DEVELOPMENT AND EVALUATION OF THE 7 & 7 SYNCH PROTOCOL FOR ENHANCED CONTROL OF THE BOVINE ESTROUS CYCLE AMONG POSTPARTUM BEEF COWS

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Dedication

This thesis is dedicated to my family for their constant love and support. Thank you for always encouraging me to chase my dreams, no matter what state or how far away it takes me. You are my favorite people to come home to.

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List of Abbreviations

AI	Artificial insemination
BCS	Body conditions core
CIDR	Controlled internal drug release
CL	Corpus luteum
d	Day(s)
E_2	Estradiol-17β
ET	Embryo transfer
FSH	Follicle stimulating hormone
FTAI	Fixed-time artificial insemination
g	Gram(s)
GnRH	Gonadotropin-releasing hormone
hrs	Hour(s)
i.m.	Intramuscular
LFD	Large follicle diameter
LH	Luteinizing hormone
mg	Milligram(s)
MGA	Melengestrol acetate
ml	Milliliter(s)
mm	Millimeter(s)
ng	Nanogram(s)
pg	Picogram
P_4	Progesterone

$PGF_{2\alpha}$, PG	Prostaglandin $F_{2\alpha}$
RIA	Radioimmunoassay
SAS	Statistical Analysis System
SE	Standard error
µg	Microgram(s)

Abstract

Experiment 1 was designed to evaluate the effect of treatments in advance of gonadotropin-releasing hormone (GnRH; 100 μg gonadorelin) administered at the start of estrus synchronization. We hypothesized that administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 500μg cloprostenol) followed by treatment with an intravaginal progesterone-releasing insert (CIDR; 1.38g progesterone) would result in increased follicle size at GnRH, thereby enhancing response to GnRH and overall response to estrus synchronization. Postpartum suckled beef cows (n = 194) in two locations were assigned to one of five treatments (Figure 1) based on age, days postpartum, and body condition score. Cows in treatment 1 (control) received the 7-d CO-Synch + CIDR protocol: administration of GnRH and CIDR insertion on day -10, and administration of $PGF_{2\alpha}$ and CIDR removal on day -3. Treatments 2-5 were designed as a two-by-two factorial. On day -17, cows in Treatments 2-5 received a CIDR insert, either with (Treatments 2 and 3) or without (Treatments 4 and 5) administration of $PGF_{2\alpha}$. On day -10, all cows were administered GnRH, and CIDR inserts were either removed (Treatments 2 and 4) or remained in place until day -3 (Treatments 3 and 5). On day -3, estrus detection aids (Estrotect) were applied and a representative subset of cows (n = 64) in each treatment were fitted with estrus detection transmitters (Accubreed). Blood samples were collected on days -27, -17, -10, -3, and 0 for determination of serum estradiol and/or progesterone concentrations via radioimmunoassay. For a representative subset of cows (n = 104), transrectal ovarian ultrasound was performed to assess ovarian follicle size and presence of corpora lutea on days -17, -10, -3, and 0. Treatment with $PGF_{2\alpha}$ and CIDR in advance of GnRH (Treatments 2 and 3) resulted in increased diameter of the largest ovarian follicle ($P < 0.001$) and increased serum concentrations of estradiol ($P < 0.0005$) on day -10. In addition, variation among cows in CL status tended to be decreased ($P = 0.08$) on

day -3, with cows more likely to have a single CL rather than no CL or multiple CL. Lastly, estrous response prior to fixed-time artificial insemination tended (P = 0.08) to be improved. Results support the hypothesis that administration of $PGF_{2\alpha}$ and treatment with a CIDR for 7 days prior to GnRH improves the likelihood of GnRH response and enhances response of mature beef cows to estrus synchronization.

Experiment 2 was designed to evaluate the effectiveness of the recently developed 7 & 7 Synch protocol to synchronize estrus and ovulation among recipients prior to embryo transfer. Postpartum beef cows (n=1,358) across thirteen locations were assigned to either the 7-d CO-Synch + CIDR protocol or the 7 & 7 Synch protocol prior to estrus detection and subsequent embryo transfer. Cows were preassigned to balanced treatments within location based on age and days postpartum, with body condition score recorded at embryo transfer. Cows assigned to the 7-d CO-Synch + CIDR protocol were administered gonadotropin-releasing hormone (GnRH; 100µg gonadorelin acetate) on Day 0, an intravaginal controlled internal drug release insert (CIDR; 1.38 g progesterone) from Day 0 to Day 7, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg dinoprost tromethamine) coincident with CIDR removal on Day 7. Cows assigned to the 7 & 7 Synch protocol were administered $PGF_{2\alpha}$ (25 mg dinoprost tromethamine) coincident with CIDR insertion on Day -7, GnRH (100 µg gonadorelin acetate) on Day 0, and $PGF_{2\alpha}$ (25 mg dinoprost tromethamine) coincident with CIDR removal on Day 7. The 7 & 7 Synch protocol was hypothesized to enhance response to GnRH administration on Day 0 among mixed groups of estrous cycling and anestrous recipient cows, ultimately resulting in improved estrous response and synchrony of estrus prior to embryo transfer. Cows were observed for visible signs of estrus following estrus synchronization, with GnRH (100 µg gonadorelin acetate) administered to cows failing to express estrus during the detection period. Embryo transfer was performed

approximately seven days after estrus or GnRH administration. Presence of corpora lutea (CL) was determined via transrectal palpation by a single veterinarian blinded to treatment, and embryos were transferred only to cows with palpable CL. Embryo transfer was performed using either fresh or frozen embryos staged and graded according to IETS recommended guidelines, with embryo information recorded for each recipient. The proportion of cows expressing estrus was improved ($P < 0.0001$) among cows assigned to the 7 & 7 Synch protocol (86% [529/615] vs 76% [488/640]). The proportion of cows expressing estrus and presenting with palpable CL at embryo transfer was greater ($P < 0.0001$) among cows following treatment with the 7 & 7 Synch protocol compared to the 7-d CO-Synch + CIDR protocol (76% [466/615] vs 65% [418/640]). Consequently, the proportion pregnant to embryo transfer was greater ($P < 0.03$) following the 7 & 7 Synch protocol (40% [263/653]) compared to the 7-d CO-Synch + CIDR protocol (34% [228/664]). In summary, the 7 & 7 Synch protocol involving administration of $PGF_{2\alpha}$ and treatment with a CIDR for 7 days prior to GnRH improved the likelihood of estrus expression in recipient cows, increased the proportion of cows eligible to receive an embryo, which resulted in a greater pregnancy rate to embryo transfer.

Chapter 1

Review of Literature

Introduction

Success in a beef cattle operation can be defined as productivity and profitability. Use of reproductive technologies such as estrus synchronization and artificial insemination allow producers the opportunity to improve the reproductive productivity of the herd and increase their profit margins. Artificial insemination provides the ability to select genetically superior sires for breeding decisions and in some cases alleviates the need for a greater number of natural service bulls. Estrus synchronization facilitates a timed breeding event and creates a shorter calving season with a greater proportion of calves being born earlier. When used in combination, beef cattle producers will reap the benefits of a well-managed breeding program with genetically elite, early-born, uniform calf crops.

Estrus synchronization and artificial insemination are among the most powerful and applicable technologies for genetic improvement of beef herds (Seidel, 1995). However, low adoption rates are seen among the beef industry. According to the National Animal Health Monitoring System survey in 2007, only 7.9% of beef cattle operations use any form of estrus synchronization, and 7.6% of beef cattle operations use artificial insemination. The survey also reported that labor and time constraints are the most common reasons for producers to not use reproductive technologies (NAHMS, 2007). Although estrus synchronization eliminates the need for estrus detection, failure of some females to express estrus or large variation in timing of estrus expression among a group of females can lead to poor pregnancy rates with fixed-time

artificial insemination. This outcome can be discouraging to beef cattle producers and therefore result in reluctance to adopt reproductive management practices.

Estrus expression is critical for a female to conceive after a timed breeding event. A review of over 10,000 animals using the top recommended estrus synchronization protocols indicated a 27% increase in pregnancy rate for those females that exhibited estrus prior to fixed-time artificial insemination (Richardson et al., 2016). Success rates in breeding programs using both conventional and sex-sorted semen can be impacted by specific timing of artificial insemination relative to estrus expression and ovulation, which occurs 25 to 30 hours after the onset of estrus (Christenson et al., 1975; Bernard et al., 1983). In comparison to conventional semen, performing artificial insemination with sex-sorted semen at a later time point following estrus expression and closer to the time of ovulation yields the highest probability of pregnancy (Sales et al., 2011; Bombardelli et al., 2016). Successful embryo transfer is also contingent on estrus expression and synchrony of estrus expression among a group of recipient females. Recipients that are exposed to higher estradiol concentrations prior to exhibiting estrus have higher pregnancy rates (Bó and Cedeño, 2018). Estrus synchronization protocols aim to control estrus expression among a group of animals, but variation in day of cycle among the group creates a logistical problem when attempting to manage the timing of estrus expression. Variation in timing of estrus expression could potentially be reduced through efforts that reduce variation in day of cycle prior to the start of an estrus synchronization protocol; however, there is still question as to whether reduced variation would result in greater success rates to timed artificial insemination or embryo transfer. This chapter reviews literature relating to the bovine estrous cycle, with an emphasis on estrus synchronization protocols that facilitate timed artificial

insemination events using sex-sorted semen or to generate synchrony in stage of cycle among recipient females in an embryo transfer program.

A Review of the Bovine Estrous Cycle

Estrous cycle

The estrous cycle defines the period of reproductive cyclicity for the bovine. The estrous cycle is typically 21 days long in beef cattle but can range from 18 days to 24 days depending on the number of follicular waves for the cow (Ginther et al., 1989; Noseir, 2003; Jaiswal et al., 2009). The length of the estrous cycle begins at estrus and ends at the following estrus. The time period between estrus can be divided into the follicular phase, which occupies 20% of the estrous cycle, and the luteal phase, which makes up 80% of the cycle. In the shorter of the two phases, the follicular phase, the reproductive tract is under the influence of estrogen produced by the developing preovulatory follicles on the ovary. This phase can be further subdivided into two phases: proestrus and estrus. During proestrus, the corpus luteum undergoes luteolysis and a decrease in progesterone allows a concurrent increase in estradiol as the preovulatory follicle progresses toward physiological maturity. Estrus is the period of sexual receptivity for the female, when estradiol reaches peak concentrations and a subsequent surge of luteinizing hormone (LH) initiates the cascade of events culminating in ovulation. The luteal phase is longer than the follicular phase and is defined as the time that a corpus luteum is present on the ovary and the reproductive tract is under the influence of progesterone. Further division of the luteal phase includes metestrus and diestrus. During metestrus, the ovulated follicle undergoes luteinization, the process of forming the corpus luteum, and progesterone secretion begins. Diestrus is characterized as the period when the corpus luteum is producing high amount of

progesterone. This is the longest phase of the estrous cycle, typically lasting 10-14 days. All of these events combined allow for the bovine female to conceive and maintain a pregnancy.

Proestrus

The proestrus portion of the follicular phase begins after the corpus luteum (CL) on the ovary undergoes luteolysis (regression). Luteolysis of the CL decreases circulating serum concentrations of progesterone (Echternkamp and Hansel, 1973). The decrease in serum progesterone concentrations relieves negative feedback on hypothalamic secretions of gonadotropin-releasing hormone (GnRH). Increasing pulse frequency of GnRH stimulates the anterior pituitary to increase secretions of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH; Schally et al., 1971a). During this period of low progesterone, the amplitude of LH pulse decrease, but frequency of these pulses increase (Rahe et al., 1980). This greater frequency of LH pulsatility drives increased production and secretion of estradiol-17β by the preovulatory follicle, because estradiol-17β production is regulated by the frequency of LH pulses (Walters et al., 1984; Rhodes et al., 1995). Through the "two cell, two gonadotropin model", LH and FSH interact with the two cell types in the follicle wall, granulosa and theca interna cells, to produce follicular estradiol (Fortune and Quirk, 1988). Theca interna cells bind LH to promote the conversion of cholesterol to androstenedione as the granulosa cells provide the precursor pregnenolone in this conversion (Fortune, 1986). Granulosa cells create testosterone from thecal androstenedione that diffuses across the basement membrane from theca interna cells. Then FSH stimulates the enzyme aromatase cytochrome P-450 to convert testosterone to estradiol within the granulosa cells (Dorrington et al., 1975, Armstrong and Papkoff, 1976; Voss and Fortune, 1993). Increasing circulating estradiol concentrations will eventually initiate the preovulatory gonadotropin surge of LH.

Estrus

The period of the follicular phase when estradiol concentrations are highest is known as estrus. The duration of estrus is usually 12 to 18 hours (Wiltbank et al., 1967; Allrich, 1994), with ovulation occurring 25 to 30 hours after the onset of estrus (Christenson et al., 1975; Bernard et al., 1983). High levels of estradiol induce behavioral estrus, which is when the cow or heifer is sexually receptive and will stand to be mounted. Estradiol also induces changes in the reproductive tract to facilitate sperm transport, prepare for copulation and maintenance of pregnancy (reviewed by Pohler et al., 2012). The secretion profile of estradiol changes in this stage of the estrous cycle. The frequency and amplitude of estradiol-17β pulses increase from the preovulatory follicle prior to the surge release of LH (Walters et al., 1984). In order for the preovulatory follicle to be responsive to the LH surge, the follicle must acquire the appropriate number of LH receptors on the granulosa cells to obtain ovulatory capacity (Sartori et al., 2001). After the LH surge, a series of events occur to allow for the follicle to ovulate. The theca interna cells of the follicle transition to producing progesterone instead of testosterone (Fortune et al., 2009). Progesterone increases the production of the enzyme collagenase, which aids in the breakdown of collagen, a major component of the connective tissue covering the outside of the follicle (Reich et al., 1991). Following the LH surge, there is also an increase in prostaglandin E_2 and prostaglandin $F_{2\alpha}$ produced by ovary (Murdoch et al., 1986; Algire et al., 1992). Prostaglandin E_2 increases blood flow to the ovary and dominant follicle (Hristovska et al., 2007). Prostaglandin $F_{2\alpha}$ increases contractions of the ovarian smooth muscle. All of these events culminate to increase tension within the follicle wall and pressure within the antrum resulting in weakening of the follicle wall. The apex of the follicle pushes outward and weakens until rupture and release of the oocyte.

Metestrus

After ovulation, the first phase of the luteal phase begins, known as metestrus. The cell types that made up the follicle prior to ovulation now become the framework for a cellular remodeling process known as luteinization. Luteinization, initiated by the LH surge, transitions a preovulatory follicle into a highly vascular structure known as a corpus luteum (CL), capable of secreting large quantities of progesterone. The granulosa cells of the follicle differentiate into large luteal cells, and the thecal cells differentiate into small luteal cells (Donaldson and Hansel, 1965; Priedkalns et al., 1968; O'Shea, 1987; Meidan et al., 1990). Luteinizing hormone is the major luteotropic hormone that is responsible for luteinization and maintenance of the corpus luteum (Hansel et al., 1973). Luteinizing hormone activates the protein kinase A second messenger pathway within the small luteal cells to stimulate secretion of progesterone. Angiogenesis occurs within the newly formed corpus luteum, making it the most vascular tissue in the body (Reynolds, 1986; Wiltbank et al., 1989). Progesterone secretions and size of the CL increase between days 4 and 7 after ovulation, due to mitosis within the luteal cells. By day 7 of the estrous cycle the remodeling process is complete, and the CL reaches maturity with elevated progesterone secretions. (Donaldson and Hansel, 1965). Serum progesterone concentrations remain high for the duration of the luteal phase, from day 7 to day 12 (Donaldson and Hansel, 1965).

Diestrus

The longest stage of the estrous cycle is diestrus and usually lasts about 10 to 14 days. During this period, the corpus luteum is fully functional and progesterone secretions are high. Elevated concentrations of progesterone are associated with preparation of the uterus for conceptus elongation, maintenance of pregnancy through interferon-tau production, and

improved pregnancy rates (Garrett et al., 1988; Mann and Lamming, 2001; Carter et al., 2008). In addition, progesterone inhibits gonadotropin secretions through negative feedback on the hypothalamus, suppressing follicular growth and estradiol secretions, and ultimately blocking estrus expression (Hansel and Convey, 1983; Allrich, 1994). Toward the end of the luteal phase, progesterone receptors decrease, and estradiol from the developing follicle upregulates the formation of oxytocin receptors in the endometrium (McCracken, 1980). Estradiol stimulates pulses of oxytocin production that initiate low levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to be released from the uterus (McCracken et al., 1996). The large luteal cells of the CL contain receptors for prostaglandin $F_{2\alpha}$ (Niswender et al., 2000). Prostaglandin $F_{2\alpha}$ acts on the large luteal cells to release more oxytocin, further amplifying the production of $PGF_{2\alpha}$ (McCracken et al., 1996). Prostaglandin $F_{2\alpha}$ is the major luteolytic hormone and is efficiently secreted in a pulsatile manner by the uterus, resulting in regression of the CL (McCracken et al., 1972). Prostaglandin $F_{2\alpha}$ is transferred from the uterine vein to the ipsilateral ovarian artery via counter-current exchange (McCracken et al., 1972). Prostaglandin $F_{2\alpha}$ vasoconstricts the arteries supplying the CL and restricts nutrients the luteal cells need for survival (Knickerbocker et al., 1988; Acosta and Miyamoto, 2004). Diestrus ends with luteolysis, the functional regression of the CL, which is characterized by a decrease in progesterone secretions and structural regression of the CL by tissue degradation on the ovary (Pate, 1994).

Folliculogenesis

Folliculogenesis is the development of follicles from primordial stage to primary, secondary, tertiary and finally a Graafian or preovulatory follicle (Britt, 2008). A heifer calf is born with approximately 133,000 primordial follicles, though that number gradually declines as

the female enters reproductive cyclicity and continues through subsequent estrous cycles (Erickson, 1966). During the estrous cycle, follicular wave development can be divided into three steps; recruitment, selection and dominance. In the bovine, follicles develop in waves during both the follicular and luteal phases (Fortune, 1994). The understanding of follicular wave development came from the technological breakthrough of ultrasonography, as it facilitated identification of individual follicles to be followed through development and regression (Sirois and Fortune, 1988). Generally, the number of follicular waves that develop during the estrous cycle varies from two to three and is correlated to the length of the estrous cycle for that female. Interovulatory intervals of equal to or less than 21 days are typically of the 2-wave pattern, whereas interovulatory intervals equal to or greater than 22 days are 3-wave pattern (Ginther et al., 1989; Noseir, 2003; Jaiswal et al., 2009). Follicular waves begin around day 2, 9, and 16 for females with three waves of follicular development and around day 2 and 11 for those with two waves of follicular development (Sirois and Fortune, 1988). Variation among a group of animals in stage of follicular development has meaningful consequences for estrous cycle control, as ovarian responses to products will differ based on whether follicular development is in the recruitment, selection, or dominance stage.

Recruitment

Initial recruitment is when primordial follicles are continuously recruited into a pool of primary follicles, and oocyte growth occurs until growth is arrested in prophase I of meiosis (McGee and Hsueh, 2000). Cyclic recruitment begins after pubertal onset, and a cohort of antral follicles are cyclically recruited from the pool of primary follicles with each surge of follicle stimulating hormone (FSH) during the estrous cycle (McGee and Hsueh, 2000). An FSH surge allows a cohort of antral follicles to emerge at 4 mm in size just before the day of ovulation

(Adams et al., 1992b; Sunderland et al., 1994; Ginther et al., 1996). In response to the growth stimulated by FSH, the follicles increase production of estradiol and inhibin. FSH secretions decrease once a sufficient concentration is obtained (Ginther et al., 1996). The granulosa cells of the ovarian follicles produce a correlated increase in inhibin as FSH concentrations decrease (Taya et al., 1996). Estradiol and inhibin play a role in the inhibition of FSH secretions. While these follicles are still FSH-dependent they are subjected to the selection process.

Selection

Selection of the dominant follicle occurs when the largest follicle reaches diameter of greater than or equal to 8.5 mm (Ginther et al., 1999). The remaining (subordinate) follicles regress or temporarily grow at a reduced rate and then regress. FSH levels begin to decrease when the follicles diverge into a dominant follicle and subordinate follicles (Adams et al., 1992b; Ginther et al., 1999). The dominant follicle grows larger even in a low FSH environment and produces more estradiol. The subordinate follicles cannot continue growth without high levels of FSH, so they go on to undergo atresia (Lucy, 2007). Establishment of follicular dominance is characterized by the follicle transitioning from FSH dependency to LH dependency (Xu et al., 1995). This important transition determines the follicle that will continue on in follicular development.

Dominance

Dominance of a follicle is characterized by the ability to grow with lower levels of circulating FSH, as the dominant follicle becomes LH-dependent. The stage of estrous cycle the dominant follicle has developed in determines its final fate, either atresia or ovulation. The dominant follicle that is selected during the follicular phase is exposed to concentrations of LH sufficient to induce ovulation. With low levels of circulating progesterone, the negative feedback

relationship on GnRH secretions from the hypothalamus is eliminated, and the positive feedback relationship between follicular estradiol and LH is increased to initiate the cascade of events for ovulation (Schally et al., 1971b; Walters et al., 1984; Rhodes et al., 1995). In the luteal phase, the dominant follicle undergoes atresia and a new follicular wave emerges because serum progesterone concentrations inhibit an LH surge capable of ovulating the dominant follicle (Fortune et al., 1991; Lucy et al., 1992).

A Review of Estrus Synchronization Products

Progesterone

Progestins were the first class of hormone evaluated for the control of estrus in estrus synchronization protocols, with the goal of establishing an extended artificial luteal phase by administering exogenous progestins (reviewed by Patterson et al., 2002). Commercially available progestins mimic the actions of progesterone produced by the corpus luteum. Progestins have no effect on the spontaneous regression of the corpus luteum on the ovary. Once the CL has regressed though, progestin treatment at a sufficient dose can maintain serum progesterone concentrations at a high enough level to result in negative feedback on GnRH and therefore LH and estradiol (Ulberg et al., 1951). This inhibits estrus and subsequent ovulation (Nellor and Cole, 1956; Hansel et al., 1961). Progestins also have a role in inducing cyclicity among prepubertal heifers or anestrous cows. Following the first ovulation in prepuberal heifers and postpartum cows, a short luteal phase usually occurs before establishment of normal, typical-length estrous cycles (Perry et al., 1991; Werth et al., 1996). Short exposure to exogenous progestins mimic this short luteal phase, and effectively reprograms the reproductive axis to initiate or resume normal estrous cycling.

Administering a low level of progestin for an extended period of time to a cycling female following luteolysis can result in maintenance of a dominant follicle on the ovary for an extended period. A dominant follicle maintained through treatment with exogenous progesterone is referred to as a persistent follicle (Sirois and Fortune, 1990). Persistent follicles are characterized by their extended life span at a large preovulatory size and at high levels of estradiol production (Sirois and Fortune, 1990; Savio et al., 1993). Formation of persistent follicles has been associated with increased LH pulse frequency when low (subluteal) concentrations of progestins are administered to cattle, in the absence of luteal tissue (Kojima et al., 1995; Kinder et al., 1996). Artificial insemination (AI) following development of a persistent follicle (e.g. AI performed based on estrus occurring after long-term progestin treatment) is not recommended due to the associated decreased fertility (Patterson et al., 1989; Mihm et al., 1994). The decreased fertility can be attributed to the extended exposure to increased estradiol secretions, as the oocyte progresses through meiosis early prior to the LH surge, due to prolonged exposure to increased LH pulse frequency (Mattheij et al., 1994; Mihm et al., 1999; Santos et al., 2016).

Progestin products

The two progestin products that are commercially available include the Controlled Internal Drug Release (CIDR) and melengestrol acetate (MGA). The EAZI-BREED CIDR (Zoetis, Parsippany, NJ) intravaginal insert is a T-shaped device with a silicone coating impregnated with 1.38 grams of progesterone. It is approved by the FDA for the synchronization of estrus in beef heifers and cows, dairy heifers, and lactating dairy cows (Food and Drug Adminstration, 2009). MGA is an orally active progestin that is incorporated into the feed at a level of 0.5 mg/day/animal (Zimbelman and Smith, 1966). MGA is approved for use only in

heifers (Food and Drug Adminstration, 2018). Both of these products are incorporated in many of the top recommended estrus synchronization protocols for heifers and cows.

Prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ is the estrus synchronization product that is incorporated into every approved protocol for heifers and cows. The goal of administering prostaglandin $F_{2\alpha}$ when the cycling heifer or cow is on day 6 through day 16 of the estrous cycle is to induce premature luteal regression (Henricks et al., 1974; Braun et al., 1988). After day 5 of the estrous cycle, the corpus luteum will have acquired the capacity to undergo luteolysis (Lauderdale, 1972; Tsai and Wiltbank, 1998). The response to exogenous $PGF_{2\alpha}$ is the same as in response to its natural occurrence during the estrous cycle: decrease in corpora lutea diameter, decline in blood serum progesterone, increased blood serum estradiol, and a surge of luteinizing hormone to initiate estrus and subsequent ovulation (Wettemann et al., 1972; Louis et al., 1975). The interval to estrus expression following $PGF_{2\alpha}$ administration varies based on the stage of cycle at which the $PGF_{2\alpha}$ is administered (Macmillan and Henderson, 1984). This difference comes from the variation in stage of follicular wave, and through the use of GnRH and progestin treatments the follicular waves can be synchronized and managed (Lauderdale, 2004).

Prostaglandin $F_{2\alpha}$ products

There are many commercially available $PGF_{2\alpha}$ products including Lutalyse®, Lutalyse HighCon®, Estrumate®, estroPLAN®, Prostamate®, In-Synch®, and Synchsure®. These $PGF_{2\alpha}$ products are either made from natural $PGF_{2\alpha}$, specifically dinoprost tromethamine or various analogs of $PGF_{2\alpha}$ such as cloprostenol sodium. All the $PGF_{2\alpha}$ products have been approved by the Food and Drug Administration/Center for Veterinary Medicine for their ability

to induce luteolysis. Several scientific trials have compared the effectiveness of these $PGF_{2\alpha}$ products and found no difference in their luteolytic capabilities or in fertility following their use (reviewed by Lauderdale, 2004).

Gonadotropin-releasing hormone

Exogenous gonadotropin-releasing hormone (GnRH) can be administered to induce premature ovulation of a follicle (Ryan et al., 1988). The hormonal action of GnRH is to release luteinizing hormone from the anterior pituitary in order to stimulate ovulation and initiate a new follicular wave (Roche et al., 1999; Lauderdale, 2015). A follicle's responsiveness, or ability to ovulate in response to administration of exogenous GnRH, is dependent on the stage of follicular growth (Geary et al., 2000). The follicle must have reached a sufficient stage of physiological maturity, having acquired ovulatory capacity via increased expression of LH receptors on granulosa cells (Sartori et al., 2001). Ovulatory capacity of a follicle is often associated with a diameter of approximately 10 mm, but physiological maturity and LH receptor acquisition are what ultimately determine a follicle's capability to ovulate (Sartori et al., 2001). Ovulation will occur between 24 and 32 hours following administration of GnRH if a responsive follicle is present on the ovary (Pursley et al., 1995).

Gonadotropin-releasing hormone products

The five GnRH products commercially available are Cystorelin®, Factrel®, Fertagyl®, GONABreed®, and Ovacyst®. These products are all FDA-approved to treat ovarian cysts in dairy cows. Only four of the five, excluding Ovacyst®, are also FDA-approved to be used with a $PGF_{2\alpha}$ product for the synchronization of estrous cycles in dairy cattle. GONABreed® and Cystorelin® are two GnRH products that are FDA-approved for use with a $PGF_{2\alpha}$ product for

synchronizing estrous cycles in beef cows (Food and Drug Adminstration and Center for Veterinary Medicine, 2019).

Development of Estrus Synchronization Protocols

The development of methods to control the estrous cycle of cows and heifers has been described as having occurred in six phases (reviewed by Patterson et al., 2002; Patterson et al., 2004). Phase I included administering exogenous progesterone in an effort to mimic an artificial luteal phase. Phase II combined the use of estrogens or gonadotropins to progestins. Phase III involved the discovery of prostaglandin $F_{2\alpha}$ as the luteolytic agent. Phase IV combined the use of progestational agents with $PGF_{2\alpha}$. These protocols demonstrated the understanding of control of the luteal phase as follicular waves were not yet recognized. The use of transrectal ultrasonography made it possible to characterize the changes that occur during a follicular wave (Sirois and Fortune, 1988). Precise control of the estrous cycle is now understood to require manipulation of both follicular waves and luteal lifespan; Phase V facilitated this through the combination of GnRH and $PGF_{2\alpha}$. Phase VI incorporated the use of progestins, GnRH and $PGF_{2\alpha}$ together. This phase was preceeded by research that described a subset of animal exhibiting estrus prior to $PGF_{2\alpha}$ administration, therefore reducing the proportion detected in estrus and inseminated during the synchronized period (Kojima et al., 2000). The understanding of how each of these exogenous hormones control the estrous cycle in a cow or heifer laid the groundwork for the development of estrus synchronization protocols.

Ovsynch was a protocol developed to synchronize the follicular phase in addition to the luteal phase. The protocol is administration of GnRH followed by $PGF_{2\alpha}$ seven days later, with a second administration of GnRH 48 hours later, and artificial insemination (AI) performed 16

hours after GnRH. If present, a dominant follicle will ovulate in response to the first GnRH administration, leading to the development of a CL (Garverick et al., 1980; Sartori et al., 2001). Two to three days later a new cohort of follicles begins a new follicular wave, and administration of $PGF_{2\alpha}$ seven days later induces luteolysis of the CL and allows the dominant follicle of the new wave to further develop (Twagiramungu et al., 1995). This protocol still forms the basis of protocols used in the dairy industry today and has resulted in improved pregnancy rates to AI without the need for estrus detection (Pursley et al., 1997).

A similar protocol, Select Synch, was developed to breed cows upon detection of estrus. This protocol continues to be a recommended option for use in beef cows (Beef Reproduction Task Force, 2018). Like Ovsynch, Select Synch includes an initial administration of GnRH, with $PGF_{2\alpha}$ administration seven days later, followed by estrus detection and AI. The majority of cows will express estrus 36 to 72 hours after $PGF_{2\alpha}$ (Stevenson et al., 2000). The protocol outlines that producers should heat detect one day prior to $PGF_{2\alpha}$ because approximately 5 to 15% of the cows can be expected to express estrus on or before the day of administration of $PGF_{2\alpha}$ (Geary et al., 2000; Kojima et al., 2000). Accuracy in estrus detection is important in the success of using this estrus synchronization protocol.

Another protocol, CO-Synch, uses the same strategy as Ovsynch and Select Synch but with fixed-time AI, requiring no estrus detection. The CO-Synch protocol includes administration of GnRH followed by $PGF_{2\alpha}$ seven days later, with administration of GnRH and AI performed 48 hours after $PGF_{2\alpha}$ administration. Published data using CO-Synch in a large number of diverse situations averaged pregnancy rates of 48% (reviewed by Kesler and Constantaras, 2004). Protocols more common in the beef industry today include the concept of CO-Synch with the addition of a progestin treatment like the CIDR.

With identifying the benefit of adding progestins to an estrus synchronization protocol came initial studies involving the CIDR, as it is approved for us in postpartum beef cows. An initial study from Lucy et al. (2001) assigned 851 postpartum beef cows to either a single administration of $PGF_{2\alpha}$, a CIDR for 7 days with $PGF_{2\alpha}$ on day 6, or no treatment. Treatment with a CIDR increased synchronization rates and enhanced pregnancy rates (Lucy et al., 2001). The limitation to the protocol was that $PGF_{2\alpha}$ was administered on day 6 after CIDR insertion, which required an additional trip through the chute for the cows. A multi-state CIDR trial evaluated the effectiveness of incorporating a CIDR into the CO-Synch protocol and reported an improvement in fixed-time AI (Larson et al., 2006). Now called the 7-d CO-Synch + CIDR protocol, it begins with administration of GnRH coincident with CIDR insertion and $PGF_{2\alpha}$ is administered seven days later coincident with CIDR removal. The recommended time to administer GnRH and perform AI is 60 to 66 hours after $PGF_{2\alpha}$ administration and CIDR removal (Busch et al., 2008). A large field trial involving 7,028 cows synchronized with the 7-d CO-Synch + CIDR protocol yielded an average 62% pregnancy rate to fixed-time AI (Patterson et al., 2011). This protocol has now become an industry standard to use on postpartum beef cows in conjunction with reproductive technologies, including artificial insemination and embryo transfer.

Another protocol that utilizes the benefit of progestin treatment is the 7-11 Synch protocol. This protocol includes feeding of MGA for seven days and administration of $PGF_{2\alpha}$ on the last day MGA is fed. Administration of GnRH occurs four days after feeding of MGA has ended and $PGF_{2\alpha}$ is administered a second time seven days after administration of GnRH. In comparison to the Select Synch protocol, the 7-11 protocol resulted in a higher degree of estrus response (91% vs. 69%) and greater AI pregnancy rate (66% vs. 40%) during a 24-hour peak

response period, occurring between 42 and 66 hours (Kojima et al., 2000). This protocol was effective in synchronizing the first wave of follicular development, therefore improving synchrony of estrus without reducing fertility in both anestrous and cyclic cows. Although MGA is no longer approved for use in beef cows, effects of progesterone in the protocol could theoretically be achieved with the use of a CIDR. However, a CIDR-based protocol modeled after 7-11 has not yet been evaluated, and differences between CIDR and MGA would need to be accommodated through alteration of the treatment schedule.

Efficacy of Estrus Synchronization Protocols

Valuable estrus synchronization protocols have been available to beef producers for several decades. These protocols can improve whole-herd reproductive management and increase genetic gains, yet adoption by the industry has been slow. The labor and time commitment associated with detecting estrus following a synchronization protocol may be one reason for reluctancy to adopt the technology (NAHMS, 2007). To address this challenge, more recent research in the field of estrus synchronization has been to develop methods that synchronize estrus effectively enough to decrease the period of time over which estrus detection is required. Ultimately, this has resulted in strategies for fixed-time artificial insemination that yield acceptable pregnancy rates. However, other challenges continue to limit adoption of this management practice. For examples, success of estrus synchronization and AI programs can be influenced by the proportion of females within the herd who have yet to resume normal estrous cycles following calving are placed on an estrus synchronization protocol. These anestrous females pose a problem in their response to the hormonal treatments of an estrus synchronization protocol.

Anestrus is defined as the condition in which a female does not exhibit regular estrous cycles. The inability to resume the regular occurrence of estrous cycles is primarily due to lactation or presence of a calf, inadequate nutrition or lower body condition score, and age or parity of the cow. These factors signal an alteration to the feedback relationships among the hypothalamus, pituitary and ovary. Postpartum cows have low serum concentrations of LH caused by low frequency pulses of GnRH from the hypothalamus (Short et al., 1972; Ingalls et al., 1973). The GnRH "pulse generator" is inhibited during the early postpartum period due to increased sensitivity of the hypothalamus to the negative feedback effects of estradiol (Acosta et al., 1983; Garcia-Winder et al., 1986). The negative effect of estradiol interacts with lactational and suckling factors and is compounded if the cow is in poor body condition score at parturition or has inadequate nutrition post calving. Until normal estrous cycles resume, the growing follicles undergo atresia, because the negative feedback effect of estradiol at the hypothalamus delays resumption of the typical pulse frequency of GnRH and LH release that is critical for a follicle to attain full maturity and ultimately ovulate (reviewed by Berardinelli, 2007).

From a management perspective, some animal characteristics contributing to anestrus are easily identified, such as body condition, parity, and days postpartum. These factors are known to affect pregnancy rate and therefore the success of a breeding season. Body condition score at calving is a good predictor of when first estrus will occur. Cows calving with a body condition score of less than 5 (1 = emaciated and 9 = obese) are at greater risk for longer interval to first estrus (Short et al., 1990). Additionally, the proportion of cows cycling increases by approximately 18% for every unit increase in body condition score, when body condition score is increased from 3.5 to 6.0 at the start of the breeding season (Stevenson et al., 2002). The effect of body condition score on anestrous females can be compounded with the effect of parity in the

case of primiparous cows. First-calf cows require the greatest amount of energy to sustain lactation as well as their own growth, therefore energy for the resumption of estrous cycles becomes low priority (Short and Adams, 1988). Parity alone contributes to the proportion of cows cycling at the start of the breeding season, as seen in a large data set from Stevenson et. al where 64% of the multiparous (n = 2547) cows were cycling in comparison to only 55% of the primiparous (n = 673) cows (Stevenson et al., 2002). Finally, days postpartum or days since the female calved can influence the timing of the female's return to normal estrous cycles. The longer the period between calving and the onset of the breeding season, the higher the percentage of cows who have resumed estrous cycles (Stevenson et al., 2002). These three variables inhibit the female's regular expression of estrus and therefore negatively impact the success of an estrus synchronization protocol and breeding season.

Besides the challenges that occur with anestrous females entering a breeding season, females in the herd that have already resumed regular estrous cycles can also have challenges on estrus synchronization protocols. Following a synchronization protocol, cows detected in estrus prior to fixed-time artificial insemination have significantly higher pregnancy rates (Richardson et al., 2016). If estrus expression is not detected, GnRH can be administered to induce ovulation, but these non-estrous cows typically achieve decreased pregnancy rates compared to the estrous cows (Busch et al., 2008; Bridges et al., 2012; Whittier et al., 2013). This decrease in fertility is associated with lower estradiol concentrations and subsequent decreased progesterone concentrations, as inducing ovulation initiates a GnRH surge before the dominant follicle reaches physiological maturity (Perry et al., 2005; Jinks et al., 2013). Additionally, in the absence of luteal tissue, cycling females can develop a persistent follicle when administered progestin for an extended period of time during an estrus synchronization protocol. The persistent follicle grows

to an increased preovulatory size and therefore has an earlier time to ovulation (Sanchez et al., 1995; Smith and Stevenson, 1995). These females do not express estrus in the same window of time as the others enrolled in the estrus synchronization protocol, causing variation in synchrony among the group that may be problematic for a timed breeding event.

As the beef cattle industry moves toward the use of more advanced reproductive technologies such as the use of sex-sorted semen and embryo transfer, the need for improved estrus synchronization protocols has become apparent. Improving estrus expression response and synchrony among a group while not giving up the convenience of a fixed-time breeding event would support not only continued adoption of estrus synchronization in general but also increase adoption of the more costly technologies of sex-sorted semen and embryo transfer. The variability in success of these technologies may be a function of the variability in response among females undergoing the estrus synchronization protocol used. The most common protocol selected to synchronize estrus in postpartum beef cows is the 7-d CO-Synch + CIDR. Its limitation is that it begins with administration of GnRH, intended to establish synchrony of ovarian follicular wave development. In a group of cycling and anestrous cows, 35% of the cows will fail to ovulate in response to GnRH just due to their stage of the estrous cycle (Vasconcelos et al., 1999; Geary et al., 2000). The other proportion of cows will ovulate in response to administration of GnRH; however, moving forward this protocol has created two subpopulations of cows undergoing estrus synchronization. Persistent follicles can develop in cows who fail to respond to GnRH undergo luteolysis during the protocol resulting in suboptimal fertility (Stock and Fortune, 1993; Jinks et al., 2013). For the proportion of cows among which GnRH induces ovulation and of those that have a CL, an additional accessory corpus luteum will form following the GnRH-induced ovulation, resulting in a higher progesterone environment in which the

growing follicle develops (Price and Webb, 1989; Rajamahendran and Sianangama, 1992; Fricke et al., 1993). High circulating concentrations of progesterone can be moderately suppressive of follicular growth (Adams et al., 1992a; Mantovani et al., 2010). Variation among the group in circulating progesterone concentrations may contribute to variation among cows in physiological maturity of the dominant follicle at the time of $PGF_{2\alpha}$ administration, in turn contributing to variance in interval to estrus. The 7-d CO-Synch + CIDR protocol might also generate different timing of estrus expression between estrous cycling and anestrous cows. The average time to estrus after $PGF_{2\alpha}$ among anestrous cows induced by the protocol to resume cyclicity is earlier than the average time to estrus among cows that were already estrous cycling at protocol initiation (Stevenson et al., 2000). Considering all these sources of variation, the 7-d CO-Synch + CIDR protocol does not appear to generate the degree of estrus expression synchrony needed to achieve maximal pregnancy rates when producers are using technologies such as sex-sorted semen and embryo transfer in timed approaches.

When using sex-sorted semen in timed artificial insemination, it is necessary to pay special attention to time at which insemination is performed. When performing artificial insemination with sex-sorted semen, it is recommended that females are inseminated on the basis of observed standing estrus rather than by appointment (Seidel, 2007). Improvements in pregnancy rates when sex-sorted semen is used with FTAI can be achieved when the proportion of non-estrous females inseminated with sex-sorted semen is reduced (Thomas et al., 2014; Thomas et al., 2017; Thomas et al., 2019). When an ejaculate undergoes the process to become sexed, about 75% of the spermatozoa are damaged, discarded, or lost due to logistical constraints, leaving only about a quarter of the sample to become sex-sorted semen of the desired sex (Seidel and Garner, 2002). This reduction in usable sperm cells results in a unit of sex-sorted

semen containing approximately 4 million sperm cells, in comparison to about 20 million sperm cells per unit of conventional semen. Additionally, cryopreservation has damaging effects on the sex-sorted semen, with sorted frozen-thawed spermatozoa possibly having a shorter period of viability within the female reproductive tract (Hollinshead et al., 2003). These stresses and others may limit the fertility of semen that has been sex-sorted and highlight the need for insemination to occur closer to the time of ovulation.

Accelerating the rate of genetic progress in a beef herd can be accomplished through implementation of embryo transfer. Many factors affect the success of this technology including management of donor females, embryo stage and quality, and estrus synchronization of recipient females. More recent research has focused on synchronizing timing of estrus expression among recipients and understanding the implications of non-estrous females. Bó and Cedeño (2018) reviewed numerous experiments that demonstrate the association between estrus expression and high pregnancy rates, as well as reduced pregnancy loss in recipients receiving both in vitro- and in vivo- produced frozen/thawed bovine embryos. Expression of estrus following a synchronization protocol resulted in greater diameter of the ovulatory follicle, larger subsequent CL area, and greater P_4 concentrations, resulting in a higher pregnancy rate to embryo transfer among recipients that expressed estrus than among recipients that did not express estrus (Baruselli et al., 2003). Some estrus synchronization protocols for recipient females have been developed to include estradiol, but the use of estradiol compounds to synchronize estrus and ovulation in cattle is illegal in the United States. Many variables play into the success of an embryo transfer breeding event, but data suggests potential improvements when using an estrus synchronization protocol that allows a greater proportion of recipients to express estrus in a synchronized manner. Additionally, improved synchrony of estrus among recipients could create

success in a fixed-time embryo transfer event where no estrus detection of recipients is performed.

As previously described, the variation in day of cycle within a group prior to the start of an estrus synchronization protocol like the 7-d CO-Synch + CIDR contributes to the variation in timing of estrus expression observed following synchronization. The dairy industry has developed presynchronization protocols to address this problem and improve fertility to fixed-time artificial insemination. The goal of the various presynchronization treatments is to enhance the response to the first GnRH administration of the Ovsynch protocol by administering $PGF_{2\alpha}$ and/or GnRH at various timepoints prior to the start of the breeding protocol (reviewed Wiltbank and Pursley, 2014). Although the dairy industry has had success in decreasing the variability in stage of cycle among cows on the Ovsynch estrus synchronization protocol, very little has been done in the beef industry to incorporate this method. Although the 14-d CIDR protocol is a presynchronization-based approach to estrous cycle control that is effective among beef heifers, mature beef cows exhibit less synchronous estrus expression, and a much lower estrous response prior to fixed-time AI following this protocol (Nash et al., 2012; Nash et al., 2013). Yet, cows undergoing long term progestin treatment (14 days of MGA or 14-d CIDR) have increased estradiol concentrations prior to fixed-time AI, which can be advantageous for pregnancy rates (Fralix et al., 1996; Abel et al., 2017). The 9-d CIDR-PG estrus synchronization protocol was developed for postpartum beef cows, evaluating administering $PGF_{2\alpha}$ at the start of the progestin treatment. Prostaglandin $F_{2\alpha}$ induces luteolysis and allows enhanced uniformity of subsequent follicular development during the progestin treatment. Among cows, this protocol was found to improve estrus expression synchrony, circulating estradiol concentrations, and pregnancy rates in comparison to the 14-d CIDR-PG protocol and the 7-d CO-Synch + CIDR protocol (Thomas et

al., 2016; Thomas et al., 2018). This presynchronization approach has the potential to significantly impact estrus synchronization protocols for mature beef cows if the limitation of the long treatment schedule can be refined. A possible solution is an estrus synchronization protocol that capitalizes on the advantage of $PGF_{2\alpha}$ before treatment with a CIDR to improve the response to GnRH among cows on a short-term protocol like the 7-d CO-Synch + CIDR. The experiments that follow explore this approach as a possible solution to estrus synchronization in mature beef cows.

Chapter 2

Treatment with prostaglandin $F_{2\alpha}$ and an intravaginal progesterone insert in advance of gonadotropin-releasing hormone enhances response to estrus synchronization in mature beef cows

Abstract

An experiment was designed to evaluate the effect of treatments in advance of gonadotropin-releasing hormone (GnRH; 100 μg gonadorelin) administered at the start of estrus synchronization. We hypothesized that administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 500μg cloprostenol) followed by treatment with an intravaginal progesterone-releasing insert (CIDR; 1.38g progesterone) would result in increased follicle size at GnRH, thereby enhancing response to GnRH and overall response to estrus synchronization. Postpartum suckled beef cows (n = 194) in two locations were assigned to one of five treatments (Figure 1) based on age, days postpartum, and body condition score. Cows in treatment 1 (control) received the 7-d CO-Synch + CIDR protocol: administration of GnRH and CIDR insertion on day -10, and administration of $PGF_{2\alpha}$ and CIDR removal on day -3. Treatments 2-5 were designed as a two-by-two factorial. On day -17, cows in Treatments 2-5 received a CIDR insert, either with (Treatments 2 and 3) or without (Treatments 4 and 5) administration of $PGF_{2\alpha}$. On day -10, all cows were administered GnRH, and CIDR inserts were either removed (Treatments 2 and 4) or remained in place until day -3 (Treatments 3 and 5). On day -3, estrus detection aids (Estrotect) were applied and a representative subset of cows (n = 64) in each treatment were fitted with estrus detection transmitters (Accubreed). Blood samples were collected on days -27, -17, -10, -3, and 0 for

determination of serum estradiol and/or progesterone concentrations via radioimmunoassay. For a representative subset of cows (n = 104), transrectal ovarian ultrasound was performed to assess ovarian follicle size and presence of corpora lutea on days -17, -10, -3, and 0. Treatment with $PGF_{2\alpha}$ and CIDR in advance of GnRH (Treatments 2 and 3) resulted in increased diameter of the largest ovarian follicle ($P < 0.001$) and increased serum concentrations of estradiol ($P < 0.0005$) on day -10. In addition, variation among cows in CL status tended to be decreased ($P = 0.08$) on day -3, with cows more likely to have a single CL rather than no CL or multiple CL. Lastly, estrous response prior to fixed-time artificial insemination tended ($P = 0.08$) to be improved. Results support the hypothesis that administration of $PGF_{2\alpha}$ and treatment with a CIDR for 7 days prior to GnRH improves the likelihood of GnRH response and enhances response of mature beef cows to estrus synchronization.

Key words: 7 & 7 Synch, estrus synchronization, beef cow, sex-sorted semen

Introduction

The 7-d CO-Synch + CIDR protocol is a commonly used estrus synchronization protocol among cows in the U.S. beef industry. This producer-friendly protocol facilitates use of reproductive technologies such as fixed-time artificial insemination (FTAI) and embryo transfer. However, a limitation of the 7-day CO-Synch + CIDR is that it begins with administration of gonadotropin-releasing hormone (GnRH), which is not universally effective among all cows undergoing estrus synchronization. In a group of estrous cycling and anestrous cows, the response rate to GnRH on a random day of the estrous cycle is approximately 65% (Vasconcelos et al., 1999; Geary et al., 2000). The remaining 35% of cows are in a stage of follicular

development that does not allow for ovulation in response to GnRH. Ultimately, the variation among cows in response rate to GnRH results in two subpopulations of cows undergoing estrus synchronization. This variation within the group in response to GnRH results in variation in timing of estrus and ovulation later in the protocol. These two subpopulations of cows create a logistical problem when attempting to generate the degree of synchrony in estrus expression needed to achieve optimal pregnancy rates to FTAI with sex-sorted semen.

The dairy industry has developed presynchronization programs that involve treatment with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and GnRH to decrease the variability in stage of cycle among cows and enhance the response to the first GnRH injection of the breeding protocol (reviewed by Wiltbank and Pursley, 2014). In contrast, the beef industry has underutilized presynchronization approaches for postpartum cows. The aim of the present work is to develop a simple presynchronization method for beef cows that would reduce variation in day of cycle prior to administrations of GnRH. We hypothesized that administration of $PGF_{2\alpha}$ at the start of progestin treatment would result in a larger proportion of cows having a physiologically mature dominant follicle at the time of GnRH administration, based on average follicle size and serum concentrations of estradiol-17β. Additionally, we hypothesized that this effect would improve the likelihood of ovulatory response to GnRH, thereby reducing variation among cows undergoing estrus synchronization and improving rates of estrus expression in advance of FTAI.

Material and Methods

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee.

Experimental Design

Estrus was synchronized for 194 postpartum beef cows across two locations. Within each location, cows were blocked based on age, days postpartum, and body condition score and assigned to one of five treatments (Figure 1). The five treatments were designed as a two-by-two factorial with a control, represented as treatment 1. On day -17 of the experiment, cows in treatment 2, 3, 4, and 5 received an Eazi-Breed CIDR insert (1.38 g progesterone; Zoetis) with $PGF_{2\alpha}$ (25 mg, i.m.; Estrumate; Merck Animal Health) administered to treatments 2 and 3. On day -10, cows in treatment 1 received a CIDR whereas the CIDR insert was removed for cows in treatments 2 and 4 and remained in place for cows in treatment 3 and 5. All treatments received GnRH (100 μg, i.m.; Fertagyl; Merck Animal Health) on day -10. On day -7, the CIDR insert was removed for treatments 1, 3, and 5 and all treatments were administered $PGF_{2\alpha}$. Estrus detection aids (Estrotect, Estrotect Inc) were applied to all cows in treatments 2 and 4 coincident with CIDR removal on day -10, in order to evaluate potential estrus expression occurring between day -10 and day -3. Estrus detection aids were evaluated and removed on day -3 and occurrence of estrus was defined as removal of at least 50% of the coating from the Estrotect patch. New Estrotect patches were applied to cows in all treatments at the time of $PGF_{2\alpha}$ administration on day -3 and were evaluated at the time of AI on day 0. A subset of cows (n = 64) also received estrus detection transmitters (AccuBreed, Estrotect, Inc), and timing of estrus onset was recorded. Fixed-time artificial insemination with X-bearing sex-sorted semen (SexedULTRA 4M, Sexing Technologies Inc) was performed on day 0, 66 hours after administering $PGF_{2\alpha}$. A single technician performed all inseminations, and a single sire was used within each location. Cows in all treatments were administered GnRH at the time of AI. Cows were exposed for natural service beginning 14 days after AI.

Blood sampling and radioimmunoassay

Blood samples were collected on days -27, -17, -10, -3 and 0 for cows in all treatments by jugular venipuncture. Blood samples were allowed to clot and stored at 4° C for 24 hours. Serum was collected by centrifugation and stored at -20° C. Blood samples from day -27 and -17 were evaluated for progesterone (P_4) concentrations via radioimmunoassay (RIA) to determine pretreatment estrous cyclicity status of each cow. Cows were classified as anestrous if serum concentrations of progesterone were below 0.5 ng/ml at both pretreatment blood samples. Blood samples from day -10 and day -3 were also evaluated for P_4 concentrations via RIA. Serum concentrations of P_4 were analyzed in duplicate samples (100 µl of sample serum per single tube) using a double-antibody RIA procedure and precipitation assay reagents (#07-170105; MP Biomedicals; Santa Ana, CA) as described by (Pohler et al., 2016). The antigen-antibody complex was precipitated by centrifugation following incubation. The supernatant was discarded and assay tubes with precipitate were counted for 1 minute on a gamma counter. Sensitivity of the assay was 0.05 ng/ml. Intra- and interassay coefficients of variation for the P_4 RIA were 1.9% and 8.0% respectively. Radioimmunoassay was performed on blood samples collected on day -10, -3, and 0 to determine serum estradiol-17β (E_2). Serum concentrations of E_2 were assayed in duplicate samples (300 µl of sample serum per single tube) using estradiol antibody (#07-138216; MP Biomedicals) and 3-^{125} Iodo-Estradiol-17β (#07138226; MP Biomedicals). Bound and free estradiol was separated with dextran-coated charcoal and centrifugation. The supernatant was poured off and counted for 5 minutes on a gamma counter (Kirby et al., 1997). Sensitivity of the assay was 0.5 pg/ml. Intra- and interassay coefficients for the E_2 RIA were 3.7% and 12.4% respectively.

Ultrasonography

Transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite, Inc., Bothwell, WA) was performed on days -17, -10, -3 and 0 on a representative subset of cows in all treatments (n = 104) to record diameter of the largest ovarian follicle present as well as presence or absence of corpora lutea. Follicle height and width were measured using electronic calipers and measurements were averaged to report largest follicle diameter (LFD). Presence of a corpus luteum was determined based on observable luteal tissue via ultrasonography.

Pregnancy Diagnosis

Pregnancy rate to AI was determined 70 to 85 days after artificial insemination by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite, Inc., Bothwell, WA). Pregnancies resulting from artificial insemination were distinguished from natural service pregnancy based on fetal size (reviewed by Poock and Wilson, 2006).

Statistical Analysis

A generalized linear model (GLM procedure of SAS) was used to confirm no difference between treatments in age, days postpartum, or body condition score of cows. To assess effectiveness of $PGF_{2\alpha}$ administration and progesterone treatment prior to GnRH administration, serum E_2 concentrations, LFD, and serum P_4 concentrations were analyzed using a mixed model (GLIMMIX procedure of SAS) with the fixed effect of treatment. To evaluate differences in mean serum E_2 concentrations and LFD on day -10, the following pre-planned contrasts were

evaluated: treatment 1 versus treatments 2 or 3, treatment 1 versus treatments 4 or 5, and treatments 2 or 3 versus treatments 4 or 5. In addition to treatment, estrous cyclicity status and location were included in the model as fixed effects in these analyses. Treatment differences in the proportion of cyclic cows prior to treatment, CL status on day -10 and day -3, estrous response, and pregnancy rate to FTAI were analyzed using chi-square (FREQ procedure of SAS). The timing of estrus expression was analyzed by the GLM procedure of SAS with the fixed effect of treatment.

Results

Treatment summary

The number of cows, estrous cyclicity status, age, days postpartum, and body condition score for each treatment are summarized in Table 2.1. The mean age, days postpartum, and body condition score did not differ across treatments. The proportion of cyclic versus anestrous cows prior to treatment initiation also did not differ across treatments.

Largest follicle diameter

Largest follicle diameter (LFD) measurements recorded via transrectal ultrasonography on day -17, day -10, day -3, and day 0 are summarized in Table 2.2. Mean LFD did not differ across all treatments at the start of the experiment (day -17). On day -10, treatments receiving presynchronization (treatments 2-5) had increased LFD ($P < 0.05$) compared to control (treatment 1). Additionally, treatments receiving presynchronization with $PGF_{2\alpha}$ and CIDR on day -17 (treatments 2 and 3) had a larger LFD ($P < 0.05$) than treatments receiving

presynchronization with only a CIDR (treatments 4 and 5). Mean LFD on day -3 and on day 0 did not differ across treatments.

Variables related to likelihood of GnRH response

Serum progesterone concentrations, LFD, proportion of cows with LFD greater than or equal to 10 mm, and serum estradiol concentrations on day -10 are summarized in Table 2.3, with results pooled for treatments that are identical in treatment schedule up until day -10. Treatments 2 and 3 were both administered $PGF_{2\alpha}$ on day -17 coincident with CIDR insertion, whereas treatments 4 and 5 only received the CIDR insert. There was no difference in serum progesterone concentrations across treatments on day -10. The LFD was larger ($P < 0.001$) for treatments 2 and 3 (13.4 mm ± 0.4) and for treatments 4 and 5 (11.3 mm ± 0.5) in comparison to treatment 1 (8.3 mm ± 0.6) (Figure 2.2). Additionally, for treatments 2 and 3, the LFD was larger ($P < 0.001$) than treatment 1 and treatments 4 and 5. The proportion of cows with LFD greater than or equal to 10 mm was greater ($P < 0.05$) for treatments 2 and 3 (83%) and treatments 4 and 5 (65%) compared to treatment 1 (25%). Serum estradiol concentrations were greater ($P < 0.005$) for treatments 2 and 3 (4.5 pg/ml ± 0.3) compared to treatments 4 and 5 (3.2 pg/ml ± 0.3) and treatment 1 (2.6 pg/ml ± 0.3) (Figure 2.3).

Corpora lutea status

Corpora lutea (CL) status and serum progesterone concentrations on day -3 are summarized in Table 2.4. The CL status was determined via transrectal ultrasonography and categorized as absence of CL, single CL, or CL with an accessory CL. Presynchronization (treatments 2-5) tended ($P = 0.08$) to decrease the variation in CL status compared to control

(treatment 1), with cows in presynchronization treatments tending to have a single CL on day -3. Serum progesterone concentrations on day -3 did not differ significantly between treatments 2-5 but were lower ($P < 0.05$) in treatments 3 and 4 compared to control (treatment 1).

Serum estradiol-17β concentrations

Serum estradiol-17β concentrations on day -3 and day 0 are summarized in table 2.5. On day -3 circulating concentrations of serum estradiol-17β were elevated ($P < 0.05$) among cows in treatment 3 (4.2 pg/ml ± 0.3) and treatment 5 (4.5 pg/ml ± 0.3) in comparison to control (3.3 pg/ml ± 0.3). Serum estradiol-17β concentrations were higher ($P < 0.05$) among cows in treatment 1 (5.0 pg/ml ± 0.3) in comparison to treatment 5 (3.8 pg/ml ± 0.3) and had a tendency ($P = 0.07$) to be greater in treatment 1 than in treatment 3 (4.2 pg/ml ± 0.3) on day 0. Additionally, on day 0, cows in treatment 4 (4.8 pg/ml ± 0.4) had a higher ($P < 0.05$) serum estradiol-17β concentrations than among cows treatment 5.

Estrous response

Estrous response for cows within each treatment is summarized in Table 2.6. Estrous response between GnRH administration and $PGF_{2\alpha}$ for treatments 2 and 4 differed ($P = 0.03$) with 15% of the cows in treatment 4 having expressed estrus in comparison to 0% of the cows in treatment 2. Estrous response by FTAI tended ($P = 0.08$) to differ among treatments. The interval from $PGF_{2\alpha}$ to estrus expression tended to differ ($P = 0.07$) based on treatment, with treatment 4 having a delayed mean time to estrus (53.7 hours ± 2.0) compared to control (45.2 hours ± 2.0).

Pregnancy rate

Pregnancy rate of cows to FTAI with sex-sorted semen based on treatment and estrous response are summarized in Table 2.7. Across all treatments, cows achieved similar pregnancy rates to FTAI with sex-sorted semen. Additionally, across all treatments there was no difference in pregnancy rate based on estrous response at the time of FTAI. The final pregnancy rate at the end of the breeding season within treatment was also not different across treatments.

Discussion

As the beef industry adopts advanced reproductive technologies such as sex-sorted semen and embryo transfer, estrus synchronization protocols may need to achieve a greater degree of uniformity in timing of estrus expression among females undergoing estrus synchronization in order to achieve profitable pregnancy rates. Currently the 7-day CO-Synch + CIDR is the most commonly used estrus synchronization protocol among postpartum beef cows in the United States. Unfortunately, the proportion of estrous cycling and anestrous cows that ovulate in response GnRH at the start of the protocol is only 65% (Vasconcelos et al., 1999; Geary et al., 2000). The other 35% do not ovulate in response to administration of GnRH due to their stage of cycle, and ultimately different subpopulations of cows undergoing estrus synchronization are created. Elevated circulating concentrations of progesterone will occur among some proportion of the cyclic cows that ovulate in response to administration of GnRH, as a proportion of these cows will develop an accessory corpus luteum (CL) in addition to a CL that is already present (Price and Webb, 1989; Rajamahendran and Sianangama, 1992; Fricke et al., 1993). Pulsatile frequency of LH release and the corresponding rate of development of the preovulatory follicle will be slightly moderated among this class of cows, due to the high circulating concentrations of progesterone (Adams et al., 1992a; Mantovani et al., 2010). Another disadvantage is that a

proportion of cyclic cows that fail to respond to GnRH could undergo luteolysis during the course of the 7-d CIDR treatment, resulting in development of a persistent follicle. This is detrimental for two reasons. First, artificial insemination following ovulation of a persistent follicle is not recommended because of the oocyte's prolonged exposure to preovulatory concentrations of estradiol and increased LH pulse frequency leading to premature resumption of meiosis, which results in poor fertility and subsequent decreased pregnancy rates (Stock and Fortune, 1993; Mattheij et al., 1994; Mihm et al., 1994; Jinks et al., 2013; Santos et al., 2016). Second, in comparison to the follicle of other cows on the protocol, persistent follicles will be at a larger size and more physiologically mature at the time of CIDR removal, therefore driving estrus expression and ovulation to occur at an earlier time (Sanchez et al., 1995; Smith and Stevenson, 1995). Additionally, the progestin treatment within the estrus synchronization protocol has the ability to induce estrous cycling among some of the cows that are anestrous at the start of the protocol (Fike et al., 1997; Stevenson et al., 2003). Although inducing cyclicity among the anestrous cows is advantageous for pregnancy rates, their timing in estrus expression varies from the cyclic cows in the synchronized group. Anestrous cows that resume cyclicity may express estrus earlier following progestin removal compared to cows that were estrous cycling at the start of the protocol (Stevenson et al., 2000). Ultimately the variation in day of cycle among cows at the start of the 7-d CO-Synch + CIDR protocol contribute to the variation in estrous response and poor synchrony of estrus expression following estrus synchronization.

The dairy industry has successfully developed and utilized presynchronization programs to decrease the variation in stage of cycle among cows prior to their enrollment in an estrus synchronization protocol. The beef industry though, has been slow to adopt presynchronization methods, with the exception of long-term progestin-based protocols used commonly in beef

heifers, such as the 14-d CIDR-PG and MGA-PG protocols (Brown et al., 1988; Deutscher, 2000; Lamb et al., 2000; Leitman et al., 2009; Mallory et al., 2010). Use of long-term progestin protocols is uncommon among mature beef cows due to the duration of the treatment schedule. However, recent research efforts from our lab have evaluated new presynchronization approaches for mature beef cows using $PGF_{2\alpha}$ combined with CIDR treatment, such as the 9-d CIDR-PG protocol. In comparison to the 14-d CIDR-PG protocol in mature beef cows, this protocol was found to improve estrous response, synchrony of estrus onset, and pregnancy rates (Thomas et al., 2016). The long treatment schedule of the 9-d CIDR-PG protocol limits opportunities for adoption, but the presynchronization approach has the potential to significantly impact estrus synchronization protocols. When developing the treatments for this experiment, labor associated with presynchronization was considered, as extra labor and time are the main reasons beef producers are reluctant to utilize reproductive technologies on their operations (NAHMS, 2007). The presynchronization approaches evaluated in the present experiment only add one additional handling of animals and one additional week in length of the protocol.

The results from this experiment demonstrate a mechanism to increase the response rate to GnRH by administering $PGF_{2\alpha}$ and treating with a progesterone insert for seven days prior to administration of GnRH. Following this treatment, increased follicle size was observed at the time of GnRH administration, along with correlating increases in serum estradiol concentrations. The positive relationship between size of the dominant follicle and serum estradiol concentrations have been previously reported by others and are suggestive of greater physiological maturity of the follicle (Ireland and Roche, 1982; Kruip and Dieleman, 1985). The proportion of cows with a follicle greater than or equal to 10 mm was also increased for the treatments utilizing this mechanism. Although physiological maturity and LH receptor

acquisition are what ultimately determine a follicle's capability to ovulate, a follicle is often considered to have reached the required threshold for potential induction of ovulation after reaching a minimum diameter of approximately 10 mm (Sartori et al., 2001). A greater proportion of cows having a larger average follicle diameter reduces the variation in response rate to GnRH at the start of the 7-d CO-Synch + CIDR protocol. Additionally, our confidence in this response rate to GnRH is strongly supported by an observed absence of estrus expression between GnRH and $PGF_{2\alpha}$ among cows assigned to treatment 2, in which the CIDR insert was removed and not in place to prevent estrus expression during this time. The increased responsiveness to GnRH facilitated through this presynchronization method also minimizes or eliminates the potential problem of persistent follicles, which can occur among a proportion of cows on the 7-d CO-Synch + CIDR protocol and contribute to suboptimal fertility. Additionally, the earlier time of estrus expression among cows who develop a persistent follicle on the 7-d CO-Synch + CIDR protocol is also not a concern, as treatment with a CIDR insert and administration of $PGF_{2\alpha}$ increases the response rate to GnRH.

Subsequent to the improved response to GnRH on day -10, we observed a tendency for decreased variation in corpora lutea status on day -3. Treatments receiving presynchronization tended to have a single CL, with lower proportions of cows having no luteal tissue or a CL with an accessory CL compared to the 7-d CO-Synch + CIDR treatment. Particularly in treatments 2 and 3, we conclude that the majority of cows with a single CL exhibited a newly formed CL that resulted from induced ovulation through administration of GnRH on day -10. This interpretation may be supported by the observation of decreased mean serum progesterone concentrations in these treatments, despite an increased proportion of cows having a CL in comparison to the 7-d CO-Synch + CIDR protocol. Presynchronization that includes administration of $PGF_{2\alpha}$ prior to

the 7-d CO-Synch + CIDR protocol reduced the proportion of cows that were late in the luteal phase during the protocol and formed an additional accessory CL in response to GnRH. Thus, in comparison to the 7-d CO-Synch + CIDR protocol, this presynchronization mechanism minimizes the proportion of cows that express estrus at a later time as a result of the preovulatory follicle that develops under high circulating progesterone concentrations and receives lower frequency pulsatile LH support during development.

To achieve subluteal concentrations of progesterone prior to GnRH administration we administered $PGF_{2\alpha}$ coincident with insertion of a CIDR in treatments 2 and 3. However, serum progesterone concentrations were not statistically different across treatments when GnRH was administered on day -10. We also hypothesized that by removing the CIDR insert for the last seven days for treatment 2, the dominant follicle could grow to a larger size in the lower circulating progesterone concentration environment; however, we did not observe a difference in average LFD on day -3 across treatments. Although we hypothesize the earlier treatment with progesterone in this approach may increase the likelihood of GnRH response among anestrous cows, we did not have the number of anestrous cows per treatment necessary to detect a statistical difference. If the progesterone from the CIDR insert induces anestrous cows to resume cyclicity as reported by others (Fike et al., 1997; Stevenson et al., 2003) and earlier induction yields an increased likelihood of ovulation in response to GnRH, this could minimize the variation in timing of estrus expression seen following the 7-d CO-Synch + CIDR protocol.

Several results support the hypothesis that administering $PGF_{2\alpha}$ prior to a progesterone treatment can increase the response rate to GnRH. Treatment tended to affect estrous response observed by FTAI. According to the intervals from $PGF_{2\alpha}$ to estrus, collected by estrus detection transmitters, the recommended time to perform FTAI at 60 to 66 hours after $PGF_{2\alpha}$ is applicable

to the protocols that include the presynchronization mechanism. The increased estrous response we observed in the appropriate time interval indicate potential success when artificially inseminating at a fixed time point with sex-sorted semen. Due to the stresses of the sorting process and cryopreservation, sex-sorted semen has limited viability within the reproductive tract of a female, resulting in impaired fertility (Seidel and Garner, 2002; Hollinshead et al., 2003). Higher pregnancy rates when using sex-sorted semen can be achieved by only inseminating females who have been observed in standing estrus (Seidel, 2007; Thomas et al., 2014; Thomas et al., 2017; Thomas et al., 2019). Typically, this requires an extended period of estrus detection or a multiple day breeding event. The treatments that included a presynchronization mechanism prior to that 7-d CO-Synch + CIDR protocol increased the proportion of cows that expressed estrus in a synchronized time period, facilitating the use of sex-sorted semen in a fixed-time breeding event. Although, we did not detect any statistical differences in pregnancy rate, this was to be expected, as we did not design this experiment with the power to do so. Finally, for all treatments, if cows did not become pregnant to AI, they had similar ability to become pregnant to natural service, as there was no difference in pregnancy rate at the end of the breeding season

The adoption of sex-sorted semen in the beef industry has been limited due to the inability to achieve success in a fixed-time breeding event. The development of an estrus synchronization protocol resulting in improved synchrony of estrus expression would facilitate the adoption of sex-sorted semen in timed breeding events and allow beef cattle producers the opportunity to reap the benefits of this advanced reproductive technology. The results from this experiment demonstrate an improvement in response rate to GnRH by incorporating a presynchronization mechanism prior to an estrus synchronization protocol that begins with administration of GnRH. Based on these results and consideration of logistics and ease of

application, we suggest that the treatment schedule used in treatment 3 offers the most potential for further research as a novel estrus synchronization protocol. In this treatment schedule, $PGF_{2\alpha}$ is administered coincident with CIDR insertion seven days prior to the 7-d CO-Synch + CIDR protocol. Although no cows expressed estrus following CIDR removal on day -10 in treatment 2, the CIDR remaining in place the last seven days ensures that no animal has the opportunity to exhibit estrus during an unfeasible time period prior to breeding. The treatment schedule used in treatment 3 is now referred to as "7 & 7 Synch" due to the additional seven days added to the treatment schedule. Future research efforts are needed to evaluate field fertility using this approach, both for timed artificial insemination with conventional and sex-sorted semen and for synchronization of recipient females prior to embryo transfer.

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Table 2.1.
Cow age, days postpartum (DPP), and body condition score (BCS) by treatment.

Treatment[1]	N	Age, y	DPP[2]	BCS[3]	Estrous Cyclicity[4] Proportion	%
Treatment 1	37	4.5 ± 2.4	77.3 ± 13.4	5.6 ± 0.6	32/37	86
Treatment 2	36	4.8 ± 2.4	72.7 ± 12.2	5.5 ± 0.6	32/36	89
Treatment 3	38	4.7 ± 2.4	77.2 ± 13.5	5.6 ± 0.6	34/38	89
Treatment 4	38	4.7 ± 2.5	76.5 ± 12.0	5.6 ± 0.7	36/38	95
Treatment 5	36	4.8 ± 2.6	75.7 ± 12.7	5.6 ± 0.7	34/36	94

Data presented as mean value (± standard deviation of mean)

[1]See Figure 1 for treatment descriptions.

[2]DPP calculated from calving date to breeding date.

[3]BCS of cows at the time of first pretreatment blood sample (day -27) on a scale of 1-9 (1 = emaciated and 9 = obese)

[4]Proportion cycling prior to the start of treatment. Cows were classified as anestrous on the basis of serum progesterone concentrations lower than 0.5 ng/ml in two samples taken ten days apart.

Table 2.2.
Average large follicle diameter (LFD) determined via transrectal ultrasonography.

Treatment[1]	LFD on Day -17[2] Mean ± SE	LFD on Day -10[3] Mean ± SE	LFD on Day -3[4] Mean ± SE	LFD on Day 0[5] Mean ± SE
Treatment 1	10.3 ± 0.6	8.3 ± 0.7[a]	11.3 ± 0.4	13.8 ± 0.6
Treatment 2	10.1 ± 0.6	13.8 ± 0.7[c]	12.1 ± 0.4	15.2 ± 0.5
Treatment 3	9.7 ± 0.7	13.0 ± 0.9[cB]	11.9 ± 0.5	14.1 ± 0.7
Treatment 4	9.8 ± 0.7	11.5 ± 0.7[b]	12.2 ± 0.5	14.9 ± 0.7
Treatment 5	9.8 ± 0.7	11.2 ± 0.8[b]	11.9 ± 0.6	13.8 ± 0.6

Data presented as mean value (± standard error of mean)
[1]See Figure 1 for treatment descriptions.
[2]LFD at the start of the treatments (P = 0.94)
[3]LFD when all cows received GnRH
[abc]Values with different superscript, p value < 0.05
[bB]Values with tendency of P = 0.08
[4]LFD when all cows received $PGF_{2\alpha}$ (P = 0.67)
[5]LFD on the day of FTAI with sex-sorted semen (P = 0.39)

Table 2.3.
Comparisons between variables for treatments that are similar on day -10

	Serum progesterone (ng/ml)[2]	Diameter of Dominant follicle[3]	Dominant Follicle ≥ 10 mm[4]		Serum estradiol-17β (pg/ml)[5]
Treatment[1]	Mean ± SE	Mean ± SE	Proportion	%	Mean ± SE
Treatment 1	2.7 ± 0.6	8.3 ± 0.6[a]	5/20	25[d]	2.6 ± 0.3[f]
Treatment 2 & 3	2.8 ± 0.4	13.4 ± 0.4[b]	34/41	83[e]	4.5 ± 0.3[g]
Treatment 4 & 5	3.6 ± 0.5	11.3 ± 0.5[c]	28/43	65[e]	3.2 ± 0.3[f]

Data presented as mean value (± standard error of mean)
[1]See Figure 1 for treatment descriptions.
[2]Values did not differ, p value > 0.16
[3] [abc]Values with different superscripts, p value < 0.001
[4] [de]Values with different superscripts, p value < 0.05
[5] [fg]Values with different superscripts, p value < 0.0005

Table 2.4.
Corpora lutea (CL) status and serum progesterone concentration on day -3.

Treatment[1]	CL Status[2]						Serum progesterone (ng/ml)[3]
	Absence of CL		Single CL		Cl & acc. CL		
	Proportion	%	Proportion	%	Proportion	%	Mean ± SE
Treatment 1	7/20	35	9/20	45	4/20	20	4.9 ± 0.7[a]
Treatment 2	3/21	14	17/21	81	1/21	5	3.8 ± 0.5[ab]
Treatment 3	2/20	10	15/20	75	3/20	15	3.3 ± 0.5[b]
Treatment 4	1/22	5	20/22	91	1/22	5	2.8 ± 0.5[b]
Treatment 5	5/21	24	14/21	67	2/21	10	3.9 ± 0.7[ab]

Data presented as mean value (± standard error of mean)
[1]See Figure 1 for treatment descriptions.
[2]CL status when all treatments received $PGF_{2\alpha}$ (P = 0.08)
[3]Serum progesterone concentration determined RIA
[ab]Values with different superscripts, p value < 0.05

Table 2.5.
Average serum estradiol-17β concentrations determined via radioimmunoassay (RIA).

Treatment[1]	Serum estradiol-17β (pg/ml) on Day -3 Mean ± SE	Serum estradiol-17β (pg/ml) on Day 0 Mean ± SE
Treatment 1	3.3 ± 0.3[a]	5.0 ± 0.3[xZ]
Treatment 2	3.8 ± 0.4[ab]	4.6 ± 0.4[xy]
Treatment 3	4.2 ± 0.3[b]	4.2 ± 0.3[xyz]
Treatment 4	3.8 ± 0.3[ab]	4.8 ± 0.4[x]
Treatment 5	4.5 ± 0.3[b]	3.8 ± 0.3[y]

Data presented as mean value (± standard error of mean)
[1]See Figure 1 for treatment descriptions.
[ab]Values with different superscripts, p value < 0.05
[xy]Values with different superscripts, p value < 0.05
[zZ]Values with tendency of (P = 0.07)

Table 2.6.
Estrous response based on treatment and timing of estrus expression.

	Estrous Response Between GnRH & $PGF_{2\alpha}$[2]		Estrous Response by FTAI[3]		Interval from $PGF_{2\alpha}$ to Estrus[4] (hrs)
Treatment[1]	Proportion	%	Proportion	%	Mean ± SE
Treatment 1			25/37	68	45.2 ± 2.0
Treatment 2	0/36[a]	0	33/36	92	49.6 ± 3.1
Treatment 3			31/38	82	46.3 ± 3.0
Treatment 4	5/34[b]	15	27/39	69	53.7 ± 2.0
Treatment 5			29/37	78	44.6 ± 2.2

Data presented as mean value (± standard error of mean)
[1]See Figure 1 for treatment descriptions.
[2]Estrotect patches were applied with CIDR removal on day -10 and estrous response was recorded on day -3
[ab]Estrous response differs between treatments (P = 0.03)
[3]Estrous response by FTAI refers to females that were detected in estrus by approximately 66 hours after $PGF_{2\alpha}$ (P = 0.08)
[4]Interval from $PGF_{2\alpha}$ to estrus expression data collected from Accubreed estrus detection transmitters (P = 0.07)

Table 2.7.
Pregnancy rates to fixed-time artificial insemination (FTAI) based treatment and estrous response.

	Pregnancy Rate[1] to FTAI		Estrous[3] Pregnancy Rate to FTAI		Non-estrous[4] Pregnancy Rate to FTAI		Final Pregnancy Rate[5]	
Treatment[1]	Proportion	%	Proportion	%	Proportion	%	Proportion	%
Treatment 1	19/37	51	15/25	60	4/12	33	35/37	95
Treatment 2	15/38	39	14/33	42	0/3	0	32/38	84
Treatment 3	24/38	63	19/31	61	3/7	43	34/38	89
Treatment 4	15/40	38	12/27	44	3/12	25	37/40	93
Treatment 5	18/39	46	16/29	55	2/8	25	34/39	87

[1]See Figure 1 for treatment descriptions
[2]Pregnancy rate to AI determined by transrectal ultrasonography 70 - 85 days after FTAI. (P = 0.16)
[3]Estrous was classified as >50% of the Estrotect patch coating removed. (P = 0.46)
[4]Non-estrous was classified as <50% of the Estrotect patch coating removed. (P = 0.73)
[5]Pregnancy rate at the end of the breeding season. (P = 0.60)

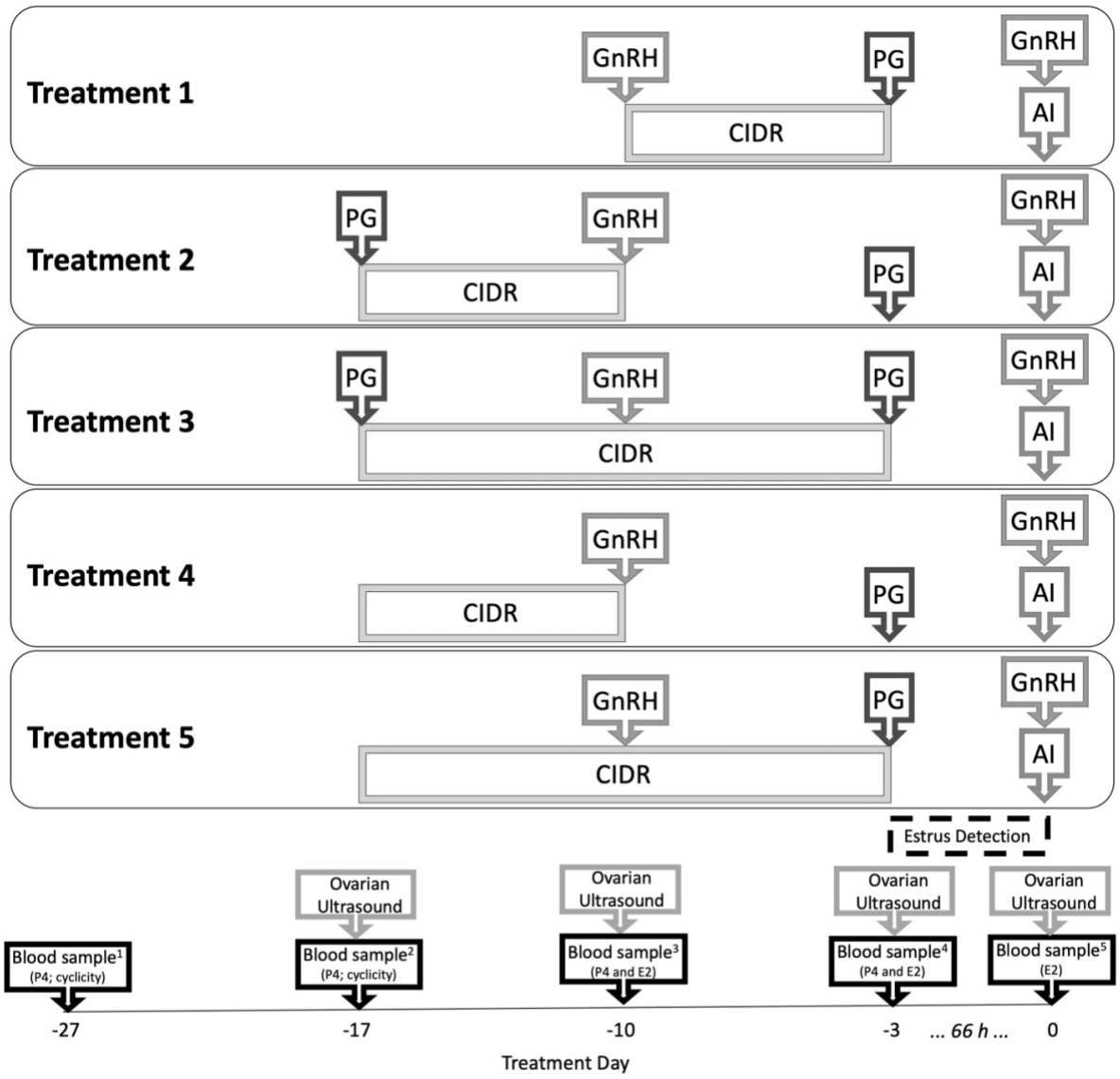

Figure 2.1. Treatment diagrams for experiment 1. Treatment 1: 7-d CO-Synch + CIDR protocol received a CIDR insert (1.38 g progesterone) and administration of GnRH (100 µg Fertagyl) on day -10. On day -3, CIDR inserts were removed, $PGF_{2\alpha}$ (25 mg Estrumate) was administered, and Estrotect patches were applied. Treatment 2 received a CIDR insert and $PGF_{2\alpha}$ was administered on day -17. On day -10 CIDR inserts were removed, GnRH was administered, and Estrotect patches were applied. $PGF_{2\alpha}$ was administered on day -3 and Estrotect patches were replaced. Treatment 3 received CIDR insert and $PGF_{2\alpha}$ was administered on day -17. GnRH was administered on day -10. On day -3, CIDR inserts were removed, $PGF_{2\alpha}$ was administered, and Estrotect patches were applied. Treatment 4 received CIDR inserts on day -17. On day -10, CIDR inserts were removed, GnRH was administered, and Estrotect patches were applied. $PGF_{2\alpha}$ was administered on day -3 and Estrotect patches were replaced. Treatment 5 received CIDR inserts on day -17 and GnRH was administered on day -10. On day -3, CIDR inserts were removed, $PGF_{2\alpha}$ was administered and Estrotect patches were applied. For all treatments on day 0, 66 hours after $PGF_{2\alpha}$, cows were inseminated with sex-sorted semen and GnRH was administered.

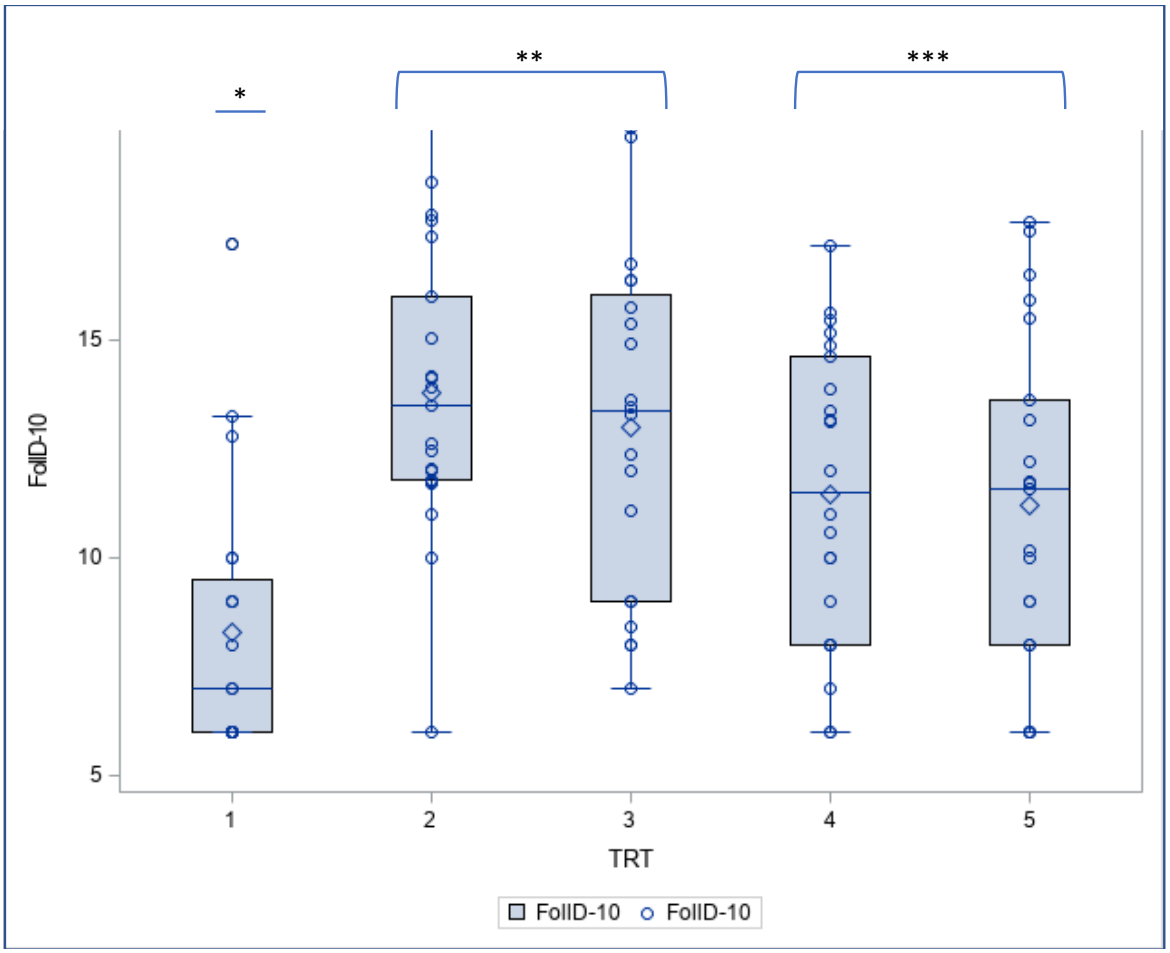

Figure 2.2. Box and whisker plot of average large follicle diameter (LFD) on day -10. Average LFD measurements were recorded via transrectal ultrasonography using electronic calipers to measure follicle height and width. The LFD for treatments 2 and 3 was larger ($P < 0.001$) than treatment 1 and treatments 4 and 5. The LFD was larger ($P < 0.001$) for treatments 2 and 3 (13.4 mm ± 0.4) and for treatments 4 and 5 (11.3 mm ± 0.5) in comparison to treatment 1 (8.3 mm ± 0.6). Additionally, for treatments 2 and 3, the LFD was larger ($P < 0.001$) than treatment 1 and treatments 4 and 5.

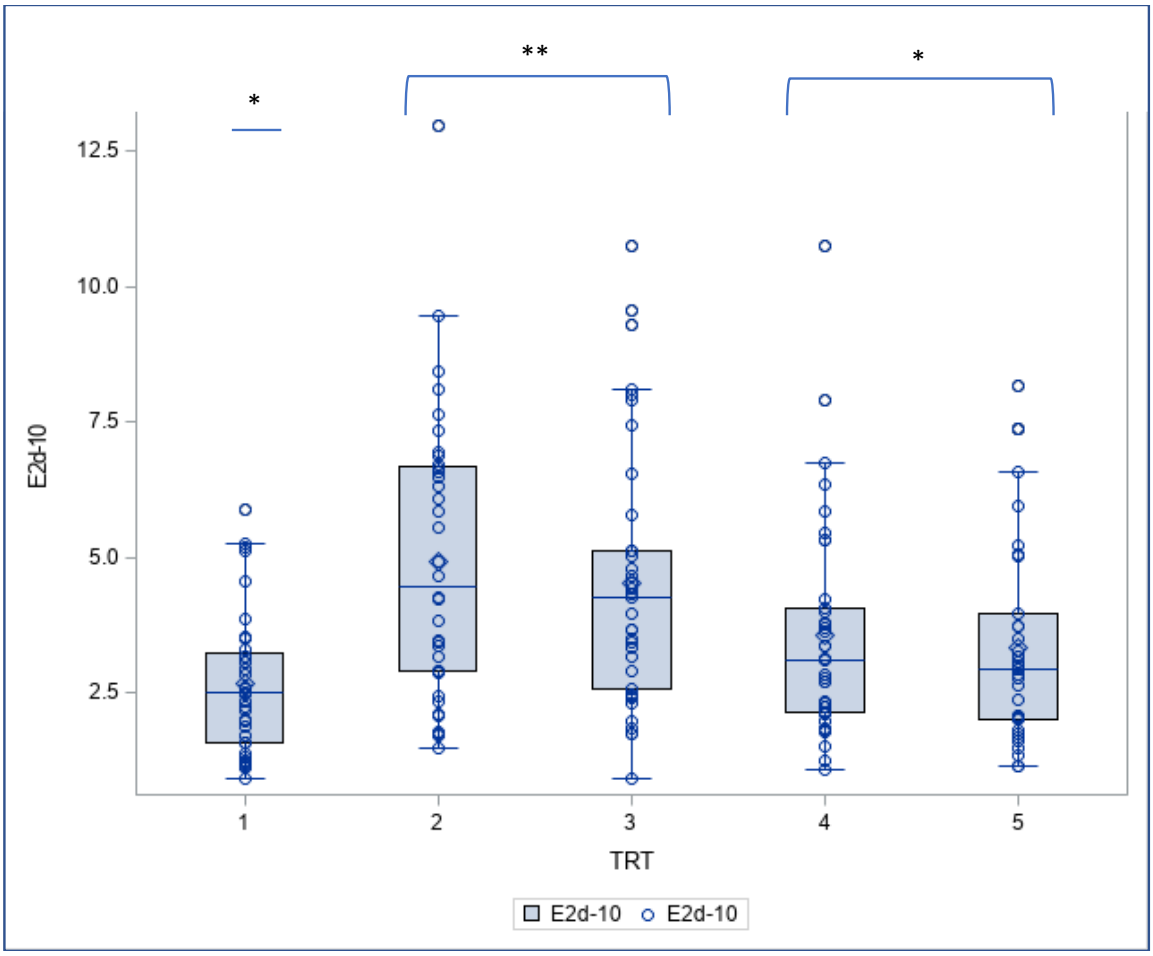

Figure 2.3. Box and whisker plot of serum estradiol-17β concentrations on day -10. Circulating serum estradiol-17β concentrations were determined via radioimmunoassay (RIA). Serum estradiol concentrations were greater ($P < 0.005$) for treatments 2 and 3 (4.5 pg/ml ± 0.3) compared to treatments 4 and 5 (3.2 pg/ml ± 0.3) and treatment 1 (2.6 pg/ml ± 0.3).

Chapter 3

Comparison of the 7 & 7 Synch protocol and the 7-day CO-Synch + CIDR protocol among recipient beef cows in an embryo transfer program

Abstract

An experiment was designed to evaluate the effectiveness of the recently developed 7 & 7 Synch protocol to synchronize estrus and ovulation among recipients prior to embryo transfer. Postpartum beef cows (n=1,358) across thirteen locations were assigned to either the 7-d CO-Synch + CIDR protocol or the 7 & 7 Synch protocol prior to estrus detection and subsequent embryo transfer. Cows were preassigned to balanced treatments within location based on age and days postpartum, with body condition score recorded at embryo transfer. Cows assigned to the 7-d CO-Synch + CIDR protocol were administered gonadotropin-releasing hormone (GnRH; 100µg gonadorelin acetate) on Day 0, an intravaginal controlled internal drug release insert (CIDR; 1.38 g progesterone) from Day 0 to Day 7, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg dinoprost tromethamine) coincident with CIDR removal on Day 7. Cows assigned to the 7 & 7 Synch protocol were administered $PGF_{2\alpha}$ (25 mg dinoprost tromethamine) coincident with CIDR insertion on Day -7, GnRH (100 µg gonadorelin acetate) on Day 0, and $PGF_{2\alpha}$ (25 mg dinoprost tromethamine) coincident with CIDR removal on Day 7. The 7 & 7 Synch protocol was hypothesized to enhance response to GnRH administration on Day 0 among mixed groups of estrous cycling and anestrous recipient cows, ultimately resulting in improved estrous response and synchrony of estrus prior to embryo transfer. Cows were observed for visible signs of estrus following estrus synchronization, with GnRH (100 µg gonadorelin acetate) administered to cows

failing to express estrus during the detection period. Embryo transfer was performed approximately seven days after estrus or GnRH administration. Presence of corpora lutea (CL) was determined via transrectal palpation by a single veterinarian blinded to treatment, and embryos were transferred only to cows with palpable CL. Embryo transfer was performed using either fresh or frozen embryos staged and graded according to IETS recommended guidelines, with embryo information recorded for each recipient. The proportion of cows expressing estrus was improved ($P < 0.0001$) among cows assigned to the 7 & 7 Synch protocol (86% [529/615] vs 76% [488/640]). The proportion of cows expressing estrus and presenting with palpable CL at embryo transfer was greater ($P < 0.0001$) among cows following treatment with the 7 & 7 Synch protocol compared to the 7-d CO-Synch + CIDR protocol (76% [466/615] vs 65% [418/640]). Consequently, the proportion pregnant to embryo transfer was greater ($P < 0.03$) following the 7 & 7 Synch protocol (40% [263/653]) compared to the 7-d CO-Synch + CIDR protocol (34% [228/664]). In summary, the 7 & 7 Synch protocol involving administration of $PGF_{2\alpha}$ and treatment with a CIDR for 7 days prior to GnRH improved the likelihood of estrus expression in recipient cows, increased the proportion of cows eligible to receive an embryo, which resulted in a greater pregnancy rate to embryo transfer.

Key words: 7 & 7 Synch, estrus synchronization, embryo transfer, recipient

Introduction

In the past 40 years, embryo transfer has become a powerful reproductive technology available to the cattle industry, surpassing artificial insemination in its ability to produce a higher quantity of genetically superior offspring in a shorter period of time (Lohuis, 1995; Hasler,

2014). In addition to being a useful tool in research, embryo transfer has proven valuable in the beef cattle industry in its ability to produce genetically elite sires, proliferate the number of elite females, establish disease free herds, and facilitate the international movement of animal genetics through embryos (Mapletoft and Bó, 2006). Although many factors influence the successful use of embryo transfer, estrus synchronization of recipient females is one critical component of a successful program. Following a synchronization protocol, a higher pregnancy rate to embryo transfer occurs among recipients that express estrus than among recipients that do not express estrus (Baruselli et al., 2003). Additionally, estrus expression following a synchronization protocol affects the number of recipients eligible to receive an embryo when those that do not express estrus are excluded from the pool of potential candidates.

As with artificial insemination, a commonly used estrus synchronization protocol among recipient females in an embryo transfer program is the 7-d CO-Synch + CIDR protocol. Although it is a producer-friendly protocol, the variation in estrous response observed following synchronization is limiting to the goal of creating a large population of recipients eligible to receive embryo transfer. The 7-d CO-Synch + CIDR protocol typically results in acceptable estrous response on average, but the variation that can occur is evident in previous literature where multiple locations are included in an experiment. As an example, in two recent experiments in which all cows were treated with the 7-d CO-Synch + CIDR protocol, the range in observed estrous response across locations was from 61% to 84% and 61% to 82% respectively (Bishop et al., 2016; Thomas et al., 2019). Although this variation is across different locations in a single year, the range in proportion of females exhibiting estrus can also occur within a single operation from one breeding season to the next. Variable estrous response observed following synchronization with the 7-d CO-Synch + CIDR protocol ultimately results

in variable pregnancy rates attained, as pregnancy rates are highly influenced by rates of estrus expression (Richardson et al., 2016).

The objective of this experiment was to compare estrous response and pregnancy rates to embryo transfer among recipient females treated with either the 7-d CO-Synch + CIDR protocol or a recently developed estrus synchronization protocol known as 7 & 7 Synch (Bonacker et al., 2019). We hypothesized that the presynchronization mechanism of the 7 & 7 Synch protocol ($PGF_{2\alpha}$ administration and progesterone treatment seven days prior to the administration of GnRH) would induce cyclicity among cows that were anestrous due to shorter days postpartum, lower body condition score, or younger age. Additionally, we hypothesized that the 7 & 7 Synch protocol would increase estrus expression and decrease variation in synchrony among recipient females.

Materials and Methods

Animals and treatments

Estrus was synchronized for 1358 multiparous and primiparous recipient beef cows across thirteen locations. Within location, cows were preassigned to one of two treatments (Figure 1) based on age and days postpartum. Body condition score was recorded for each recipient on day of embryo transfer (scale of 1 to 9; 1 = emaciated and 9 = obese). On day 0, cows assigned to the 7 & 7 Synch protocol received an Eazi-Breed intravaginal controlled internal drug release insert (CIDR; 1.38 g progesterone; Zoetis) with administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg dinoprost tromethamine i.m.; Lutalyse; Zoetis). On day 7, cows in both treatments were administered gonadotropin-releasing hormone (GnRH; 100 ug gonadorelin acetate i.m.; Fertagyl; Merck Animal Health), and cows assigned to the 7-d CO-

Synch + CIDR protocol received a CIDR insert. On day 14, all cows were administered $PGF_{2\alpha}$ and CIDR inserts were removed.

Estrus detection

An estrus detection period occurred following $PGF_{2\alpha}$ for approximately 132 hours. To record timing of estrus onset, cows were visually appraised for behavioral signs of estrus twice daily during the estrus detection period. Estrotect patches were applied at $PGF_{2\alpha}$ to aid in estrus detection. At locations 1, 2, 5, 7, and 9 where non-estrous cows were potential recipients, GnRH was administered to the non-estrous recipients following the period of estrus detection.

Embryo transfer

Embryo transfer was performed by a veterinarian approximately seven days after estrus detection or GnRH administration. Presence of a corpus luteum (CL) on the ovary of each recipient was determined via transrectal palpation by a single veterinarian at each location prior to embryo transfer. Embryos were transferred into the uterine horn ipsilateral to the CL. Fresh or frozen embryos were staged and graded according to IETS recommended guidelines, and embryo information was recorded for each recipient upon embryo transfer.

Pregnancy diagnosis

Pregnancy rate to embryo transfer was determined approximately 75 days after embryo transfer by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0-5.0 MHz linear-array transducer; SonoSite, Inc., Bothwell, WA) for all locations except locations 5 and 9, where pregnancy diagnosis was performed via palpation by an experienced veterinarian.

Statistical Analysis

Overall estrous response was analyzed using the GLIMMIX procedure of SAS with age, body condition score, and days postpartum included in the model as fixed effects. Location was included in the model as a random effect. All potential two-way interactions were evaluated and removed from the model if not significant. Chi-Square (FREQ procedure of SAS) was used for the comparison of cumulative proportions of estrus expression in 12-hour intervals, utilization rate for estrous and non-estrous recipients, and pregnancy rate for recipients who received embryo transfer and all recipients that received synchronization.

Results

Treatment summary

The number of cows, age, days postpartum, and body condition score for each treatment within each location are summarized in Table 3.1. Age, days postpartum, and body condition score did not differ between treatments.

Estrous response

Estrous response and timing of estrus expression for each treatment are summarized in Table 3.1 Location 10 is not included in estrous response results as no estrus detection was performed, and all recipients were administered GnRH. The total estrous response following estrus detection was higher ($P < 0.001$) for recipients treated with the 7 & 7 Synch protocol (86%) compared to recipients treated with the 7-d CO-Synch + CIDR protocol (76%). Additionally, for every 12-hour interval of estrus expression, each cumulative proportion was

greater ($P < 0.005$) for recipients treated with the 7 & 7 Synch protocol compared to recipients treated with the 7-d CO-Synch + CIDR protocol. Estrous response was affected by days postpartum ($P < 0.01$) and body condition score ($P < 0.0001$); however, estrous response was not affected by either a treatment x days postpartum or a treatment x body condition score interaction. Figure 3.3 illustrates the increase in estrous response as days postpartum increases, and Figure 3.4 illustrates the increase in estrous response as body condition score of recipients increase. Additionally, Figure 3.2 is the distribution of estrus expression in 12-hour intervals for both the 7 & 7 Synch and the 7-d CO-Synch + CIDR protocols.

Utilization rate

Rates of successful embryo transfer based on treatment for all recipients who expressed estrus and/or were administered GnRH are summarized in Table 3.3. There was no difference ($P = 0.3$) in utilization rate between treatments among recipients that expressed estrus following synchronization (7-d CO-Synch + CIDR = 86%; 7 & 7 Synch = 88%). There was also no difference ($P = 0.12$) in utilization rate between treatments for those recipients that were administered GnRH (7-d CO-Synch + CIDR = 59%; 7 & 7 Synch = 49%). The overall utilization rate for both estrous recipients and those that were administered GnRH did not differ ($P = 0.6$) between treatments (7-d CO-Synch + CIDR = 80%; 7 & 7 Synch = 81%). However, the proportion of cows that expressed estrus and received embryo transfer was greater ($P < 0.0001$) among cows treated with the 7 & 7 Synch protocol (76% [466/615]) compared to cows treated with the 7-d CO-Synch + CIDR protocol (65% [418/640]) (Figure 3.5).

Pregnancy rate

Table 3.4 reports the pregnancy rates between treatments for recipients that expressed estrus and received embryo transfer, recipients that were non-estrous and administered GnRH prior to embryo transfer, and the pregnancy rate for all recipients receiving embryo transfer. A proportion of cows from Location 3 are excluded from pregnancy results as they received split embryos, which is not consistent with the fresh or frozen embryos transferred to recipients at all other locations. Treatments did not differ ($P = 0.4$) in pregnancy rate of recipients that expressed estrus and received embryo transfer (7-d CO-Synch + CIDR = 50%; 7 & 7 Synch = 53%). Also, there was no difference ($P = 0.7$) between treatments in pregnancy rate of recipients that were non-estrous and administered GnRH prior to embryo transfer (7-d CO-Synch + CIDR = 44%; 7 & 7 Synch = 47%). There was no difference ($P = 0.3$) in pregnancy rate between treatments among all recipients that received embryo transfer (7-d CO-Synch + CIDR = 49%; 7 & 7 Synch = 52%). Table 3.5 summarizes pregnancy rates to embryo transfer based on treatment of all cows receiving estrus synchronization. The pregnancy rate for all cows synchronized was greater ($P < 0.03$) among recipients synchronized with the 7 & 7 Synch protocol (40%) compared to recipients synchronized with the 7-d CO-Synch + CIDR protocol (34%).

Discussion

In the time from when the first live calf was successfully produced from embryo transfer to present day, improved success rates with embryo transfer have been the result of efforts both from scientists who developed the procedures and techniques and from practitioners who have adapted the technology for practical commercial application (Willett et al., 1951; Hasler, 2014). Much to the credit of both, beef cattle producers have been provided the opportunity to reap the substantial benefits of embryo transfer in their reproductive management programs. In order to

increase utilization of the technology, continued improvements are necessary to address a multitude of challenging factors that influence success of an embryo transfer program. These factors include the donor's response to superovulation, number of viable embryos recovered, as well as synchrony of stage of cycle between the donor and recipients. This experiment focuses on the subject of recipient estrus synchronization. In beef cattle operations using embryo transfer, a veterinarian often has negligible influence over the selection of the donor, the number of viable embryos collected, or other decisions made prior to transfer. In contrast, the veterinarian can have a significant influence on recipient management and the estrus synchronization protocol utilized.

Ultimately, two aspects of estrus synchronization are important for the success of an embryo transfer program. First, the recipients need to successfully express estrus following synchronization in order to achieve optimal pregnancy rates (Baruselli et al., 2003; Bó and Cedeño, 2018). One key factor limiting estrous response is the proportion of anestrous females in the group. The inability for these females to resume the regular occurrence of estrous cycles is influenced by lactation or presence of a calf, nutrition and body condition, and age or parity of the cow. As days postpartum or days since the female calved can influence the resumption of cyclicity, the proportion of cows that resume cyclicity can be improved with a longer period between calving and the onset of the breeding season (Stevenson et al., 2002). Additionally, at higher planes of nutrition and higher body condition scores, the interval to first estrus decreases and proportion of estrous cycling cows increases (Short et al., 1990; Stevenson et al., 2002). A continuation of the effect of nutrition and body condition score are that primiparous cows require the greatest amount of energy to resume their estrous cycles; therefore, special considerations are

required for management of primiparous cows to ensure resumption of estrous cyclicity prior to the start of the breeding season (Stevenson, 2002).

Secondly, the recipients need to express estrus in a sufficiently narrow window of time in order for a fixed-time embryo transfer program to result in acceptable pregnancy rates. Establishment of pregnancy among recipients requires tight synchrony between the stage of the embryo and the uterine environment of the recipient following estrus synchronization (Moore and Shelton, 1964). Variation in timing of estrus expression can be a result of the estrus synchronization protocol utilized. A commonly used estrus synchronization protocol among beef cattle recipients is the 7-d CO-Synch + CIDR. Although easy to implement and producer friendly, it results in variable estrous response. The differing response to the first GnRH administration within a group of estrous cycling and anestrous cows creates populations of cows that express estrus at a different time points within the group. Situations where persistent follicles develop, an accessory CL is formed, or anestrous cows are induced to resume cyclicity with the progestin treatment, all contribute to the variation in timing of estrus expression following the 7-d CO-Synch + CIDR protocol. Therefore, an estrus detection period is required in order to successfully match the stage of embryo to the uterine environment of the recipient. The labor associated with estrus detection limits the adoption of embryo transfer, and the range of time over which estrus expression occurs limits potential success rates if performing fixed-time embryo transfer.

This experiment aimed to increase the estrous response and decrease the variation in timing of estrus expression among recipients treated with the 7 & 7 Synch protocol (Bonacker et al., 2019). This protocol successfully improved the response rate to GnRH by incorporating a presynchronization approach of $PGF_{2\alpha}$ administration coincident with progesterone treatment

seven days prior to administration of GnRH. Results suggested treatment with the 7 & 7 Synch protocol may minimize variation in follicular development and luteal status, both of which contribute to variation in timing of estrus expression. Additionally, we hypothesized that progesterone treatment prior to administration of GnRH may improve the likelihood of ovulatory response to GnRH among anestrous cows. Anestrous cows that fail to ovulate and form a CL in response to GnRH administration express estrus early, in response to progestin withdrawal following CIDR removal, rather than at a time more similar to the rest of the group undergoing estrus synchronization. The present results support the hypothesis that the 7 & 7 Synch protocol results in enhanced rates of estrous response, thereby increasing the number of embryos transferred into estrous recipients and increasing pregnancy rate per female receiving estrus synchronization. Taking into consideration that labor is a limiting factor to producers for the adoption of reproductive technologies, this novel estrus synchronization protocol only adds one additionally trip through the chute in comparison to the 7-d CO-Synch + CIDR protocol. There is potential for the increased estrous response, increased pregnancy rate, and increased number of ET calves born to compensate for the added time and labor associated with this additional trip through the chute.

Following treatment with the 7 & 7 synch protocol, an increase in total estrous response was observed among recipients. This resulted in more eligible candidates for embryo transfer and fewer recipients receiving GnRH administration. In addition to the increase in estrous response, in the 12-hour intervals of estrus expression, each cumulative proportion was significantly greater for the 7 & 7 Synch protocol in comparison to the 7-d CO-Synch + CIDR protocol. These results raise questions as to whether fixed-time embryo transfer could be performed effectively following synchronization with the 7 & 7 Synch protocol. Although cyclicity status of each cow

at the start of treatments is unknown in this experiment, factors associated with anestrus were characterized, such as age, days postpartum, and body condition score. The 7 & 7 Synch protocol resulted in increased estrous response across body condition score and days postpartum ranges, including an 83% estrous response for cows 0 to 45 days postpartum. Although estrous response was not significantly affected by the interaction of treatment and body condition score, the observation across treatments of an increase in estrous response with each increase in body condition score underscores the importance of proper nutritional management of recipients.

In a one-day embryo transfer event, the number of pregnancies that can result from embryo transfer is limited by the number of embryos transferred, which is ultimately limited by the number of recipients with an acceptable corpus luteum. In the present data, the likelihood that a recipient would express estrus and receive embryo transfer was greater for cows recieving the 7 & 7 Synch protocol. This protocol generated a larger pool of potential candidates for embryo transfer, which can be beneficial when transferring fresh embryos and when the number of embryos needing to be transferred is unknown until the day of the collection. In the same scenario, more eligible recipients for embryo transfer decreases the number of fresh embryos that must be cryopreserved rather than immediately transferred.

We observed no difference in pregnancy rate of recipients receiving embryo transfer, nor was a difference anticipated. The pregnancy rate of all cows receiving estrus synchronization was significantly higher following the 7 & 7 Synch protocol due to the increase in total number of estrous recipients for transfer. A successful day of embryo transfer is not just about the day the veterinarian transfers embryos into recipients. The management of the recipients prior the start of the breeding season can have a tremendous influence on the number of eligible candidates to potentially receive embryo transfer, as well as the number of embryos transferred. In contrast,

future success among a group of recipients is dependent on the day of embryo transfer, as those recipients that conceive early in the breeding season have a better chance to be estrous cycling, suitable candidates for embryo transfer the next year. These results suggest the potential for the 7 & 7 Synch protocol to increase the number of eligible candidates for embryo transfer through an increased estrous response. Future research is needed to evaluate the efficacy of using the 7 & 7 Synch protocol to synchronize estrus among recipients prior to fixed-time embryo transfer.

Acknowledgements

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Table 3.1.
Cow age, days postpartum (DPP), and body condition score (BCS) by location and treatment.

Location	Treatment[1]	N	Age, y	DPP[2]	BCS[3]
Location 1	7-d CO-Synch + CIDR	128	4.8 ± 2.3	61.3 ± 12.3	5.1 ± 0.7
	7 & 7 Synch	130	4.9 ± 2.4	60.5 ± 13.0	5.1 ± 0.7
Location 2	7-d CO-Synch + CIDR	128	6.0 ± 1.9	52.8 ± 13.3	5.5 ± 0.6
	7 & 7 Synch	122	6.0 ± 1.9	53.1 ± 13.6	5.5 ± 0.5
Location 3	7-d CO-Synch + CIDR	44	5.2 ± 2.4	47.8 ± 10.8	6.1 ± 0.6
	7 & 7 Synch	37	5.8 ± 2.3	41.5 ± 10.2	5.9 ± 0.7
Location 4	7-d CO-Synch + CIDR	31	4.5 ± 0.5	53.3 ± 14.6	6.2 ± 0.4
	7 & 7 Synch	31	4.5 ± 0.5	55.1 ± 13.1	6.2 ± 0.5
Location 5	7-d CO-Synch + CIDR	46	5.0 ± 2.1	43.2 ± 17.3	6.0 ± 0.6
	7 & 7 Synch	48	5.1 ± 2.1	43.9 ± 15.5	6.2 ± 0.7
Location 6	7-d CO-Synch + CIDR	56	3.9 ± 1.7	47.9 ± 10.3	5.2 ± 0.7
	7 & 7 Synch	37	3.7 ± 2.0	52.4 ± 11.5	5.0 ± 0.8
Location 7	7-d CO-Synch + CIDR	41	5.0 ± 2.1	61.3 ± 10.2	4.8 ± 0.5
	7 & 7 Synch	41	5.3 ± 2.1	59.4 ± 10.1	4.9 ± 0.5
Location 8	7-d CO-Synch + CIDR	28	6.4 ± 2.6	56.4 ± 9.7	4.9 ± 0.7
	7 & 7 Synch	29	6.6 ± 2.8	56.9 ± 12.9	5.0 ± 0.7
Location 9	7-d CO-Synch + CIDR	44	2.0 ± 0	76.9 ± 15.0	5.6 ± 0.5
	7 & 7 Synch	45	2.0 ± 0	72.4 ± 14.2	5.5 ± 0.5
Location 10	7-d CO-Synch + CIDR	21	4.1 ± 1.8	57.0 ± 9.1	5.2 ± 0.7
	7 & 7 Synch	21	4.2 ± 2.0	59.1 ± 9.7	5.2 ± 0.9
Location 11	7-d CO-Synch + CIDR	50	5.0 ± 2.3	61.1 ± 15.2	5.1 ± 0.7
	7 & 7 Synch	53	4.6 ± 2.3	64.2 ± 14.7	5.0 ± 0.7
Location 12	7-d CO-Synch + CIDR	51	3.3 ± 1.0	62.1 ± 13.3	5.8 ± 0.8
	7 & 7 Synch	50	3.3 ± 0.9	62.2 ± 12.6	5.7 ± 0.7
Location 13	7-d CO-Synch + CIDR	22	5.7 ± 2.5	55.2 ± 11.4	4.6 ± 0.6
	7 & 7 Synch	24	5.8 ± 2.0	54.5 ± 14.6	4.5 ± 0.8
Overall	7-d CO-Synch + CIDR	690	4.8 ± 2.2	56.9 ± 14.3	5.4 ± 0.8
	7 & 7 Synch	668	4.8 ± 2.3	56.6 ± 14.6	5.4 ± 0.8

Data presented as mean value (± standard deviation of mean)
[1]See Figure 1 for treatment descriptions.
[2]DPP calculated from calving date to day 0 of treatments.
[3]BCS of cows at the time of embryo transfer on a scale of 1-9 (1 = emaciated and 9 = obese)

Table 3.2.
Cumulative estrous response and timing of estrus expression for each treatment.

Estrus expression (by 12 h intervals)	[1]7-d CO-Synch + CIDR		7 & 7 Synch	
	Cumulative		Cumulative	
	Proportion	%	Proportion	%
24-36	62/640	10[a]	92/615	15[b]
36-48	145/640	23[a]	199/615	32[b]
48-60	324/640	51[a]	367/615	60[b]
60-72	400/640	63[a]	454/615	74[b]
72-84	444/640	69[a]	492/615	80[b]
84-96	468/640	73[a]	510/615	83[b]
96-108	477/640	75[a]	521/615	85[b]
108-120	484/640	76[a]	529/615	86[b]
120-132	488/640	76[c]	529/615	86[d]
Total	488/640	76[c]	529/615	86[d]
Non-estrous, GnRH	77/640	12	57/615	9
Non-estrous, excluded	75/640	12[a]	29/615	5[b]

[1]See Figure 1 for treatment descriptions.
[ab]Values within row differ, p value < 0.005
[cd]Values within row within differ, p value < 0.0001

Table 3.3.
Rates of successful embryo transfer based on treatment for all recipients who expressed estrus and/or were administered GnRH.

Treatment[1]	Utilization Rate for Estrous Recipients		Utilization Rate for Non-Estrous Recipients Administered GnRH		Utilization Rate Overall	
	Proportion	%	Proportion	%	Proportion	%
Treatment 1	418/488	86[a]	75/127	59[b]	493/615	80[c]
Treatment 2	466/529	88[a]	54/110	49[b]	520/639	81[c]

[1]See Figure 1 for treatment descriptions.
[a]Values within column did not differ (P = 0.3)
[b]Values within column did not differ (P = 0.12)
[c]Values within column did not differ (P = 0.6)

Table 3.4.
Pregnancy rates to embryo transfer based on treatment for all recipients who expressed estrus and received embryo transfer or were administered GnRH and received embryo transfer.

Treatment[1]	Pregnancy Rate for Estrous Recipients		Pregnancy Rate for Non-Estrous Recipients Administered GnRH		Pregnancy Rate of All Recipients Receiving Transfer	
	Proportion	%	Proportion	%	Proportion	%
7-d CO-Synch + CIDR	196/394	50[a]	32/73	44[b]	228/467	49[c]
7 & 7 Synch	239/454	53[a]	24/51	47[b]	263/505	52[c]

[1]See Figure 1 for treatment descriptions.
[a]Values within column did not differ (P = 0.4)
[b]Values within column did not differ (P = 0.7)
[c]Values within column did not differ (P = 0.3)

Table 3.5.
Proportion of potential estrous recipients and pregnancy rates to embryo transfer based on treatment for all potential recipients receiving estrus synchronization.

Treatment[1]	Recipients that expressed estrus and received embryo transfer		Pregnancy Rate of Recipients Receiving Synchronization	
	Proportion	%	Proportion	%
7-d CO-Synch + CIDR	418/640	65[a]	228/664	34[c]
7 & 7 Synch	466/615	75[b]	263/653	40[d]

[1]See Figure 1 for treatment descriptions.
[ab]Values within column differ, p value < 0.0001
[cd]Values within column differ, p value < 0.03

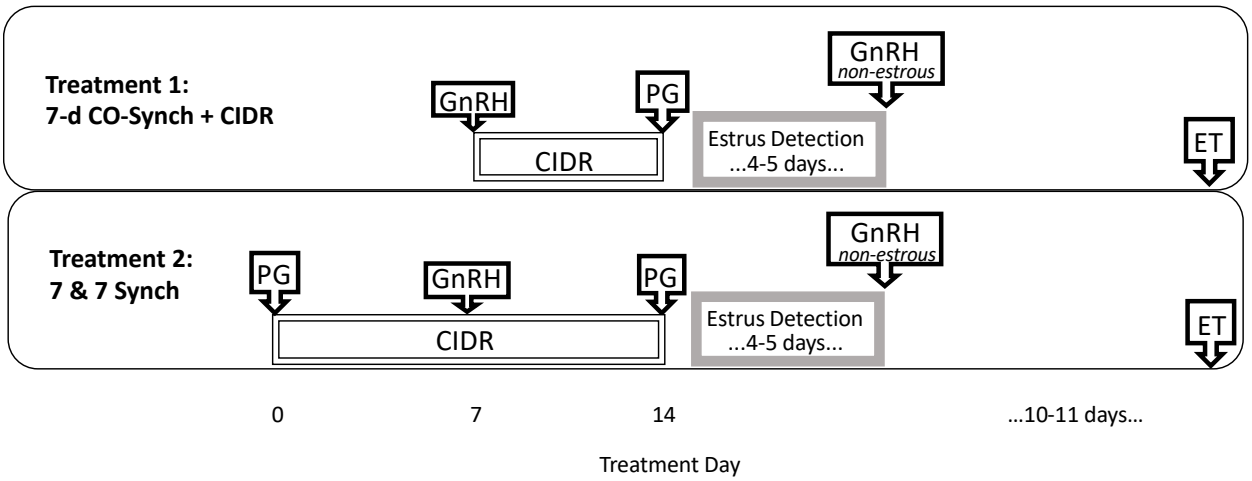

Figure 3.1. Treatment diagrams for experiment 2. Treatment 1: 7-d CO-Synch + CIDR protocol received a CIDR insert (1.38 g progesterone) and administration of GnRH (100 µg Fertagyl) on day 7. On day 14, CIDR inserts were removed, $PGF_{2\alpha}$ (25 mg Lutalyse) was administered, and Estrotect patches were applied. Estrus detection was performed for approximately 132 hours, and GnRH was administered to non-estrous cows in locations where non-estrous cows were potential recipients. Embryo transfer was performed by a veterinarian approximately seven days after estrus detection or GnRH administration. Treatment 2 received CIDR insert and $PGF_{2\alpha}$ was administered on day 0. GnRH was administered on day -10. On day 14, CIDR inserts were removed, $PGF_{2\alpha}$ was administered, and Estrotect patches were applied. Estrus detection was performed for approximately 132 hours, and GnRH was administered to non-estrous cows in locations where non-estrous cows were potential recipients. Embryo transfer was performed by a veterinarian approximately seven days after estrus detection or GnRH administration.

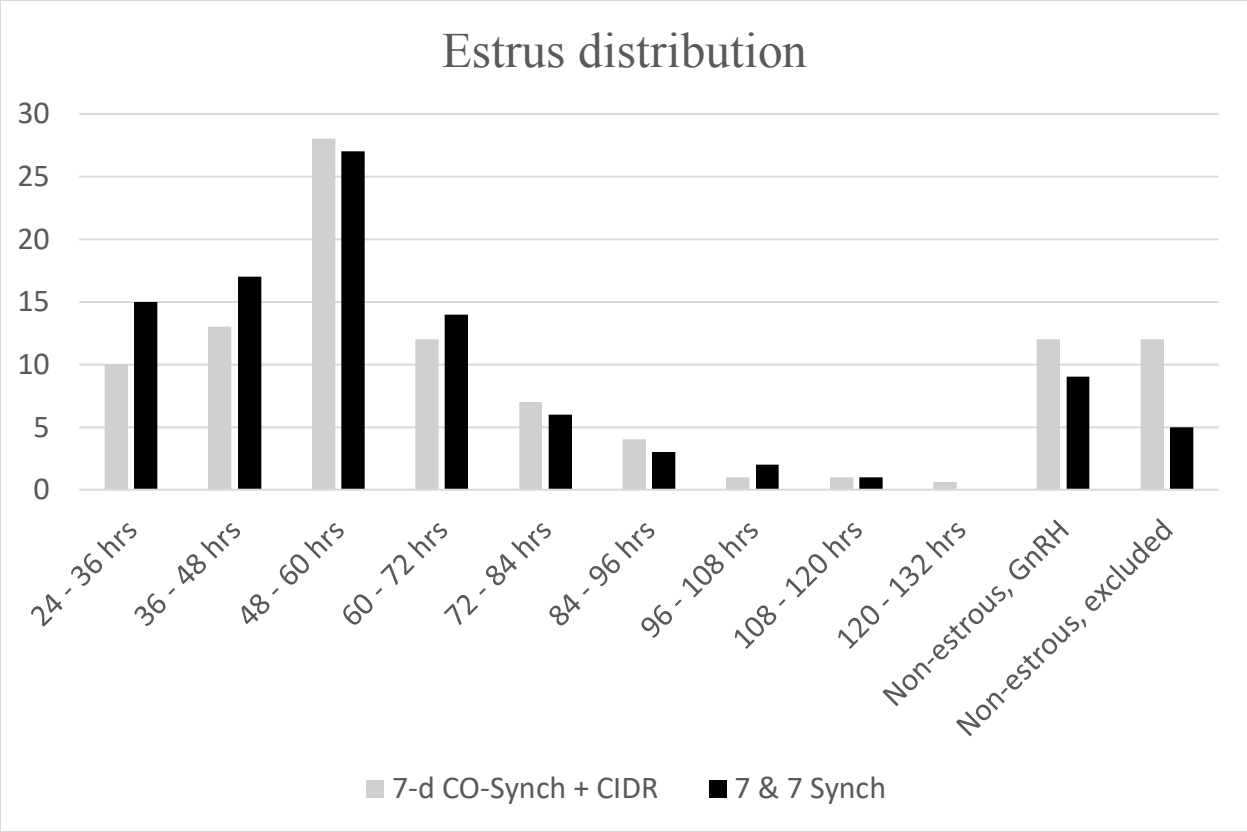

Figure 3.2. Estrus expression in 12-hour intervals following administration of $PGF_{2\alpha}$.

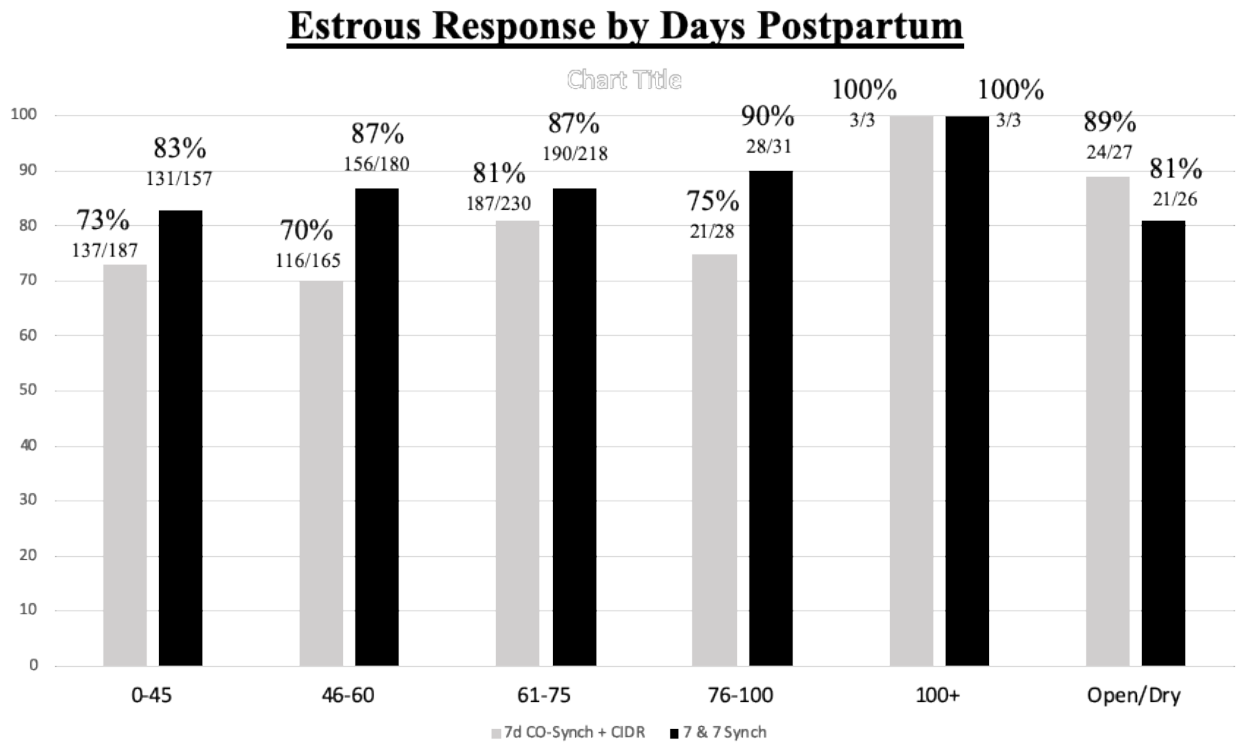

Figure 3.3. Estrous response based on days postpartum ranges.

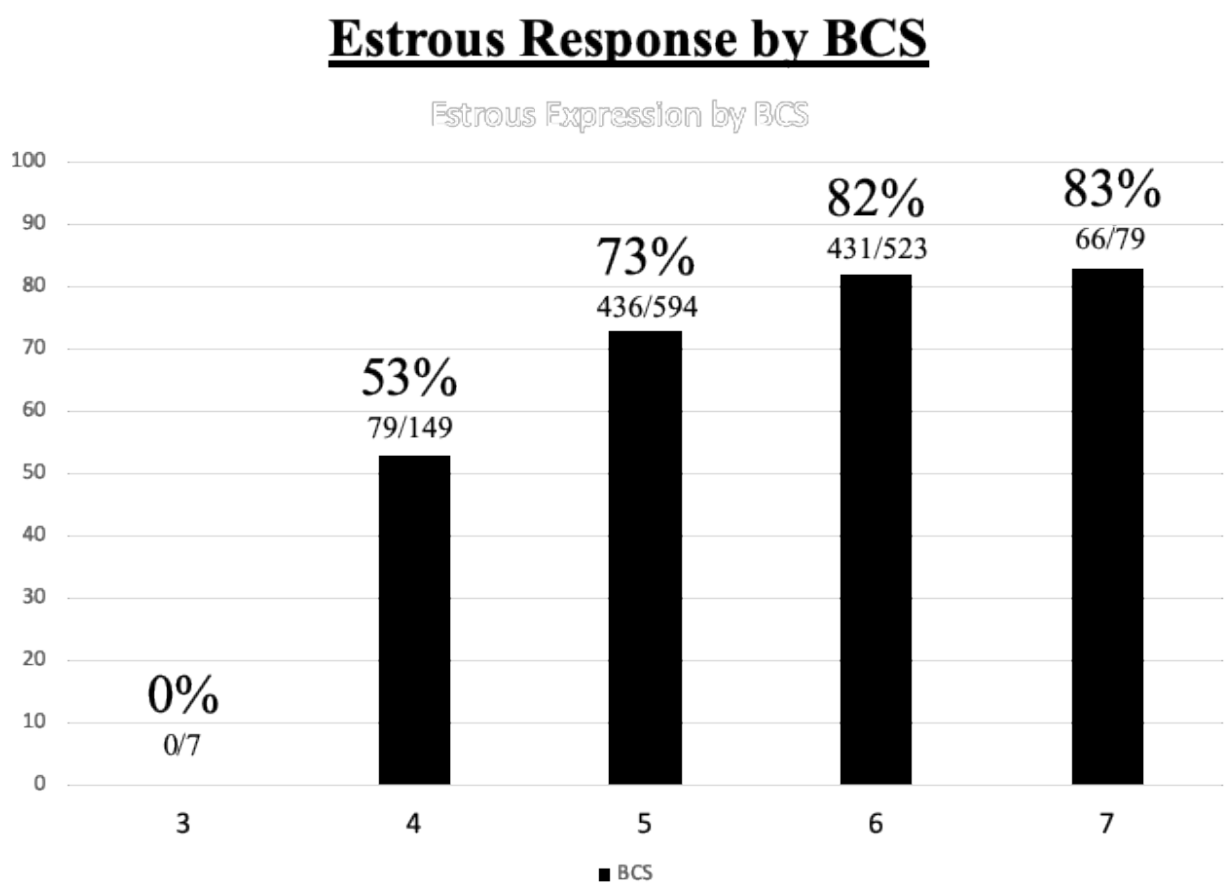

Figure 3.4. Estrous response based on body condition score. The effect of body condition score on estrous response was significant ($P < 0.0001$).

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Vita

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