action. After careful comparison of both growth methods, we saw encouraging results for continued study of facultative anaerobes and obligate anaerobes in the future. By compiling preliminary and experimental data on the antibiotic susceptibility of anaerobes in anaerobic conditions, it could help clinicians to more accurately diagnose infections and prescribe accurate antimicrobials. Antibiotic susceptibility testing of anaerobes in anaerobic conditions could provide clinicians a more accurate way to dose and treat infections, while reducing diagnostic costs.