Reproductive performance of beef heifers following timed-artificial insemination: the role of temperament and acclimation to human handling and the handling facility

by

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Abstract

A goal of beef cattle production is for each cow to produce one calf per year. This standard is vital for the efficiency of cow-calf operations. Achieving this goal requires strategic management of heifer development to optimize reproductive success in their first year. The success or failure of a heifer to become pregnant early in her first breeding season affects her performance in the subsequent year. Furthermore, heifers that calve early in their first calving season stay in the herd longer and wean heavier calves than those that calve later. The timing of a heifer's first parturition can influence her productivity for up to the next 6 calving seasons, ultimately impacting her ability to produce one calf per year. Therefore, becoming pregnant early in her first breeding season is of great importance. An effective management strategy to increase the number of heifers that become pregnant early in the breeding season is to implement an estrus synchronization (ES) protocol and timed artificial insemination (TAI). However, the success of ES and TAI can be influenced by many factors, including heifer temperament. Previous studies have demonstrated that heifers with excitable temperaments have greater plasma cortisol concentrations and decreased conception rates to TAI compared with calm heifers. However, plasma cortisol concentrations decrease as the handling events of an ES protocol progress. Moreover, acclimation to human handling and the facility has been shown to improve reproductive outcomes following ES and TAI in cows and natural service breeding in heifers. The objectives of this study were to investigate the effects of acclimating heifers to the human handling and the facility during the handling events of an ES protocol on temperament and conception rates to TAI. We hypothesized that acclimating heifers to human handling and the facility during the handling events of the ES protocol would improve temperament and lower plasma cortisol concentrations by TAI, and acclimated heifers would have greater conception rates to TAI than non-acclimated heifers. Before enrollment in the study, 622 *Bos taurus* yearling commercial beef heifers from five herds in the spring of 2023 and three herds in the spring of 2024 (reported as 8 locations in total) were evaluated for reproductive tract score (RTS), chute score (CS), and exit velocity (EV). Heifers at each location were stratified based on CS and RTS to be acclimated to human handling and the facility during the handling events of the ES (TRT; n = 307) or serve as control (CTRL; n = 315). They were tagged according to treatment and managed as one group at each location. All heifers were enrolled in the 7-day CO-Synch + CIDR ES protocol and received TAI. The CIDR was inserted on d 0 of the study, removed on d 7, and TAI occurred on d 10. Before the ES protocol handling events (d 0, 7, and 10), TRT heifers were sorted off and acclimated to the handling facility by moving them through the tub, ally, and chute without restraint. After acclimation, they were commingled with CTRL heifers and returned to the facility for the ES event of that day. To assess temperament, CS and EV were collected for all heifers on d 0, 7, and 10 of the study. Additionally, blood samples were collected for 120 heifers from 2 locations, to analyze plasma cortisol concentrations (d 0, 7, 10). Estrus detection patches were placed on d 7 and scored on d 10. Pregnancy status was determined between 40 days and 91 days post-TAI by transrectal ultrasonography. No treatment effects were observed for RTS (P = 0.78), CS (P = 0.99), or EV (P = 0.87) on d -10. Exit velocities decreased as the protocol progressed for all heifers (P < 0.0001) but did not differ based on treatment (P = 0.27). Additionally, no differences were observed for estrus patch scores (P = 0.46) between treatments. There were no differences in cortisol concentrations between TRT and CTRL heifers (P = 0.64). However, TRT heifers had decreased CS on d 7 (P = 0.01) and d 10 (P = 0.01) when compared to CTRL, and greater conception rates to TAI (54% for TRT and 45% for CTRL; P = 0.02). These results suggest that acclimating heifers to human handling and the facility during

the handling events of the 7-day CO-Synch + CIDR ES protocol led to improved chute scores, indicating a potential improvement in temperament, and effectively improved conception rates to TAI.

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Chapter 1 - Literature Review

Bovine Estrous Cycle

Hypothalamic-Hypophyseal-Gonadal-Uterine Axis

The bovine estrous cycle consists of two distinct phases, each defined by the dominant ovarian structure present during that phase. It is regulated by several hormones through intricate feedback loops. The cycle is on average 21 days in length and begins at estrus (day 0) and ends at the onset of the subsequent estrus (reviewed by Forde et al., 2011). The two phases of the estrous cycle, follicular and luteal, can be further divided into four stages. The follicular phase consists of proestrus and estrus (Forde et al., 2011). The luteal phase is made up by metestrus and diestrus. The phases of the cycle are regulated by the type and relative abundance of steroid hormones, primarily estradiol and progesterone.

The hypothalamus is a small and complex region of the brain that is key in regulating homeostasis through endocrine function (Hansel and Convey, 1983). It is made up of clusters of nerve cell bodies. These clusters in the preoptic area (POA) and the arcuate nucleus (ARC) have axons that extend into the pituitary stalk and terminate on blood vessels located there (Leshin et al., 1988). This portal system is known as the hypothalamic-hypophyseal portal system, as it delivers blood from the hypothalamus to the anterior pituitary (adenohypophysis; Hansel and Convey, 1983). The anterior pituitary is a small endocrine gland located within the cranial cavity, directly inferior to the hypothalamus (Hansel and Convey, 1983).

The POA and ARC are primarily responsible for the synthesis and secretion of gonadotropin releasing hormone (GnRH; Leshin et al., 1988; Clarke et al., 2001). This neuropeptide hormone is released into the hypothalamic-hypophyseal portal system and delivered to its target cells within the anterior pituitary, the gonadotroph cells (Kakar et al., 1993). The gonadotroph cells of the anterior pituitary are the source of synthesis and secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH; Schally et al., 1971). The hormones GnRH, LH, and to a lesser degree, FSH, are released in a pulsatile fashion (Clarke and Cummins, 1982; Adams et al., 1992). Recent research has identified additional regulatory factors involved in this process, including the neuropeptide kisspeptin (Garcia-Galiano et al., 2012). Neurons in the POA and ARC that synthesize the neuropeptide kisspeptin mediate GnRH secretion with GnRH neurons having membrane bound G protein coupled receptors for kisspeptin (Garcia-Galiano et al., 2012).

Estrus is the period of reproductive receptivity and can be observed visually through changes in behavior. Notably, behaviors such as standing to be mounted, vocalization, restlessness, secretion of clear vaginal mucous, and congregation can be expressed by the cow in estrus (Reith and Hoy, 2018). These behaviors are driven by elevated concentrations of estradiol (Boer et al., 2010). Under positive feedback of estradiol, the POA of the hypothalamus secretes GnRH pulses that are high in both amplitude and frequency, leading to the LH surge needed to induce ovulation of the large antral follicle (Moenter et al., 1991). In the cow, ovulation occurs typically around 31 hours after the onset of estrus, in early metestrus (White et al., 2002).

Metestrus is a period of transition, characterized by the formation and growth of the corpus luteum (CL) from the ovulated follicle, and the start of progesterone secretion from the CL. Under influence from progesterone, GnRH neurons in the POA are suppressed and GnRH returns to basal levels of secretion from the ARC (Kesner et al., 1982). Once the CL is fully functional, it maintains progesterone secretion. This stage is known as diestrus. During diestrus, progesterone not only suppresses estrous behavior through negative feedback on the hypothalamus, but promotes endometrial secretions, creating a habitable environment for early embryonic development (Brooks et al., 2014). Diestrus ends with luteolysis, or the regression of the CL. The hormone responsible for luteoysis is prostaglandin $F_{2\alpha}$ from the uterine endometrium. With the regression of the CL and decrease in progesterone, negative feedback on POA of the hypothalamus is lifted, prompting the start of proestrus.

During proestrus, antral follicles either die or mature leading to typically one dominant follicle. With the growth of the dominant follicle, there is an increase in estradiol concentration in the blood (Evans and Fortune, 1997). Estradiol is a steroid hormone derived from cholesterol. Estradiol stimulates the release of GnRH from the POA of the hypothalamus and it is proposed that it also works upstream of GnRH as well, on kisspeptin neurons (Smith et al., 2009). In response to GnRH, LH and FSH are secreted from the anterior pituitary through a positive feedback loop driven by estradiol (Kesner et al., 1982). Essentially, as estradiol increases, so does GnRH, and so do the gonadotropins that "feed" the antral follicle that produces estradiol.

It should be clarified that while GnRH pulse frequency directly mediates the release of LH, FSH is controlled in a slightly different manner. Estradiol and inhibin from large antral follicles have an inhibitory effect on FSH secretion (Kaneko et al., 1993; Cupp et al., 1995). Thus, while the pulsatile release of GnRH and LH continues into estrus, release of FSH does not (Cupp et al., 1995). In this way, hormones produced and secreted by the hypothalamus, pituitary, ovary, and uterus work together dynamically during the bovine estrous cycle.

Follicular Dynamics

The earliest stages of follicular development, from activation to secondary stage, are gonadotropin-independent (McNatty et al., 1999), difficult to study, and are not well understood. The development and use of transrectal ultrasound techniques, however, have allowed for better tracking, measuring, and research of individual antral follicles that are greater than 3 mm in diameter. Antral follicles are ovarian follicles that have a fluid filled cavity (antrum; reviewed by Rodgers and Irving-Rodgers, 2010). The follicle is made up of an outer layer of cells known as theca interna cells. This layer is separated from a more internal layer known as the granulosa cell layer by a basement membrane. Granulosa cells are the source of estradiol (Fortune, 1986) and inhibin (Henderson and Franchimont, 1981). The fluid in the antrum is known as follicular fluid and supports the maturation of the oocyte (Romero-Arredondo and Seidel, 1994), also housed within the antrum of the follicle.

Follicular dynamics refers to the continuous cycle during which antral follicles grow and then regress, ultimately leading to the preovulatory follicle. During a single estrous cycle, most cows have 2 or 3 waves of follicular growth (Rajakoski, 1960; Sirois and Fortune, 1988; Knopf et al., 1989), with the preovulatory follicle emerging from the last wave. Follicular dynamics consists of four processes: recruitment, selection, dominance, and atresia. During recruitment, a group of small antral follicles begins to grow and secrete a minor amount of estradiol. At this point, follicles grow at a similar rate, this period is sometimes referred to as the common growth phase (Lopez et al., 2005). Throughout the process of selection, most small antral follicles undergo atresia, while a few continue to grow. Near the end of the common growth phase, divergence of the selected follicles into either dominance or subordination occurs (Lopez et al., 2005). These large follicles produce estradiol and inhibin in great enough concentrations to suppress FSH from the anterior pituitary (Adams et al, 2008). In turn, growth of small antral follicles is ceased leading to atresia and no new follicles are recruited.

The fate of the dominant follicle is determined by the level of progesterone from the corpus luteum. If progesterone is elevated, the dominant follicle quits growing and undergoes atresia and a new wave emerges (Bergfelt et al., 1991). This is the case for the first follicular wave during diestrus. Likewise, it has been reported that removing a dominant follicle via follicular ablation resulted in a surge of FSH and a new follicular wave (Bergfelt et al., 1994). However, when progesterone is reduced as it is following luteolysis, the growth of the dominant follicle and its production of estradiol continues (Araujo et al., 2009). With the removal of negative feedback on the hypothalamus, increased estradiol production from the preovulatory follicle elicits a rise in GnRH pulse frequency, followed by increases in LH pulse frequency and culminating in the LH surge and ovulation (Kesner et al., 1981).

Dominance is largely depended on growth rate of the follicle (Lopez et al., 2004). Deviation is the point of the greatest growth rate difference between the 2 largest follicles and occurs 2 to 3 days after wave emergence (Ginther et al., 1997). The average diameter of the largest follicle at the start of deviation is 8.5 mm. When this threshold is met, it has been reported that FSH concentrations decrease to a point that can no longer sustain the growth of the other follicles (Ginther et al., 1999). At this point, the dominant follicle has acquired sufficient LH receptors on the surface of its granulosa cells to switch from FSH-dependent growth to LH-dependent growth (Zhenzhong et al., 1995; Adams et al., 2008).

Estradiol production follows a 2-cell, 2-gonadotropin pathway (Dorrington et al., 1975). The binding of LH to its membrane bound receptor on theca interna cells (Zhenzhong et al.,1995) of the follicle initiates the pathway that converts cholesterol into testosterone and androstenedione (another androgen; Fortune, 1986). Testosterone is a lipid-soluble steroid hormone capable of diffusing out of the theca interna cells, across the basement membrane, and into the granulosa cells of the follicle. Granulosa cells have membrane bound FSH receptors on their surface (Zhenzhong et al., 1995). When FSH is bound, the granulosa cells synthesize the enzymes necessary to convert androgens like testosterone into estradiol. The enzyme necessary for the final conversion of testosterone into estradiol is aromatase (CYP19A1; Bao and Garverick, 1998). Estradiol, also a steroid hormone, can leave the granulosa cells and travel through the blood to its target tissues in the hypothalamus and the reproductive tract. The continuation of this pathway, in the absence of negative feedback of progesterone on the hypothalamus, is necessary for estradiol concentrations to reach the threshold for the LH surge (Kesner et al., 1981). The preovulatory LH surge promotes necessary ovarian events that lead to ovulation: specifically, elevated blood flow, breakdown of connective tissue, and contractions of the ovary (Espey, 1980).

Luteinization

Luteinization refers to the morphological and functional changes undergone by the granulosa and theca interna cells, after ovulation, when they form the CL. As its name would suggest, LH is a driver of this transformation. Luteinization is characterized by rapid cellular proliferation, cell differentiation, angiogenesis, and switching from production of estradiol to progesterone. Angiogenesis is the process by which new blood vessels form from existing ones. This process occurs rapidly and extensively during CL formation to support luteinization (Reynolds et al., 2000).

During its formation, the CL doubles in size and cell number every 60 hours (Reynolds et al., 1994). Granulosa cells undergo hypertrophy and develop into large luteal cells (Alila and Hansel, 1984). Theca cells become slightly larger but mainly undergo hyperplasia as they become small luteal cells. Both small and large luteal cells are steroidogenic and have membrane bound receptors for LH. Interestingly though, when LH is bound to its receptor on small luteal cells, the pathway for progesterone synthesis is stimulated, but when bound to its receptor on large luteal cells, the pathway is not stimulated (Niswender, 2002). Furthermore, while left seemingly unregulated, large luteal cells still have the capacity to secrete progesterone (Fitz et al., 1982). The large luteal cells are also responsible for luteal synthesis and storage of large amounts of oxytocin. Luteal oxytocin is a peptide hormone synthesized in secretory granules of large luteal cells (Flint et al., 1990).

The pathway for progesterone synthesis is dependent on basal levels of LH and cholesterol. Low (LDL) and high density (HDL) lipoprotein deliver cholesterol to the luteal cells (Niswender, 2002). When LH is bound to its receptor on the cell membrane, it activates a G-protein that in turn activates adenylate cyclase. From there, ATP is converted to cyclic AMP (cAMP) and the protein kinase A (PKA) second messenger pathway is activated. This leads to cholesterol entering the mitochondria of the cell where it is enzymatically converted to pregnenolone and exits the mitochondria. In the cytoplasm of the cell, pregnenolone is enzymatically converted to progesterone. At this point, progesterone can diffuse across the cell membrane, into the blood and travel to target tissues.

Luteolysis

Effective signaling between the corpus luteum and the uterine endometrium is essential for the success of luteolysis. Luteolysis is the process during which the CL loses the ability to produce progesterone followed by structural regression of the CL tissue. Wiltbank and Casida (1956) demonstrated the necessity for communication between the uterus and the CL for luteolysis. In this study, complete removal of the uterus in the cow allowed the CL to be maintained for up to 154 days. Luteolysis is vital for reproduction as the removal of negative feedback effects by progesterone is necessary for increased GnRH pulse frequency, the LH surge, and ovulation.

The hormone responsible for luteolysis is prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). This 20-carbon fatty acid hormone is secreted by the uterine endometrium and targets the CL as well as the uterine myometrium. The hormone $PGF_{2\alpha}$ is secreted in a pulsatile fashion (Ginther et al., 2009). Amplitude and frequency of $PGF_{2\alpha}$ increase around day 16 of the estrous cycle in the non-pregnant cow (Wolfenson et al., 1985). The most accepted model for the stimulation of $PGF_{2\alpha}$ secretion is that oxytocin of both luteal and pituitary origin are triggers (McCracken et al., 1999).

When stimulated by oxytocin, great concentrations of $PGF_{2\alpha}$ are secreted and leave the uterus via the utero-ovarian vein (Mapletoft et al., 1976). A prostaglandin transport protein is responsible for transferring $PGF_{2\alpha}$ by countercurrent exchange to the ovarian artery (Mapletoft et al., 1976). Exchange of $PGF_{2\alpha}$ occurs on the same side as the CL (ipsilateral; Collins et al., 1966; Inskeep and Butcher, 1966). This allows great concentrations of $PGF_{2\alpha}$ to get to the ovary without dilution in circulation and pulmonary metabolism. When $PGF_{2\alpha}$ reaches luteal cells, it binds to its membrane bound G-protein coupled receptor, mainly found on large luteal cells (Pate, 2018). When bound, it stimulates more oxytocin secretion from the large luteal cells. This additional oxytocin secretion has two paths of action. It can stimulate more $PGF_{2\alpha}$ secretion resulting in a positive feedback loop, and it can stimulate pathways locally to shut down progesterone production as well as pathways for cell death.

When oxytocin binds to its receptor on small luteal cells, it activates protein kinase C (PK-C; Orwig et al., 1994). When the PK-C pathway is activated, it is likely that the process, within the mitochondria of luteal cells, that converts cholesterol to pregnenolone is disrupted, causing a decrease in progesterone production. Moreover, increased oxytocin leads to increased intracellular free calcium, resulting in reduced cholesterol availability and apoptosis, or programmed cell death (Niswender et al., 2007). In addition, the PK-C pathway activates mitogen-activated protein kinase (MAPK) signaling pathways that also lead to apoptosis. It has been hypothesized that immune cells known as cytokines may also play a role in luteolysis (Pate and Keyes, 2001). The specific intra- and intercellular mechanisms for luteolysis are still being investigated.

The use of exogenous prostaglandin injections has been studied for the purpose of inducing premature luteolysis of the CL (Lauderdale et al., 1972; Burfening, et al 1978). The developing CL, however, does not regress in response to $PGF_{2\alpha}$ injection before about day 5 of the estrous cycle (Henricks et al., 1974; Braun et al., 1988). After day 5 or 6, $PGF_{2\alpha}$ does induce luteolysis, this is because the CL has acquired the capacity for luteolysis. Some differences between the developing CL and the developed CL have been described. Notably, the developing CL has no calcium signaling, does not induce an immune response, and there is no mRNA encoding genes for apoptosis (Pate, 2018). While the developing CL does not undergo luteolysis when challenged by $PGF_{2\alpha}$, it is not insensitive to $PGF_{2\alpha}$ (Tsai and Wiltbank, 1998) and has high-affinity $PGF_{2\alpha}$ receptors present (Wiltbank et al., 1995).

Luteal Rescue

The CL is maintained or rescued when an embryo is present (Godkin et al., 1984). The embryo sends signals that prevent luteolysis, this is known as the maternal recognition of pregnancy. In addition, for pregnancy to be maintained, a functional CL producing progesterone is necessary. In this way, the embryo and the CL are dependent on each other for survival. Early studies demonstrated this by removing the conceptus from the uterus and tracking CL lifespan (Betteridge et al., 1978). Maternal recognition of pregnancy in the cow occurs around day 16 of the estrous cycle (Godkin et al., 1984; Farin et al., 1990). At this time, the trophoblastic cells of the blastocyst secrete a protein that signals maternal recognition and extends CL function

(Knickerbocker et al., 1986). These proteins are part of a class called interferons and are specifically known as bovine interferon tau (bIFN-τ) and ovine interferon tau (oIFN-τ). The signal from IFN-τ inhibits oxytocin receptor production in the uterine endometrium. Through blocking production of oxytocin receptors, IFN-τ has an anti-luteolytic effect on the endometrium of the uterus (Spencer and Bazer, 1996). In addition, supplementing high doses of IFN-τ in bovine endometrial culture systems increased prostaglandin E_2 (PGE_2) production, but did not affect $PGF_{2\alpha}$ (Guzeloglu et al., 2004). The effects of PGE_2 are luteotropic and are likely involved in luteal rescue. In ewes, it has been demonstrated that IFN-τ also has action at the level of the CL as well (Bott et al., 2009). Here, IFN-τ upregulates IFN-stimulated genes (ISGs). The effect of upregulation of ISGs results in increased survival gene expression (Basavaraja et al., 2019) and enhanced cellular viability (Basavaraja et al., 2017). While it is not clear whether IFN-τ in the CL facilitates luteal rescue, the CL of pregnant ewes is less sensitive to the $PGF_{2\alpha}$ than that of non-pregnant ewes (Silva and Niswender, 1984).

Artificial Insemination and Timed-Artificial Insemination

Importance of Estrus Detection for Successful Artificial Insemination

Major developments in semen storage, collection, and evaluation in the mid 20th century took artificial insemination (AI) from a topic of research in animal biotechnology to an industry-wide practice of importance (Foote, 2002). The success or failure of AI is greatly dependent on the accurate detection of first standing estrus. The best indication of a cow in estrus is seeing her standing to be mounted by another animal, yet not all animals display this behavior. Hurnik and King reported that 86% of confined beef cattle expressed estrus through standing to be mounted after returning to normal cyclicity postpartum (Hurnik and King, 1987). It is important to observe for secondary indications of estrus such as vocalization, restlessness, secretion of clear vaginal mucous, or congregation. Furthermore, these secondary signs can also indicate that a female is in proestrus and can even be observed in cows that were recently in standing estrus. Estrus detection aids are available such as mounting-activity detectors and tailhead markers; but observation and time spent with the cattle are still the indisputable best ways to accurately detect first standing estrus, even though continuous observation is rarely feasible. Producers should aim for observing cattle twice a day and spending at least 30 minutes to do so each time.

Acute observation is necessary for improving fertility to AI. Intensive management systems where cattle are easy to access, observe, and are handled often are, intrinsically, more conducive to the adoption of AI. This is particularly evident in the dairy industry where AI is the primary method utilized for breeding cattle. In contrast, beef cattle are typically managed in extensive grazing systems, making estrus detection and AI implementation more difficult (Foote, 2002). Despite these challenges, estrus detection remains a key limiting factor in the success of AI for beef cow herds. The development of timed-AI (TAI) protocols, which use pharmacologic methods to synchronize ovulation, has addressed this limitation by allowing females to be bred within a controlled timeframe. Extensive research has been conducted on pharmacologic control of the estrous cycle.

Pharmacologic Control of the Estrous Cycle

The estrous cycle consists of two phases: the luteal phase and follicular phase. Thorough understanding of cycle dynamics and the physiological changes associated with each phase has enabled the development of biotechnology to effectively manipulate the cycle. Pharmacologic control is achieved using exogenous hormones. The application of hormones approved for use in the United States to initiate a new follicular wave, induce premature luteolysis, extend or simulate the luteal phase, and trigger ovulation has been studied extensively.

Initial methods of estrous cycle control were primarily focused on inducing premature luteolysis of the corpus luteum (CL) in efforts to synchronize estrous. This was done through single (Lauderdale et al., 1972) or multiple (Burfening, et al 1978) injections of $PGF_{2\alpha}$. When a single injection of $PGF_{2\alpha}$ was explored by Lauderdale and others, cattle were randomly assigned to one of three groups: a control group that did not receive $PGF_{2\alpha}$, with breeding occurring at estrus detection within 18 to 25 days (Treatment 1), a group that received $PGF_{2\alpha}$ and breeding at estrus detection within 7 days of the $PGF_{2\alpha}$ injection (Treatment 2), and a group that received $PGF_{2\alpha}$ and breeding at 72 and 90 hours post-injection (Treatment 3). It was reported that 88% of cattle that received $PGF_{2\alpha}$ injection were detected in estrus 2 to 4 days later. Conception rates of the cattle in this study to Treatments 1, 2, and 3 were reported as 42%, 30%, and 40%, respectively. One major pitfall to the $PGF_{2\alpha}$-exclusive protocols is that the CL must acquire the capacity for luteolysis. As a result, females that are on day 1 to day 5 of their estrous cycle will not respond well, if at all, to $PGF_{2\alpha}$ and the CL will not undergo luteolysis (Henricks et al., 1974; Braun et al., 1988). Furthermore, for $PGF_{2\alpha}$ to be effective, there must be a functional CL with the capacity for luteolysis present. Cows that are in postpartum anovulatory anestrous as well as heifers that are prepubertal will not respond to $PGF_{2\alpha}$ injections alone (Lucy et al., 2001).

Extensive research has been conducted to maximize response to pharmacologic strategies for estrous cycle control. Notably, the addition of progestins, like a controlled internal drug release (CIDR) device, to the protocol resulted in improved synchronization rates and greater conception rates for beef females when compared to a $PGF_{2\alpha}$ injection alone (Lucy et al., 2001). Utilizing exogenous progestins ensures plasma progesterone levels are greater than 1 ng/mL, delaying estrus and the LH surge until after removed (Perry et al., 2004). Furthermore, it has been demonstrated that progestin treatment can support resumption of luteal function in postpartum anestrous cows (Fike et al., 1997; Perry et al., 2004) and can hasten the onset of puberty in prepubertal heifers (Anderson et al., 1996; Imwalle et al., 1998).

Another key factor to pharmacologic control of the estrous cycle is the ability to control follicular waves, whether it is for initiating the emergence of a new follicular wave, or for the purpose of inducing ovulation. Exogenously induced, through GnRH injection, the gonadotropins LH and FSH surge, turning over the current follicular wave and ovulating dominant antral follicles that are greater than 10 mm (Sartori et al., 2001; Atkins et al., 2010).

Estrus synchronization (ES) and ovulation synchronization (OS) protocols for beef cattle have since been developed that strategically utilize a combination of GnRH, $PGF_{2\alpha}$, and progestins before AI or TAI, respectively. Treating cows with GnRH 7 days before $PGF_{2\alpha}$ was successful in synchronizing 70-83% of cows (Twagiramungu et al., 1995). The estrous synchronization strategy that includes a GnRH injection followed by a $PGF_{2\alpha}$ injection 7 days later, plus a second injection of GnRH administered 48 to 66 hours later at TAI is known as the CO-Synch protocol. The addition of a CIDR to the CO-Synch protocol has been shown to improve synchronization in beef cows by suppressing estrus and the LH surge, allowing for extension or simulation of the luteal phase. In one study, 560 suckled beef cows were randomly assigned to one of two treatments: a CO-Synch protocol without a CIDR and insemination at a fixed time, or a CO-Synch + CIDR protocol with insemination at a fixed time. When comparing the CO-Synch breeding protocol to the CO-Synch + CIDR breeding protocol, there was an 11% increase in pregnancy rates when a CIDR was included in the protocol (48% vs 59% respectively; Lamb et al., 2001). Another study compared the efficacy of the CO-Synch + CIDR with TAI protocol to other protocols that require estrus detection (Larson et al., 2006). The study concludes that the CO-Synch + CIDR protocol results in similar pregnancy rates compared to those requiring estrus detection for beef cows.

Other studies have explored the efficacy of OS protocols and TAI protocols for beef heifers. Lamb and others (2010) reported that the three most reliable TAI protocols include CO-Synch + CIDR, MGA-$PGF_{2\alpha}$, and CIDR Select. It is worth noting that CIDR Select is rarely used anymore, with the 14-day CIDR protocol being more commonly used instead. Regardless, each comes with its unique advantages and disadvantages. One study compared the CO-Synch + CIDR TAI protocol to the CIDR Select protocol (14-day CIDR, GnRH 9 days later, $PGF_{2\alpha}$ 7 days after that, and GnRH and TAI 72 hours after the $PGF_{2\alpha}$ injection). In this study, 217 crossbred replacement beef heifers were assigned within reproductive tract score (RTS), a measurement of reproductive maturity for beef females, and by age and body weight to one of the two treatments (Busch et al., 2007). Heifers that were pre-synchronized with progesterone (CIDR Select protocol) had greater TAI pregnancy rates (62%) compared with those treated with CO-Synch + CIDR protocol (47%). Additionally, heifers in the CIDR Select protocol had comparatively improved estrous synchrony and estrous response than those in the CO-Synch + CIDR protocol. Vraspir and others (2013) compared two long term progesterone-based protocols for beef heifers: melengestrol acetate (MGA) with $PGF_{2\alpha}$ and the CIDR Select protocol. In this study, 1,385 nulliparous angus-influenced yearling beef heifers were randomly assigned to one of the two groups. Heifers had similar pregnancy rates to TAI (MGA=62% and CIDR=61%). Although long term progesterone-based protocols that utilize CIDRs can improve pregnancy rates in beef heifers, they require more time, additional handling events, and increased labor, making them less appealing to many beef producers (Lamb et al., 2006). Pharmacologic control of the estrous cycle has been achieved through extensive research and continuous refinement of estrus and ovulation synchronization protocols. The adoption of these breeding protocols depends on their consistency and effectiveness in improving pregnancy rates, economic feasibility, and the required time and labor commitment.

Recent efforts to improve pregnancy rates to TAI while keeping cost of the protocol, and time and labor commitments in mind have been made. Pre-synchronization treatments with $PGF_{2\alpha}$ or a combination of $PGF_{2\alpha}$ and GnRH are common in the dairy industry to improve the uniformity in stage of the estrous cycle prior to ovulation synchronization and TAI (reviewed by Wiltbank and Pursley, 2014). While the pre-synchronization protocols for dairy cattle are quite intensive, progress has been made in developing a pre-synchronization protocol for beef cattle that requires fewer handling events than those utilized in the dairy industry; one such protocol is the 7 and 7 Synch protocol. This protocol involves a CIDR inserted for 14 days, injections of $PGF_{2\alpha}$ at the time of CIDR insertion and when pulled, as well as injections of GnRH after 7 days post-CIDR insertion and at the time of AI (Bonacker et al., 2020a; Andersen et al., 2020; French et al., 2013). Andersen and others (2020) reported a greater proportion of cows expressing estrus 82% vs 64%) and greater pregnancy rates to TAI (72% vs 61%) when cows were enrolled in the 7 and 7 Synch protocol and received AI with conventional semen compared to those enrolled in the 7-day CO-Synch + CIDR protocol and received AI with conventional semen. While the 7 and 7 Synch protocol involves an additional handling event than the 7-day CO-Synch + CIDR protocol, the improved results may prove to make it a worthwhile effort for beef producers.

Benefits of Estrous Synchronization and TAI

Although artificial insemination has been used in cattle for many decades, beef producers have been more hesitant than dairy producers to adopt AI into their reproductive management strategies. As of 2020, the USDA reported that less than 15% of beef cows and heifers are bred using AI (USDA, 2020). It's been suggested that early studies with poor pregnancy rates may be overshadowing the refined modern protocols we have available today. Years of research have improved breeding protocols, providing a valuable set of tools for beef producers to enhance reproductive efficiency, hasten genetic progress, and contribute to increased profitability.

A goal of beef cattle production is for each cow to produce one calf per year. This standard is vital for the productivity of the beef cattle sector. Achieving this goal requires strategic management of heifer development to optimize their reproductive success in their first year. The success or failure of a heifer to become pregnant early in her first breeding season affects her performance in the subsequent year (Burris and Priode. 1958). Furthermore, heifers that calve early in their first calving season stay in the herd longer and wean heavier calves than those that calve later (Cushman et al., 2013). The timing of a heifers first parturition can influence her productivity for up to the next 6 calving seasons, ultimately impacting her ability to produce one calf per year. Therefore, becoming pregnant early in her first breeding season is of great importance. An effective management strategy to increase the number of heifers that become pregnant early in the breeding season is to implement an OS protocol and TAI (reviewed by Kasimanickam et al., 2025).

A milestone event in the yearlong production cycle of a female beef cow is the timely return to estrus following parturition. After calving, cows enter a period of anestrus and anovulation (Short et al., 1990). To meet the goal of producing one calf per year in a cow-calf production system, cows must overcome postpartum anestrus in a timely manner. While successful uterine involution is essential for endometrial healing, it is not the primary limiting factor for the return of cyclicity, as beef cattle typically do not exhibit estrus before uterine involution is complete (Kiracofe, 1980). Instead, the resumption of normal hormonal patterns, along with factors such as suckling and nutrition, plays a more critical role (Humphrey et al., 1983; Short et al., 1990).

For cows to remain in production, they are expected to return to estrus within 50 to 80 days postpartum (Larson, 2020). For cows experiencing postpartum anestrus, hormonal treatment with progesterone supplementation can expedite the return to estrus (Fike et al., 1997; Rhodes et al., 2003). Therefore, it is beneficial to enroll both cyclic and acyclic postpartum beef cows in ES or OS protocols. However, according to the manufacturer's label, cows must be at least 20 days postpartum before receiving a CIDR.

Rodgers et al. (2012) demonstrated the economic benefits of ES and TAI in a commercial cow-calf setting. In this study, 1,197 suckled beef cows were randomly assigned to either TAI after synchronization of ovulation using the CO-Synch + CIDR protocol or mating by natural service (NS) without synchronization. Cows that received TAI had a greater weaning rate (84%) compared to those that were mated by NS (78%). Additionally, the mean calving date from initiation of the calving season was significantly shorter for TAI compared to NS groups (26.8 d vs 31.3 d respectively). Moreover, when looking at the calving distribution by noncumulative 10-d intervals, a greater percentage of cows receiving TAI calved in the 1st and 2nd 10-d intervals than did NS cows. This resulted in the cows that were in the TAI group weaning heavier calves (193.4 kg) than the NS cows (175.9 kg). Furthermore, in the same study, Rodgers and others conducted a partial budget analysis to study the economic effects of using ES and TAI. There was a $49.14 advantage per cow exposed to TAI compared with NS. The main driver of financial difference was kg of calf weaned per cow exposed to breeding. Other factors contributing to increased returns include older, more uniform calves at weaning due to a shorter calving distribution, resulting in calves of a similar age and weight. Furthermore, when utilizing synchronization and TAI, fewer clean-up bulls are required to cover the remainder of the breeding season (Timlin et al., 2021). Selecting AI sires with favorable expected progeny differences (EPD) for preweaning growth provides further advantages (Johnson and Jones, 2005). The use of AI sires with superior EPDs not only increases calf value but also reduces labor costs associated with dystocia when choosing to breed to calving-ease sires (Johnson and Dahlke, 2016). By leveraging ES and TAI, producers can push genetic progress, leading to increased weaning weights, improved preweaning growth, and lower dystocia rates. Research has consistently shown that ES and TAI improve reproductive efficiency, calf age and weight uniformity, and overall profitability in well managed herds, making them valuable tools for beef producers.

Factors Affecting Fertility to TAI

Nutrition and Body Condition

Nutrition and reproduction are the two most critical factors influencing profitability in the cow-calf industry. Various interactions between nutrients and metabolic factors affect the hypothalamic-hypophyseal-ovarian axis, influencing reproduction, some of which are well understood, while others remain unclear. The relationship between nutrition and reproduction is especially important when assessing factors that impact fertility to timed artificial insemination (TAI). Body condition score (BCS) and certain nutritional interventions, specifically, can play a significant role in improving fertility outcomes in TAI programs.

Body condition score influences response and fertility to TAI in beef cows. One study, designed to investigate the difference in pregnancy rates between OS protocols with TAI and ES protocols with estrus detection and AI, enrolled 2,598 suckled beef cows for synchronization. It was demonstrated retrospectively that each one-unit increase in BCS, within the range of 3.0 to

9.0 (on a 1 through 9 scale), led to an 11.5% increase in the proportion of cows cycling at the start of the treatment (synchronization; Larson et al., 2006). This did not, however, lead to an increase in the percentage of cows that became pregnant to TAI when split into 3 BCS groups (< 5, from 5 to 5.9, and ≥ 6). Another study was conducted to evaluate reproductive and productive performance of beef cows according to BCS at the start of the breeding season (Cooke et al., 2021). Likewise, pregnancy rates to TAI did not differ between cows with a BCS ≥ 5 or BCS<5 at the start of the breeding season. Calving rate, weaning rate, weaning weight, and weaning age, however, were all greater for cows that had a BCS ≥ 5 compared with those with a BCS<5 at the start of the breeding season. In contrast, a study comparing the efficacy of two TAI protocols for Angus cross beef cows (n=1817) found that TAI pregnancy rates were lesser for cows with a body condition ≤ 4 compared with those with a BCS > 5 (on a 1 to 9 scale; Whittier et al., 2013). Moreover, cows that were a BCS > 4 had increased expression of estrus compared to cows with a BCS ≤ 4, but not pregnancy rates in a meta-analysis that was conducted using data from 10,116 beef females in studies that used TAI (Richardson et al., 2016). In contrast, another study reported that pregnancy rates to TAI, following an estradiol and progestin-based synchronization protocol, were greater for cows with a high or moderate BCS compared with those with a low BCS (on a 1 to 5 scale; Nishimura et al., 2018). Pregnancy rates were reported as 57.8%, 59.1%, and 41.5% for high, moderate, and low respectively. While results for the effect of BCS on fertility to TAI directly are mixed, it is likely that the differences in BCS in studies that did not report differences in fertility were minute. There is evidence, however, to support that adequate BCS, and proper nutritional management are important factors for TAI success.

One nutritional management strategy that has demonstrated enhanced fertility to TAI is the supplementation of calcium salts of purified unsaturated fatty acids (PUFA), such as soybean oil, during the early embryonic period (Lopes et al., 2011; Cooke et al., 2014; Pickett et al., 2023). In one study, a total of 554 multiparous, lactating, non-pregnant Angus-influenced cows were enrolled in the experiment (Pickett et al., 2023). On day −10, cows were ranked by BCS and days postpartum (DPP), then allocated into 12 groups (46 ± 4 cows/group) to ensure similar averages of BCS and DPP across groups. Groups were randomly assigned to receive a self-fed low-moisture block (LMB) enriched with calcium salts of soybean oil or a non-enriched LMB control from day −10 to day 100. Cows receiving calcium salts of soybean oil had greater pregnancy rates to TAI (67.2%) compared with the control (59.3%), experienced less pregnancy loss, and weaned greater kilograms of calf weaned/cow exposed to breeding.

Another study investigated the physiologic pathway of calcium salts of soybean oil on pregnancy establishment in *Bos indicus* cows. Beef cows supplemented with calcium salts of soybean oil after AI effectively incorporated omega-6 fatty acids into their endometrium, CL, and conceptus in early gestation (Cooke et al., 2014). In addition, plasma progesterone concentrations were increased significantly and IFN tended to increase in cows supplemented calcium salts of soybean oil after TAI until 19 days post AI. This innovative nutritional management strategy highlights the potential impact of diet on improving fertility outcomes to TAI.

Exhibiting Estrus

Widespread implementation of AI in the beef industry is reliant on three factors: limited handling events, consistent acceptable pregnancy rates, and the elimination of the need to detect estrus (Mercadante and Lamb, 2024). Nonetheless, research has demonstrated that females that exhibit estrus before TAI have enhanced reproductive outcomes. Estradiol levels rise prior to the onset of estrus behavior, the first standing estrus, and ovulation (Perry and Perry, 2008). It is not surprising, therefore, when estrus is exhibited from 24 hours prior to or at TAI, pregnancy rates are increased for cows and heifers (Perry et al., 2005, 2007). In the meta-analysis of beef females enrolled in TAI protocols (Richardson et al., 2016), those that were detected in estrus prior to TAI had a 27% increase in conception rate in comparison to those that were not detected in estrus.

Protocols that include two TAI events (split-time AI; STAI) along with estrus detection have been studied. These protocols are designed to increase the number of females bred after exhibiting estrus, while still having a fixed time for AI to occur for those that don't exhibit estrus. In a study comparing the efficacy of a STAI protocol for cows with the 7-day CO-Synch + CIDR and TAI protocol, expression of estrus and pregnancy rate to AI were not different between the two (Bishop et al., 2016). Current protocols that attempt to breed more females that have expressed estrus, such as STAI protocols, do not seem to yield greater pregnancy rates to AI when inseminating with conventional semen. As reviewed earlier, pre-synchronization and use of the 7 and 7 Synch protocol have, however, proven to be a valid strategy to increase the estrus response (Andersen et al., 2020; Ketchum et al., 2023) and yield greater pregnancy rates to TAI (Andersen et al., 2020). While TAI protocols have effectively eliminated the need to detect for estrus, it is of note that exhibiting estrus prior to TAI is a factor that affects reproductive outcomes, and strategies to enhance the estrus response are being studied.

Suckling Stimulus and Presence of the Calf

The postpartum anestrous interval can be altered for most cows through nutritional and pharmacologic means. One factor that drives reproductive function postpartum is the offspring-to-dam interaction. A foundational study included 34 pregnant Angus cows allocated to one of three groups: suckled, non-suckled intact, and non-suckled mastectomized (Short et al., 1972). In this study, the calves were removed at birth from both non-suckled groups. Cows that were not suckled had a shorter interval from calving to first estrus compared to those that were suckled. Likewise, when calves were weaned early (at 3 or 10 days after birth) postpartum interval to first estrus was shortened compared with cows whose calves were weaned 90 days after birth (Bellows et al., 1974).

While the presence of the calf and suckling stimulus affects the postpartum interval, it also affects fertility to TAI protocols. Geary and others (2001) demonstrated this by exploring the differences in conception rate to the Ovsynch and CO-Synch protocols with and without calf removal. In this study, 473 beef cows were blocked by breed, postpartum interval, age, and AI sire and were randomly assigned to one of four treatments: Ovsynch with calf removal for 48 hours from day 7 to 9 of the protocol, or not, and CO-Synch with calf removal for 48 hours from day 7 to 9 of the protocol, or not. When conception rates for both protocols were pooled, the percentage of cows conceiving with ovulation synchronization and calf removal for 48 hours (on day 7 to 9) was greater than when calves were not removed from their dam (62% vs 53%). When calf removal from $PGF_{2\alpha}$ injection until post-TAI was added to variations of the CO-Synch + CIDR protocol, pregnancy rates to TAI was increased (41% vs 12%) compared with when the calves were left with their dam (Marquezini et al., 2013). These results suggest improved fertility to TAI when the calf is removed for a period. The application of this strategy, however, is not widely adopted in the US cow-calf industry today due to the increased labor and management requirements as well as the negative impact of stress on both the dam and the calf.

Effects of Temperament on Cattle Production

Stress

Stress is the result of an event or condition that places strain on a biological system (Collier et al., 2017). To cattle, stressors (the event or condition stimulating the response) can be physical such as temperatures outside of the thermal neutral zone or feed restriction. Stressors can also be psychological such as social isolation, weaning, or human handling. Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis. When cattle perceive a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH), and vasopressin (VP; HPA axis physiology reviewed by Lucy et al., 2022). These neuropeptide hormones are released into the hypothalamic-hypophyseal portal system and are delivered to corticotrope cells of the anterior pituitary. Here, they stimulate the release of adrenocorticotropic hormone (ACTH). When released into circulation, ACTH has its main action on the adrenal gland, stimulating the secretion of cortisol from the adrenal cortex. The HPA axis and stress response in cattle have been investigated thoroughly through challenge with exogenous CRH (Veissier et al., 1999; Gupta et al., 2004; Curley et al., 2008; Cooke and Bohnert, 2011) and ACTH (Verkerk et al., 1994; Verkerk and Macmillan, 1997; Curley et al., 2008). Cortisol in circulation is a key mediator of the stress response and helps the body suppress non-essential functions.

Glucocorticoids like cortisol stimulate gluconeogenesis in the liver (Bassett, 1968) and decrease insulin sensitivity (Sanchez et al., 2016), resulting in elevated blood glucose concentrations during times of stress. Acute stress occurs when the HPA axis is activated and increases in cortisol are short-term or low magnitude (Burnett et al., 2014). An animal can experience chronic stress when the HPA axis is excessively activated or when activated for an extended period. Individual differences in how cattle perceive and respond to stressors play a crucial role in their overall well-being and productivity. One key factor influencing these responses is temperament.

Temperament Assessment

Cattle temperament is defined as the behavioral response to human handling (Fordyce et al., 1988). Low-stress handling techniques and exposure to human handling have been shown to alleviate the animals' physiological stress response and improve their temperament (Brandão and Cooke, 2021). There is no single perfect measurement of temperament. Temperament, however, can be measured using a combination of techniques including chute score and pen score. Temperament can also be measured by the velocity at which the individual exits the chute, known as chute exit velocity. Furthermore, measurements of cortisol concentrations in hair, blood, saliva, feces, urine, and even milk can aid in assessing temperament as physiologic biomarkers.

Multiple measurements of temperament can be assessed chute side. Chute score is one such example. A 5-point scoring system has been used to measure chute score while the animal is restrained in a chute. A single technician determines the chute score based on the individual animal's behavior where 1 = calm, no movement, 2 = restless movement, 3 = frequent movement and vocalization, 4 = constant movement, vocalization, shaking of the chute, 5 = violent, continuous struggling (Cooke et al., 2011, 2017; Dias et al., 2022). Along with chute score, chute exit velocity can also be measured while the animal is exiting the chute. This can be done using a timer activated by infrared beams. One beam is positioned just outside the head catch of the chute, and a second beam is placed a set distance beyond the first. When the animal crosses the first beam, after being released from the chute, the timer begins; it stops when the animal crosses the second beam. Exit velocity is then calculated by dividing the distance between the beams by the recorded exit time. Exit velocity can be used as continuous data or can further be divided into quintiles to create an exit score. Animals are assigned a score from 1 through 5 based on the quintile their exit velocity falls into where 1 = the slowest animals and an exit score of 5 = the fastest animals (Cooke et al., 2011; Dias et al., 2022). From there, it is possible to create a temperament score for individuals. Temperament score is typically calculated as the average of 2 or more of an individual's temperament measurements, such as chute score and chute exit velocity (Cooke et al., 2009; Dias et al., 2022).

Some novel work has been done in investigating other potential predictors of temperament: eye white (EW) percentage and eye temperature (Core et al., 2009; Chen et al., 2021). Eye white percentage can be measured with a video camera by placing it to the side of the animal's head while restrained in the chute (Core et al., 2009). Eye white percentage can then be calculated by choosing two still images from the video for each animal to evaluate using an image analysis program. Such programs can calculate the area of an image in pixels. While chute side, chute score and flight speed can be collected of the animals. Core et al. (2009) recorded such measurements in this way for three separate groups: 48 heifers, 39 bulls, and 60 steers. Correlation coefficients for EW percentage and chute scores were 0.67 (heifers), 0.95 (bulls), and 0.70 (steers). Correlation coefficients for EW percentage and flight speeds were 0.42 (heifers), 0.33 (bulls), and 0.29 (steers). There is potential for EW percentage as a predictor of temperament because some correlations in the Core et al (2009) study were moderate to strong between measurements of temperament and EW percentage. Another study investigated the use of infrared thermography eye temperature (IRT) and EW percentage to predict cattle temperament (Chen et al., 2021). In contrast, the results yielded from this study failed to show a strong relationship between IRT, EW percentage, and cattle temperament. It is notable that they concluded future studies should include more cattle with greater variations in temperament.

Other studies have included pen score as a measurement of temperament (Arthington et al., 2008). Pen score can be assessed after the animal is released from the chute and enters its pen where a technician is standing along the fence line. Once the animal enters the pen and notices the technician, the technician takes 3 steps in the animal's direction and assesses the response. The score of the response is determined using a 5-point scale, where 1 = unalarmed and unexcited walking slowly away from the technician, 2 = slightly alarmed, moving moderately quickly away from the technician, 3 = moderately alarmed and excited, moving away from the technician quickly, 4 = very alarmed and excited by the presence of the technician, moving very quickly and with head held high, 5 = very excited and aggressive toward the technician requiring the technician to avoid contact between the animal and themselves (Arthington et al., 2008). A similar unrestrained measurement of temperament is the use of a docility test. The docility test, sometimes called the yard test, is different from a pen score in that the technician singles off an animal from the group and herds it to a corner of the pen where it is held for a predetermined amount of time. Observing the animal the entire time, the technician scores the animal on a 5-point scale similar to that for the pen score (Haskell et al., 2014).

Measuring temperament based on behavioral traits alone fails to fully encompass the physiological mechanisms that govern temperament. Physiological control of stress is primarily measured via concentrations of cortisol from various sample types including hair, blood, saliva, feces, urine, and milk. Measuring the concentrations of cortisol during handling can provide insightful information on the stress level of the animal and is linked to temperament (Curley et al., 2008). Blood samples are typically collected through jugular venipuncture. In contrast, a less stressful method of collection is via the coccygeal vein or artery, accessible along the ventral portion of the animal's tailhead. Cortisol can also be measured from hair of the tail switch (Burnett et al., 2014). Hair should be cut as close to the skin as possible using scissors. It is worth noting, that hair cortisol concentrations are more indicative of chronic stress than acute stress (Moya et al., 2013). For instance, after branding, plasma cortisol concentrations only remained elevated from 5.5 to 25.5 minutes (Lay et al., 1992). When no longer perceiving a stressor (HPA axis is not activated) cortisol is cleared from circulation, with one study reporting clearance around 30 minutes (Dunlap et al., 1981). In contrast, cortisol can remain in the hair follicle much longer and is not cleared as quickly making it a good biomarker for chronic stress. Cortisol concentrations can be evaluated in many different sample types and are good biomarkers of stress when used in combination with other behavior-related measurements to assess temperament in cattle.

Temperament and Production Efficiency

Beef cattle exhibiting greater excitability, and therefore having a poorer temperament, are less productive than their calmer cohorts in many scenarios. In a study of 120 *Bos indicus* crossbred steers, those with poorer temperament had lower average daily gain (ADG), but not poorer carcass qualities (Petherick et al., 2002). One study clearly details some notable differences among temperament of feedlot cattle (Voisinet et al., 1997). The study included 292 steers and 144 heifers with representation of a range of breeds. On average, animals with Brahman influence were more excitable. Heifers exhibited greater excitability than steers. Within breed group, animals with less desirable temperament had poorer ADG. These differences in ADG between more excitable than calmer cattle are likely attributed to the increased time excitable cattle spend inspecting their surroundings (e.g., less time spent eating; Nkrumah et al., 2007). Differences in ADG between these two temperament groups can also be explained by the effects of greater cortisol concentrations on metabolism (Bassett, 1968; Sanchez et al., 2016). Fell and others (1999) investigated associations between temperament, performance, and immune function in 209 feedlot steer calves. Upon entering the feedlot, calves were classified as nervous or calm based on flight time and chute crush score (similar to exit velocity and chute score). The results of this study indicated that nervous calves had greater cortisol concentrations, poorer ADG, and greater morbidity rates than calm calves, highlighting the adverse effects of excitable temperament in a feedlot setting.

Carcass quality is also affected by temperament. Café and others (2011) investigated the effects of temperament (as measured by flight speed and chute score) on performance traits of Brahman and Angus steers in New South Whales (NSW) and Western Australia (WA). As flight speed increased, Brahman cattle in NSW had significant reductions in carcass weight and rib fat. Likewise, Brahman cattle in WA had reduced carcass weight, smaller longissimus lumborum muscle area (commercially available as ribeye and striploin steaks), and darker meat color as flight speed increased. The associations between temperament and performance traits in Angus cattle were weaker than in Brahman cattle. An interesting study evaluated feedlot steers for temperament and classified them as docile, restless, and nervous-flighty, and further investigated the effects of temperament on carcass quality and consumer sensory evaluation (Yang et al., 2019). Steers that were classified as nervous-flighty had lighter hot carcass weights than their calmer cohorts. In addition, a sensory panelist group that was blinded to the animal's temperament classification claimed steaks from cattle in the docile and the restless group were significantly more tender, had less perceived connective tissue, and were juicier than steaks from the nervous-flighty steers. Although methods to assess temperament were not all similar, the research points to a link between poor temperament and counterproductivity on multiple fronts.

Temperament and Reproductive Efficiency

Many studies have demonstrated negative impacts of poor temperament on reproductive efficiency in cattle. In one such study, 953 lactating multiparous Nelore cows were evaluated for temperament and allocated to two temperament groups (Cooke et al., 2017). One group was defined to have adequate temperament (temperament score ≤ 3), whereas the other group was defined to have excitable temperament (temperament score > 3). The breeding season consisted of an ovulation synchronization and TAI protocol followed by pregnancy diagnosis 30 days later via transrectal ultrasonography. At that point, if cows were diagnosed as non-pregnant, they were either exposed to bulls for 60 days, assigned to a second synchronization and TAI protocol, or culled. Excitable cows tended to have poorer pregnancy rates to TAI compared with those with adequate temperament. Pregnancy loss and calving rates were also negatively impacted in cattle with excitable temperaments. Although excitable temperament is more common in *Bos indicus* breeds, the effects of poor temperament on reproduction extend to *Bos taurus* breeds as well. Cooke and others (2012) investigated the effects of temperament and acclimation to human handling on reproductive performance of *Bos taurus* females. In this study, 433 multiparous Angus x Hereford cows were evaluated for temperament and defined as either aggressive or adequate. Cows with aggressive temperament had greater plasma cortisol concentrations and impaired pregnancy rates and calving rates in comparison with those with adequate temperament. Likewise, cows that were considered aggressive had reduced kilogram of calf born per cow exposed to breeding and tended to have reduced kilogram of calf weaned per cow exposed to breeding (Cooke et al., 2012).

Effect of temperament on reproduction of heifers has been previously studied. Angus influenced heifers (n=297) were enrolled in the 7-day CO-Synch + CIDR synchronization protocol with TAI (Dias et al., 2022). The heifers were evaluated for temperament and classified as either calm or excitable on the first day of the protocol. Approximately 40 days after TAI, pregnancy status was determined via transrectal ultrasonography. Although only 29% of the heifers enrolled were considered excitable, plasma cortisol concentrations during all handling events were greater and conception rates to TAI were reduced in excitable heifers compared with calm heifers (36%, and 55%, respectively). Furthermore, when looking at cortisol concentrations at TAI by temperament and by pregnancy outcome, non-pregnant excitable heifers had greater cortisol concentrations compared with pregnant excitable heifers. In addition, no differences in cortisol concentrations at the TAI were detected between pregnant and non-pregnant calm heifers on the day of TAI. These results from both *Bos indicus* and *Bos taurus* breeds indicate a reduction in reproductive efficiency among cattle with poor temperaments when plasma cortisol concentrations are greater.

Echternkamp and others (1984) were the first to study the relationship between circulating concentrations of luteinizing hormone (LH) and cortisol in cows. In the study, 8 ovariectomized Hereford cows were evaluated for cortisol and LH concentrations while physically restrained; 4 of the cows had previously been acclimated to the stanchions and restraint while the other 4 had not. Cows that were acclimated to being physically restrained had significantly lesser circulating concentrations of cortisol and greater concentrations of LH in addition to greater frequency of LH pulses, demonstrating the influence of restraint stress on LH secretion.

Strategies to Improve Temperament in Cattle

With the substantial evidence of the negative impacts of poor temperament on production (Petherick et al., 2002), reproduction (Cooke et al., 2017; Dias et al., 2022), and general welfare of cattle and their handlers, strategies for improving temperament would be of great value. Moreover, docility is a moderately heritable trait (Haskell et al., 2014). Genetic selection through culling aggressive animals and breeding to sires with favorable docility EPDs offers an effective approach to modifying the temperament profile of a cow herd (Brandão and Cooke, 2021). Nevertheless, selecting for docility through genetic selection is a gradual process. In contrast, acclimating cattle to human handling serves as an effective short-term strategy to improve temperament. Acclimation of female beef cattle to human handling and the handling facility has been well documented for both *Bos indicus* and *Bos taurus* breeds and crosses (Cooke et al., 2009a, b, 2012). Cooke and others (2009a) randomly allocated 160 Braford cows and 235 Brahman x Angus cows during two breeding seasons to either receive acclimation or not. Acclimation consisted of 15 minutes of one person walking among the cattle and hand offering range cubes 2 times a week for 5 months. Although no difference in temperament or plasma cortisol was detected between cows that were acclimated and controls, acclimated cows from the first year of the study had greater pregnancy rates compared with non-acclimated cohorts. Nevertheless, these results were inconsistent from year to year and this method of acclimation leaves room for improvement.

The same research group investigated the effects of acclimation on a group of 37 Braford heifers and 43 Brahman x Angus heifers that were weaned at approximately 7 months of age (Cooke et al., 2009b). Acclimation occurred 3 times weekly for 4 weeks by moving the heifers individually though the handling facility. On week 1, heifers were moved through the tub, ally, and chute without restraint. During week 2, 3, and 4, heifers were moved through and restrained in the chute for 5 seconds, 30 seconds and sent back to pasture immediately, and 30 seconds and sent back to pasture after 1 hour in a holding pen respectively. Acclimation decreased adrenal steroidogenesis, hastened puberty attainment and pregnancy status for heifers that were acclimated compared with non-acclimated heifers. In contrast, acclimated heifers had poorer ADG compared with controls. This reduction in ADG was attributed to the distance the acclimated heifers traveled from their pasture to the handling facility for each acclimation event (2 kilometers round trip).

Although these two previous studies (Cooke et al., 2009a, b) specifically investigated the effects of acclimation on temperament for *Bos indicus*-influenced breeds, Cooke and others (2012) also studied the effects of acclimation on *Bos taurus* heifers. In this subsequent study, 88 Angus x Hereford heifers that were weaned at 6 months of age were randomly allocated to receive acclimation or no treatment. Acclimation occurred 3 times weekly for 4 weeks by moving the heifers through the handling facility utilizing the same methods described earlier (Cooke et al., 2009b). In contrast, in this study, the round-trip distance traveled by acclimated heifers from their pasture to the handling facility and back was 0.6 kilometers. In this study, ADG was not reduced by acclimation and acclimated heifers had partial improvements in temperament as indicated by slower exit velocities, decreased circulating concentrations of cortisol and haptoglobin, and hastened puberty attainment (Cooke et al., 2012).

Overall, there is substantial evidence that acclimation to human handling and the handling facility can positively impact temperament as well as reproduction in heifers. The methods of acclimation must be strongly considered. Additional research in this area is needed to determine the effectiveness of acclimation methods that can be easily applied in a production setting with limited time and labor.

Chapter 2 - Effects of Acclimation During the Handling Events of the 7-day CO-Synch + CIDR Protocol on Temperament and Reproductive Performance of *Bos taurus* Beef Heifers

Abstract

It has been previously demonstrated that heifers with excitable temperament have greater plasma cortisol concentrations and decreased conception rates after timed-artificial insemination (TAI). Nevertheless, plasma cortisol concentrations are decreased as the handling events of an estrus synchronization (ES) or ovulation synchronization (OS) protocol progress. The objectives of this study were to investigate the effects of acclimating heifers to human handling and the facility during the handling events of an OS protocol on temperament and conception rates to TAI. Ten days before enrollment in the study, 622 *Bos taurus* yearling commercial beef heifers from five herds in the spring of 2023 and three herds in the spring of 2024 (reported as 8 locations in total) were evaluated for reproductive tract scores (RTS), chute score (CS), and chute exit velocity (EV). To determine RTS, the following scale was used based on uterine horn diameter and tone and palpable ovarian structures: 1 = immature, < 20mm diameter, no tone, and no palpable structures ; 2 = 20-25mm diameter and no tone; 3 = 20-25mm diameter with slight tone; 4 = 30mm diameter with good tone; 5 = > 30mm diameter with good tone and a corpus luteum present. Chute scores were recorded on a scale where 1 = calm and motionless; 2 = restless movements; 3 = frequent movement and vocalization; 4 = constant movement, vocalization, shaking, and struggling; 5 = violent and continuous struggling. Chute exit velocity was measured using infrared sensors. Heifers were stratified based on CS and RTS to be acclimated during the handling events of the breeding protocol (TRT; n = 307) or serve as control (CTRL; n = 315), tagged according to treatment and managed as one group at each location. All heifers were enrolled in the 7-day CO-Synch + CIDR protocol and received TAI. Before the breeding protocol handling events (d 0, 7, and 10), TRT heifers were sorted off and acclimated by moving them through the tub, ally, and chute without restraint. After acclimation, they were commingled with CTRL heifers and returned to the facility for the OS event of that day. To assess temperament, CS and EV were collected for all heifers on the day the CIDR was inserted (d 0), the day the CIDR was removed (d 7) and the day of TAI (d 10). In addition, blood samples were collected from 120 heifers from 2 locations, to analyze plasma cortisol concentrations (d 0, 7, 10). Estrus detection patches were placed on d 7 and scored on d 10. Pregnancy status was determined 40 to 91 days post-TAI by transrectal ultrasonography. No treatment effects were observed for RTS ($P = 0.78$), CS ($P = 0.99$), or EV ($P = 0.87$) prior to the start of the breeding protocol (d -10). Exit velocities decreased as the protocol progressed for all heifers ($P < 0.0001$), but did not differ based on treatment ($P = 0.27$). There were no differences in cortisol concentrations between TRT and CTRL heifers ($P = 0.64$). In addition, no difference was observed for estrus patch scores ($P = 0.46$) between treatments. In contrast, TRT heifers had decreased CS on d 7 ($P = 0.01$) and d 10 ($P = 0.01$) when compared with CTRL, and greater pregnancy rates to TAI (54% for TRT and 45% for CTRL; $P = 0.02$). These results suggest that acclimating heifers to human handling and the facility during the handling events of the 7-day CO-Synch + CIDR synchronization protocol led to improved chute scores, indicating a potential improvement in temperament, and effectively improved pregnancy rates to TAI.

Introduction

A goal of beef cattle production is for each cow to produce one calf per year, a standard that is vital for the efficiency of cow-calf operations. Achieving this goal requires strategic management of heifer development to optimize their reproductive success in their first year. The success or failure of a heifer to become pregnant early in her first breeding season affects her performance in subsequent years (Burris and Priode, 1958). Furthermore, heifers that calve early in their first calving season stay in the herd longer and wean heavier calves than those that calve later (Cushman et al., 2013). Timing of first parturition can influence heifer productivity for up to 6 subsequent calving seasons, ultimately determining her ability to produce one calf per year. Therefore, becoming pregnant early in her first breeding season is of great importance. An effective management strategy to increase the number of heifers that become pregnant early in the breeding season is to implement an estrus synchronization (ES) or ovulation synchronization (OS) protocol and artificial insemination (AI) or timed artificial insemination (TAI), respectively (Rodgers et al., 2012).

The success of OS and TAI is influenced by many factors, including heifer temperament. Cattle temperament is defined as the behavioral response to human handling (Fordyce et al., 1988). Previous studies have demonstrated that heifers with excitable temperament have greater plasma cortisol concentrations and decreased conception rates after TAI compared with calm heifers (Dias et al., 2022). Plasma cortisol concentrations in heifers, however, decrease with acclimation (Cooke et al., 2009b) and as the handling events of a breeding protocol progress (Dias et al., 2022). Moreover, acclimation to human handling has been shown to improve reproductive outcomes after TAI in cows (Cooke et al., 2009a) and natural service breeding in heifers (Cooke et al., 2009b). The objectives of this study were to investigate the effects of acclimating heifers to human handling and the facility during the handling events of an OS protocol on temperament and pregnancy rates to TAI. We hypothesized that acclimating heifers to human handling and the facility during the handling events of the OS protocol would effectively decrease temperament excitability at TAI and thus increase the percentage of heifers pregnant to TAI compared with non-acclimated control heifers.

Materials and Methods

The animals utilized in this study were handled and cared in accordance with the procedures approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) #4858.

Animals and Temperament Evaluation

This experiment took place over 2 consecutive spring breeding seasons of 2023 (Year 1) and of 2024 (Year 2). A total of 622 commercial *Bos taurus* yearling beef heifers from 5 herds in Year 1 and 3 herds in Year 2 (treated as 8 locations) were enrolled in the experiment (Table 2.1). Ten days before enrollment in the breeding protocol, all heifers were evaluated for reproductive tract score (RTS), chute score (CS), and exit velocity (EV). In addition, heifers from 6 locations were evaluated for body condition score (BCS; 1-9 scale). Reproductive tract scores were assessed using a 5-point scoring system based on uterine horn tone and diameter, and presence of palpable ovarian structures where 1 = immature, < 20-mm diameter, no tone, no palpable structures, 2 = 20- to 25-mm diameter, no tone, 3 = 25- to 30-mm diameter, slight tone, 4 = 30-mm diameter, good tone, 5 = > 30-mm diameter, good tone, corpus luteum present (adapted from Anderson et al., 1991 and Holm et al., 2009). Heifers with an immature tract and no palpable structures (RTS of 1) were excluded from the study. To evaluate CS, the methods utilized were previously described (Cooke et al., 2011). While restrained in the chute, heifers were scored based on movement and vocalization, where 1 = calm, no movement, 2 = restless movement, 3 = frequent movement and vocalization, 4 = constant movement, vocalization, and shaking of the chute, and 5 = violent, continuous struggling. To measure EV, exit time was first recorded chute-side using a timer activated by infrared beams. One beam was positioned just outside the head catch of the chute, and a second beam was placed 2 meters beyond the first. When a heifer exited the chute and activated the first beam, the timer began; it stopped once the heifer activated the second beam. To calculate the final EV, the 2-meter distance between the beams was divided by the recorded exit time in seconds.

Before release from the chute on the day RTS was evaluated (d -10), heifers were stratified by RTS and CS to either receive acclimation (TRT; n=307) or not (CTRL; 315) at each location. Stratification occurred chute side while the heifer was still restrained. We utilized a pivot table to balance our treatments based on RTS and CS before releasing the animal from the chute. In addition, heifers were tagged with a colored ear tag indicating their respective treatment and then released from the chute. Body weight (BW; 7 locations) and body condition score (BCS; 6 locations) were recorded on d -10 as well. After data collection on each handling day of the experiment (d -10, 0, 7, 10), TRT and CTRL heifers were grouped together at each location.

Reproductive Management

All heifers at all locations were enrolled in the 7-day CO-Synch + CIDR TAI breeding protocol for beef heifers (Lamb et al., 2006). All heifers received 2 mL i.m. of gonadotropin releasing hormone (100 μg; GnRH; Factrel; Zoetis Animal Health, Parsippany, NJ) and a CIDR (EAZI-BREED CIDR; 1.38 g of progesterone; Zoetis Animal Health) was inserted vaginally on d 0 of the breeding protocol. The CIDR was removed, and heifers were given 2 mL i.m. of prostaglandin $F_{2\alpha}$ (25 mg; $PGF_{2\alpha}$; Lutalyse HighCon; Zoetis Animal Health, Parsippany, NJ) on d 7. On d 10 (54 hours ± 2 hours following CIDR removal), 2 mL of Factrel was administered i.m. and TAI was performed. A schematic of the treatment description as well as the breeding protocol can be found in Figure 2.1.

Treatment Description

Before each breeding protocol event (d 0, 7, and 10), all heifers were brought to a holding pen near the handling facility and sorted by treatment. Treated heifers were then acclimated to human handling and the handling facility. Acclimation occurred by moving heifers from the tub, into the ally, and out through the chute without restraint. Following acclimation, TRT heifers were commingled with CTRL heifers and then all heifers were brought to the handling facility for the breeding protocol event for that day. In addition, on d 0, 7, and 10, all heifers were evaluated for CS and EV to assess temperament. On d 7, an estrus detection patch (Estrotect Breeding Indicator, Rockway Inc., Spring Valley, WI) was applied to the heifer's tailhead and on d 10, was evaluated and scored based on the percentage of the patch surface that was activated. Patches were scored on a 5-point system; 0 = lost patch, 1 = less than 25% activated, 2 = 26% to 50% activated, 3 = 51% to 75% activated, 4 = more than 75% activated. After TAI, heifers were returned to the pasture and exposed to bulls 14 days post-TAI (experimental d 24). Pregnancy status was determined between 40 and 91 days after TAI by transrectal ultrasonography. Conception rate was defined as the number of heifers that became pregnant to TAI divided by the total number of heifers that were inseminated on the day of TAI.

Blood Collection and RIA

Blood samples were collected from all heifers at one location in the spring of 2023 (n = 60) and one location in the spring of 2024 (n = 60). Blood was collected via coccygeal venipuncture into tubes containing sodium heparin (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) on d -10, 0, 7, and 10, and stored on ice until transported to the lab by 6 hours after collection for processing. Blood was centrifuged at 1,500 x g for 20 minutes and plasma was recovered and stored at -20 °C. Plasma cortisol concentrations were determined using ImmuChem Coated Tube Cortisol ^{125}I kits (MP Biomedicals, LLC, Solon, OH), and analyzed in duplicates. Although blood samples were collected on d -10, they are not included in this manuscript. In the absence of assay results from d -10 samples, the reported intra- and inter-assay coefficients of variation (CV) were 6.1% and 9.8%, respectively. These values are derived from assays 1 and 2, which analyzed samples collected on d 0, 7, and 10. The assay sensitivity was 0.03 ng/mL.

Statistical Analysis

All data were analyzed using the SAS 9.4 software (SAS Inst., Inc., Cary, NC). The GLIMMIX procedure of SAS was used to analyze conception rates to TAI. The model included treatment, estrus detection patch score, and AI technician as fixed effects. Random effects included AI sire and location to account for variability due to these factors. Degrees of freedom were adjusted using the Satterthwaite approximation. Least squares means comparisons were conducted for treatment effects with pairwise differences assessed. Repeated measures, including, CS, EV, and plasma cortisol concentrations were analyzed using the MIXED procedure in SAS. The model included treatment, day, and their interaction as fixed effects, with heifer nested within treatment as a random effect. An autoregressive covariance structure (AR(1)) was applied to account for correlation between repeated measures. Degrees of freedom were adjusted using the Satterthwaite approximation. Least squares means were compared using Tukey's adjustment for multiple comparisons. In addition, the model for plasma cortisol analysis included the fixed effect of location. The level of significance was set at $P \leq 0.05$ and tendencies were determined if $0.05 < P \leq 0.10$.

Results and Discussion

Before enrollment in the ovulation synchronization protocol (d -10), no differences were detected between TRT and CTRL heifers in RTS (P = 0.78) or CS (P = 0.99), as was the design of the experiment. In addition, EV (P = 0.87) and BCS (P = 0.88) for both groups did not differ.

Reproductive Performance

In the present study, acclimation to human handling and the handling facility was explored as a potential strategy to improve temperament and enhance reproductive performance in heifers. Acclimated heifers had greater conception rates (P = 0.02) after TAI compared with controls (Figure 2.2). Results from previous studies investigating the effects of acclimation on heifer reproductive performance are mixed. While puberty was hastened in Angus x Hereford heifers that were acclimated 3 times weekly during a 4-week period after weaning, conception rates did not differ when insemination occurred 166 days after the final day of acclimation (Cooke et al., 2012). Puberty was hastened when Brahman-crossbred heifers were acclimated utilizing a similar method, and acclimated heifers became pregnant earlier in the breeding season than controls (Cooke et al., 2009b). In the previous study, the final day of acclimation was 92 days before the start of the breeding season, and heifers were bred by natural service. These differing responses may be attributed to breed and timing of acclimation. In the current study, estrus detection patch scores did not differ (P = 0.46) between treatments. Our results indicate that acclimation during the handling events of the 7-day CO-Synch + CIDR breeding protocol is associated with improved reproductive performance in *Bos taurus* yearling commercial beef heifers.

Temperament

For the purposes of assessing temperament on a behavioral basis, we collected CS and EV for all heifers on all handling days of the experiment (d -10, 0, 7, 10). Our results demonstrate that acclimation effectively improved CS on d 7 ($P = 0.01$) and d 10 ($P = 0.01$; Figure 2.3). Whereas acclimation failed to decrease EV ($P = 0.27$) in TRT heifers, EV for all heifers decreased from d 0 to the day of TAI (Day 10; Figure 2.4). Brahman-crossbred heifers acclimated to the handling facility and human handling for 4 weeks post-weaning had improved CS, but EV did not differ between treatments (Cooke et al., 2009b). In contrast, a 4-week post-weaning acclimation period did not produce differences in CS between acclimated and non-acclimated Angus x Hereford heifers (Cooke et al., 2009b). In the study by Cooke et al. (2012), a significant treatment x day interaction was also observed, with acclimated heifers exhibiting significantly decreased EV on d 200 post-weaning compared with non-acclimated heifers.

For producers to adopt the use of acclimation three criteria must be met: the method must be time-efficient, labor-conscious, and yield a significant positive impact on a production outcome. In the present study, the outcome of interest was conception rate after TAI. Acclimation, achieved by moving heifers through the tub, ally, and chute without restraint, satisfied all three criteria. The time required for the acclimation procedure was recorded at 6 locations for all three handling event days of the breeding protocol. At these locations, acclimation required no more than 18 minutes to complete (n = 38; Table 2.2), and resulted in improved conception rates (Figure 2.2).

In both studies discussed previously (Cooke et al., 2009b, 2012), and the present study, plasma cortisol concentrations were analyzed in addition to CS and EV as a physiologic surrogate for temperament. Concentrations of plasma cortisol can provide insightful information on the stress level of the animal and is correlated to temperament (Curley et al., 2008). Acclimation effectively decreased plasma cortisol concentrations in Brahman-crossbred heifers (Cooke et al., 2009b) and Angus x Hereford heifers (Cooke et al., 2012). Moreover, heifers enrolled in the 7-day CO-Synch + CIDR OS protocol had decreased plasma cortisol concentrations by the day of TAI compared with the first day of the breeding protocol, regardless of the heifer's temperament status (excitable vs calm; Dias et al., 2022). Those authors suggest that the handling events of the 7-d CO-Synch + CIDR breeding protocol may have an acclimatory effects on *Bos taurus* heifers.

In the present study the objectives were to investigate the effects of acclimation to human handling and the facility during the breeding protocol on temperament. For this purpose, plasma cortisol concentrations were analyzed in 120 heifers from 2 locations. Despite partially improved temperament in TRT heifers, indicated by reduced CS, plasma cortisol concentrations did not differ ($P = 0.64$). Our findings suggest, however, that acclimation may improve the heifers' ability to cope with stress at a physiological level that is not reflected by plasma cortisol concentrations. These findings are not statistically analyzed, but the potential for this is demonstrated in Figure 2.5. Plasma cortisol concentrations were considered to be outliers and deleted when concentrations were 1.5 times greater than the interquartile range (IQR) of quartile 3 or 1.5 times less than the IQR of quartile 1. When examining the probability of conception as cortisol concentration on the day of TAI (d 10) increase, an interesting pattern emerges. The probability of conception decreases as cortisol concentrations increase among CTRL heifers. This observation is consistent with previous studies that have indicated that elevated plasma cortisol concentrations may impair fertility in beef females (Dias et al., 2022; Cooke et al., 2017). In contrast, TRT heifers did not show the same decline in probability of conception as cortisol concentration increased. In fact, the probability of conception for heifers that were acclimated remained relatively stable across cortisol ranges, including at greater cortisol concentrations on the day of TAI. While acclimation did not significantly affect plasma cortisol concentrations, acclimated heifers are potentially more resilient to the effects of stress at the day of TAI, allowing them to still become pregnant to TAI despite similar plasma cortisol concentrations as controls.

Conclusions

The present study demonstrates the effects of acclimation during the handling events of the 7-day CO-Synch + CIDR breeding protocol on temperament, plasma cortisol concentrations, and reproduction of *Bos taurus* beef heifers. Our results indicate that acclimating heifers to human handling and the facility led to improved CS but not EV or plasma cortisol concentrations. These measures indicate a potential improvement in temperament. In addition, acclimation potentially improved the ability of heifers to cope with stress, mitigating its detrimental effects on conception rates after TAI, as acclimated heifers had greater conception rates to TAI compared with controls.

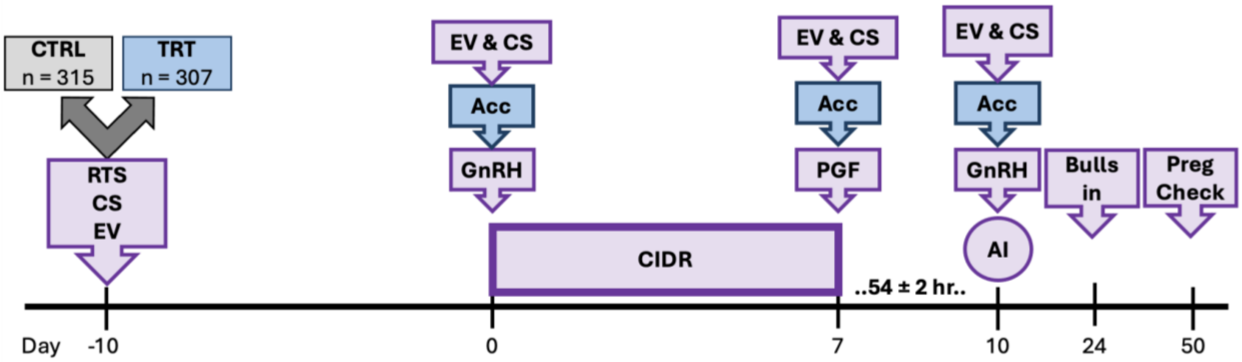

Figure 2.1 Schematic of the treatment description and the breeding protocol used in this study. Anything in a purple box was applied to all heifers. Acclimation (Acc) in blue boxes was only applied to treatment heifers. Heifers were stratified by reproductive tract score (RTS) and chute score (CS) to receive acclimation or serve as a control. On each handling day of the breeding protocol, CS and exit velocity (EV) was collected for all heifers.

Table 2.1 Characteristics of heifers for each location, including number of heifers treated, mean body weight (BW), body condition score (BCS), and reproductive tract score (RTS)

Location	**Year** [1]	**n**	**BW, kg** [2]	**BCS** [2,3]	**RTS** [2,4]
1	1	125	336.5 ± 3.1	5.3 ± 0.04	3.56 ± 0.06
2	2	125	327.8 ± 2.8	5.3 ± 0.04	3.22 ± 0.07
3	1	60	354.7 ± 3.7	6.3 ± 0.07	3.12 ± 0.11
4	2	76	402.9 ± 3.3	6.9 ± 0.07	3.47 ± 0.06
5	1	61	366.7 ± 3.8	6.0 ± 0.05	3.74 ± 0.10
6	2	60	359.1 ± 3.4	---	3.57 ± 0.10
7	1	66	377.9 ± 3.4	6.0 ± 0.06	3.15 ± 0.08
8	1	49	---	---	4.71 ± 0.08
Overall	---	622	355.7 ±1.6	5.8 ± 0.03	3.50 ± 0.03

[1]Year 1 = Spring 2023, Year 2 = Spring 2024.

[2]Reported as Mean ± SEM.

[3]Body condition score is based on 0-9 scale.

[4]Reproductive tract score is based on 1-5 scale.

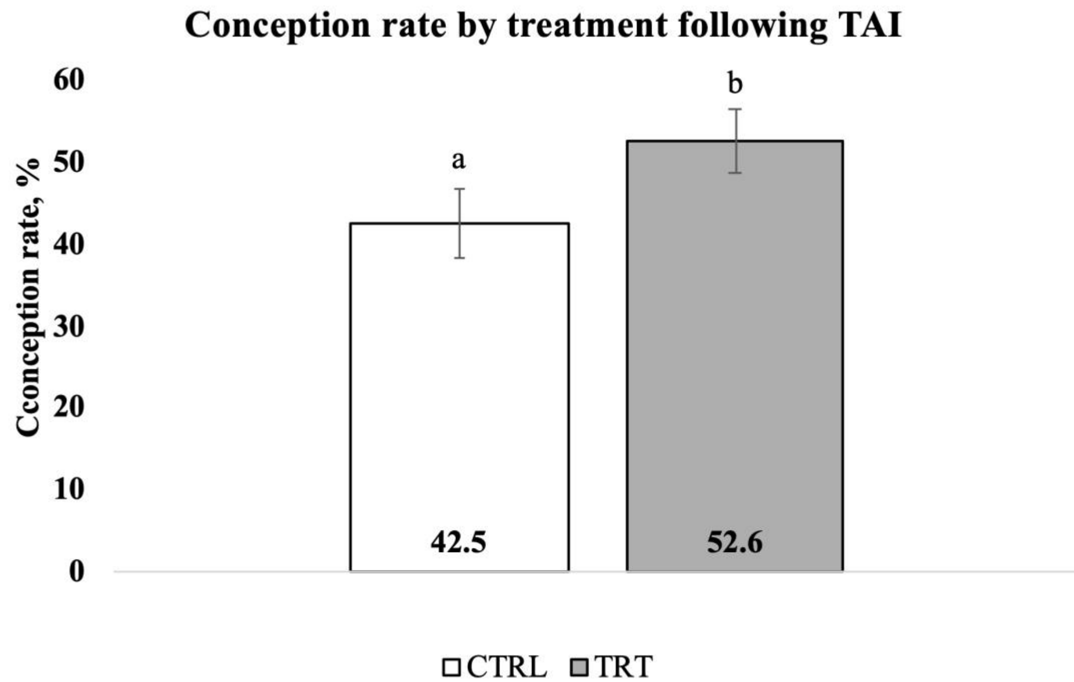

Figure 2.2 Conception rates according to treatment. [a-b] Means with different superscript letters differ ($P \leq 0.05$) between acclimated (TRT) and control (CTRL). Conception rate was defined as the number of heifers that became pregnant to TAI divided by the total number of heifers that were inseminated on the day of TAI**.** Acclimated heifers had increased conception rates compared with controls ($P = 0.02$).

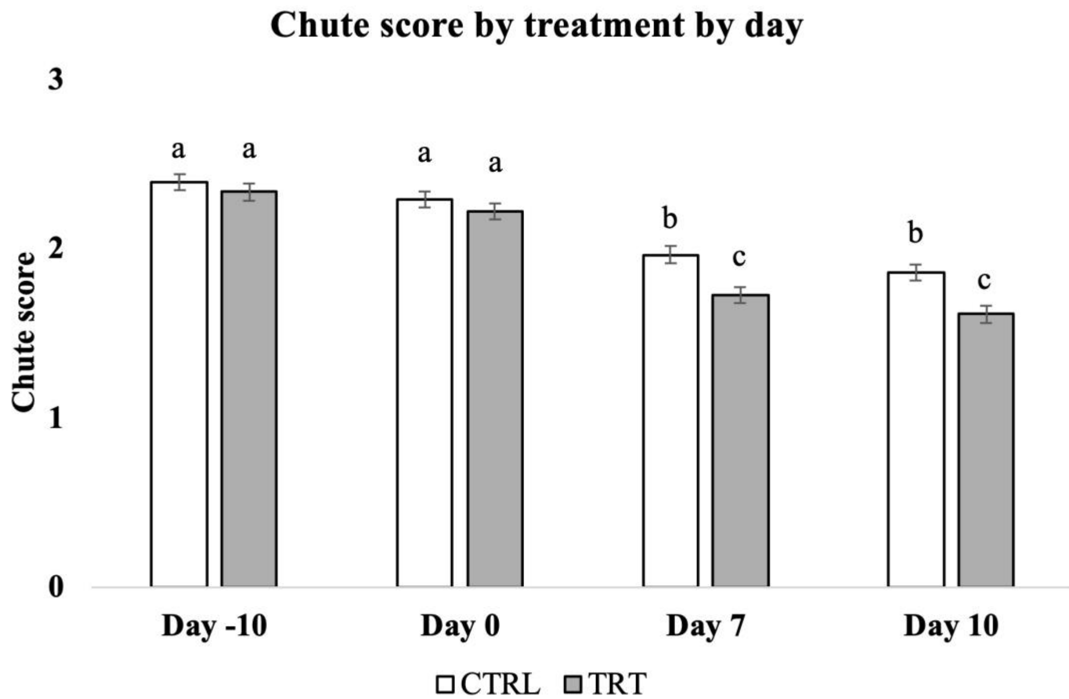

Figure 2.3 Chute scores (1-5 scale; reported as mean ± SEM) by treatment and by day of the experiment. Heifers that were acclimated had improved chute scores on d 7 ($P = 0.01$) and d 10 ($P = 0.01$) compared with those that were not acclimated. [a-c] Superscripts indicate differences within and across days at $P \leq 0.05$. A treatment effect was detected ($P = 0.001$). A day effect was detected ($P < .0001$). A treatment x day interaction was detected as well ($P = 0.04$).

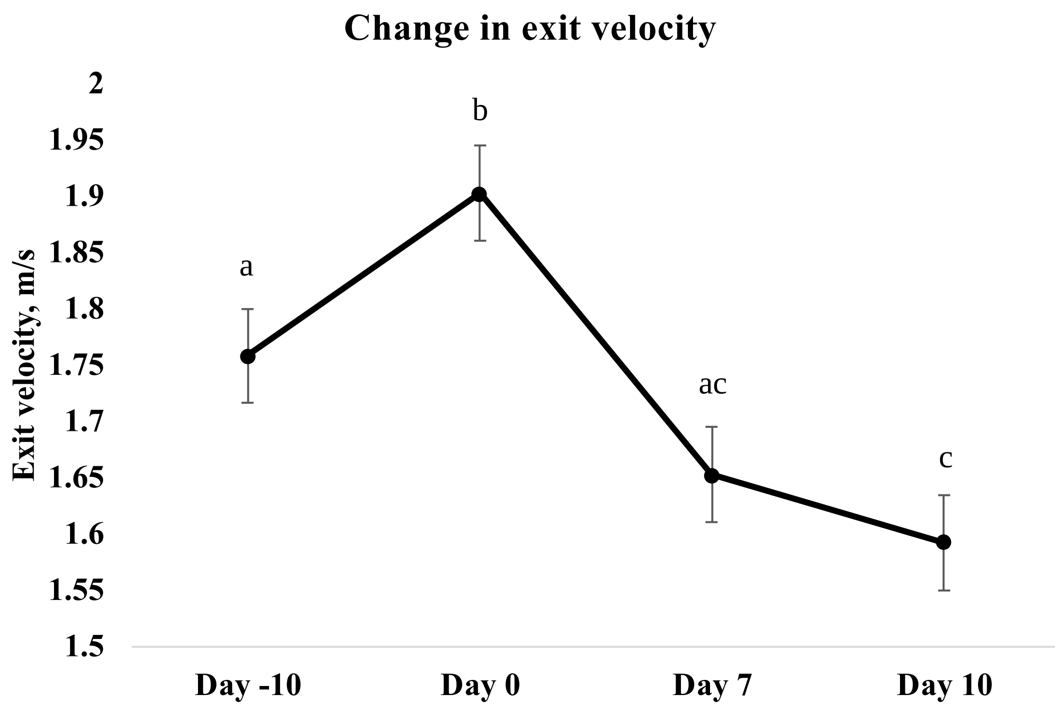

Figure 2.4 Exit velocities (mean ± SEM) by treatment across handling event days. All heifers experienced decreased exit velocities by the day of TAI (effect of day; $P < 0.0001$), but there were no treatment effects of acclimation on exit velocity ($P = 0.27$) or treatment x day interactions ($P = 0.88$). $^{a\text{-}c}$ Superscripts indicate significant differences across days at $P \leq 0.05$.

Table 2.2 Time (reported as "min:sec") required for performing the acclimation protocol [1] at 6 locations for each handling event and number of heifers acclimated at those locations

	Location					
	1	**2**	**4**	**5**	**6**	**7**
Day 0	7:16	8:17	12:18	6:40	7:36	5:38
Day 7	9:20	11:54	17:28	4:57	6:30	5:27
Day 10	8:38	8:11	7:15	6:17	7:13	4:23
n	62	62	38	30	30	30

[1]Acclimation procedure involved processing the heifers through the tub, alley, and chute without restraint. The time started when the first heifer entered the alley from the tub and stopped when the last heifer exited the chute.

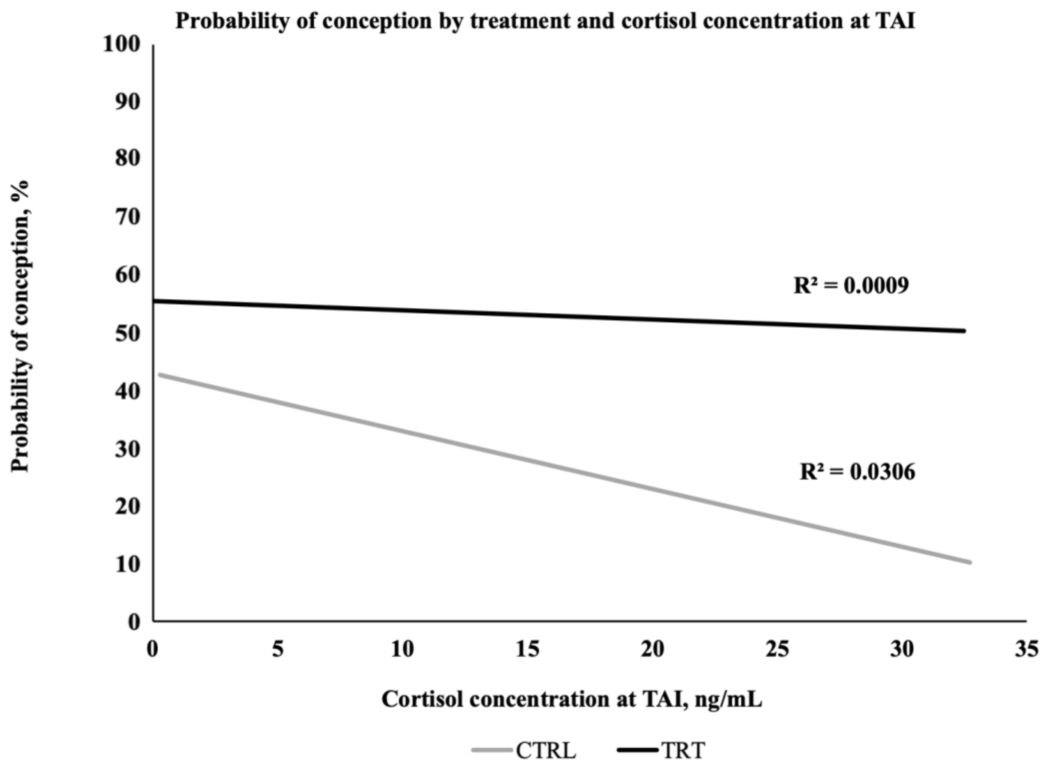

Figure 2.5 Probability of conception by treatment based on plasma cortisol concentrations on the day of timed-artificial insemination (TAI). Non-acclimated heifers (CTRL) have decreased probability of conception as plasma cortisol concentrations on the day of TAI increase. Acclimated heifers (TRT) have relatively stable probability of conception as cortisol concentrations on the day of TAI increase.

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