COMPARISON OF LONG-TERM CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND OVULATION PRIOR TO FIXED-TIME ARTIFICIAL INSEMINATION IN POSTPARTUM BEEF COWS

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

COMPARISON OF LONG-TERM CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND OVULATION PRIOR TO FIXED-TIME ARTIFICIAL INSEMINATION IN POSTPARTUM BEEF COWS

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a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

This thesis is dedicated to my parents, Nathan and Susan, who have always supported and believed in me, who have instilled the values of honesty, integrity, responsibility, and dedication in me, and who inspired my passion for agriculture, and to my siblings, Brian and Elaine, my girlfriend, Ali, and to my other family and friends for their continual love and support during my education and throughout life.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
BCS	Body condition score
CIDR	Controlled internal drug release insert
CL	Corpus luteum/ Corpora lutea
cm	Centimeter(s)
d	Day(s)
E_2	Estradiol-17β
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
FTAI	Fixed-time artificial insemination
g	Gram(s)
GnRH	Gonadotropin-releasing hormone
h	Hour(s)
hd	Head
i.m.	Intramuscular
kg	Kilogram(s)
LH	Luteinizing hormone
mg	Milligram(s)
mL	Milliliter(s)
MGA	Melengestrol acetate
ng	Nanogram(s)

P_4	Progesterone
PG	Prostaglandin $F_{2\alpha}$
pg	Picogram(s)
RIA	Radioimmunoassay
s	Second(s)
SAS	Statistical Analysis System
SE	Standard error
µg	Microgram(s)
U.S.	United States
yr	Year(s)

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ABSTRACT

Estrous synchronization and artificial insemination (AI) are management tools that cattle producers can use to improve reproductive performance within their herds. Few cattle producers however, implement these technologies, generally due to increased time and labor commitments. In recent years, our research group has focused on the development of estrous synchronization protocols that facilitate the use of fixed-time AI (FTAI), which greatly reduce labor requirements by eliminating the need for estrus detection. Currently, short-term CIDR-based protocols are the preferred method to synchronize estrus prior to FTAI in postpartum beef cows. Alternatively, long-term CIDR-based protocols have been used with greater success in beef heifers. This experiment compared two long-term CIDR-based protocols to synchronize estrus and ovulation prior to FTAI in postpartum beef cows. The hypothesis tested was that extending the interval from CIDR removal to PG from 16 to 19 d would improve estrous response after PG and improve pregnancy rates resulting from FTAI. Cows assigned to

the 14-19 d CIDR-PG protocol (n = 196) received CIDR inserts (1.38 g progesterone) from d 0 to 14 and $PGF_{2\alpha}$ (PG; 25 mg, i.m.) 19 d after CIDR removal on d 33. Cows assigned to the 14-16 d CIDR-PG protocol (n = 195) received CIDR inserts from d 3 to 17, and PG 16 d after CIDR removal on d 33. Cows in both treatments were artificially inseminated on d 36, 72 h after PG, with GnRH (100 μg i.m.) at FTAI. The overall objectives of the experiment were to evaluate treatments on the basis of estrous response after PG and FTAI pregnancy rate, and to identify potential differences in response to these protocols among various age classes of females. Estrous response after PG was higher for cows assigned to the 14-19 d CIDR-PG protocol. Estrous response after PG among cows ≥ 4 yr was higher for cows assigned to the 14-19 d CIDR-PG protocol, but there were no differences between treatments for cows ≤ 3 yr. There were no differences between treatments for FTAI pregnancy rate. However, pregnancy rate after FTAI among cows ≥ 4 yr tended to be higher for cows assigned to the 14-19 d CIDR-PG protocol. In summary, both protocols worked effectively to synchronize estrus and ovulation prior to FTAI in postpartum beef cows, suggesting that a range in interval from CIDR removal to PG may be feasible when using long-term CIDR-based protocols. Higher estrous response rates after PG and improvements in FTAI pregnancy rate for 14-19 d treated cows ≥ 4 yr of age warrants further consideration. In conclusion, long-term CIDR-based protocols provide an alternative method to synchronize estrus prior to FTAI in postpartum beef cows, and allow beef producers to select a protocol best suited to their management style.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

The beef cattle industry is a mainstay of U.S. agriculture. In 2011, cash receipts from cattle were $63 billion and defined 17% of total farm receipts and 38% of livestock and product receipts. In comparison, U.S. cash receipts for dairy and swine totaled $39.5 billion and $22 billion, respectively. Cash receipts from cattle are spread broadly across the U.S. in contrast to swine and dairy which are concentrated into a small number of animal owners and states (USDA ERS, 2012). Many factors affect profitability of a beef operation, but within the cow-calf sector, reproductive performance is a key driver of economic viability (Trenkle and Willham, 1977; Wiltbank, 1990). There are several ways to measure the reproductive performance of a cow-calf operation including pregnancy rate, calving interval, weaning weight of calves per cow exposed, percent calf crop weaned, and/or culling/replacement rate. Genetics and selection can be utilized to improve reproduction; however, improvements in reproduction are more rapidly influenced by changes in management.

Estrous synchronization and artificial insemination (AI) are management tools that were developed to improve reproductive efficiency. Estrous synchronization can be used to increase the number of females that become pregnant early in the breeding season, which leads to decreased calving intervals, shorter calving seasons, and a more uniform calf crop at weaning (Dzuik and Bellows, 1983). Schafer et al. (1990) concluded that females that conceive during a synchronized estrus period wean calves that are on

average 13 d older and 9.5 kg heavier than calves from nonsynchronized females. Estrous synchronization increases the number of opportunities for a female to conceive during the breeding season, as cows or heifers that are synchronized should have a greater number of estrous cycles within a defined breeding season compared to non-synchronized females (e.g. four vs. three estrous cycles in a 65 d breeding season). This is an important consideration since the greatest losses in reproductive efficiency result from females that fail to become pregnant (Bellows and Short, 1990). Females that conceive early in the breeding season also have a greater number of days postpartum prior to the subsequent breeding season. This increases the probability for future pregnancy success and longer retention in the herd.

Estrous synchronization is often used to facilitate AI, which beef producers can utilize to increase the genetic merit of their cattle and potential for increased profit. Artificial insemination allows for the use of sires that are genetically superior for economically relevant traits (e.g., calving ease, growth, carcass merit), without the expense of owning a bull (Trenkle and Willham, 1977). In combination, estrous synchronization and AI are generally considered as the most important and widely applicable reproductive biotechnologies available for beef cattle production (Seidel, 1995).

Although many studies have outlined the efficacy and potential benefits of estrous synchronization and AI programs, beef producers have been slow to adopt these technologies. The National Animal Health Monitoring System of the United States Department of Agriculture (NAHMS, 2009) reports that only 7.9% of producers utilize estrous synchronization methods, and only 7.6% of producers use AI in their herds. The

most common reasons beef producers cite for not incorporating these technologies into their management programs typically include time and labor concerns and/or the technology is viewed as being too difficult or complicated to implement (NAHMS, 2009). Estrous synchronization protocols should be designed to reduce time and labor requirements by limiting cattle handling and reducing the need for estrus detection in order to expand the use of AI (Larson et al., 2006). Protocols that utilize fixed-time AI (FTAI) offer a solution to decrease time and labor inputs by eliminating the need for estrus detection. The development of economical and effective methods to synchronize estrus and ovulation to facilitate use of FTAI should result in increased adoption of these technologies in the U.S. beef herd (Patterson et al., 2003).

A REVIEW OF THE BOVINE ESTROUS CYCLE

The estrous cycle. The estrous cycle of the cow is a continuous succession of physiological changes regulated by the interaction of reproductive hormones that begins at puberty and continues throughout life. The bovine is a polyestrous species, meaning that estrous cycles occur regularly throughout the year. The typical length of the estrous cycle of the cow is 21 d, but ranges from 17 to 24 d (Hansel and Convey, 1983; Senger, 2005). The estrous cycle is divided into two distinct phases, the follicular phase and the luteal phase. The follicular phase comprises approximately 20% of the estrous cycle, while the luteal phase encompasses the remaining 80% of the estrous cycle (Senger, 2005). The follicular phase is defined as the period from regression of the corpus luteum (CL) to ovulation, and is further divided into two stages known as proestrus and estrus. Proestrus begins with luteolysis, which causes a decline in progesterone (P_4) and ends with the onset of estrus. Estrus is the period of sexual receptivity that is characterized by

behavioral changes caused by an increase in estradiol (E_2). The luteal phase is defined as the period from ovulation to regression of the CL, and is subdivided into two stages referred to as metestrus and diestrus. Metestrus is the period from ovulation to formation of a functional CL, encompassing the transition in hormonal dominance from E_2 to P_4. During diestrus, P_4 reaches maximal concentrations to prepare the uterine environment for early embryonic development. If the female fails to conceive, luteolysis occurs and the estrous cycle continues.

The follicular phase. During the follicular phase of the estrous cycle, gonadotropin release from the anterior lobe of the pituitary supports a series of events that stimulates follicular growth leading to sexual receptivity of the female and ovulation. The follicular phase begins with proestrus, a period marked by CL regression and a subsequent decline in P_4 secretion. Reduced P_4 secretion causes an increase in pulse frequency of gonadotropin releasing hormone (GnRH) from the hypothalamus. Secretion of GnRH in the female is controlled by clusters of nerve cell bodies in the hypothalamus referred to as hypothalamic nuclei. The ventromedial nucleus and the arcuate nucleus comprise the tonic center, which is responsible for basal secretions of GnRH that occur throughout the estrous cycle. The preoptic nucleus, anterior hypothalamic area, and suprachiasmatic nucleus comprise the surge, or preovulatory center (Eurell and Frappier, 2006; Herbison, 2008; Knobil, 1988; Senger, 2005). When P_4 concentrations are high during the luteal phase, E_2 exerts a negative feedback effect on GnRH. However, when P_4 concentrations are low during the follicular phase, E_2 has a positive feedback effect on GnRH (Schallenberger et al., 1984; Spicer and Echternkamp, 1986; Stumpf et al., 1989; Walters and Schallenberger, 1984). Parvicellular neurons within the surge center respond

to the decrease in P_4 and increased E_2 by secreting high frequency pulses of GnRH (Hansel and Convey, 1983; Schallenberger et al., 1984; Spicer and Echternkamp, 1986). Gonadotropin releasing hormone then triggers the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. Follicle stimulating hormone stimulates growth of ovarian follicles, and LH initiates the production of E_2 through the two-cell, two-gonadotropin model. Luteinizing hormone binds to its membrane receptor on cells of the theca interna within the developing follicle leading to a cascade of intracellular events that result in conversion of cholesterol to androstenedione through the delta five pathway. Androstenedione then diffuses out of the theca interna cells, across the basement membrane of the follicle, and into the granulosal cells. The granulosal cells contain receptors for FSH. Binding of FSH to its receptor leads to the conversion of adrostenedione to testosterone via 17β-hydroxysteroid dehydrogenase; testosterone is then converted to estradiol-17β (E_2) via P450 aromatase (Fortune and Quirk, 1988; Hansel and Convey, 1983; Richards and Midgley, 1976). This two-cell, two-gonadotropin model continues until E_2 reaches sufficient threshold levels to stimulate the preovulatory LH surge.

The synthesis of LH receptors on the granulosal cells of the preovulatory follicle is an important step in preparing the follicle for ovulation (Sartori et al., 2001). The preovulatory surge of LH leads to ovulation through a series of biochemical pathways. Factors and mechanisms that may affect the process of ovulation include prostaglandins, P_4, FSH, histamine, collagenases, proteinases, hyperemia, hydrostatic pressure, and smooth muscle contraction (Epsey and Lipner, 1994; Murdoch et al., 1986; Nalbandov et al., 1973; Szego and Gitin, 1964). Locally produced collagenases and matrix

metalloproteinases break down the extracellular matrix of the tunica albuginea and thecal cell layers, causing the apex of the follicle to push outward and weaken (Curry and Osteen, 2003; Epsey and Lipner, 1994). Together, these factors lead to the eventual rupture of the follicle and release of the oocyte into the reproductive tract for fertilization.

Concurrent to the events that lead up to ovulation, elevated concentrations of E_2 cause behavioral changes associated with estrus and prepare the reproductive tract for mating. These behavioral changes include increased movement, vocalization, and nervousness. An obvious sign of estrus is the female's attempt to mount other animals or standing to be mounted by other animals. In the cow, standing estrus (when the cow will accept a bull for mating) typically lasts 12 to 18 hours (Hammond, 1927; Nalbandov and Casida, 1942; Wiltbank et al., 1967), and ovulation normally occurs 24 to 32 hours after the onset of estrus (Christenson et al., 1975; Senger, 2005; Wiltbank et al., 1967). In preparation for mating, E_2 causes a thickening of the mucosa within the caudal vagina, increased mucus production within the cranial vagina and cervix, development of uterine glands, and increased secretion of fluid and movement of cilia within the oviduct. Estradiol also increases blood flow to the reproductive organs, facilitating increased secretion throughout the tract and swelling of the vulva, a visual indicator of estrus (Bishop, 1956; Ford et al., 1979; Resnik et al., 1974; Senger, 2005).

The luteal phase. Ovulation marks the end of the follicular phase and the beginning of the luteal phase. When the follicle ovulates, surrounding blood vessels are ruptured and blood clots to form the corpus hemorrhagicum. The corpus luteum is formed by luteinization of cells from the ovulatory follicle. Luteinizing hormone controls this process transforming granulosal cells and theca interna cells into large and small luteal

cells, respectively (Smith et al., 1994). Corpora lutea increase in size and become fully functional around day five of the estrous cycle and begin producing high concentrations of P_4 (Garverick et al., 1992; Hansel and Convey, 1983; Smith et al., 1994; Wiltbank, 1994). Negative feedback of progesterone on the hypothalamus reduces frequency of basal secretions of GnRH from the tonic center. This prevents subsequent development of preovulatory follicles and maintains relatively low levels of E_2, which in turn prevents the preovulatory surge of GnRH and LH, and behavioral estrus (Driancourt, 2001; Garverick et al., 1992; Hansel and Convey, 1983; Smith et al., 1994; Wiltbank, 1994). Progesterone also acts on the uterus by decreasing contractions of the myometrium and stimulating secretions from the endometrial glands to support a newly formed conceptus (Senger, 2005; Spencer and Bazer, 2002). The CL continues to grow until midway through the estrous cycle when maximal P_4 concentrations are achieved (Donaldson and Hansel, 1965).

Progesterone down regulates its own receptor during the late luteal phase of the estrous cycle, which reduces action on the hypothalamus and uterus, and mitigates the inhibitory effect of P_4 on E_2. Increased E_2 activity produces a series of intermittent episodes of oxytocin secretion in the hypothalamus (McCracken et al., 1996) and also leads to upregulation of oxytocin receptors within the uterus. Oxytocin acts on the uterus to cause pulsatile secretions of prostaglandin $F_{2\alpha}$ (PG). In the bovine, oxytocin is stored by luteal cells, and released in synchrony with PG pulses. This may serve to amplify the production of uterine PG (McCracken et al., 1999). A counter current exchange mechanism between the uterus and ovary allows for PG to exert a local effect on the corpus luteum (Hixon and Hansel, 1974; McCracken et al., 1999). Prostaglandin $F_{2\alpha}$ acts

on the CL to cause vascular constriction, which decreases blood flow and deprives luteal cells of nutrients, substrates essential for steroidogenesis, and luteotropic support (Pharriss et al., 1970). This process of CL regression, or luteolysis, results in death of cells that form the CL, forming a scar like tissue referred to as the corpus albicans. Luteolysis signals the end of the luteal phase around d 18 of the estrous cycle, and the beginning of the follicular phase.

Folliculogenesis and follicular waves. Folliculogenesis is the developmental process that results in the formation of Graafian (pre-ovulatory) follicles from a pool of primordial follicles (Spicer and Echternkamp, 1986). Follicular development progresses through the following stages: Primordial, primary, secondary, tertiary, and Graafian. The pool of primordial follicles present in the cortex of the bovine ovary is set near birth, and is estimated to be in the range of 150,000 primordial follicles (Erickson, 1966). Throughout the life of the cow, primordial follicles gradually enter the pool of growing follicles, but greater than 99% of follicles that enter the growing pool undergo atresia (Ireland, 1987). The mechanisms that stimulate primordial follicles to enter the growing cohort are not completely understood; however, growth and differentiation factors produced by the ovary and within the follicle are believed to be involved (Elvin et al., 2000). Follicular development in the bovine occurs in a wave-like manner, with cows exhibiting two, three, or four follicular waves during their estrous cycles (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a). Follicular waves occur in three distinct stages: Recruitment, selection, and dominance. Recruitment is characterized by growth of a cohort (normally one to six) of small, antral follicles (4 to 5 mm in diameter), which receive sufficient pituitary gonadotropic stimuli to progress towards ovulation.

Emergence of a follicular wave is preceded by an increase in FSH concentrations that begins approximately 2.5 d before the cohort of growing follicles appears (Adams et al., 1992; Hamilton et al., 1995). Follicles continue growing over the next 36 to 48 h, after which one follicle (8 to 9 mm in diameter) is selected for further development, while other follicles in the cohort become atretic (Adams et al., 1992; Hamilton et al., 1995). The selected follicle establishes dominance by producing E_2 and inhibin to reduce FSH release from the anterior pituitary (Fortune, 1994; Ginther et al., 2001; Knight and Glister, 2001). The dominant follicle reaches maximum size and maintains dominance for 3 to 6 d before regression during the luteal phase (Ginther et al., 1989b; Knopf et al., 1989). Following atresia of the dominant follicle, negative feedback from E_2 and inhibin is lost, which allows FSH concentrations to increase and a new follicular wave is initiated (Adams et al; 1992). However, if luteolysis occurs during the growth phase of the dominant follicle, the follicle matures and ovulates under the influence of LH (Kastelic et al., 1990; Lucy et al., 1992; Sirois and Fortune, 1990).

FACTORS AFFECTING ANESTRUS IN CATTLE

Anestrus. Anestrus is defined as the absence of estrous cyclicity. Anestrus occurs after parturition, due to insufficient hormonal stimuli, and normally ranges from 46 to 104 d in suckled beef cows (Casida, 1971; Wiltbank, 1970). When postpartum anestrus is prolonged, cows have fewer opportunities to conceive during the subsequent breeding season. Factors that affect the length of postpartum anestrus include lactational status or suckling, body condition, parity, dystocia, and other minor factors (Dunn et al., 1969; Short et al., 1990; Stagg et al., 1998; Wiltbank and Cook, 1958).

Endocrine changes. A transient rise in FSH leads to resumption of follicular growth within 7 to 10 d following parturition in most cows (Crowe, 2008). However, these follicles do not mature to the preovulatory stage, due to the absence of LH pulses. Early postpartum anestrus in cows is primarily a function of decreased LH pulsatility, caused by depletion of LH stores in the anterior pituitary (Nett et al., 1988; Yavas and Walton, 2000). After parturition, the anterior pituitary is less responsive to GnRH, a contributing factor leading to decreased LH pulsatility (Crowder et al., 1982; Short et al., 1990). Although, stores of LH are replenished around d 15 to 30 postpartum (Labhsetwar et al., 1964; Lamming et al., 1981; Moss et al., 1985; Wagner et al., 1969), circulating concentrations of LH remain low in postpartum cows a result of low LH pulsatility (Arije et al., 1974; Convey et al., 1983; Gauthier et al., 1982; Nett et al., 1988; Schallenberger, 1985). Low LH pulsatility is a result of negative feedback from E_2 on the hypothalamic GnRH pulse-generator. Negative feedback from E_2 is modulated by suckling and calf stimulus. The GnRH pulse-generator eventually loses sensitivity to negative feedback from E_2 as length of the postpartum interval extends (Yavas and Walton, 2000). This allows for resumption of GnRH production and the pulsatile pattern of LH release, which normally occurs around 25 to 32 d postpartum in cows (Lamming et al., 1981; Rawlings et al., 1980; Riley et al., 1981). The increase in LH pulsatility causes increased release of E_2 from the preovulatory follicle, which induces a positive feedback effect on GnRH. This results in the preovulatory LH surge and subsequent ovulation. The first postpartum ovulation is often followed by a shortened luteal phase due to premature luteolysis (Short et al., 1990). This is caused by premature release of PG from the endometrium, possibly

amplified by the suckling-induced release of oxytocin from the posterior pituitary (Yavas and Walton, 2000).

Suckling. Suckling stimulus of a calf significantly influenced the duration of postpartum anestrus in beef cows (Edgerton, 1980). Suckled beef cows tend to take longer to resume estrous cyclicity than non-suckled cows (40 vs. 21 d; Carruthers and Hafs, 1980; Williams, 1990). Multiple neuronal pathways in the cow exert suppressive effects on LH secretion. One of the first of these pathways to be studied involved the somatosensory signal through tactile stimulation of the udder or teat by the calf (Short et al., 1972; Williams et al., 1987). Other factors such as maternal olfaction, visual stimulation by the calf, and physical presence of the calf have been shown to exert a suppressive effect on LH pulsatility (Griffith and Williams, 1996; McVey and Williams, 1991; Montiel and Ahuja, 2005; Mukasa-Mugerwa et al., 1991; Stagg et al., 1998; Williams et al., 1984; 1993). Studies have shown that calf removal for a period of 24 h results in increased LH pulse frequency, and temporary calf removal improves estrous response when used in conjunction with estrous synchronization protocols (Geary et al., 1998; Smith et al., 1983; Stagg et al., 1998; Walters et al., 1982; Walters and Schallenberger, 1984; Williams, 1990; Wiltbank and Cook, 1958).

Body condition. Body condition of a cow serves as an indicator of energy reserves and nutritional status. Body condition score (BCS; 1 to 9 scale, 1 = emaciated, and 9 = obese) is a subjective, visual measurement based on rib visibility, fat thickness along the spine and over the transverse spinous processes, and fat deposits in the brisket and tail head areas (Richards et al., 1986). The mature postpartum cow prioritizes energy use to first meet maintenance requirements, followed by lactation, before utilizing energy for

reproductive processes. (Grimard et al., 1997; Guedon et al., 1999; Short et al., 1990). Body condition at parturition is positively correlated with LH pulse frequency and follicular development during the early postpartum period (Ryan et al., 1994), LH stores in the anterior pituitary at 30 d postpartum (Connor et al., 1990), and postpartum interval after early weaning (Bishop et al., 1994). Reduced body condition (general recommendation BCS 6) prior to parturition seems to impact length of the postpartum interval more severely compared to nutritional deficiencies post-calving (Short et al., 1990). Still, postpartum nutrition is important, as a loss in body condition during the postpartum period can lead to decreased conception rates to first service, prolonged calving to conception intervals, and increased numbers of inseminations per conception (Gillund et al., 2001).

Parity. Parity, or age, can also have a significant effect on reproductive success. Young cows ($\leq$ 3 yr) and older cows ($\geq$ 7 yr) often experience reduced fertility, longer postpartum anestrous periods, and increased incidence of dystocia compared to middle-aged cows (Bellows and Short, 1978; Doornbos et al., 1984; Laster et al., 1973; Short et al., 1990). Primiparous cows commonly have higher energy requirements because they have not reached mature size and are still growing. If nutrient requirements are not met, postpartum LH pulse frequency can be decreased (Grimard et al., 1995; Randel et al., 1996). This results in postpartum anestrous periods that are 1 to 4 weeks longer in primiparous versus multiparous cows (Fajersson et al., 1999; Grimard et al., 1995; Guedon et al., 1999; Lamming et al., 1981; Randel et al., 1996)

Dystocia. Dystocia is defined as difficult or abnormal parturition. Cows that experience dystocia often experience increased length of the postpartum interval,

decreased conception rates to first service AI, and reduced weaning weights of calves during subsequent years, compared to cows that do not experience calving difficulty (Brinks et al., 1973; Doornbos et al., 1984; Laster et al., 1973). Several management techniques may be used to reduce the incidence and severity of dystocia. These practices include prebreeding pelvic area measurements of heifers, prepartum nutritional management for body condition, use of calving ease sires, and appropriate obstetrical assistance.

Minor factors. Several other minor factors influence length of postpartum anestrus in beef cows. These include genetics, disease, retained placenta, twin births, stress, presence of bulls, and season (Doornbos et al., 1984; Echternkamp and Gregory, 1999*;* Edgerton, 1980; Short et al., 1990*;* Tauck et al., 2010; Wheeler et al., 1982). Careful consideration and management of these factors is necessary to optimize reproductive performance and efficiency of a beef cow-calf operation.

A REVIEW OF PROGESTINS

Progesterone, produced by the CL, is the dominant hormone of the luteal phase of the estrous cycle. Progesterone suppresses the release of GnRH from the hypothalamus to prevent the maturation and ovulation of a dominant follicle. It has long been known that the estrous cycle can be manipulated through the use of exogenous P_4 or synthetic progestins (Makepeace et al., 1937; Kind and Dorfman, 1963). Progestins can be used to synchronize estrus, by preventing ovulation and behavioral estrus during administration. Progestins have also been shown to be effective to induce estrous cyclicity in prepubertal heifers and anestrous cows. Two progestins are approved for use in cattle in the United

States including, the feed additive, melengestrol acetate (MGA), and the intravaginal device, Controlled Internal Drug Release insert (CIDR).

Research has demonstrated that new CIDR inserts (Savio et al., 1993; Sirois and Fortune, 1990; Stock and Fortune, 1993) and MGA can extend the lifespan of a dominant follicle in the absence of a CL. Long term treatment with a progestin can lead to an increased occurrence of persistent follicles. Still, ovulation typically occurs shortly after removal of the progestin treatment (Savio et al., 1993; Stock and Fortune, 1993). This results in reduced fertility at the first synchronized estrus, following progestin removal; however, normal fertility can be achieved at the second estrus (Patterson et al., 1989).

Progestins can induce puberty in peripubertal heifers (Gonzalez-Padilla et al., 1975; Patterson et al., 1990) by simulating the natural increase in P_4 that occurs before the onset of puberty (Berardinelli et al., 1979). Progestins induce cyclicity by increasing LH pulse frequency (Imwalle et al., 1998; Smith and Day, 1990) and down-regulating E_2 receptors in the hypothalamus (Anderson and Day, 1996; Anderson et al., 1996). Endogenous P_4 also rises prior to resumption of estrous cyclicity in postpartum cows (Rawlings et al., 1980), and progestins are believed to stimulate resumption of estrous cyclicity through a similar method as that observed in heifers to induce puberty.

Melengestrol acetate. Melengestrol acetate (MGA; Figure 1.1), an orally active progestin, was developed in 1962 to improve growth rate in feedlot heifers. Melengestrol acetate functions to suppress estrus and ovulation, but does not inhibit follicular growth and production of E_2. Current recommendations for use of MGA in estrous synchronization protocols suggest feeding MGA at a rate of 0.5 mg/hd/d to suppress

estrus, inhibit ovulation, and induce puberty in heifers (Imwalle et al., 2002; Patterson et al., 1990; Zimbelman and Smith, 1966a; 1966b). Melengestrol acetate is effective at low concentrations because it has an 11.1-fold higher binding affinity for the P_4 receptor compared to endogenous P_4 (Perry et al., 2005a). Consistent daily intake of MGA is important for effective synchrony of estrus. This can be accomplished by utilizing a grain or protein supplement fed at a rate of 1.4 to 2.3 kg/hd/d to administer the proper dosage of MGA. Adequate bunk space (60 linear cm/hd) and a routine daily feeding schedule are also recommended (Patterson et al., 2006).

Controlled Internal Drug Release. The EAZI-BREED™ Controlled Internal Drug Release insert (CIDR; Pfizer Animal Health, New York, NY), is an intravaginal device that continuously releases P_4 which is then absorbed into the bloodstream. The CIDR was approved for use by the FDA for estrous synchronization in suckled beef cows and beef and dairy heifers, resumption of estrous cyclicity in dairy cows, induction of puberty in beef and dairy heifers, (FDA, 2002) and estrous synchronization in lactating dairy cows (FDA, 2010). Each insert is impregnated with 1.38 g of P_4 in elastic silicone molded over a T-shaped, nylon spine. The CIDR's T-shaped design has two wings that can be pushed forward into a straight, flattened position, to facilitate insertion into the vaginal vestibule. An applicator is used to properly position the CIDR inside the vaginal vestibule. Once inserted, the wings of the CIDR bend back into the original T-shape to hold the CIDR in place. Retention rates, or the percentage of animals that retain a CIDR intravaginally for the desired time period, range from 96 to 99% for beef heifers (Lucy et al., 2001; Macmillan et al., 1988; Macmillan and Thatcher, 1991).

Progesterone release from the CIDR maintains circulating concentrations > 2.0 ng/ml in the absence of a CL (Chenault et al., 2003). Research indicates that P_4 concentrations reach peak blood concentrations within 1 h after CIDR insertion and decrease 12 to 24 h following CIDR removal (Lamb et al., 2006; Perry et al., 2004). Progesterone from CIDR inserts clears the circulation faster upon removal as compared to removal of melengestrol acetate from feed (Tauck et al., 2007), indicating that MGA remains biologically active in the body for a longer period of time. Due to the CIDRs' ability to deliver a constant, consistent dose of P_4, and its faster rate of clearance after removal, synchrony of estrus is improved in beef heifers treated with CIDR-based versus MGA-based estrous synchronization protocols (Kojima et al., 2004; Tauck et al., 2007). The CIDR-based protocols also produce similar pregnancy rates resulting from FTAI when compared to MGA treatment, while reducing labor required to feed MGA on a daily basis (Schafer et al., 2007).

DEVELOPMENT OF ESTROUS SYNCHRONIZATION PROTOCOLS

Development of GnRH-PG protocols. Exogenous administration of PG has been shown to be effective in regressing luteal tissue in estrous-cycling cows that are between d 5 and 18 of their estrous cycles. However, PG is not effective in eliciting an estrous response in anestrous cows or cows lacking a functional CL (Inskeep, 1973; Lauderdale, 1974). When PG is administered, the CL (if present) is regressed, and the cow will subsequently come into estrus. The stage of the estrous cycle or stage of follicular development at the time of PG injection leads to variation in the interval to estrus

following PG (Macmillan and Henderson, 1984; Sirois and Fortune, 1988). This variation can be reduced by an injection of GnRH prior to PG administration. Exogenous GnRH was shown to induce a preovulatory-like surge of LH (Bao and Garverick, 1998; Garverick et al., 1980), which can induce ovulation of follicles ≥ 10 mm in diameter (Sartori et al., 2001). A new or accessory CL will form and begin producing P_4 if the cow responds to GnRH and ovulation occurs (Garverick et al., 1980; Pursley et al., 1995). Within 3 to 4 d following GnRH administration, a new follicular wave emerges (Twagiramungu et al., 1992; 1995). Injecting PG 6 to 7 d following GnRH administration was shown to regress newly formed luteal tissue (Thatcher et al., 1989; Twagiramungu et al., 1992; 1995).

Development of the Select Synch protocol. The Select Synch protocol (Figure 1.2) utilizes an injection of GnRH on d 0 followed by an injection of PG on d 7. The interval between GnRH and PG administration allows for growth and development of a new dominant follicle and allows time for the new or accessory CL to become responsive to PG, which leads to improved synchrony of estrus following PG (Thatcher et al., 1989; Twagiramungu et al., 1992). Effective use of this protocol requires estrus detection beginning 2 d prior to PG and continuing 6 d following PG, with AI performed 12 h after detected estrus. This protocol requires an extended period of estrus detection because a proportion (5 to 15%) of cows exhibit estrus prior to PG administration (Dejarnette et al., 2001; Kojima et al., 2000); however, despite these considerations, the protocol has been used effectively in postpartum beef cows.

Development of the CO-Synch protocol. The CO-Synch protocol (Figure 1.3) is similar to the Select Synch protocol with GnRH administration on d 0 and PG 7 d later.

These protocols differ in that cows that are assigned to the CO-Synch protocol are artificially inseminated at a predetermined fixed-time, 48 h following PG, and receive a second injection of GnRH at AI. The CO-Synch protocol reduces time and labor requirements compared to an estrus detection protocol, and pregnancy rates resulting from FTAI typically range from 43 to 49% (Geary and Whittier, 1998; Lamb et al., 2001; Larson et al., 2006).

Development of the 7-d Select Synch and CO-Synch + CIDR protocols. The 7-d Select Synch and CO-Synch + CIDR protocols (Figure 1.4) are analogous to the previously described Select Synch and CO-Synch protocols, only that a CIDR is inserted at the time of GnRH injection on d 0 and removed coincident with PG on d 7. Combining a CIDR with these protocols reduces the number of cows that display estrus prior to PG and improves fertility in anestrous cows (Lamb et al., 2001; Larson et al., 2006). Pregnancy rates resulting from synchronized AI can range from 56 to 72% for cows treated with the 7-d Select Synch + CIDR protocol (Bridges et al., 2008; Wilson et al., 2010). Similar results have been reported for cows synchronized with the 7-d CO-Synch + CIDR protocol, with FTAI pregnancy rates averaging 66% (Busch et al., 2008; Schafer et al., 2007; Wilson et al., 2010).

Development of the 5-d Select Synch and CO-Synch + CIDR protocols. The 5-d Select Synch and CO-Synch + CIDR protocols (Figure 1.5) are similar to the previously mentioned 7-d protocols, except the interval from GnRH to PG is reduced to 5 d, as the name implies. The 5-d protocols also recommend a second injection of PG, 12 h after the first injection, to facilitate complete luteolysis of accessory CL formed in response to GnRH (Bridges et al., 2008). The theory behind development of the 5-d protocols was

that a younger, growing dominant follicle (3 to 4 d after wave emergence), would be capable of producing higher intra-follicular and circulating concentrations of E_2 at the time of AI, which may lead to increased success rates after FTAI (Bridges et al., 2008).

Development of CIDR Select. Pregnancy rates in heifers following administration of short-term CIDR-based protocols are less consistent (Busch et al., 2007; Colazo et al., 2004; Lamb et al., 2006; Leitman et al., 2008; Martinez et al., 2000; 2002) compared to postpartum beef cows (Bridges et al., 2008; Busch et al., 2008; Lamb et al., 2001; Larson et al., 2006; Schafer et al., 2007; Wilson et al., 2010). This is likely due to the fact that heifers are less responsive to a single injection of GnRH compared to cows (Geary et al., 2000; Macmillan and Thatcher, 1991; Moreira et al., 2000; Pursley et al., 1995; Thompson et al., 1999). This results in reduced synchrony of follicular waves, which in all likelihood reduces success rates from AI following administration of the short-term protocols. Research has shown that ovulatory response to GnRH in heifers may be influenced by day or stage of the estrous cycle when GnRH is administered (Atkins et al., 2008; Leitman et al., 2008; Schafer et al., 2006). This led to the idea that pre-synchronization with a progestin prior to a GnRH-PG protocol may lead to increased efficiency and success rates after AI.

Kojima et al. (2004) evaluated two long-term progestin-based estrous synchronization protocols in beef heifers on the basis of estrous response, timing of AI, and pregnancy rate resulting from AI. Heifers were presynchronized with either MGA (MGA Select; Figure 1.6) or a CIDR insert (14-d CIDR; Figure 1.6). Results from this trial showed a similar overall estrous response between protocols after PG, but heifers treated with the 14-d CIDR protocol exhibited a more highly synchronized estrus and

improved pregnancy rates resulting from AI compared to MGA treated heifers. This prompted several subsequent studies with heifers comparing short and long-term CIDR-based protocols.

Busch et al. (2007) compared short- (7-d CO-Synch + CIDR) versus long-term (CIDR Select; Figure 1.7) CIDR-based protocols in beef heifers. Heifers assigned to the long-term protocol (CIDR Select) showed an increase in estrous response and synchrony of estrus and improved FTAI pregnancy rates compared to heifers synchronized with a short-term protocol. Leitman et al. (2008) then compared the long-term CIDR Select protocol with two short-term protocols (7-d Select Synch, 7-d Select Synch + CIDR), and reported that heifers synchronized with the CIDR Select protocol had an improved synchrony of estrus and ovulation following treatment in both estrous-cycling and prepubertal heifers compared to heifers treated with either of the two short-term protocols. Together, these studies (Kojima et al., 2004; Busch et al., 2007; Leitman et al., 2008) demonstrated that presynchronization with a 14-d CIDR treatment prior to administration of the GnRH-PG protocol was an effective means of synchronizing estrus in beef heifers.

Development of 14-d CIDR-PG. A study by Tauck et al. (2007) compared long-term progestin-based protocols (14-d CIDR vs. 14-d MGA) with PG 17 d (CIDR) or 19 d (MGA) after progestin removal. Heifers were observed for estrus after PG and inseminated according to estrus, however FTAI was performed 72 h after PG for heifers that failed to exhibit estrus by 60 h. Estrous response up to 120 h following progestin removal and 60 h following PG administration was higher among CIDR-treated heifers, however there were no differences between treatments in pregnancy rates resulting from

AI. These protocols differ from CIDR Select, as they did not include GnRH following progestin removal. Studies by Leitman et al. (2009a; 2009b) evaluated the timing and necessity of administering GnRH within a long-term CIDR-based protocol. These results showed that heifers that did not receive GnRH (14-d CIDR-PG; Figure 1.7) exhibited an improvement in synchrony of estrus following PG and increased pregnancy rates resulting from AI compared to heifers that received GnRH (CIDR Select). Mallory et al. (2011) then compared pregnancy rates after FTAI among heifers treated with the 14-d CIDR-PG and CIDR Select protocols (Figure 1.8). Heifers were inseminated at 66 and 72 h after PG for the 14-d CIDR-PG and CIDR Select treated groups, respectively, with GnRH administered to all heifers at AI. Results reported from this experiment showed similarities in estrous response between treatments and a tendency toward improved pregnancy rates after FTAI among 14-d CIDR-PG treated heifers. Collectively, these studies demonstrated that GnRH within a 14-d CIDR-PG protocol is not required to synchronize estrus and ovulation prior to FTAI in beef heifers.

The previous experiments demonstrated the efficacy of long-term CIDR-based protocols in heifers; however, little published research is available regarding use of these protocols in cows. Recently, Nash et al. (2011) compared the 14-d CIDR-PG and CIDR Select protocols (Figure 1.9) in postpartum beef cows. Two experiments were performed to evaluate use of these protocols in cows; the first of which involved a comparison of treatments on the basis of detected estrus and the second involved FTAI. In both experiments, estrous response after CIDR removal and PG was similar between treatments. Interestingly, the mean interval to estrus after CIDR removal in cows was 48.3 h, which tends to be longer than the interval reported in heifers following treatment

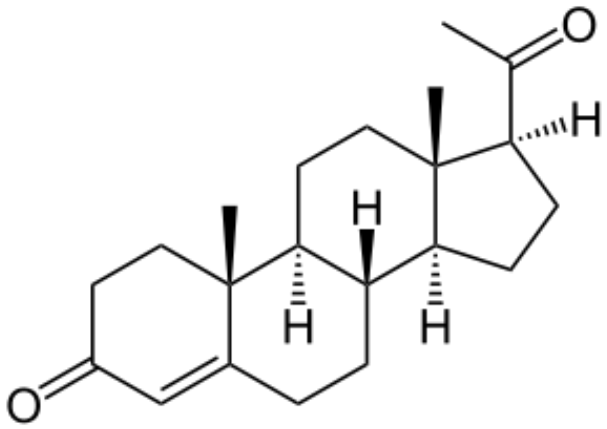
with a 14-d CIDR protocol (31.1 h, Leitman et al., 2008; 37.8 h, Mallory et al., 2010). In the first experiment (Nash et al. 2011), pregnancy rates were similar between treatments when AI was performed on the basis of detected estrus. However, in the second experiment, FTAI pregnancy rates were higher for cows assigned to the 14-d CIDR-PG protocol (79%) compared to CIDR Select (42%). Although the numbers of cows involved in these studies were limited, these results support previous studies from our lab in work with heifers demonstrating that GnRH is not required in long-term CIDR-based protocols to facilitate synchrony of estrus and ovulation prior to FTAI. Nash et al. (2012) performed a larger field trial comparing the short-term 7-d CO-Synch + CIDR protocol with the 14-d CIDR-PG protocol in postpartum beef cows. Estrous response after PG and prior to FTAI was reduced in cows assigned to the 14-d CIDR-PG protocol (23%) compared to the 7-d CO-Synch + CIDR (49%) treated cows. Despite differences in estrous response, pregnancy rates resulting from FTAI were similar between treatments. Estrous response after PG and prior to FTAI observed in cows treated with the 14-d CIDR-PG protocol (23%; Nash et al., 2012) is lower compared to heifers (78%; Mallory et al., 2011). Together, these studies suggest that differences may exist in length of estrous cycles or follicular waves in heifers compared to cows following administration of a long-term CIDR-based protocol.

SUMMARY

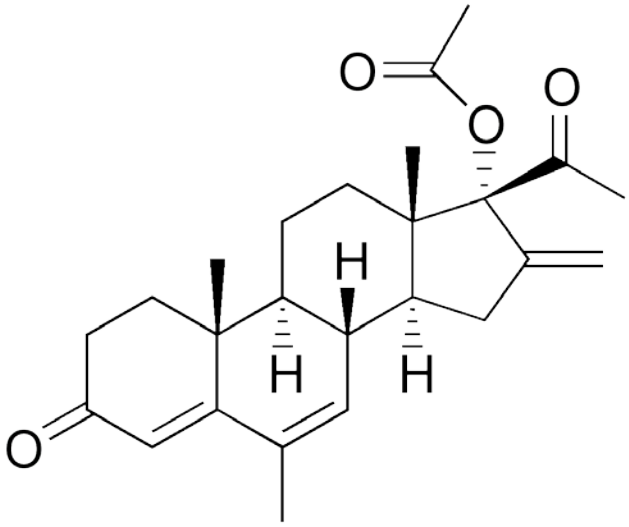
Effective use of estrous synchronization and artificial insemination provides cattle producers with the opportunity to reduce the length of breeding and calving seasons, create more uniform calf crops, increase pounds of calf weaned per cow exposed, and effectively integrate superior genetics into beef herds. A better understanding of the

physiological mechanisms and characteristics of the bovine estrous cycle has resulted in the development of protocols to synchronize estrus and ovulation to facilitate use of artificial insemination. Many protocols have been developed for use in both cows and heifers, and these protocols vary on the basis of cost, labor, efficacy, and convenience.

In recent years, short-term CIDR-based protocols have been the preferred method to synchronize estrus in postpartum beef cows, while long-term CIDR-based estrous synchronization protocols were shown to result in higher pregnancy rates in beef heifers. Long-term CIDR-based protocols also offer labor-reducing management options relative to animal health considerations. Few studies however, have examined the use of long-term CIDR-based protocols in postpartum beef cows or potential differences among different age classes of females. These considerations provide the rationale for the experiments presented in this thesis.



Progesterone (P_4)



Melengestrol acetate (MGA)

Figure 1.1. Chemical structure of progesterone (P_4) and melengestrol acetate (MGA).

Select Synch

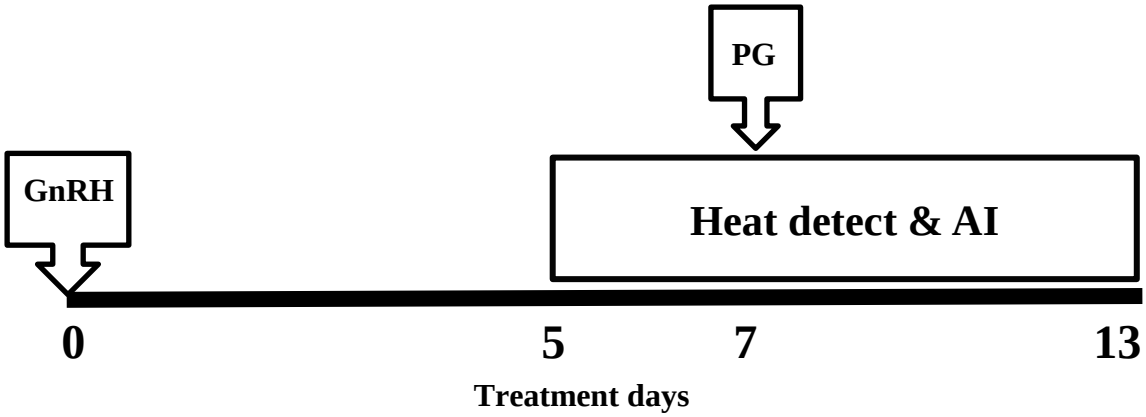


Figure 1.2. Treatment schedule for the Select Synch protocol (from Geary et al., 2000).

CO-Synch

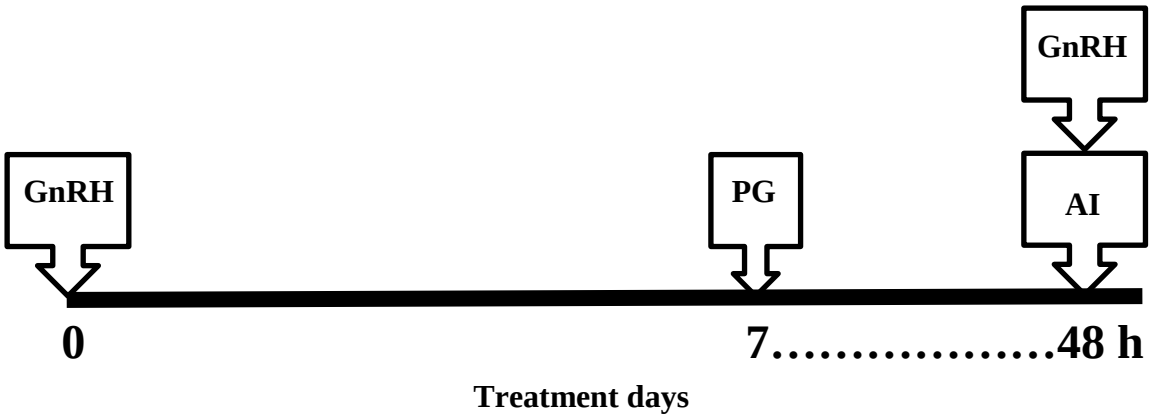
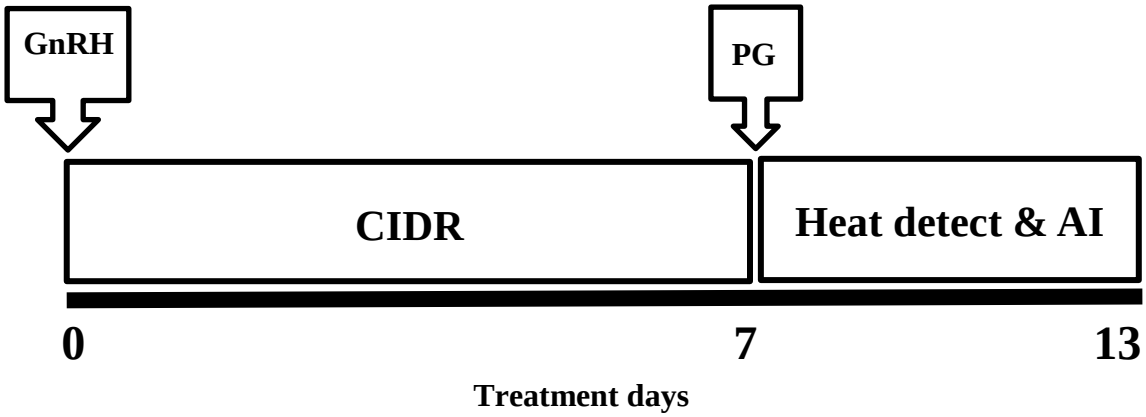


Figure 1.3. Treatment schedule for the CO-Synch protocol (from Geary and Whittier, 1998)

7-d Select Synch + CIDR



7-d CO-Synch + CIDR

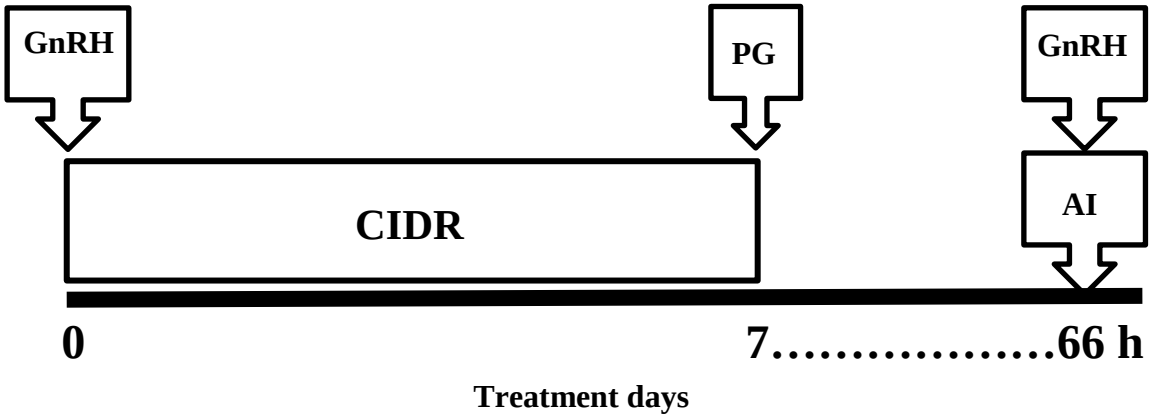
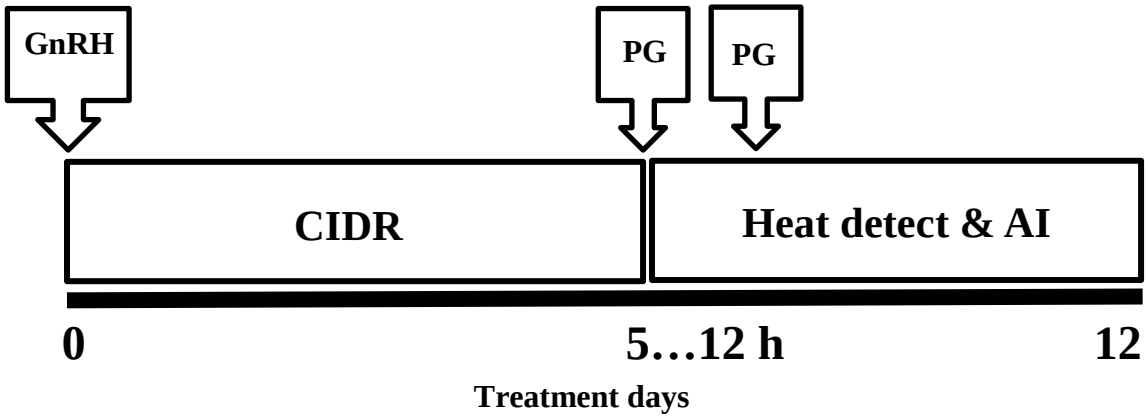


Figure 1.4. Treatment schedule for the 7-d Select Synch and CO-Synch + CIDR protocols (from Lamb et al., 2001; Schafer et al., 2007).

5-d Select Synch + CIDR



5-d CO-Synch + CIDR

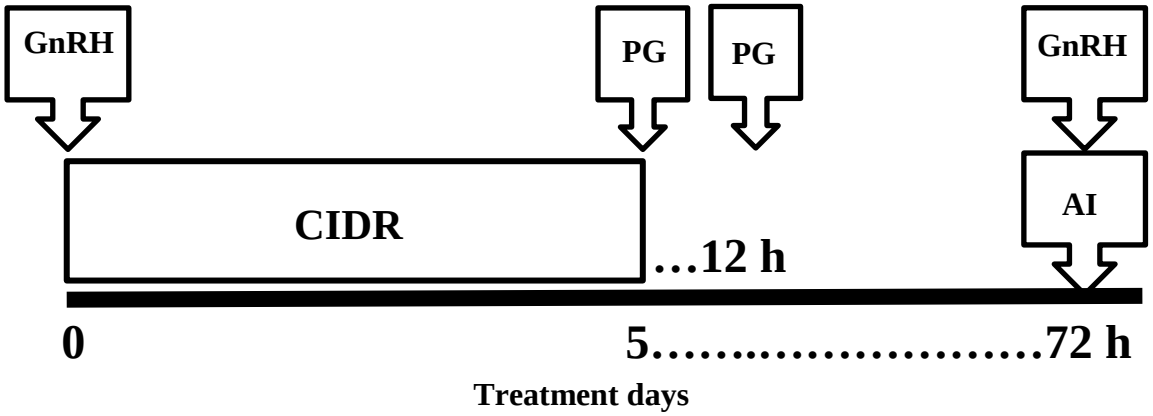
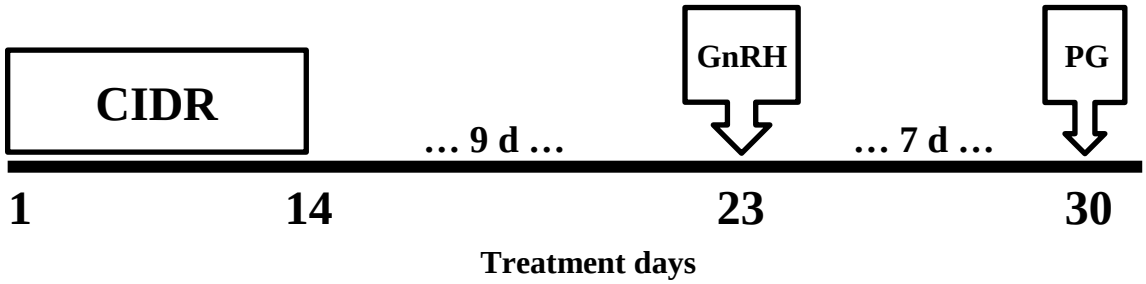


Figure 1.5. Treatment schedule for the 5-d Select Synch and CO-Synch + CIDR protocols (from Bridges et al., 2008).

14-d CIDR



MGA Select

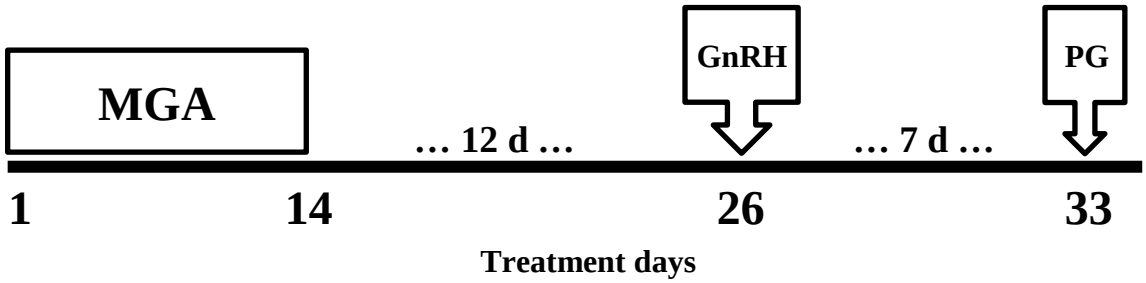


Figure 1.6. Treatment schedule for the 14-d CIDR and MGA Select protocols in heifers (from Kojima et al., 2004).

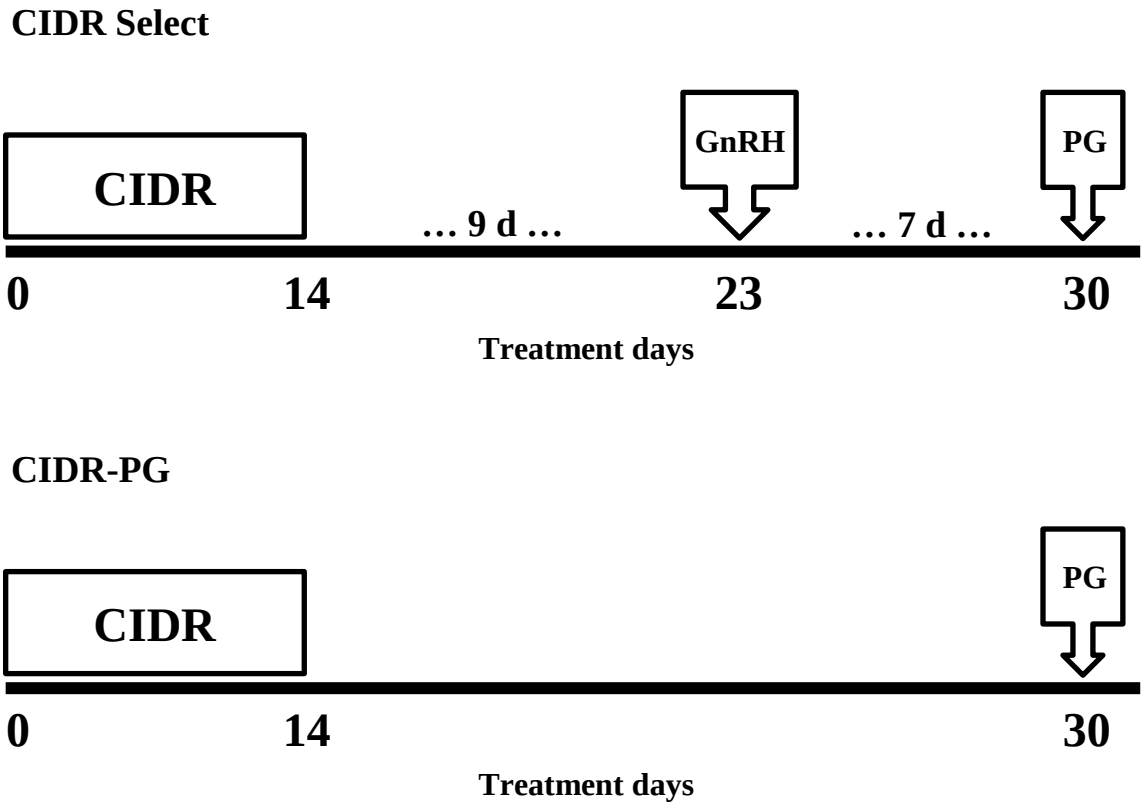


Figure 1.7. Treatment schedule for the CIDR Select and CIDR-PG protocols in heifers (from Busch et al., 2007; Leitman et al., 2009b).

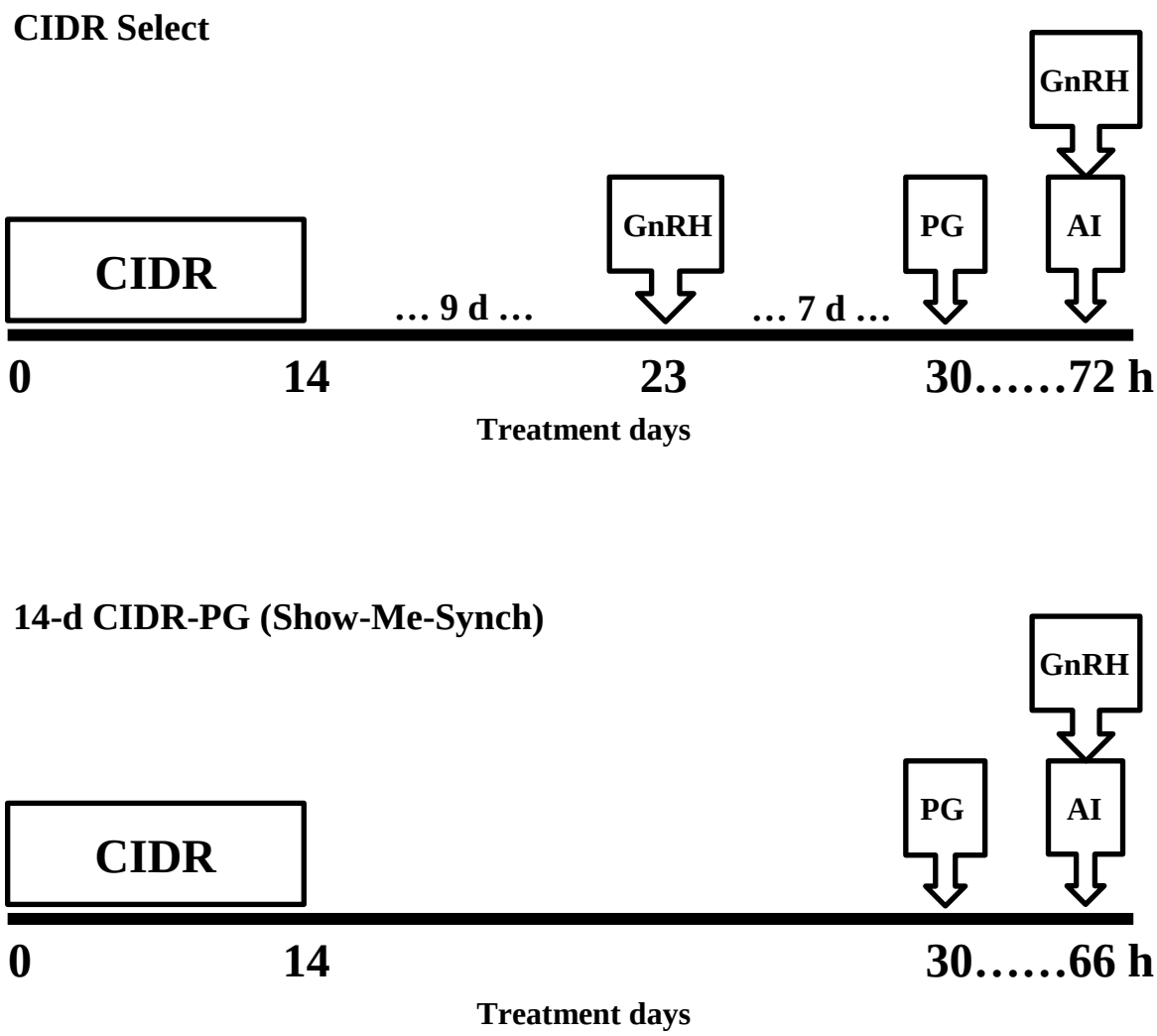


Figure 1.8. Treatment schedule for the CIDR Select and 14-d CIDR-PG (Show-Me-Synch) protocols in heifers (from Mallory et al., 2011).

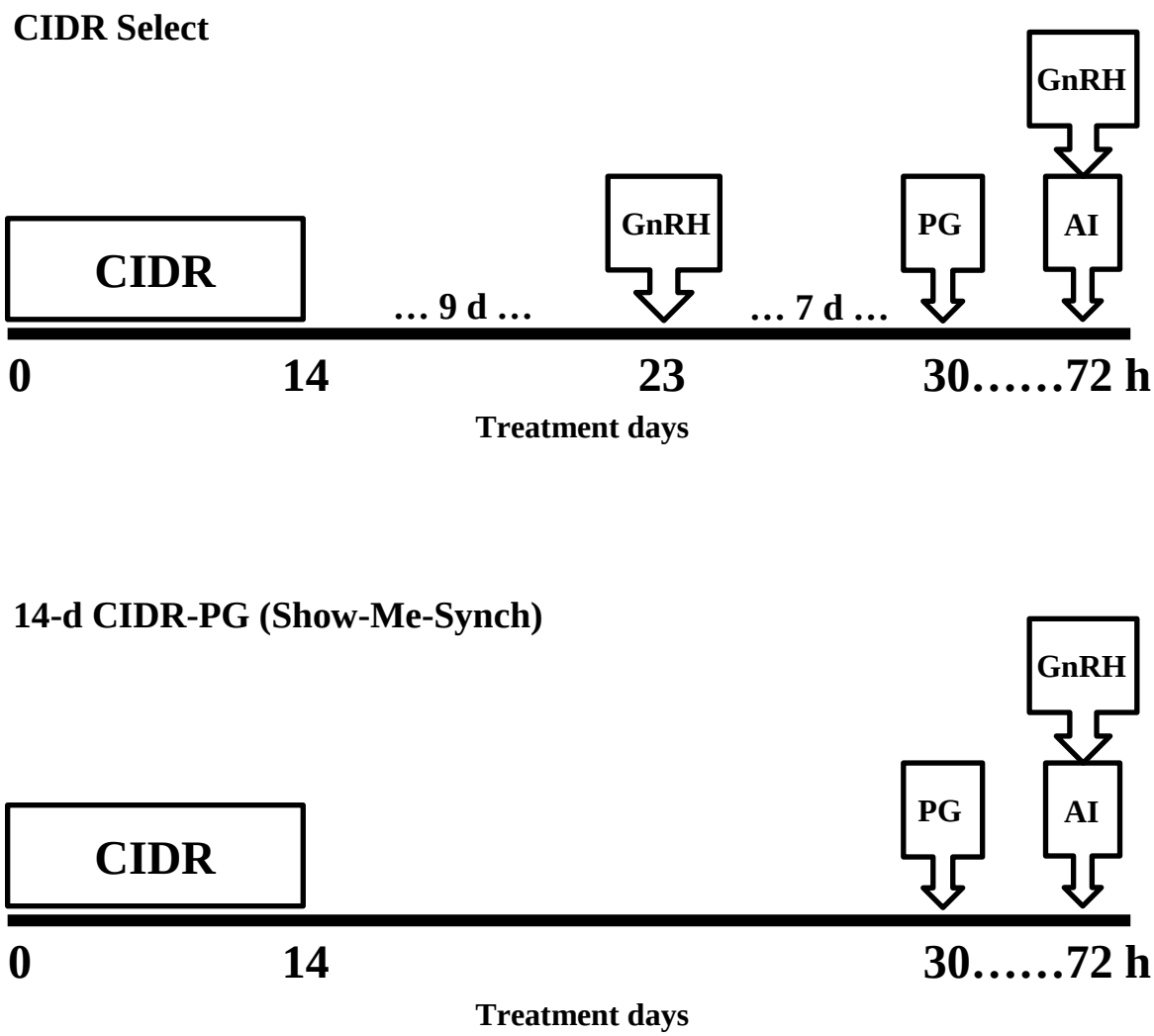


Figure 1.9. Treatment schedule for the CIDR Select and 14-d CIDR-PG (Show-Me-Synch) protocols in cows (from Nash et al., 2011, 2012).

CHAPTER 2

COMPARISON OF LONG-TERM CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND OVULATION PRIOR TO FIXED-TIME ARTIFICIAL INSEMINATION IN POSTPARTUM BEEF COWS

ABSTRACT

This experiment compared two long-term CIDR-based protocols to synchronize estrus and ovulation prior to fixed-time AI (FTAI) in postpartum beef cows. Cows were assigned to treatments by age, body condition score (BCS), and days postpartum (DPP). Cows assigned to the 14-19 d CIDR-PG protocol (n = 196) received CIDR inserts (1.38 g progesterone) from d 0 to 14 and an injection of prostaglandin $F_{2\alpha}$ (PG; 25 mg, i.m.) 19 d after CIDR removal on d 33. Cows assigned to the 14-16 d CIDR-PG protocol (n = 195) received CIDR inserts from d 3 to 17, and PG 16 d after CIDR removal on d 33. Cows in both treatments were artificially inseminated on d 36, 72 h after PG, with GnRH (100 μg, i.m.) at FTAI. Cows were exposed to natural service sires 14 d after FTAI for the remainder of a 75 d breeding season. Blood samples for progesterone (P_4) were collected at d −10 and d 0 to determine pretreatment estrous cyclicity status, and again at PG. Blood samples for estradiol (E_2) were collected at PG and FTAI. HeatWatch estrus detection transmitters were utilized from CIDR removal until FTAI to determine onset of estrus following CIDR removal and PG. Dominant follicle diameter was determined via transrectal ultrasonagraphy at PG and FTAI. Pregnancy diagnosis was performed via

transrectal ultrasonagraphy 70 d after FTAI and confirmed at d 140 of gestation. Age, DPP, and BCS were analyzed by PROC TTEST. Follicle diameters, P_4 and E_2 concentrations were analyzed by PROC MIXED. Pretreatment estrous cyclicity status, estrous response after CIDR removal and PG, FTAI pregnancy rate, final pregnancy rate, and differences in these response variables between age groups (cows aged ≤ 3 vs. ≥ 4 yr) were analyzed by PROC GLIMMIX. There were no differences between treatments for age, DPP, BCS, or pretreatment estrous cyclicity status. Estrous response after CIDR removal was similar between treatments. Cows in both treatments had similar size dominant follicles on d 33 at PG, and d 36 at FTAI. Progesterone concentrations at PG were higher ($P = 0.03$) for 14-16 d CIDR-PG treated cows compared to 14-19 d treated cows (3.5 ng/mL vs. 2.8 ng/mL, respectively). Mean concentrations of E_2 were similar between treatments at PG. Mean concentrations of E_2 at FTAI were higher ($P = 0.01$) for 14-16 d CIDR-PG treated cows compared to 14-19 d treated cows (7.3 pg/mL vs. 6.6 pg/mL, respectively), but there were no differences between treatments in mean concentrations of E_2 at FTAI for cows that failed to exhibit estrus after PG. Overall, estrous response after PG was higher ($P < 0.01$) for cows assigned to the 14-19 d CIDR-PG protocol compared to 14-16 d treated cows (47.4 vs. 29.7%, respectively). Estrous response after PG among cows ≥ 4 yr was higher ($P < 0.01$) for cows assigned to the 14-19 d CIDR-PG protocol compared to the 14-16 d protocol (51.4 vs. 28.3%, respectively), but there were no differences between treatments for cows ≤ 3 yr. Despite differences in estrous response after PG, there was no difference between treatments for pregnancy rate resulting from FTAI ($P = 0.22$; 14-19 d CIDR-PG, 56.6%; 14-16 d CIDR-PG, 51.5%). However, pregnancy rate after FTAI among cows ≥ 4 yr tended to be higher ($P = 0.06$)

for cows assigned to the 14-19 d CIDR-PG protocol compared to the 14-16 d treated cows (66.7 vs. 54.3%, respectively). Final pregnancy rates were similar between treatments (P = 0.82; 14-19 d CIDR-PG, 84.6%; 14-16 d CIDR-PG, 83.9%). In summary, both protocols worked effectively to synchronize estrus and ovulation prior to FTAI in postpartum beef cows, suggesting that a range in interval from CIDR removal to PG may be feasible when using long-term CIDR-based protocols. Additionally, higher estrous response rates after PG and prior to FTAI, and associated improvements in pregnancy rates resulting from FTAI for 14-19 d treated cows ≥ 4 yr of age warrants further consideration.

INTRODUCTION

Estrous synchronization and artificial insemination (AI) are management tools that can be used to improve reproductive performance and efficiency of a cow-calf operation. Estrous synchronization protocols that facilitate fixed-time AI (FTAI) decrease time and labor inputs by eliminating the need for estrus detection, which should result in increased adoption of these technologies in the U.S. beef herd (Patterson et al., 2003). Short-term CIDR-based protocols are generally the preferred method to synchronize estrus in postpartum beef cows, while long-term CIDR-based protocols have been shown to result in higher pregnancy rates in beef heifers (Busch et al., 2007; Leitman et al., 2008). Few studies however, have examined the use of long-term CIDR-based protocols in postpartum beef cows or potential differences in response to these protocols among different age classes of females.

This experiment was designed to compare two long-term CIDR-based protocols on their effectiveness to synchronize estrus and ovulation prior to FTAI. The objectives of the experiment were to evaluate treatments on the basis of estrous response after CIDR removal, estrous response after PG and prior to FTAI, dominant follicle diameters at PG and FTAI, steroid hormone concentrations, FTAI pregnancy rate, and final pregnancy rate. Previous research (Nash et al., 2012) indicates that long-term CIDR based protocols yield comparable pregnancy rates resulting from FTAI in postpartum beef cows compared to short-term CIDR-based protocols. Nash et al. (2012) however, reported that estrous response after PG and prior to FTAI was reduced in cows assigned to a long-term CIDR-based protocol (23%; 14-16 d CIDR-PG) compared to a short-term CIDR-based protocol (49%; 7-d CO-Synch + CIDR). Additionally, estrous response after PG and prior to FTAI among 14-16 d CIDR-PG treated cows (23%; Nash et al., 2012) is lower compared to heifers (78%; Mallory et al., 2011). Heifers tend to exhibit a more highly synchronized distribution of estrus after CIDR removal and a shorter mean interval to estrus compared to cows (Leitman et al., 2008; Mallory et al., 2010; Nash et al., 2011). Collectively, these studies suggest that potential differences in length of estrous cycles or follicular waves among various age groups of females warrants more thorough evaluation of long-term CIDR-based protocols in postpartum beef cows. Given these considerations, the objective herein was to evaluate 14-d CIDR protocols in postpartum beef cows on the basis of potential changes in estrous response after PG and pregnancy rates after FTAI that may occur as a result of lengthening the interval from CIDR removal to PG. The hypothesis tested was that extending the interval from CIDR removal to PG from 16 to

19 d would improve estrous response after PG and improve pregnancy rates resulting from FTAI.

MATERIALS AND METHODS

The experimental procedures involved in this study were approved by the University of Missouri Animal Care and Use Committee.

Experimental design. This experiment was conducted over two years at the University of Missouri Thompson Farm Research Center (Spickard, MO, USA). Primiparous and multiparous, Angus-cross, postpartum beef cows (n = 391) were randomly assigned to one of two treatments that were balanced for age, days postpartum (DPP), and body condition score (BCS; 1 to 9 scale, 1 = emaciated, 9 = obese; Richards et al., 1986). Blood samples were collected on d -10 and d 0 to determine pretreatment estrous cyclicity status (progesterone ≥ 0.5 ng/mL at one or both sampling times, estrous cycling). Cows assigned to the 14-19 d CIDR-PG protocol (n = 196; Figure 2.1) received an EAZI-BREED^{TM} Controlled Internal Drug Release insert (CIDR; 1.38 g progesterone; Pfizer Animal Health, New York, NY) from d 0 to 14 followed by an injection of prostaglandin $F_{2\alpha}$ (PG; 25 mg, i.m.; Lutalyse® Sterile Solution, Pfizer Animal Health) on d 33. Cows assigned to the 14-16 d CIDR-PG protocol (n = 195; Figure 2.1) received a CIDR insert (1.38 g progesterone) from d 3 to 17 and PG (25 mg) on d 33.

Estrus detection and artificial insemination. Cows were fitted with HeatWatch estrus detection transmitters (DDx Inc., Denver, CO) from the time of CIDR removal until FTAI for continuous estrus detection. Estrus was defined as cows receiving ≥ 3 mounts of ≥ 2 s in duration within a 4 h period, with the onset of estrus determined as the

first mount within that time period (Busch et al., 2007). Cows were artificially inseminated on d 36, 72 h after PG, coincident with an injection of GnRH (100 μg, i.m.; Cystorelin®, Merial, Athens, GA). The times for which PG was administered and FTAI was performed were recorded for each cow to determine whether the 72 h interval from PG to FTAI was maintained. Artificial insemination was performed by four experienced technicians and three Angus sires were used artificially. Cows were exposed to natural service sires 14 d after FTAI for the remainder of a 75 d breeding season.

Ultrasonography of dominant follicles. Dominant follicle diameters were measured via transrectal ultrasonography (Aloka 500V equipped with a 7.5 MHz linear array transducer, Aloka, Wallingford, CT) on d 33 at the time of PG injection, and again on d 36 at FTAI. A subset of cows within each treatment were used to characterize follicular dynamics between CIDR removal and PG. Ovaries were mapped every other day beginning 10 d after CIDR removal, coincident with d 24 for cows assigned to the 14-19 d CIDR-PG protocol (n = 41; Figure 2.3) and d 27 for cows assigned to the 14-16 d protocol (n = 43; Figure 2.3). This schedule resulted in two more scans for cows assigned to the 14-19 d protocol compared to the 14-16 d protocol, but allowed for better comparison of treatments, as cows should be at similar time points in their estrous cycles 10 d after CIDR removal. Subsets of cows from each treatment that exhibited estrus and with CL confirmed by ultrasonagraphy 10 d after CIDR removal were examined as described. Cows from each treatment were evenly distributed on the basis of pretreatment estrous cyclicity status and age group (cows aged ≤ 3 vs. ≥ 4 yr). Ovarian structures were mapped and follicles ≥ 5 mm in diameter were recorded to characterize follicular waves and track growth of dominant follicles.

Pregnancy diagnosis. Pregnancy diagnosis was performed by transrectal ultrasonography (Aloka 500V equipped with a 5.0 MHz linear array transducer) 70 d after FTAI. Final pregnancy rate was determined by rectal palpation 140 d after FTAI.

Blood collection and RIA. Blood samples were collected via jugular venipuncture on d -10 and d 0 relative to treatment initiation to determine pretreatment estrous cyclicity status; cows were considered to be estrous cycling if P_4 concentrations were ≥ 0.5 ng/mL at one or both of the pretreatment blood sampling times. Additionally, blood samples were collected on d 33 at PG, and again on d 36 at FTAI. Blood samples were allowed to clot and stored at 4°C for 24 h. Blood samples were centrifuged and serum was separated. Serum samples were stored at -20°C until hormone analyses were performed. Serum concentrations of P_4 and E_2 were determined via radioimmunoassay (RIA) from blood samples collected at PG for all cows, and serum concentrations of E_2 were determined from blood samples collected at FTAI. Serum P_4 concentrations were measured with a Coat-A-Count kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with intra- and inter-assay coefficients of variation of 7.2 and 4.1%, respectively, with an assay sensitivity of 0.1 ng/mL (Kirby et al., 1997). Serum E_2 concentrations were measured by validated extraction assay (Kirby et al., 1997). Intra- and inter-assay coefficients of variation were < 10 and 6.2%, respectively, with an assay sensitivity of 0.5 pg/mL.

Statistical analyses. Differences between treatments in age, DPP, BCS, and interval from PG to FTAI were analyzed by PROC TTEST (SAS Institute Inc., Cary, NC). Differences in mean interval to estrus after CIDR removal and PG were analyzed by ANOVA using the linear statistical model of treatment, year, estrous cyclicity status, and

two-way interactions; and the Bartlett test was used to assess homogeneity of variance (PROC GLM of SAS). The variances for the mean interval to estrus after CIDR removal and PG were found to be heterogeneous between treatments. After attempting a $\log_{10}$ transformation, which failed to stabilize variance, a rank transformation was utilized for testing differences (Conover and Iman, 1981). However, all tables and figures are presented with non-transformed values. Variances associated with the interval to estrus after CIDR removal and after PG were compared by F-test (greater variance divided by the smaller variance; Snedecor and Cochran, 1989). Differences in dominant follicle diameters at PG and FTAI, serum concentrations of P_4 at PG, and serum concentrations of E_2 at PG and FTAI were analyzed using a mixed model (PROC MIXED of SAS) arranged as a 2 x 2 x 2 x 2 factorial (treatment x year x pretreatment estrous cyclicity status x estrous response after PG). Diameters of dominant follicles recorded for the respective time points in the intensive study were analyzed using repeated measures over time using the mixed model procedures of SAS (PROC MIXED) as outlined by Littell et al. (1998). Pretreatment estrous cyclicity status, estrous response after CIDR removal and PG, FTAI pregnancy rate, and final pregnancy rate were analyzed using a generalized linear mixed model (PROC GLIMMIX of SAS), using a binomial distribution and the link function of logit. The model for pretreatment estrous cyclicity status included the main effects of treatment and year and the interaction of treatment x year. The models for estrous response after CIDR removal and PG included the main effects of treatment, year, and pretreatment estrous cyclicity status and two-way interactions. The models for FTAI pregnancy rate and final pregnancy rate included the main effects of treatment, year, and pretreatment estrous cyclicity status and two-way interactions. Treatment differences

among age groups (cows aged ≤ 3 vs. ≥ 4 yr) and differences in FTAI pregnancy rate due to estrous response after PG, AI sire, or AI technician were analyzed using PROC GLIMMIX.

RESULTS

The results from both years of the experiment were similar (no significant treatment x year interactions) and data for the two years were pooled. The number, proportion of estrous cycling cows, age, DPP, and BCS of cows before the initiation of treatments are shown in Table 2.1. There were no differences between treatments for pretreatment estrous cyclicity status (P = 0.60), age (P = 0.82), DPP (P = 0.62), or BCS (P = 0.75).

Estrous response after CIDR removal. Figure 2.2 illustrates the distribution of estrus after CIDR removal. The overall estrous response after CIDR removal was similar between treatments (P = 0.38; Table 2.2). A greater proportion of estrous cycling cows exhibited estrus after CIDR removal compared to anestrous cows (P = 0.001; 70.3% vs. 53.3%). The mean interval to estrus after CIDR removal was similar between treatments (P = 0.19; Table 2.2) and there were no differences between treatments in variances for mean interval to estrus after CIDR removal. There was an effect of estrous cyclicity status on mean interval to estrus, with estrous cycling cows exhibiting longer intervals to estrus after CIDR removal compared to anestrous cows (P = 0.02; 65.4 vs. 54.4 h, respectively).

Dominant follicle diameters. Cows in both treatments had similar size dominant follicles on d 33 at PG (P = 0.79) and on d 36 at FTAI (P = 0.50; Table 2.3). Dominant

follicle diameters were larger ($P < 0.001$) at PG and FTAI for cows that exhibited estrus after PG and prior to FTAI compared to cows that failed to exhibit estrus. In the intensive study, no treatment differences were found in mean follicular diameters at corresponding time points. As well, the slopes of the lines, equivalent to the growth patterns of the dominant follicles, were similar between treatments prior to PG and FTAI (data not shown).

Serum steroid hormone concentrations. Mean concentrations of P_4 at PG are shown in Table 2.4 and mean concentrations of E_2 at PG and FTAI are shown in Table 2.5. Progesterone concentrations were higher ($P = 0.03$) for 14-16 d CIDR-PG treated cows compared to 14-19 d treated cows (3.5 vs. 2.8 ng/mL, respectively). Cows that became pregnant after FTAI had higher ($P < 0.001$) mean concentrations of P_4 at PG compared to cows that failed to become pregnant after FTAI. Cows in year one had higher mean concentrations of P_4 at PG compared to year two ($P < 0.001$). Cows that were estrous cycling prior to the initiation of treatments had higher ($P < 0.001$) concentrations of P_4 at PG than anestrous cows. There were no differences between treatments in the mean concentrations of E_2 at PG ($P = 0.12$). Overall, mean concentrations of E_2 at FTAI were higher ($P = 0.01$) for 14-16 d CIDR-PG treated cows compared to 14-19 d treated cows, but there were no differences between treatments in mean concentrations of E_2 at FTAI for cows that failed to exhibit estrus after PG ($P = 0.11$). Estradiol concentrations were higher ($P < 0.001$) at PG and FTAI for cows that exhibited estrus after PG and prior to FTAI compared to cows that failed to exhibit estrus. Mean concentrations of E_2 at PG and FTAI were higher in year one compared to year two ($P < 0.001$).

Estrous response after PG and prior to FTAI. The distribution of estrus after PG and prior to FTAI is illustrated in Figure 2.4. Estrous response after PG and prior to FTAI was higher (P = 0.001) for cows assigned to the 14-19 d CIDR-PG protocol compared to the 14-16 d CIDR-PG protocol (47.4 vs. 29.7%, respectively; Table 2.6). Mean interval to estrus after PG was similar between treatments (P = 0.33; Table 2.6) and there were no differences in the variances for mean intervals to estrus after PG. Mean interval to estrus after PG was slightly longer in year one compared to year two (P = 0.01; 62.9 vs. 57.3 h, respectively). When data were analyzed within age groups, there was no difference between treatments in estrous response after PG for cows ≤ 3 yr (P = 0.13; Table 2.9); however, there was a significantly higher estrous response among cows ≥ 4 yr that were assigned to the 14-19 d CIDR-PG protocol compared to 14-16 d treated cows (P = 0.001; 51.4 vs. 28.3%; Table 2.9).

Pregnancy rates. Pregnancy rates after FTAI and final pregnancy rates at the end of the breeding season are shown in Table 2.7. Overall, pregnancy rates resulting from FTAI were similar between treatments (P = 0.22) with no differences based on pretreatment estrous cyclicity status (P = 0.16). Final pregnancy rates were also found to be similar between treatments (P = 0.82). The average interval from PG to FTAI for both treatments was 72.7 h ± 0.0 (mean ± SE). There were no differences in pregnancy rates resulting from FTAI based on AI technician (P = 0.85). Pregnancy rates after FTAI were lower (P < 0.001) for cows inseminated with semen from one AI sire used in the experiment, however there was no treatment x AI sire interaction (P = 0.97). Based on this analysis, cows inseminated with semen from this sire remained in the study. The effect of estrous response after PG on pregnancy rates resulting from FTAI is shown in

Table 2.8. Cows that exhibited estrus prior to FTAI achieved higher (P = 0.003) pregnancy rates than those that did not (68.7 vs. 45.0%, respectively), but there were no differences between treatments in FTAI pregnancy rates based on estrous response. When the data were analyzed within age groups, there was no difference between treatments in pregnancy rates resulting from FTAI for cows aged ≤ 3 yr (P = 0.60; Table 2.9). However, among cows ≥ 4 yr, pregnancy rates resulting from FTAI tended to be higher for cows assigned to the 14-19 d CIDR-PG protocol compared to 14-16 d treated cows (P = 0.06; 66.7 vs. 54.3%; Table 2.9).

DISCUSSION

This experiment was conducted to evaluate long-term (14-d) CIDR-based protocols in postpartum beef cows on the basis of extending the interval from CIDR removal to PG. Two long-term CIDR-based protocols were compared on the basis of estrous response after CIDR removal, estrous response after PG and prior to FTAI, dominant follicle diameter at PG and FTAI, steroid hormone concentration patterns, pregnancy rate resulting from FTAI, and final pregnancy rate at the end of the breeding season.

There were no differences between treatments in estrous response, interval to estrus, or associated variances in interval to estrus following CIDR removal. Estrous cycling cows displayed a higher estrous response compared to anestrous cows, but this should be expected as these cows have likely resumed normal LH pulsatility patterns (Arije et al., 1974) that would increase the likelihood of estrus after CIDR removal. Estrous cycling cows exhibited longer mean intervals to estrus after CIDR removal

compared to anestrous cows. This pattern is consistent with trends reported by Nash et al. (2011) and may be explained by variation in stage of the estrous cycle at the time CIDRs were inserted. For example, if estrous cycling cows had a CL present at the time of CIDR removal, expression of estrus would not be possible until the CL was regressed. Overall, the mean interval to estrus after CIDR removal was 59.2 h. This interval was longer than that seen in 14-d CIDR-PG treated heifers (31.1 h, Leitman et al., 2008; 37.8 h, Mallory et al., 2010), which supports the rationale for extending the interval from CIDR removal to PG. It is also important to note that a large proportion of anestrous cows displayed estrus after CIDR removal, indicating that cows either resumed normal estrous cyclicity or were induced to cycle after progestin exposure.

Despite differences between protocols in the interval from CIDR removal to estrus, mean diameters of dominant follicles were similar between treatments on d 33 at PG and d 36 at FTAI. Mean follicle diameters increased by approximately 2.3 mm from PG to FTAI, suggesting that both treatments had similar growth patterns of dominant follicles between these time points. Dominant follicles appear to acquire ovulatory capacity at a diameter around 10 mm (Sartori et al., 2001). Perry et al. (2005b) reported that GnRH-induced ovulation of follicles $\leq$ 11 mm resulted in decreased pregnancy rates and increased late embryonic mortality in heifers. In the current study, cows that exhibited estrus after PG and prior to FTAI had larger dominant follicles at PG and FTAI compared to those that did not exhibit estrus. Still, mean dominant follicle diameters for cows that failed to exhibit estrus after PG measured $\geq$ 13.2 mm, suggesting they have acquired ovulatory capacity and potential for acceptable fertility after GnRH-induced ovulation and FTAI. Subsets of cows from each treatment were used to characterize

follicular growth patterns after CIDR removal and prior to PG. Ultrasound was performed on an every other day schedule beginning 10 d after CIDR removal. These data failed to provide any conclusive evidence to suggest differences between treatments in follicular dynamics prior to PG. Daily ovarian scans may have provided a more descriptive characterization of treatments, but the every other day schedule was used in an attempt to prevent compromised fertility at FTAI that may have occurred from stress related to a daily scanning schedule.

Mean concentrations of P_4 at PG were higher for cows assigned to the 14-16 d CIDR-PG protocol compared to the 14-19 d treated cows. This may be due to the extended interval from CIDR removal to PG for the 14-19 d treated cows, in which case a proportion of these cows may have initiated normal CL regression, leading to reduced P_4 concentrations at PG. Estrous cycling cows were more likely to exhibit estrus after CIDR removal, which may explain differences in P_4 concentrations at PG based on estrous cyclicity status, as cows that exhibited estrus following CIDR removal should have functional CL at the time of PG. Elevated concentrations of P_4 during the estrous cycle preceding AI are associated with increased pregnancy rates (Bello et al., 2006; Corah et al., 1974; Folman et al., 1973). In the current study, mean concentrations of P_4 at PG were higher for cows that became pregnant after FTAI compared to those that failed to become pregnant. Mean concentrations of E_2 at PG were similar between treatments, while mean concentrations of E_2 at FTAI were higher for cows assigned to the 14-16 d CIDR-PG protocol compared to 14-19 d treated cows. However, mean concentrations of E_2 at FTAI were similar between treatments for cows that failed to exhibit estrus after PG. The increase in E_2 from PG to FTAI was consistent between treatments and

corresponds to the increase in mean diameters of dominant follicles at these times. Additionally, elevated concentrations of E_2 at PG and FTAI for cows that exhibited estrus after PG and prior to FTAI corresponds with larger size dominant follicles observed at the respective time points.

Nash et al. (2012) reported that pregnancy rates resulting from FTAI were similar for cows assigned to long-term CIDR-based protocols compared to short-term CIDR-based protocols. However, estrous response after PG and prior to FTAI was reduced in cows assigned to the long-term (23%; 14-d CIDR-PG) compared to the short-term protocol (49%; 7-d CO-Synch + CIDR; Nash et al., 2012). These data supported the rationale for this experiment in extending the interval from CIDR removal to PG, which was hypothesized to increase estrous response after PG and potentiate improvements in pregnancy rates after FTAI. The results presented here indicate that a greater proportion of cows assigned to the 14-19 d CIDR-PG protocol exhibited estrus after PG and prior to FTAI compared to the 14-16 d treated cows. Estrous response after PG for 14-16 d CIDR-PG treated cows in this study was comparable to response rates reported by Nash et al. (2012); whereas higher estrous response rates for cows assigned to the 14-19 d CIDR-PG protocol were comparable to those observed in cows following treatment with the short-term, 7-d CO-Synch + CIDR protocol (Busch et al., 2008, Nash et al., 2012). One of the goals of this experiment was to characterize differences between treatments among various age classes of females. The fact that estrous response after PG was only improved among cows ≥ 4 yr assigned to the 14-19 CIDR-PG protocol, points to an interesting trend in differences between the two treatments.

Although the 14-19 d CIDR-PG protocol was successful in improving estrous response after PG, there were no differences between treatments in pregnancy rates resulting from FTAI. This suggests that both protocols were successful in synchronizing estrus and ovulation to facilitate FTAI. Cows in both treatments that exhibited estrus prior to FTAI had higher pregnancy rates than those that failed to exhibit estrus, consistent with previous studies (Busch et al., 2008, Nash et al., 2012; Perry et al., 2005b). When differences between age groups were compared, there was a trend toward higher pregnancy rates after FTAI among cows ≥ 4 yr that were assigned to the 14-19 d protocol compared to similar age cows assigned to the 14-16 d protocol. These differences were reflected in differences between treatments in estrous response after PG for cows in the respective age groups. These results provide evidence that a 14-16 d CIDR-PG schedule, used successfully in heifers (Leitman et al., 2009b; Mallory et al., 2011), may be a more appropriate schedule for younger age cows (≤ 3 yr), whereas a 14-19 d schedule is perhaps better suited for cows ≥ 4 yr. Further studies are needed to more carefully characterize differences among various age groups of females on the basis of differences in length of estrous cycles or follicular waves following a 14-d CIDR treatment.

In conclusion, both protocols worked effectively to synchronize estrus and ovulation prior to FTAI in postpartum beef cows, indicating that a range in interval from CIDR removal to PG may be feasible when using long-term CIDR-based protocols. Beef producers may be reluctant to use long-term CIDR-based protocols, as these protocols are more challenging to implement in herds with extended calving periods. Furthermore, long-term CIDR-based protocols require that cows are handled one extra time compared

to short-term schedules (7-d CO-Synch + CIDR). Long-term protocols provide a unique opportunity however, to combine animal health and reproduction in a single management step, by administering pre-breeding vaccinations at the time of CIDR insertion. These results agree with previous studies (Nash et al., 2011; 2012) suggesting that long-term CIDR-based protocols provide an alternative method of synchronizing estrus prior to FTAI in postpartum beef cows, which at the same time provide labor-reducing management options pertaining to animal health-related considerations.

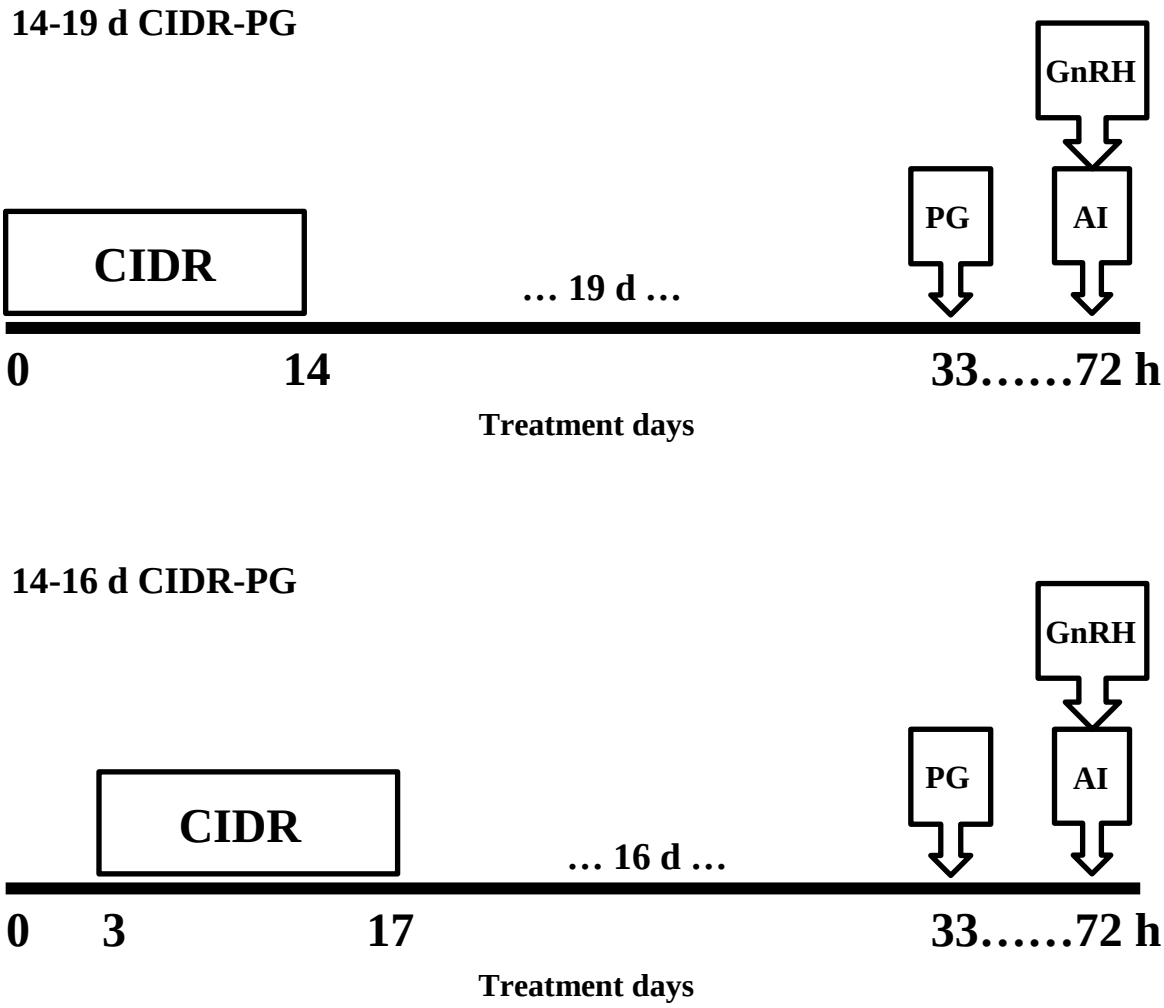


Figure 2.1. Treatment schedule for the 14-19 d CIDR-PG and 14-16 d CIDR-PG protocols. Cows assigned to the 14-19 d CIDR-PG protocol received CIDR inserts (1.38 g progesterone) from d 0 to 14 and an injection of prostaglandin F2α (PG; 25 mg, i.m.) 19 d after CIDR removal on d 33. Cows assigned to the 14-16 d CIDR-PG protocol received CIDR inserts from d 3 to 17, and PG 16 d after CIDR removal on d 33. Cows in both treatments were artificially inseminated on d 36, 72 h after PG, with GnRH (100 μg i.m.) at FTAI.

Table 2.1. Number, estrous cyclicity status, age, days postpartum (DPP), and body condition score (BCS) of cows prior to treatment initiation (mean ± SE).

	Treatment[1]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
No. of cows	196	195	---
Cows with elevated progesterone[2]	74/196 = 37.8%	71/195 = 36.4%	0.60
Age[3], yr	5.2 ± 0.2	5.0 ± 0.2	0.82
DPP[4], d	44.8 ± 1.3	45.7 ± 1.3	0.62
BCS[5]	5.3 ± 0.0	5.2 ± 0.0	0.75

[1]See Figure 2.1 for a description of treatment protocols.

[2]Estrous cyclicity is equal to the number of cows with elevated (≥ 0.5 ng/mL) concentrations of progesterone in blood serum prior to treatment initiation. Cows were considered to be estrous cycling if progesterone was elevated in either one or both blood samples collected on d -10 and d 0.

[3]Age (yr) of cows at initiation of treatments.

[4]Number of days postpartum on d 0.

[5]Body condition scores of cows on d 0 (1 to 9 scale; 1 = emaciated, 9 = obese).

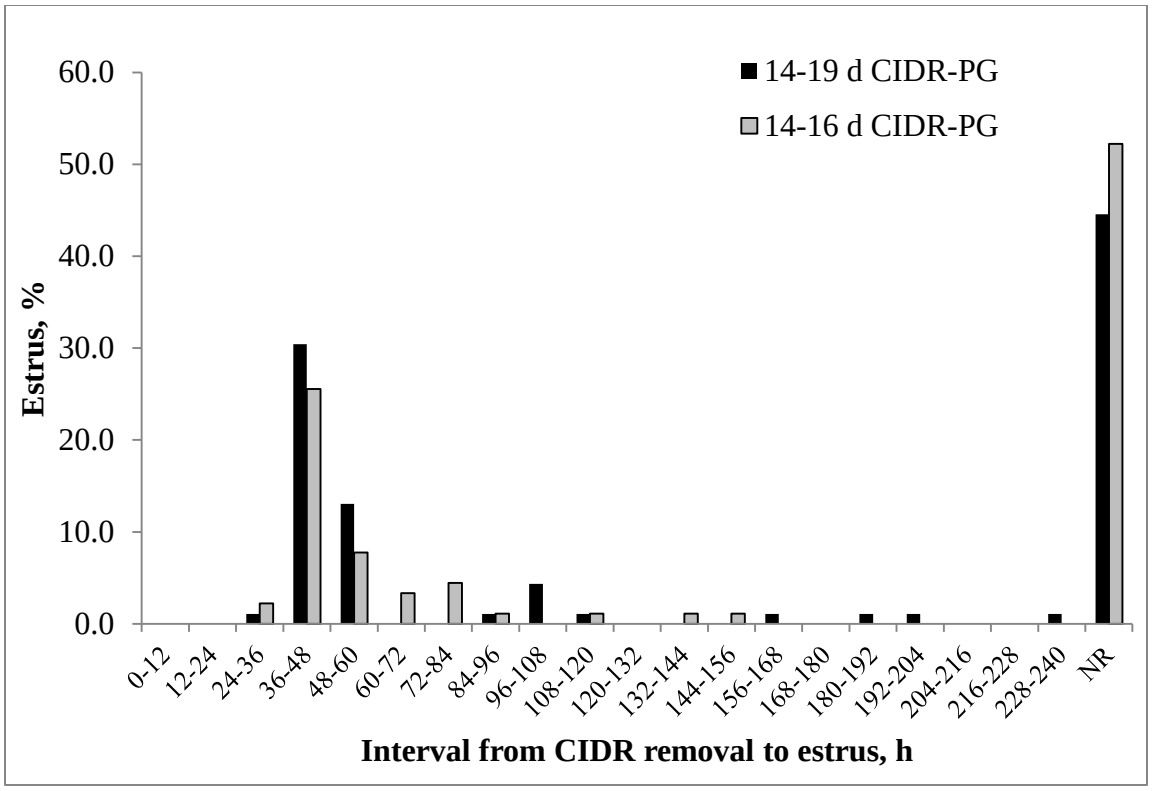


Figure 2.2. Distribution of estrus after CIDR[1] removal. Percentage of cows that exhibited estrus within each 12 h interval following CIDR removal; graphed by treatment: 14-19 d CIDR-PG (black bar) and 14-16 d CIDR-PG (gray bar); NR = no estrous response. See Figure 2.1 for a description of treatment protocols.

[1]CIDR = EAZI-Breed CIDR insert (1.38 g of progesterone; Pfizer Animal Health, New York, NY).

Table 2.2. Estrous response after CIDR[1] removal.

	Treatment[2]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Overall estrous response	124/196 = 63.3%	109/195 = 55.9%	0.38
Estrous-cycling	52/74 = 70.3%	50/71 = 70.4%	0.98
Anestrus	72/122 = 59.0%	59/124 = 47.6%	0.11
Interval to estrus, h; mean ± SE	63.2 ± 3.4	54.7 ± 2.2	0.19
Estrous-cycling; mean ± SE	73.5 ± 4.4	57.1 ± 4.7	0.18
Anestrus; mean ± SE	55.8 ± 3.8	52.7 ± 4.1	0.64

[1]CIDR = EAZI-Breed CIDR insert (1.38 g of progesterone; Pfizer Animal Health, New York, NY).

[2]See Figure 2.1 for a description of treatment protocols.

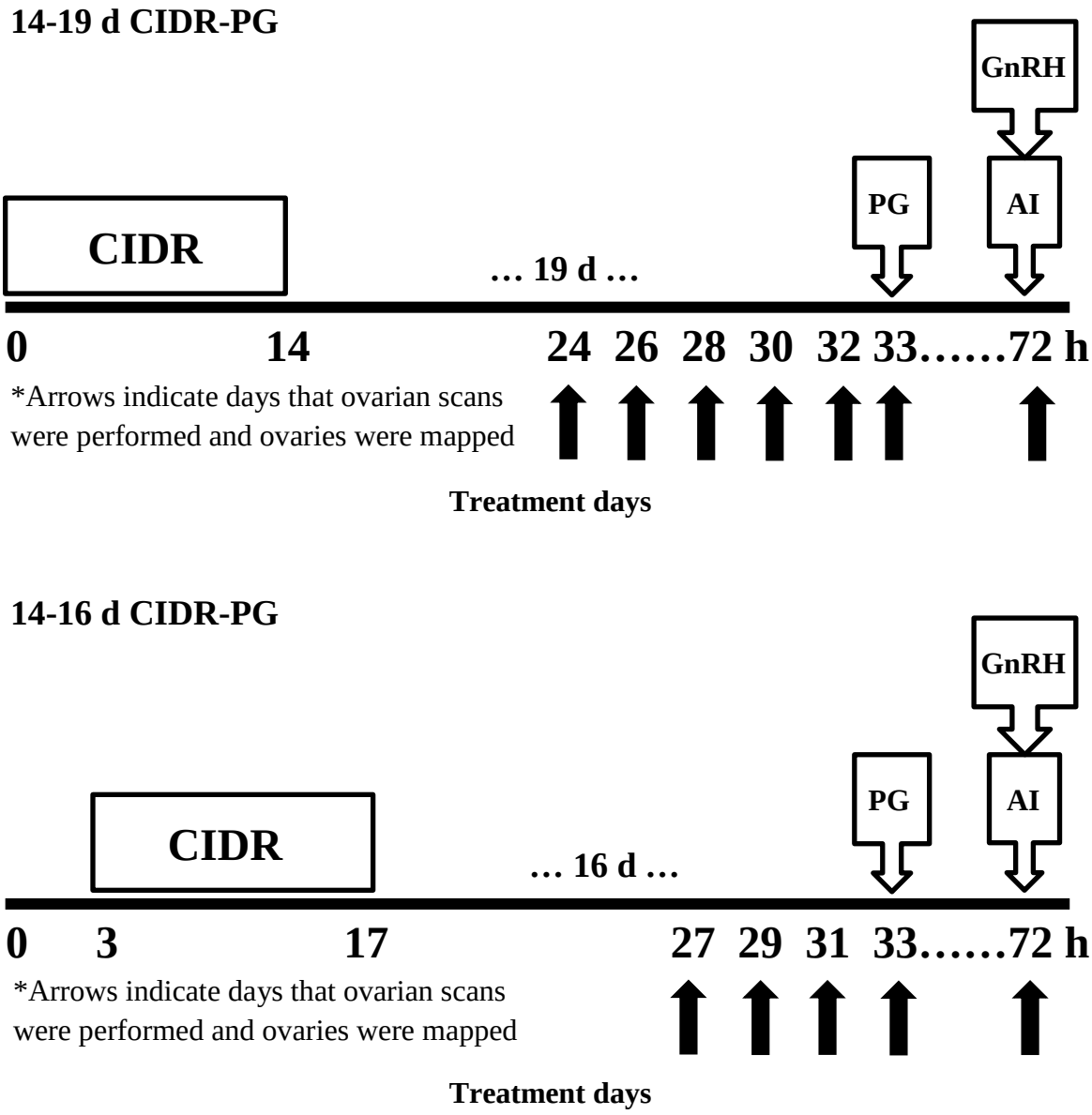


Figure 2.3. Treatment schedule for the intensive study to characterize follicular dynamics between CIDR removal and PG for the 14-19 d CIDR-PG and 14-16 d CIDR-PG protocols. Ovarian scans were performed and ovaries were mapped every other day beginning 10 d after CIDR removal; beginning on d 24 for cows assigned to the 14-19 d CIDR-PG protocol and d 27 for cows assigned to the 14-16 d CIDR-PG protocol. Arrows indicate days on which ovarian scans were performed and ovaries were mapped.

Table 2.3. Dominant follicle diameters at PG[1] and FTAI[2].

	Treatment[3]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Follicle diameter at PG, mm (mean ± SE)	11.5 ± 0.2	11.4 ± 0.2	0.47
Exhibited estrus after PG and prior to FTAI	12.1 ± 0.2[y]	12.3 ± 0.3[y]	0.38
Failed to exhibit estrus after PG and prior to FTAI	10.9 ± 0.2[z]	11.0 ± 0.2[z]	0.95
Follicle diameter at FTAI, mm (mean ± SE)	13.8 ± 0.1	13.7 ± 0.1	0.56
Exhibited estrus after PG and prior to FTAI	14.5 ± 0.2[y]	14.7 ± 0.2[y]	0.50
Failed to exhibit estrus after PG and prior to FTAI	13.2 ± 0.2[z]	13.3 ± 0.2[z]	0.94

[1]PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).[1]

[2]FTAI = Fixed-time artificial insemination.

[3]See Figure 2.1 for a description of the treatment protocols.

[y,z]Means within columns with different superscripts differ ($P < 0.05$).

Table 2.4. Serum concentrations of progesterone at PG[1].

	Treatment[1]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Serum concentrations of progesterone at PG (ng/mL) (mean ± SE)	2.9 ± 0.2[a]	3.5 ± 0.2[b]	0.03
Exhibited estrus after PG and prior to FTAI	3.0 ± 0.3	3.3 ± 0.3	0.33
Failed to exhibit estrus after PG and prior to FTAI	2.7 ± 0.2[a]	3.5 ± 0.2[b]	0.02
Became pregnant after FTAI	3.4 ± 0.2[a, y]	4.4 ± 0.2[b, y]	0.002
Failed to become pregnant after FTAI	2.1 ± 0.3[z]	2.5 ± 0.2[z]	0.25

[1]See Figure 2.1 for a description of the treatment protocols.

[2]PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).

[3]FTAI = Fixed-time artificial insemination.

[a,b]Means within rows with different superscripts differ ($P < 0.05$).

[y,z]Means within columns with different superscripts differ ($P < 0.05$).

Table 2.5. Serum concentrations of estradiol-17β at PG[1] and FTAI[2].

	Treatment[3]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Serum concentrations of estradiol-17β at PG (pg/mL) (mean ± SE)	3.5 ± 0.2	3.7 ± 0.2	0.12
Exhibited estrus after PG and prior to FTAI	4.1 ± 0.2^{y}	4.0 ± 0.3^{y}	0.63
Failed to exhibit estrus after PG and prior to FTAI	3.0 ± 0.2^{z}	3.5 ± 0.2^{z}	0.07
Serum concentrations of estradiol-17β at FTAI (pg/mL) (mean ± SE)	6.6 ± 0.2^{a}	7.3 ± 0.2^{b}	0.01
Exhibited estrus after PG and prior to FTAI	7.0 ± 0.3^{y}	8.3 ± 0.4^{y}	0.06
Failed to exhibit estrus after PG and prior to FTAI	6.3 ± 0.3^{z}	6.8 ± 0.3^{z}	0.11

[1]PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).[1]

[2]FTAI = Fixed-time artificial insemination.

[3]See Figure 2.1 for a description of the treatment protocols.

[a,b]Means within rows with different superscripts differ ($P < 0.05$).

[y,z]Means within columns with different superscripts differ ($P < 0.05$).

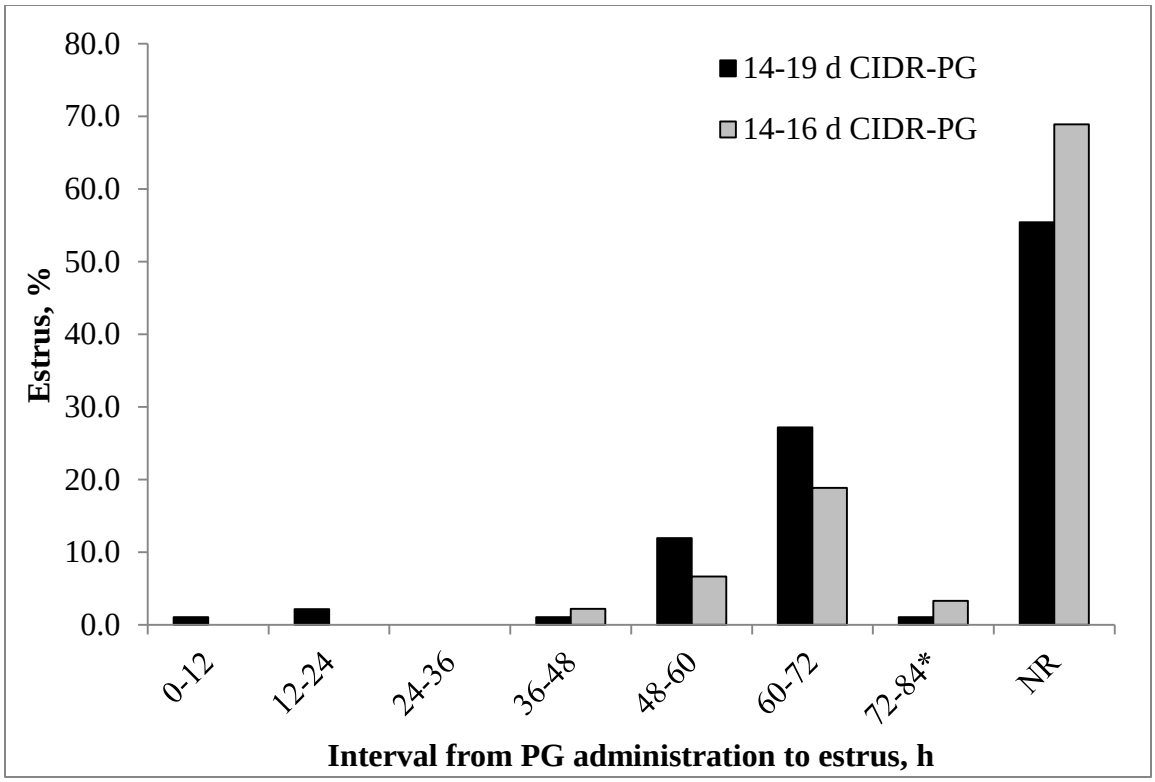


Figure 2.4. Distribution of estrus after PG[1] and prior to FTAI[2]. Percentage of cows that exhibited estrus within each 12 h interval following PG administration up to FTAI; graphed by treatment: 14-19 d CIDR-PG (black bar) and 14-16 d CIDR-PG (gray bar); NR = no estrous response. See Figure 2.1 for a description of treatment protocols.

[1]PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).

[2]FTAI = Fixed-time artificial insemination

*Heatwatch estrus detection transmitters were removed at FTAI. Due to random order of treatment at PG and FTAI, the interval between PG and FTAI was slightly greater than 72 h for a small number of cows. The longest recorded interval from PG administration to estrus was 72.3 h. See Table 2.5 for interval mean interval to estrus after PG and prior to FTAI.

Table 2.6. Estrous response after PG[1] and prior to FTAI[2].

	Treatment[3]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Estrous response	93/196 = 47.4%[a]	58/195 = 29.7%[b]	0.001
Estrous-cycling	34/74 = 45.9%	22/71 = 31.0%	0.07
Anestrus	59/122 = 48.4%[a]	36/124 = 29.0%[b]	0.003
Interval to estrus, h; mean ± SE	59.3 ± 1.1	61.4 ± 1.4	0.33
Estrous-cycling; mean ± SE	60.3 ± 1.4	63.3 ± 2.4	0.33
Anestrus; mean ± SE	57.9 ± 1.8	60.2 ± 1.8	0.75

[1]PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).

[2]FTAI = Fixed-time artificial insemination

[3]See Figure 2.1 for a description of treatment protocols.

[a,b]Means within rows with different superscripts differ ($P < 0.05$).

Table 2.7. FTAI[1] pregnancy rate and final pregnancy rate.

	Treatment[2]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
FTAI pregnancy rate[3]	111/196* = 56.6%	100/194* = 51.5%	0.22
Estrous cycling	48/74 = 64.7%	38/71 = 53.5%	0.16
Anestrus	63/122 = 51.6%	62/123 = 50.4%	0.91
Final pregnancy rate[4]	165/195* = 84.6%	161/192* = 83.9%	0.82

[1]FTAI = Fixed-time artificial insemination.

[2]See Figure 2.1 for a description of treatment protocols.

[3]FTAI pregnancy rate determined by ultrasound 70 d after FTAI.

[4]Final pregnancy rate determined by rectal palpation 140 d after FTAI.

*Discrepancies in totals result from cows in which FTAI pregnancy or final pregnancy diagnosis was not performed due to unrelated morbidity or mortality.

Table 2.8. Effect of estrous response after PG[1] on FTAI[2] pregnancy rate.

	Treatment[3]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Overall estrous response after PG	93/196 = 47.4%[a]	58/195* = 29.7%[b]	0.001
FTAI pregnancy rate[4]			
Cows that exhibited estrus	64/93 = 68.8%[y]	39/57* = 68.4%[y]	0.96
Cows that failed to exhibit estrus	47/103 = 45.6%[z]	61/137* = 44.5%[z]	0.86

[1] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).

[2]FTAI = Fixed-time artificial insemination.

[3]See Figure 2.1 for a description of treatment protocols.

[4]FTAI pregnancy rate determined by ultrasound 70 d after FTAI.

[a,b]Means within rows with different superscripts differ ($P < 0.05$).

[y,z]Means within columns with different superscripts differ ($P < 0.05$).

*Discrepancies in totals result from cows in which FTAI pregnancy diagnosis was not performed due to unrelated morbidity or mortality.

Table 2.9. Estrous response after PG[1] and FTAI[2] pregnancy rate by age group.

	Treatment[3]		
	14-19 d CIDR-PG	14-16 d CIDR-PG	P-value
Overall estrous response after PG	93/196 = 47.4%[a]	58/195* = 29.7%[b]	0.001
Cows aged ≤ 3 years	36/85 = 42.4%	28/89 = 31.5%	0.13
Cows aged ≥ 4 years	57/111 = 51.4%[a]	30/106* = 28.3%[b]	0.001
Overall FTAI pregnancy rate[4]	111/196 = 56.6%	100/194* = 51.5%	0.22
Cows aged ≤ 3 years	37/85 = 43.5%	43/89 = 48.3%	0.60
Cows aged ≥ 4 years	74/111 = 66.7%[c]	57/105* = 54.3%[d]	0.06

[1] PG = $PGF_{2\alpha}$ (25 mg i.m., Lutalyse; Pfizer Animal Health, New York, NY).

[2]FTAI = Fixed-time artificial insemination.

[3]See Figure 2.1 for a description of treatment protocols.

[4]FTAI pregnancy rate determined by ultrasound 70 d after FTAI.

[a,b]Means within rows with different superscripts differ ($P < 0.05$).

[c,d]Means within rows with different superscripts tended to differ ($P = 0.06$).

*Discrepancies in totals result from cows in which FTAI pregnancy diagnosis was not performed due to unrelated morbidity or mortality.

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VITA

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