THESIS

SYNCHRONIZING FOLLICULAR WAVES USING 14 DAY CIDR INSERT PROTOCOLS IN BEEF COWS AND ASSESSING RETICULO-RUMEN TEMPERATURE CHANGES FOR DETECTION OF OVULATION IN DAIRY COWS

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ABSTRACT

SYNCHRONIZING FOLLICULAR WAVES USING 14 DAY CIDR INSERT PROTOCOLS IN BEEF COWS AND ASSESSING RETICULO-RUMEN TEMPERATURE CHANGES FOR DETECTION OF OVULATION IN DAIRY COWS

In the first experiment, objectives were to determine the effectiveness of an extended controlled internal drug release (**CIDR**) insert estrus synchronization protocol to produce 2 follicular waves, induce cyclicity in anestrus cows, and evaluate the efficacy of a single 50 mg dose of prostaglandin $F_{2\alpha}$ (**PG**) at CIDR removal. This experiment included 779 primiparous and multiparous lactating beef cows at 3 locations (n = 779) that were randomly assigned to 1 of 3 treatments. Cows in the 14-d 50 PG treatment received a CIDR (1.38 g progesterone) with 100 μg GnRH analogue im on d 0, 100 μg GnRH analogue im on d 9, and CIDR removal with 50 mg PG im on d 14. Cows in the 14-d 6 h PG treatment were assigned the same protocol as the 14-d 50 PG treatment except that 25 mg PG im was given on d 14, plus 25 mg PG im 6 ± 1 h later. Cows in the 5-day CO-Synch + CIDR (**5-d CO-Synch**) treatment, received a CIDR with 100 μg GnRH analogue im on d 9, CIDR removal with 25 mg PG im on d 14, and 25 mg PG im 6 ± 1 h after first PG injection. Cows in all treatments received 100 μg GnRH analogue im with TAI 72 ± 3 h after CIDR removal. Pregnancy status to TAI was determined by ultrasonography 37 to 40 d after TAI. Pregnancy rate to TAI was higher ($P < 0.05$) in 14-d 50 PG treatment than 14-d 6 h PG and 5-d CO-Synch treatments.

In the following year, 2 experiments were conducted at 6 locations. Our objectives were to: 1) determine the efficacy of an extended CIDR protocol with 2 induced follicular waves, and 2) determine the ability of initiating the CIDR protocol with GnRH analogue (Factrel) or PG. In

exp. one, 588 primiparous and multiparous lactating beef cows at 2 locations were randomly assigned to 1 of 3 treatments. Cows in the 14-d GnRH-9 treatment (n = 202) received the same treatment as the 14-d 50 PG as described earlier. Cows in the 14-d GnRH-7 treatment received a CIDR insert and 100 µg GnRH analogue im on d 0, 100 µg GnRH analogue im on d 7, and CIDR removal with 25 mg PG im on d 14. Cows in the 7-day CO-Synch + CIDR (**7-d CO-Synch**) treatment, received a CIDR insert and 100 µg GnRH analogue im on d 7, and CIDR removal concurrent with 25 mg PG im on d 14. Cows in all treatments received 100 µg GnRH analogue im with TAI at either 72 ± 3 h (14-d GnRH-9 treatment) or 63 ± 3 h (14-d GnRH-7 and 7-d CO-Synch treatments). Combined across all locations, pregnancy rates to TAI were not different ($P > 0.05$) between 14-d GnRH-9 (54.8%), 14-d GnRH-7 (54.4%), and 7-d CO-Synch (52.3%) treatments.

In exp. two, 625 primiparous and multiparous lactating beef cows across 4 locations were randomly assigned to 1 of 3 treatments. Cows in the 14-d GnRH treatment (n = 205) received the same treatment as the 14-d 50 PG treatment described earlier. Cows in the 14-d PG treatment (n = 214) received the same treatment as 14-d GnRH cows except that 25 mg PG im was given on d 0 instead of GnRH analogue. Cows in the 5-day CO-Synch treatment (n = 206), received the same treatment as described previously. Cows in all treatments received 100 µg GnRH analogue im with TAI 72 ± 3 h after CIDR removal. Combined across all locations, pregnancy rates to TAI were higher ($P < 0.05$) in the 14-d PG treatment (70.4%) than both the 14-d GnRH (54.4%) and 5-d CO-Synch (53.5%) treatments.

The final experiment assessed changes in reticulo-rumen temperature to detect ovulation in lactating dairy cows. Lactating dairy cows (n = 494) ≥ 46 days in milk at 1 location were enrolled in a standard presynchronization protocol which included 2 PG injections 14 d apart.

Twelve d later, cows were enrolled in an ovulation synchronization protocol of 100 μg GnRH im (d -10), 500 μg cloprostenol im (d -3), and 100 μg GnRH im 48 h later. All cows received TAI 16 to 19 h after the second GnRH injection. Blood was collected throughout the synchronization period to determine cycling status, response to synchronization treatments, and ovulation around the time of TAI. Reticulo-rumen temperature (**Trr**) was recorded by temperature recording reticulo-rumen boluses administered to each cow via balling gun within 24 h of calving. Each Trr reading was recorded every time animals entered the milking parlor to establish a 7 d baseline. The single maximum Trr rise (°C) from baseline on d of TAI (**T_{rr}MAX**) and the average of all (1 to 3) Trr readings on d of TAI (**T_{rr}AVG**) were used for analysis of Trr change related to ovulation. Mean (± SE) T_{rr}MAX rise from the baseline tended to be higher ($P = 0.06$) in ovulatory (n = 446; 0.180 ± 0.023°C) than anovulatory (n = 48; 0.094 ± 0.042°C) cows. Mean (± SE) T_{rr}AVG was higher ($P < 0.01$) in ovulatory (0.064 ± 0.011°C) than anovulatory (-0.047 ± 0.046°C) cows. The use of changes in Trr in ovulatory cows has been validated to pinpoint animals that do in fact ovulate in an estrus synchronization protocol.

Key Words: Beef cows, Dairy cows, CIDR, Estrus synchronization, Reticulo-rumen temperature

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CHAPTER I

REVIEW OF LITERATURE

Early Discoveries in AI and Usage

The implementation of AI as a practical reproductive tool began with innovating research performed in 1899 by Russian scientist E. I. Ivanoff who utilized AI in domestic farm animals, namely horses (Foote, 2002). However, tracing back to the late 1600's, the earliest research in AI began with the discovery of the sperm cell under a homemade microscope by Anton van Leeuwenhoek and his student Johan Hamm (Foote, 2002). Later, in 1784, Italian scientist Lazzaro Spallanzani took Leeuwenhoek's research and uncovered that spermatozoa are essential for fertilization (Foote, 2002) in which he conducted the first successful AI in a dog.

The basis of practical AI research in animals spread throughout the world with Ivanoff's early findings during the first half of the 20th century. Such experimental findings included revolutionizing methods of semen collection (Foote, 2002), and successfully shipping ram semen for AI in ewes (Walton, 1933). Further refinements of AI techniques were established by scientists in Denmark who developed the method of semen deposited deeply within the cervix and uterus using semen enclosed in small pipettes. This research revolutionized AI techniques in cattle, especially dairy herds (Foote and Bratton, 1949), and further elevated interest in research to improve success rates of AI. Throughout the 1940's, vast strides in improving AI techniques were made by several groups of researchers. Semen extenders were included to improve fertility and survivability (Foote and Bratton, 1949), and use of antibiotics within semen to prevent disease transmission avoided damage of spermatozoa from contamination during semen handling.

One of the most monumental discoveries in worldwide adoption of AI was cryopreservation of rooster semen (Polge et al., 1949) by the inclusion of glycerol within the extender to preserve spermatozoa. Further modifications in semen extenders and cryoprotectants have improved overall frozen-thawed semen used in AI techniques to the current products, and has allowed for worldwide trade of superior bull sires for genetic diversity of cow herds.

Since the refinement of AI, this technique has been widely adopted worldwide and according to the USDA is used by approximately 78.3% of dairy herds in the U.S (USDA, 2007). The feasibility of applying AI as a reproductive management tool in dairies is high due to ease of access to these animals, which are regularly in confinement. However, there has been less adoption of AI in beef cow-calf operations due to the labor, facility requirements, and costs associated with estrus synchronization and AI (NAHMS, 2009). According to NAHMS, only around 8% of beef operations currently utilize these reproductive technologies with the majority being seedstock cattle operations. Also, in beef cows, use of AI is half that in beef heifers, although less than 20% of all nulliparous beef heifers are submitted to AI (Hall, 2011). However, recent findings (Lamb et al., 2010) have shown that the number of units of beef sire semen used has steadily increased in the past 10 years along with overall herd size, while the number of beef herds have continued to decline.

Development of Estrus Synchronization Protocols as a Reproductive Tool

While the innovating research and adoption of AI was widespread throughout the 1940's, little was actually known about manipulation of the estrous cycle in order to mass mate a large number of animals at the same time with successful results. At the time, the only implemented protocol for AI included establishment of the A.M./P.M. rule in dairy cows which optimized fertility and conception to AI from breeding animals 12 h after visual observation of estrus

(Trimberger, 1948). This process is still applicable today, resulting in high conception rates, but requires constant monitoring of animals and increased labor requirements from breeding animals in a very spread out period of time.

The earliest reports of understanding the mechanisms underlying the estrous cycle began with studying sheep during the 1920's (Mckenzie et al., 1934); reproductive tract histology encompassed much of the early research. It was during this time period that the mean length of a cow's estrous cycle was documented and averaged 20 to 22 d (Chapman and Casida, 1935; Nalbandov and Casida, 1942; Nellor and Cole, 1956). Regulation of the estrous cycle in cows remains one of the obstacles that researchers still attempt to control.

During the 1940's, many discoveries underlying the hypothalamic-pituitary-gonadal axis were uncovered, which led to the basis for the majority of estrus synchronization protocols used currently. It was during this period that researchers found injection of sheep pituitary extract gonadotropins into cows resulted in ovulation of a follicle and corpus luteum (**CL**) formation (Casida et al., 1943). Along with the understanding of follicular turn over (Casida et al., 1943), the ability of progesterone (**P4**) to inhibit estrus via negative feedback mechanisms in a dose dependent manner in heifers was determined, and that different doses acted to either allow or inhibit follicular growth (Ulberg et al., 1951). Also uncovered was the fact that upon P4 removal, estrus ensued, which was of particular interest and value for future estrus synchronization protocols. The use of estrogens, namely estradiol-17β, was also of interest to researchers during this time period. This steroid hormone was shown to have luteolytic activity based on a decrease in P4 levels, CL weight, and CL regression upon estradiol-17β im injection (Kaltenbach et al., 1964; Niswender et al., 1965; Wiltbank et al., 1961).

The first research using orally active synthetic progestogens incorporated the use of medroxyprogesterone acetate (sold as Repromix) as a synchronization tool in the 1960's (Hansel et al., 1961) and led to the first results of successful synchronization of estrus in beef cows. However, due to its high cost and variability in synchronization of estrus in studies conducted using Repromix, production was terminated shortly after production. Shortly after the efforts of Hansel and others, further use of orally active progestogens (16-α-17 dihydroxyprogesterone acetophenonide, **DHPA**) in conjunction with estradiol valerate (**EV**) provided valuable insight on estrus synchronization. The use of DHPA and EV in these protocols increased synchrony of estrus and AI conception rates in beef heifers when compared to control groups (Wiltbank and Kasson, 1968). These findings in synchronization of estrus led to further use of progestogens placed into a slow release capsule, implanted subcutaneously, and coupled with EV im injections to synchronize estrus in beef cattle (Wiltbank et al., 1971; Miksch et al., 1978). This idea was summarized by numerous researchers and labeled as the Syncro-Mate-B protocol (Wiltbank and Gonzalez-Padilla, 1975).

The elimination of P4 implants for estrus synchronization occurred after development of intra-vaginal P4 release that could be easily administered and removed. This concept was first investigated using a stainless steel vaginal insert with a progesterone-infused silastic elastomer wrapped around the steel insert (Roche, 1976). The progesterone releasing intra-vaginal device (**PRID**) was used in both dairy and beef cattle and resulted in an increase in synchrony of estrus compared to control animals. Pregnancy rates were similar in PRID synchronized beef cows when compared to animals solely detected in estrus and administered AI (Roche, 1976).

The use of a progesterone releasing controlled internal drug release (**CIDR**) insert was approved by the FDA in 1997 (Food and Drug Administration, 1997). The CIDR was first

developed in New Zealand for synchronization of estrus in sheep, and its structure lacked the stainless steel base and concerns associated with the earlier PRID intra-vaginal models. The functions of CIDRs in estrus synchronization have been used to increase pregnancy rates, synchrony of estrus, initiate cyclicity in previously anestrous animals, and prevent premature ovulation in cows (Lucy et al., 2001b).

Melengestrol Acetate (**MGA**), another oral synthetic progestogen, has also been extensively studied as a reproductive management tool to control follicular growth and inhibit estrus, and arose mainly from feedlot settings in which prevention of estrus was necessary. Melengestrol Acetate is one of many tools to synchronize estrus in current protocols, and is cheaper to use than CIDR. However, the use of a CIDR ensures that animals are receiving the necessary dose of P4 to inhibit ovulation compared to MGA, which is administered in feed.

As research to both prevent estrus and induce luteolysis was progressing, so were experiments to identify what controlled ovarian follicles and ovulation. The discovery of gonadotropin releasing hormone (**GnRH**) and its release was first identified from hypothalamic extracts isolated in sheep (Burgus et al., 1972) and pigs (Matsuo et al., 1971). This discovery and isolation led to understanding the role of GnRH as an immensely important peptide hormone for the control of ovarian follicles and inducing ovulation. It was soon after the discovery of GnRH that its role in directly triggering the release of both follicle stimulating hormone (**FSH**) and luteinizing hormone (**LH**) to control follicular growth and ovulation was identified (Mauer and Rippel, 1972; Kaltenbach et al., 1974). The understanding that injections of GnRH would stimulate release of LH and ovulation of a dominant follicle immediately led to implementation of GnRH injections within estrus synchronization protocols.

While estradiol-17β was described earlier as having mainly luteolytic effects in the 1930's, research also identified smooth muscle contractions of the uterus from insertion of semen (Kurzroc and Lieb, 1930), caused by an unknown substance within the seminal fluid termed PG (later identified to be a prostaglandin). However, it was not until the 1970's that prostaglandin $F_{2\alpha}$ (**PG**) was identified as having major luteolytic effects in cattle (Lauderdale, 1972; Liehr et al., 1972). One conclusion of major interest was the responsiveness of a CL to PG based on d of the estrous cycle. These findings (Lauderdale et al., 1974) described a CL being non-responsive to PG on d 2 to 4 of the estrous cycle, but responsive by d 6 to 9 and 13 to 16. Response to PG on these days of the estrous cycle was also associated with initiation of estrus within 2 to 4 d of PG injection. Research relating the effect of PG to induce estrus in cows became one of the pivotal aspects of estrus synchronization success. This discovery was also coupled with progestogen usage to prevent estrus, and GnRH injections to cause the ovulation of a dominant follicle. The prevention of premature ovulation of a dominant follicle during the synchronization period by use of progestins is important to maintain the synchrony of estrus in a large number of animals that are being synchronized.

Benefits of Progesterone Incorporated into Estrus Synchronization

Incorporation of P4 into estrus synchronization protocols is a well-established reproductive management tool. The ability to artificially mimic the activity of an active CL has numerous advantages to provide more cows the opportunity to conceive to AI.

One of the major benefits of P4 usage, especially in presynchronization, is the ability to induce cyclicity in early postpartum anestrous cows especially since as many as 50% of beef cows are anestrus at the beginning of breeding season (Lamb and Dahlan, 2002). There are many early postpartum factors that prevent resumption of cyclicity, resulting in failure to respond to

estrus synchronization protocols. Near the end of a bovine pregnancy, the negative feedback mechanism within hypothalamic-pituitary-gonadal axis is extremely high from suppression exhibited by the placenta and fetus. This early inhibition results in abnormally high accumulated levels of FSH and severe depletion of LH levels in the anterior pituitary during the period of involution of the uterus (Yavas and Walton, 2000a). It is believed to be these low levels of circulating LH that inhibit the ovulation of a follicle (Yavas and Walton, 2000b). Prolonged exposure to exogenous P4 can act to decrease the negative feedback of estradiol and stimulate the pulsatile secretions of LH and resumption of normal cyclicity (Yavas and Walton, 2000a; Lamb et al., 2001).

Another essential advantage for the success of P4 in an estrus synchronization protocol is blocking an LH surge causing ovulation (Savio et al., 1993). This is important for animals that initiate an estrus synchronization protocol towards the end of the estrous cycle where premature luteal regression and ovulation of a follicle can occur prior to PG administration.

Identification of Follicular Waves and Follicular Response to GnRH in Cattle

The idea that multiple follicular waves within a single estrous cycle occur in cows was first described in the 1960's by identification of follicles at various stages of the cycle from examination of abattoir ovaries (Rajakoski, 1960). Here, beef heifers were first described as having 2 follicular waves occurring within the normal estrous cycle (Rajakoski, 1960). It was not until the development and application of ultrasonography that follicular growth, atresia, and ovulation could be tracked closely throughout the estrous cycle. It was this technological advancement that allowed further research into identification of a third follicular wave in some animals, mainly seen in heifers (Sirois and Fortune, 1988; Savio et al., 1988; Noseir, 2003). The difference in length of the luteal phase between 2 and 3 wave cycles was also identified using

ultrasonography (Taylor and Rajamahendran, 1991). Control of whether cows exhibit 2 or 3 follicular waves is still not well understood, but some underlying factors include heat stress and nutrition level (Lucy et al., 1992). Cows fed on a lower plane of nutrition have shown to have increased incidences of 3 follicular waves (Adams, 1998).

Heat stress during summer months has also showed to have altering effects on follicular growth and viability due to decreases in granulosa cell aromatase activity (Putney et al., 1988a,b; Lew et al., 1993). These results also show tendencies towards 3 follicular waves.

Understanding the stages of the estrous cycle with maximum response to GnRH is important in determining the optimal time to initiate estrus synchronization protocols. The ability to initiate a new follicular wave at the initiation of a protocol is a factor for success in achieving high conception rates to AI due to the high likelihood of a dominant follicle's presence when PG is administered to induce estrus (Thatcher et al., 1989). This response to GnRH also allows for synchronization of estrus in a large number of animals for mass mating (Twagiramungu et al., 1992; Pursley et al., 1995). However, response to GnRH administered to a large number beef cows in random stages of the estrous cycle results in only approximately 66% of cycling cows having a responsive follicle and a sufficient surge of LH to cause ovulation of the dominant follicle (Geary et al., 2000). This response to GnRH corresponds to other studies in dairy cows that report similar response to GnRH administered to those seen in beef cows with highest response on d 5 of the estrous cycle (Vasconcelos et al., 1999). This lack of response in beef and dairy cows has been of interest for research in developing protocols to maximize the number of animals that have a responsive follicle when GnRH is administered by the use of presynchronization.

Factors such as d of the estrous cycle, number of follicular waves, and diameter of follicles all play roles in determining the responsiveness of follicles of cows administered GnRH. Results from targeting the day of the cycle and subsequent response to GnRH was highest from d 4 to 9 and 18 to 20 in lactating beef cows although mean follicle size did not differ between cows at the targeted days of the estrous cycle (Geary et al., 2000; Atkins et al., 2010). Size of bovine follicles also has effects on ovulatory capacity dependent on layers of granulosa and theca cells providing the components of a mature follicle. Based on ovulatory response to GnRH, a follicle ≥ 10 mm has been identified as a GnRH responsive follicle (Martinez et al., 1999; Sartori et al., 2001).

Results evaluating responsiveness of a dominant follicle have led to much research in the dairy industry to maximize the proportion of animals that are in the early luteal phase (d 5 to 12) of the estrous cycle when initiating a synchronization protocol with GnRH to ensure ovulation of a dominant follicle. Targeting of this stage of the estrous cycle has been accomplished through the use of presynchronization protocols by administering 2 doses of PG 14 d apart followed by initiation of an estrus synchronization protocol using GnRH 12 d later (Moreira et al., 2001). The ability to presynchronize positions the majority of these cows in this stage of the estrous cycle has been widely accepted in dairy estrus synchronization protocols due to its ability to maximize conception rates to AI.

Development and Benefits of Timed-AI in Cows

The use of AI in estrus synchronization protocols, while widely accepted as a reproductive management tool, requires extensive labor, attention to detail, and proper facilities for success. The extra labor required is increased with the need to detect estrus in animals after synchronization for successful timing of AI for portions of animals as they come into estrus.

However, the variability of the interval from PG induced luteolysis to estrus is highly variable (Ferguson and Galligan, 1993; Kastelic and Ginther, 1991) and depends on maturity of the dominant follicle when the CL is lysed. This leaves many producers with lengthy periods of time to breed animals that are spread out over several days depending on when animals exhibit estrus. The application of heat detection and AI after the end of synchronization will maximize pregnancy rates to AI, but extra labor is necessary to accomplish this.

The ability to effectively concentrate a large percentage of animals to ovulate around the same time interval from PG injection for TAI was developed first in the dairy industry via the Ovsynch protocol, which targeted the mean interval from PG to estrus. Once this interval was determined, GnRH was given at the end of that interval to force ovulation of any dominant follicle present and TAI 16 to 18 h after GnRH injection (Pursley et al., 1995 and 1997). Utilizing the second GnRH injection after PG has had immensely positive effects on increasing TAI pregnancy rates in dairy cows and has been widely adopted by the dairy industry along with modifications for usage in beef cows (Pursley et al., 1995; Geary and Whittier, 1998a). The main advantage of this modified synchronization protocol is elimination of the need for detection of estrus and the ability to AI a large number of animals at the same time by synchronizing ovulation rather than solely estrus.

The idea of administering GnRH after estrus synchronization concurrent with TAI has also been applied for beef cow synchronization, but with a slight modification. The Ovsynch protocol has proven to be highly successful for estrus synchronization, but the extra handling of animals required for the second GnRH prior to TAI in not practical for many beef operations. The implementation of the CO-Synch protocol utilizes the same idea as that of Ovsynch except that TAI is performed at the same time as GnRH administration. The TAI pregnancy rates of the

CO-Synch protocol are about 8 percentage points lower than those seen in the Ovsynch protocol, but for most producers the small drop in pregnancy rates are not worth the extra handling of animals (Geary and Whittier, 1998a). This protocol also utilized the mean interval from PG administration to estrus to denote the proper interval for TAI.

Current Applications of Estrus Synchronization Protocols and TAI

From the early implementation of estrus synchronization using the Syncro-Mate-B protocol to the current state of applied reproductive physiology, thousands of experiments and numerous protocols have been tested and developed to determine the best method for synchronization of estrus or ovulation in both dairy and beef cows. The many protocols in current use have taken into consideration the multitude of research conducted to understand the mechanisms of controlling the bovine estrous cycle dating back to the 1940's. The Beef Reproduction Task Force has identified successful protocols and compiled them into a database for access to producers (Beef Reproduction Task Force, 2012).

The major protocol of emphasis with great success used by both dairy and beef operations described earlier was the Ovsynch protocol. The idea behind GnRH at the start of the Ovsynch protocol is to ovulate a dominant follicle initiating a new follicular wave, administer $PGF_{2\alpha}$ 7 d later to lyse any existing CL, and trigger ovulation of the dominant follicle approximately 48 h later with a second GnRH injection. Modifications to the Ovsynch protocol have been made for the beef industry by administering the second GnRH injection at TAI, which removes the extra time handling animals and has been identified as the CO-Synch protocol (Geary et al., 1998b). Another modification to the CO-Synch protocol, the Select-Synch protocol, utilizes estrus detection and AI, if feasible at facilities, and can increase AI pregnancy rates compared to TAI protocols (Geary et al., 2000). The benefits of P4 described above gave

rise to the inclusion of a CIDR within the CO-Synch protocols for TAI to increase pregnancy rates and is identified as the 7-day CO-Synch + CIDR protocol (Lucy et al., 2001b). Implementation of these simple protocols has been widely adopted for synchronization due to their ease of application and low number of times handling animals, but overall adoption in the commercial beef cow industry is still low.

The application and acceptance of 7 d GnRH-CIDR-PG estrus synchronization protocols have remained highly recommended (Beef Reproduction Task Force, 2012). However, of recent interest has been shortening the CIDR interval in the 7-day CO-Synch + CIDR protocol to 5 d, known as the 5-day CO-Synch + CIDR protocol (Bridges et al., 2008; Gunn et al., 2009). The theory behind shortening the CIDR protocol to 5 d was to increase the length of proestrus from CIDR removal and PG injection to TAI from research investigating the increased fertility of younger bovine follicles exhibiting higher levels of estradiol from a longer proestrus period than older follicles (Mussard et al., 2003a,b). The longer proestrus period is due to removal of P4 influence two days earlier with CIDR removal and PG allowing for the dominant follicle to ovulatory capacity. This theory of increased fertility and conception rates to AI from increased follicular estradiol secretion has also been documented in dairy cows (Lopes et al., 2007). The decrease in length of synchronization to 5 d extended the period of proestrus and increased the interval from PG to TAI to 72 h versus 60 to 66 h in previous 7-day CO-Synch + CIDR protocols. This modification of the 7-day CO-Synch + CIDR protocol has resulted in TAI pregnancy rates of up to 70% (Bridges et al., 2008). However, results from other investigations have found no differences in TAI pregnancy rates between 7-day CO-Synch + CIDR and 5-day CO-Synch + CIDR protocols (Johnson et al., 2009; Wilson et al., 2010). Also, a major drawback to this protocol is the lack of PG responsiveness of a 5 d CL formed from forced ovulation of a

dominant follicle at the initiation of the protocol. Preliminary research investigating the effects of PG in luteolysis described CL responsiveness between d 6 to 9 and 13 to 16, but lacked responsiveness to PG from d 0 to 5 (Lauderdale et al., 1972). Therefore, original data in the 5-day CO-Synch + CIDR protocol required a single PG injection at CIDR removal and a second PG injection 12 h later to ensure complete luteolysis in beef cows. Despite this major drawback, the increase in pregnancy rates has stimulated a multitude of research to investigate the benefits and drawbacks of this new estrus synchronization protocol, namely the requirement of two 25 mg doses of PG at 12 h intervals (Bridges et al., 2008; Kasimanickam et al., 2009).

The focus of investigating PG dosage required for inducing luteolysis targeted the potential of administering twice the normal dosage of PG concurrent with CIDR removal to be sufficient versus the previously described 2 doses at 12 h intervals. It has been previously described that a single dose of PG at CIDR removal with the 5-day CO-Synch + CIDR protocol was not sufficient in inducing luteal regression and decreased TAI pregnancy rates when compared to 2 PG injections at 12 h intervals in lactating beef cows (Bridges et al., 2008; Kasimanickam et al., 2009). Further investigation determined the appropriate interval for 2 PG injections could be as short as 6 h apart when using the 5-day CO-Synch + CIDR protocol (Peel et al., 2010). In addition, a multi-state experiment was conducted in 2010 to compare the efficacy of a double dose of PG administered concurrently with CIDR removal versus a single dose of PG at CIDR removal with a second dose of PG 8 h later (Bridges et al., 2011). Preliminary results found no differences in TAI pregnancy rates between the 2 treatments, but the 5-day CO-Synch + CIDR protocol with a single 50 mg dose of PG at CIDR removal has yet to be accepted as an addition to the current protocol by the Beef Reproduction Task Force.

The sensitivity of the bovine CL has obviously been of much interest in relation to the success of the shorter 5-day CO-Synch + CIDR, but sensitivity appears to not be correlated with lack of PG receptors present on the CL. The developing CL already possesses high affinity PG receptors (Wiltbank et al., 1995), but within the cascade initiating luteal regression lies other possible inhibitors of this process. For example, increased expression of protein kinase C inhibitors present in a developing CL could inhibit the cascade of events from $PGF_{2\alpha}$ binding to its receptor and initiating luteolysis (Madhusudan et al., 2009). A higher expression of the protein kinase C epsilon gene, responsible for translation of protein kinase C, in the d 10 bovine CL has also been associated with a higher sensitivity to PG if administered during this stage of the estrous cycle (Madhusudan et al., 2009).

Effective Applications of Estrus Synchronization in Dairy Cow Reproduction

The use of estrus synchronization for TAI has been a widely accepted tool for manipulation of the bovine estrous cycle especially in the dairy industry, where breeding for high milk production has decreased the fertility of these animals (Beam and Butler, 1999; Lucy, 2001a). Proper detection of estrus for timely and effective AI is still a key factor for successful conception in some AI protocols. Timely detection of estrus can increase the effectiveness of conception to AI and profitability of estrus synchronization usage (Pecsok et al., 1994). However, the confinement involved and hard surfaces adopted by the majority of dairy operations have limited exhibition of outward signs of estrus for detection (Britt et al., 1986; Vailes and Britt, 1990). This led to the implementation of estrus synchronization protocols to control the estrous cycle of many animals, but there is still variability in the actual induction of estrus and ovulation from cow to cow (King et al., 1982; Stevenson et al., 1984). This issue has been partially alleviated by the inclusion of GnRH 16 to 18 h prior to TAI for synchronization of

ovulation as seen in the Ovsynch protocol (Pursley et al., 1995), but there is still variability in timing of ovulation with this protocol.

The lack in control from PG injection on d -3 and initiation of luteolysis to the actual ovulation of a dominant follicle for AI on d 0 is particularly variable and depends on the developmental stage of the follicle when PG is given (Kastelic et al., 1991; Wenzel, 1991; Ferguson and Galligan, 1993). This area has been a topic for research of estrus synchronization protocols for many years. The use of GnRH injections 48 h after PG has alleviated some of these problems in control of timing of ovulation with the Ovsynch protocol (Pursley et al., 1995), but variability in actual timing still exists.

Benefits of Incorporating the 5-day CO-Synch + CIDR protocol into an Extended P4 Estrus Synchronization Protocol

Many of the recent GnRH-CIDR-PG estrus synchronization protocols developed (i. e. 5-day and 7-day CO-Synch + CIDR protocols) aim to solely initiate one new follicular wave with a GnRH injection at the initiation of the protocol. Animals that have a responsive follicle ≥ 10 mm have a high probability of ovulation and starting of a new follicular wave 2 to 2.5 d later (Twagiramungu et al., 1994; Pursley et al., 1995). However, as discussed previously, approximately only 66% of beef cows are in a stage of the estrous cycle where dominant follicle response to GnRH will occur (Geary et al., 2000). Failure in response to GnRH results in poor synchrony of estrus due to the lack of a fresh follicular wave generated. When PG is given concurrently with CIDR removal at the end of the estrus synchronization, these animals will ovulate an aged follicle with decreased fertility from P4 inhibition preventing ovulation or natural regression. Also, lack of response could result in too immature of a follicle being present and offset the normal interval from PG to estrus. Complete lack of dominant follicle presence could also likely result in poor TAI pregnancy rates.

The benefits of long-term presynchronization with P4 prior to initiation of standard estrus synchronization protocols have the ability to induce cyclicity in previously anestrous cows and heifers (Smith et al., 1987; Lamb et al., 2001). Protocols such as CIDR Select have incorporated the extended 14 d P4 influence prior to initiation of the 7-day CO-Synch protocol to induce cyclicity and increase synchrony of estrus. However, the need to set up a fresh follicular wave with GnRH after P4 influence is necessary due to the poor fertility among oocytes grown during extended P4 influence from persistent follicular growth (Sirois and Fortune, 1990). This results in the CIDR Select and other protocols that mimic this principle to be quite lengthy to complete.

A novel idea in the current experiments investigated the melding of ideas to utilize the benefits of both short and long-term estrus synchronization protocols using a 14 d CIDR protocol with multiple GnRH injections concurrent with the 14 d CIDR. This experimental protocol first aimed to increase the likelihood of animals having a GnRH responsive follicle to ovulate and initiate a new follicular wave. This included GnRH injections at CIDR insertion and 9 d later. The belief would be that 60 to 70% of cows would ovulate and start a new follicular wave with the first GnRH injection (Geary et al., 2000). By the time the second GnRH injection is given 9 d later, these animals are expected to have another responsive follicle to ovulate and initiate a second wave of follicular growth. Those that did not ovulate to the GnRH on d 0 would continue follicular growth and have a GnRH responsive follicle by the time of the second GnRH injection and initiate a new follicular wave prior to PG administration and CIDR removal. Anestrus cows without ovarian follicular activity due to early postpartum interference at the initiation of the protocol would likely fail to respond to the first GnRH injection. However, the influence of P4 for the first 9 d of the protocol could stimulate LH pulse frequencies enough to initiate cyclicity and induce ovulation of a follicle to the GnRH administered by d 9, effectively setting up a new

follicular wave. This is one of the first investigations to determine the ability to successfully induce 2 follicular waves within 14 d of exposure to P4.

With the second GnRH injection occurring 5 d prior to PG and CIDR removal, this protocol also mimics the dynamics of the 5-day CO-Synch + CIDR protocol, but could increase the number of animals responding to the GnRH administered. While this protocol takes advantage of the increased fertility of forcing ovulation of a younger follicle, the approach to effectively lyse the 5 d accessory CL formed from ovulation was an additional matter of interest. This question was investigated by administering either a single 50 mg dose of PG at CIDR removal or a 25 mg dose of PG at CIDR removal with a second 25 mg dose 6 h later.

Potential Manipulations in Estrus Synchronization to Increase Response to GnRH and PG Responsiveness of a D 5 of the Estrous Cycle CL

Results from the beef cow experiment utilizing the 14 day CIDR protocol conducted previously (2010) were encouraging enough to further investigate the mechanisms underlying synchronization of 2 follicular waves, but with some modifications. A single 50 mg dose of PG at CIDR removal was sufficient for luteal regression resulting in acceptable TAI pregnancy rates, and two 25 mg doses at 6 h intervals was not investigated further.

Altering the interval from first GnRH injection and CIDR insertion to second GnRH by 2 d was an area of interest to potentially increase the number of animals responding to the second GnRH. This theory was supported by research to determine the day of the estrous cycle with the highest probability of having a follicle responsive to GnRH. The shifting of second GnRH 2 d back in a 14 d CIDR would mimic the dynamics of the 7-day CO + Synch + CIDR protocol by administering this GnRH 7 d prior to CIDR removal. Cows ovulating to the first GnRH with CIDR insertion would initiate a new follicular wave within 2 to 2.5 d (Bentley et al., 1998; Martinez et al., 2000) and likely be around d 5 of the estrous cycle at time of second GnRH

injection. This would hypothetically set cows up to be in the stage of the estrous cycle with the highest probability of having a GnRH responsive follicle to ovulate and initiate a second follicular wave of growth (Vasconcelos et al., 1999). This altering to increase response to second GnRH administered could increase TAI pregnancy rates when compared to the 14 d CIDR protocol with GnRH on d 9 of P4 influence.

Replacing GnRH with PG at the initiation of the protocol was a new modification to the existing protocol to determine its effects on synchronizing follicular waves. During the luteal phase, a spontaneously forming CL will elicit enough P4 to suppress LH pulse frequencies (Roberson et al., 1989), but exogenous P4 (e.g. CIDR or MGA) does not release enough P4 to inhibit the same LH release. Therefore, presence of low levels of exogenous P4 in the absence of a spontaneously formed CL will mimic the endocrinological environment of the follicular phase of the estrous cycle rather than the luteal phase (Kinder et al., 1996). However, the threshold level of P4 maintained still prevents ovulation or atresia of a dominant follicle and could force growth of a persistent follicle (Kinder et al., 1996). Establishing this environment of increased LH pulse frequency on a dominant follicle was potentially demonstrated during the first 9 days of the protocol by inclusion of PG at the initiation of the 14 d CIDR instead of GnRH. Also, the current protocol could ensure the presence of a GnRH responsive follicle to increase the chance of ovulation and initiation of a fresh follicular wave with the GnRH injection 9 d after PG and CIDR.

The potential benefits of creating this environment would be the increased LH pulse frequency acting on the existing follicle during the first 9 d of the 14 d CIDR. Exposure of follicles to these high levels of LH could increase presence of LH receptors on the follicle that has been growing which would agree with previous research on persistent follicular growth.

Investigators discovered higher numbers of granulosa cell LH receptors on persistent follicles when compared to normal dominant follicles (Cupp et al., 1993). Upon ovulation on d 9, this increase in presence of LH receptors on the early CL could allow for a faster rate of luteinization. The faster rate luteinization could ensure PG responsiveness of all animals that ovulated a follicle 5 d prior to PG in which a single 50 mg dose of PG could be sufficient for complete luteolysis. This effect might increase the synchrony of estrus from all animals initiating and completing luteal regression in a tighter window, resulting in a larger group of animals ovulating at the designated TAI.

Temperature Rises Associated with Initiation and Detection of Estrus in Cattle

Vaginal temperature rises (0.3 to 0.8°C) have been associated with initiation of estrus in lactating dairy cows (Bobowiec et al., 1990). This increase in vaginal temperature can last for 7 to 12 h after initiation of estrus (Fisher et al., 2008). Detection of ovulation based on rises in core temperature (**Tc**) of animals has been studied as a potential tool for increasing success rate of AI systems in the dairy herd (J. A. Small, 2011). However, there have been very few studies to validate this aspect of timing of ovulation on a large scale. The ability to detect estrus and ovulation based on rises in Tc without the need for visual confirmation could be a huge benefit as a reproductive management tool in the dairy industry.

Previous research in cattle and other species have revealed animals having a circadian rhythm of body temperature with peaks and troughs throughout the d (Bitman et al., 1983). This rhythmicity has the highest temperatures being recorded at dusk and lowest temperatures just before dawn (Piccione et al., 2003). Incorporation of this circadian rhythm has also been associated with an estrous rhythmicity as animals' progress through the estrous cycle (Piccione et al., 2003). While P4 has been previously described as having a thermogenic action in rats

(Freeman et al., 1970), initiation of estrus has also been associated with thermogenicity with peaks within 8 h of initiation (Cooper-Prado et al., 2010). Rises in rumen temperature from predetermined baselines of 0.61°C recorded from reticulo-rumen boluses at the initiation of estrus and can be effectively used to time ovulation in beef cows (Cooper-Prado et al., 2010). This experiment generated baselines of rumen temperature while incorporating the circadian rhythm into the model, and appears to be quite important to detect this rise associated with estrus.

Other research has used milk temperature measures to predict estrus within dairy cows (Fordham et al., 1988). While, this experiement successfully detected estrus in 76% of animals, they also had a false positive rate of 11%. The ability to predict estrus within this experiment proved to be difficult due the daily fluctuations of cows that show this estrous rhythmicity.

Conclusions

From the early observations of sperm cells under a microscope in the 1600's to the current technologies applying our knowledge in perfection of estrus synchronization protocols, the multitude of research has resulted in thousands of experiments and publications in the field of reproductive physiology. The ability to control the estrous cycle in both dairy and beef cows is a testament to the hard work and intelligence of numerous researchers to complete this task. Discovering the hormones that control the estrous cycle, and timing of LH and FSH rises relative to declining P4 to trigger ovulation of a follicle has allowed for understanding of the specific mechanisms and applications to control the estrous cycle of each animal. Modifications to manipulate the timing of these hormonal events have resulted in the ability to control the estrous cycle in thousands of cows at a single time point. While slight difficulties still exist to maximize

the efficacy of estrus synchronization, progress continues to be made to perfect these technologies for application.

New technologies, such as temperature recording rumen boluses, is one such recent technology that could prove to uncover even more knowledge on understanding the estrous cycle and control of it. Detecting rises in rumen temperature related to ovulation of a dominant follicle could sway the application of estrus synchronization, namely the use of TAI. Accurate rumen temperature rises associated with ovulation could be immensely useful to target appropriate timing of AI relative to these rises that are detected.

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CHAPTER II

Administration of a GnRH analogue on day 9 of a 14-day controlled internal drug release insert with timed AI in lactating beef cows[1]

SUMMARY: Many estrus synchronization protocols aim to induce a new follicular wave. Our objectives were to determine the effectiveness of GnRH analogue administered d 0 and 9 during an extended controlled internal drug release (**CIDR**) protocol to produce 2 follicular waves, induce cyclicity in anestrus cows, and evaluate the efficacy of a single 50 mg dose of prostaglandin $F_{2\alpha}$ (**PG**) to initiate luteal regression upon CIDR removal. Lactating beef cows at 3 locations (n = 247, location 1; n = 395, location 2; n = 137 location 3) were randomly assigned to one of 3 treatments. Cows in the 14-d 50 PG treatment received a CIDR (1.38 g progesterone) with 100 μg GnRH analogue im on d 0, 100 μg GnRH analogue im on d 9, and CIDR removal concurrent with 50 mg PG im on d 14. Cows in the 14-d 6 h PG treatment were assigned the same protocol as the 14-d 50 PG treatment except that 25 mg PG im was given on d 14, plus 25 mg PG im 6 ± 1 h later. Cows in the control treatment, 5-day CO-Synch + CIDR (**5-d CO-Synch**), received a CIDR concurrent with 100 μg GnRH analogue im on d 9, CIDR removal concurrent with 25 mg PG im on d 14, and 25 mg PG im 6 ± 1 h after first PG injection. Cows in all treatments received 100 μg GnRH analogue im and timed-AI (**TAI**) 72 ± 3 h after CIDR removal. Pregnancy status to TAI was determined by ultrasonography 37 to 40 d after TAI. Averaged over all locations, pregnancy rates to TAI for 14-d 50 PG, 14-d 6 h PG, and 5-d CO-

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Synch treatments were 58.2, 46.8, and 41.9%, respectively. Pregnancy rates to TAI were higher ($P < 0.05$) in 14-d 50 PG treatment than 14-d 6 h PG and 5-d CO-Synch treatments. Cycling status at 2 locations (n = 243, location 1; n = 391, location 2) was determined from blood collected on d -7 and 0; cows with serum progesterone concentrations > 1 ng/mL at either bleed (or both) were considered cycling. Averaged over the 2 locations, there was a tendency ($P = 0.06$) for a greater number of cycling animals to become pregnant to TAI in the 14-d 50 PG treatment (64.4%) than 5-d CO-Synch treatment (50.2%). The 14-d CIDR with GnRH analogue on d 0 and 9, and a single 50 mg dose of PG im at CIDR removal, was a more efficacious protocol to maximize TAI pregnancy rates than the standard 5-day CO-Synch + CIDR.

Key Words: Beef cows, CIDR, Estrus synchronization, $PGF_{2\alpha}$, Timed-AI

INTRODUCTION

Recent estrus synchronization protocols for timed-AI (**TAI**) such as the 5-day CO-Synch + CIDR have achieved pregnancy rates up to 70% in beef cows. This is a marked increase compared to previous protocols (Bridges et al., 2008; Gunn et al., 2009). These protocols attempt to induce one new follicular wave by injecting GnRH at initiation of the protocol. This approach does not address the stage of the estrous cycle when cows are non-responsive to GnRH. At the start of most estrus synchronization protocols, approximately 66% of beef cows can be in a stage of the estrous cycle where GnRH causes a surge of LH that will ovulate an existing follicle and induce a new follicular wave, likely resulting in a reduction in pregnancy rate to TAI in cows not responding to this GnRH (Geary et al., 2000).

The use of progestins in estrus synchronization protocols may induce cyclicity in anestrus cows, but these protocols are extensive in labor and time (Perry et al., 2002, 2004; Smith et al., 1987). The need to initiate a new follicular wave to produce a fertile ovulation after long-term progestin removal lengthens these protocols because of the poor fertility among oocytes in resulting persistent follicles (Mihm et al., 1994; Patterson et al., 1989).

The objective of the current study was to assess the effectiveness of adding an additional GnRH injection within a 14 d CIDR program, which may initiate two follicular waves while under the influence of progesterone. We hypothesized that this would position more cows in a stage of the estrous cycle responsive to GnRH prior to progestin removal. This protocol also mimicked the 5-day CO-Synch + CIDR by giving the second GnRH injection 5 d prior to CIDR removal. The second objective was to evaluate the ability of a 14-d CIDR protocol to induce cyclicity in anestrus cows. The final objective was to assess the efficacy of a single 50 mg dose of prostaglandin $F_{2\alpha}$ at (**$PGF_{2\alpha}$**) CIDR removal compared to two 25 mg doses 6 h apart with this CIDR protocol.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures with cows were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment. Multiparous and primiparous Angus, Angus cross, and Hereford cross beef cows at 3 ranches in Wyoming and Colorado (location 1; n = 247, location 2; n = 395, location 3; n = 137) were assigned to one of 3 treatments based on the last (location 1) or last plus second to last (location 3) integer of ear tag

(Figure 2.1). At location 2, cows were blocked by age and post partum interval (**PPI**, d from calving to TAI) prior to assignment of treatments. All animals were evaluated for BCS by a single evaluator throughout the experiment on d 9 of treatments using a 1 to 9 BCS system (Richards et al., 1986). The d of initial GnRH analogue (Factrel®, Fort Dodge Animal Health, Fort Dodge, IA) injection and CIDR (CIDR, 1.38 g progesterone, EAZI-BREED CIDR®, Pfizer Animal Health, NewYork, NY) insertion was considered d 0 of the 14 d CIDR treatments. Cows in the 14-d 50 PG treatment received a CIDR insert concurrent with 100 μg GnRH analogue im on d 0, 100 μg GnRH analogue im on d 9, and CIDR removal concurrent with a single 50 mg dose of $PGF_{2\alpha}$ im (Lutalyse®, Pfizer Animal Health) on d 14. Cows in 14-d 6 h PG treatment received a CIDR insert concurrent with 100 μg GnRH analogue im on d 0, 100 μg GnRH analogue im on d 9; CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ im on d 14, with another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later. Cows in the control treatment, 5-day CO-Synch + CIDR, received a CIDR insert concurrent with 100 μg GnRH analogue im on d 9; CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ im was on d 14, with another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later (Figure 2.1). This interval was used as the most efficacious interval for maximizing TAI pregnancy rates from our previous research using the 5-day CO-Synch + CIDR protocol (Peel et al., 2010). Cows in all treatments received 100 μg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal. For all treatments, estrus detection patches (Estrotect®, Estrotect, Inc., Spring Valley, WI) were placed on the tail head of each cow at CIDR removal to aid in visual detection of estrus. Estrus detection began 36 h after CIDR removal at 12 h intervals and continued until TAI at locations 1 and 2. Visual observation of estrus was not possible at location 3, but estrus detection patches were applied. Patches were scored at TAI on d 17 at all 3 locations using a 1 to

4 scale based on amount of film on each patch that was removed (1 = completely untouched, 2 = approximately 50% removed, 3 = almost all or 100% removed, and 4 = missing patch).

Blood Collection

Reproductive cycling status was determined from blood obtained by coccygeal venipuncture on d -7 and 0 for serum concentrations of progesterone. Blood was collected in 10 mL serum vacutainer tubes (BD Vacutainer^{TM}, Becton, Dickinson and Company, Franklin Lakes, NJ) and placed directly on ice within 10 min of collection. Samples were centrifuged at 2800 x *g* for 10 min within 8 h after collection, and stored at -20°C. Concentrations of progesterone were determined using the RIA procedure as previously described (Niswender et al., 1973). Cows with progesterone levels > 1 ng/mL at either bleeding date (or both) were identified as cycling at the initiation of treatments, and cows with progesterone levels < 1 ng/mL for both bleeding dates were identified as anestrus at initiation of treatments. Inter-assay and intra-assay CV were 9.3% and 3.1%, respectively. Average sensitivity of assays was 0.03 ng/mL. Blood collection was only feasible at locations 1 and 2 (n = 243, location 1; n = 391, location 2).

Pregnancy Diagnosis

Pregnancy status to TAI was diagnosed between 37 and 40 d after TAI using transrectal ultrasonography (3.5 MHz linear transducer GP-DV, E.I. Medical, Loveland, CO). Cows were exposed to intact bulls 9 to 10 d after TAI.

Statistical Analyses

Data were analyzed via logistic regression using the GLIMMIX procedure in SAS (SAS Inst., Inc., Cary, NC). The initial model included location, treatment, parity (primiparous or multiparous), BCS, PPI, sire, artificial insemination technician, and location × treatment. However, the final model used only significant factors ($P < 0.10$), which were, treatment,

location × treatment, parity, BCS, and PPI. For cycling status data at locations 1 and 2, cycling and cycling × treatment were added to the final model.

RESULTS AND DISCUSSION

Numbers of cows, PPI, BCS, and parity are presented in Table 2.1. The mean (± SE) BCS for 14-d 50 PG, 14-d 6 h PG, and 5-day CO-synch + CIDR treatments was 4.8 ± 0.05, 4.9 ± 0.04, and 5.0 ± 0.05, respectively. The mean (± SE) PPI at TAI for 14-d 50 PG, 14-d 6 h PG, and 5-day CO-Synch + CIDR treatments was 76 ± 1.1, 76 ± 1.2, and 76 ± 1.2 d, respectively, with a range of 38 to 115 d. Mean PPI did not differ among treatments within location ($P > 0.10$), but differed by location ($P < 0.05$). Similarly, mean BCS did not differ among treatments within location ($P > 0.10$), but differed by location ($P < 0.05$).

Overall pregnancy rate to TAI did not differ between treatments ($P > 0.05$; Table 2.2) at locations 1 or 2. At location 3, pregnancy rate to TAI was higher ($P < 0.01$) in the 14-d 50 PG treatment (60.7%) than both the 14-d 6 h PG (32.0%) and 5-day CO-Synch + CIDR treatments (26.5%), but there was no difference ($P > 0.10$) between the 14-d 6 h PG and 5-day CO-Synch + CIDR treatments. There was a treatment × location interaction ($P < 0.05$) for combined results across locations. Averaged over all locations, pregnancy rate to TAI for 14-d 50 PG, 14-d 6 h PG, and 5-day CO-Synch + CIDR treatments were 58.2, 46.8, and 41.9%, respectively (Table 2.2); pregnancy rate to TAI was higher ($P < 0.05$) in the 14-d 50 PG treatment than both the 14-d 6 h PG and 5-day CO-Synch + CIDR treatments, but the 14-d 6 h PG treatment did not differ from the 5-day CO-Synch + CIDR treatment ($P > 0.10$).

In the current study, the potential to induce two follicular waves within a 14-d CIDR estrus synchronization protocol resulted in an increased pregnancy rate to TAI compared to an

estrus synchronization protocol recommended by the Beef Reproduction Task Force, the 5-day CO-Synch + CIDR (Beef Reproduction Task Force, 2012). However, results must be interpreted cautiously due to the treatment × location interaction; it is not clear why pregnancy rates were very low for two of the treatments at location 3, which was likely responsible for the interaction effect. This location had the fewest number of animals per treatment; the GLIMMIX procedure should ensure that these lower numbers did not influence overall least square means inappropriately, but caution is still advised.

Averaged over locations 1 and 2, pregnancy rate to TAI for both cycling and non-cycling cows (Table 2.3) did not differ ($P > 0.05$) by treatment. However, there was a tendency ($P = 0.06$) for cycling animals to have a higher TAI pregnancy rate in the 14-d 50 PG treatment than the 5-day CO-Synch + CIDR treatment when data were combined across locations 1 and 2. Data on cycling status at location 3 could not be collected.

Postpartum anestrus in beef cows results in infertility and poor response to certain estrus synchronization protocols (Short et al., 1990; Perry et al., 2004). However, at the two locations that cycling status was evaluated in the current study, pregnancies to TAI in cows determined to be anestrus at the initiation of the protocols were similar ($P > 0.10$) among treatments. Surprisingly, the 14 d CIDR treatments were not different ($P > 0.10$) from the 5-day CO-Synch + CIDR treatment in resulting TAI pregnancy rates in anestrus animals. The extended (14 d) influence of progesterone on prepubertal beef heifers has been shown to induce cyclicity due to the longer progesterone exposure needed to elicit an effect on follicular growth (Leitman et al., 2009), but this progesterone was given about two wk earlier relative to TAI than in the current study.

Short postpartum cows (n = 63; PPI at TAI of 38 to 44 d) had acceptable TAI pregnancy rates [14-d 50 PG: 13/24 (54.2%), 14-d 6 h PG: 10/19 (52.6%), 5-day CO-Synch + CIDR: 17/20 (85.0%)]. However, there were limited numbers of animals in this category.

The third objective was to evaluate the difference in effectiveness of a single 50 mg dose of $PGF_{2\alpha}$ to two 25 mg im doses at 6 h intervals within the 14 d CIDR treatments for maximizing TAI pregnancy rates. A single dose would reduce the number of times animals must go through the chute if effective at lysing the corpus luteum (**CL**). At locations 1 and 2, there were no differences ($P > 0.10$) between the two 14 d CIDR treatments in pregnancy rate to TAI. However, at location 3, pregnancy rate to TAI was higher ($P < 0.05$) in the 14-d 50 PG treatment than the 14-d 6 h PG treatment. In combined data across locations, pregnancy rate to TAI was higher ($P < 0.05$) in the14-d 50 PG treatment than the 14-d 6 h PG treatment. The ability of PG to induce luteal regression of a CL induced by exogenous GnRH 5 d prior to progestin removal has been previously documented in both beef cows and heifers (Bridges et al., 2008; Kasimanickam et al., 2009; Peel et al., 2010). However, within these protocols it has still been recommended (Beef Reproduction Task Force, 2012) to give a second 25 mg $PGF_{2\alpha}$ injection 8 ± 2 h after progestin removal and initial $PGF_{2\alpha}$ injection to ensure complete luteal regression for effective timing of ovulation and successful conception to TAI. This can be a burden due to the need for processing animals through the chute an additional time.

In the present study, a single 50 mg dose of $PGF_{2\alpha}$ at CIDR removal for the 14-d 50 PG treatment appeared to be sufficient in inducing luteal regression of any CL formed due to GnRH analogue on d 9 of the treatment. This was verified indirectly from comparison of TAI pregnancy rates between the 14 d CIDR treatments. However, the true physiological response of luteal regression requires further verification, for example, including repeated ultrasonography.

Acceptable TAI pregnancy rates in the 14-d 50 PG treatment seen in the current study were similar to those in a 5-day CO-Synch + CIDR protocol with two 25 mg doses of $PGF_{2\alpha}$ at CIDR removal in a multistate trial (Bridges et al., 2011). In both experiments, no differences in TAI pregnancy rates were seen between a single 50 mg dose of $PGF_{2\alpha}$ im or two 25 mg im doses at 6 h intervals. However, preceding research (Bridges et al., 2008; Kasimanickam et al., 2009) found that the 8 h interval in between two 25 mg doses of $PGF_{2\alpha}$ was necessary for maximum success in TAI pregnancy rates. The d 5 bovine CL has been originally described as non-responsive to $PGF_{2\alpha}$ (Lauderdale et al., 1972). However, research had determined that 2 injections of 25 mg of $PGF_{2\alpha}$ at 6 h intervals is as effective as 2 injections of 25 mg $PGF_{2\alpha}$ at 12 h intervals (Peel et al., 2010) and that 6 h intervals were more effective than 2 or 4 h intervals (Seabrook et al., 2010) in order to achieve maximum TAI pregnancy rates within the 5-day CO-Synch + CIDR protocol.

At locations 1 and 2, few cows (n = 31) were observed in estrus at 36 and 48 h after CIDR removal before TAI. No cows at either location came into estrus by 36 h after CIDR removal and $PGF_{2\alpha}$. Those animals observed in estrus at 48 h still had acceptable pregnancy rates to TAI 24 h later [14-d 50 PG: 6/11 (54.5%), 14-d 6 h: 8/12 (66.7%), 5-day CO-Synch + CIDR: 5/8 (62.5%)]. There were no differences in pregnancy rate to TAI among treatments ($P > 0.10$) due in part to limited numbers.

Estrous response of treatments was determined by amount of film removed from estrus detection patches on d of TAI and are presented in Table 3.4. A higher percentage of animals ($P < 0.05$) had a patch score of 3, indicative that estrus behavior occurred during the interval from CIDR removal to TAI, in both the 14-d 50 PG (58.3%) and 14-d 6 h PG (54.6%) treatments than the 5-day CO-Synch + CIDR treatment (44.0%). Similarly, a higher percentage of cows ($P <$

0.05) in the 5-day CO-Synch + CIDR treatment (36.5%) had a patch score 1, indicative of lack of exhibiting estrus, than both the 14-d 50 PG (24.1%) and 14-d 6 h PG (24.9%) treatments. There were no differences ($P > 0.10$) between 14-d 50 PG and 14-d 6 h PG treatments in either estorus response (patch score 1 and 3 comparisons). However, solely using estrus detection patch scores as an indicator of estrus response has limitations because it was not determined exactly when patches were tripped during the 72 h interval from CIDR removal to TAI. The increase in estrous response in the 14-d 50 PG treatment is consistent with increased TAI pregnancy rates when compared to the 5-day CO-Synch + CIDR treatment, but the increased estrus response in the 14-d 6 h treatment did not result in an increased TAI pregnancy rate vs. the 5-day CO-Synch + CIDR treatment.

The increased estrous response seen in the 14 d CIDR treatments could be explained by the different endocrinological environment these cows were exposed to during the 9 d of progesterone influence prior to the second GnRH analogue injection. Circulating levels of progesterone that resulted from the 14 d CIDR treatments during this period could have increased the amount of circulating estradiol-17β secreted by the dominant follicle compared to cows in the 5-day CO-Synch + CIDR treatment; if a spontaneously formed CL was not present on d 0. Higher levels of estradiol-17β produced could have led to increased estrus behavior in animals from the 14 d CIDR treatments. This increased estradiol-17β could be explained by increased LH pulse frequencies and would agree with previous research investigating long-term progesterone estrus synchronization (Kinder et al., 1996). In the absence of a spontaneously formed CL, progesterone absorbed from a CIDR is high enough to prevent ovulation but low enough to mimic the high LH pulse frequency of the follicular phase rather than the luteal phase (Kinder et al., 1996). This environment could have occurred in the 14 d CIDR treatments and

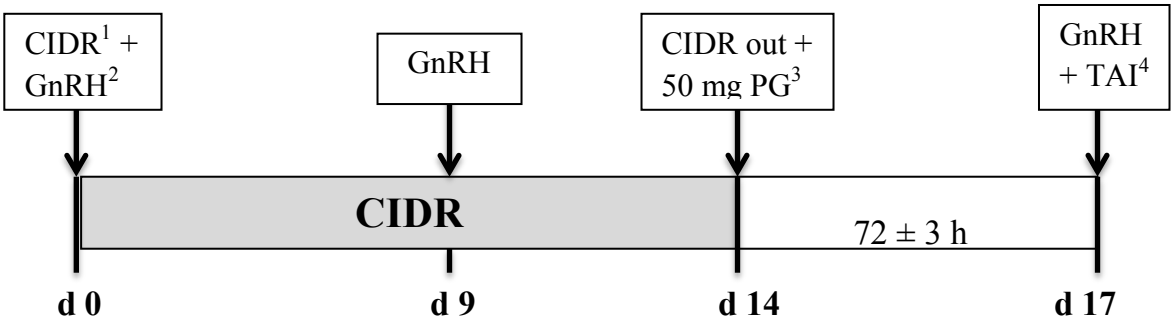
could explain the increased estrus response. Due to the lack of information on actual follicular turn over in response to GnRH analogue injections, failure to ovulate in response to the initial GnRH analogue injection on d 0, under the influence of progesterone, could have effectively produced follicles with extended follicular growth for 9 d or longer depending on what stage of growth the follicles were in on d 0. This would occur if a spontaneously formed CL were not present on d 0. Growth of persistent follicles has been proven to produce high levels of estradiol-17β, and result in low fertility due to ovulation of an aged oocyte (Revah et al., 1996; Savio et al., 1993). Early stages of this type of follicle could have been present in the current study from d 0 to 9 and contributed to increased estradiol-17β levels. However, the ovulatory capability of these follicles would not be diminished due to the potentially increased number of LH receptors present on these follicles from the high LH pulse frequency environment. Though, they were not considered true persistent follicles, this theory would agree with research finding increased numbers of LH receptors on granulosa cells of persistent follicles compared to dominant follicles with normal follicular growth (Cupp et al., 1993). However, the second GnRH injection on d 9 would have avoided persistent follicles by forcing ovulation, and initiated a new wave of follicular growth. This would explain the high estrous response of the 14 d CIDR treatments, and low TAI pregnancy rate of the 14-d 6 h PG treatment at location 3 (32%), but these low results were not seen at any other location in either of the two 14 d CIDR treatments. Incorporation of ultrasonography into the 14 d CIDR treatments in future experiments could be beneficial to determine whether follicular turn over was induced in the 14 d CIDR treatments. Increased estrus response was also seen with increased TAI pregnancy rates. As patch score increased, TAI pregnancy rates increased (Table 3.5). However, there were no differences ($P > 0.10$) between treatments and TAI pregnancy rates by estrous response.

As previously mentioned, response to the initial GnRH injection in 5 to 7 d CIDR-based estrus synchronization protocols is a key factor in initiating a new follicular wave to ovulate a fertile oocyte after CIDR removal. The ability to increase the likelihood of cows responding to GnRH has been previously documented with presynchronization of estrous cycles (Leitman et al., 2009; Schafer et al., 2006; Stegner et al., 2004) and has been proven to increase pregnancy rates in both beef cows and heifers. That concept was used in the current experiment with a different approach, by inclusion of two GnRH injections concurrent with 14 d of progestin influence. Cows with a follicle responsive to GnRH on d 0 should ovulate and start a new follicular wave. As a result of this protocol, they would then have a new follicle that would be responsive to GnRH on d 9, and ovulating that follicle while under progestin influence would initiate a second follicular wave until CIDR removal on d 14. We expected some cows that did not respond to the initial GnRH on d 0 to have a follicle that was responsive to GnRH by d 9, and thus initiate a new follicular wave to ovulate after CIDR removal. The GnRH given on d 9 addressed the issue of poor fertility of oocytes in follicles during extended (14 d) progestin influence by ovulating or luteinizing persistent follicles or follicles on the way to becoming persistent. Unfortunately, TAI pregnancy rates in non-cycling animals were not increased using the 14 d CIDR protocols compared to the shorter 5-day CO-Synch + CIDR protocol. Therefore, additional cyclicity was not induced in the 14 d CIDR treatments compared to the 5-day CO-Synch + CIDR treatment. A single 50 mg dose of PG at CIDR removal produced similar TAI pregnancy rates to two 25 mg doses at 6 h intervals.

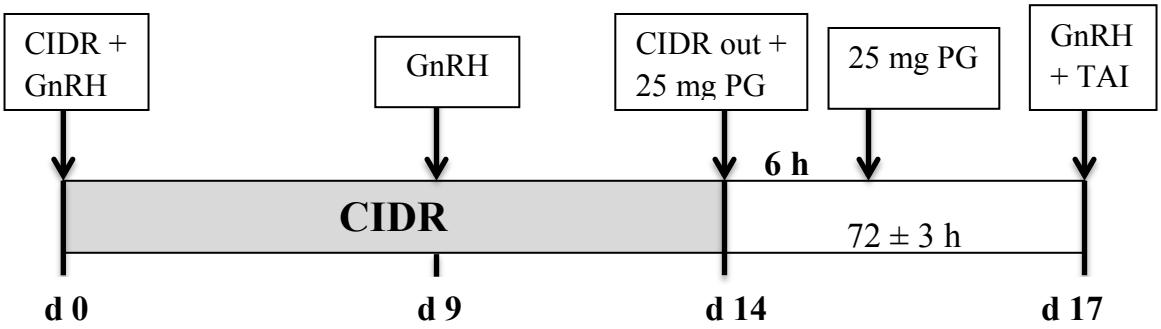
IMPLICATIONS

Pregnancy rates to TAI with 14 d CIDR estrus synchronization protocols using a GnRH analogue on d 9 were promising. Further research involving ultrasonography is needed to quantify the rate that 2 follicular waves were induced within the 14 d CIDR protocols. However, the 14-d 50 PG treatment increased TAI pregnancy rates compared to the beef industry accepted 5-day CO-Synch + CIDR protocol, and the single 50 mg dose of $PGF_{2\alpha}$ im was sufficient in the 14-d 50 PG treatment versus two 25 mg doses of $PGF_{2\alpha}$ at 6 h intervals. Future studies to assess the ovulation/luteinization rate of persistent follicles potentially formed within this protocol are needed along with optimizing timing of the GnRH injection within the 14 d of progesterone influence.

Treatment 1: 14-d 50 PG



Treatment 2: 14-d 6 h PG



Treatment 3: 5-day CO-Synch + CIDR

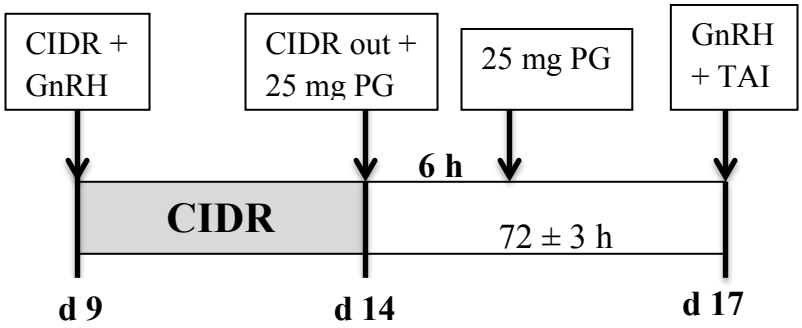


Figure 2.1. Estrus synchronization treatments administered to lactating beef cows
[1]CIDR = controlled internal drug release device inserted intra-vaginally (1.38 g progesterone, EAZI-BREED CIDR®, Pfizer Animal Health).
[2]GnRH = 100 µg GnRH analogue administered im (Factrel®, Fort Dodge Animal Health).
[3]PG = prostaglandin $F_{2\alpha}$ administered im (Lutalyse®, Pfizer Animal Health).
[4]TAI = Timed-AI.

Table 2.1. Post partum interval (PPI, d at TAI), BCS, and parity of lactating beef cows for 3 treatments at 3 locations (LS mean ± SE)

Location and treatment[4]	n =	PPI (d)	BCS	% Parity Primiparous	% Multiparous
Location 1					
14-d 50 PG[1]	103	80 ± 1.7	5.0 ± 0.07	20.4	79.6
14-d 6 h PG[2]	70	82 ± 2.0	4.9 ± 0.08	25.7	74.3
5-day CO-Synch + CIDR[3]	74	82 ± 2.0	5.1 ± 0.08	31.1	68.9
Total for location 1	247	81 ± 1.1[a]	5.0 ± 0.05[a]	25.1	74.9
Location 2					
14-d 50 PG	128	75 ± 1.9	4.3 ± .04	18.0	82.0
14-d 6 h PG	133	75 ± 2.0	4.6 ± .04	13.5	86.5
5-day CO-Synch + CIDR	134	74 ± 1.7	4.7 ± .05	13.4	86.6
Total for location 2	395	75 ± 1.1[b]	4.5 ± .03[b]	14.9	85.1
Location 3					
14-d 50 PG	47	71 ± 2.3	5.6 ± .07	8.5	91.5
14-d 6 h PG	46	73 ± 2.0	5.6 ± .07	13.0	87.0
5-day CO-Synch + CIDR	44	72 ± 2.5	5.6 ± .08	13.6	86.4
Total for location 3	137	72 ± 1.3[c]	5.6 ± .04[c]	11.7	88.3
Combined across locations					
14-d 50 PG	278	76 ± 1.1	4.8 ± 0.05	17.3	82.7
14-d 6h PG	249	76 ±1.2	4.9 ± 0.04	16.9	83.1
5-day CO-Synch + CIDR	252	76 ± 1.2	5.0 ± 0.05	18.7	81.3
Total for all treatments across locations	779	76 ± 0.7	4.9 ± 0.01	17.6	82.4

[abc]Within a column only across location totals, means without common superscripts differ ($P < 0.05$).

[1]14-d 50 PG = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 with 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[2]14-d 6 h PG = receive same protocol as 14-d 50 PG treatment except they received 25 mg $PGF_{2\alpha}$ im at CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

[3]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ im with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

[4]Cows in all treatment groups received 100 μg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal.

Table 2.2. LS means (± SE) for timed-AI (TAI) pregnancy rates (PR) of lactating beef cows by treatment and location, and across at locations

Location and treatment[1]	n =	TAI PR[4] (%)
Location 1		
14-d 50 PG[2]	103	54.7 ± 5.4
14-d 6h PG[3]	70	60.5 ± 6.3
5-day CO-Synch + CIDR[4]	74	47.0 ± 6.1
Location 2		
14-d 50 PG	128	59.1 ± 5.0
14-d 6h PG	133	48.6 ± 4.9
5-day CO-Synch + CIDR	134	54.0 ± 4.9
Location 3		
14-d 50 PG	47	60.7 ± 8.3[a]
14-d 6h PG	46	32.0 ± 7.3[b]
5-day CO-Synch + CIDR	44	26.5 ± 6.8[b]
Combined across locations[5]		
14-d 50 PG	278	58.2 ± 3.9[x]
14-d 6h PG	249	46.8 ± 4.2[y]
5-day CO-Synch + CIDR	252	41.9 ± 4.1[y]

[ab]Within a location or combined across locations, means without common superscripts differ within location ($P < 0.05$).

[1]Cows in all treatment groups received 100 μg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal.

[2]14-d 50 PG: 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 with 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[3]14-d 6 h PG: receive same protocol as 14-d 50 PG treatment except they received 25 mg $PGF_{2\alpha}$ im at CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

[4]5-day CO-Synch + CIDR: 5 d CIDR with 100 μg GnRH analogue im d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ im with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

[5]There was a treatment × location interaction ($P < 0.05$).

Table 2.3. LS means (± SE) for timed-AI (TAI) pregnancy rates (PR) of lactating beef cows by cycling status and treatment within 2 locations, by location and combined across 2 locations

Location and treatment[1]	Cycling cows[2]		Non-cycling Cows[3]	
	n =	TAI PR[3] (%)	n =	TAI PR (%)
Location 1				
14-d 50 PG[4]	40	66.1 ± 7.9	61	48.8 ± 6.4
14-d 6h PG[5]	32	69.5 ± 8.8	37	55.6 ± 8.2
5-day CO-Synch + CIDR[6]	26	49.2 ± 9.8	47	47.6 ± 7.3
Total	98	-	145	-
Location 2				
14-d 50 PG	78	62.6 ± 5.7[a]	50	51.8 ± 7.1
14-d 6h PG	69	45.4 ± 6.0[b]	62	50.3 ± 6.3
5-day CO-Synch + CIDR	73	51.1 ± 5.9[a,b]	59	55.6 ± 6.5
Total	220	-	171	-
Combined across 2 locations[7]				
14-d 50 PG	118	64.4 ± 4.6[x]	111	50.3 ± 4.8
14-d 6h PG	101	57.9 ± 5.3[x,y]	99	53.0 ± 5.1
5-day CO-Synch + CIDR	99	50.2 ± 5.1[y]	106	51.6 ± 4.9
Total for all treatments at 2 locations	318	-	316	-

[a,b]Within location and cycling status, means without common superscripts differ ($P < 0.05$).
[x,y]TAI pregnancy rates pooled across locations for the 14-d 50 PG treatment had a tendency ($P = 0.06$) to be higher than the 5-day CO-Synch + CIDR treatment.
[1]Cows in all treatment groups received a 100 µg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal.
[2]Cycling cows = cows with progesterone concentration ≥ 1 ng/ml on d -7 and/or 0.
[3]Non-cycling cows = cows with progesterone concentration ≤ 1 ng/ml on d -7 and 0.
[4]14-d 50 PG = 14 d CIDR 100 µg GnRH analogue im d 0 and 9 with 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[5]14-d 6 h PG = receive same protocol as 14-d 50 PG treatment except they received 25 mg $PGF_{2\alpha}$ im at CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
[6]5-day CO-Synch + CIDR = 5 d CIDR with 100 µg GnRH analogue im d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ im with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
[7]There was no treatment × location interaction ($P > 0.10$).

Table 2.4. Estrous response by treatment using estrus detection patches evaluated on day of timed-AI in lactating beef cows across all locations

Treatment[5]		Patch Score[1] (% of treatment)			
	n =	1	2	3	4
14-d 50 PG[2]	278	24.1[a]	13.3	58.3[a]	4.3
14-d 6 h PG[3]	249	24.9[a]	16.9	54.6[a]	3.6
5-day CO-Synch + CIDR[4]	252	36.5[b]	16.7	44.0[b]	2.8

[ab]Within columns, patch score means without common superscripts differ ($P < 0.05$).
[1]Patch Score = Based on amount of film removed from Estrotect patch on d of TAI (1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch).
[2]14-d 50 PG = 14 d CIDR with 100 µg GnRH analogue im d 0 and 9 with 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]14-d 6 h PG = receive same protocol as 14-d 50 PG treatment except they received 25 mg $PGF_{2\alpha}$ im at CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
[4]5-day CO-Synch + CIDR = 5 d CIDR with 100 µg GnRH analogue im d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ im with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
[5]Cows in all treatment groups received 100 µg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal.

Table 2.5. LS means for timed-AI (TAI) pregnancy rates (PR) by patch score and treatment using estrus detection patches evaluated on day of TAI in lactating beef cows across all locations

Patch Score[1]	TAI PR (%) by treatment		
	14-d 50 PG[2]	14-d 6 h PG[3]	5-day CO-Synch + CIDR[4]
1	52.2 (35/67)	43.5 (27/62)	42.4 (39/92)
2	48.6[a] (18/37)	35.7[ab] (15/42)	28.6[b] (12/42)
3	67.3 (109/162)	62.5 (85/136)	65.8 (73/111)
4	66.7 (8/12)	66.7 (6/9)	57.1 (4/7)

[ab]Within rows, means without common superscripts tend ($P = 0.06$) to differ.
[1]Patch Score = Based on amount of film removed from estrus detection patch on d of TAI (1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch).
[2]14-d 50 PG = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 with 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]14-d 6 h PG = receive same protocol as 14-d 50 PG treatment except they received 25 mg $PGF_{2\alpha}$ im at CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
[4]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9 with CIDR insertion, 25 mg $PGF_{2\alpha}$ im with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
Cows in all treatment groups received a 100 μg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal.

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CHAPTER III

Fixed-time AI in lactating beef cows after GnRH on day 9 of a 14-day CIDR estrus synchronization protocol[1]

SUMMARY: Failure to conceive to timed-AI (**TAI**) in many 5 to 8 d controlled internal drug release (**CIDR**)-based estrus synchronization protocols for lactating beef cows may occur due to lack of response to the GnRH given at the initiation of the protocol. Our objectives were to: 1) determine the efficacy of an extended controlled internal drug release (**CIDR**) protocol with 2 induced follicular waves, and 2) compare the efficacy of an extended progestin treatment with GnRH analogue (Factrel) or prostaglandin $F_{2\alpha}$ (**PG**) injections to maximize TAI pregnancy rates. In exp. 1, lactating beef cows (n = 588) at 2 locations were randomly assigned to one of 3 treatments. Cows in the 14-d GnRH-9 treatment (n = 202) received a CIDR (1.38 g progesterone) and 100 μg GnRH im on d 0, 100 μg GnRH im on d 9, and CIDR removal with 50 mg PG im on d 14. Cows in the 14-d GnRH-7 treatment (n = 204) received the same protocol as 14-d GnRH-9 cows except that 100 μg GnRH im was given on d 7 instead of d 9, and a single 25 mg im dose of PG was given at CIDR removal. Cows in the control treatment (n = 182), 7-day CO-Synch + CIDR (**7-d CO-Synch**), received a CIDR and 100 μg GnRH im on d 7, and CIDR removal with 25 mg PG im on d 14. Cows in all treatments received 100 μg GnRH im with TAI at either 72 ± 2 h (14-d GnRH-9) or 63 ± 3 h (14-d GnRH-7 and 7-d CO-Synch) after CIDR

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removal. Pregnancy status to TAI was determined 37 to 40 d after TAI by ultrasonography. Pregnancy rate to TAI across locations were not different ($P > 0.10$) among 14-d GnRH-9 (54.8%, n = 202), 14-d GnRH-7 (54.4%, n = 204), and 7-d CO-Synch (52.3%, n = 182) treatments. In exp. 2, lactating beef cows (n = 630) at 4 locations were randomly assigned to one of 3 treatments. Cows in the 14-d GnRH treatment received a CIDR and 100 μg GnRH im on d 0, 100 μg GnRH im on d 9, and CIDR removal with 50 mg PG im on d 14. Cows in the 14-d PG treatment were assigned the same protocol as 14-d GnRH cows except that 25 mg PG im was given at CIDR insertion instead of GnRH. Cows in the control treatment, 5-day CO-Synch + CIDR (**5-d CO-Synch**), received a CIDR and 100 μg GnRH im on d 9, CIDR removal and 25 mg PG im on d 14, and 25 mg PG im 6 ± 1 h later. Cows in all treatments received 100 μg GnRH im with TAI 72 ± 2 h after CIDR removal. In exp. 2, pregnancy rate to TAI was higher ($P < 0.05$) in 14-d PG (70.4%) than 14-d GnRH (54.4%) and 5-d CO-Synch (53.5%) treatments. There was no increase in pregnancy rate to TAI with inclusion of 2 GnRH injections within a 14-d CIDR in exp. 1 or 2. However, replacement of GnRH with PG at the initiation of the 14-d PG treatment, in exp. 2, improved the TAI pregnancy rate and was also superior to the 5-d CO-Synch protocol.

Key Words: Beef cows, CIDR, Estrus synchronization, $PGF_{2\alpha}$, Timed-AI

INTRODUCTION

Research incorporating multiple follicular waves within an estrus synchronization protocol, such as the 14-d controlled internal drug release (**CIDR**)-prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) protocol in heifers (14-day CIDR-PG), allows multiple follicular waves to grow without induced

ovulation using exogenous GnRH (Leitman et al., 2009). While this protocol results in acceptable timed-AI (**TAI**), it is quite time consuming (33 d).

Failure to respond to GnRH is a common problem with many short-term progestin based estrus synchronization protocols that initiate only one follicular wave since only 66% of cycling cows are in a stage of the estrous cycle where there is a follicle responsive to GnRH (Geary et al., 2000). The inclusion of GnRH injections on d 0 and 9 of a 14 d CIDR insert increased TAI pregnancy rates compared to a standard 5-day CO-Synch + CIDR protocol in beef cows (Giles et al., 2011). Altering the timing of a second GnRH injection within the 14 d CIDR could also increase follicular response with the second GnRH injection. This could be possible due to the animals being on d 5 of the estrous cycle when given, which has the highest chance of ovulating to the GnRH (Vasconcelos et al., 1999).

During the luteal phase, a spontaneously formed corpus luteum (**CL**) will elicit enough progesterone (**P4**) to suppress LH pulse frequencies (Roberson et al., 1989), but low levels of exogenous P4 (e.g. CIDR) does not similarly suppress the same LH pulses. This environment could be created in a 14 d CIDR protocol by inclusion of $PGF_{2\alpha}$ at the initiation of exogenous P4 (Kinder et al., 1996). Increasing LH pulse frequency would theoretically increase the number of LH receptors on the growing follicle (Cupp et al., 1993). This could ensure ovulation from GnRH, CL formation, and responsiveness to $PGF_{2\alpha}$ leading to a more synchronized estrus.

The objective of exp. 1 was to compare the effect of interval from CIDR insertion to GnRH on either d 7 or 9 in a 14 d CIDR protocol on TAI pregnancy rates. The effectiveness of these 14 d CIDR protocols were also compared to the control 7-day CO-Synch + CIDR protocol.

The first objective of exp. 2 was to to compare TAI pregnancy rates between two 14 d CIDR estrus synchronization protocols with PG or GnRH at the time of CIDR insertion to a

recommended short-term estrus synchronization protocol, the 5-day CO-Synch + CIDR. The second objective was to validate synchronization of multiple follicular waves and compare PG responsiveness of an accessory CL formed as a result of ovulation of a follicle with extended follicular growth to those formed by a normal growing follicle within the 14 d of CIDR treatment.

MATERIALS AND METHODS

Animals

All experimental procedures with cows were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiments. Cows in both experiments were managed as commercial herds, and therefore included cows that were older in age (> 6 yr), thin BCS, primiparous, and < 45 d postpartum (**PPI**).

Experiment 1

Experimental Design

Primiparous and multiparous Angus and Angus cross beef cows at 2 locations (n = 588) in Colorado and Wyoming (location 1; n = 202, location 2; n = 386) that would be at least 35 d post partum by d of TAI were randomly assigned to one of 3 treatments (Figure 3.1). All animals were evaluated for BCS on d 7 using a 1 to 9 BCS system (Richards et al., 1986) by one evaluator. The d of initial GnRH analogue (Factrel®**,** Fort Dodge Animal Health, Fort Dodge, IA) injection and CIDR (EAZI-BREED CIDR®, Pfizer Animal Health, NewYork, NY; 1.38 g of progesterone) insert was considered d 0 for the two 14 d CIDR treatments (14-d GnRH-7 and 14-

d GnRH-9). Cows in the control treatment (7-day CO-Synch + CIDR) received their initial GnRH analogue injection and CIDR insert on d 7 relative to the 14 d CIDR treatments. Cows in the 14-d GnRH-9 received 100 μg GnRH analogue im on d 9, CIDR removal concurrent with a single 50 mg dose of $PGF_{2\alpha}$ (Lutalyse®, Pfizer Animal Health) im on d 14, and 100 μg GnRH analogue im concurrent with TAI at 72 ± 3 h after CIDR removal. Cows in the 14-d GnRH-7 treatment received 100 μg GnRH analogue im on d 7, CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ im on d 14, and 100 μg GnRH analogue im concurrent with TAI at 63 ± 3 h after CIDR removal. Cows in the control treatment, 7-day CO-Synch + CIDR, received a CIDR insert concurrent with 100 μg GnRH analogue im on d 7, CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ im on d 14, and 100 μg GnRH analogue im concurrent with TAI at 63 ± 3 h after CIDR removal (Figure 3.1). For all treatments, estrus detection patches (Estrotect®, Estrotect, Inc., Spring Valley WI) were placed on the tail head of each cow upon CIDR removal for aiding in estrous response. Patches were scored on d of TAI (d 17) using a 1 to 4 scale (1 = completely untouched, not-rubbed; 2 = partially or approximately 50% rubbed off; 3 = almost all or 100% rubbed off; and 4 = missing patch).

Pregnancy Diagnosis

Pregnancy status to TAI was diagnosed between 37 and 40 d after TAI using transrectal ultrasonography (3.5 MHz linear transducer GP-DV, E.I. Medical, Loveland, CO). Cows were exposed to intact bulls beginning 9 to 10 d after TAI.

Statistical Analyses

Data were analyzed via logistic regression using the GLIMMIX procedure in SAS (SAS Inst., Inc., Cary, NC) to determine differences in TAI pregnancy rates, BCS, and PPI (d from calving to TAI) among treatments. The initial model included the factors location, treatment,

parity (primiparous vs. multiparous), BCS, PPI, service sire, AI technician, estrus detection patch score, BCS × treatment, parity × treatment, PPI × treatment, estrus detection patch score × treatment, and location × treatment. However, the final model only included location ($P = 0.57$), treatment ($P = 0.66$), and location × treatment ($P = 0.69$) to determine LS means. Data for estrous response by treatments were analyzed via linear regression using the GLM procedure in SAS to determine difference in estrus detection patch scores among treatments.

Experiment 2

Experimental Design

Angus, Angus cross, and Hereford cows (n = 630) at 4 locations (n = 264, location 1; n = 99, location 2; n = 139, location 3; n = 128, location 4) that were at least 33 d post partum by d of TAI were randomly assigned to one of 3 treatments (Figure 3.2) on d 0. All animals were evaluated for BCS on d 9 as described previously. Cows in the 14-d GnRH treatment received a CIDR concurrent with 100 μg GnRH analogue im on d 0, 100 μg GnRH analogue im on d 9, and CIDR removal concurrent with a single 50 mg dose of $PGF_{2\alpha}$ im on d 14. Cows in the 14-d PG treatment were treated identically to the 14-d GnRH cows except 25 mg $PGF_{2\alpha}$ im was injected on d 0 instead of 100 μg GnRH analogue im. Cows in the control treatment, 5-day CO-Synch + CIDR, received a CIDR concurrent with 100 μg GnRH analogue im on d 9 relative to the 14 d CIDR treatments, CIDR removal concurrent with 25 mg $PGF_{2\alpha}$ im on d 14, and 25 mg PG im 6 ± 1 h later (Figure 3.2). Cows in all treatments received 100 μg GnRH analogue im concurrent with TAI 72 ± 3 h after CIDR removal. For all treatments, estrus detection patches were placed

on the tail head of each cow upon CIDR removal for aiding in estrus response, and were scored as previously described.

Pregnancy Diagnosis

Pregnancy status to TAI was diagnosed between 37 and 40 d after TAI as described above. Cows were exposed to intact bulls beginning 9 to 10 d after TAI.

Ovarian Response

Ovarian structures and responses to treatments were determined via transrectal ultrasonography (as described above) performed in a random subset of cows at location 1 (n = 126) and all cows at location 2 (n = 99) on d 0, 3, 9, 12, 14, and 17 (Figure 3.3). All responses to treatments administered are shown in Figure 3.4 relative to ultrasonography performed. Visible left and right ovarian structures (CL and follicles) ≥ 5 mm were recorded. A successful response to GnRH analogue on d 0 (for 14-d GnRH treatment) was defined as presence of a follicle ≥ 9 mm on d 0 and absence of that follicle by d 3 and(or) replacement with a CL on d 9. A lack of response to GnRH analogue on d 0 was defined as a cow having no follicle present on d 0 or presence of a follicle ≥ 9 mm on d 0 that had grown or remained statically present by d 3. Lack of replacement of a follicle with a CL by d 9 was also considered a lack of response to GnRH analogue on d 0. A successful response to $PGF_{2\alpha}$ on d 0 (for 14-d PG treatment) was defined as presence of CL on d 0 and absence by d 3 and absence on d 9 as well. Cows with absence of CL on d 0, 3, and 9 were also grouped with the positive response to $PGF_{2\alpha}$ cows for some analyses because they were within the same endocrinological background of hormones for the purposes of the experiment. Lack of a response to $PGF_{2\alpha}$ on d 0 was defined as a cow having presence of a CL on d 0 and continued presence of the CL on d 3 and 9. Another lack of response to $PGF_{2\alpha}$ was defined as cows with absence of a CL on d 0, but presence of a new CL by d 3 and(or) 9.

These cows were denoted as having recently ovulated and did not have a visibly formed CL by d 0 of the treatment. No data from ultrasonography on d 0 or 3 were accumulated from cows in the 5-day CO-synch + CIDR treatment since no treatments were administered to animals on those days. A successful response to GnRH analogue on d 9 was defined (for all 3 treatments) as a cow having a follicle ≥ 9 mm on d 9 and absence of that follicle by d 12 and(or) replacement with a CL on d 14. A lack of response to GnRH analogue on d 9 was defined as a cow having no follicle present on d 9 or presence of a follicle ≥ 9 mm on d 9 that had grown or remained statically present by d 12. Lack of replacement of a follicle with a CL by d 14 was also considered a lack of response to GnRH on d 9. Ultrasonography performed on d 14 was used to determine size of dominant follicles along with relative size and presence of CL at time of PG administration. Ultrasonography performed on d 17 with TAI was used to determine response to $PGF_{2\alpha}$ administered on d 14 and size of potentially ovulatory follicles.

In the subset of cows at location 1 and all cows at location 2, estrus behavior was visually observed daily for 1 h in the morning and evening beginning 22 d prior to initiation of treatments. Cows were classified as being cyclic at initiation of treatments if standing estrus behavior was identified prior to initiation of treatments and (or) if a CL was present on either ovary on d 0 of treatments. Based on visual observation of estrus, cows were classified into 4 groups based on stage of the estrous cycle. The d of estrous cycle groups were identified as d 0 to 6, 7 to 12, 13 to 17, or 18 to 21 to determine differences in success of each treatment by stage of the estrous cycle treatments were initiated on.

Statistical Analyses

Data were analyzed via logistic regression using the GLIMMIX procedure in SAS for determining differences in TAI pregnancy rates, response to GnRH, follicle sizes (mm), BCS,

and PPI among treatments. The initial model included the factors location, treatment, parity (primiparous vs. multiparous), BCS, PPI, sire, AI technician, estrus detection patch score, BCS × treatment, parity × treatment, PPI × treatment, estrus detection patch score × treatment, and location × treatment. However, the final model only used the significant factors ($P < 0.05$) treatment and PPI, and non-significant factors ($P > 0.10$) location and location × treatment. Data for estrous response by treatments were analyzed via linear regression using the GLM procedure in SAS to determine difference in estrus detection patch scores among treatments. For the cows subjected to ultrasonography at locations 1 and 2, determining differences in response to treatments were also analyzed via the GLM procedure in SAS.

RESULTS

Experiment 1

Numbers of cows, BCS, and PPI for treatments are presented by location and combined across locations in Table 3.1. Mean BCS did not differ ($P > 0.10$) among treatments across or within locations ($P > 0.10$). Mean PPI for 14-d GnRH-9, 14-d GnRH-7, and 7-day CO-Synch + CIDR treatments did not differ within location ($P > 0.10$), but differed by location ($P < 0.05$). Mean pregnancy rates to TAI did not differ ($P > 0.10$; Table 3.2) for 14-d GnRH-9, 14-d GnRH-7, and 7-day CO-Synch + CIDR treatments at either locations or across locations. There was no treatment x location interaction ($P = 0.69$).

Short postpartum cows (≤ 45 days postpartum) had acceptable TAI pregnancy rates in the 14-d GnRH-7 (n = 7; 57.1%) and 7-day CO-Synch + CIDR (n = 6; 50.0%) treatments, but were low in the 14-d GnRH-9 treatment (n = 8; 12.5%). However, there were few cows (n = 21) in this category across both locations.

Estrous response of treatments was determined by amount of film removed on Estrotect patches on d of TAI (Table 3.3). Estrous response from Estrotect patch scores was analyzed by patch score (1 to 4) percentages of each treatment. A greater percentage ($P < 0.05$) of cows with a patch score of 1 (completely untouched, not rubbed) occurred in the 7-day CO-Synch + CIDR treatment (31.3%) than the 14-d GnRH-9 (19.8%) and 14-d GnRH-7 (22.1%) treatments, which did not differ from each other ($P > 0.10$; Table 3.3). There were also a greater percentage ($P < 0.05$) of cows with a patch score of 3 (almost all or 100% rubbed off) in 14-d GnRH-9 (44.1%) and 14-d GnRH-7 (42.6%) treatments than the 7-day CO-Synch + CIDR (29.7%) treatment. It is important to note that there were a high percentage of cows in all 3 treatments with a patch score 4 (missing patch; 27.7%, 31.9%, and 20.3%). This was due to a large rainstorm at location 2 on d 14 which resulted in poor adhesion of Estrotect patches to the tail heads of cows when applied. As patch score increased from 2 to 3, TAI pregnancy rates also increased ($P < 0.05$) among all 3 treatments. However, across patch scores 1 to 4, TAI pregnancy rates were not different ($P > 0.05$) between treatments (Table 3.4).

Experiment 2

Numbers of cows, BCS, and PPI for treatments are presented by location and combined across locations in Table 3.5. Mean BCS for 14-d GnRH, 14-d PG, and 5-day CO-Synch + CIDR treatments did not differ ($P > 0.10$) among treatments, or among treatments within each location. However, mean BCS differed ($P < 0.05$) by location. Mean PPI for 14-d GnRH, 14-d PG, and 5-day CO-Synch + CIDR treatments did not differ ($P > 0.10$) among treatments, or among treatments within each location. However, mean PPI differed ($P < 0.05$) by location. Pregnancy rates to TAI did not differ ($P > 0.05$; Table 3.6) among treatments at location 1 or 2. However, at

locations 3 and 4, pregnancy rates to TAI were higher ($P < 0.05$) in the 14-d PG treatment (76.6%, location 3; 75.8%, location 4) than either the 14-d GnRH (55.3%, location 3; 55.8%, location 4) or 5-day CO-Synch + CIDR (46.7%, location 3; 52.4%, location 4) treatments. There were no differences ($P > 0.10$) between the 14-d GnRH and 5-day CO-Synch + CIDR treatments at locations 3 or 4. There was no treatment x location interaction ($P = 0.53$). For combined data from all four locations, pregnancy rates to TAI were higher ($P < 0.05$) in the 14-d PG treatment (70.4%, n = 217) than either the 14-d GnRH (54.4%, n = 205) or 5-day CO-Synch + CIDR (53.5%, n = 208) treatments.

Estrous response of treatments was determined by amount of film removed on Estrotect patches on d of TAI (Table 3.7). Estrous response from Estrotect patch scores was analyzed by patch score (1 to 4) percentages of each treatment. A greater percentage ($P < 0.05$) of cows had a patch score 1 (completely untouched, not rubbed) in the 5-day CO-Synch + CIDR treatment (29.6%) than either the 14-d GnRH (20.5%) or 14-d PG (19.6%) treatments, which did not differ from each other ($P > 0.10$; Table 3.7). As patch score increased from 1 to 3, TAI pregnancy rates increased ($P < 0.05$) among all 3 treatments. TAI pregnancy rates of patch scores 1 and 3 (Table 3.8) were higher ($P < 0.05$) in the 14-d PG treatment (59.5%, patch score 1; 80.0%, patch score 3) than either the 14-d GnRH (31.0% patch score 1; 63.3%, patch score 3) or 5-day CO-Synch + CIDR (37.7%, patch score 1; 64.4%, patch score 3) treatments. There was no difference in TAI pregnancy rates ($P > 0.05$) between patch scores 1 and 3 in 14-d GnRH and 5-day CO-Synch + CIDR treatments.

There were no differences ($P > 0.05$) in CL presence at CIDR insertion between 14-d GnRH (59.7%; 43/72), 14-d PG (65.8%; 52/79) and 5-day CO-Synch + CIDR (73.7%; 56/76) treatments from ultrasonography performed in a subset of cows at location 1 and all cows at

location 2 (Table 3.9). Luteolysis in response to $PGF_{2\alpha}$ administered to the 14-d PG treatment (n = 79) on d 0 occurred in 76.9% (39/52) of all cows with presence of a CL (n = 52) on d 0. By d 9, 63.3% (50/79) of all cows in the 14-d PG treatment had absence of a CL; this included cows that lysed a CL by d 3 (n = 40) or had absence of CL from d 0 to 9 (n = 10). The TAI pregnancy rate of these animals was 70.0% (35/50), but was not different ($P > 0.10$) from cows with presence of a CL on d 9 (62.1%; 18/29). Ovulation or follicle turnover in response to GnRH analogue administered at CIDR insertion for the 14-d GnRH treatment (n = 72) occurred in 54.2% (39/72) of all cows (Table 3.10). All 3 treatments received GnRH analogue on d 9, and response to GnRH analogue was higher ($P < 0.05$) in the 14-d GnRH (76.4%) and 14-d PG (83.5%) treatments than the 5-day CO-Synch + CIDR (57.9%) treatment. There were no differences ($P > 0.10$) in response to GnRH analogue on d 9 between 14-d GnRH and 14-d PG treatments. Similarly, the percentage of cows with a follicle ≥ 10 mm present on d 9 was higher ($P < 0.05$) in the 14-d PG treatment (89.9%) than 5-day CO-Synch + CIDR (68.4%) treatment, but not different ($P > 0.10$) from the 14-d GnRH treatment (80.6%). There was a marginally significant difference ($P = 0.09$) between d 9 follicle presence percentages of 14-d GnRH and 5-day CO-Synch + CIDR treatments (Table 3.10). While overall responses to d 9 GnRH analogue were higher in the 14-d CIDR treatments than the 5-day CO-Synch + CIDR treatment, TAI pregnancy rates by response to d 9 GnRH analogue were not different ($P > 0.05$) between the 14-d GnRH (56.4%), 14-d PG (69.7%), and 5-day CO-Synch + CIDR (56.8%) treatments (Table 3.10).

Mean (± SE) follicle sizes (mm) on d 9 for the 14-d GnRH, 14-d PG, and 5-d CO-Synch + CIDR treatments were 13.5 ± 0.43, 14.7 ± 0.47, and 11.9 ± 0.34 mm, respectively (Table 3.10). Mean d 9 follicle size was higher ($P < 0.05$) in the 14-d PG treatment than the 5-day CO-Synch

+ CIDR and tended ($P = 0.06$) to be higher than the 14-d GnRH treatments. D 9 follicle size was higher ($P < 0.05$) in the 14-d GnRH treatment than the 5-day CO-Synch + CIDR treatment. There were no differences ($P > 0.05$) in mean (± SE) follicle size on d 14 in the 14-d GnRH (10.7 ± 0.21 mm), 14-d PG (10.9 ± 0.23 mm), and 5-day CO-Synch + CIDR (11.0 ± 0.27 mm) treatments. Similarly, there were no differences in mean follicle size or percentage of cows with presence of follicle ≥ 12 mm on d 17 between 14-GnRH (16.0 ± 0.21; 86.1%), 14-d PG (16.3 ± 0.39; 83.5%), and 5-day CO-Synch + CIDR (15.2 ± 0.38; 88.2%) treatments (Table 3.10).

Synchronization of 2 follicular waves from response to GnRH analogue d 0 and 9 occurred in 40.3% (29/72) of cows in the 14-d GnRH treatment. However, of the 14-d GnRH treatment cows that ovulated in response to d 0 GnRH analogue (n = 39), 74.4% (29/39) ovulated in response to d 9 GnRH analogue as well, forming two synchronized follicular waves with TAI pregnancy rates of 55.2% (16/29). These results were similar ($P > 0.10$) to cows that did not ovulate in response to d 0 GnRH analogue (n = 33), but ovulated in response to d 9 GnRH analogue (n = 26) with TAI pregnancy rates of 57.7% (15/26). There were few cows (n = 10) that ovulated in response to d 0 GnRH analogue, but did not ovulate in response to d 9 GnRH analogue with TAI pregnancy rates of 50.0% (5/10). There were few cows (n = 7) in the 14-d GnRH treatment that failed to ovulate in response to GnRH analogue on d 0 and 9 with TAI pregnancy rates of 28.6% (2/7). There were no differences ($P > 0.10$) between percentage of cows in 14-d GnRH (88.9%), 14-d PG (86.1%), and 5-day CO-Synch + CIDR (86.8%) treatments with CL presence on d 14. However, a higher percentage ($P < 0.05$) of cows in 14-d GnRH (12.5%) and 5-day CO-Synch + CIDR (19.7%) treatments had a detectable CL on d 17 at TAI than the 14-d PG treatment (3.8%). Pregnancy rate to TAI of cows with presence of a follicle ≥ 12 mm on d 17 were higher ($P < 0.05$) in the 14-d PG treatment (72.7%) than 14-d

GnRH (53.2%) treatment, but not different ($P > 0.05$) than the 5-day CO-Synch + CIDR (64.2%) treatment (Table 3.9). There were no differences ($P > 0.10$) in this category between 14-d GnRH and 5-day CO-Synch + CIDR treatments.

Short postpartum cows (≤ 45 d postpartum) had acceptable TAI pregnancy rates in the 14-d GnRH (n = 12; 50.0%), 14-d PG (n = 10; 60.0%) and 5-day CO-Synch + CIDR (n = 9; 88.9%). However, there were few cows (n = 31) in this category across locations.

Cycling status determined in the subset of cows at location 1 and all cows at location 2 is presented in Table 3.11. At both locations, there were no differences ($P > 0.10$) in the proportion of animals cycling and non-cycling among all 3 treatments at CIDR insertion d 0. Within locations 1 and 2, there were no differences ($P > 0.10$) in TAI pregnancy rates between cycling and non-cycling animals in any of the 3 treatments. There was no treatment × location interaction ($P > 0.10$). Combined at both locations, pregnancy rates to TAI were not different ($P > 0.05$) among 14-d GnRH (cycling – 58.3%; non-cycling – 45.8%), 14-d PG (cycling – 69.0%; non-cycling – 61.9%), and 5-day CO-Synch + CIDR (cycling – 66.1%; non-cycling – 45.0%) treatments. While there were few non-cycling cows among the 3 treatments (n = 65), TAI pregnancy rates of non-cycling cows were numerically, but not significantly higher ($P = 0.20$) in the 14-d PG (61.9%) treatment than the 14-d GnRH (45.8%) and 5-day CO-Synch + CIDR (45.0%) treatments (3.11).

Pregnancy rates from TAI by d of the estrous cycle by treatment of cows at locations 1 and 2 are presented in Table 3.12. There were no differences ($P > 0.10$) in TAI pregnancy rates between the 14-d GnRH, 14-d PG, and 5-day CO-Synch + CIDR treatments by each d of the estrous cycle group (d 0 to 6, 7 to 12, 13 to 17, 18 to 21, or combined), but there were very few numbers of animals in each group.

DISCUSSION

Pregnancy rates from TAI between 5-day CO-Synch + CIDR and 7-day CO-Synch + CIDR protocols have varied. The novel theory behind the 5-day CO-Synch + CIDR protocol utilizes the greater estradiol-17β levels and fertility in ovulation of younger follicles resulting in increased TAI pregnancy rates by up to 10% compared to the standard 7-day CO-Synch + CIDR protocol in beef cows (Bridges et al., 2008 and Gunn et al., 2009). However, additional research investigating these 2 protocols revealed no differences in TAI pregnancy rates between the 2 protocols (Johnson et al., 2009 and Wilson et al., 2010). Modifications of these protocols were studied in exp. 1. The thinking behind the 14-d GnRH-9 treatment mimicked the 5-day CO-Synch + CIDR protocol by administration of GnRH analogue under P4 influence with CIDR removal 5 d after GnRH analogue administration. The same design was used in the 14-d GnRH-7 treatment to mimic the 7-day CO-Synch + CIDR by administration of GnRH analogue under P4 influence with CIDR removal 7 d after GnRH analogue administration. Both ideas would hypothetically set up two follicular waves by inducing ovulation or follicle turnover to the first GnRH analogue injection and inducing a second follicular wave 1.5 to 2 d after the second GnRH injection (Bentley et al., 1998; Martinez et al., 2000). However, by shifting the second GnRH analogue injection 2 d earlier (14-d GnRH-7 treatment), cows that ovulated to the first GnRH analogue injection would be set up with an equivalent d 5 (of the estrous cycle) follicle, which has the highest probability of responding to the GnRH analogue (Geary et al., 2000 and Vasconcelos et al., 1999).

In a previous study, we showed that a single injection of 50 mg $PGF_{2\alpha}$ was just as efficacious for luteolysis as two 25 mg injections at 6 h intervals with the 14-d GnRH-9 protocol (Seabrook et al., 2010). The benefit of overlaying extended P4 influence (CIDR) for either 9 d

(14-d GnRH-9 treatment) or 7 d (14-d GnRH-7 treatment) and inclusion of a second GnRH analogue injection were investigated in experiment 1. The efficacy of these two 14 d CIDR treatments to increase TAI pregnancy rates were compared to the Beef Reproduction Task Force recommended 7-day CO-Synch + CIDR protocol. While in a previous study the 14-d GnRH-9 treatment increased TAI pregnancy rates compared to another recommended protocol, the 5-day CO-Synch + CIDR (58.2 vs. 47.0 %; Giles et al., 2011), a similar advantage was not observed in exp. 1 when the 14-d GnRH-9 treatment was compared to the 7-d CO-Synch + CIDR treatment (54.8 vs. 52.3%). Similarly, TAI pregnancy rates were not different ($P > 0.10$) between 14-d GnRH-7 (54.4%) and 7-day CO-Synch + CIDR (52.3%) treatments.

While changing the interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 d in a short-term CIDR estrus synchronization protocol increased TAI pregnancy rates in some previous studies (Bridges et al., 2008), the same increase was not seen when comparing the long-term CIDR synchronization 14-d GnRH-9 (54.8%) and 14-d GnRH-7 (54.4%) treatments. One of the main differences between these two 14 d CIDR treatments was the age of follicles forced to ovulate upon CIDR removal. While the increased fertility of ovulating a d 8 follicle (Ahmad et al., 1997) from GnRH analogue administered on d 9 was hypothetically utilized in the 14-d GnRH-9 treatment, there was no increase in TAI pregnancy rates compared to ovulation of a d 10 follicle from GnRH analogue administered on d 7 in the 14-d GnRH-7 treatment. Lack of ultrasonographic monitoring for the response to GnRH analogue in the 14 d CIDR treatments did not allow comparison of TAI pregnancy rates between cows that ovulated in response to the GnRH analogue administered on either d 9 in the 14-d GnRH-9 treatment or d 7 in the 14-d GnRH-7 treatment. Cows in the 14-d GnRH-9 treatment that ovulated a follicle to d 9 GnRH analogue may have had increased TAI pregnancy rates compared to cows in the 14-d GnRH-7

treatment that ovulated a follicle to the d 7 GnRH analogue. Another factor that could have resulted in similar TAI pregnancy rates between the 14 d CIDR treatments in experiment 1 was response to the second GnRH analogue administered on either d 9 (14-d GnRH-9 treatment) or d 7 (14-d GnRH-7 treatment). Cows in the 14-d GnRH-7 treatment that ovulated in response to the GnRH analogue administered on d 0 would theoretically have had a d 5 (of the estrous cycle) follicle that emerged 1.5 to 2 d after ovulation (Bentley et al., 1998; Martinez et al., 2000). These cows would be synchronized to potentially have the highest response to the second GnRH analogue administered on d 7 resulting in successful synchronization of 2 follicular waves (Vasconcelos et al., 1999). The increased response to the second GnRH analogue administered in the 14-d GnRH-7 protocol could lead to higher response to the treatment upon CIDR removal and synchronize ovulation of a larger number of animals in this treatment compared to the 14-d GnRH-9 treatment. Investigating the response to the second GnRH analogue administered within the 14-GnRH-9 treatment could have led to the similar results between these two 14-d CIDR treatments. There could have been an increased fertility in follicles ovulated in response to the d 9 GnRH analogue administered in the 14-d GnRH treatment, but decreased percentage of cows that ovulated in response to this GnRH analogue. This could be due to the interval of days between GnRH analogue administered on d 0 and 9. Further investigation into response between synchronizing two follicular waves using these two 14-d CIDR protocols with use of ultrasonography could increase understanding response to GnRH analogue administered at different intervals.

While there were no differences in TAI pregnancy rates between the 3 treatments in exp. 1, estrous responses among treatments differed. The increased number of cows in the 14-d GnRH-9 and 14-d GnRH-7 treatments with a patch score 3 compared to the 7-day CO-Synch +

CIDR would hypothetically have increased TAI pregnancy rates since pregnancy rate increased as patch score increased from 1 to 3. However, differences in the interval from CIDR removal and $PGF_{2\alpha}$ to standing estrous behavior between treatments could be the underlying determinant in successfully conceiving to TAI within these 3 treatments. The 60 to 66 h interval has been well documented and adopted in the 7-day CO-Synch + CIDR protocol (Dobbins et al., 2006 and Larson et al., 2006), but has not been verified as optimal within the 14-d GnRH-7 treatment. Hypothetically, the interval from CIDR removal and $PGF_{2\alpha}$ to estrus in the 14-d GnRH-7 treatment should be similar to that of the 7-day CO-Synch + CIDR treatment, but differential response from the extra 7 d of P4 influence in the 14-d GnRH-7 treatment could have had an effect on this interval. Also, a possible lack of response to the GnRH analogue administered on d 7 could have offset the interval from CIDR removal to standing estrous behavior in these animals. Similarly, the use of 68 to 72 h as the interval from CIDR removal and $PGF_{2\alpha}$ to estrus in the 14-d GnRH-9 treatment was used to coincide to the interval used in the 5-day CO-Synch + CIDR protocol, but could have been offset for similar reasons as the 14-d GnRH-7 treatment (Bridges et al., 2008). However, quite good TAI pregnancy rates were achieved using this 68 to 72 h interval within the 14-d GnRH-9 protocol in a previous experiment (Giles et al., 2011). Possibly, the GnRH analogue administered at the time of AI compensated for the delay in estrous behavior with the 7-day CO-Synch + CIDR treatment.

Data on the possibility and efficacy of inducing 2 synchronized follicular waves using a GnRH analogue has not been documented in recent literature. However, inducing three follicular waves within a GnRH-$PGF_{2\alpha}$ based synchronization protocol has increased TAI conception rates in dairy cows (Friedman et al., 2011). However, this experiment lacked ultrasonographic tracking of follicular growth and ovulation for validating induction of multiple follicular waves.

Previous research on synchronizing two follicular waves under the influence of P4 increased TAI pregnancy rates in lactating beef cows compared to the 5-day CO-Synch + CIDR protocol (Giles et al., 2011), but they did not evaluate ovarian responses with ultrasonography, which is necessary for further understanding of follicular dynamics.

Within exp. 2, tracking follicular waves in a subset of cows at location 1 and all cows at location 2 confirmed that most cows that responded to d 0 GnRH analogue injection (n = 39) also responded (74.4%) to d 9 GnRH analogue synchronizing two follicular waves. The overall response in synchronization of two follicular waves in the 14-d GnRH treatment (40.3%; 29/72) was lower than expected, but lack of success could be explained by the overall response of animals to the GnRH analogue on d 0 (54.2%; 39/72). This lack of response could have led to poor synchronization of follicular waves for the succeeding GnRH analogue administration on d 9. However, most cows not responding to the d 0 GnRH analogue (45.8%; 33/72), ovulated in response to the GnRH analogue administered on d 9 (78.8%; 26/33), and had acceptable TAI pregnancy rates (57.7%; 15/26). While there were few cows that failed to respond to GnRH analogue on both d 0 and 9 (9.7%; 7/72), as expected TAI pregnancy rates were lower (28.6%; 2/7) than for cows responding to GnRH analogue.

The most important objective was to end up with a follicle that ovulated a fertile oocyte for TAI, and having a follicle responsive to GnRH 5 or 14 d prior to CIDR removal is an important step for that objective. Within exp. 2, data from ultrasonography at locations 1 and 2 reinforced the idea that successful response to GnRH within an estrus synchronization protocol would increase TAI pregnancy rates. However, the increased response to d 9 GnRH analogue in the 14-d GnRH treatment (76.4%) compared to the 5-day CO-Synch + CIDR treatment (57.9%) was not associated with higher TAI pregnancy rates within these treatments, which was further

verified by the similarities between the two treatments at locations 3 and 4. Apparently, the 5-day CO-Synch + CIDR treatment, in some way compensates for the low response to the initial GnRH injection.

The reduced fertility of oocytes in persistent bovine follicles has been documented repeatedly (Mihm et al., 1994 and Sanchez et al., 1993). This decreased fertility has led to estrus synchronization protocols that avoid ovulation of these poor quality oocytes for AI. The persistent follicle formed from administering prolonged low biological doses of P4 (e. g. CIDR or melengestrol acetate) has been avoided in currently recommended protocols. However, within experiment 2, the 14-d PG treatment takes advantage of these follicles by forcing ovulation via GnRH and setting up a fresh follicular wave.

Presence of low levels of exogenous P4, in the absence of a spontaneously formed CL, is more similar to the endocrinological environment of the follicular phase of the estrous cycle rather than the luteal phase (Kinder et al., 1996). However, the threshold level of P4 maintained from low dose progestins still prevents ovulation or atresia of a dominant follicle and forces extended growth of a persistent follicle (Kinder et al., 1996). Creating this environment, while preventing ovulation of the dominant follicle, could increase exposure of the existing follicle to frequency of LH pulses, which would agree with research finding higher number of LH receptors on granulosa cells of persistent follicles than normal dominant follicles (Cupp et al., 1993). Therefore, in the 14-d PG treatment, along with ensuring the presence of a responsive follicle at time of GnRH analogue on d 9 of a 14 d CIDR, the follicle that ovulated also might have a higher number of LH receptors and luteinize more rapidly than a normal ovulated follicle. This could ensure luteinization and complete responsiveness to $PGF_{2\alpha}$ at CIDR removal, leading to a more synchronized estrus for TAI.

GnRH analogue responsiveness at locations 1 and 2 in exp. 2 was validated from the increased response to d 9 GnRH analogue in the 14-d PG treatment (83.5%; 66/79) compared to the 5-day CO-Synch + CIDR treatment (57.9%; 44/76). The increased response to d 9 GnRH analogue in the 14-d PG treatment was associated with a non-significantly ($P = 0.16$) higher TAI pregnancy rate (69.7%; 46/66) than for cows responding to d 9 GnRH analogue in the 5-day CO-Synch + CIDR treatment (56.8%, 25/44). However, within the 14-d PG treatment as a whole at locations 1 and 2, TAI pregnancy rates were higher in cows that responded to d 9 GnRH analogue (58.2%; 46/79) compared to the lower response of the 5-day CO-Synch + CIDR treatment (34.2%; 26/76). The overall higher TAI pregnancy rates ($P < 0.01$) in the 14-d PG treatment than the 5-day CO-Synch + CIDR treatment across all locations also support this interpretation.

Coincident with the benefits of increased response to d 9 GnRH analogue within the 14 d CIDR treatments in exp. 2 at locations 1 and 2, the measure of CL responsiveness within the 3 treatments at CIDR removal and $PGF_{2\alpha}$ also affected their TAI pregnancy rates. While the presence of high affinity $PGF_{2\alpha}$ receptors are found on the early developing bovine CL (Wiltbank et al., 1995), responsiveness of the early CL (d 0 to 5) appears to be inhibited by blocks within the downstream cascade of events of $PGF_{2\alpha}$ binding to its receptor on the ovary. Inclusion of two 25 mg $PGF_{2\alpha}$ injections at 8 ± 2 h intervals with CIDR removal in the 5-day CO-Synch + CIDR protocol (Bridges et al., 2008) ensures luteal regression of a d 5 CL; a single 50 mg dose of $PGF_{2\alpha}$ has also been investigated with varying efficacy (Bridges et al., 2011; Bridges et al., 2008 and Kasimanickam et al., 2009). Within the 14-d GnRH treatment, a single 50 mg dose of $PGF_{2\alpha}$ was as efficacious as two 25 mg injections at 6 h intervals (Giles et al., 2011) and a single 50 mg dose of $PGF_{2\alpha}$ apparently was also efficacious for luteolysis in the 14-d

PG treatment. Follicles forced to ovulate on d 9 in the 14-d PG treatment may have had additional LH receptors to enhance the luteinization rate and achieve $PGF_{2\alpha}$ responsiveness at CIDR removal. While these variables of responsiveness were not measured in exp. 2, the increased CL presence on the d of TAI in the 5-day CO-Synch + CIDR treatment (19.7%; 15/76) compared to the 14-d PG treatment (3.8%; 3/79) is consistent with increased CL responsiveness to $PGF_{2\alpha}$ of cows in the 14-d PG treatment. The 14-d GnRH treatment also had a higher percent presence of a CL (12.5%; 9/72) than the 14-d PG treatment. While mean follicle size and percentage of cows with presence of follicle ≥ 12 mm on d 17 did not differ between the 14-d GnRH (16.0 mm, 86.1%), 14-d PG (16.3 mm, 83.5%), and 5-day CO-Synch + CIDR (15.2 mm, 88.2%) treatments, the sustained CL presence could offset timing of ovulation and decrease successful conception to TAI, and may be a partial explanation for treatment differences in TAI pregnancy rates in exp. 2.

Estrous response results in exp. 2 were similar to those seen in exp. 1 with a greater percentage of cows with a patch score 1, indicative of lack of exhibiting estrus, in the 5-day CO-Synch + CIDR (29.6%) treatment than the 14-d GnRH (20.5%) and 14-d PG (19.6%) treatments. While the higher percentages of patch scores 2, 3, and 4 were associated with increased TAI pregnancy rates in the 14-d PG treatment, these higher percentages did not reflect increased TAI pregnancy rates in the 14-d GnRH treatment when compared to the 5-day CO-Synch + CIDR treatment. However, within exp. 2, there were no differences in percentages of patch score 3 among any of the treatments which could explain the lack of increased TAI pregnancy rates in the 14-d GnRH treatment compared to the 5-day CO-Synch + CIDR treatment.

In exp. 2, the two 14 d CIDR treatments at locations 1 and 2 had no added benefit in initiating cyclicity in previously anestrus cows when compared to the shorter 5-day CO-Synch +

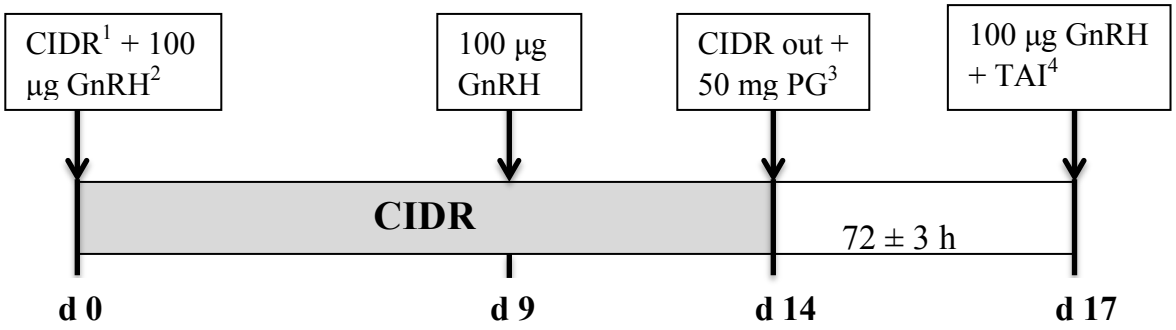
CIDR treatment. Pregnancy rate to TAI in anestrus cows were numerically, but not statistically significantly higher in the 14-d PG treatment (61.9%) than both the 14-GnRH (45.8%) and 5-day CO-Synch + CIDR (45.0%) treatments. Why the same results in anestrus cows were not seen in the 14-d GnRH treatment as the 14-d PG treatment might be due to the lack of accessory CL formed in the 14-d PG treatment compared to the 14-d GnRH treatment. Administration of exogenous GnRH has had added benefits in anestrus beef cows to induce ovulation and luteal function (Dejarnette et al., 2001 and Stevenson et al., 2000). Although LH levels were not measured, the hypothetically increased LH pulse frequencies without accessory CL formation while under P4 influence in the 14-d PG treatment could have stimulated follicular growth and hasten resumption of cyclicity more so than exogenous GnRH. This would agree with previous experiments, which determined that an LH analogue could reduce the anestrus interval in suckled beef cows (Riley et al., 1981 and Roberge et al., 1992). The increased follicular activity prior to GnRH administration in the 14-d PG treatment could have induced a higher response to the GnRH analogue administered on d 9.

IMPLICATIONS

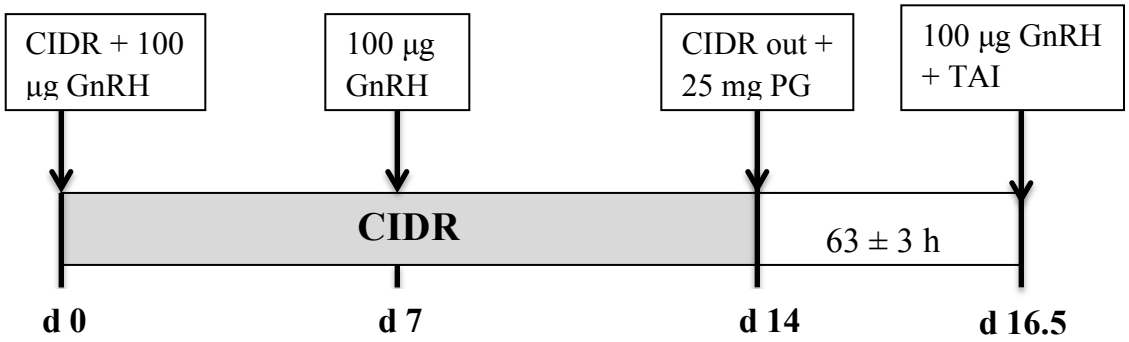
Determining the value of incorporating estrus synchronization protocols for TAI into a beef operation requires analysis of labor requirements and other costs. When considering adoption, the benefits of the 14-d PG treatment include increasing TAI pregnancy rates by 17 percentage points (32%) and are substantial when compared to short-term Beef Reproduction Task Force recommended 5-day CO-Synch + CIDR protocol; both protocols require processing cows 4 times. Coinciding with the improved d 9 response to GnRH, the 14-d PG treatment also had the

best response to $PGF_{2\alpha}$ upon CIDR removal, resulting in an overall superior estrus synchronization protocol for maximizing TAI pregnancy rates in lactating beef cows.

Treatment 1: 14-d GnRH-9



Treatment 2: 14-d GnRH-7



Treatment 3: 7-day CO-Synch + CIDR

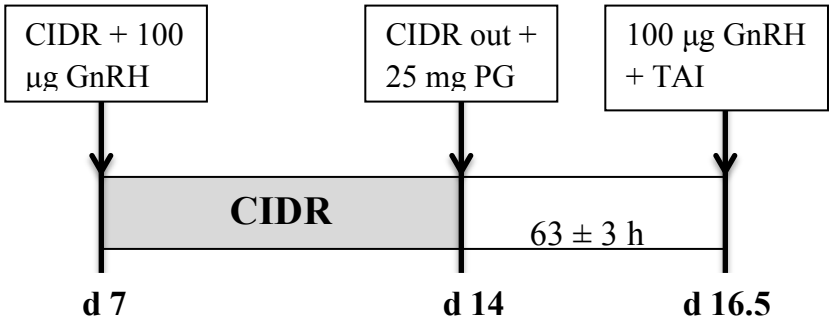
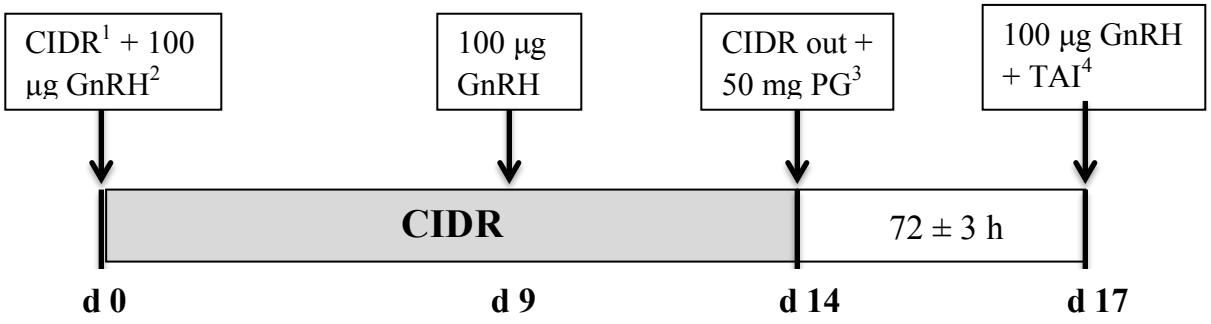
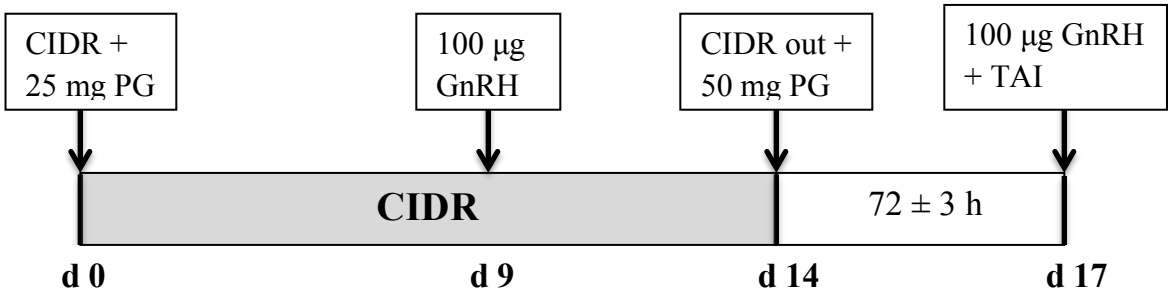


Figure 3.1. Estrus synchronization treatments administered to lactating beef cows in exp. 1
[1]CIDR: controlled internal drug release device inserted intra-vaginally (CIDR; 1.38 g progesterone, EAZI-BREED CIDR®, Pfizer Animal Health).
[2]GnRH: GnRH analogue administered im (Factrel, Fort Dodge Animal Health).
[3]PG: prostaglandin $F_{2\alpha}$ administered im (Lutalyse, Pfizer Animal Health).
[4]TAI: Timed-AI.

Treatment 1: 14-d GnRH



Treatment 2: 14-d PG



Treatment 3: 5-day CO-Synch + CIDR

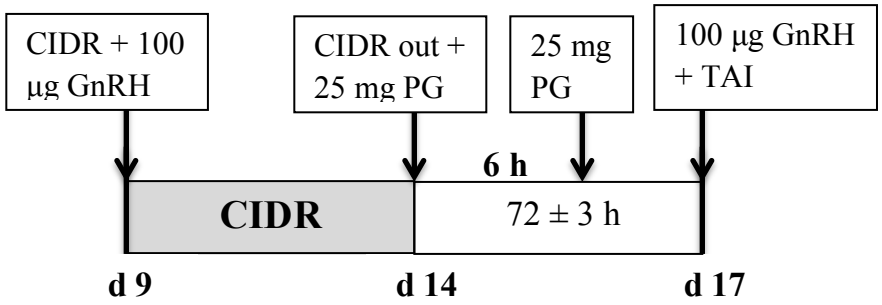


Figure 3.2. Estrus synchronization treatments administered to lactating beef cows in exp. 2
[1]CIDR: controlled internal drug release device inserted intra-vaginally (CIDR; 1.38 g progesterone, EAZI-BREED CIDR®, Pfizer Animal Health).
[2]GnRH: GnRH analogue administered im (Factrel, Fort Dodge Animal Health).
[3]PG: prostaglandin $F_{2\alpha}$ administered im (Lutalyse, Pfizer Animal Health).
[4]TAI: Timed-AI.

Treatment

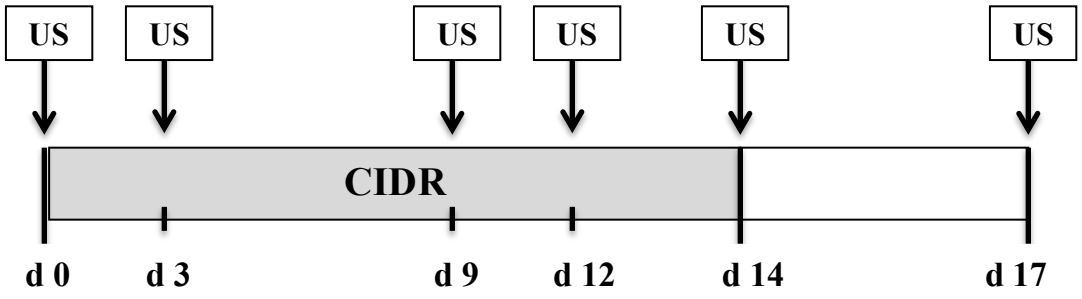


Figure 3.3. Ultrasonography relative to treatments performed in a random subset of cows from location 1 and all cows at location 2 to determine responses to treatments in exp. 2
US = Ultrasonography.

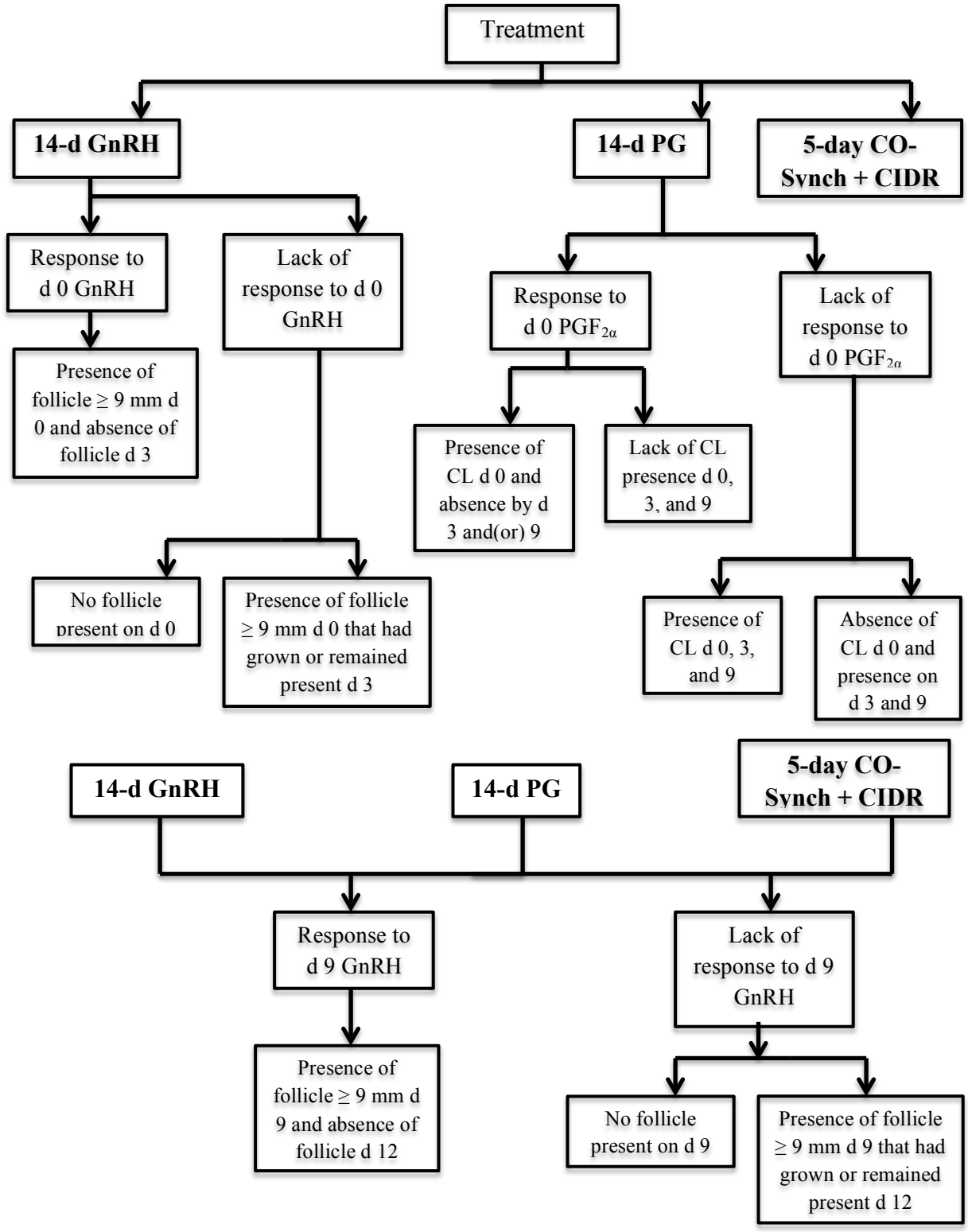


Figure 3.4. Determining response to treatments administered from ultrasonography performed in a random subset of cows at location 1 and all cows at location 2 in exp. 2

Table 3.1. Number, post partum interval (PPI, d from calving to timed-AI on d 17), BCS, and parity of lactating beef cows for 3 treatments at 2 locations in exp. 1 (LS mean ± SE)

Location and treatment[1]	n =	PPI (d)	BCS	% Parity Primiparous	% Multiparous
Location 1					
14-d GnRH-9[3]	71	105 ± 1.21	4.4 ± 0.07	0	100
14-d GnRH-7[4]	65	108 ± 1.04	4.6 ± 0.09	0	100
7-day CO-Synch + CIDR[5]	66	105 ± 1.14	4.5 ± 0.10	0	100
Total for location 1	202	106 ± 0.67[a]	4.5 ± 0.05	0	100
Location 2					
14-d GnRH-9	131	76 ± 1.72	4.5 ± 0.06	17.6	82.4
14-d GnRH-7	139	75 ± 1.58	4.5 ± 0.07	15.8	84.2
7-day CO-Synch + CIDR	116	77 ± 1.83	4.6 ± 0.07	17.2	82.8
Total for Location 2	386	76 ± 0.98[b]	4.5 ± 0.04	16.8	83.2
Combined across locations[5]					
14-d GnRH-9	202	86 ± 1.54	4.5 ± 0.05	11.4	88.6
14-d GnRH-7	204	86 ± 1.57	4.5 ± 0.05	10.8	89.2
7-day CO-Synch + CIDR	182	87 ± 1.58	4.6 ± 0.06	11.0	89.0
Total for all treatments at all locations	588	87 ± 0.90	4.5 ± 0.05	11.1	88.9

[ab]Within columns across locations, means without common superscripts differ ($P < 0.05$).
[1]PPI, BCS, and parity did not differ between treatments within location ($P > 0.10$).
[2]14-d GnRH-9 = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]14-d GnRH-7 = 14 d CIDR with 100 μg GnRH analogue im d 0 and 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[4]7-day CO-Synch + CIDR = 7 d CIDR with 100 μg GnRH analogue im d 7 and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[5]There was no treatment × location effect ($P = 0.69$).
Cows in all treatments received 100 μg GnRH analogue im with timed-AI at either 72 ± 3 h (14-d GnRH-9 treatment) or 63 ± 3 h (14-d GnRH-7 and 7-day CO-Synch + CIDR treatments) after CIDR removal.

Table 3.2. LS means (± SE) for timed-AI (TAI) pregnancy rates of lactating beef cows by treatment at both locations in exp. 1

Location and treatment	n =	TAI pregnancy rate (%)
Location 1		
14-d GnRH-9[1]	71	62.0 ± 5.8
14-d GnRH-7[2]	65	58.5 ± 6.1
7-day CO-Synch + CIDR[3]	66	57.6 ± 6.1
Location 2		
14-d GnRH-9	131	47.3 ± 4.4
14-d GnRH-7	139	50.4 ± 4.2
7-day CO-Synch + CIDR	116	46.6 ± 4.6
Combined across all locations[4]		
14-d GnRH-9	202	54.8 ± 3.5
14-d GnRH-7	204	54.4 ± 3.3
7-day CO-Synch + CIDR	182	52.3 ± 3.1

Means do not differ ($P > 0.10$).

[1]14-d GnRH-9 = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[2]14-d GnRH-7 = 14 d CIDR with 100 μg GnRH analogue im d 0 and 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[3]7-day CO-Synch + CIDR = 7 d CIDR with 100 μg GnRH analogue im d 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[4]There was no treatment × location interaction ($P = 0.69$); therefore means were combined across locations.

Cows in all treatments received 100 μg GnRH analogue im with TAI at either 72 ± 3 h (14-d GnRH-9 treatment) or 63 ± 3 h (14-d GnRH-7 and 7-day CO-Synch + CIDR treatments) after CIDR removal.

Table 3.3. Estrous response by treatment using estrus detection patch scores evaluated on d of timed-AI (TAI) in lactating beef cows across both locations in exp. 1

Treatment		Patch Score[1] (% of treatment)			
	n =	1	2	3	4
14-d GnRH-9[2]	202	19.8[a]	8.4[a]	44.1[a]	27.7[ab]
14-d GnRH-7[3]	204	22.1[a]	3.4[b]	42.6[a]	31.9[b]
7-day CO-Synch + CIDR[4]	182	31.3[b]	18.7[c]	29.7[b]	20.3[a]

[ab]Within columns, means without common superscripts differ ($P < 0.05$).
[1]Patch score = Amount of film removed from estrus detection patch on d of TAI (1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch).
[2]14-d GnRH-9 = 14 d CIDR with 100 µg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]14-d GnRH-7 = 14 d CIDR with 100 µg GnRH analogue im d 0 and 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[4]7-day CO-Synch + CIDR = 7 d CIDR with 100 µg GnRH analogue im d 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
Cows in all treatments received 100 µg GnRH analogue im with TAI at either 72 ± 3 h (14-d GnRH-9 treatment) or 63 ± 3 h (14-d GnRH-7 and 7-day CO-Synch + CIDR treatments) after CIDR removal.

Table 3.4. LS means (± SE) for timed-AI (TAI) pregnancy rates by estrous response and treatment using estrus detection patches evaluated on d of TAI in lactating beef cows across locations in exp. 1

Patch Score[1]	TAI pregnancy rate (%)		
	14-d GnRH-9[2]	14-d GnRH-7[3]	7-day CO-Synch + CIDR[4]
1	37.5 ± 7.7 (15/40)	42.2 ± 7.4 (19/45)	33.3 ± 6.2 (19/57)
2	29.4 ± 11.1 (5/17)	28.6 ± 17.1 (2/7)	41.2 ± 8.6 (14/34)
3	62.9 ± 5.1 (56/89)	60.9 ± 5.3 (53/87)	74.1 ± 6.0 (40/54)
4	53.6 ± 6.7 (30/56)	52.3 ± 6.2 (34/65)	51.4 ± 8.3 (19/37)

Means within score do not differ ($P > 0.10$).

[1]Patch Score: Based on amount of film removed from Estrotect patch on day of TAI with 1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch.

[2]14-d GnRH-9: 14 d CIDR with 100 μg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[3]14-d GnRH-7: 14 d CIDR with 100 μg GnRH analogue im d 0 and 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[4]7-day CO-Synch + CIDR: 7 d CIDR with 100 μg GnRH analogue im d 7, and 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

Cows in all treatments received 100 μg GnRH analogue im with TAI at either 72 ± 3 h (14-d GnRH-9 treatment) or 63 ± 3 h (14-d GnRH-7 and 7-day CO-Synch + CIDR treatments) after CIDR removal.

Table 3.5. Number, post partum interval (PPI, d from calving to TAI on d 17), BCS, and parity of lactating beef cows for 3 treatments at 4 locations in exp. 2 (LS mean ± SE)

Location and treatment[1]	n =	PPI (d)	BCS	% Parity Primiparous	% Multiparous
Location 1					
14-d GnRH[2]	85	80 ± 1.78	4.4 ± 0.05	27.1	72.9
14-d PG[3]	91	80 ± 1.79	4.4 ± 0.05	26.4	73.6
5-day CO-Synch + CIDR[4]	88	83 ± 1.62	4.5 ± 0.06	26.1	73.9
Total for location 1	264	81 ± 1.00[a]	4.4 ± 0.03[a]	26.5[a]	73.5[a]
Location 2					
14-d GnRH	30	68 ± 2.56	4.5 ± 0.11	20.0	80.0
14-d PG	36	72 ± 2.14	4.6 ± 0.12	15.2	84.8
5-day CO-Synch + CIDR	33	72 ± 2.74	4.7 ± 0.14	16.1	83.9
Total for location 2	99	71 ± 1.43[b]	4.6 ± 0.07[a]	17.0[b]	83.0[b]
Location 3					
14-d GnRH	47	59 ± 2.04	5.3 ± 0.09	8.5	91.5
14-d PG	47	61 ± 2.34	5.5 ± 0.08	19.1	80.9
5-day CO-Synch + CIDR	45	63 ± 2.49	5.3 ± 0.09	26.7	73.3
Total for location 3	139	61 ± 1.32[c]	5.4 ± 0.05[b]	18.0[b]	82.0[b]
Location 4					
14-d GnRH	43	87 ± 0.55	5.6 ± 0.09	0	100
14-d PG	43	86 ± 0.68	5.6 ± 0.08	0	100
5-day CO-Synch + CIDR	42	87 ± 0.69	5.6 ± 0.09	0	100
Total for location 4	128	87 ± 0.37[d]	5.6 ± 0.05[b]	0[c]	100[c]
Combined across locations					
14-d GnRH	205	75 ± 1.21	4.8 ± 0.05	16.1	83.9
14-d PG	217	76 ± 1.16	4.9 ± 0.05	17.8	82.2
5-day CO-Synch + CIDR	208	78 ± 1.16	4.9 ± 0.05	19.4	80.6
Total for all treatments at all location	630	76 ± 0.68	4.9 ± 0.03	17.8	82.2

[a-d]Within columns, means without common superscripts differ ($P < 0.05$).

[1]PPI, BCS, and parity did not differ between treatments within location ($P > 0.10$).

[2]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[3]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[4]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

Cows in all treatments received 100 μg GnRH analogue im with TAI at 72 ± 3 h after CIDR removal.

Table 3.6. LS means (± SE) for timed-AI (TAI) pregnancy rates of lactating beef cows by treatment at all locations in exp. 2

Location and treatment	n =	TAI pregnancy rate (%)
Location 1		
14-d GnRH[1]	85	56.5 ± 5.5
14-d PG[2]	91	65.6 ± 5.1
5-day CO-Synch + CIDR[3]	88	53.4 ± 5.3
Location 2		
14-d GnRH	30	51.7 ± 9.3
14-d PG	36	61.8 ± 8.3
5-day CO-Synch + CIDR	33	61.3 ± 8.4
Location 3		
14-d GnRH	47	55.3 ± 7.3[a]
14-d PG	47	76.6 ± 6.4[b]
5-day CO-Synch + CIDR	45	46.7 ± 7.5[a]
Location 4		
14-d GnRH	43	55.8 ± 7.6[a]
14-d PG	43	75.8 ± 6.8[b]
5-day CO-Synch + CIDR	42	52.4 ± 7.9[a]
Combined across all locations[4]		
14-d GnRH	205	54.4 ± 4.0[a]
14-d PG	217	70.4 ± 3.6[b]
5-day CO-Synch + CIDR	208	53.5 ± 3.9[a]

[ab]Within location and combined across all locations, means without common superscripts differ ($P < 0.05$).

[1]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[2]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[3]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

[4]There was no treatment × location effect ($P = 0.53$); therefore means were combined across locations.

Cows in all treatments received 100 μg GnRH analogue im with TAI at 72 ± 3 h after CIDR removal.

Table 3.7. Estrous response by treatment using estrus detection patches evaluated on d of timed-AI (TAI) in lactating beef cows across all locations in exp. 2

Treatment		Patch Score[1] (% of treatment)			
	n =	1	2	3	4
14-d GnRH[2]	205	20.5[a]	16.6	29.3	33.6[b]
14-d PG[3]	214	19.6[a]	17.3	35.1	28.0[ab]
5-day CO-Synch + CIDR[4]	206	29.6[b]	17.5	31.5	21.4[a]

[ab]Within columns, means without common superscripts differ ($P < 0.05$).
[1]Patch Score = Based on amount of film removed from estrus detection patch on d of TAI (1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch).
[2]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[4]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.
Cows in all treatments received 100 μg GnRH analogue im with TAI at 72 ± 3 h after CIDR removal.

Table 3.8. LS means (± SE) for timed-AI (TAI) pregnancy rates by estrus detection patch score and treatment using estrus detection patches evaluated on d of TAI in lactating beef cows across all locations in exp. 2

Patch Score[1]	TAI pregnancy rate (%)		
	14-d GnRH[2]	14-d PG[3]	5-day CO-Synch + CIDR[4]
1	31.0[a] (13/42)	59.5[b] (25/42)	37.7[a] (23/61)
2	44.1[a] (15/34)	70.3[b] (26/37)	52.8[ab] (19/36)
3	63.3[a] (38/60)	80.0[b] (60/75)	64.6[a] (42/65)
4	68.1 (47/69)	60.0 (36/60)	56.8 (25/44)

[ab]Within rows, means without common superscripts differ ($P < 0.05$).

[1]Patch score = Based on amount of film removed from estrotect patch on d of TAI (1 = completely untouched, 2 = approximately 50% rubbed off, 3 = almost all or 100% rubbed off, and 4 = missing patch).

[2]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[3]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9, and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[4]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal, and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h later.

Cows in all treatments received 100 μg GnRH analogue im with TAI at 72 ± 3 h after CIDR removal.

Table 3.9. Responses to treatment associated with corpora lutea dynamics based on changes to ovarian structures based on ultrasonography at locations 1 and 2 combined for cows in exp. 2

		Treatment		
		14-d GnRH[1]	14-d PG[2]	5-day CO-Synch + CIDR[3]
Responses (± SE)	n =	72	79	76
Corpus luteum (**CL**) presence on d 0 (%)		59.7 ± 5.8 (43/72)	65.8 ± 5.4 (52/79)	73.7 ± 5.2 (56/76)
CL lysed due to d 0 $PGF_{2\alpha}$ (%)[4]		-	75.0 ± 6.1 (39/52)	-
Absence of CL on d 9 (%)		47.2 ± 5.9[a] (34/72)	63.3 ± 5.5[b] (50/79)	31.6 ± 5.4[c] (24/76)
Timed-AI (TAI) pregnancy rate for cows with no of CL on d 9		38.2 ± 13.7[a] (13/34)	70.0 ± 6.5[b] (35/50)	50.0 ± 10.4[b] (12/24)
TAI pregnancy rate for cows with a CL on d 9		65.8 ± 7.8 (25/38)	62.1 ± 9.2 (18/29)	63.5 ± 6.7 (33/52)
CL presence by d 14 (%)		88.9 ± 4.3 (64/72)	86.1 ± 3.9 (68/79)	86.8 ± 3.9 (66/76)
CL presence d 17 (%)		12.5 ± 3.9[a] (9/72)	3.8 ± 2.2[b] (3/79)	19.7 ± 4.6[a] (15/76)

[ab]Within rows, means without common superscripts differ ($P < 0.05$).
[1]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[2]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h.
[4]Percentage of cows in 14-d PG treatment with presence of a CL d 0 that lysed CL by d 3.
Cows in all treatments received 100 μg GnRH analogue im with timed-AI at 72 ± 3 h after CIDR removal.

Table 3.10. Responses to treatment associated with response to GnRH analogue injections based on changes to ovarian structures based on ultrasonography at locations 1 and 2 combined for cows in exp. 2

		Treatment		
		14-d GnRH[1]	14-d PG[2]	5-day CO-Synch + CIDR[3]
Responses (± SE)	n =	72	79	76
Response to d 0 GnRH (%)[5]		54.2 ± 5.9 (39/72)	-	-
Response to d 9 GnRH (%)[5]		76.4 ± 5.0[a] (55/72)	83.5 ± 4.2[a] (66/79)	57.9 ± 5.7[b] (44/76)
Response to both d 0 and 9 GnRH (%)[6]		74.4 ± 7.1 (29/39)	-	-
TAI pregnancy rate for cows responding to d 9 GnRH		56.4 ± 6.7 (31/55)	69.7 ± 5.8 (46/66)	56.8 ± 7.5 (25/44)
TAI pregnancy rate for cows not responding to d 9 GnRH		41.2 ± 12.3 (7/17)	61.5 ± 14.0 (8/13)	62.5 ± 8.7 (20/32)
Presence of follicle ≥ 10 mm d 9 (%)		80.6 ± 4.7[ab] (58/72)	89.9 ± 3.4[a] (71/79)	68.4 ± 5.4[b] (52/76)
Follicle size on d 9 (mm)		13.5 ± 0.43[a]	14.7 ± 0.47[a]	11.9 ± 0.34[b]
Follicle size on d 17 (mm)		16.0 ± 0.21	16.3 ± 0.39	15.2 ± 0.38
Presence of follicle ≥ 12 mm on d 17 (%)		86.1 ± 4.1 (62/72)	83.5 ± 4.2 (66/79)	88.2 ± 3.7 (67/76)
TAI pregnancy rate of cows with follicle ≥ 12 mm on d 17 (%)		53.2 ± 6.4[a] (33/62)	72.7 ± 5.5[b] (48/66)	64.2 ± 5.9[ab] (43/67)
TAI pregnancy rate of cows without follicle ≥ 12 mm on d 17 (%)		50.0 ± 16.7 (5/10)	38.5 ± 14.0 (5/13)	33.3 ± 16.7 (3/9)

[ab]Within rows, means without common superscripts differ ($P < 0.05$).
[1]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[2]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h.
[5]A successful response to GnRH given on d 0 or 9 was defined as presence of a follicle ≥ 9 mm on either d 0 or 9 and absence of that follicle 3 d later.
[6]A successful response to GnRH both on d 0 and 9 only included those cows eligible to synchronize 2 follicular waves (i.e. responded to d 0 GnRH).
Cows in all treatments received 100 μg GnRH analogue im with timed-AI at 72 ± 3 h after CIDR removal.

Table 3.11. LS means (± SE) for timed-AI (TAI) pregnancy rates of lactating beef cows by cycling status and treatment by location and combined across locations in exp. 2

Location and treatment	Cycling cows[1]		Non-cycling Cows[2]	
	n =	TAI pregnancy rate (%)	n =	TAI pregnancy rate (%)
Location 1				
14-d GnRH[3]	31	61.3 ± 8.7	11	36.6 ± 14.5
14-d PG[4]	32	74.2 ± 7.9	11	63.6 ± 14.5
5-day CO-Synch + CIDR[5]	31	67.7 ± 8.4	11	36.6 ± 14.5
Location 2				
14-d GnRH	17	52.9 ± 12	12	53.8 ± 13.8
14-d PG	26	65.4 ± 9.3	10	60.0 ± 15.5
5-day CO-Synch + CIDR	25	64.0 ± 9.6	9	55.6 ± 16.6
Combined across locations[6]				
14-d GnRH	48	56.3 ± 7.2	24	45.8 ± 10.2
14-d PG	58	69.0 ± 6.1	21	61.9 ± 10.6
5-day CO-Synch + CIDR	56	66.1 ± 6.3	20	45.0 ± 11.1

No differences in TAI pregnancy rates by cycling status among treatments ($P > 0.10$).

[1] Cycling cows = observed in visual estrus from d -22 to 0 or presence of corpus luteum on ovary on d 0.

[2] Non-cycling cows = not observed in visual estrus from d -22 to 0 and lack of corpus luteum on ovary d 0.

[3]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[4]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.

[5]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h.

[6]No treatment × location interaction ($P > 0.10$).

Cows in all treatment groups received a 100 μg GnRH analogue im and TAI 72 ± 3 h after CIDR removal.

Table 3.12. At onset of treatments, LS means for timed-AI (TAI) pregnancy rates of lactating beef cows by d of cycle combined from a subset of cows at location 1 and 2 combined for cows in exp. 2 (Mean ± SE)

Treatment	TAI pregnancy rate (%) by d of estrous cycle				
D of estrous cycle group[1]	0-6	7-12	13-17	18-21	Combined
14-d GnRH[2]	80.0 (4/5)	75.0 (3/4)	40.0 (2/5)	37.5 (3/8)	46.2 (12/26)
14-d PG[3]	40.0 (2/5)	100.0 (8/8)	33.3 (2/6)	80.0 (8/10)	69.0 (20/29)
5-day CO-Synch + CIDR[4]	66.7 (8/12)	50.0 (4/8)	50.0 4/4)	100.0 (1/1)	68.0 (17/25)

No differences in TAI pregnancy rates by d of cycle between treatments ($P > 0.10$).
[1]D of estrous cycle group: Cows grouped by d of estrous cycle range among 3 treatments from visual observation of standing estrus from d -22 to 0.
[2]14-d GnRH = 14 d CIDR with 100 μg GnRH analogue im d 0 and 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[3]14-d PG = 14 d CIDR with 25 mg $PGF_{2\alpha}$ im d 0, 100 μg GnRH analogue im d 9 and 50 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal.
[4]5-day CO-Synch + CIDR = 5 d CIDR with 100 μg GnRH analogue im d 9, 25 mg $PGF_{2\alpha}$ im on d 14 with CIDR removal and another 25 mg $PGF_{2\alpha}$ im 6 ± 1 h.
Cows in all treatment groups received a 100 μg GnRH analogue im and TAI 72 ± 3 h after CIDR removal.

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CHAPTER IV

Assessing reticulo-rumen temperature change and ovulation in lactating dairy cows[1]

SUMMARY: Variability in the interval from prostaglandin $F_{2\alpha}$ injection to ovulation in dairy cows results in decreased conception rates from timed-AI (**TAI**). Previous research indicated a temperature rise related to ovulation that might be used as a reproductive management tool for proper timing of AI. The objective of this experiment was to compare changes in reticulo-rumen temperature as a predictor or indicator of ovulation in lactating dairy cows. Primiparous and multiparous lactating dairy cows (n = 494) ≥ 46 days in milk at one location were enrolled in an estrus presynchronization protocol 36 d prior to TAI (d -36); which included 500 μg cloprostenol i.m. on d -36 and a second 500 μg cloprostenol i.m. 14 d later. On d -10, cows were enrolled in an ovulation synchronization protocol in which they received 100 μg GnRH i.m. (d -10), 500 μg cloprostenol i.m. (d -3), and 100 μg GnRH i.m. 48 h later. All cows received TAI 16 to 19 h after the second GnRH injection. Blood was collected throughout the synchronization period to determine cycling status, response to synchronization treatments, and ovulation during the TAI period. Reticulo-rumen temperature (**Trr**) was recorded by temperature recording reticulo-rumen boluses administered to each cow via balling gun within 24 h of calving. Each Trr reading was recorded every time animals entered the milking parlor to establish a 7 d baseline. The single maximum Trr rise (°C) from baseline on d of TAI (**T_{rr}MAX**) and the average of all (1 to 3) Trr

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readings on d of TAI (**T_{rr}AVG**) were the two readings used for analysis of Trr change related to ovulation. Mean (± SE) T_{rr}MAX rise from the baseline tended to be higher ($P = 0.06$) in ovulatory (n = 446; 0.180 ± 0.02°C) than anovulatory (n = 48; 0.094 ± 0.04°C) cows. Mean (± SE) T_{rr}AVG was higher ($P < 0.01$) in ovulatory (0.064 ± 0.01°C) than anovulatory (-0.047 ± 0.04°C). The early use of changes in Trr in ovulatory cows was validated to pinpoint animals that do in fact ovulate in an estrus synchronization protocol. These are some of the earliest data to identify Trr change from a baseline in relation to ovulation.

Key Words: Dairy cows, Estrus synchronization, Reticulo-rumen temperature, Timed-AI

INTRODUCTION

Estrus synchronization is a widely accepted tool for manipulation of the bovine estrous cycle, especially in the dairy industry where genetic selection for high milk production has been associated with decreased fertility (Beam and Butler, 1999; Lucy et al., 2001). Proper detection of estrus for timely and effective artificial insemination (**AI**) is still a key factor for successful conception in any AI protocol. However, the confinement involved and hard surfaces adopted by the majority of dairy operations have limited exhibition of outward signs of estrus for reliable detection (Britt et al., 1986; Vailes et al., 1990). This led to the implementation of estrus synchronization protocols to control the estrous cycle of many animals, but there is still variability in the actual induction of estrus and ovulation (King et al., 1982; Stevenson et al., 1984). This issue has been partially alleviated by the inclusion of GnRH 16 to 18 h prior to TAI for synchronization of ovulation as seen in the Ovsynch protocol (Pursley et al., 1995), but there is still variability in timing of ovulation within this protocol.

Detection of ovulation based on rises in core temperature of animals has been studied as a potential tool for predicting the variation in timing of ovulation and increasing success rate of AI systems in beef herds (Cooper-Prado et al., 2010; Piccione et al., 2003). However, there have been very few studies to evaluate this aspect of timing of ovulation on a large scale. The ability to detect estrus and ovulation based on rises in reticulo-rumen temperature (**Trr**) without the need for visual confirmation could be a beneficial reproductive management tool in the dairy industry.

The first objective of this study was to evaluate if Trr rises from a predetermined 7 d baseline are related to ovulation of a follicle. A rise in Trr could be detected using a reticulo-rumen bolus. A second objective was to test the quality of temperature monitoring data received from the reticulo-rumen bolus, and its efficacy as a reproductive management tool.

MATERIALS AND METHODS

Animals and Collection of Data

All experimental procedures with cows were approved by the Colorado State University Animal Care and Use Committee prior to initiation of the experiment (protocol #10-2176A). Primiparous and multiparous lactating Holstein dairy cows (n = 608) in northern Colorado (Lasalle, CO) were housed in free-stall pens between October 2010 and March 2011, and milked three times daily at approximately 8 h intervals. Within 24 h post calving, cows were administered a temperature recording reticular-rumen bolus (Phase IV Engineering, Boulder, CO) via balling gun. Upon milking, cows entering the parlor passed through dual fixed panel readers located along the entrance rails. Once cows passed by the panel readers, Trr readings were recorded and downloaded into the TempTrack® computer software database (DVM

Systems, Greeley, CO).

Estrus Synchronization Method

Cows were assigned by week of calving date into estrus synchronization groups. If animals were ≥ 46 d in milk (**DIM**, d post partum) on the designated d for initiation of presynchronization, they were enrolled into an estrus presynchronization (**presynch**; Moreira et al., 2001) protocol on d -36 from the d of TAI. This protocol began on d -36 when animals received 500 μg cloprostenol i.m. (**PGF**, Estrumate®; Intervet/Schering Plough Animal Health, Kirkland, Quebec) and a second 500 ug PGF i.m. 14 d later. On d -10, cows were started on a synchronization of ovulation protocol (**Ovsynch**, Pursley et al., 1995) by receiving 100 μg GnRH i.m. (Cystorelin; Merial Co., Athens, GA) followed by 500 μg PGF i.m. on d -3, 100 μg GnRH i.m. 48 h after PGF injection, and TAI 16 to 19 h later (Figure 4.1). The above estrus synchronization protocol and AI procedures were conducted by employees at the dairy. Reproductive tract abnormalities such as pyometra, metritis, or tract adhesions were diagnosed on d -10, and data from these cows were removed from the study and excluded from any further analyses. On d -10, ovarian structures were also diagnosed via transrectal ultrasonography (3.5 MHz linear transducer GP-DV, E.I. Medical, Loveland, CO). Throughout the experiment, a single qualified reproductive ultrasonography technician determined both reproductive tract abnormalities and ovarian structures. Cows were culled from the dairy at certain times throughout the study prior to pregnancy diagnosis for various health reasons or death. These animals were also removed from the entire analyses.

Blood Collection

Reproductive cycling status and responses to hormone treatments were determined through blood collections for serum progesterone concentration at various times throughout the

synchronization period, as noted in Figure 1. Blood was collected by coccygeal venipuncture on d -22, -15, and -10 to confirm reproductive cycling status prior to Ovsynch protocol. Blood was also collected on d -3, 0, and 7 to determine response to hormone treatments and ovulation (Figure 1). Blood was collected in 10 mL serum vacutainer tubes (BD Vacutainer^{TM}, Becton, Dickinson and Company, Franklin Lakes, NJ) and placed directly on ice within 10 min after collection. Samples were centrifuged at 2800 x *g* for 10 min within 5 h after collection, and stored at -20°C. Total progesterone concentration was determined in each sample using RIA (Progesterone Coat-A-Count Kit, Siemens Healthcare Diagnostics, Deerfield, IL). Sixteen progesterone RIAs were completed with an inter-assay CV of 10.3%. Sensitivity was calculated at 0.l ng/mL. Inability to locate animals on certain days of blood collection, hormone treatments, and TAI resulted in removal of cows from the analyses.

Classification of Synchronization Response

Cows with serum progesterone concentrations > 1 ng/mL on any (or all) of the bleeding dates on d -22, -15, -10, or presence of a corpus luteum (**CL**) from ultrasonography diagnoses on d -10, were classified as cycling at the initiation of the Ovsynch protocol. Cows with serum progesterone concentrations < 1 ng/mL on all 3 bleeding dates (d -22, -15, and -10), and absence of any CL structure on the ovary (d -10), were classified as anestrus at the initiation of the Ovsynch protocol. Blood was also collected on d -3, 0, and 7 to evaluate responses to hormone treatments and ovulation. Cows with serum progesterone concentrations < 1 ng/mL on d 0 (d of TAI) and > 1 ng/mL on d 7 (7 d post-TAI) were classified as having ovulated to the synchronization protocol. Cows with serum progesterone concentrations > 1 ng/mL on d 0 and > 1 ng/mL on d 7, < 1 ng/mL on d 0 and < 1 ng/mL on d 7, or > 1 ng/mL on d 0 and < 1 ng/mL on d 7 were classified as anovulatory to the Ovsynch protocol.

Temperature Recording Statistical Analysis

For full analyses, all weeks were grouped together. The Trr baseline used was a 7 d baseline. The baseline created for each d was generated from the mean Trr readings from the previous d and built upon each previous d during the 7 d period. Throughout the study, the consistency of Trr readings varied due to panel reader malfunctions and inconsistent milking schedules. This led to inability to obtain 3 consistent Trr readings each day from some cows at certain points during the estrus synchronization period. So, baselines were determined with some missing values as described further on. These baselines were used to determine Trr rises associated with ovulation and determined Trr rises from the baseline on d of TAI. For the purposes of this segment of the experiment, diurnal variation in Trr of each cow was ignored in the model.

Cows have been described as having a rhythmicity or diurnal variation in body temperature throughout the day (Piccione et al., 2003). Thus, adjusting values for diurnal variation in calculating the 7 d baseline could aid in extracting the 0.6°C rise associated with ovulation found in previous research (Cooper-Prado et al., 2010). Generation of this 7 d baseline accounting for diurnal variation used the same Trr readings when ignoring diurnal variation as previously described and was done using the regression procedure in SAS (SAS Inst., Inc., Cary, NC). This procedure created a harmonic regression model, which included the sine and cosine of h of the day. The equation used to fit the model was $y = \beta^0 + \beta^1 * st + \beta * ct$. This fit one cycle/d of Trr readings. The major limitation in creating the 7 d baseline for each cow when diurnal variation was accounted for prior to expected ovulation was the fact that a maximum of only 21 potential Trr recordings (3 milkings/d) were possible to determine this variation of Trr of each cow throughout the day. Along with this limitation, failure of Trr readings to be recorded from

the TempTrack® panel readers resulted in missing data throughout the experiment. This problem occurred due to mechanical failures in the ability of the panel readers to record Trr readings. Because of this, very few animals (n = 88) had complete Trr readings during the period that the 7 d baseline data were formulated, and most animals' averaged only 15 Trr readings during this period. Therefore, the harmonic regression model in SAS fit predicted diurnal variation that had to be generated to account for Trr changes in between Trr recordings when animals were milked. A second limitation within the data set was the consistency in which cows were milked during the day to record Trr. The inability to milk cows 3 times/d at near the same 8 h interval throughout the day made determining the 7 d baseline when accounting for diurnal variation difficult.

Two possible criteria used to determine proper removal of Trr readings associated with water consumption or Trr reading malfunction from the panel readers. These included Trr readings below 37.5°C, which was deemed the threshold drop of normal biological temperatures from previous research (J. A. Small protocol). Also, removal of Trr readings that were ≥ 2 standard deviations from the baseline was also attempted. After both approaches were conducted, removal of Trr readings that were < 37.5°C provided a more consistent baseline curve to account for diurnal variation throughout the day.

The temperature data to predict ovulation were analyzed on 2 levels; the first included Trr changes between ovulatory and anovulatory cows when accounting for diurnal variation by d of each cow during the 7 d baseline generation period, and the second, ignoring the diurnal variation by d within each cow during this period. When accounting for diurnal variation, the REG procedure in SAS was used to determine R^2 values based on accuracy of the harmonic regression model generated from Trr readings of each cow during the 7 d baseline generation

period. To determine potential cows that exhibited this diurnal variation throughout the 7 d baseline generation period, two separate analyses were done. The first analysis included cows with R^2 values ≥ 0.4 for fit of data to the harmonic regression model. The second analysis used R^2 values ≥ 0.3. The TTEST procedure in SAS was used to determine differences in Trr changes between ovulatory and anovulatory cows both when diurnal variation was accounted for and ignored in the model.

The main area of interest for changes in Trr was on the d of TAI. The first analysis used was the single maximum rise from the 7 d baseline out of all Trr (**T_{rr}MAX**) readings on the d of TAI. The second analysis used was the average rise from the 7 d baseline of all (1, 2, or 3) Trr readings (**T_{rr}AVG**) on the d of TAI. These were the two readings used for analysis of Trr change as a method for determining differential rises from the baseline between ovulatory and anovulatory cows. All animals included in the 7 d baseline had at least 14 Trr recordings during the 7 d prior to d of TAI and expected ovulation.

RESULTS

Six-hundred-eight cows were originally entered into the study between October 2010 and March 2011. Data from 35 cows were removed due to reproductive tract abnormalities, being culled from the dairy, or death. Also, data from an additional 35 cows were removed from the analyses from lack of Trr readings due to malfunction in bolus reading on the panel reader, or failure to properly administer a bolus. Failure of complete blood sample collection for progesterone concentration resulted in data being removed from 42 cows. After removal of data from the cows described above, data on 494 cows remained with complete data throughout the

estrus synchronization period, including serum progesterone concentrations and Trr readings. However, of these 494 cows, data for TAI pregnancy rate was missing on 37 cows. Four hundred fifty-seven of the cows in the experiment had complete pregnancy diagnosis data. Of these animals, the overall TAI pregnancy rate was 44.4% (203/457). Of the 494 cows with Trr readings and progesterone data, 94.5% (467/494) were deemed cycling at the initiation of the Ovsynch protocol based on serum progesterone concentrations. The TAI pregnancy rate for cycling cows (n = 432), was 59.5% (257/432). The TAI pregnancy rate for non-cycling cows was 28.0% (7/25). Of the 494 cows, 90.3% (446/494) were deemed ovulatory to the estrus synchronization protocol.

Accounting for diurnal variation using the harmonic regression model with an R^2 value ≥ 0.40 resulted in only 55 cows (ovulatory, n = 46; anovulatory, n = 9) that fit the criteria for proper diurnal variation. A limitation to the model was that only 1 to 3 Trr readings/d were possible when fitting the harmonic regression resulting in few cows fitting the criteria. There was no difference ($P > 0.10$) in $T_{rr}MAX$ between ovulatory and anovulatory cows, which averaged (± SE) 0.067 ± 0.05 and 0.002 ± 0.11°C, respectively (Table 4.1). When the harmonic regression model included cows with an R^2 value ≥ 0.30, there were 105 cows (ovulatory, n = 91; anovulatory, n = 14) that fit these criteria for proper diurnal variation. There was no difference ($P > 0.10$) in $T_{rr}MAX$ between ovulatory and anovulatory cows, which averaged 0.239 ± 0.38 and 0.015 ± 0.10°C, respectively. While accounting for diurnal variation with no R^2 value as a cut-off, the $T_{rr}MAX$ was higher ($P < 0.01$) in ovulatory cows (n = 446) than anovulatory cows, which averaged 0.040 ± 0.02 and -0.118 ± 0.06°C, respectively (Table 4.1).

The second analysis, which ignored diurnal variation and did not use the harmonic regression model, evaluated the $T_{rr}MAX$ and $T_{rr}AVG$ between ovulatory and anovulatory cows.

There was a tendency ($P = 0.06$) for a higher mean (± SE) $T_{rr}MAX$ in ovulatory cows (0.180 ± 0.02°C) than anovulatory cows (0.094 ± 0.04°C) on d of TAI. In the same analysis, ovulatory cows had a higher ($P < 0.01$) mean $T_{rr}AVG$ than anovulatory cows on d of TAI and averaged 0.064 ± 0.01 and -0.047 ± 0.04°C (Table 4.2).

DISCUSSION

In the current study, the use of reticulo-rumen boluses and TempTrack® software generated a baseline temperature using the Trr collected up to 3 times daily for 7 d in SAS, which provided a multitude of data for analyses on Trr changes in the bovine. The Trr changes identified on the d of ovulation in the current experiment provided critical data for identifying a rise in Trr associated with ovulation. These Trr changes observed occurred during the short time frame of the follicular phase within the estrous cycle that has major implications on the efficacy of estrus synchronization.

In the present study, the high percentage of cows (90.3%) that actually ovulated in response to the estrus synchronization protocol is a testament to work done over the past 20 years to control the reproductive cycle of a lactating dairy cow. However, the lack of control from $PGF_{2\alpha}$ injection on d -3 (i.e. initiation of luteolysis) to the actual ovulation of a dominant follicle is particularly variable from cow to cow depending on the developmental stage of the follicle when $PGF_{2\alpha}$ is given (Kastelic et al., 1991; Wenzel et al., 1991; Ferguson et al., 1993). The use of GnRH injections 48 h after $PGF_{2\alpha}$ has alleviated some of the variability in controlling timing of ovulation with the Ovsynch protocol (Pursley et al., 1995), but variability still exists. The diagnosis of true ovulations to GnRH injections given 48 h after $PGF_{2\alpha}$ was estimated through serum progesterone concentrations seen in cows used for the experiment at time of TAI and

thereafter. From this, our analyses provided some insight into actual occurrence and timing of ovulation seen in Trr rises associated with the d of ovulation (i.e. d of TAI).

The ability to successfully generate an accurate 7 d baseline from 2 to 3 Trr readings/d to track and target rises in Trr associated with ovulation has not been well documented in dairy cows. Initiation of estrus in beef cows has been associated with a 0.61°C increase from Trr readings at the same time period from the previous d. The Trr rise occurred during an 8 h period from initial visual observation of estrous behavior (Cooper-Prado et al., 2011). The visual observation of initiation of estrous behavior enabled targeting periods of time to focus on observing rises in Trr. This 0.61°C rise was recorded from Trr readings collected every 15 min during this period, and appeared to be a key factor in pinpointing these Trr rises while accounting for and determining the diurnal variation exhibited by these animals. Constant monitoring of body temperature in cattle and other species has revealed circadian rhythms with peaks and troughs throughout the day (Piccione et al., 2003; Bitman et al., 1983). In the current experiment, the maximum of only three potential Trr readings/d and inconsistency of Trr readings due to variable milking schedules resulted in difficulties generating the 7 d baseline data when accounting for diurnal variation to determine the Trr increase associated with ovulation. These factors caused the 7 d baseline data from the harmonic regression model to only be consistent in 11% of cows using the R^2 cut-off of 0.40 and only 21% of cows using 0.30 within the current experiment. With these few numbers and limited number of Trr readings to use, extracting this 0.5°C or greater rise associated with estrus and ovulation in the small group of animals was not observed. However, our early findings in animals using the R^2 cut-off value of 0.30 showed ovulatory cows having a 0.24°C rise in Trr on d of TAI. While this was not significantly different (possibly due to low number of cows) from anovulatory cows (0.015°C),

the marked increase within this subset of cows had the highest deviation from the 7 d baseline of all analyses. This finding could be further extrapolated with more Trr readings throughout the day to diagnose more ovulatory cows with this circadian rhythm (i.e. R^2 value ≥ 0.30) compared to anovulatory cows. Currently, the bolus used in the experiment has been remodeled to collect Trr readings hourly throughout the d.

When the R^2 cut-off value was ignored, including all cows in the harmonic regression model, results showed a significant rise in Trr from the baseline in ovulatory cows than anovulatory cows. Although, this Trr rise associated with ovulation was significantly higher compared to anovulatory cows, the 0.040°C rise in the T_{rr}MAX was smaller than the rise seen in other reports associated with estrus and ovulation (Cooper-Prado et al., 2010; Piccione et al., 2003).

However, when diurnal variation throughout the day was ignored in the model, though small, there was a significant ($P < 0.01$) 0.064°C increase from baseline in the T_{rr}AVG associated with ovulatory cows compared to the -0.047°C drop in T_{rr}AVG of anovulatory cows. The T_{rr}MAX also tended ($P = 0.06$) to be higher in ovulatory cows than anovulatory cows as well as when diurnal variation was ignored.

Hence, the hypothesis that ovulatory cows would have a rise in temperature from baseline concurrent with ovulation was observed in the current study. The authors recognize that there are possibilities to refine this technology for practical application on a dairy operation. The inability of the harmonic regression model to track diurnal variation posed problems with only 1 to 3 daily Trr observations, but with more Trr readings throughout the day, the ability to pick up this rhythm might be greatly enhanced. Nonetheless, when accounting for diurnal variation, with the low numbers of Trr readings/d throughout the estrus synchronization period, we were still able to

find subtle rises in the T_{rr}MAX of ovulatory animals compared to anovulatory cows. This is quite remarkable with only 2 to 3 Trr readings/d. However, the Trr rises were small relative to results when body temperature was recorded every 15 min or hourly (Cooper-Prado et al., 2011; Piccione et al., 2003) throughout the day.

These results provide insight on the timing of ovulation with average Trr rise (T_{rr}AVG) and maximum single rise (T_{rr}MAX) on d of TAI. However, with the T_{rr}AVG as the overall average Trr rise from baseline and only ≤ 3 Trr readings/d, it was impossible to accurately diagnose the exact time of ovulation. This rise only demonstrates day relative to ovulation. The T_{rr}AVG rise during the day is relative to the 8 h period of Trr rise at the initiation of estrus in beef cows in other studies (Cooper-Prado et al., 2011). However, the single peak rise from baseline (T_{rr}MAX) on d of TAI is a more exact determinant of timing of ovulation. In the current study, this single peak rise from baseline seen in ovulatory cows both when accounting for diurnal variation ($P < 0.01$) and ignoring it ($P = 0.06$) compared to anovulatory cows could be further developed for practical usage with further research. With more Trr readings, a larger Trr peak from the baseline might be evident for proper timing of insemination relative to ovulation.

However, the small rises observed within this experiment could be difficult to detect for proper reproductive management practices. This is a serious limitation for a real world setting using the current instrumentation due to normal fluctuations in the Trr occurring on such a regular basis. Differentiation of Trr rises associated with ovulation could be easily masked with physical actions of the cow along with physiological activities that would mimic this rise in Trr, potentially to a greater extent than seen in the current study.

The use of receiver operating characteristic (**ROC**) curves to determine differences in Trr rises on day of TAI between ovulatory and anovulatory cows could be possible to determine

potential true and false positives, and true and false negatives in Trr rises related to ovulation. However, within the current experiment, the very small differences in these Trr readings between ovulatory and anovulatory cows would likely lead to a lower than acceptable sensitivity when using the ROC curves and relative tests. This decrease in sensitivity would lead to the inability of the test to determine a high percentage of true positives relative to the total number of tests administered. This would also have an effect on the predictive value of positive, which determines the likelihood of a positive test for ovulation detected in a cow that actually ovulates. These small differences in Trr rises would also likely have an effect on the specificity of the test resulting in false positive results as well. For these reasons, the use of ROC was not investigated in the current experiment.

Another major factor in the experiment was the quality of Trr readings used to generate the 7 d baseline. The failure of readings to consistently register in the TempTrack® software 3 times/d was the main area of concern pertaining to consistency of 7 d baselines generated. This led to Trr readings for some cows' T_{rr}AVG actually being their T_{rr}MAX on TAI date as well from only 1 (or in some cases 0) Trr reading on TAI date. The lack of consistency in Trr readings for each cow may have also had negative impacts on the quality of the baseline generated. Also, panel reader malfunction led to complete days without Trr recordings, and related to lack of accuracy in Trr results.

The proximity of the watering device in relation to where Trr readings were recorded upon entrance to the milking parlor likely affected the validity of Trr readings. Water consumption significantly drops reticular temperatures (Bewley et al., 2008). Although given that the time from cow movement from pen to milking parlor and entering the parlor for Trr readings could be upwards of 2 h, reticular temperature 3 h post water consumption may still

remain low depending on water temperature (Bewley et al., 2008). This issue can also be of greater concern as cows drink more water during summer months. The removal of any Trr recordings below 37.5°C was done to alleviate some of the issues associated with water consumption, but did not completely alleviate the problem. The number of cows that had poor or complete absence of Trr readings throughout the experiment remain as evidence of necessary improvement in software and equipment functionality to allow this technology to be more useful as a reproductive tool.

IMPLICATIONS

Although results were promising, the validity of Trr rises correlating to ovulation needs further research and refinement before widespread industry application is practical. Many environmental and physiological aspects pertaining to the animal, such as drinking cold water, have an affect on temperature and need to be accounted for before rises in Trr can be matched to ovulation reliably.

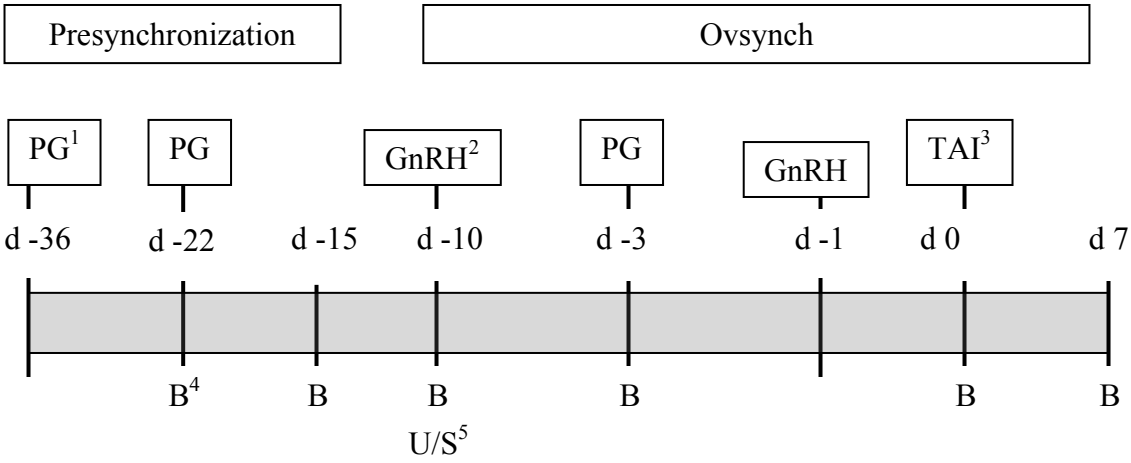

Figure 4.1. Estrus synchronization protocol administered to lactating dairy cows and periods of data collection
[1]GnRH = 100 μg given im of the GnRH analogue (Cystorelin; Merial Co.)
[2]PGF = 500 μg prostaglandin $F_{2\alpha}$ given im (Intervet/Schering Plough Animal Health)
[3]TAI = timed-AI performed 16-19 h after 2nd GnRH injection
[4]B = blood collection via coccygeal venipuncture
[5]U/S = ultrasonography performed transrectally for ovarian structures

Table 4.1. Comparison of reticulo-rumen temperature (Trr) changes between cows that showed progesterone profiles consistent with ovulation or anovulation during the period of Timed-AI (TAI) d. Mean (± SE) for maximum rise ($T_{rr}MAX$) in °C from t-Test comparison are presented as LS means from harmonic regression models created

R^2 Value[1]		**Ovulatory**	**Anovulatory**
0.30	n =	91	14
$T_{rr}MAX$[2]		0.239 ± 0.38	0.015 ± 0.10
0.40	n =	46	9
$T_{rr}MAX$		0.067 ± 0.05	0.002 ± 0.11
Overall[3]	n =	446	48
$T_{rr}MAX$		0.040 ± 0.02[a]	-0.118 ± 0.06[b]

[ab]Within a row, means without common superscripts differ ($P < 0.05$).
[1]R^2 Value = Used as cut-off for determining consistency of harmonic regression model.
[2]$T_{rr}MAX$ = mean of the single maximum rise from baseline of all T_{rr} readings on d of TAI.
[3]Overall = All cows grouped together with no cut-off for R^2 value in harmonic regression model.
The harmonic regression model fit cows to the model using the equation $y = \beta^0 + \beta^1 * st + \beta * ct$.

Table 4.2. Averages for maximum rise and average of all T_{rr} rises in °C on timed-AI (TAI) date of ovulatory and anovulatory cows from T-Test comparison when ignoring harmonic regression model for diurnal variation. Presented as LS means (mean ± SE)

Variable		**Ovulatory**	**Anovulatory**
	n =	446	48
$T_{rr}MAX$[1]		0.180 ± 0.02^{a}	0.094 ± 0.04^{b}
$T_{rr}AVG$[2]		0.064 ± 0.01^{x}	-0.047 ± 0.04^{y}

[ab]Within a row, means without common superscripts have a tendency to differ ($P = 0.06$).
[xy]Within a row, means without common superscripts differ ($P < 0.01$).
[1]$T_{rr}MAX$; mean of the single maximum rise from baseline of all Trr readings on d of TAI.
[2]$T_{rr}AVG$; mean rise from baseline of all Trr readings on the d of TAI.

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APPENDIX I

SAS Code for Chapter II

SAS Code for analyzing differences in BCS, PPI, estrus detection patch score, and parity among treatments:

```
proc glm;
class tx;
model age/BCS/PPI=tx;
lsmeans tx/pdiff;
run;
```

SAS Code for analyzing differences in TAI pregnancy rates among treatments:

```
proc glimmix; class loc  trt age;
 model _9d(ref=first) = loc trt loc*trt age bcs  ppi /dist=binary solution;
  lsmeans trt|loc age/ ilink pdiff;
  slice loc*trt /sliceby=loc pdiff ilink ;
 run;
```

SAS Code for analyzing differences in TAI pregnancy rates by cycling status among treatments:

```
proc glimmix; class loc  trt age cycling;
 model _9d(ref=first) = loc trt age cycling bcs ppi /dist=binary;
  lsmeans loc trt age cycling/ilink;
 run;
```

SAS Code for Chapter III

SAS Code for analyzing differences in BCS, PPI, estrus detection patch score, and parity among treatments in exp. 1 and 2:

```
proc glm;
class tx;
model age/BCS/PPI=tx;
lsmeans tx/pdiff;
run;
```

SAS Code for analyzing differences in TAI pregnancy rates among treatments in exp. 1:

```
proc glimmix; class location BCS PPI tx age;
 model preg(ref=first) = location tx location*tx age /dist=binary solution;
  lsmeans tx|location/ ilink pdiff;
  slice location*tx /sliceby=location pdiff ilink ;
 run;
```

SAS Code for analyzing differences in TAI pregnancy rates among treatments in exp. 2:

```
proc glimmix; class location BCS PPI tx age;
 model preg(ref=first) = location tx location*tx  ppi /dist=binary solution;
  lsmeans trt|loc ppi/ ilink pdiff;
  slice loc*trt /sliceby=loc pdiff ilink ;
 run;
```

SAS Code for analyzing differences in all responses to treatments from Table 3.9 in exp. 2:

```
proc glm;
class tx;
model follicle_size/follicle_presence/response_gnrh/cl_presence/cl_absence/TAI_preg=tx;
lsmeans tx/pdiff;
run;
```

SAS Code for Chapter IV

SAS code for creating harmonic regression model to account for diurnal variation:

```
proc sort data=baseline2; by bolusID tempread hour;
data baseline3; set baseline2; by  bolusID tempread;
retain initial_baseline;
if first.bolusID then initial_baseline=tempread;
baseline_days = tempread - initial_baseline;
hour=hour+baseline_days*24;

st = sin(2*3.14*hour/24);
ct = cos(2*3.14*hour/24);

proc freq data=baseline3; tables bolusID;
ods output onewayfreqs=onewayfreq;
run;

data allhours; set onewayfreq; baseline=0;do hour=1 to 200;
st = sin(2*3.14*hour/24);
ct = cos(2*3.14*hour/24);output;end;
keep bolusID hour st ct baseline;
run;

proc sort data=baseline3; by  hour bolusID;run;
proc sort data=allhours;  by  hour bolusID;run;
data baseline3; merge allhours baseline3 ;by hour bolusID;
if bolusid;run;
proc sort data=baseline3; by bolusID hour ;run;

proc reg data=baseline3; where baseline=0 ; by bolusid;
model tempreadc = st ct;output out=residuals p=pred;run;

proc sort data=residuals; by bolusid hour;run;
proc means data=residuals noprint nway; by bolusid hour;
 var pred tempreadc; output out=means mean=;run;

data baseline4; set baseline;/*observed data vs pred*/
if tempreadc >=37.5;
if baseline=2;/*post AI*/
if f8='PM' then f7=f7+12;
if f8='PM' then hour=hour+12;

st = sin(2*3.14*hour/24);
ct = cos(2*3.14*hour/24);
run;
```

SAS code for analyzing differences between ovulatory and anovulatory cows when accounting for diurnal variation using R^2 values:

```
data obs_pred; merge baseline4 (keep= bolusid TempreadC hour ovul) means (keep=bolusid pred hour); by bolusid hour;run;
 data obs_pred2; set obs_pred;
 if not missing(pred);
 diff_temp = round(tempreadc-pred,0.1);
 run;
 proc ttest data=obs_pred2; class ovul; var diff_temp;run;

 proc glimmix data=obs_pred2; class ovul bolusID; model diff_temp=ovul;random bolusID; lsmeans ovul;run;
 proc sgpanel data=obs_pred2; panelby preg; histogram diff_temp/scale=count; run;
```

SAS code for analyzing differences between ovulatory and anovulatory cows when ignoring diurnal variation:

```
data baseline2; set baseline;
if tempreadc >=37.5;
if baseline=0;/*select only baseline data*/

run;

proc sort data=baseline2; by bolusID;
proc means data=baseline2 noprint; var tempreadc; output mean=mean_temp std=std_temp out=means; by bolusid;run;
proc sort data=baseline; by bolusID;run;
data baseline3; merge baseline means; by bolusid;
if tempreadc >=(mean_temp-0.7) and tempreadc <=(mean_temp+0.7);
if baseline=2;

diff_temp = tempreadc-mean_temp;
run;

 proc glimmix data=baseline3; class ovul bolusID; model diff_temp=ovul;random bolusID; lsmeans ovul;run;
 proc sgpanel data=baseline3; panelby ovul; histogram diff_temp/scale=count; run;
```