COMPARISON OF CONTROLLED INTERNAL DRUG RELEASE (CIDR)-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND FACILITATE ARTIFICIAL INSEMINATION (AI) IN POSTPARTUM BEEF COWS

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

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DEDICATION

I dedicate this thesis to my family and friends that have supported me in my continued education. First to my daughter, Faith Wilson, who gave me the strength to keep going; to my parents Dean Wilson and Diana Jones for always believing in me and instilling in me the values of hard work, honesty, and responsibility; to Christy Rader for your friendship and patience; to Dr. David Patterson for giving me the opportunity to continue my education and the confidence and support to complete it. To all of you and many others that helped me to fully realize my goals and aspirations; it would not have been possible without your support and understanding.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
BCS	Body condition score
CL	Corpus luteum/Corpora lutea
cm	Centimeter(s)
CIDR	Controlled Internal Drug Release
d	Day(s)
DPP	Days postpartum
E_2	Estradiol-17β
FSH	Follicle stimulating hormone
FTAI	Fixed-time insemination
GnRH	Gonadotropin releasing hormone
h	Hour(s)
kg	Kilogram(s)
LH	Luteinizing hormone
MGA	Melengestrol acetate
mL	Milliliter(s)
mm	Millimeter(s)
ng	Nanogram(s)
P_4	Progesterone

PG	Prostaglandin $F_{2\alpha}$
RIA	Radioimmunoassay
s	Second(s)
T	Treatment
US	United States

COMPARISON OF CIDR-BASED PROTOCOLS TO SYNCHRONIZE ESTRUS AND FACILITATE AI IN POSTPARTUM BEEF COWS

Dallas James Wilson

Dr. David J. Patterson, Thesis Advisor

ABSTRACT

Artificial insemination (AI) and estrous synchronization are reproductive technologies that enable beef producers to efficiently improve herd genetics. To date, however, beef producers have been reluctant to adopt these technologies, citing a lack of time and labor as the primary reason for failure of adoption. Effective protocols to synchronize estrus should minimize the number and frequency of cattle handlings, while reducing or eliminating estrus detection. Development of effective estrous synchronization protocols is necessary for the expanded acceptance and use of AI in U.S. beef herds. The recent development of protocols designed to synchronize estrus and ovulation enable producers to utilize fixed-time AI (**FTAI**), thus reducing time and labor required to detect estrus.

The 7-d Select Synch and CO-Synch + CIDR protocols provide an efficient and effective means to synchronize estrus in postpartum beef cows. The Select Synch and CO-Synch + CIDR protocols are similar except that insemination is performed on the basis of a recorded estrus rather than at a predetermined fixed-time for the Select Synch, and CO- Synch + CIDR protocols, respectively. Recently a 5-d Select Synch and CO-Synch + CIDR protocol was developed. To date, however, the literature is devoid of

information comparing the 7-d and 5-d protocols on the basis of their practical application in the field.

Experiment 1 was designed to fully characterize and compare the 7-d and 5-d Select Synch + CIDR protocols on the basis of follicular dynamics and timing and synchrony of estrus following treatment administration. There were no differences between treatments for estrous response, interval to estrus, or the variance for interval to estrus. Additionally, response to GnRH and follicle size at GnRH did not differ between the two treatments, nor were there any differences in synchronized conception or pregnancy rates resulting from AI. The results from Experiment 1 indicate that the 5-d and 7-d Select Synch + CIDR protocols performed similarly on the basis of inducing and synchronizing estrus in postpartum beef cows with no resulting differences between treatments in pregnancy rates resulting from AI. Experiment 2 was designed to compare pregnancy rates resulting from FTAI following administration of the 7-d and 5-d CO-Synch + CIDR protocols with insemination performed 66 and 72 h following treatment, for cows assigned to the two treatments, respectively. There was no effect of treatment, technician, sire, or location on FTAI pregnancy rates. The results from this experiment demonstrate that the two protocols performed comparably on the basis of pregnancy rates resulting from FTAI.

Given these observations, the 5-d protocol provides an alternative to the 7-d protocol for use in facilitating FTAI, however beef producers must seriously consider the increased labor and treatment costs associated with this protocol, compared with the 7-d protocol in which cows are inseminated 66 h following treatment administration.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Reproductive efficiency is an important determinant of profitability in a beef cow-calf operation (Dickerson, 1970). The pounds of calf weaned per cow exposed for breeding is used to gauge reproductive efficiency in a cow herd, and can be greatly affected by cows that fail to become pregnant by the end of the breeding season. Cows failing to conceive during the breeding season account for nearly one-half of the losses in potential calves (Wiltbank, 1983). Estrous synchronization and AI are tools that enhance reproductive management in beef cattle. Estrous synchronization allows for more cows to become pregnant early in the breeding season, which is essential to maintaining a 365 d calving interval. This also allows more time for cows to resume estrous cyclicity before the beginning of the subsequent breeding season. Estrous synchronization improves uniformity of a calf crop (Dziuk and Bellows, 1983). A concentrated calving period that results in a more uniform calf crop allows producers to more efficiently allocate labor at calving over a few weeks instead of months. There is also a greater economic value associated with more uniform calves at sale. Improvements to beef cow herds are expanded through the use of AI as a result of superior and varied genetics that beef producers may utilize without owning an expensive bull (Trenkle and Willham, 1977).

These management techniques have been available for over 30 yr allowing producers to increase pounds of calf weaned per cow exposed, shorten the calving season

and improve calf uniformity at weaning (Dziuk and Bellows, 1983). For these reasons, artificial insemination and estrous synchronization are generally regarded as the most important and applicable of all available biotechnologies to the beef cattle industry (Seidel, 1995).

Currently, however, beef producers have been reluctant to apply these reproductive technologies on their farms or ranches. Recent surveys indicate that only 13.3% of beef operations utilize AI and 11.9% of operations use available methods of estrous synchronization (NAHMS, 1998). Major reasons cited for a failure to adopt these technologies include "time and labor constraints", followed by the perception that the technologies are too "complicated" or "costly" to implement (NAHMS, 1998). Low adoption rates of these practices lead one to question the future competitive position of the U.S. beef cattle industry, especially when one considers the rate of technology adoption in other parts of the world (Kojima, 2003). Countries such as Brazil have adopted AI and estrous synchronization at a much faster pace than that of the United States. In the last 20 yr domestic sales of beef semen in the U.S. increased from 800,000 to 1.35 million units, an increase of 59% (NAAB, 2007). However, in Brazil during that same time, sales of beef semen increased from 1.5 to 4.6 million units, a staggering 212% increase (ASBIA, 2007). All of the advantages associated with estrous synchronization and AI, lead one to believe that the Brazilian beef cattle herd is improving at a rapid pace.

It is important that effective estrous synchronization protocols are developed in order to increase the use of AI. Estrous synchronization protocols should be designed to reduce time and labor inputs by limiting cattle handlings and reducing or eliminating estrus detection (Larson et al., 2006). Recently, the development of estrous

synchronization protocols was expanded to include protocols that facilitate fixed-time artificial insemination (**FTAI**). Protocols that facilitate FTAI drastically reduce labor inputs by reducing or eliminating the need to detect estrus and at the same time accommodating high pregnancy rates resulting from AI. The development of methods that facilitate expanded use of FTAI for beef cows and heifers is dependent upon protocols to synchronize estrus and ovulation that are practical to implement, affordable to use, and that result in highly acceptable pregnancy rates following treatment administration (Patterson et al., 2003).

A REVIEW OF THE BOVINE ESTROUS CYCLE

Endocrinology of the Bovine Estrous Cycle

The mean interval from parturition to the initiation of estrous cyclicity in mature, postpartum cows may vary from 34 to 84 d (Casida, 1971; Wiltbank, 1970). The estrous cycle of the cow is based on the continuous succession of physiological events that begin and end with estrus. The estrous cycle of the bovine female lasts an average of 21 d, but may vary from 18 to 24 d depending on the animal (Hansel and Convey, 1983). The estrous cycle of the cow can be categorized in three distinct phases comprised of estrus, the follicular, and luteal phases. Estrus refers to the period from the onset of estrus to ovulation. The follicular phase includes the time period from regression of the corpus luteum (**CL**) to the onset of estrus. The luteal phase comprises 80% of the estrous cycle, lasting from ovulation and CL development to CL regression (Senger, 2003). When applied to the original mouse model, the luteal phase is comprised of metestrus and

diestrus, while proestrus makes up the follicular phase. The estrous cycle begins and ends with estrus.

Estrus. Estrus, the period of sexual receptivity, may last from 12 to 18 h in the cow (Nalbandov and Casida, 1942; Wiltbank et al., 1967). Various hormones work to control both the physiological and behavioral changes that occur during estrus. Estradiol-17β (**E_2**) is a steroid hormone that is produced by growing follicles located on the ovary. The functions of E_2 at estrus include the display of behavioral estrus, preparation of the vaginal and uterine environments for mating, and stimulation of the preovulatory surge of lutenizing hormone (**LH**; Schallenberger et al., 1984; Walters et al., 1984). When concentrations of E_2 reach threshold levels, positive feedback activates the preovulatory surge center in the hypothalamus, which results in the "surge release" of LH and FSH from the anterior pituitary in response to the increased episodic release of gonadotropin-releasing hormone (**GnRH**) from the hypothalamus. (Cupp et al., 1995; Moenter et al., 1990; Schallenberger et al., 1984; Spicer and Echternkamp, 1986; Walters et al., 1984). The LH surge, more correctly defined as frequent, high amplitude LH pulses (Rahe et al., 1980), concludes in spite of lingering GnRH secretion, suggestive of pituitary desensitization and/or depletion of LH secretory capacity (Moenter et al., 1991). The preovulatory LH surge is required for the last phase of maturation of the dominant follicle and ovulation of the oocyte (Garverick and Smith, 1986; Ginther et al., 2000; Schallenberger et al., 1984).

Metestrus. After the preovulatory LH surge, the subsequent ovulation occurs 24 to 32 hr later (Senger, 2003; Wiltbank et al., 1967). Luteinizing hormone concentrations steadily decline after ovulation (Cupp et al., 1995; Garverick et al., 1971; Hansel and

Convey, 1983; Schallenberger et al., 1984; Stumpf et al., 1989), but LH continues to play an important role in ovarian function. Luteinizing hormone controls the transformation or luteinization of the theca and granulosa cells into small and large luteal cells, respectively (Garverick and Smith, 1986; Garverick et al., 1992; Niswender et al., 1986). These luteal cells make up the newly forming endocrine gland, the corpus luteum. The luteinized granulosa cells or large luteal cells are responsible for most progesterone (**P_4**) secretion, although small luteal cells produce 20% of total CL progesterone (Garverick et al., 1988; Garverick and Smith, 1986; Garverick et al., 1992; Smith et al., 1994; Wiltbank, 1994).

Diestrus. Follicles, prior to ovulation, secrete E_2 from granulosa and thecal cells. These cells then differentiate into the luteal cells of the corpus luteum, which are responsible for the production of P_4 (Garverick et al., 1992; Hansel and Convey, 1983; Smith et al., 1994; Wiltbank, 1994). The steroid hormone, progesterone ($P_{4)}$), is a potent regulator of the bovine estrous cycle. Progesterone regulates the estrous cycle by causing the suppression of E_2 and LH secretion, inhibiting behavioral estrus, and maintaining pregnancy (Driancourt, 2001; Garverick et al., 1992; Hansel and Convey, 1983; Smith et al., 1994; Wiltbank, 1994). The elevation of progesterone begins around day 4 to 5 of the estrous cycle with peak concentrations around day 7 to 12. These concentrations remain high until CL regression occurs between days 18 and 19 (Donaldson and Hansel, 1965; Hansel and Convey, 1983). Follicular waves are preceded by a transient rise in FSH which declines at follicular selection. At this time, LH dependent dominant follicles may continue to ovulation or become atretic (Adams et al., 1992; Hamilton et al., 1995). Collectively, metestrus and diestrus make up the luteal phase of the bovine estrous cycle,

involving the development of the corpus luteum and the resulting secretion of progesterone.

Proestrus. As the luteal phase ends, down-regulation of progesterone receptors occurs due to the initial rise in P_4 at CL formation. This concludes the inhibitory effect of P_4 on LH pulsatility. An increase in LH pulsatility drives the production of E_2 from the developing preovulatory follicle. The increase in E_2 concentration signals the high-frequency pulsatile release of low levels of oxytocin from the posterior pituitary and upregulates endometrial oxytocin receptors in the uterus (McCracken et al., 1996; 1999). Oxytocin stimulates the production of small amounts of prostaglandin $F_{2\alpha}$ (**PG**) from the uterus. Prostaglandin $F_{2\alpha}$ travels from the uterus to the CL on the ovary by means of a countercurrent exchange mechanism involving the utero-ovarian plexus (McCracken et al., 1999). Secretion of luteal oxytocin stimulated by uterine PG then acts to increase uterine PG secretion causing luteolysis (McCracken et al., 1999). Regression of the CL occurs around day 18 of the estrous cycle, signaling the end of the luteal phase and the beginning of the follicular phase of the estrous cycle.

Regression of the corpus luteum causes an immediate and significant decrease in P_4 concentrations which ends the negative feedback that P_4 exerts on the preovulatory LH surge center (Hansel and Convey, 1983; Savio et al., 1990). Gonadotropin releasing hormone is then secreted in high frequency pulses from the hypothalamus; this signals the release of gonadotropins, LH and FSH, from the anterior pituitary (Hansel and Convey, 1983; Schallenberger et al., 1984; Spicer and Echternkamp, 1986). The "two-cell, two gonadotropin model" proposes that the binding of LH to thecal cells stimulates the production of testosterone in the ovary. Testosterone then diffuses through the

basement membrane to the granulosa cell layer. When FSH reaches its receptors on the granulosa cells, the granulosa cell converts testosterone into E_2 via aromatase (Richards and Midgley, 1976). Increased concentrations of E_2 exert a positive feedback effect on the hypothalamus and anterior pituitary gland (Schallenberger et al., 1984; Spicer and Echternkamp, 1986; Stumpf et al., 1989; Walters et al., 1984). Three days after the decrease in P_4, E_2 reaches peak threshold concentrations (Cupp et al., 1995; Garverick et al., 1992; Savio et al., 1990). This stimulates the production of gonadotropins from the hypothalamus and pituitary gland. The increased concentrations of both E_2 and LH permit the rapid preovulatory follicular growth and maturation necessary for the expression of estrus and ovulation. Proestus and estrus, together, make up the follicular phase of the bovine estrous cycle.

Folliculogenesis. Folliculogenesis describes the process whereby ovarian follicles develop from primary into secondary and eventually Graafian follicles on the path to ovulation (Senger, 2003). There are five categories in which follicles are classified: primordial, primary, secondary, tertiary and Graafian follicles. Cows have a fixed number of primordial follicles from birth, with estimates around 150,000 follicles. This number steadily decreases to around 3,000 follicles as a cow ages to 15 to 20 yr of age (Erickson, 1966; Webb et al., 1992). Primordial follicles are recruited to become primary follicles continuously throughout the life of the animal (Hansel and Convey, 1983). Folliculogenesis is a process that is repeated as follicular waves are formed during the bovine estrous cycle.

Follicular waves. The sequence of follicular growth and development is referred to as follicular waves. Cows may experience two or three follicular waves per estrous

cycle (Sirois and Fortune, 1988). Follicular waves have three distinct phases that ultimately lead to the selection of a dominant follicle; these three phases include recruitment, selection and dominance (Fortune et al., 1988; Ginther et al., 2001).

Cyclic recruitment. Follicular cyclic recruitment is a process whereby follicles are selected and grouped into a cohort and mature in an environment of gonadotropic stimulation to permit progress toward ovulation (Hodgen, 1982). A cohort or group of 4 to 5 mm follicles, normally 1 to 6, are recruited from the pool of primordial follicles, and begin to grow in response to an increase in FSH (Bao and Garverick, 1998; Sirois and Fortune, 1988). The regulation of recruitment of primordial follicles is not fully understood (Elvin et al., 2000). Recruitment occurs on days 2 and 11, or days 2, 9, and 16 for cows having two or three follicular waves, respectively (Pierson and Ginther, 1984; Sirois and Fortune, 1988). Concentrations of FSH transiently rise 2.5 d preceding the emergence of the first follicular wave and decline with the emergence of the developing cohort (Adams et al., 1994; Hamilton et al., 1995). Developing follicles in a cohort are dependent on FSH secretion for maturation (Adams, 1999; Driancourt, 2001; Ginther et al., 2000) until those follicles become atretic or attain dominance.

Selection. After the recruitment of a cohort, selection of the dominant follicle occurs. Selection is a process occurring approximately 36 to 48 h after recruitment in which a single follicle of a cohort is chosen to avoid atresia and develop toward ovulation of a competent oocyte (Bao and Garverick, 1998; Hodgen, 1982). Selection of follicles occurs on d 4 to 5 and 13 to 14 for two wave cows and around d 3, 10, and 17 for three wave cows. As the selected follicle grows, it continues to secrete increasing amounts of E_2 and inhibin exerting negative feedback on the pituitary and decreasing release of FSH

(Knight and Glister, 2001). The selected follicle then transitions to being dependent upon LH instead of FSH (Roche et al., 1998; Ginther et al., 1996).

Dominance. After a follicle is selected for continued growth it achieves dominance. Dominance is the process by which the dominant follicle inhibits the emergence of a new follicular wave (Ginther et al., 1996; Hodgen, 1982). The maximum size of the dominant follicle ranges between 10 and 15 mm (Webb et al., 1992). Dominance occurs approximately 1 d after selection, which falls around d 6 and 15 and d 7, 14, and 21 in cows with two and three waves, respectively (Driancourt, 2001; Lucy et al., 2001; Pierson and Ginther, 1984; Savio et al., 1990). Once the dominant follicle reaches maximum size, the dominant follicle persists for 3 to 6 days (Ginther et al., 1989; Knopf et al., 1989). The fate of the dominant follicle is either atersia or ovulation depending on the stage of the estrous cycle.

Luteinizing hormone plays a vital role in the growth of the dominant follicle (Garverick and Smith, 1986; Schallenberger et al., 1984; Sirois and Fortune, 1988). Once the follicle reaches dominance, LH receptors in the granulosa cell and to a lesser extent, the thecal cell layer, are expressed, making the dominant follicle LH dependent (Bao and Garverick, 1998; Webb et al., 1999). Luteinizing hormone stimulates the production of androgen precursors from the thecal cells; this controls E_2 synthesis, influencing the overall health of the developing dominant follicle (Garverick et al., 1988; Garverick and Smith, 1986; Garverick et al., 1992). Pulse frequency of LH determines whether the dominant follicle will proceed to ovulation or become atretic (Driancourt, 2001; Roche et al., 1998). Pulse frequency of LH is controlled by negative feedback of P_4 and positive feedback of E_2 (Cupp et al., 1995; Schallenberger et al., 1984; Stumpf et al., 1989). Thus,

determination of whether ovulation occurs depends upon the stage of the estrous cycle. The dominant follicle will undergo regression during the luteal phase in the presence of an active CL secreting P_4. However, if the dominant follicle undergoes LH dependent growth after the CL undergoes regression, that follicle should go on to ovulate (Kastelic et al., 1990). In order for ovulation of the dominant follicle to occur, there must be an increase in LH receptor expression within the granulosa cell layer, highlighting the role of LH in determining ovulation (Sartori et al., 2001).

FACTORS AFFECTING ANESTRUS IN CATTLE

Anestrus. Anestrus is the lack of estrous cyclicity, which results in the absence of estrus and ovulation. Anestrus poses a significant problem for beef producers and accounts for major economic losses. When anestrus occurs after parturition it may impair a cow's ability to conceive early in the breeding season. When cows conceive early in the breeding season, resulting calves are born earlier and are generally heavier at weaning (Dunn et al., 1969). Anestrus reduces the lifetime number of calves a cow produces which is an important determinant of reproductive efficiency (Duffy et al., 2000). Factors that contribute to extended anestrus in postpartum beef cows include body condition, lactational status or suckling, dystocia, parity and other minor factors (Dunn et al., 1969; Stagg et al., 1998; Wiltbank, 1970; Wiltbank and Cook, 1958).

Endocrine considerations. Steroids and peptides secreted from the ovary exert negative feedback on both the hypothalamus and pituitary gland (Yavas and Walton, 2000). This leads to the inhibition of FSH release (Crowe et al., 1998; Labhsetwar et al.,

1964), the depletion of LH stores (Nett et al., 1988), and a resulting suppression of ovarian follicular growth (Casida, 1968; Casida et al., 1943; Choudary et al., 1968; Labhsetwar et al., 1964).

Follicle stimulating hormone requires only minimal GnRH stimulation to elicit its' release (Menge et al., 1962). This leads to a rise in circulating FSH concentrations (Beam and Butler, 1997), and allows for the recruitment and early development of a new follicular wave shortly following parturition. The necessary hormonal stimulation required for ovulation, however is not achieved (Yavas and Walton, 2000). Follicles become atretic and fail to ovulate in large part as a result of inadequate LH pulsatility (Yavas and Walton, 2000).

Post-calving anestrus is in large part a function of inadequate LH pulse frequency. Secretion of GnRH from the hypothalamus influences LH pulse frequency from the anterior pituitary, which is generally regarded to be less responsive to GnRH after parturition (Crowder et al., 1982; Short et al., 1990). Inadequate secretion of LH may result from the lack of sufficient GnRH required to increase LH pulsatility.

Stores of luteinizing hormone are not replenished until 15 to 30 d post-calving (Cermak et al., 1983; Labhsetwar et al., 1964; Lamming et al., 1981; Moss et al., 1985; Nett et al., 1988; Wagner et al., 1969), and circulating concentrations of LH remain low due to low LH pulsatility (0.8 to 2.3/h; Arije et al., 1974; Convey et al., 1983; Humphrey et al., 1983; Jagger et al., 1987; Nett et al., 1988; Schallenberger, 1985; Williams et al., 1983). Beginning 25 to 32 d postpartum the pulsatile pattern of LH resumes (Lamming et al., 1981; Peters and Lamming, 1984; Rawlings et al., 1980; Riley et al., 1981; Webb et al., 1980). The frequency of LH pulsatility increases ½ to 2 h prior to the time a cow

exhibits estrus and ovulates following calving (Carruthers et al., 1980; Carruthers and Hafs, 1980; Schallenberger, 1985); FSH pulses synchronize with the LH pulses (Schallenberger, 1985). Recovery of pulsatile release of gonadotropins is seen among cows during the postpartum interval; among heifers prior to pubertal onset; and among seasonal breeders that transition from anestrus to estrous cycling (Bronson, 1988; Foster, 1988; Thatcher and Hansen, 1993). The increase in LH causes increased ovarian release of E_2, which in turn induces positive feedback on LH release, the LH surge, ovulation, and the resumption of estrous cyclicity (Kesler et al., 1977). This cascade of hormonal events beginning with the increase in LH pulsatility is paramount for the resumption of normal estrous and ovulatory cycles (Humphrey et al., 1983; Peters et al., 1981).

Suckling. Suckling is a major contributing factor that influences the length of the postpartum interval in beef cows. Although LH stores are replenished by d 15 to 30 postpartum (Cermak et al., 1983; Labhsetwar et al., 1964; Lamming et al., 1981; Moss et al., 1985; Nett et al., 1988; Wagner et al., 1969), the suckling stimulus inhibits LH pulsatility by suppressing GnRH secretion from the hypothalamus (Carruthers et al., 1980; Lamming et al., 1981; Nett, 1987). Length of anestrus is then dependent upon suckling stimulus after LH stores are replenished (Nett, 1987). For this reason suckled beef cows require longer periods of time to resume estrous cycles following calving than non-suckled cows (38 vs. 14 d; Carruthers and Hafs, 1980; Duffy et al., 2000; Williams, 1990). Neuronal pathways that suppress LH secretion include: Tactile stimulation of the udder or teat by the calf (Short et al., 1972; Williams et al., 1987), maternal vision and olfaction of the calf (Griffith and Williams, 1996), and the physical presence of the calf (Macmillan, 1983; McVey and Williams, 1991; Stagg et al., 1998; Williams et al., 1984;

1993). Studies indicate that calf removal for a period of 24 h is long enough for a subsequent increase in LH pulse frequency. This was associated with an improvement in estrous response when used in conjunction with various estrous synchronization protocols (Geary et al., 1998; Smith et al., 1983; Stagg et al., 1998; Walters et al., 1984; Williams, 1990; Wiltbank and Cook, 1958).

Body condition. The body condition of the cow is a good indicator of nutritional status. Body condition score (**BCS**; 1 to 9 scale, 1 = emaciated, and 9 = obese) is a subjective, visual assessment based on rib visibility, rump-fat thickness and fat thickness around the hooks and pins (Richards et al., 1986). Basal metabolism, activity, growth, and basic energy reserves take precedence over any reproductive processes including estrous cyclicity status, and the establishment or maintenance of pregnancy (Grimard et al., 1997; Guedon et al., 1999; Short et al., 1990). Studies have shown that acceptable BCS at parturition resulted in increased follicular development, replenished LH stores 30 d postpartum, and increased LH pulse frequency early postpartum (Connor et al., 1990; Ryan et al., 1994). Low body condition has also been found to decrease the likelihood of first service conception, while increasing the number of inseminations per conception (Gillund et al., 2001). Deficiencies in BCS prior to calving impact length of the postpartum interval to a higher degree than deficiencies post calving (Short et al., 1990). Since prepartum nutritional status is an important indicator of postpartum BCS (DeRouen et al., 1994; Spitzer et al., 1995), it is important that adequate nutrition and resulting BCS are achieved during the prepartum gestational period rather than postpartum (Houghton et al., 1990; Killen et al., 1989; Morrison et al., 1999; Short et al., 1990).

Parity. Cow age, or parity was shown to have a major effect on estrous cyclicity status of postpartum beef cows. Young cows (≤ 3 years of age) and older age cows (≥ 7 years) experience an increased incidence of dystocia, longer postpartum intervals, and reduced fertility compared to middle age cows (Bellows and Short, 1978; Cavestany and Galina, 2001; De Kruif, 1978; Doornbos et al., 1984; Laster et al., 1973; Short et al., 1990). Nutrient requirements are generally higher among primiparous cows which leads to reduced LH pulse frequency (Grimard et al., 1995; Randel et al., 1996). Primiparous cows experience postpartum intervals that are 1 to 4 wk longer compared to multiparous cows (Fajersson et al., 1999; Guedon et al., 1999; Lamming et al., 1981; Randel et al., 1996; Sharpe et al., 1986; Tervit et al., 1977; Walton et al., 1992). As a result, producers must nutritionally manage younger and older age groups of females to better control reproductive inefficiencies of these respective age groups.

Dystocia. Dystocia is defined as parturition that is prolonged and difficult for the cow (Senger, 2003). Dystocia or calving difficulty increases the length of time to first estrus following parturition (Brinks et al., 1973; Doornbos et al., 1984; Laster et al., 1973). Cows that experience dystocia at parturition exhibit longer postpartum intervals, reduced first service conception rates, and wean fewer pounds of calf than cows that do not experience dystocia (Brinks et al., 1973; Doornbos et al., 1984; Dziuk and Bellows, 1983; Laster et al., 1973). Management techniques, including utilization of calving ease sires, pelvic examinations for heifers, proper nutrition, and vigilant observation at calving may help to decrease the incidence and resulting negative effects of dystocia on postpartum reproductive performance.

Minor factors. Numerous other factors influence the duration of anestrus following parturition in beef cows. These include, but are not limited to: Within breed genetic variation, presence of bulls, twin births, stress, disease, or retained placenta (Browning et al., 1996; Edgerton, 1980; Peters and Riley, 1982; Short et al., 1990; Vandeplassche, 1985). It is important for producers to understand and correctly manage these factors to ensure maximum reproductive efficiency.

REVIEW OF PROGESTINS

Progestins played an important role in the development of methods to synchronize estrus since the discovery that P_4 inhibits ovulation and maturation of ovarian follicles (Hansel et al., 1961; Lamond, 1964; Nellor and Cole, 1956; Ulberg et al., 1951). Endogenous P_4 produced by the CL regulates the bovine estrous cycle by inhibiting the hypothalamic release of GnRH (Hansel and Convey, 1983). Today, however, exogenous progestins are used effectively to induce estrous cyclicity and synchronize estrus. The two exogenous progestins approved for use in the U.S. include the feed additive, melengestrol acetate (MGA; Brown et al., 1988; Jaeger et al., 1992; King et al., 1994; Mauck et al., 1988; Patterson et al., 1994; Yelich et al., 1995), and the intravaginal insert or CIDR device (Controlled Internal Drug Release; Bridges et al., 1999; Hanlon et al., 1996; Hansel and Beal, 1979; Lammoglia et al., 1998; Lucy et al., 1990; Rivera et al., 1998; Roche, 1978; Roche et al., 1981; Savio et al., 1993; Sirois and Fortune, 1990; Smith et al., 1984; Stock and Fortune, 1993).

Long-term progestin treatments were found to increase LH pulse frequency and the subsequent development of persistent follicles (Bergfeld et al., 1996; Custer et al., 1994; Duchens et al., 1994; Sanchez et al., 1995; Taylor et al., 1993). Ovulation of persistent follicles takes place once the progestin treatment ends. Although synchrony of estrus and ovulation following withdrawal or removal of the progestin are considered to be good, the associated fertility is generally regarded as poor (Hansel et al., 1961). For this reason, protocols to synchronize estrus that involved only progestins were abandoned.

Progesterone concentrations increase prior to the resumption of estrous cycles in postpartum beef cows (Rawlings et al., 1980), and immediately before the onset of puberty in heifers (Berardinelli et al., 1979).This illustrates the significant role P_4 plays in the resumption of normal estrous cycles in the female bovine. Progestins may be used to successfully induce puberty in heifers (Gonzalez-Padilla et al., 1975; Patterson et al., 1990) by increasing LH pulse frequency and down-regulating E_2 receptors in the hypothalamus; with the greatest effect generally seen after progestin removal (Anderson and Day, 1996; Anderson et al., 1996; Hall et al., 1997; Imwalle et al., 1998; Smith and Day, 1990). The resumption of estrous cycles in postpartum beef cows may occur through a similar mechanism.

Melengestrol Acetate. Melengestrol acetate or MGA (6-methyl-17-alpha-acetoxy-16-methylene-preg-4, 6-diene-3, 20-dione) is an orally active progestational steroid. Melengestrol acetate was developed in 1962 for the purpose of improving rate of gain and feed efficiency among heifers in feedlots by suppressing estrus and ovulation, while at the same time allowing ovarian follicular development to occur (Zimbelman and

Smith, 1966). Melengestrol acetate demonstrated superior ability to block estrus and ovulation compared to other orally active progestational compounds (Zimbelman and Smith, 1963). Melengestrol acetate has the potential to maintain pregnancy when fed to ovariectomized heifers at the rate of 4.0 $mg/hd^{-1}/d^{-1}$; a dosage eight times higher than the dosage required to block estrus and ovulation (Zimbelman and Smith, 1966; 1963). Current recommendations for feeding MGA to effectively suppress estrus, block the LH surge and ovulation, induce puberty in heifers, and synchronize estrus is a dosage of 0.5 $mg/hd^{-1}/d^{-1}$ (Imwalle et al., 2002; Patterson et al., 1990; Zimbelman, 1963; Zimbelman and Smith, 1966). Melengestrol acetate successfully blocks estrus and ovulation at low concentrations as a result of having an 11.1-fold higher binding affinity for the progesterone receptor than natural progesterone (Perry et al., 2005). To ensure optimal results MGA should be fed at a 1:4 MGA to carrier ratio, with the carrier being a grain or protein supplement (Patterson et al., 2003). Management considerations for feeding MGA include: Once per day feeding at approximately the same time every day, and providing adequate bunk space (60 linear cm/hd^{-1}) to ensure that each animal consumes the necessary daily intake (Patterson et al., 2003).

Controlled Internal Drug Release. The EAZI-Breed™ Controlled Internal Drug Release (**CIDR**) insert (1.38 g progesterone; Pfizer Animal Heath, New York, NY) obtained approval from the Food and Drug Administration (**FDA**) for use in postpartum beef cows, and beef and dairy heifers in 2002 and for lactating dairy cows in 2003. Approval was authorized by FDA for use in synchronizing estrus in beef cows and beef and dairy heifers and for the resumption of estrous cyclicity and induction of puberty in dairy cows and beef and dairy heifers, respectively (FDA, 2002). The structure of CIDR

inserts includes a layer of silicone that contains 10% P_4 by weight that is formed over a T-shaped nylon structure (Chenault et al., 2003). This structure has flexible wings that can be pulled back straight when inserted into the vagina. These wings bend back into the T-shape after insertion and hold the CIDR in place in the vagina. Retention rates of the CIDR range from 96 to 99% in beef heifers (Lucy et al., 2001; Macmillan et al., 1991; 1988). The silicone layer of the CIDR is impregnated with 1.38g of P_4. When inserted into the vagina, P_4 from the CIDR device is continuously secreted and absorbed through the vaginal wall. The P_4 released from the CIDR insert is sufficient to increase and maintain P_4 concentrations in blood at > 2.0 ng/mL in the absence of a CL on the ovary (Chenault et al., 2003). Progesterone concentrations were shown to rapidly increase to peak blood concentrations within 1 h after CIDR insertion and, likewise, P_4 concentrations decrease rapidly to 0 from 12 to 24 h once the CIDR is removed (Lamb et al., 2006; Perry et al., 2004). Progesterone in CIDR inserts clears the body much faster after removal than when MGA is removed from the feed (Tauck et al., 2007). Similar to MGA, CIDR inserts may be used to initiate estrous cycles among anestrous postpartum beef cows (Perry et al., 2004; Wheaton and Lamb, 2007). The CIDR-based protocols were shown to facilitate comparable pregnancy rates with MGA resulting from FTAI following treatment, without the extra labor required to feed MGA on a daily basis (Schafer et al., 2007).

DEVELOPMENT OF GNRH-PG-GNRH ESTROUS SYNCHRONIZATION PROTOCOLS FOR POSTPARTUM BEEF COWS

Development of the GnRH-PG protocol. Prostaglandin $F_{2\alpha}$ has been used effectively to synchronize estrus among estrous cycling cows and heifers. Although one injection of PG initiates CL regression in cows with functional corpora lutea between days 6 to 16 of the estrous cycle, this method is ineffective among anestrous cows (Lauderdale, 1974). Additionally, the stage of follicular development among cows that do respond to PG is directly responsible for the variation in time between PG and estrus (Macmillan and Henderson, 1984; Sirois and Fortune, 1988). These findings lead to the development of protocols that call for the administration of GnRH prior to PG in the GnRH- PG protocol.

When single injections of GnRH are administered to cows in varying stages of the estrous cycle, LH is released, which causes the ovulation or atresia of large dominant follicles ($\geq$ 10 mm). This also results in the emergence of a new follicular wave 3 to 4 d following administration (Twagiramungu et al., 1995; 1992). Administration of GnRH 6 to 7 d before PG allows for synchronization of follicular waves. Prostaglandin $F_{2\alpha}$ can then be used to regress both the natural and GnRH-induced accessory CL resulting in a more synchronous estrous response (Thatcher et al., 1989; Twagiramungu et al., 1995; Twagiramungu et al., 1992).

Ovsynch protocol. The Ovsynch protocol is a variation of the GnRH-PG protocol, involving GnRH on d 0, PG on d 7, GnRH 48 h after PG (d 9), and FTAI 16 to 24 hr after the last injection of GnRH (Pursley et al., 1995). Timing of the second GnRH

injection induces ovulation in 87 to 100% of cows, which occurs 24 to 32 h after GnRH is administered (Pursley et al., 1997). The Ovsynch protocol is a reliable and effective means of synchronizing estrus in postpartum lactating dairy cows (Burke et al., 1996; Pursley et al., 1997; 1995; Schmitt et al., 1996). Pregnancy rates resulting from fixed-time AI in lactating dairy cows vary from 32 to 45% following administration of Ovsynch (Pursley et al., 1997; 1998). The use of Ovsynch in postpartum beef cows is limited; however, pregnancy rates resulting from FTAI have ranged from 42 to 57% following administration of this protocol (Geary and Whittier, 1998; Geary et al., 1998a).

Select Synch protocol. The Select Synch protocol utilizes GnRH and PG 7 d apart to synchronize development of follicular waves and luteal regression. Artificial insemination is performed 12 h after detected estrus. The 6 to 7 d interval between GnRH and PG was found to enhance the synchrony of estrus following treatment administration (Thatcher et al., 1989; Twagiramungu et al., 1992). The Select Synch protocol was used effectively to synchronize estrus in postpartum beef cows, however one of the major limitations of the protocol involves the 5 to 15% of cows that exhibit estrus after GnRH and prior to PG (Dejarnette et al., 2001; Kojima et al., 2000).

CO-Synch protocol. The CO-Synch protocol is similar to the Select Synch protocol except that cows are inseminated on the basis of a predetermined fixed-time rather than after a recorded estrus. In the CO-Synch protocol cows are inseminated with an injection of GnRH 48 h following the administration of PG. The CO-Synch protocol differs from Ovsynch in that cows are inseminated at the time GnRH is administered, and not 16-24 h afterward, as is the case with Ovsynch. The CO-Synch protocol requires one less trip through the chute than Ovsynch; however, pregnancy rates among CO-Synch

treated cows following FTAI were lower compared to Ovsynch treated cows (Geary and Whittier, 1998). Pregnancy rates resulting from FTAI range from 43 to 49% following administration of the CO-Synch protocol (Geary and Whittier, 1998; Lamb et al., 2001; Larson et al., 2006).

DEVELOPMENT OF SHORT-TERM CIDR-BASED ESTROUS SYNCHRONIZATION PROTOCOLS FOR POSTPARTUM BEEF COWS

7-d CO-Synch and Select Synch + CIDR protocols. The CO-Synch and Select Synch protocols were shown to have marginal success in synchronizing estrus prior to FTAI due to the fact 5 to 15% of estrous cycling cows exhibit estrus prior to the time PG was administered (Kojima et al., 2000; Twagiramungu et al., 1995). The addition of the CIDR to these protocols prevents cows from exhibiting estrus prior to PG and enhanced fertility among anestrous cows (Lamb et al., 2001; Larson et al., 2006). Pregnancy rates resulting from fixed-time AI were improved when progestins were used to presynchronize cows (Perry et al., 2002; Schafer et al., 2007) prior to the administration of GnRH and PG, or were included as part of the CO-Synch protocol (Lamb et al., 2001; Larson et al., 2006). Schafer et al. (2007) and Busch et al. (2008) reported that pregnancy rates resulting from FTAI in the range of 66% were achievable following treatment with the 7d CO-Synch + CIDR protocol when insemination was performed 66 h following CIDR removal and PG.

5-d CO-Synch and Select Synch + CIDR protocol. The 5-d CO-Synch + CIDR and Select Synch + CIDR protocols shorten the interval from GnRH to PG and CIDR administration from 7 to 5 d. In addition, the 5-d protocol requires two injections of PG 12 h apart to facilitate luteolysis of accessory corpora lutea that form after the initial injection of GnRH. Kasimanickam et al. (2008) demonstrated that cows assigned to the 5-d protocol that received only one injection of PG had significantly lower pregnancy rates resulting from FTAI compared to cows assigned to the 5-d protocol that received two injections of PG 12 h apart.

Bridges et al. (2008) hypothesized that shortening the duration of CIDR treatment with the 5-d protocol would result in improvements in pregnancy rates following FTAI. This hypothesis is based on the premise that d 4 dominant follicles have higher intrafollicular E_2 concentrations and a greater ability to produce E_2 compared to older age follicles (Valdez et al., 2005). Increased follicular concentrations of E_2 are believed to result in higher AI pregnancy rates (Lopez et al., 2005). Hence, if CIDR removal and AI are more accurately timed with the 5-d protocol to coincide with follicular development, higher AI pregnancy rates may be achieved. Further studies should be conducted to fully elucidate the validity of this hypothesis.

SUMMARY

There are a number of protocols available to beef producers that may be used to facilitate estrous synchronization and AI. These protocols vary on the basis of treatment

cost, the amount of labor required to facilitate administration of the protocol, and the overall effectiveness of the protocol in successfully synchronizing estrus and ovulation.

The addition of the CIDR to the CO-Synch and Select Synch protocols resulted in the development of an effective and efficient means to synchronize estrus and ovulation and facilitate fixed-time AI. The 7-d protocols have been used extensively in postpartum beef cows with a high degree of success. The 5-d protocol was recently developed to potentially improve estrous synchronization rates and resulting fixed-time AI pregnancy rates in postpartum beef cows by lengthening the proestrus period and increasing estrogenic capacity of preovulatory follicles (Bridges et al., 2008). However, to date there have been no studies designed to compare these protocols on the basis of application and performance in the field based on pregnancy rates resulting from FTAI. These considerations form the basis for the studies reported in this thesis.

Table 1.1. A compilation of results from studies in postpartum beef cows inseminated at fixed-times following treatment.

Treatment	Time of AI (h)	No.	Pregnancy rate (%)
Ovsynch[1]		220	42
Ovsynch[2]		402	57
Ovsynch + calf removal[3]		112	62
CO-synch[2]	48	369	49
CO-synch + calf removal[3]	48	119	63
CO-synch[4]	48	287	48
CO-synch[5]	72	112	47
CO-synch[6]	60	551	43
7-d CO-Synch + CIDR[4]	48	273	58
7-d CO-Synch + CIDR[6]	60	547	54
7-d CO-Synch + CIDR[7]	66	323	66
7-d CO-Synch + CIDR[8]	54	424	61
7-d CO-Synch + CIDR[8]	66	426	67
7-d CO-Synch + CIDR[9]	60	312	60
5-d CO- Synch + CIDR[9]	60	111	57
5-d CO- Synch + CIDR[9]	72	304	70

[1] Geary et al., 1998a; [2] Geary and Whittier, 1998; [3] Geary et al., 1998b; [4] Lamb et al., 2001; [5] Perry et al., 2002; [6] Larson et al., 2006; [7] Schafer et al., 2007; [8] Busch et al., 2008; [9] Bridges et al., 2008.

Ovsynch

Figure 1.1. Treatment schedule for the Ovsynch protocol (from Pursley et al., 1995).

Select Synch

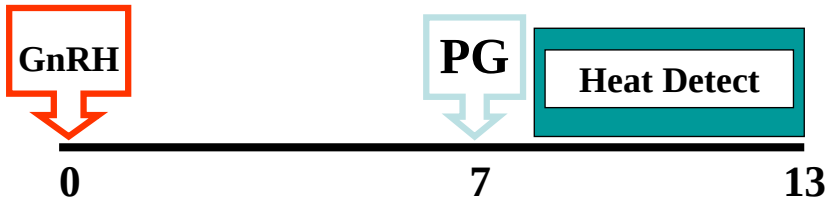


Figure 1.2. Treatment schedule for the Select Synch protocol (from DeJarnette et al., 2001).

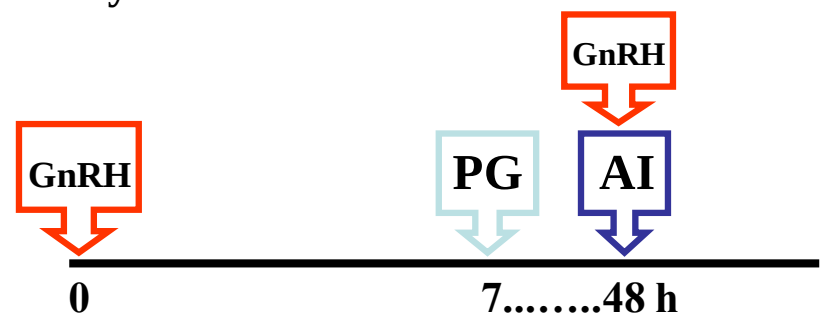


Figure 1.3. Treatment schedule for the CO-Synch protocol (from Geary et al., 1998b).

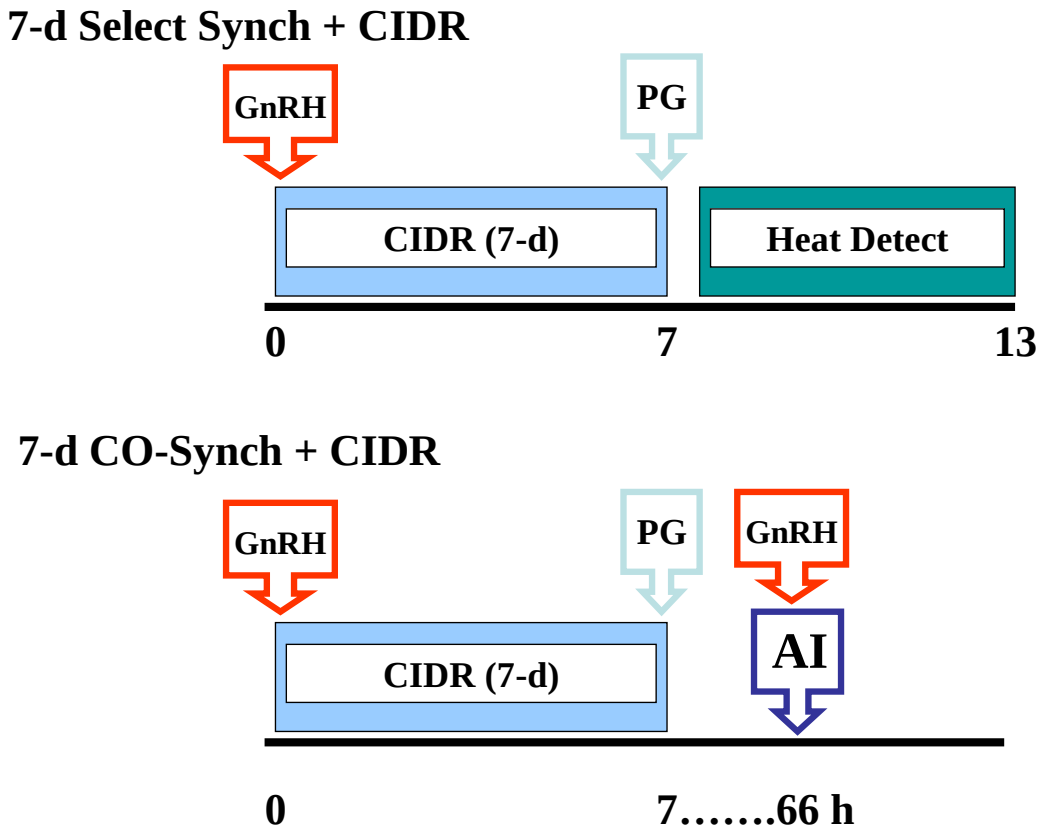
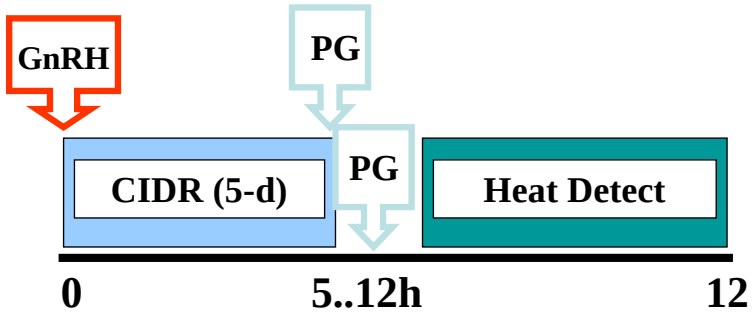


Figure 1.4. Treatment schedule for the 7-d Select Synch and CO-Synch + CIDR protocols (from Lamb et al., 2001; Schafer et al., 2007).

5-d Select Synch + CIDR



5-d CO-Synch + CIDR

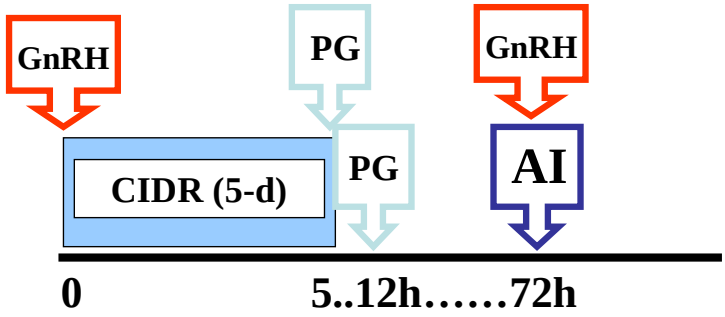


Figure 1.5. Treatment schedule for the 5-d Select Synch and CO-Synch + CIDR protocols (from Bridges et al., 2008).

CHAPTER 2

CHARACTERIZATION AND COMPARISON OF THE 7-DAY AND 5-DAY SELECT SYNCH + CIDR PROTOCOLS IN POSTPARTUM BEEF COWS

ABSTRACT

In this study two short-term controlled internal drug release (CIDR)-based estrous synchronization protocols were characterized and compared on the basis of follicular dynamics and timing and synchrony of estrus following treatment administration. Primi- and multiparous, crossbred, lactating beef cows (n = 117) were randomly assigned to two treatments by age, calving date (days postpartum, **DPP**), and body condition score (**BCS**). Blood samples were collected via jugular venipuncture 10 d before and immediately prior to treatment administration (d 0) to determine estrous cyclicity status. Cows assigned to the 7-d Select-Synch + CIDR treatment (**7-d**; n = 59) received an injection of GnRH (100 µg i.m.) and CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m) was administered and CIDR inserts were removed on day 7. Cows assigned to the 5-d Select-Synch + CIDR treatment (**5-d**; n = 58) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 5; a second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All cows were fitted with HeatWatch estrus detection transmitters at CIDR removal. AI was performed approximately 12 h after detected estrus. Estrus was

detected and AI was performed for both treatments over the 144 h synchronized period. Transrectal ultrasonography on d 0 and 1 was performed to map ovaries and visualize follicular dynamics for each cow. Ultrasonography on d 2 and 3 was used to determine which animals responded to GnRH by ovulating a dominant follicle (**DF)**. Conception rate to AI was determined by transrectal ultrasonography on d 72. There was no difference in the estrous response ($P = 0.85$), interval to estrus ($P = 0.09$), or the variance for interval to estrus ($P = 0.75$) between treatments. The response to GnRH (P = 0.75) and follicle size at GnRH ($P = 0.96$) did not differ between the two treatments. There was an effect of estrous cyclicity status prior to treatment administration ($P < 0.05$) on estrous response, with 75/82 (91.5%) estrous cycling cows exhibiting estrus and 21/35 (60%) anestrous cows exhibiting estrous. Pregnancy rate resulting from AI was affected by estrous cyclicity status of cows prior to treatment administration ($P = 0.05$) with 57/82 (69.5%) estrous cycling cows and 12/35 (34.2%) anestrous cows becoming pregnant to AI. In summary, the 5-d and 7-d Select Synch + CIDR protocols performed similarly on the basis of inducing and synchronizing estrus and facilitated comparable synchronized conception and pregnancy rates resulting from AI.

INTRODUCTION

Artificial insemination (AI) and estrous synchronization are reproductive technologies that enable beef producers to efficiently improve herd genetics. Current surveys indicate that only 13.3% of beef operations utilize AI and 11.9% of operations use available methods of estrous synchronization to facilitate AI (NAHMS, 1998). Major reasons cited for a failure to adopt these technologies include “time and labor

constraints", followed by the perception that the technologies are too "complicated" or "costly" to implement (NAHMS, 1998).

Development of effective estrous synchronization protocols is necessary for the expanded acceptance and use of AI in U.S. beef herds. Estrous synchronization enables producers to utilize fixed-time AI (**FTAI**), thus reducing time and labor required to detect estrus. The development of methods that will facilitate expanded use of FTAI for beef cows and heifers is dependent upon protocols to synchronize estrus and ovulation that are practical to implement, affordable to use, and that result in highly acceptable pregnancy rates following treatment administration (Patterson et al., 2003).

The Select Synch protocol was used effectively to synchronize estrus in postpartum beef cows (Dejarnette et al., 2001; Kojima et al., 2000). The inclusion of a progestin [controlled internal drug releasing (**CIDR**) inserts] as part of the Select Synch protocol improved success rates based on pregnancy rates in postpartum beef cows compared to cows that did not receive a CIDR (Lamb et al., 2001; Larson et al., 2006). To date, however, there have been no comprehensive studies designed to fully characterize the 7-d Select Synch + CIDR protocol. The 5-d Select Synch + CIDR protocol was recently developed to potentially improve estrous synchronization rates and resulting fixed-time AI pregnancy rates in postpartum beef cows by lengthening the proestrus period and increasing estrogenic capacity of preovulatory follicles (Bridges et al., 2008).

This study was designed to fully characterize and compare these two estrous synchronization protocols on the basis of follicular dynamics and timing and synchrony of estrus following administration.

MATERIALS AND METHODS

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

Experimental design. Primi- and multiparous, crossbred, lactating beef cows (n = 117) were randomly assigned to two treatments by age, calving date (days postpartum, **DPP**), and body condition score (**BCS**; 1 to 9 scale, 1 = emaciated, and 9 = obese; Richards et al., 1986). Cows assigned to the 7-d Select-Synch + CIDR treatment (**7-d**; n = 59) received an injection of GnRH (100 µg i.m.; Cystorelin®, Merial, Athens, GA) and EAZI-Breed™ CIDR inserts (1.38 g progesterone ; Pfizer Animal Heath, New York, NY) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse® Sterile Solution, Pfizer Animal Heath) was administered and CIDR inserts were removed on day 7. Estrus detection and AI were performed within a 144 h synchronized period. Cows assigned to the 5-d Select-Synch + CIDR treatment (**5-d**; n = 58) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 5; a second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. Estrus was detected and AI was performed over the 144 h synchronized period. Calves from both treatments were sorted from cows before and returned directly to their dams after each working to minimize effects of calf removal. Cows were inseminated by one of two experienced AI technicians 12 h after the recorded estrus. Technicians were assigned to cows within treatment by age, calving date and BCS. A single AI sire was used in the experiment. Cows were exposed to fertile bulls 12 d following AI for the remainder of a 60 d breeding season.

Ultrasonography and pregnancy diagnosis. Transrectal ultrasonography (Aloka 500V equipped with 7.5 MHz linear array transducer, Aloka, Wallingford, CT) was performed on day 0 and day 1, and day 2 and 3 for 7-d and 5-d treated cows, respectively. Ultrasonography on d 0 and d 1 was performed to map ovaries and visualize follicular dynamics for each cow. Ultrasonography on d 2 and 3 was used to determine which animals responded to GnRH, by ovulating a dominant follicle (**DF)**. Cows were considered to have responded to GnRH if the DF observed on d 0 or 1 was no longer visible on d 2 or 3 for the respective treatments. Conception rate to AI was determined by transrectal ultrasonography (Aloka 500V equipped with 5.0 MHz linear array transducer) on d 72. Final pregnancy rate was determined by rectal palpation 90 d after the end of the breeding season. Conception rate at first service was calculated as the number of cows that became pregnant divided by the number of cows inseminated. Pregnancy rate was the proportion of cows diagnosed pregnant of those assigned to treatment.

Ertrus detection. All cows were fitted with HeatWatch estrus detection transmitters (DDx Inc. Denver,CO) that were capable of detecting and continuously monitoring estrus. Transmitters were fitted at the time of CIDR removal and were removed at the time of AI or the end of the synchronized period. Estrus was defined as cows receiving ≥ 3 mounts, each of which were ≥ 2 s in duration within a 4 h period.

Blood collection and RIA. Blood samples were collected via jugular venipuncture 10 d before and immediately prior to treatment administration (d 0) to determine estrous cyclicity status. Blood samples were allowed to clot and stored at 4ºC for 24 h. Serum collection was performed by centrifugation and stored at -20º C until hormone analyses

were performed. Cows were considered to be estrous cycling when progesterone concentrations were ≥ 0.5 ng/ml at one or both of the pretreatment blood sampling times. Serum progesterone concentrations were determined by RIA using a Coat-a-Count kit (Diagnostic Products Corp., Los Angeles, CA; (Kirby et al., 1997) with an intraassay coefficient of variation of 2.22% and an assay sensitivity of 0.1 ng/mL.

Statistical analyses. Differences in age, days postpartum, BCS and interval to estrus between treatments were analyzed by PROC TTEST (SAS Institute Inc. Cary, NC). Pretreatment estrous cyclicity, estrous response, response to GnRH, AI technician, AI pregnancy rate and final pregnancy rate were analyzed using a generalized linear models method (PROC GLIMMIX of SAS) using a binomial distribution and the link function of logit. The model included the main effects of treatment and estrous cyclicity status and the 2-way interaction. Variances associated with the interval to estrus were compared by performing an F-test (the greater variance divided by the smaller variance (Snedecor and Cochran, 1989). These variances were calculated to provide comparisons of the degree of synchrony of estrus.

RESULTS

There were no differences in age, BCS, DPP, or estrous cyclicity status prior to treatment administration between the two treatments (Table 2.1). Additionally, response to GnRH (P = 0.75) and follicle size at GnRH (*P* = 0.96) did not differ between treatments (Table 2). There was no difference between treatments (Table 2.2) in the interval to estrus (*P* = 0.09), or the variance for interval to estrus (*P* = 0.75). Figure 2.2

illustrates the respective patterns of estrus distribution for the two treatments. There was no effect of treatment ($P = 0.85$) on estrous response (Table 2.2). There was, however, an effect of estrous cyclicity status prior to treatment administration ($P < 0.05$) on estrous response, with 75/82 (91.5%) of the estrous cycling cows exhibiting estrus and 21/35 (60%) of the anestrous cows exhibiting estrus during the synchronized period. There was no effect of technician ($P = 0.42$, $P = 0.58$) or treatment ($P = 0.85$, $P = .91$) on synchronized conception or pregnancy rates resulting from AI, respectively (Table 2.3). Pregnancy rate resulting from AI was affected by estrous cyclicity status of cows prior to treatment administration ($P = 0.05$), with 57/82 (69.5%) of the estrous cycling cows and 12/35 (34.2%) of the anestrous cows that conceived as a result of AI.

DISCUSSION

Estrous synchronization and AI are tools that enhance reproductive management in beef cattle. These management techniques have been available for over 30 yr allowing producers to shorten the calving season, increase pounds of calf weaned per cow exposed for breeding, and improve calf uniformity at weaning (Dziuk and Bellows, 1983). Many U.S. producers, however, have been reluctant to apply reproductive management technologies in their operations. Low adoption rates of these practices lead one to question the future competitive position of the U.S. beef cattle industry, especially when one considers the rate of technology adoption in other parts of the world (Kojima, 2003). Estrous synchronization protocols should be designed to reduce time and labor inputs by

limiting cattle handlings and reducing or eliminating estrus detection (Larson et al., 2006).

The Select Synch protocol utilizes GnRH and PG to synchronize the development of follicular waves and luteal regression. The Select Synch protocol is similar to the CO-Synch protocol except that cows are inseminated on the basis of a recorded estrus rather than at a predetermined fixed-time. The 6 to 7 d interval from GnRH to PG was confirmed to enhance the synchrony of estrus following treatment administration (Thatcher et al., 1989; Twagiramungu et al., 1992). Pursley et al., (1995) demonstrated that GnRH and PG, administered 7 d apart, may be used to effectively synchronize estrus and facilitate FTAI in lactating dairy cows. The CIDR device, containing progesterone, was added to this protocol to overcome the 5 to 15% of cows that may be expected to exhibit estrus before PG (Kojima et al., 2000; Lamb et al., 2001).

In this study the 5-d and 7-d Select Synch + CIDR protocols were characterized and compared on the basis of estrous response, timing and synchrony of estrus, and pregnancy rates resulting from AI. The 7-d Select Synch + CIDR protocol involves the administration of GnRH and CIDR insertion on d 0, followed by the administration of PG and CIDR removal on d 7. The 5-d protocol shortens the interval from GnRH to PG and CIDR administration from 7 to 5 d. In addition, the 5-d protocol involves two injections of PG 12 h apart to facilitate luteolysis of accessory corpora lutea that form after the initial injection of GnRH. Kasimanickam et al. (2008) demonstrated that cows assigned to the 5-d protocol that received only one injection of PG had significantly lower pregnancy rates resulting from FTAI compared to cows assigned to the 5-d protocol that received two injections of PG 12 h apart.

Bridges et al. (2008) hypothesized that shortening the duration of CIDR treatment with the 5-d protocol would result in improvements in pregnancy rates following FTAI. This hypothesis is based on the premise that d 4 dominant follicles have higher intrafollicular estradiol-17β (**E_2**) concentrations and a greater ability to produce E_2 compared to older age follicles (Valdez et al., 2005). Increased follicular concentrations of E_2 are believed to result in higher AI pregnancy rates (Lopez et al., 2005). Hence, if CIDR removal and AI are more accurately timed with the 5-d protocol to coincide with follicular development, higher AI pregnancy rates may be achieved.

The results from this study clearly indicate that there were no differences between the 5-d and 7-d protocols on the basis of pregnancy rates of cows that were inseminated after a detected estrus. Additionally, there were no differences between treatments in estrous response, or the interval to or synchrony of estrus. There were however, numeric differences in the mean intervals to estrus following PG between treatments (71.2 and 64.8 h for the 5-d and 7-d protocols, respectively). Although these times did not differ significantly ($P = 0.09$), the data are useful in providing a reference point regarding the appropriate timing of AI when cows are inseminated at predetermined fixed times.

Bridges et al. (2008) reported significantly higher FTAI pregnancy rates for 5-d treated cows compared to cows assigned to the 7-d protocol. This study (Bridges et al., 2008) involved a 5-d CIDR treatment with two injections of PG 12 h apart and fixed-time insemination at 72 h after the first injection of PG. The 7-d CO-Synch + CIDR treatment utilized two injections of PG with fixed-time AI at 60 h. These results are in contrast, however, to the study by Wilson et al. (2008b), who reported no difference in FTAI

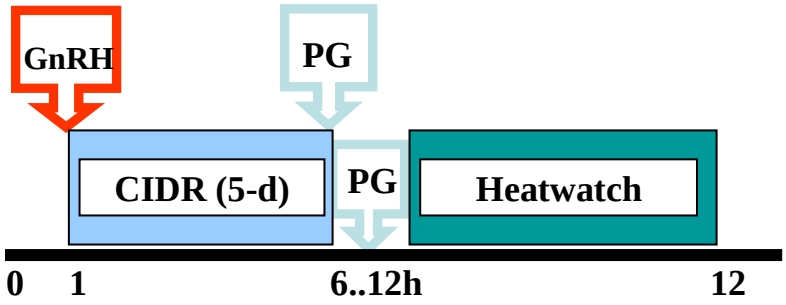
pregnancy rates between 5-d and 7-d treated cows. The Wilson et al. (2008b) study used the same 5-d protocol as Bridges et al. (2008), however cows assigned to the 7-d protocol received only one injection of PG with fixed-time insemination at 66 h.

These conflicting results may be explained by the mean intervals to estrus reported in this study. Timing of AI is important relative to pregnancy outcome following FTAI. Busch et al. (2008) confirmed that FTAI pregnancy rates were higher when AI was performed at 66 versus 54 h after administration of the 7-d CO-Synch + CIDR protocol. The 66 h interval would appear to be the near optimal timing of AI based on the results from this study. In addition, insemination is recommended to be performed at 72 h following administration of the 5-d protocol (Bridges et al., 2008), which also coincides with what would be considered to be the near the optimal timing of AI from results reported here. As previously stated, when pregnancy rates following fixed-time AI for the 5-d and 7-d CO- Synch + CIDR protocols were compared, no differences were found between treatments when cows were inseminated at 72 and 66 h, respectively. These data suggest that length of the proestrus period is an important consideration relative to pregnancy outcome following administration of both the 5-d and 7-d protocols.

There were no differences between the 5-d and 7-d Select Synch + CIDR protocols when considering AI pregnancy rates of cows classified as estrous cycling and anestrus prior to treatment. The authors acknowledge the potential for misclassification of cows on the basis of estrous cyclicity determined from two blood samples 10 d apart before treatment administration and the use of progesterone values $\geq$ 0.5 ng/mL to confirm cyclicity. However, the potential for committing a type II error is greatly minimized, if not negated, in describing cows as anestrus when using a 0.5-ng/mL cutoff.

The 5-d and 7-d Select Synch + CIDR protocols performed similarly on the basis of inducing and synchronizing estrus in this study. The two protocols also facilitated similar synchronized conception and pregnancy rates resulting from AI. The 5-d Select Synch + CIDR protocol provides an alternative to the 7-d protocol for use in facilitating FTAI, however labor and treatment costs should be considered when comparing the two protocols given the similarity in pregnancy rates that resulted following treatment.

5-d Select Synch + CIDR



7-d Select Synch + CIDR

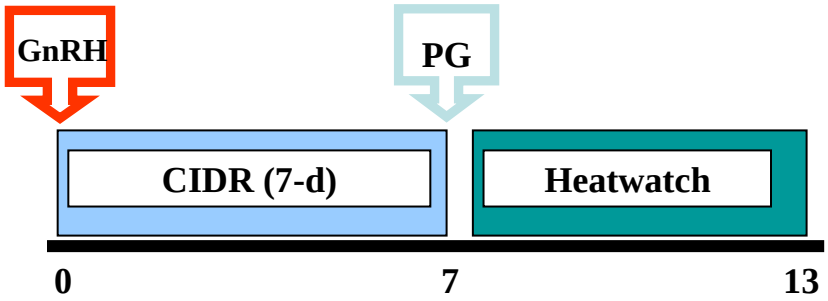


Figure 2.1. Cows were assigned to the 7-d or 5-d Select-Synch + CIDR protocols. Cows in the 7-d Select-Synch + CIDR treatment (**7-d**; n = 59) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. Estrus detection and AI were performed within a 144 h synchronized period. Cows in the 5-d Select-Synch + CIDR treatment (**5-d**; n = 58) received an injection of GnRH (100 µg) and a CIDR insert (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 5. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. Heat detection and AI were performed within a 144 h synchronized period.

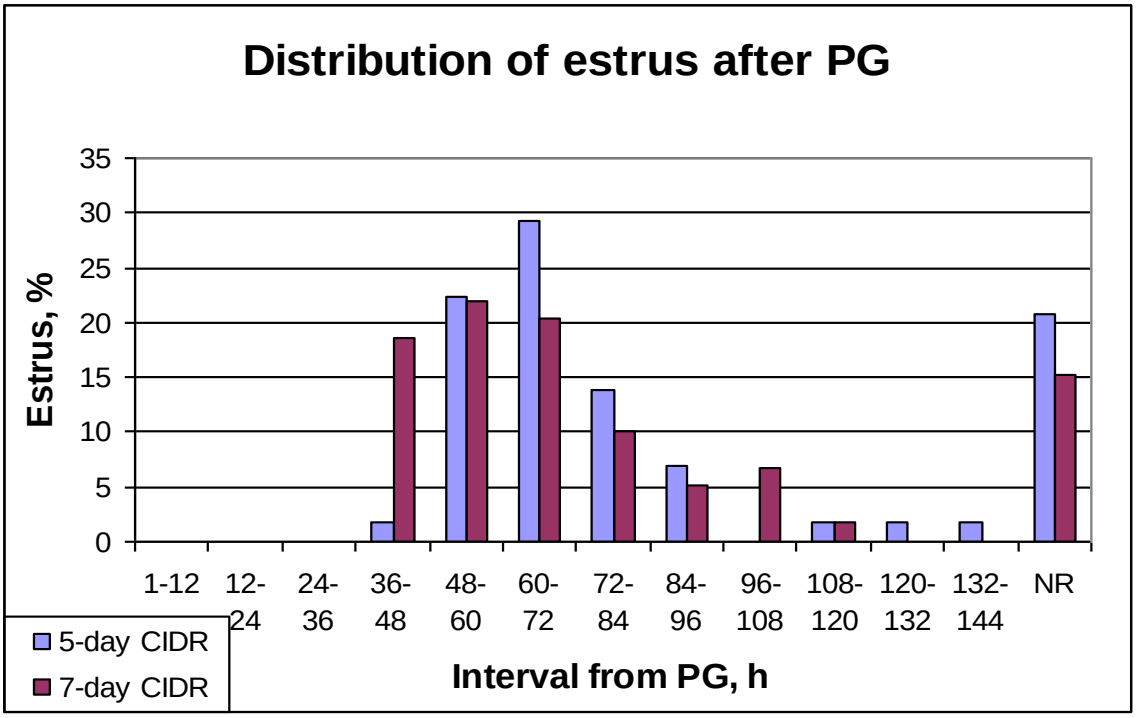


Figure 2.2. Distribution of estrus following PG administration (0 to144 h).

NR = no response

Table 2.1. Number of cows, age, days postpartum, body condition score (BCS) and estrous cyclicity status for cows in each treatment (Mean ± SE).

Treatment[1]	No	Age (Yr)	Days postpartum[2](d)	BCS[3]	Cows with elevated progesterone[4] Proportion	%
5d Select Synch + CIDR	58	2.9 ± 0.1	78.2 ± 3.0	4.9 ± 0.05	37/58	64
7d Select Synch + CIDR	59	2.9 ± 0.1	78.9 ± 2.8	4.8 ± 0.05	45/59	76
Overall	117	2.9 ± 0.2	78.5 ± 4.1	4.8 ± 0.07	82/117	70

[1]Cows in the 7-d Select-Synch + CIDR treatment (**7-d**; n = 59) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. Estrus detection and AI were performed within a 144 h synchronized period. Cows in the 5-d Select-Synch + CIDR treatment (**5-d**; n = 58) received an injection of GnRH (100 µg) and a CIDR insert (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 5. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. Estrus detection and AI were performed within a 144 h synchronized period.

[2]Number of days postpartum at day 0 for the 5-d and 7-d protocols.[3]Body condition scores of cows at the time of the first blood sample before initiation of treatments (1 to 9 scale, where 1 = emaciated, and 9 = obese).

[4]Estrous cyclicity = the percentage of cows with elevated (≥ 0.5 ng/mL) concentrations of progesterone in serum before treatment initiation. Cows were considered cyclic if progesterone was elevated in either or both of two blood samples collected 10 d before, and immediately prior to the initiation of treatments.

Table 2.2. Estrous response, interval to estrus, response to GnRH, and average follicle size at GnRH.

Treatment[1]	Estrous[2] response		Interval[3] to estrus (h)	Variance for interval to estrus	Response[4] to GnRH		Follicle size at GnRH (mm)
	Proportion	%			Proportion	%	
5d Select Synch + CIDR	46/58	79	71.2 ± 2.6	316	41/58	71	11.9 ± 0.4
7d Select Synch + CIDR	50/59	85	64.8 ± 2.6	348	42/59	71	11.9 ± 0.4

[1]Cows in the 7-d Select-Synch + CIDR treatment (**7-d**; n = 59) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. Estrus detection and AI were performed within a 144 h synchronized period. Cows in the 5-d Select-Synch + CIDR treatment (**5-d**; n = 58) received an injection of GnRH (100 µg) and a CIDR insert (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 5. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. Estrus detection and AI were performed within a 144 h synchronized period.
[2]Estrous response = number of cows that exhibited estrus during the synchronized period (0 to 144 h).
[3]Interval to estrus = number of hours from administration of PG to estrus.
[4]Cows were considered to have responded to GnRH if the dominant follicle observed by ultrasonagraphy on day 0 or 1 was no longer visible on d 2 or 3 for the respective treatments.

Table 2.3. Synchronized conception rates to AI, synchronized pregnancy rates to AI and final pregnancy rates.

Treatment[1]	Synchronized conception[2]		Synchronized pregnancy[3]		Final pregnancy[4]	
	Proportion	%	Proportion	%	Proportion	%
5d Select Synch + CIDR	33/46	72	33/58	57	52/58	90
7d Select Synch + CIDR	36/50	72	36/59	61	51/59	86

[1]Cows in the 7-d Select-Synch + CIDR treatment (**7-d**; n = 59) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. Estrus detection and AI were performed within a 144 h synchronized period. Cows in the 5-d Select-Synch + CIDR treatment (**5-d**; n = 58) received an injection of GnRH (100 µg) and a CIDR insert (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 5. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. Heat detection and AI were performed within a 144 h synchronized period.
[2]Synchronized conception rate = number of cows that conceived of those exhibiting estrus and inseminated during the synchronized period (0 to 144 h).
[3]Synchronized pregnancy rate = number of cows that conceived of the total number treated.
[4]Final pregnancy rate = number of cows that conceived by the end of the 60 d breeding season

CHAPTER 3

A COMPARISON OF THE 5-DAY AND 7-DAY CO-SYNCH + CIDR PROTOCOLS IN FACILITATING FIXED-TIME ARTIFICIAL INSEMINATION (FTAI) IN POSTPARTUM BEEF COWS

ABSTRACT

The objective of this study was to compare pregnancy rates resulting from fixed-time AI following administration of the 7-d and 5-d CO-Synch + CIDR protocols with insemination performed 66 and 72 h following treatment, respectively, for cows assigned to the two treatments. Crossbred, lactating, beef cows at three locations (n = 118, 202, and 99 at the three locations, respectively) were assigned to two treatments within age group (2 to 16 y), and by calving date (days postpartum; **DPP**) and body condition score (**BCS**). Blood samples were collected via jugular venipuncture 10 d before and immediately prior to treatment administration (d 0) to determine estrous cyclicity status. Cows assigned to the 7-d CO-Synch + CIDR treatment (**7-d**; n = 209) received an injection of GnRH (100 µg i.m.) and CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.) was administered and CIDR inserts were removed on day 7. All 7-d treated cows were fixed-time inseminated 66 h following treatment with GnRH (100 µg) administered at AI. Cows assigned to the 5-d CO-Synch + CIDR treatment (**5-d**; n = 210) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 2. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts

were removed on day 7. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All 5-d treated cows were fixed-time inseminated 72 h following treatment, with GnRH (100 µg) administered at AI. Pregnancy rate resulting from AI was determined by transrectal ultrasonography between d 60 and 67 following AI. There was no effect of treatment (P = 0.85), technician (P = 0.20), sire (P = 0.25), or location (P = 0.06) on FTAI pregnancy rates. Estrous cyclicity status (estrous cycling versus anestrus) prior to treatment administration had no effect on pregnancy rates resulting from FTAI for the 5-d or 7-d CO-Synch + CIDR protocols (5-d P = 0.51 and 7-d P = 0.26). The results from this study demonstrate that the two treatments performed comparably on the basis of pregnancy rates resulting from FTAI.

INTRODUCTION

Reproductive efficiency is a major economic trait that impacts profitability in a cow-calf enterprise (Dickerson, 1970). Artificial insemination and estrous synchronization are management practices that support improvements in reproductive efficiency by shortening the breeding season, and increasing calf age and uniformity at weaning. Genetically superior sires with proven, high accuracy EPD may be utilized to enhance the genetic potential of resulting offspring. For these reasons, artificial insemination and estrous synchronization are generally regarded as the most important and applicable of all available biotechnologies to the beef cattle industry (Seidel, 1995). To date, however, less than 12 % of beef producers report utilizing these technologies, citing a lack of time and labor as the primary reason for failure of adoption (NAHMS,

1998). Effective estrous synchronization protocols should minimize the number and frequency of cattle handlings, while reducing or eliminating estrus detection (Busch et al., 2008). These protocols must also successfully synchronize estrus to facilitate acceptable pregnancy rates resulting from fixed-time AI (**FTAI)**.

The CO-Synch protocol + CIDR protocol is currently the most widely used estrous synchronization protocol available for use in postpartum suckled beef cows (Larson et al., 2006). Schafer et al. (2007) and Busch et al. (2008) reported that pregnancy rates in the range of 66% resulting from fixed-time AI were achievable following treatment with the 7 d CO-Synch + CIDR protocol when insemination was performed at 66 h following CIDR removal and PG administration. Bridges et al. (2008) reported recently, however, that shortening the length of CIDR administration from 7-d to 5-d and lengthening the proestrus period from 60 h to 72 h resulted in improvement in pregnancy rates resulting from fixed-time AI compared with cows assigned to a 7-d protocol that were inseminated at 60 h following treatment.

The objective of this study was to compare pregnancy rates resulting from fixed-time AI following administration of the 7-d and 5-d CO-Synch + CIDR protocols with insemination performed 66 and 72 h following treatment, respectively.

MATERIALS AND METHODS

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

Experimental design. Crossbred, lactating, beef cows at three locations (n = 118, 202, and 99 at the three locations, respectively) were assigned to two treatments within age groups (2 to 16 yr), and by calving date (days postpartum; **DPP**) and body condition score (**BCS**; 1 to 9 scale, 1 = emaciated and 9 = obese; Richards et al., 1986). Cows assigned to the 7-d CO-Synch + CIDR treatment (**7-d**; n = 209) received an injection of GnRH (100 µg i.m.; Cystorelin®, Merial, Athens, GA) and EAZI-Breed™ CIDR inserts (1.38 g progesterone; Pfizer Animal Heath, New York, NY) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse® Sterile Solution, Pfizer Animal Heath) was administered and CIDR inserts were removed on day 7. All 7-d treated cows were fixed-time inseminated 66 h following treatment with GnRH (100 µg) administered at AI. Cows assigned to the 5-d CO-Synch + CIDR treatment (**5-d**; n = 210) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 2. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 7. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All 5-d treated cows were fixed-time inseminated 72 h following treatment, with GnRH (100 µg) administered at AI. Calves from both treatments were sorted from cows before and returned directly to their dams after each working to minimize effects of calf removal. All cows were inseminated by one of two experienced AI technicians at location 2 and 3, and one of three technicians at location 1. Technicians were assigned to cows within treatment by cow age, calving date and BCS. One AI sire was used at location 1, two AI sires were used at location 2, and three AI sires were used at location 3. Four sires were used overall. Cows were exposed to fertile bulls, 12 to 15 d following AI, for the remainder of a 60 d breeding season.

Pregnancy diagnosis. FTAI pregnancy rate was determined by transrectal ultrasonography (Aloka 500V equipped with 5.0 MHz linear array transducer, Aloka, Wallingford, CT) between day 60 and 67 following AI. Final pregnancy rate was determined by rectal palpation 90 d following the end of the breeding season.

Blood collection and RIA. Blood samples were collected via jugular venipuncture 10 d before and immediately prior to treatment administration (d 0) to determine estrous cyclicity status. Blood samples were allowed to clot and stored at 4º C for 24 h. Serum collection was performed by centrifugation and stored at -20º C until hormone analyses were performed. Cows were considered to be estrous cycling when progesterone concentrations were ≥ 0.5 ng/ml at one or both of the pretreatment blood samples. Serum progesterone concentrations were determined by RIA using a Coat-a-Count kit (Diagnostic Products Corp., Los Angeles, CA; Kirby et al., 1997) with intra- and interassay coefficients of variation of 2.39 and 13.65%, respectively, and an assay sensitivity of 0.1 ng/mL.

Statistical analyses. Differences in age, days postpartum, and BCS between treatments were analyzed by ANOVA using the linear statistical model of location, treatment and the interaction of location X treatment (PROC MIXED, SAS Institute Inc. Cary, NC). Differences based on pretreatment estrous cyclicity, AI sire, AI technician, FTAI pregnancy rate and final pregnancy rate were analyzed using a generalized linear models method (PROC GLIMMIX of SAS) using a binomial distribution and the link function of logit. The antilog of the average logit from GLIMMIX produced the odds.

RESULTS

The number of cows, and their mean age, DPP, BCS and estrous cyclicity status prior to treatment administration for each location and overall are shown in Table 3.1. There were no differences between treatments at the respective locations for age, DPP, BCS or estrous cyclicity status prior to treatment administration; however, there were differences among locations (Table 3.1).

The interval from PG to FTAI (mean ± SD, h) was 72.6 ± 0.6 and 66.4 ± 1.2 h for the 5-d and 7-d treatment groups, respectively. There was no effect of treatment (P = 0.85), technician (P = 0.20), sire (P = 0.25), or location (P = 0.06) on pregnancy rates resulting from FTAI (Table 3.2). Estrous cyclicity status prior to treatment administration had no effect on pregnancy rates resulting from FTAI among cows assigned to the 5-d or 7-d CO-Synch + CIDR protocols (5-d P = 0.51 and 7-d P = 0.26; Table 3.3). Based on odds, cows synchronized with the 5-d CO-Synch + CIDR and 7-d CO Synch + CIDR protocols were 1.87 and 2.04 times, respectively, more likely to become pregnant than not.

DISCUSSION

Estrous synchronization affords beef producers the opportunity to increase the uniformity of a calf crop by reducing the length of a calving season (Dziuk and Bellows, 1983). The labor required to detect estrus may also be reduced with estrous synchronization, which more readily facilitates use of AI. It will be essential in order to

increase use of AI in the U.S. beef cattle industry that estrous synchronization protocols are not only effective, but practical and economical to implement. Recently, estrous synchronization protocols were developed that eliminate the need to detect estrus, allowing AI to be performed at predetermined fixed times without reducing pregnancy rates resulting from AI (Perry et al., 2002; Stegner et al., 2004; Bader et al., 2005; Larson et al., 2006; Schafer et al., 2007; Busch et al., 2008; Bridges et al, 2008).

The CO-Synch protocol uses GnRH and PG to synchronize estrus and ovulation prior to FTAI (Perry et al., 2002; Lamb et al., 2001; Larson et al., 2006). The 7-d interval between GnRH administration and PG was used successfully to provide the time that is necessary for a new follicular wave to be recruited, along with selection and maturation of the dominant follicle to a point where successful ovulation of a healthy oocyte may be achieved (Thatcher et al., 1989; Pursley et al., 1995). The CO-Synch protocol was shown to have marginal success in synchronizing estrus before FTAI due to 5 to 15% of estrous cycling cows expressing estrus prior to the time PG was administered (Twagiramungu et al., 1995; Kojima et al., 2000). Addition of the CIDR to the CO-Synch protocol prevents cows from exhibiting estrus prior to the time PG is administered and enhanced fertility among anestrous cows (Lamb et al., 2001; Larson et al., 2006). Pregnancy rates resulting from fixed-time AI were improved when progestins were used to presynchronize cows (Perry et al., 2002; Schafer et al., 2007) prior to the administration of GnRH and PG or were included as part of the CO-Synch protocol (Lamb et al., 2001; Larson et al., 2006).

The 5-d and 7-d CO-Synch + CIDR protocols are similar except for differences in the interval from GnRH to PG (5 vs 7-d), the length of CIDR treatment, and that two injections of PG are required. The first PG injection is administered at the time CIDR

inserts are removed, and a second PG injection is administered 12 h later. Additionally, FTAI is performed at 72 h from the first injection of PG for 5-d treated cows versus 66 h for 7-d treated cows. Two injections of PG are required with the 5-d protocol to effectively regress accessory corpora lutea that form as a result of GnRH-induced ovulations at the initiation of treatment (Bridges et al., 2008; Kasimanickam et al., 2008). Bridges et al. (2008) hypothesized that shortening the duration of CIDR treatment from 7 to 5-d would better time CIDR removal coincident with optimal follicular development. Valdez et al. (2005) reported that d 4 follicles have higher intra-follicular concentrations of estradiol-17β (**E_2**) and a greater ability to produce E_2 compared to older age follicles, and that increased follicular E_2 correlated to higher pregnancy rates resulting from AI (Lopez et al., 2005). Based on these data, Bridges et al. (2008) hypothesized that lengthening the period of proestrus (the time between PG and AI) would result in higher pregnancy rates following fixed-time AI.

Bridges et al. (2008) reported that pregnancy rates following fixed-time AI were significantly higher for cows assigned to the 5-d protocol compared to cows that were assigned to a 7-d protocol. In the current study there was no difference in pregnancy rates resulting from fixed-time AI between 5-d and 7-d treated cows. It is important to contrast these results with those reported by Bridges et al. (2008) for cows assigned to the 7-d protocol. Cows assigned to the 7-d protocol in this experiment received a single injection of PG and were inseminated 66 h after PG, whereas 7-d treated cows in the study reported by Bridges et al. (2008) received two injections of PG with insemination performed 60 h after the first PG injection.

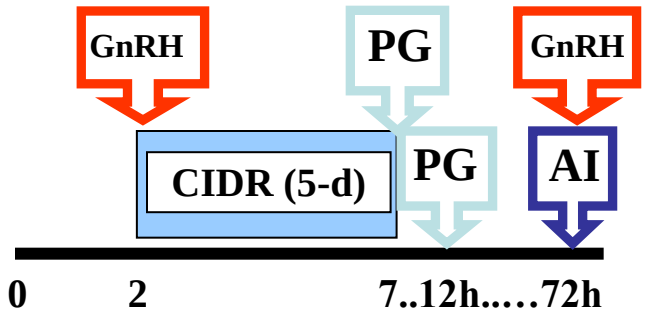
Wilson et al. (2008a) reported that there were no differences between 5-d and 7-d Select Synch + CIDR treated cows when compared on the basis of estrous response, interval to or synchrony of estrus, and synchronized conception or pregnancy rates resulting from AI. A possible explanation for differences in results between this study and Bridges et al. (2008) may be related to the differences in timing of AI for the 7-d treated cows in each study. It should be noted that timing of insemination for cows in this study that were assigned to the 5-d and 7-d protocols (72 and 66 h, respectively) were nearly coincident with the mean intervals to estrus reported by Wilson et al. (2008a). Mean intervals to estrus for cows assigned to the 5-d and 7-d Select Synch + CIDR protocols were 71.2 and 64.8 h, respectively. These data suggest that pregnancy rates resulting from FTAI are perhaps more a function of properly timed AI in relation to proestrus, rather than timing CIDR removal and follicular development. Additionally, cows assigned to the 5-d protocol are required to receive two injections of PG, 12 h apart, which requires additional animal handling compared with cows that were assigned to the 7-d protocol. It is difficult to assess the impact of additional animal handling and resulting stress as it relates to pregnancy outcome.

It is also important to note that there were no differences within or between treatments in FTAI pregnancy rates among cows that were considered to be estrous cycling or anestrus prior to treatment administration. There were, however, numeric differences in pregnancy rates resulting from FTAI between 5-d and 7-d treated cows that were anestrus versus estrous cycling. Pregnancy rates following FTAI for estrous cycling and anestrous cows were 70 and 63%, versus 64 and 73% for the 5-d and 7-d treated cows, respectively. The numerically higher pregnancy rates among anestrous cows

assigned to the 7-d protocol in this study are similar to those reported by Schafer et al. (2007). These data suggest that field trials with larger numbers of cows are perhaps warranted to better evaluate potential differences between treatments on the basis of efficacy among mixed groups of estrous cycling and anestrous cows. The data furthermore raise the question as to whether two additional days of progesterone exposure are beneficial in terms of improving the likelihood of pregnancy outcome following treatment with the CO-Synch + CIDR protocol among anestrous cows.

Finally, the 5-d and 7-d CO-Synch + CIDR protocols were compared in this study on the basis of pregnancy outcome and practical application in the field. The results from this study clearly demonstrate that the two treatments perform comparably on the basis of pregnancy rates resulting from FTAI. Given this observation, the 5-d protocol provides an alternative to the 7-d protocol for use in facilitating FTAI, however beef producers must seriously consider the increased labor and treatment costs associated with this protocol, compared with the 7-d protocol in which cows are inseminated 66 h following treatment administration.

5-d CO-Synch + CIDR



7-d CO-Synch + CIDR

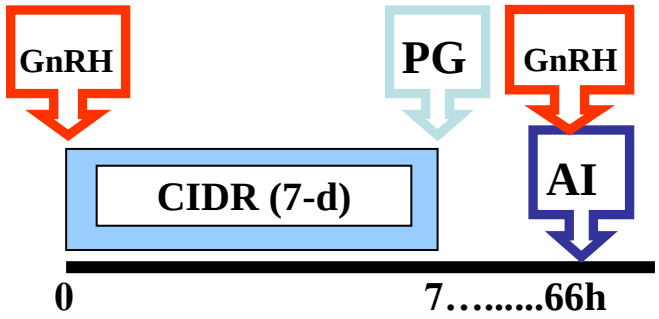


Figure 3.1. Cows were assigned to the 7-d or 5-d CO-Synch + CIDR protocols. Cows in the 7-d CO-Synch + CIDR treatment (**7-d**) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. All 7-d treated cows were fixed-time inseminated 66h following treatment with GnRH (100 µg) administered at AI. Cows in the 5-d CO-Synch + CIDR treatment (**5-d**) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 2. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 7. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All 5-d treated cows were fixed-time inseminated 72 h following treatment with GnRH (100 µg) administered at AI.

Table 3.1. Number of cows at each location, days postpartum, BCS and estrous-cycling status for cows before initiation of each treatment (means ± SE).

Location and Treatment[1]	No.	Age, yr	Time postpartum,[2] d	BCS[3]	Cows with elevated progesterone[4]	
					Proportion	%
Location 1						
5d	60	7.9 ± 0.4	59.2 ± 1.6	5.5 ± 0.05	55/60	92
7d	58	7.5 ± 0.4	59.1 ± 1.7	5.5 ± 0.05	53/58	91
Combined	118	7.7 ± 0.3[a]	59.1 ± 1.2[a]	5.5 ± 0.03[a]	108/118	92[a]
Location 2						
5d	100	4.4 ± 0.3	78.6 ± 1.3	5.8 ± 0.04	38/100	38
7d	102	4.4 ± 0.3	78.6 ± 1.3	5.8 ± 0.04	41/102	40
Combined	202	4.4 ± 0.2[b]	78.6 ± 0.9[b]	5.8 ± 0.03[b]	79/202	39[b]
Location 3						
5d	50	6.2 ± 0.4	67.4 ± 1.8	5.6 ± 0.05	29/50	58
7d	49	6.0 ± 0.4	68.1 ± 1.8	5.6 ± 0.05	34/49	69
Combined	99	6.1 ± 0.3[c]	67.8 ± 1.3[c]	5.6 ± 0.04[c]	63/99	64[c]
Overall						
5d	210	5.8 ± 0.2	70.4 ± 1.0	5.7 ± 0.03	122/210	58
Overall						
7d	209	5.6 ± 0.2	70.7 ± 1.1	5.7 ± 0.03	128/209	61

[1]Cows in the 7-d CO-Synch + CIDR treatment (**7-d**) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. All 7-d treated cows were fixed-time inseminated 66h following treatment with GnRH (100 µg) administered at AI. Cows in the 5-d CO-Synch + CIDR treatment (**5-d**) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 2. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 7. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All 5-d treated cows were fixed-time inseminated 72h following treatment with GnRH (100 µg) administered at AI.

[2]Number of days postpartum at day 0 for both the 5-d and 7-d protocols.

[3]Body condition scores of cows at the time of the first blood sample before initiation of treatments (1 to 9 scale, where 1 = emaciated, and 9 = obese).

[4]Estrous cyclicity = the percentage of cows with elevated (≥ 0.5 ng/mL) concentrations of progesterone in serum before treatment initiation. Cows were considered cyclic if progesterone was elevated in either or both of two blood samples collected 8 to 10 days before, and immediately prior to the initiation of treatments.

[a,b,c]Within a column, means without a common superscript differ ($P < 0.05$).

Table 3.2. Pregnancy rates after fixed time AI and at the end of the breeding season.

Location and Treatment[1]	Pregnancy rate to fixed-time AI[2]		Pregnancy rate at the end of the breeding season[3]	
	Proportion	%	Proportion	%
Location 1				
5d	36/60	60	57/60	95
7d	38/58	66	57/58	98
Location 2				
5d	72/100	72	99/100	99
7d	73/102	72	97/102	95
Location 3				
5d	32/50	64	45/50	90
7d	29/49	59	46/49	94
Overall				
5d	140/210	67	201/210	96
Overall				
7d	140/209	67	200/209	96

[1]Cows in the 7-d CO-Synch + CIDR treatment (**7-d**) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. All 7-d treated cows were fixed -time inseminated 66h following treatment with GnRH (100 µg) administered at AI. Cows in the 5-d CO-Synch + CIDR treatment (**5-d**) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 2. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on d 7. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All 5-d treated cows were fixed-time inseminated 72h following treatment with GnRH (100 µg) administered at AI.

[2]Pregnancy rate to fixed-time AI was determined by ultrasound 60 -70 d after AI.

[3]Pregnancy rate at the end of the breeding season was determined 80-90 d after the end of a 60 d breeding season

Table 3.3. Pregnancy rates after fixed-time AI based on estrous cyclicity before initiation of the treatments.

	5d CO-Synch + CIDR[1]				7d CO-Synch + CIDR[1]			
	Estrous cycling[2]		Anestrus[2]		Estrous cycling[2]		Anestrus[2]	
Location	Proportion	%	Proportion	%	Proportion	%	Proportion	%
1	34/55	62	2/5	40	35/53	66	3/5	60
2	31/38	82	41/62	66	27/41	66	46/61	75
3	20/29	69	12/21	57	20/34	59	9/15	60
Combined	85/122	70	55/88	63	82/128	64	58/79	73

[1] Cows in the 7-d CO-Synch + CIDR treatment (**7-d**) received an injection of GnRH (100 µg i.m.; Cystorelin®) and EAZI-Breed™ CIDR inserts (1.38 g progesterone) on day 0. Prostaglandin $F_{2\alpha}$ (**PG**; 25 mg i.m.; Lutalyse®) was administered and CIDR inserts were removed on day 7. All 7-d treated cows were fixed-time inseminated 66h following treatment with GnRH (100 µg) administered at AI. Cows in the 5-d CO-Synch + CIDR treatment (**5-d**) received an injection of GnRH (100 µg) and CIDR inserts (1.38 g progesterone) on day 2. Prostaglandin $F_{2\alpha}$ (25 mg) was administered and CIDR inserts were removed on day 7. A second injection of PG (25 mg) was administered to all 5-d treated cows 12 h after the first PG injection. All 5-d treated cows were fixed-time inseminated 72h following treatment with GnRH (100 µg) administered at AI.

[2]Estrous cyclicity = the percentage of cows with elevated (≥ 0.5 ng/mL) concentrations of progesterone in serum before treatment initiation. Cows were considered cyclic if progesterone was elevated in either or both of two blood samples collected 8 to 10 days before, and immediately prior to the initiation of treatments.

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VITA

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