

Multistate Research Activity Accomplishments Report

Project Number: NC-1201

Project Title: Methods to Increase Reproductive Efficiency in Cattle

Period Covered: July 20, 2021 to July 12, 2022

Date of This Report: September 11, 2022

Annual Meeting Dates: July 12 and 13, 2022

Meeting Participants: Cushman, Bob (bob.cushman@usda.gov) and Snider, Alex (alex.snider@usda.gov) - USDA Meat Animal Research Center; Fontes, Pedro (pedrofontes@uga.edu) - University of Georgia; Fricke, Paul (pmfricke@wisc.edu) and Khatib, Hasan (hkhatib@wisc.edu) - University of Wisconsin; Larson, Jamie (JLarson@ads.msstate.edu) – Mississippi State University; McLean, Kyle (kmclea10@utk.edu) – University of Tennessee; Perry, George (george.perry@ag.tamu.edu) – Texas A&M University; Richard Pursley (pursleyr@msu.edu) – Michigan State University; Rhodes, Shelly (rhodesm@vt.edu) – Virginia Tech; Smith, George (smithge7@msu.edu) -Administrative Advisor; Lima, Fabio (Falima@ucdavis.edu) – UC Davis; Rosasco, Shelby (srosasco@wyo.edu) – University of Wyoming; Funston, Rick Funston (rfunston2@unl.edu) – University of Nebraska; Christensen, Dempster- University of Nebraska; Gomez Leon, Victor (vgomezleon@k-state.edu) – Kansas State University; Dahlen, Carl (carl.dahlen@ndsu.edu) – North Dakota State University.

Brief summary of minutes of annual meeting:

The 2022 Annual Meeting was held in person in Starkville, MS; however, some members participated remotely through using the WebEx platform. The meeting was called to order by Pedro Fontes. Meeting began with introductions followed by station reports. Members Pedro Fontes (GA), Victor Leon Gomez (KS), Richard Pursley (MI), Jamie Larson (MS), George Perry (TX), Paul Fricke (WI) presented their station reports. First day of meeting was then adjourned by Pedro Fontes. Meeting was called to order on the second day by Pedro Fontes followed by a business discussion. Group elected Kyle McLean and Fabio Lima to serve as chair and secretary, respectively. George Smith provided an update on the successful proposal renewal, as well as an overview of the expectations for reports moving forward. Members Hasan Khatib, Shelby Rosasco, Bob Cushman, Alex Snider, Kyle McLean, Carl Dahlen, and Shelby Rhoades presented their stations reports. The states of MO and NE did not present their station reports; however, a report was submitted and included herein. Meeting was then adjourned by Pedro Fontes.

Accomplishments (Research; Objectives 1 and 2):

Short Term Outcomes of Research from NC-1201 participants:

Beef cattle

- Virus isolation and RT-PCR results were negative in all samples for all animals following vaccination with MLV vaccines (**Texas, USMARC**).
- Vaccination with MLV around estrus negatively influenced luteal cell populations, P4 concentrations, and increased concentrations of IL-6 (**Texas, USMARC**).

- A greater degree of apoptosis in new CL which formed after an abnormal cycle following MLV administration indicates the ability of MLV to induce estrous cycle dysfunction and extend its effects to luteal cell development and function (**Texas, USMARC**).
- Preovulatory estradiol were associated with gene expression in the oviduct that likely influences fertilization and early embryonic development through components of metabolic, growth factor signaling, hormone signaling, and cell adhesion pathways (**Texas, USMARC**).
- Incubation of sperm in capacitation media and flow cytometry analysis for viability and zinc signatures seems promising to estimate in vivo fertility differences amongst bulls (**Texas, USMARC**).
- Effectiveness of pre-synchronization method depends on a cows' physiological status at the beginning of the protocol. The 7&7 protocol increased estrus response compared to PG6d, but there was no difference in pregnancy success (**Texas, Georgia**).
- Luteal color Doppler ultrasonography is an accurate method to diagnose pregnancy earlier than industry-standard practices (**Georgia, Virginia**).
- Heifers that experienced late embryonic mortality had decreased luteal volume on day 20 of gestation compared with heifers that maintained pregnancy and a greater proportion of heifers that experienced late embryonic mortality had luteal cavities on days 20 and 22 of gestation (**Georgia, Virginia**).
- No differences were determined between heifers exposed to the PG-7 7-day CO-Synch + CIDR protocol and those presynchronized with both PGF and a CIDR insert when inseminated with either conventional or sexed semen. (**Texas, Georgia**).
- Exposing cows to an early resynchronization protocol starting on day 15 on gestation (Resynch) results in more synchronized estrus expression after the first TAI compared with natural service (Control). (**Georgia, Virginia**).
- Cows exposed to early resynchronization protocol (Resynch) tended have more TAI pregnancies compared with cows exposed to natural service. (**Georgia, Virginia**).
- Cumulative pregnancy rates during the first 25 days of the breeding season were greater in cows exposed to natural service after the first TAI compared with cows exposed to early resynchronization with color Doppler and a second TAI on day 22 of gestation. (**Georgia, Virginia**).
- Administration of Bovine Appeasing Substance to beef heifers at the initiation of a TAI protocol did not alter temperament, measured as CS and EV, and failed to increase pregnancy rates. (**Virginia, Texas**).
- Suckled beef cows enrolled in the 7&7 TAI protocol had increased estrus expression, but similar pregnancy rate when compared with cows enrolled in the 7-day CO-Synch+CIDR TAI protocol. (**Virginia, Georgia**).
- Developing heifers on a stair-step nutritional program resulted in a similar number of primordial follicles in beef heifers, differing from results of previous research which has suggested development of heifers utilizing a stair-step nutritional regime resulted in a larger ovarian reserve. The impact of specific nutrients and timing of supplementation on ovarian function and physiology in beef heifers needs to be further evaluated. (**Wyoming, Nebraska**).
- A longer MGA feeding period did not significantly alter timing of expression of estrus or pregnancy rates resulting from AI when using the MGA-PG protocol (**Missouri, Nebraska**).
- Nutritional plane did not appear to impact the morphological abnormalities seen in spermatozoa of mature bulls (Tennessee).
- Protein supplementation can impact presence and overall profile of a variety of cytokines in uterine fluid during heifer development (Tennessee).
- Concentration of amino acids in uterine fluid is impacted more by pubertal status than protein supplementation (Tennessee).

- Growth rate can impact presence and overall profile of a variety of cytokines in uterine fluid during heifer development (Tennessee).
- Continue to elucidate the bacterial and immunological environment associated with the establishment of pregnancy (Tennessee).
- Results indicate that performing FTAI later following the 14-d CIDR®-PG protocol increases the proportion of heifers that express estrus and are serviced with sex-sorted semen, but does not improve conception rates of estrous heifers inseminated at later timepoints. (Missouri)
- The GnRH-based 7 & 7 Synch protocol, involving PG administration and P4 device treatment seven days prior to GnRH administration, resulted in an enhanced P/AI compared to estradiol-based protocols and thus may be an effective option in locations where estradiol products are (or may soon be) unavailable for use in synchronization programs from a regulatory standpoint. (Missouri)
- Cattlemen may consider a reduced number of bulls needed for natural service breeding after FTAI, which can decrease bull related costs and increase the economic feasibility of adopting FTAI protocols (Virginia)
- Ipsi and contra-lateral uterine horns microbiome analyses show similar beta diversity indexes between horns on day 15 of the estrous cycle; however, there were differences in microbial community between uterine horns (Georgia).
- Differences in follicular development and behavioral estrus in synchronized Brahman females may be due to seasonality, endogenous progesterone production, and potential differences in sensitivity to GnRH and progesterone (Texas).
- The lateral flow pregnancy test compares very well to transrectal ultrasonography for diagnosis of pregnancy status. To decrease the likelihood of false positive results, the test should be utilized at greater intervals post calving as false positive results decreased as postpartum interval increased (Texas).
- Preovulatory estradiol may be eliciting an effect on early uterine attachment or alter histotroph in a way that better prepares the conceptus to transition its nutritional source to choriovitelline thus improving pregnancy maintenance through day 30 (Texas).
- Estrus expression prior to d 0 may enhance earlier attachment of the fetal/maternal interface, and supplemental E2 on d 0 may improve attachment by d 26 (Texas).
- DAG1 and SERPINA5 do not seem to be a putative fertility marker. In vitro embryo production is not a good predictor of SCR, since High-SCR and Low-SCR bulls may have Good or Poor in vitro embryo developments (Texas).
- Prenatally stressed Brahman bulls do not have an obvious problem to fertilize but may produce sperm with epigenetic modifications that hinder blastocyst formation (Texas).

Dairy cattle

- Characterized that approximately ¼ of dairy cows with conceptus attachment had pregnancy loss and 20% had pregnancy loss prior to d 35 post-AI. These are significant and novel findings considering these are losses that were not understood until now. (Michigan)
- Characterized that lactating dairy cows that have conceptus attachment \geq d 22 post-ovulation have a 60 % chance of pregnancy loss. (Michigan)
- Inducing new accessory CL in late 1st follicular wave development to increase progesterone (P4) during elongation did not improve conceptus attachment or decrease time to conceptus attachment. (Michigan)
- Increasing P4 during elongation appeared to reduce the percent of cows with conceptus attachment. (Michigan)

- Cows with conceptus attachment after 21 days post-ovulation had greater chances for pregnancy loss compared to cows with earlier conceptus attachment. (Michigan)
- Progesterone was not different in cows with conceptus attachment that maintain pregnancy vs. cows with conceptus attachment and pregnancy loss until day 27 after ovulation when a few cows initiated luteolysis. (Michigan)
- The fertility program Double Ovsynch did not result in different time to conceptus attachment compared to cows receiving AI following detected estrus. (Michigan)
- Cows that had conceptus attachment after mean time to conceptus attachment had a greater likelihood of pregnancy loss compared to cows that attached earlier. (Michigan)
- Cows receiving AI following detected estrus had greater chance for pregnancy loss compared to cows receiving Double Ovsynch. (Michigan).
- Increasing the dose of GnRH, gonadorelin hydrochloride, at first GnRH of breeding OvSynch from 2 ml (100 µg) to 4 ml (200 µg) increased P/AI, but the magnitude of the effects was contingent on parity with a robust response among multiparous cows. (California)
- The benefits of ovulation at first GnRH of the breeding OvSynch of cows receiving a Double OvSynch for a higher dose of 4 mL of gonadorelin hydrochloride differ conditionally on parity and P4. (California)
- The effects on 250 ug of NGF i.m. purified from Bull seminal plasma in lactating Holstein cows were contingent on parity for progesterone, and no improvement in ISG relative abundance and P/AI were observed. (California, Virginia)
- Inhibin A at prostaglandin of the OvSynch differed according to parity and GDPR, but neither Inhibin A nor B could be used to predict ovulation and pregnancy per AI.
- Anti-Mullerian hormone was only affected by parity and could not be used to predict GDPR, ovulation, or pregnancy. (California)
- The use of color Doppler ultrasonography when discriminating between luteal and follicular ovarian cysts in dairy cattle resulted in higher diagnostic accuracy when compared to using B-mode ultrasonography alone. (California)
- NGF from seminal plasma may interact directly with the theca and granulosa cells of the bovine pre-ovulatory follicle to stimulate testosterone production, which may be secondary to theca cell proliferation. Additionally, decreased FGF2 expression in NGF-treated follicle wall cells suggests hastened onset of follicle wall cellular remodeling that occurs during early luteal development. (California)
- Allocation of sexed and beef semen to inseminate U.S. Holsteins and Jerseys increased over the past 3 years particularly in larger herds that allocate semen type differentially based on parity and service number. (Wisconsin)
- Lactating Jersey cows submitted to a Double-Ovsynch protocol for TAI at 1st service had more P/AI than cows inseminated after synchronization of estrus. (Wisconsin)
- Although pregnancy outcomes did not differ between treatments, timed-embryo transfer (TET) cows had more pregnancy losses than timed artificial insemination (TAI) cows, and multiparous cows had more pregnancy losses than primiparous cows. (Wisconsin)
- Future investigations to increase embryo survival after TET are critical to optimize the efficiency of embryo transfer in lactating Holstein cows. (Wisconsin).
- Short-term, high-dose chromium supplementation of dairy cattle is beneficial for postpartum uterine health and progesterone production (Virginia)

Outputs:

This group combined for 128 publications including 51 peer-reviewed journal articles last year. Of the journal articles published, 15 are collaborative projects from at least 2 institutions represented in NC1201. Additionally, several papers are from institutions with multiple investigators that are member of this group. Articles that are not peer-reviewed focused on extending research findings to stakeholders through the U.S. and the world and contain numerous collaborations among the group that should progress to peer-reviewed publication status.

Activities:

- The Missouri Show-Me-Select Replacement Heifer Program continues to provide an educational conduit for beef producers and allied industry in Missouri, resulting in the enhanced adoption of reproductive and genetic/genomic technologies across the state.
- The FB Miller Internship at the University of Missouri was successful in training students in reproductive management by providing the knowledge, tools, and competency for them to become future leaders in the genetic improvement of beef and dairy herds.
- The University of Missouri dual DVM-MS program provided further training and expertise in the area of beef cattle reproduction to veterinary students that intended to offer specialized reproductive services as part of a food animal practice.

Milestones: This group of reproductive biologists and reproductive management specialists have now become the leaders in the creation of new knowledge relating to the manipulation of ovarian development to enhance fertility and reproductive management of cattle and the translation of that information to key stakeholders in the U.S. This is evident in the combination of the numbers of publications, presentations, and grants funded in this report. While the overarching goal of multistate research is collaboration across stations (highlighted in this report), projects conducted independently at various stations also contribute collectively to the body of knowledge driving development of new reproductive management programs applied within beef and dairy industries.

Impacts:

Key grants funded during the reporting period (only collaborative group efforts are included):

Year	Value	Funding Agency	State Included
2021	150,007	Kemin	CA
2022	20,000	California Food Animal Health - Hatch	CA
2022	20,000	California Food Animal Health - Hatch	CA
2020-2022	149,597	California Dairy Research Foundation	CA
2022	92,695	USDA NIFA	CA
2022	650,000	USDA NIFA	GA, TX
2022	299,552	USDA NIFA	MO
2022	150,000	Michigan Alliance for Animal Agriculture	MI
2021	154,000	Vetagro	MI
2021	150,000	Boehringer Ingelheim Animal Health	MI

2021	150,000	Michigan Alliance for Animal Agriculture	MI
2022	299,941	USDA NIFA	TX, USMARC
2022	499,991	USDA NIFA	TX
2022	143,761	HelixNano	TX
2022	40,905	ABS Global	TX
2021	500,000	USDA NIFA	VA, TX

Accomplishments (long term industry impacts and communication of results; Objective 3)

Activities:

This group combined for more than 71 presentations of those data at either industry or academia events during the reporting period. The audiences included academicians, veterinarians, farm consultants and of course producers and their management teams. Additionally, three members of this group (V. Mercadante, Virginia Tech; S. Rosasco, University of Wyoming; G. Perry, Texas A&M) serve as members of the Beef Reproduction Task Force. This 9-member group serves to promote and disseminate information to beef cattle producers, academics, and veterinarians to improve cattle reproduction. Through a yearling meeting (Applied Reproductive Strategies for Beef Cattle) and monthly webinars, members of this group shared new findings and technologies with interested audiences from around the world with significant outreach.

Milestones:

Members of the Beef Reproduction Task Force participated in a virtual Applied Reproductive Strategies in Beef Cattle meeting in November 2021. This meeting had 1,871 total registrations from around the world. Recordings for this meeting have over 3,000 views online. Additionally, a total of 5 monthly webinars were offered by this group in 2021-2022 and have reached over 1,600 viewers. Moreover, the YouTube channel for the Beef Reproduction Task Force created in 2020 has now over 15,700 views.

Indicators:

- Dairy cow milk production per cow continues to increase across the United States. Efforts of NC-1201 participants efforts, for example in the state of Michigan, has led to the greatest average milk production per cow ever recorded in the United States at 26,340 lbs. per year in one state.
- Reproductive efficiencies have a much greater impact on average milk production per cow than any other management area. Fertility programs developed by members increased the percent of cows that became pregnant at the ideal time in lactation so low or no milk production in late gestation was minimized.
- Development and continued refinement of estrus synchronization programs by this group have facilitated the use of artificial insemination and embryo transfer in the beef industry. An indicator of the impact of these reproductive programs is the continued increase in beef semen sales over the last decades. Greater adoption of these reproductive technologies also contributed to the ongoing U.S. herd genetic improvement. An indicator of the ongoing beef herd genetic improvement is the unprecedented increases in carcass quality observed in the beef industry.

Publications:

Refereed Journal Articles

1. Byrd MKH, Arneson AG, Soffa DR, Stewart JW, Rhoads ML. Human continuous glucose monitors for measurement of glucose in dairy cows. *J Dairy Sci Comm.* 2022, 3(1): 78-83. <https://doi.org/10.3168/jdsc.2021-0147>.
2. Hardin KN, Roqueto dos Reis B, Dias NW, Fiske DA, Mercadante VRG, Rhoads ML, Wilson TB, White RR. Growth and reproductive responses of heifers consuming endophyte-infected tall fescue seed with or without sodium bicarbonate supplementation. *App An Nutr.* Accepted.
3. Stewart JW, Arneson AG, Byrd MKH, Negron-Perez V, Newberne H, White RR, El-Kadi S, Ealy AD, Rhoads RP, Rhoads ML. Comparison of production-related responses to hyperinsulinemia and hypoglycemia induced by clamp procedures or heat stress of lactating dairy cattle. *J Dairy Sci.* Accepted.
4. Wooldridge LK, Keane JA, Rhoads ML, Ealy AD. Bioactive supplements influencing bovine in vitro embryo development. *J Anim Sci.* 2022 Jul 1;100(7):skac091. doi: 10.1093/jas/skac091.
5. Timlin, C. L.*, N. W. Dias*, L. Hungerford, T. Redifer, J. F. Currin, V. R. G. Mercadante. A retrospective analysis of bull:cow ratio effects on pregnancy rates of beef cows previously enrolled in fixed-time artificial insemination protocols. 2021. *Transl. Anim. Sci.* 2021.10.1093 <https://doi.org/10.1093/tas/txab129>
6. Hubner A, IF Canisso, PGM Peixoto, WC Meireles Filho, BM Aldridge, FS Lima. 2022. Randomized controlled trial of propylene glycol and cyanocobalamin treatment on naturally occurring disease, milk yield, and reproduction of dairy cows with concurrent hyperketonemia and hypoglycemia. *J Dairy Sci.* (Accepted).
7. Hubner A, IF Canisso, PGM Peixoto, WC Meireles Filho, BA Aldridge, P Menta, VS Machado, FS Lima. 2022. Characterization of metabolic profile, health, milk production, and reproductive outcomes of dairy cows diagnosed with concurrent hyperketonemia and hypoglycemia *J Dairy Sci.* (Accepted).
8. Hubner A, IF Canisso, PGM Peixoto, WM Coelho Jr., LL Cunha, L Ribeiro, S Crump, FS Lima. 2022. Effects of Nerve Growth Factor- β on progesterone concentration, conceptus development, and pregnancy outcomes. *J Dairy Sci.* 105:6353-6363.
9. Monteiro H, Z Zhou, PMG Peixoto, MS Gomes, ECR Bonsaglia, IF Canisso, JL Stewart, FC Cardoso, FS Lima. 2022. Rumen and lower gut microbiomes relationship with feed efficiency and production traits throughout the lactation of Holstein dairy cows. *Sci Reports* 12:4904. <https://doi.org/10.1038/s41598-022-08761-5>
10. Garzon A, G Having, FS Lima, N Silva-del-Rio, F Samah, RV Pereira. 2022. Defining clinical diagnosis and treatment of puerperal metritis in dairy cows: A scoping review. *J. Dairy Sci.* 105:3440–3452.
11. Stewart JL, G Liying, JA Flaws, VRG Mercadante, NW Dias, IF Canisso, FS Lima. 2022. Effects of nerve growth factor- β from bull seminal plasma on steroidogenesis and angiogenic markers of the bovine pre-ovulatory follicle wall cell culture. *Front Vet Sci* 8 786480. DOI: 10.3389/fvets.2021.786480. (Virginia, California)
12. Hubner A, IF Canisso, PM Peixoto, AJ Conley, FS Lima. 2021. Effect of GnRH administered at the time of AI for cows detected in estrus by conventional estrus detection or an automated activity monitoring system. *J Dairy Sci* 105(1):831-841. doi: 10.3168/jds.2021-21011.
13. Oliveira EB, FC Ferreira, VY Jaesung, I Tagkopoulos, KN Galvão, N Silva-del-Rio, RVV Pereira, VS Machado, FS Lima. 2021. Integration of statistical inferences and machine learning algorithms improves prediction for metritis cure in dairy cows. *J Dairy Sci* 104:12887-12899. doi: 10.3168/jds.2021-20262
14. Andersen CM, Bonacker RC, Smith EG, Spinka CM, Poock SE, and Thomas JM. Evaluation of the 7 & 7 Synch and 7-day CO-Synch + CIDR treatment regimens for control of the estrous cycle

among beef cows prior to fixed-time artificial insemination with conventional or sex-sorted semen. *Animal Reproduction Science* 2021;235:106892.
<https://doi.org/10.1016/j.anireprosci.2021.106892>.

15. Ketchum JN, Bonacker RC, Andersen CM, Smith EG, Stoecklein KS, Spinka CM, and Thomas JM. Evaluation of later timepoints for split-time artificial insemination when using sexsorted semen among beef heifers following the 14-d CIDR®-PG protocol. *Animal Reproduction Science* 2021;224:106649. <https://doi.org/10.1016/j.anireprosci.2020.106649>.
16. Ault-Seay, T. B., R. R. Payton, S. E. Moorey, K. G. Pohler, F. N. Schrick, E. A. Sheperd, B. H. Voy, K. H. Lamour, D. J. Mathew, P. R. Myer, and K. J. McLean. 2022. Endometrial Gene Expression in Response to Lipopolysaccharide between Estrous Cycle Phases and Uterine Horns in Cattle. *Front. Anim. Sci.* (Accepted)
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18. Harrison, T. D.*, E. M. Chaney*, K. J. Brandt*, L. G. Schneider, L. G. Strickland, R. R. Payton, F. N. Schrick, and K. J. McLean. 2022. The effects of differing planes of nutrition and adiposity on seminal and physiological characteristics of spermatozoa in mature beef bulls. *Translational Animal Science*.
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21. Menegatti Zoca, S., E. J. Northrop-Albrecht, J. A. Walker, R. A. Cushman, and G. A. Perry. Proteomics dataset of epididymal fluid, seminal plasma, and proteins loosely attached to epididymal and ejaculated sperm from Angus bulls. *Data Br.* 42:108150 doi: doi.org/10.1016/j.dib.2022.108150. (Texas, USMARC)
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26. Northrop-Albrecht E. J., J. J. J. Rich, R. A. Cushman, R. Yao, X. Ge, and G. A. Perry. 2021. Influence of conceptus presence and preovulatory estradiol exposure on uterine gene transcripts and proteins around maternal recognition of pregnancy in beef cattle. *Mol. Cell Endocrinol.* 540:111508.. DOI: 10.1016/j.mce.2021.111508 (Texas, USMARC)
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None in 2022.