**Basic Information**

Project No. and Title: NE1727: Influence of ovary, uterus, and embryo on pregnancy success in ruminants

Period Covered: 10/01/2021 to 09/30/2022

Date of Report:

Annual Meeting Dates: 05/17/2022 to 05/18/2022

**Participants**

Michelle Rhoads, Phillip Bridges, Rafael Fissore, Amanda Patterson, Cecily Bishop, Francisco Diaz, Jianbo Yao, Joy Pate, Julio Giordano, Milo Wiltbank, Paul Tsang, Rodolfo Cardoso, Troy Ott, Ronald Butler, Soon Hon Cheong, Vimal Selvaraj, Jennifer Wood, Sarah Moorey, Alvaro Garcia-Guerra, Yi Ren, Joanne Fortune, Dave Townson, Fernando Biase, Bob Dailey, Kamilah Grant (USDA), and Mark Mirando (USDA).

Graduate students: Dallas Soffa, Savannah Speckhart, Jessica Keane, India MacKensie, Jadon Hicks, Becca Cockrum, Abby Maucieri, Adam Beard, Sarah Carr, Katie Peterson, Kirsten Senn, Chase Wilson, and John Maghee.

**Brief Summary of Minutes of Annual Meeting**

(see document with meeting minutes)

**Accomplishments**

Demonstrated that a targeted reproductive management program for first service that combined insemination at detected estrus after automated estrus alerts and timed AI led to similar reproductive performance than an all-TAI program. Also, demonstrated that cows with at least one automated estrus alert during the voluntary waiting period had improved reproductive performance. Thus, automated estrus detection systems can be used to identify cows with different expected level of reproductive success for targeted management.

Demonstrated in an experiment with multiple farms that lactating dairy cows of superior genetic merit for fertility have better reproductive performance than cows of inferior genetic for fertility regardless of the method of submission to insemination.

Evaluated and demonstrated the reproductive and economic value of targeted reproductive management strategies that used genomically enhanced predictions for fertility to identify subgroups of cows for targeted management.

Demonstrate that cows of inferior genetic merit for fertility as determined by genomic daughter pregnancy rate are more likely to have atypical estrous cycles due to a multitude of physiological failures including premature luteal regression, extended luteal phases, anestrus, anovulation, and development of follicular cysts.

Determined the effect of form of Se on the transcriptome of the early luteal phase corpus luteum (CL) with the goal of elucidating form of Se-regulated processes affecting luteal steroidogenesis and function.

Determined effects of form of Se on the development of the bovine conceptus and the endometrium using targeted qPCR on day 17 of gestation, the time of maternal recognition of pregnancy (MRP).

Determined the effect of form of Se on the expression of mRNA encoding selenoproteins in the corpus luteum (CL), and whether the previously reported MIX-induced increase in P4 is the result of increased luteal expression of key steroidogenic transcripts. Overall, we observed that the form of Se provided to cows is reported to affect the expression of mRNA encoding several selenoproteins in the CL, and that the form of Se-induced effects on luteal production of P4 appears to be the result of changes in cholesterol uptake, rather than a direct effect on the expression of steroidogenic enzymes within the CL.

Generated evidence to support the hypothesis of a physiological mechanism, involving increased uterine blood flow, is responsible for maintenance of the CL during the second month of pregnancy in cattle.

The 5-day CO-Synch is an extensively used TAI protocol; however, it requires multiple administrations of prostaglandin F2α (PGF). We conducted a study to evaluate the effect of increasing progesterone (P4) device insertion from 5 to 6 days during a TAI protocol on ovarian dynamics and fertility in beef cows. Cows in the 6-day group had a larger maximum preovulatory follicle diameter and a greater CL volume seven days after ovulation than cows in the 5-day group. Furthermore, cows assigned to the 6-day group had greater estrus expression and pregnancy rate than cows in the 5-day group.

Demonstrated that increasing the length of P4 during a prolonged proestrus CO-Synch protocol by one day improved fertility to ET by reducing pregnancy loss.

Demonstrated that pregnancies per AI (P/AI) on day 35 after TAI were greater for cows presynchronized with a progesterone device and prostaglandin compared to cows presynchronized with prostaglandin alone or cows that did not undergo presynchronization.

Determined the effects of prostaglandin F2alpha (PGF2α) on CCN1 expression in ovarian adenocarcinoma 8 (OVCAR8) cells.

Determined the effects of estradiol (E2) on CCN1 expression in ovarian tumor granulosa (KGN) cells.

Determined the effects of a protein kinase A (PKA) inhibitor (PKI), and peptide 17, an inhibitor of yes-associated protein/transcriptional enhanced associate domain (YAP/TEAD) protein-protein interaction, on CCN1 expression in steroidogenic luteal cells obtained from the 4-day-old bovine corpus luteum.

Determined the effects of the phytoestrogen, Daidzein, on CCN1 and VEGFA expression in steroidogenic luteal cells obtained from the 4-day-old bovine corpus luteum.

Determined the effects of angiotensin II (Ang II) on CCN1 expression in steroidogenic luteal cells obtained from the 4-day-old bovine corpus luteum.

Localized expression of ZIP9 to mural and cumulus granulosa cells in mouse and bovine

Identified ZIP9 localization to the mitotic spindle in granulosa cells.

Provided evidence that pregnancy in dairy cattle is associated with altered expression of molecules related to immune tolerance in peripheral blood leukocytes.

Demonstrated that AMH levels were not different in females by pregnancy status either 1 week before, or on day of AI. Similarly, serum levels of inhibin A were not significantly different by pregnancy status when analyzed pre-breeding, however on the day of AI there was a weak tendency for females who did not become pregnant to have lower levels of inhibin A.

Identified 29 follicular fluid metabolites with a positive relationship with pre-ovulatory follicle diameter and 22 metabolites that had a positive relationship with serum estradiol concentration at the time of GnRH injection to induce ovulation.

Determined that there is a positive relationship between serum estradiol concentration at the time of GnRH injection to induce ovulation and oocyte ATP level ~18 hours later.

Identified 1,387 and 2,765 mRNA transcripts with unique expression and 2,553 and 1,745 mRNA transcripts with upregulated expression in *in vivo* matured bovine oocytes or cumulus cells, respectively. This improves our understanding of oocyte and cumulus function during *in vivo* maturation.

Generated new knowledge highlighting that heat-induced increases in body temperature in lactating dairy cows differentially impacts the cumulus and granulosa cell transcriptome of the periovulatory follicle.

Characterized expression of galectin molecules, a family of lectins with essential functions during establishment of pregnancy in rodents, in bovine and ovine endometrium and conceptus tissues.

Tested and found that bovine galectin-1 has immunomodulatory effects within bovine endometrium related to maternal-conceptus immune tolerance.

Identified progesterone stimulated bovine endometrial epithelial and stroma fibroblast cell specific mRNAs (better understand uterine support of early embryonic development).

Tested and found an advantage of progesterone stimulated bovine endometrial epithelial and stroma fibroblast cell conditioned media on *in vitro* produced (IVP) bovine blastocyst development.

Identified 21 serum metabolites, from blood collected in the pre-ovulatory time period, that differed among beef cows with thin, moderate, and obese body condition scores. This helps understand the impact of body condition on fertility.

Showed that there is a positive relationship of rectal temperature at fixed timed artificial insemination on pregnancy outcomes in beef cattle during a regular breeding season where pregnancy outcomes are expected to exceed 50%.

Demonstrated that Chromium propionate supplementation during the early postpartum period (days 20-44 in milk) tends to increase the number of 6-9 mm ovarian follicles in multiparous Holstein cows. It also increases the mean diameter of follicles in this category.

Reported that early lactation dairy cattle fed a chromium propionate supplement have a greater ratio of plasma progesterone to corpus luteum volume.

Examined the role of cytokines in the initial development of the inner cell mass with blastocyst stage embryos. This served to establish the lowest effective concentration and determined the recombinant human IL6 concentration that is biologically active in bovine embryos.

Developed a CRISPR-Cas9 gene editing strategy that blocks expression of IL6 signal transducer, the predominant signaling component of the IL6 family receptor complex, reduces bovine blastocyst development.

Evaluated the effects of IL6 cytokine family members on bovine oocyte maturation.

Demonstrated that Chromium propionate supplementation during early lactation may protect against a resurgence in uterine inflammation as cows at 44 days in milk experienced no change in uterine polymorphonuclear leukocyte percent when consuming the supplement while control cows exhibited an increase in uterine polymorphonuclear leukocyte percent.

Improved understanding of the mechanisms involved in selection of a single dominant follicle in cattle. This will be key for the rational design of new reproductive management strategies and reproductive biotechnologies.

Discovered the mechanism that produces increased ovulation rate in carriers of the Trio allele. This mechanism may also underlie double ovulation in dairy cattle and in other species. This should allow us to manipulate the ovulation rate in cattle and perhaps other species. We have now determined how to practically utilize Trio to reliably produce bilateral twins in beef cattle. This may be of great practical value to beef cattle producers in the future.

Demonstrated that the corpus luteum is maintained during early pregnancy by suppression of pulses of prostaglandin F2a by interferon-tau, based on our measurements of PGFM surges. We have also discovered that there is a second period of corpus luteum maintenance after Day 25 when interferon-tau secretion ends and PGF pulses are initiated. The resolution of the mechanisms that allow maintenance of the corpus luteum during this second period will be key for reducing pregnancy loss in cattle.

Demonstrated that increasing circulating progesterone can reduce pregnancy loss in lactating dairy cows and in recipients of in-vitro produced embryos. This provides an easy method to reduce pregnancy loss by treatment with either GnRH or hCG.

Identified profiles of gut microbial families that are associated with increased ovarian inflammation and reduced reproductive performance in beef cows.

Determine the mechanistic effects of inflammation and oxidative stress on bovine somatic cell steroidogenesis, oocyte maturation and pre-implantation embryo development.

**Impacts**

Low pregnancy rates in beef and dairy cows result in increased costs to producers associated with increased rebreeding or culling of animals, and reduced likelihood of recouping heifer development costs. Reduced fertility is also a major cause of reduced milk (dairy) and meat (beef) production which impacts the food supply. The focus of the NE1727 multistate project “Influence of ovary, uterus, and embryo on pregnancy success in ruminants”, is to understand how altered ovarian function, impaired oocyte quality, and disruptions of conceptus-uterine interactions contribute to infertility, and devise management strategies that will overcome these factors in order to optimize the chances that animals successfully achieve a pregnancy. To achieve this goal, the following three objectives were performed: (1) Determine the impact of altered ovarian function on reproductive performance; (2) Identify alterations in embryo development and uterine and CL function associated with declining pregnancy establishment; and (3) Identify changes in genetics and reproductive management that lead to improved pregnancy rates. As investigators in NE1727, we have used our collective expertise to broaden the current understanding of the mechanisms by which internal and external forces modify these important physiological processes. Furthermore, this information about core biological processes was used to rationally develop new and innovative tools to increase cyclicity and breeding efficiency, and reduce early embryonic failures. The outcome of these accomplishments was improving reproductive efficiency of both dairy and beef cattle operations in the United States.

**Publications**

Peer-reviewed journal articles reporting research from this project

Crites, B.R., S.N. Carr, J.C. Matthews and P.J. Bridges. 2022. Form of dietary selenium affects mRNA encoding cholesterol biosynthesis and immune response elements in the early luteal phase bovine corpus luteum. Journal of Animal Science. In Press.

Crites, B.R., S.N. Carr, L.H. Anderson, J.C. Matthews and P.J. Bridges. 2022. Form of dietary selenium affects mRNA encoding interferon-stimulated and progesterone-induced genes in the bovine endometrium and conceptus length at maternal recognition of pregnancy. Journal of Animal Science. In Press.

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Rial C., Laplacette A.L., and Giordano J.O.\* 2022. Effect of a targeted reproductive management program designed to prioritize insemination at detected estrus and optimize days to insemination on the reproductive performance of lactating dairy cows. J. Dairy Sci. (In Press)

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*Abstracts for scientific conferences*

Matthews J.C., Q. Li, K.C. Chen and P.J. Bridges. 2022. Sensitivity of pituitary and hepatic selenoprotein transcriptomes to consumption of ergot alkaloids in growing cattle. 12th International Symposium on Selenium in Biology and Medicine.

Webb K., R.J. Trotta, P. Bridges and J.C. Matthews. July 2021. Ad libitum consumption of a 1:1 blend of inorganic:organic Se by steers grazing endopyte-infected tall fescue increases serum prolactin and alkaline phosphatase activity, but not average daily gain. ASAS-CSAS Annual Meetings & Trade Show, Louisville, KY.

Hutchings DW, Elder D, Tsang PCW. 2022. Does the phytoestrogen Daidzein stimulate angiogenic factors in bovine steroidogenic luteal cells? 56th Annual Meeting of the Society for the Study of Reproduction, Spokane, Washington.

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**Theses/Dissertations**

Crites, Benjamin Ryne, "THE EFFECTS OF FORM OF SELENIUM ON THE BOVINE CORPUS LUTEUM, UTERINE ENDOMETRIUM, AND DEVELOPMENT OF THE CONCEPTUS" (2021)”. Theses and Dissertations--Animal and Food Sciences. 135. https://uknowledge.uky.edu/animalsci\_etds/135 https://doi.org/10.13023/etd.2021.424

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Oli, N. 2022. Effects of the estrous cycle and early pregnancy on proportions and functions of peripheral immune cells in dairy cows and heifers. M.S. Thesis. The Pennsylvania State University. (Ott)

Master’s Thesis: “Increasing vitamin D levels to improve fertilization rates in cattle.” Vanessa Peixoto de Souza, M.S., D.V.M. 06/14/2021. Major Professors C. Estill and C. Bishop.

Casey Read. Effects of pre-ovulatory follicle physiological status on oocyte metabolic capacity. (PhD Dissertation, 2022; SEM PhD student).

Mary Ali Oliver. The effect of endometrial cell conditioned media on *in vitro* cultured bovine embryo development. (MS Thesis, 2021; DJM MS student)

Sarah Singleton. Novel postpartum reproductive phenotypes in beef cattle. (Undergraduate Chancellor’s Honor’s Thesis, 2021; SEM undergraduate student)

Alicia Arneson (2021). Evaluating the impact of heat stress and altered glycemic state on plasma gamma-aminobutyric acid (GABA) in lactating Holstein cows (MS thesis).

Mary-Kathryn Harrod-Byrd (2021). Serial measurements of circulating glucose and luteinizing hormone concentrations in lactating dairy cattle (MS Thesis).

Michelle L. Kott (2021). Effects of mid-gestational L-citrulline supplementation to twin-bearing ewes on umbilical blood flow, placental development, and lamb production traits (MS Thesis).

Dallas Soffa (2022). Effects of feed additives on uterine morphology and selected reproductive attributes (MS thesis).

Jacob Stewart (2021). Physiological consequences of exposure to heat stress and the mycotoxin zearalenone (MS thesis).

**Active collaboration within the group**

OSU and WI: evaluated and currently working on projects to evaluate the effect of dose of GnRH at initiation of a 5-day synchronization protocol for FTET.

NY and WI: working collaboratively on projects to determine physiological differences among cows of different genetic merit for fertility.

PA and WV: Samples shared for analysis of molecular regulators of luteal function.

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