The effects of resynchronization of estrus using the 5 d CO-Synch + CIDR system in beef heifers

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ABSTRACT

Recent efforts have improved synchronization systems that facilitate timed insemination in beef cattle. However, synchronization systems utilizing a single fixed-time artificial insemination (FTAI) frequently result in 25-40% non-pregnant heifers. The purpose of this study was to determine the effectiveness and define economic parameters of a FTAI resynchronization protocol in beef heifers after synchronization using a 5d CO-Synch + CIDR system. Estrus was synchronized in crossbred heifers (n=176) using 5 d CO-Synch + CIDR with FTAI at 72 h. After the initial AI, open heifers received either resynchronization (RS) or natural service (NS) return service treatments. The RS treatment was diagnosed for pregnancy 29 d after the initial AI, and all open heifers were resynchronized using the 5 d CO-Synch + CIDR with FTAI at 72 h. Heifers diagnosed pregnant following initial AI received no further treatment. Heifers in the NS treatment were exposed to fertile bulls from d 14 to d 66 following initial AI. Return to estrus data were collected using the Heat Watch Estrus Alert System. Total AI pregnancies tended to be higher (P=0.07) for RS (69.7%) than NS (56.5%) heifers. Overall pregnancy rate was greater for NS (89.4%) than for RS (69.7%) at the end of the breeding season (P < 0.01). The cost of RS was $128.63 and for NS was $82.50 per pregnancy. The expected average calf value per heifer exposed was $195.84 for RS treatment and $357.62 for NS

treatment. This difference was attributed to the increased number of open heifers in the RS treatment. The resynchronization of estrus after the initial FTAI yielded a limited number of pregnancies in the breeding season in this study. However, the resynchronization program also cost more per pregnancy. Further investigation into resynchronization should focus on both biological and economic impacts.

“I can do all things through Christ who strengthens me”

Philippians 4:13

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Introduction

Yearly income in a cow/calf herd is derived primarily from calf sales. These sales are based on pounds of calf sold. Heifers that conceive early in the breeding season will produce more pounds of calf per year than heifers conceiving late in the breeding season (Lesmeister et al., 1973), primarily as a result of calf age and associated weight advantages. Cows that calve earlier in the season also rebreed earlier than their late calving counterparts (Osoro and Wright, 1992) and have higher rebreeding rates (Selk et.al, 1988). Heifers that conceive early in the breeding season and thus calve earlier in the calving season have a higher average annual calf production in their lifetime than heifers that calve late (Lesmeister et al., 1973). This is because calves are typically weaned on the same date regardless of age or weight, and consequently calves born earlier in the calving season weigh more; therefore increasing the lifetime productivity of their dams. Hence, it is important to have heifers conceive early in the breeding season.

Age at puberty is an important factor in reproductive success. Puberty is the point in time at which the heifer is willing and able to reproduce successfully (when she first expresses estrus and ovulates), normally between 9-14 mo of age. The number of heifers that exhibit estrus early in the breeding season affects the number of heifers that become pregnant during the breeding season (Short and Bellows, 1971).

Use of estrus synchronization can increase the percentage of heifers conceiving early in the breeding season. These heifers will calve early in the

calving season the next year, thus the calves will be older and typically heavier at weaning. The heifers that calve early will also have a longer time to recover before the next breeding season and thus have a better chance of rebreeding during the subsequent breeding season. Artificial insemination allows the use of genetically superior bulls and reduces the number of bulls on the farm.

Puberty

Senger (2003) defines puberty for males and females as the ability to reproduce successfully. The hypothalamus is the primary component of the reproductive system that controls ovarian function and the timing of the onset of puberty (Schams et al., 1981; Kinder et al., 1987). Puberty is initiated when gonadotropin-releasing hormone (GnRH) secretion reaches sufficient concentration and frequency to stimulate release of gonadotropins by the anterior pituitary. The limiting factor for onset of puberty is the presynaptic neurons ability to transfer information to the GnRH neurons so that there will be a subsequent increase in GnRH secretion. The ability of these neurons to function is affected by three factors: 1) nutrition, 2) environment, and 3) genetics.

Nutrition

Variations in nutritional levels will affect the age at which beef heifers reach puberty. Age at puberty is decreased with an increase in ADG from birth to weaning (Wiltbank et al., 1966). However, Nelsen and colleagues (1982) found that there is a minimum age requirement that must be met regardless of ADG. Age and weight at puberty are also affected by weaning weight; a high preweaning growth rate and heavy weaning weight results in earlier puberty and

a heavier weight at puberty (Arije and Wiltbank, 1971). Several studies have reported that enhanced nutrient intake increases body size and fatness at puberty (Arije and Wiltbank, 1971; Short and Bellows, 1971; Hall et al., 1994; Hall et al., 1995). However, other studies have reported that enhanced nutrient intake results in decreased BW (Grass et al., 1972), similar BW (Crichton et al., 1972) or similar BW:height (Crichton et al., 1972; Nelsen et al., 1982) at puberty. In 1990, Kurz and colleagues found that when heifers were fed a restricted diet the pulsatile release of LH was suppressed.

Heifers with rapid growth and large frame are heavier and taller at puberty (Laster et al., 1972; Ferrell, 1982; Hall et al., 1995). After a certain weight is reached variations in ADG did not affect age of puberty (Wiltbank et al., 1966; Short and Bellows, 1971). In 1976, Frisch proposed a theory of a critical body composition or fat:lean for the onset of puberty. However, this theory has been challenged with results from several studies (Short and Bellows, 1971; Brooks et al., 1985; Yelich et al., 1992; Hopper et al., 1993; Hall et al., 1995) thus reinforcing the contention that body composition has little effect on the onset of estrous cycles (Bronson and Manning, 1991). Although body stores of fat are not critical for onset of puberty, they are related to the maintenance of estrous cycles in cattle (Imakawa et al., 1986; Richards et al., 1989).

Genetics

There are genetic differences among breeds for several traits, including age of puberty (Laster et al., 1979). Heterosis increases the rate of sexual maturity (Wiltbank et al., 1966). Crossbred heifers fed at high nutritional levels

were heavier at puberty than their straightbred counterparts even though age at puberty was the same (Wiltbank et al., 1969). Breed differences, sire and dam effects within a breed, and heterosis contribute to the age at puberty (Short et al., 1990).

Environment

Effect of season of birth on age at puberty has been reported in dairy heifers. Several studies have reported that spring-born heifers reached puberty at a younger age than heifers born at other times of the year (Hawk et al., 1954; Menge et al., 1960; Roy et al., 1980). In 1971, Arije and Wiltbank reported that beef heifers born late in the spring calving season were lighter and younger at puberty than those born earlier in the calving season. However, fall-born Angus x Holstein heifers have been reported to attain puberty at a younger age than heifers born in the spring (Schillo et al., 1982; 1983). In 1985, Hansen reported that differences in the previous studies could be due to factors other than season of birth such as season of sexual maturity and interactions with other environmental factors like nutrition. In 1992, Schillo and colleagues stated that the effect of season on the onset of puberty can be attributed to daylength and ambient temperatures. The authors reported that birth during autumn conditions and exposure to spring conditions after 6 mo of age reduced the age at onset of puberty.

There have been contradicting reports in regards to the effect of social interaction with bulls on onset of puberty. It has been reported that neither short-term (Berardinelli et al., 1978) nor long-term (Roberson et al., 1987) exposure to

bulls affected the onset of puberty in beef heifers. However, after a 2-yr study Pennel and colleagues (1986) reported a reduced age at puberty in beef heifers exposed to bulls. Likewise, in 1991 Roberson and colleagues reported a reduced age at puberty in beef heifers exposed to bulls in 3 of 4 yr. In 1982, Izard and Vandenburgh reported that treatment of heifers with bull urine accelerated puberty.

It is recommended that heifers be bred to calve as two yr-olds and that they calve earlier than their mature counterparts thus allowing for longer postpartum interval prior to the following breeding season. However, this may cause heifers to be bred on their pubertal estrus. Heifers bred on pubertal estrus have been reported to have lower fertility compared to heifers bred on their third estrus (Byerley et al., 1987). Therefore, heifers should be managed so that puberty is reached prior to the start of the breeding season. This ensures that more heifers are cycling by the beginning of the breeding season in order to reduce the negative effects on fertility that are associated with the pubertal estrus.

The Estrous Cycle

The typical bovine estrous cycle is 21 d long with a range of 17-24 d and is divided into two phases: luteal and follicular. The follicular phase begins with regression of the corpus luteum (CL) and ends with ovulation. The time from ovulation until regression of the CL is called the luteal phase. Each of these phases contains two of the four stages of the estrous cycle. The proestrus and

estrus stages are subdivisions of the follicular phase and the metestrus and diestrus stages are subdivisions of the luteal phase.

Follicular Phase

This stage is a time of transition from progesterone dominance to estrogen dominance. Estradiol is the dominant hormone in the follicular phase and is produced by the growing follicles. Estradiol initiates estrus and controls the onset of the preovulatory LH surge. This LH surge causes ovulation.

Proestrus

The first stage of the follicular phase is proestrus. The beginning of proestrus is marked by a decline of progesterone signaling the regression of the CL known as luteolysis (Senger, 1999). This decrease in progesterone causes GnRH pulse frequency to increase, resulting in an increased secretion of LH and FSH. The pulse frequency increase of LH is associated with the pulse frequency increase of GnRH (Rodriguez and Wise, 1971). Reduced progesterone concentrations cause a decline in the negative feedback on pituitary LH secretion, increasing LH pulse frequency (Smith et al., 2005). Increases in LH pulse frequency stimulate follicular maturation, thus causing an increase in follicular estradiol secretion (Kojima, 2003). During this phase of the cycle when the ovarian follicles are secreting greater amounts of estradiol, the frequency of LH pulses is increased (Rahe, 1980 and Kinder, 1991). This estradiol secretion creates a positive feedback on the hypothalamus which then secretes GnRH which in turn acts on the anterior pituitary causing release of LH (Schally, 1971).

The immature and smallest follicle is called a primordial follicle and all primordial follicles are present in the female at birth. Primordial follicles continuously enter a group of growing follicles. The reduction in progesterone and subsequent increase in GnRH causes an increase in LH and FSH release in early proestrus thus beginning the recruitment of follicles. During recruitment, a group of antral follicles develop past the early stages and begin to produce estradiol and inhibin. The amount of estradiol and inhibin increases as the follicles approach the selection of the follicle that will become dominant. Those follicles not selected undergo atresia. When the inhibin levels increase, the negative feedback on the anterior pituitary increases, thus causing a decline in FSH production.

Estrus

Proestrus ends at the onset of estrus, the most recognizable stage of the estrous cycle; the second stage of the follicular phase. Estrus is the period of sexual receptivity in the female which ranges from 6-24 h with the average being 15 h (Senger, 2003). Estradiol is at its peak during estrus causing the surge center of the hypothalamus to activate, releasing a large quantity of GnRH, which in turn causes the release of a surge of LH from the pituitary, resulting in ovulation of the dominant follicle. This is only possible when the inhibition of GnRH is removed due to the regression of the CL and is different from the pulses of GnRH that the tonic center of the hypothalamus releases. Ovulation occurs 25-30 h after the onset of estrus.

At any given time during the estrous cycle, all stages of follicles are present on the ovary (primordial, primary, secondary, antral), but the presence of the corpus luteum depends on the stage of the cycle (Senger, 2003).

Luteal Phase

The luteal phase begins with formation of the corpus luteum (originating from the ovulatory follicle) and ends with luteolysis. This phase includes metestrus and diestrus. The corpus luteum produces progesterone, its major function is to prepare the uterine endometrium for implantation and to maintain pregnancy. The CL also prevents ovulation by inhibiting release of gonadotrophins.

Metestrus.

During ovulation the follicle ruptures, causing a red structure that is called corpus hemorrhagicum. This structure begins to grow and produce progesterone, however, during metestrus both estradiol and progesterone levels are low. During this time, when progesterone levels are low, the LH pulses are high frequency (Rahe et al., 1980). Before ovulation, luteal cells (from the theca and granulose cells) are prepared for the production of progesterone (Smith et al., 1994). Large luteal cells are formed from granulose cells and small luteal cells are formed from the theca cells (O'Shea 1987 and Priedkans, 1968). However, there have been some reports of small luteal cells differentiating into large luteal cells and vice versa (Smith, 1994).

Diestrus.

The CL continues increasing in size and progesterone production until the mid-luteal phase where it peaks in size and progesterone production plateaus.

At this time of elevated progesterone, LH pulses are less frequent (Rahe et al., 1980). During this time it is bright orange or yellow and is called a corpus luteum (Kojima, 2003). Towards the end of the luteal phase, luteolysis (regression of the CL) occurs. After luteolysis there is an increase of LH pulse frequencies along with a decrease in FSH concentrations (Kojima, 2003). After a CL is lysed it is then called a corpus albicans. Because the negative feedback due to progesterone is no longer present, the female begins a new follicular phase and starts back at proestrus. However, remnants of the CL can still be seen on the surface of the ovary for several cycles.

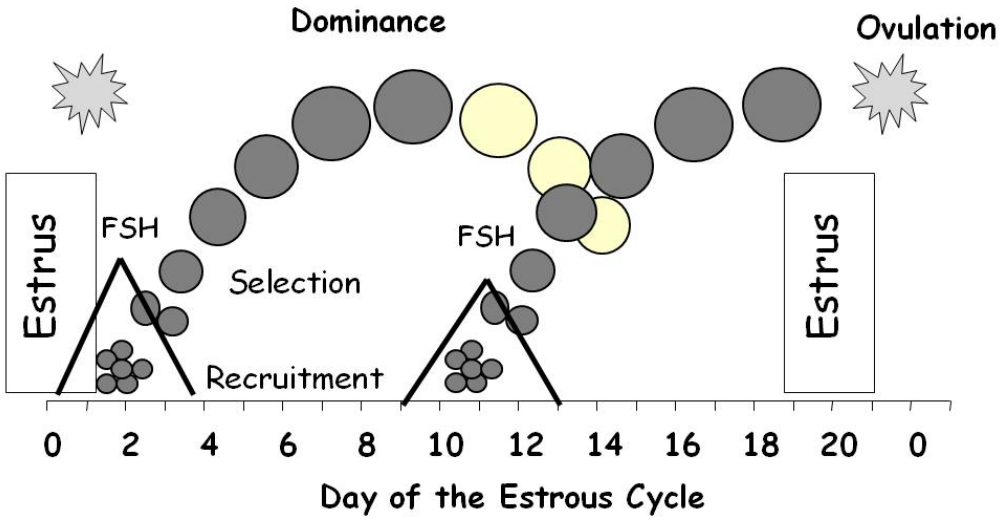
Follicular Waves

In cattle, as well as sheep and horses, dominant follicles develop in sequential waves during both the follicular and luteal phases of the cycle (Smith et al., 2005). The estrous cycle in cattle consists of two to three follicular waves. Cows that have three follicular waves generally have a longer estrous cycle (20-24 d) than cows with two follicular waves (18-20 d; Smith et al., 2005). There are three stages of follicular waves in cattle: recruitment, selection and dominance.

Recruitment is a period of time with high levels of FSH, low levels of LH and no measurable inhibin or estradiol. During this time a group of small (about 3 mm in diameter; Smith et al., 2005) follicles begin to grow and produce low amounts of estradiol (Senger, 2003). Ginther and colleagues (1996) reported that FSH peaks when the future dominant follicle reaches 4 mm in diameter and returns to basal levels by the time selection occurs (Ginther et al., 2000a). After recruitment follicles either become atretic or are selected to continue growing.

As the follicle continues growing it increases production of estradiol and inhibin which inhibit FSH secretion from the anterior lobe of the pituitary and increasing LH secretion (Ginther et al., 2000b; Senger, 2003). After a follicle has been selected (Figure 1.1) it becomes dominant, producing a large amount of estradiol, causing an LH surge.

Figure 1.1. Physiology of Follicular waves[a]



[a]Adapted from Smith et al., 2005

The continued low concentration of FSH (because of the high secretions of inhibin and estradiol from the dominant follicle) is believed to cause the follicles remaining after selection to become atretic (Senger, 2003). Ginther (1986) reported that the dominant follicle grows faster than the next largest follicle and therefore stops the initiation of a new follicular wave. The dominant follicle will either go on to ovulate or will become atretic; thus initiating a new follicular wave.

Hormonal Control of the Cycle

Artificial insemination allows beef cattle producers to use genetically superior bulls and reduces the risk of sexually transmitted diseases. The use of AI can reduce the chances of dystocia through the selection of proven sires with high reliability for calving ease and low birth weights (Bennett and Gregory, 2001). However, AI is only used by 13.3% of operations (APHIS, 1998). The labor involved in estrus detection makes AI impractical for many producers, thus the development of synchronization systems that allow for AI over a period of a few d or at a fixed-time may increase the adoption of AI.

Estrus synchronization has the potential to increase the uniformity of the calf crop, shorten the calving season, and enhance the opportunities to utilize AI (Larson et al., 2006). Protocols that reduce time and labor in a cost effective manner and produce satisfactory pregnancy rates are more likely to be used by producers. Evaluation of estrus synchronization programs should be based on their effect on pregnancy rates (Patterson et. al, 1989), as well as animal handling and labor. Synchronization protocols need to be effective in mixed populations of both cyclic and acyclic cows. Heifers may or may not have reached puberty and postpartum cows tend to be mixed populations because some are nutritionally challenged and others have had insufficient time to resume cycles after calving.

Progestins

The discovery that progesterone inhibited ovulation and maturation of preovulatory follicles (Ulberg et al., 1951; Nellor and Cole, 1956; Hansel et al.,

1961) led to the process of developing synchronization protocols to control the estrous cycle of the cow. Progesterone functions to prevent maturation and ovulation of follicles by suppressing the release of GnRH and LH from the anterior pituitary (Hansel and Convey, 1983). Final maturation and ovulation of the oocyte depends on the preovulatory surge of LH (Schallenbrger et al., 1984; Garverick and Smith, 1986; Ginther et al., 2000). Exposure to progestins is thought to be a prerequisite for establishment of normal cycles because progesterone levels increase before puberty in heifers (Berardinelli et al., 1979) and before resumption of cyclicity in postpartum cows (Short et al., 1974; Rawlings et al., 1980).

Progesterone is normally secreted from the CL. However, there are two approved exogenous sources of progesterone: melengestrol acetate (MGA) and the controlled internal drug release (CIDR). Both provide an artificial source of progestin, even when there is no CL present, thus inhibiting ovulation.

Historically other progestational compounds have been used. An orally active progestational compound, medroxyprogesterone (MAP) was reported to have a decreased potency for inhibiting estrus and ovulation when compared to MGA (Zimbelman and Smith, 1963).

In 1979, Heersche and colleagues reported 93% of heifers displaying estrus when treated with a 7 d norgestomet implant along with an injection of $PGF_{2\alpha}$ given either at implant removal or 24 h before implant removal. Although there was a high percentage of animals showing estrus (77 to 100%) after

treatment with the Syncro-Mate B system, reported conception rates to the first service have been variable (33 to 68%; Odde, 1990).

Pregnancy rates to timed insemination in beef heifers synchronized using 14-17 d MGA-$PGF_{2\alpha}$ (43%, Kesler et al., 1996; 36%, Larson et al., 1996) have been less than other synchronization systems. In 2004, Johnson and Day reported a similar pregnancy rate (49%) when using 19-d MGA- $PGF_{2\alpha}$ system.

When exposed to long term treatment of progestins a reduction in fertililty has been reported, but treatment for less than 14 d does not reduce conception (Larson,1996). However, this short term exposure must be accompanied with a luteolytic agent.

Persistent Follicles.

A persistent follicle occurs when the dominant follicle life span is extended and there is increased production of estradiol (Zimbelman and Smith, 1966a; Siriosis and Fortune, 1990). When heifers were treated with low levels of progesterone following luteolysis, persistent follicles that had a large diameter, extended lifespan, and increased production of estradiol were formed (Zimbelman and Smith, 1966b; Siriosis and Fortune, 1990; Fortune et al., 2001). According to Duffy et al. (2000), the formation of persistent follicles has been associated with increased LH frequency, and Kinder et al. (1996) found that administration of low level progestins increase LH pulse frequency, in the absence of luteal tissue.

Mihm et al. (1994) found that long term progestin treatment and ovulation of a persistent follicle followed immediately by insemination is associated with

decreased fertility. The decreased fertility after ovulation of persistent follicles may be caused by changes in the uterine environment due to the increased estradiol (Butcher and Pope, 1979). Another theory about the reduced fertility associated with persistent follicles was linked to LH. Revah and Butler (1996) suggested that there may be an increased frequency of LH pulses that oocytes in persistent follicles are exposed to, thus inducing the first meiotic division prematurely. Reported fertilization rates are similar for normal and persistent follicles, but early embryonic death occurred at a greater frequency with persistent follicles (Wehrman et al., 1996).

Puberty Induction.

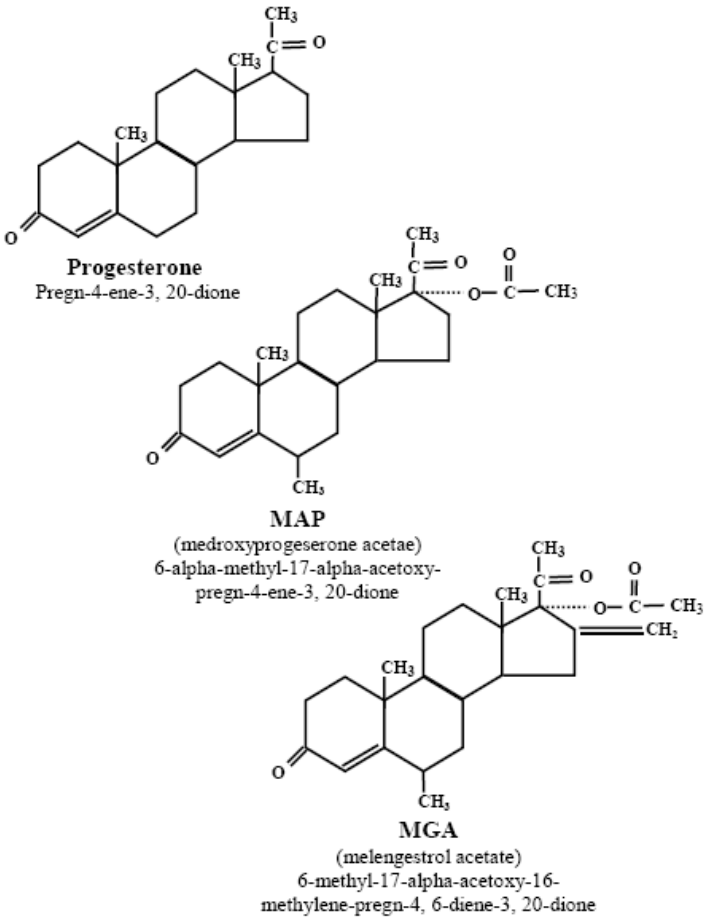
In addition to not reducing fertility, short term exposure to progestins has been reported to cause some anestrus females to begin cycling. Utilization of progestins in synchronization protocols induces puberty in heifers (Gonzalez-Padilla et al., 1975; Patterson et al., 1990). Hall et al. (1997) found that treatment with a progestin caused an increased frequency of LH pulses with the greatest occurring after progestin removal. Treatment with a progestin was also linked with an increase in the diameter of the largest follicle (Hall et al., 1997). Treatment with a progestin was also associated with an increase in follicular growth, causing an increase in estradiol production by the follicles (Hendricks et al., 1973; Wetteman and Hafs, 1973; Sheffel et al., 1982; Garcia-Winder et al., 1986).

The occurrence of a short luteal phase in prepuberal heifers and postpartum cows usually follows the first ovulation (Perry et al,. 1991). According

to Smith et al. (2005), the short term exposure to progesterone helps resume normal cycling. Therefore, the addition of progesterone before the induction of ovulation can reduce the instance of short cycling in the herd.

The most commonly used progestins in estrus synchronization systems are melengestrol acetate (MGA) and Controlled Internal Drug Release (CIDR) inserts.

Figure 1.2. Chemical structures of progesterone, medroxyprogesterone acetate (MAP), and melengestrol acetate (MGA).



Melengestrol acetate

Melengestrol acetate is progestin source that is a feed additive that was developed in 1962 for use in feedlot heifers to improve feed efficiency and rate of gain by preventing estrus (Figure 1.2; Zimbelman and Smith, 1966a). Melengestrol acetate can be used to suppress estrus, prevent preovulatory surge of LH, and inhibit ovulation when fed at the recommended level of 0.5 mg/hd/day (Zimbelman et al., 1966a; Imwalle et al., 2002). It can also induce estrous cycling in prepubertal heifers (Patterson et al, 1990; Imwalle et al., 1998). In order for MGA to work effectively, it must be consumed at the rate of 0.5 mg/hd/d. To ensure proper consumption and adequate circulating levels of progestin, feeding should be around the same time every day and adequate bunk space must be provided (Patterson et al., 2003b). However, MGA is not approved for use in postpartum beef cows. Variable pregnancy rates (36-67%) have been reported while using MGA to synchronize estrus (Patterson et al., 2005).

Controlled Internal Drug Release Inserts

The CIDR was approved in the United States in 2002 (DeJarnette, 2003) and is the only currently available FDA approved progestin source for estrus synchronization of both heifers and postpartum beef cows in the United State (FDA, 2002). The EAZI-BREED™ CIDR is a “T-shaped” intravaginal progestin insert with flexible wings that is inserted into the vagina of a heifer or cow in order to synchronize estrus. The nylon backbone of the CIDR is covered with a soft rubber compound impregnated with 1.38g of progesterone (Figure 1.2). After

insertion, concentrations of progesterone in the blood rise rapidly to peak concentrations approximately 1 h after insertion (Lamb and Larson, 2005). The progesterone concentrations in the blood decline rapidly in the 12-24 h period following CIDR removal (Perry et al., 2004). Macmillian et al. (1991) found that in ovariectomized beef heifers, plasma progesterone concentrations peaked at 8.7 ng/ml 6 h after CIDR insertion. During the 15-d treatment there was an average concentration of 5.7 ng/ml (Macmillian et al., 1991). CIDR retention rates average 96-99% in heifers treated 4 to 15 d (Lucy et al., 2001; Macmillian et al., 1988; 1991).

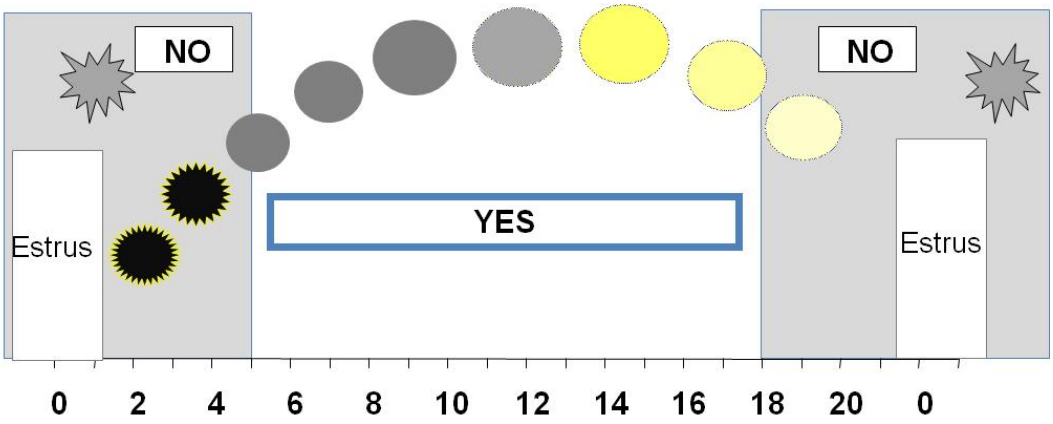
Improvements in the synchrony of estrus for beef heifers have been reported in protocols using CIDRs when compared to MGA systems (Kojima et al., 2004; Tauck et al., 2007). Tauck et al. (2007) reported that following CIDR removal, progesterone clears faster than MGA. CIDRs may also be used to initiate cyclicity in postpartum beef cows (Perry et al., 2004). When using CIDRs as the progestin in synchronization systems, Lamb and Larson (2005) reported variable pregnancy rates in postpartum beef cows (26-59%).

Prostaglandins

Prostaglandin F2α ($PGF_{2\alpha}$) causes the regression (luteolysis) of the CL and subsequent decline in progesterone. This then eliminates the inhibitory effect of progesterone and will allow follicles to grow and ovulate. When given an injection of $PGF_{2\alpha}$ or its analogs, females with a CL on their ovary will usually exhibit estrus within 2-5 d. The CL is only responsive to $PGF_{2\alpha}$ between d 5 and 18 of the cycle (Figure 1.3). In prepubertal heifers or anestrous cows, an

injection of $PGF_{2\alpha}$ is not effective due to an absence of luteal tissue (Smith et al., 2005). Therefore, females must be cycling in order for synchronization protocols using prostoglandins to be successful. Studies had previously reported that prostaglandin was more effective when given during the late luteal phase (King et al., 1982; Tanabe and Hann, 1984; Watts and Fuquay, 1985).

Figure 1.3. $PGF_{2\alpha}$ – Effect of stage of the cycle[a]



[a]Effect of the day of the cycle on luteal responsiveness to $PGF_{2\alpha}$. (Adapted from Smith et al., 2005).

Gonadotropin-releasing Hormone

GnRH from the hypothalamus acts on the pituitary causing the release of LH and FSH. Luteinizing hormone and FSH aid in follicular growth. The follicle produces estrogen, and elevated levels of estrogen are associated with ovulation and estrus. Gonadotropin-releasing hormone (GnRH) induces ovulation of dominant follicles and starts new follicular waves. GnRH causes the ovulation of follicles >10mm in size because it stimulates the preovulatory surge of LH that causes ovulation.

When evaluated across all stages of the estrous cycle a single injection of GnRH is only able to induce ovulation (thus sequencing the initiation of the next follicular wave) in 66% of cows (Geary et al., 2000) and 55% of heifers (Pursley et al., 1995). Silcox and colleagues (1993) reported that ovulatory response to GnRH of a dominant follicle during the growth phase, plateau phase, and atretic phase was 100, 33, and 0%, respectively. Geary and colleagues (2000) concluded that responses are variable and dependent on the stage of the estrous cycle at the time of the injection of GnRH. In order for timed insemination protocols to be efficient, controlling the timing of ovulation is required. A new dominant follicle emerged within 3 to 4 d after GnRH treatment (Twagiramungu et al., 1994). When combined with $PGF_{2\alpha}$ induced luteolysis (administration 6 d after GnRH) this follicle became the ovulatory follicle (Twagiramungu et al., 1994).

However, Perry and colleagues (2005) reported lower pregnancy rates in cows when follicles ≤ 11 mm were induced to ovulate using GnRH. The authors concluded that the administration of GnRH likely induced ovulation before the dominant follicle had attained physiological maturity (Perry et al., 2005). Decreased pregnancy rates after a GnRH-induced ovulation have also been reported in heifers (Perry et al., 2007). It is important to note that Perry and colleagues (2005) reported that ovulatory size had no effect on pregnancy rate when ovulation occurred spontaneously. Atkins and colleagues (2008) hypothesized that the physiological maturity of a follicle and not its diameter was more important in determining the likelihood of establishing pregnancy.

Synchronization Systems

Historically investigators have developed dozens of different estrus synchronization systems (Lamb and Larson, 2005; Odde, 1990; Patterson et al., 1989). Regulation of the estrous cycle was originially associated with control of the CL (Thimonier et al., 1975). Methods to synchronize estrus have occurred in six distinct phases (Table 1.1) Thimonier et al., 1975; Patterson et al., 2003a). In general, systems that employ only one compound (progestin, PGF, GnRH) either have poor synchronization responses or unsatisfactory pregnancy rates. This section concentrates on the development of synchronization systems to control interval and timing of estrus more precisely.

Table 1.1. Development of methods to control of estrous cycle in the cow[a].

Phase	Method
I (Progesterone phase)	Exogenous progesterone was administered to prolong the luteal phase or establish an artificial luteal phase
II (Progesterone-estrogen phase)	Progestins were combined with estrogens or gonadotropins
III (Prostaglandin $F_{2\alpha}$ (PG) phase)	PG and it's analogues were used as luteolytic agents
IV (Progesterone-PG phase)	Progestational agents were combined with PG
V (GnRH=PG phase)	GnRH and PG used to control follicular and luteal lifespan
VI (Progesterone-GnRH-PG phase)	Combination of progestins, gonadotropins, and PG to control the interval and timing of estrus more precisely

[a]Adapted from Thimonier et al., 1975; Patterson et al., 2003a; Leitman, 2007.

MGA-PG Protocol

When fed orally, MGA successfully suppresses estrus and ovulation (Zimbelman and Smith, 1966a). The group fed MGA for 10 to 18 d had a similar number of heifers expressing estrus over a 6-d period after the treatment when compared to the 20-d period for the control group. However, Zimbelman et al. (1970) reported a reduction in first service conception rate for the MGA-treated animals. The combination of norgestomet (a progestin) with estradiol valerate in the Syncro-Mate B treatment resulted in a high estrus response, but variable first service conception rates (Odde, 1990).

In 1972, the luteolytic properties of $PGF_{2\alpha}$ and its analogues were discovered (Laduerdale, 1972; Rowson et al., 1972; Louis et al., 1972). Lauderdale (1980) reported 5-d pregnancy rates of 30-36% for beef cows and 17-28% for beef heifers when a single injection of $PGF_{2\alpha}$ was administered and followed by estrus detection and AI. . When the system was preceded by estrus detection and AI for 4 d prior to PGF administration, pregnancy rates were 39% and 45% for cows and heifers, respectively (Lauderdale, 1980). In 1982, King and colleagues reported that beef cattle injected with prostaglandin between d 10 to d 15 of the estrous cycle had a greater estrus response than those injected d 5 and d 9. They concluded that the stage of the estrous cycle when prostaglandin was administered affects the interval to estrus and the proportion showing estrus. This has been confirmed with another report using dairy heifers (Stevenson et al., 1984).

In 1979, Hesche et al. treated beef heifers with a norgestomet implant for 7 d followed by an injection of prostaglandin 6 or 7 d after insertion. Heersche et al. (1979) found that over a 5-d period, 93% of the heifers displayed estrus and 62% of those that displayed estrus conceived. This study indicated that the combination of prostaglandins and progestins resulted in better control of the estrous cycle; however, the additional labor and handling of animals in this treatment was considered a disadvantage.

In 1986 (Beal and Good) and 1989 (Patterson et al.) studied a treatment of short term feeding of MGA with an injection of prostaglandin at the end of the feeding of MGA. However, a reduction of fertility was reported for heifers that began the short term MGA-PG protocol late in their estrous cycles. The formation of persistent follicles may have caused the reduced conception rate at first service (Beal et al., 1988; Patterson et al., 1989).

Brown et al., (1988) studied a treatment that was designed to address the reduced fertility by having heifers in the late luteal phase of the estrous cycle when prostaglandin was administered. The authors concluded that early methods of estrus synchronization failed to manage follicular waves, resulting in longer periods of estrus detection being necessary.

The MGA-PG protocol consists of MGA being fed for 14 d followed by an injection of PG given 16 to 18 d after the last day MGA was fed (Brown et al., 1988). Brown et al. (1988) found that when comparing MGA-PG to Syncro-Mate B, heifers on the MGA-PG protocol had greater synchronized conception and pregnancy rates. In 1995, Patterson and coworkers found that the MGA-PG

protocol resulted in a higher estrus response and increased synchronized conception rates when compared to prostaglandin treatment alone. When compared to studies administering prostaglandin 17 d following MGA removal, protocols modified to administer prostaglandin 19 d following MGA removal resulted in improved estrus response and synchrony of estrus (Lamb et al., 2000).

MGA Select

In 2001, Wood et al. amended the MGA-PG protocol to include GnRH. This protocol (MGA Select) consisted of feeding MGA for 14 d, followed 12 d later by an injection of GnRH. Seven d after the injection of GnRH, an injection of prostaglandin was administered (Figure 1.4). In this experiment all heifers in the MGA Select group ovulated following GnRH and started a new follicular wave 2 d after the injection of GnRH (Wood et al., 2001). When compared to the MGA-PG group, the MGA Select heifers had a reduced synchronized period, leading to improved synchrony in the development of follicular waves (Wood et al., 2001). However, Wood-Follis et al. (2004) determined that pubertal status of heifers at the beginning of the treatment affected the degree of synchrony following the MGA Select protocol.

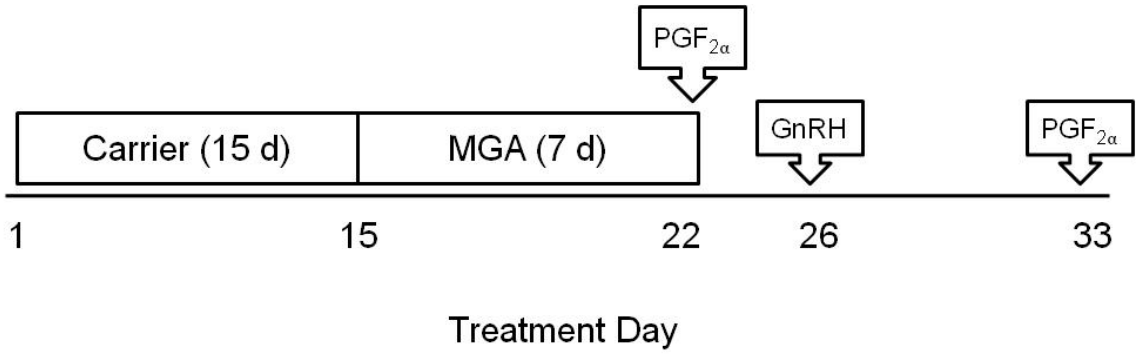
Figure 1.4. MGA Select treatment[a]

[a]Adapted from Wood et al., 2001.

7-11 Synch

In 2000, Kojima et al., found that the 7-11 Synch protocol (Figure 1.5) effectively synchronized estrus in mixed populations of cycling and anestrous postpartum beef cows. On the basis of observed estrus, there were no reported differences in pregnancy rates between MGA Select and 7-11 Synch (Kojima et al., 2000; Patterson et al., 2001; Stegner et al., 2004b).

Figure 1.5. 7-11 Synch treatment[a]



[a]Adapted from Stegner et al., 2004.

MGA Use in Timed Insemination Protocols

The time to inseminate at a fixed time was reported for MGA Select and 7-11 Synch protocols(Perry et al., 2002; Kojima et al., 2003; Stegner et al., 2004a). In 2005, Bader et al. compared MGA Select to 7-11 Synch and found that reducing the length of fixed-time AI (FTAI) protocols using MGA yield similar (61% and 67%, respectively) pregnancy rates. Further, Bader et al. (2005) determined that pregnancy rates resulting from FTAI compared favorably to

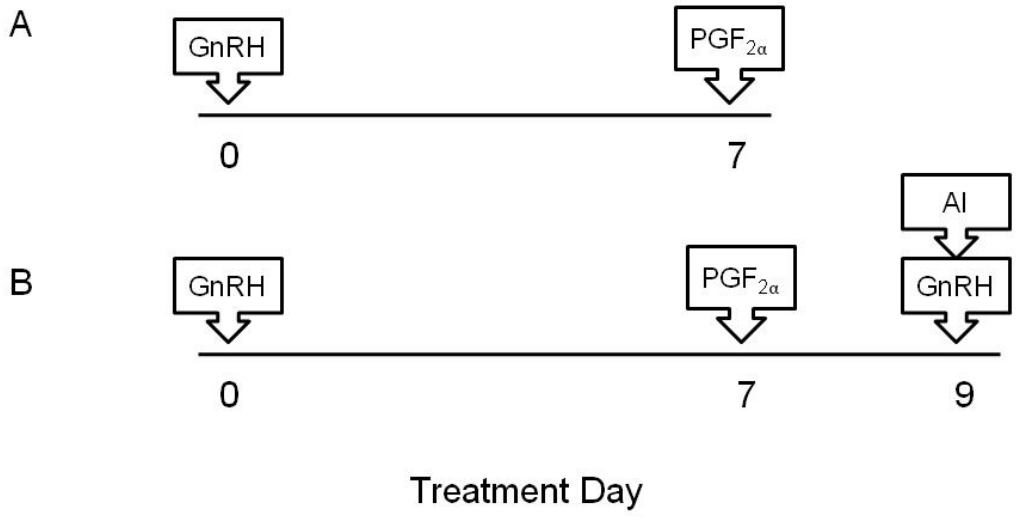
pregnancy rates of cows that were inseminated after detected estrus (Kojima et al., 2000; Patterson et al., 2002; Stegner et al., 2004b).

Select Synch & CO-Synch

When administered at random stages of the estrous cycle, an injection of GnRH causes a preovulatory-like surge of LH and can induce ovulation of follicles ≥ 10 mm in diameter, thus leading to formation of the CL and production of progesterone (Pursley et al., 1995; Bao and Garverick, 1998; Sartori et al., 2001). Twagiramungu et al. (1995) found that the newly formed CL was able to undergo prostaglandin induced regression 6 to 7 d later.

These findings led to the development of the CO-Synch and Select Synch protocols. In these protocols an injection of GnRH was administered followed by an injection of prostaglandin 7 d later (Figure 1.6).

Figure 1.6. Select Synch and CO-Synch treatments[a]



[a]Treatment schedule for Select Synch (A) and CO-Synch (B) protocols. Adapted from Geary et al., 2001

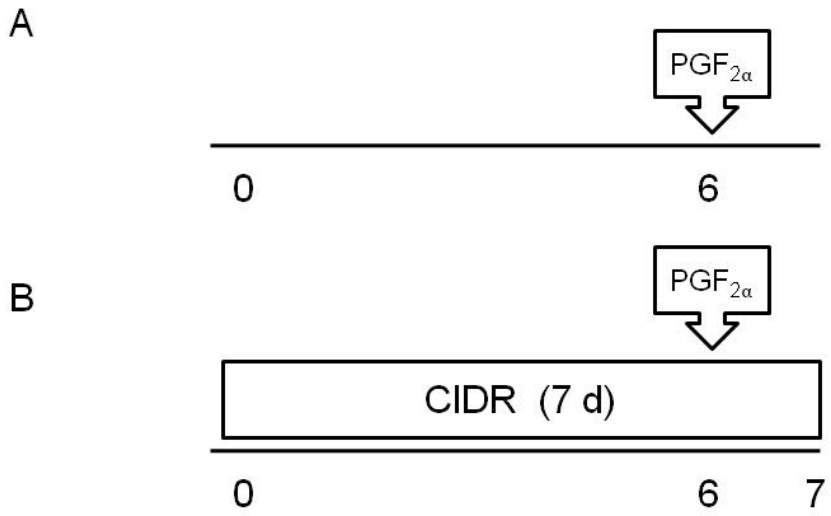
For the CO-Synch protocol, cows were inseminated and administered an injection of GnRH 48 h after prostaglandin administration (Geary et al., 2001). In the Select Synch protocol cows were inseminated based on observed estrus. In 1999, Stevenson et al., found that the Select Synch protocol was less effective in synchronizing estrus in beef heifers than the MGA-PG protocol. Kojima et al. (2000) reported that approximately 5-15% of females that are in the late luteal phase of the cycle when administered GnRH will exhibit estrus before administration of prostaglandin. Therefore, Kojima et al. (2000) recommended that detection of estrus begin 4 d after the administration of GnRH and continues until 6 d after administration of prostaglandin. However, this increases the amount of time and labor required.

CIDR use in synchronization

In 2001, Lucy et al., evaluated a CIDR-PG protocol (Figure 1.7). In this study, heifers received one of three treatments: 1) 7 d CIDR with prostaglandin administered 6 d after CIDR insertion; 2) PG; and 3) untreated control.

For the CIDR-PG group, an improvement in the 3-d synchrony of estrus rate was reported for both prepubertal and cycling heifers when compared to the PG and control treatment groups. The CIDR-PG treatment group also had a higher pregnancy rate. The CIDR-PG also had a large proportion of prepubertal heifers that exhibited estrus after the treatment and these heifers maintained this advantage throughout the breeding period over the other protocols (Lucy et al., 2001).

Figure 1.7. CIDR-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ treatments[a]

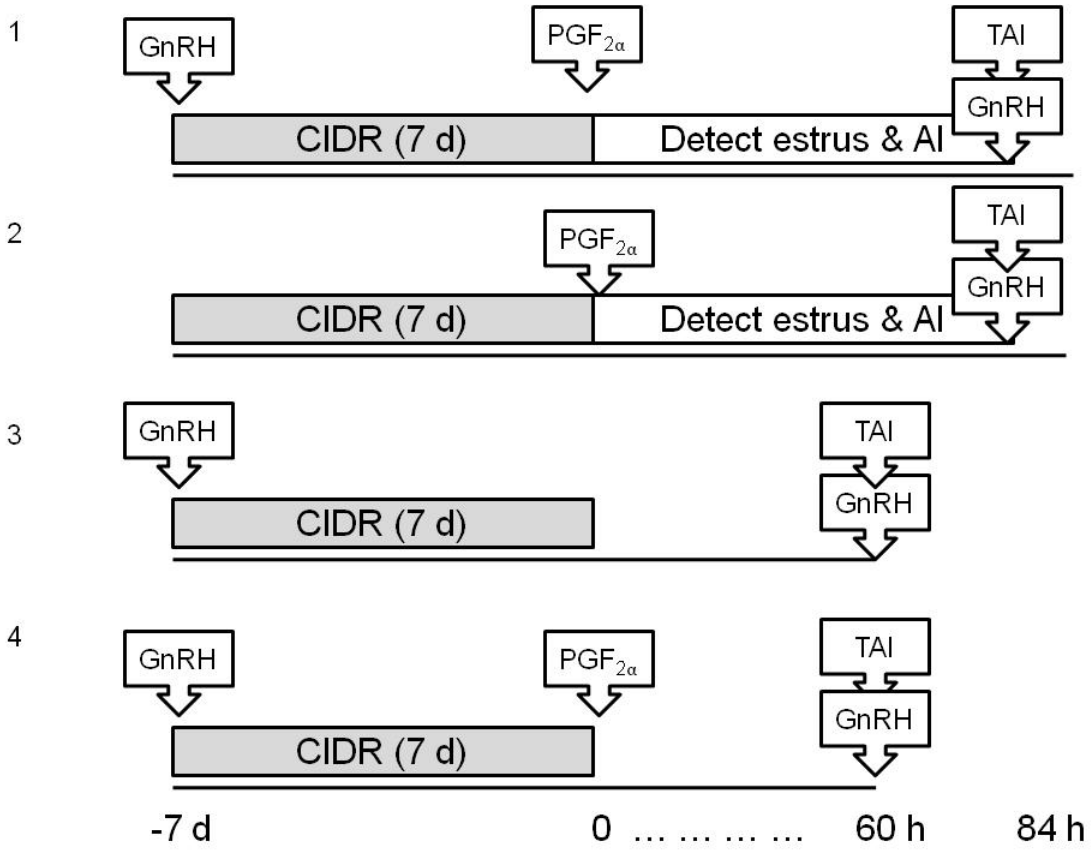


[a]Treatment schedule for $PGF_{2\alpha}$ (A) and CIDR-$PGF_{2\alpha}$ (B) protocols (Adapted from Lucy et al., 2001).

However, the CIDR-PG treatement required additional handling and labor because the prostaglandin was administered 1 d before the CIDR removal. The disadvantage of additional time and labor requirements in this synchronization protocol led to the development of protocols that would eliminate the need for estrus detection and minimizing handling.

In 2006, Lamb et al. conducted a study to determine if a synchronization protocol with FTAI could yield pregnancy rates similar to ones requiring detection of estrus and if an injection of GnRH at CIDR insertion would enhance pregnancy rates in beef heifers. The four treatments studied were: 1) CO-Synch + CIDR; 2) CIDR-PG; 3) CO-Synch + CIDR with FTAI; and 4) CIDR-PG with FTAI (Figure 1.8).

Figure 1.8. Treatment schedules for CO-Synch + CIDR, CIDR-$PGF_{2\alpha}$, CO-Synch + CIDR & FTAI, and CIDR-$PGF_{2\alpha}$ &FTAI



[a]Treatment schedule for (1) CO-Synch + CIDR, (2) CIDR-$PGF_{2\alpha}$, (3) CO-Synch + CIDR & FTAI, and (4) CIDR-$PGF_{2\alpha}$ &FTAI. Adapted from Lamb et al., 2006.

Heifers in the CO-Synch + CIDR treatment were observed for estrus for 72 h after administration of prostaglandin. Eighty-four h after administration of prostaglandin any heifer that failed to exhibit estrus was administered GnRH and inseminated. Heifers in the CIDR-PG treatment received the same treatment as the CO-Synch + CIDR group minus the initial injection of GnRH. Heifers in the CO-Synch + CIDR with FTAI received the initial CO-Synch + CIDR treatment with timed insemination and an additional injection of GnRH at 60 h after

administration of prostaglandin. Heifers in the CIDR-PG with FTAI received the initial CIDR-PG treatment with timed insemination and an additional injection of GnRH at 60 h after administration of prostaglandin. The authors found that the addition of GnRH when estrus was resulted in no difference in estrus response, synchrony of estrus or pregnancy rates. Lamb et al., (2006) concluded that the CO-Synch + CIDR with FTAI protocol was an effective protocol that yielded similar results to those requiring estrus detection in heifers.

In 2006, Larson et al., studied whether a TAI protocol could yield pregnancy rates similar to those requiring estrus detection in postpartum cows and whether the inclusion of a CIDR in a GnRH-PG protocol would enhance fertility. Cows were assigned to one of five treatments (Figure 1.9).

The control treatment consisted of CIDR insertion for 7 d followed by injection of 25 mg of $PGF_{2\alpha}$ at CIDR removal. Estrus detection and AI were performed during the 84 h following the $PGF_{2\alpha}$ injection. Cows not detected in estrus by 84 h received GnRH and were inseminated at that time. Cows in the CO-Synch treatment started with an injection of GnRH at d -7 followed by $PGF_{2\alpha}$ at d 0. At 60 h after $PGF_{2\alpha,}$ a second injection of GnRH was given, and cows were inseminated. The CO-Synch + CIDR protocol was the same as the CO-Synch treatment, except that a CIDR was inserted at d -7 and removed at d 0. Cows in the Select Synch & TAI treatment were injected with GnRH followed 7 d later by an injection of $PGF_{2\alpha}$. Cows were detected for estrus during a 84 h period and inseminated 12 h after detected estrus.

Figure 1.9. Treatment schedules for Control, CO-Synch, CO-Synch + CIDR, Select Synch & TAI, and Select Synch + CIDR & TAI.

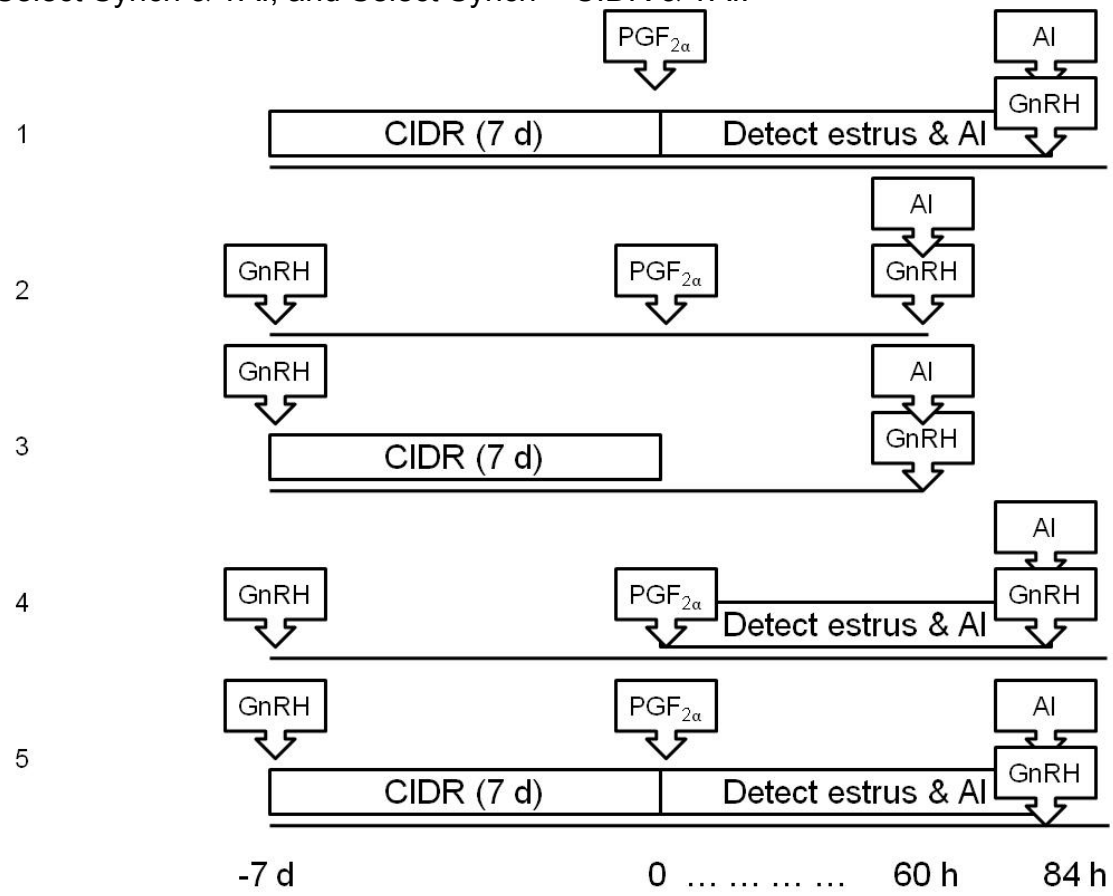


Figure 1.9. Treatment schedule for (1)Control, (2) CO-Synch, (3) CO-Synch + CIDR, (4) Select Synch & TAI, and (5) Select Synch + CIDR & TAI. Adapted from Larson et al., 2006.

Any animals not inseminated after the period of estrus detection were administered an injection of GnRH and inseminated at 84 h. Finally, the Select Synch + CIDR & TAI treatment was the same as the Select Synch + CIDR, except there was an addition of CIDR between the first injection of GnRH and $PGF_{2\alpha}$. Pregnancy rates from timed AI were similar for treatments Control (52.5%), CO-Synch + CIDR (53.8%), Select Synch & TAI (53.0%), and Select Synch + CIDR & TAI (58.0%), but all of these were greater ($P<0.01$) than the

CO-Synch treatment (43.4%). Pregnancy rates from AI were greater ($P < 0.05$) for cycling cows (54.7%) than noncycling cows (45.9%). The authors found that pregnancy rates were greater for cows that were cycling at the beginning of the breeding season, confirming other findings (Stevenson et al., 2000; Lamb et al., 2001; Stevenson et al., 2003). The additional progesterone provided by the CIDR improved pregnancy rates (CO-Synch, 43%; CO-Synch + CIDR, 54%). They concluded that the inclusion of a CIDR in a timed insemination protocol is an effective way to optimize pregnancy rate in postpartum beef cows.

Schafer et al., (2007) compared the MGA Select or CO-Synch + CIDR synchronization protocols with TAI. They found that timed insemination protocols using MGA or CIDRs had comparable pregnancy rates. Although estrus synchronization protocols utilizing TAI have been successfully developed for postpartum beef cows (Bader et al., 2005; Larson et al., 2006; Schafer et al., 2007), the same degree of success has not been found for heifers (Dahlen, et al., 2003; Colazo et al., 2004; Walker et al., 2005; Lamb et al., 2006; Busch et al., 2007)

Decreasing the interval between GnRH and PG in FTAI protocols.

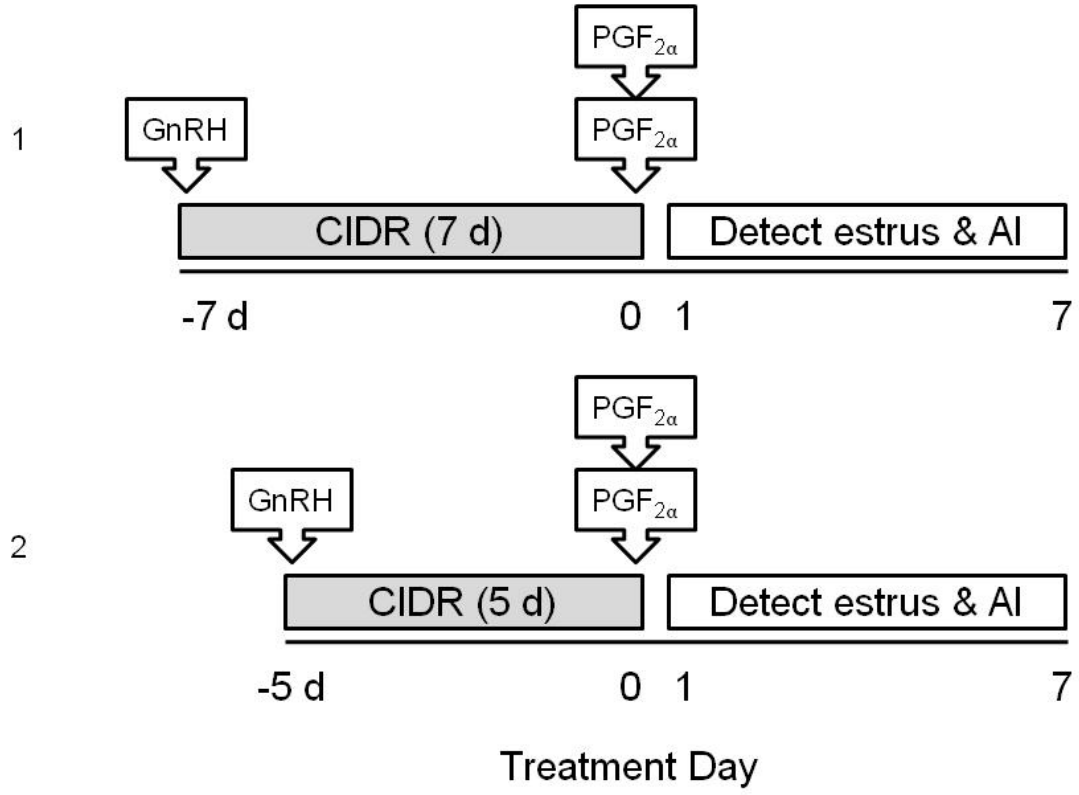
The CO-Synch protocol is the most commonly used timed-AI protocol in beef cows (Bridges et al., 2008). Although the pregnancy rates are acceptable to many beef producers, there are still 40-50% of females that do not conceive. Therefore, the potential for improvement exists. The CO-Synch protocol controls ovarian follicles, luteolysis, and ovulation through GnRH, $PGF_{2\alpha}$, and often a progestin.

The purpose of the initial injection of GnRH is to induce ovulation and start a new follicular wave. However, Geary et al., (2000) reported that this initial injection of GnRH synchronized growth in only 66% of beef cows. It has also been reported that in a proportion of cows the second GnRH injection in the CO-Synch protocol induces ovulation of smaller follicles and that these cows are less likely to become pregnant to the timed-AI (Lamb et al., 2001).

In 2008, Bridges and colleagues hypothesized that reducing the length of exposure of progestins resulting in an increase in the length of the follicular phase would increase the oocyte quality and enhance pregnancy rates. The authors believed that this alteration would increase the secretion of estradiol from the ovulatory follicle and increase timed-AI pregnancy rates.

For Experiment 1, 156 postpartum beef cows were assigned by parity and d postpartum to either the 7-d Select-Synch + CIDR (7SS) or 5-d Select-Synch + CIDR (5SS) protocol (Figure 1.10). Cows in the 7SS group received GnRH and a CIDR on d -7. Cows in the 5SS group received GnRH and a CIDR on d -5. On day 0, cows were given 25 mg of $PGF_{2\alpha}$ and CIDRs were removed from all cows. It was unknown if luteolysis of the accessory CL would occur after the single $PGF_{2\alpha}$ injection because the interval from GnRH to $PGF_{2\alpha}$ was 5 d. Therefore, a second injection $PGF_{2\alpha}$ was given 12 h after the first. Animals that exhibited estrus were inseminated approximately 12 h after detected estrus from d 1 to d 7. First service pregnancy rates for both treatments were 46.8%. Synchronization period pregnancy rate for 7SS protocol (51.4%) and 5SS protocol (56.3%) did not differ.

Figure 1.10. Experiment 1: Treatment schedules for 7 d Select Synch + CIDR and 5 d Select Synch + CIDR[a].

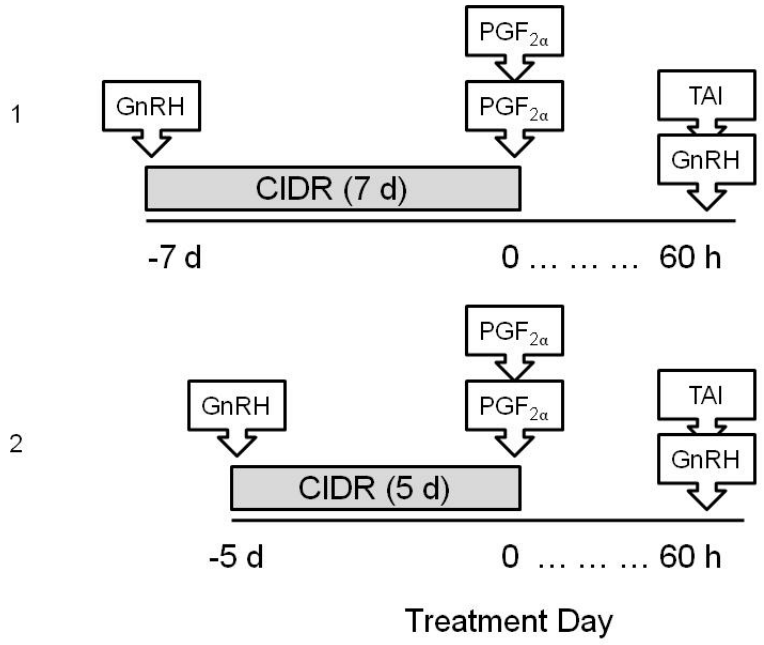


[a]Treatment Schedule for (1) 7 d Select-Synch + CIDR, (2) 5 d Select-Synch + CIDR. Adapted from Bridges et al., 2008.

In Experiment 2, 223 postpartum beef cows were assigned by parity and d postpartum to either 7-d CO-Synch + CIDR with FTAI at 60 h (7CO-60) or 5-d CO-Synch + CIDR protocol with FTAI at 60 h (5CO-60; Figure 1.11). Cows in 7CO-60 group were given an injection of GnRH and had a CIDR inserted on day -7. Cows in 5CO-60 group were given an injection of GnRH and had a CIDR inserted on day -5. On day 0, cows were injected with $PGF_{2\alpha}$ and CIDRs were removed from all cows. A second injection of $PGF_{2\alpha}$ was given approximately 12 h after CIDR removal. Insemination with an administration of GnRH was

completed at h 60. Pregnancy rates for timed insemination (7CO-60, 52.7%; 5CO-60, 56.8%) and 25 d AI (7CO-60, 69.4%; 5CO-60, 67.3%) did not differ between the treatments.

Figure 1.11. Experiment 2: Treatment schedules for 7 d CO-Synch + CIDR & FTAI at 60 h and 5 d CO-Synch + CIDR & FTAI at 60 h[a].

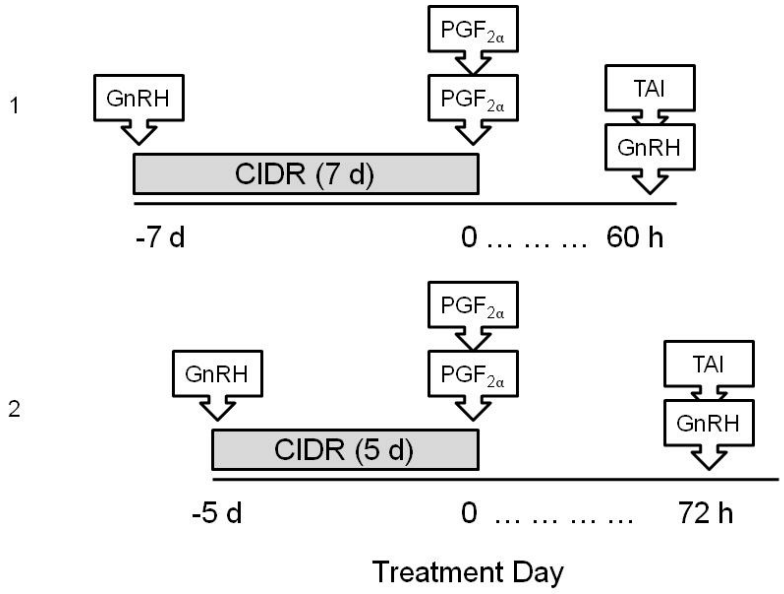


[a]Treatment Schedule for (1) 7 d CO-Synch + CIDR & FTAI at 60 h, (2) 5 d CO-Synch + CIDR & FTAI at 60 h. Adapted from Bridges et al., 2008.

In Experiment 3, 223 postpartum beef cows were assigned by parity and d postpartum to either 7-d CO-Synch + CIDR with insemination at 60 h (7CO-60) or 5-d CO-Synch + CIDR protocol with insemination at 72 h (5CO-72; Figure 1.12). On d-7 (7CO-60) or d-5 (5CO-72) cows received an injection of GnRH and a CIDR. On day 0, all cows received an injection of $PGF_{2\alpha}$ and CIDRs were removed. A second injection of $PGF_{2\alpha}$ was given 12 h after CIDR removal. Animals were inseminated and injected with GnRH at 60 h (7CO-60) or 72 h

(5CO-72). Timed insemination pregnancy rate was approximately 13% greater (P<0.05) for 5CO-72 (80.0%) than for the 7CO-60 protocol (66.7%). However, breeding season (5CO-72, 96.2%; 7CO-60, 93.7%) pregnancy rates were similar.

Figure 1.12. Experiments 3 and 4: Treatment schedules for 7 d CO-Synch + CIDR & FTAI at 60 h and 5 d CO-Synch + CIDR & FTAI at 72 h[a].



[a]Treatment Schedule for (1) 7 d CO-Synch + CIDR & FTAI at 60 h, (2) 5 d CO-Synch + CIDR & FTAI at 72 h. Experiment 3 and 4 were the same, except in experiment 4 the prostaglandin analogue cloprostenol was used. Adapted from Bridges et al., 2008.

In Experiment 4, 400 postpartum beef cows, were assigned by parity and d postpartum to the same treatments as used in Experiment 3. Instead of using $PGF_{2\alpha}$, cloprostenol was injected on d 0, followed by a second shot 12 h later. Animals were inseminated and injected with GnRH at 60 h (7CO-60) or 72 h (5CO-72). In the 5CO-72 protocol group, timed insemination pregnancy rate

(65.3%) was greater (P<0.05) than the 7CO-60 (56.2%). The protocols that reduced the exposure to progestins had an increase in pregnancy rates and are therefore effective for estrus synchronization in postpartum beef cows.

RESYNCHRONIZATION

Reproductive efficiency increases by females conceiving early in the breeding season, obtaining high pregnancy rates to first service, and early detection and reinsemination of nonpregnant animals. To maintain high reproductive performance, non pregnant cows need to be detected as early as possible after the first service and then resynchronized and inseminated (Stevenson et al., 2003). Most females that do not conceive to the first insemination are expected to return to estrus 15-25 d after the insemination (Van Cleff et al., 1996; Chenault et al., 2003), thus requiring extensive labor to maximize the number pregnant to AI. Use of ultrasonography allows early pregnancy diagnosis and detection of nonpregnant females (Pierson and Ginther, 1984; Kastelic et al., 1988).

Protocols to synchronized the return to estrus in cattle inseminated at the preceeding estrus is called the resynchronization of estrus (Chenault et al, 2003). For large dairy herds the detection of estrus at the first estrus after insemination is difficult for cows in confinement, housed on concreted and with increasing levels of milk production (Lucy, 2001). Without adequate estrus detection after the first insemination, females are not identified as having failed to conceive until scheduled pregnancy examinations 35-60 d after insemination; resulting in a minimum of a 35 day interbreeding interval (Chenault et al., 2003).

CIDR B inserts (Macmillan and Peterson, 1993; Macmillan and Burke, 1996; Van Cleff et al., 1996) and previously used CIDR inserts (El-Zarkouny et al., 2001) have been used to resynchronize females. Inserts were administered 12 to 17 d after insemination and were removed 20 to 21 d after insemination (for a 3 to 8 d exposure period). All inseminated cows received an insert, without the knowledge of whether or not they conceived to the first service. Cows receiving the resynchronization insert returned to estrus 1 to 4 d after the removal of the insert. Cows that did not receive the insert treatment returned to estrus 18 to 24 d after insemination (approximately a 7-d window).

In 2003, Chenault et al., evaluated the effectiveness of using a CIDR to resynchronize previously inseminated lactating dairy cows with an unknown pregnancy status. Administration of CIDRs for resynchronization resulted in a more precise to return to estrus, consistent with results from other studies (Macmillan and Peterson, 1993; Macmillan and Burke, 1996; Van Cleff et al., 1996). The authors concluded that CIDRs were “safe and effective for synchronization of returns to estrus of previously inseminated lactating dairy cows with unknown pregnancy status” (Chenault et al., 2003) and allow a concentration of labor needed to detect estrus and inseminate cows.

Resynchronization protocols using CIDRs (Van Cleff et al., 1996; Macmillan and Peterson, 1993; Stevenson et al., 2003) or MGA (Stevenson et al., 2003) may prolong the growth and dominance of the ovulatory follicle. When luteal regression occurs prior to progestin withdrawal, fertility may be compromised because of formation of a persistent follicle (Stevenson et al.,

2003). Treatments that fail to initiate a new follicular wave will compromise fertility because of ovulation of a persistent follicle (Savio et al., 1993). Therefore, initiation of a new follicular wave may be essential in a resynchronization protocol. Since pregnancy diagnosis is not usually performed for at least 26 d after initial insemination, resynchronization protocols designed to initiate a new follicular wave should not jeopardize established pregnancy (Colazo et al., 2006).

ECONOMICS

Artificial insemination allows beef cattle producers to use genetically-superior bulls and reduces the risk of sexually transmitted diseases. However, AI is only used by 13.3% of operations (APHIS, 1998). The most common reasons for not adapting reproductive technologies such as AI and estrus synchronization include: time/labor, cost, and complication (APHIS, 1998). Patterson and colleagues (2003a) concluded that the development of economical and convenient protocols to synchronize estrus and ovulation for a FTAI with favorable results would increase the adoption of AI. Therefore, it is important to evaluate the effect of estrus synchronization protocols on factors such as: pregnancy rate, labor, time, and economics.

The cost of estrous synchronization and artificial insemination includes many factors, such as: drugs, semen, technician fees, labor, and supplies. The cost of natural service breeding includes purchase and maintenance of the bull. In 2005, Anderson et al. reported that the cost of bull ownership and maintenance per year for a bull costing $2,500 would be $1150. This value was

reached from assuming a useful period of 3 y, annual maintenance costs of $600, and a salvage value of $850 (Anderson et al., 2005). The authors concluded that the increased management for estrous synchronization and AI would result in calves that were older (and thus heavier) at weaning and an increased calving percentage of 5% (Table 1.2; Anderson et al., 2005).

They assumed a 25 lb. per hd increase in weaning weight through the use of genetically superior and proven AI sires. The 5% increase in calving percentage was attributed to an increase in the number of cows bred early in the breeding season. When compared to natural service, the cost and revenues of AI resulted in a net change in profit advantage of $1,440 per 100 females (Table 1.2; Anderson et al., 2005).

In 2005, Sutphin reported a breeding cost of $13.91 more per AI calf compared to natural service. However, the increase in value from a 2% increase in live calves and a 2% increase in pregnancy rates added returns of $12.21 and $11.60 per AI calf, respectively (Sutphin, 2005). Sutphin (2005) concluded that the improvement of genetic quality of the herd and the boost to the net return made the benefits of estrus synchronization and AI clear.

Table 1.2. AI vs. Natural Service[a]

Increased Costs		**Increased Revenue**	
Drug costs[b]	$830	Additional weaning weight[f]	25 lbs/hd
Semen costs[c]	$2,000	Value of additional weaning weight[g]	$2,061
Technician fees[d]	$750	Change in calving percentage[h]	5%
Additional labor[e]	$432	Additional calves	$2,756
Total Increased Costs	*$4,012*	*Total Increased Revenue*	*$4,817*
Reduced Revenue		**Reduced Costs**	
Reduced cull bull sales	$850	Cleanup bulls required	3 bulls
		Lower bull ownership/maintenance	$1,485
Total Decrease in Profits	**$4,862**	**Total Increase in Profits**	**$6,302**
Net Change in Profits = $1,440			

[a] Adapted from Anderson et al., 2005.
[b] Total drug costs for synchronizing 85 mature cows (CO-Synch;$8/hd) and 15 heifers (MGA-Lutalyse; $10/hd).
[c] Total semen costs for 100 straws at $20/straw.
[d] Total AI technician costs at $7.50/hd.
[e] For additional labor costs, it is assumed that 100 hd of cattle can be worked in 4.5 h using four hired workers at an $8/h wage rate. This is separate from the technician fee.
[f] 25 lb. per hd increase in weight at weaning through the use of genetically superior and proven AI sires.
[g] The value of additional weight at $105 per cwt for 550 lb. calves.
[h] The expected increase of 5% in calving percentage is because of the enhanced management of estrous synchronization and AI and the increased number of cows bred early in the breeding season.

Johnson and Jones (2005) reported financial costs of several synchronization systems for various herd sizes and pregnancy rates, summarized the returns and costs for estrus synchronization and AI (Table 1.3). The authors also reported that the evaluative tool of cost per pregnancy did not adequately compare breeding systems because it failed to account for added value of AI sired calves. The authors concluded that the variety of synchronization systems available allows for successful application when implemented properly to a specific production system; however, the producers who will profit most from these technologies are those that can identify and market high value genetics (Johnson and Jones, 2005).

Table 1.3. Summary of costs and returns for synchronization and AI[a].

Budget Effect	Source	Budget Effect	Source
Increased returns	Heavier calves (earlier average birth date) Improved genetics (calves and replacement females) Uniformity of calf crop (fewer sires could be used, total breeding season could be shorter)	Decreased returns	Fewer cull bulls to sale
Decreased costs	Fewer bulls to purchase and maintain Less labor for more concentrated calving season More predictable calving ease	Decreased costs	Planning and management for synchronization of estrus and AI Synchronization products and supplies Labor Possible facility improvements

[a] Adapted from Johnson and Jones, 2005.

Anderson (2005) found that synchronization and AI has both short-term and long-term benefits. Short-term returns were reported at $69.74 per cow (Table 1.4) through increasing reproductive efficiency and in turn pounds of calf marketed.

Table 1.4. Increased revenues from estrus synchronization and AI[a].

	Revenue
Weaning Weight	72.6 lb. x $80 cwt. = $58.08
% Calf crop	9% more calves x $80 cwt. = $41.54
Total revenue	= $99.62
Return on investment	$99.62 - 29.88 = $69.74

[a]Adapted from Anderson, 2005.

To evaluate long-term impacts, a 10 yr average of a 60 d natural service breeding season were compared to 2 y of estrus synchronization (Table 1.5).

Table 1.5. Effects of synchronization and AI on herd profitability[a].

	10 Year NS Average	Year 1 ESAI	Year 2 ESAI
No. of females exposed	45	45	44
Calving rate percentage[b]	82%	95%	93%
% calf crop weaned	74.50%	91%	86%
WW Average (lb.)			
Steers	525	542	556
Heifers	484	514	482
Sale Weight[c]			
Steers	554	588	600
Steer sale price (per cwt.)	$77.00	$88.00	$83.00
Lbs. of calf weaned per cow exposed	381.2	481.4	448.2
Number of cows sold	5	9	6
Cash cow costs	$235.38	$285.82	$292.26
Net profit per cow exposed[d]	$57.75	$116.62	$76.83

[a]Adapted from Anderson, 2005.
[b]Number of cows calving divided by the number of cows exposed.
[c]Calves were backgrounded for approximately 25 d prior to marketing.
[d]Cash sales per cow minus cow cost.

The synchronization and AI protocol consisted of timed insemination followed 10 d later by a natural service clean up for 50 d. Long-term returns for estrus synchronization and AI were also improved through increases in the percentage of cows that calved and percent calf crop weaned; thus increasing net profit per cow exposed. Anderson (2005) agreed with Johnson and Jones (2005) that the greatest potential profit was through marketing the superior product, however the author concluded that use of synchronization and AI was a profitable venture even in a commodity market.

SUMMARY

Previous advances in science and research led to many studies to develop estrus synchronization protocols that allow producers to use superior genetics in their beef herds through the use of AI. Although these synchronization systems continue to improve pregnancy rates to initial AI, systems utilizing FTAI in heifers commonly result in 25-40% open heifers after FTAI. To date, no studies have been conducted to evaluate return to estrus distribution and efficacy of heifer resynchronization after the 5 d CO-Synch + CIDR with FTAI at 72 h. Therefore, the purpose of this study was to determine the effectiveness of a FTAI resynchronization protocol after synchronization to CO-Synch + CIDR (5 d) in beef heifers

Experimental Objectives

The purpose of this research was to determine the effectiveness of a FTAI resynchronization protocol after synchronization to CO-Synch + CIDR (5 d). In order to accomplish the purpose of the study had several objectives:

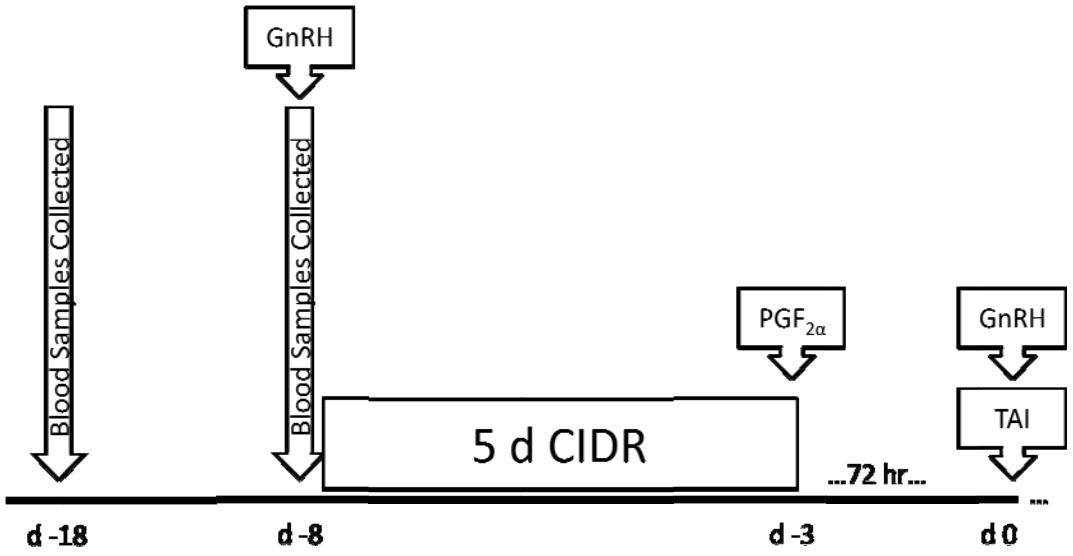
- To evaluate the return to estrus distribution in beef heifers after application of fixed-time AI system of CO-Synch + CIDR (5 d)
- To compare the efficacy of a resynchronization protocol to AI based on synchronized return to estrus versus natural service clean up.
- To evaluate the costs of resynchronization (effects of pregnancy rates, monetary costs of resynchronization)

MATERIALS AND METHODS

Animals

Crossbred, replacement heifers (n=176) at Southampton Correctional Center (36° 43' 20.2" N, 77° 15' 19.6" W) with a minimum reproductive tract score (RTS) of 3 (1=immature, 5=cycling; Anderson et al., 1991; Patterson et al., 1999), a minimum weight of 295 kg, and a mean age of 408 d one month prior to the beginning of the study were synchronized using the CO Synch + CIDR (5 d) protocol (Figure 2.1; Bridges et al., 2008).

Figure 2.1. Initial Synchronization Treatment CO-Synch + CIDR (5 d)[a].



[a] At d -8 all heifers received GnRH (100 µg i.m. of Cysotrelin®, Merial, Athens, GA) and an intravaginal-progesterone releasing insert (CIDR®, Pfizer Animal Health, New York, NY). At d -3 CIDRs were removed and 25 mg dinoprost tromethamine administered i.m. (Lutalyse®, Pfizer Animal Health, New York, NY). All heifers were administered a dose of GnRH (100 µg i.m. Cysotrelin®) 72 h following CIDR removal (d 0) and were inseminated.

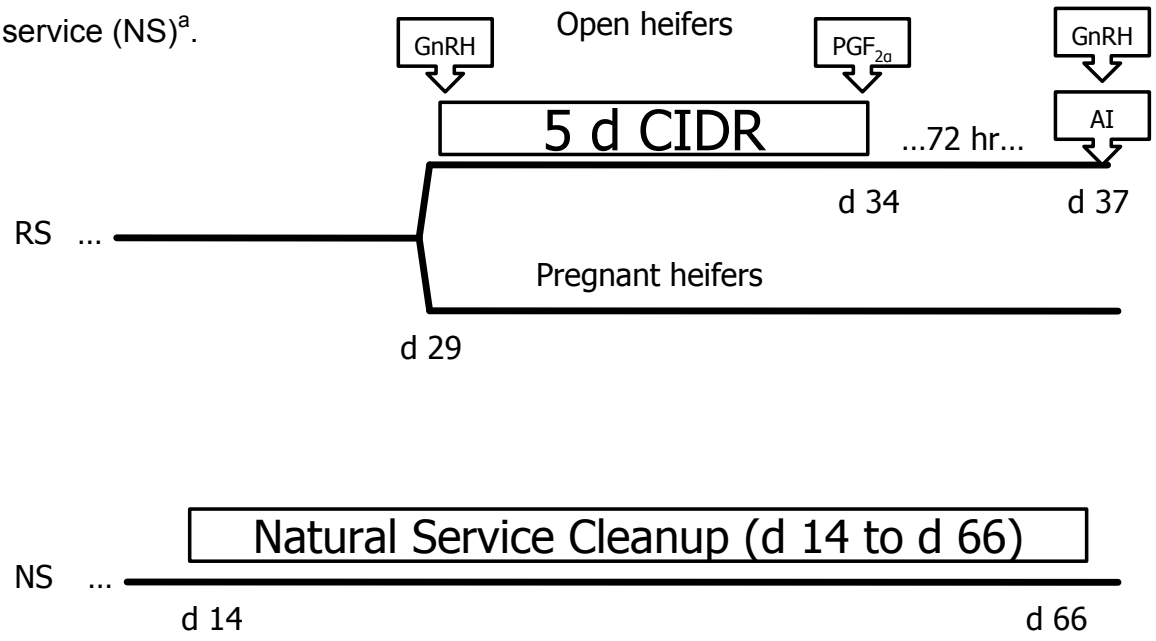
Heifers were kept in a dry lot where they were fed a corn silage based diet supplemented with soybean meal with free access to Bermuda grass hay and free choice trace mineral mix for the duration of the trial. Heifers were fed to meet NRC requirements for growing heifers with a 0.75 kg/d gain. At d -8 all heifers received GnRH (100 µg i.m. of Cysotrelin®, Merial, Athens, GA) and an intravaginal-progesterone releasing insert (CIDR®, Pfizer Animal Health, New York, NY); as well as fitted with a HeatWatch® Estrus Detections System (Cow Chips, LLC, Denver, CO) transmitter. At d -3 CIDRs were removed and a single dose of 25 mg dinoprost tromethamine administered i.m. (Lutalyse®, Pfizer Animal Health, New York, NY). A single dose of PGF was given based on previous data from our laboratory (Hall et al., unpublished data) which indicated no advantage to a second dose of prostaglandin at CIDR removal. All heifers were administered a dose of GnRH (100 µg i.m. Cysotrelin®) 72 h following CIDR removal (d 0) and were inseminated. Heifers were inseminated to one of two sires by one of two trained technicians at initial AI.

Heifers were then randomly assigned based on detection of estrus prior to initial AI to one of two return service treatments: Resynchronization (RS) and natural service (NS). For RS heifers, ultrasonographic scanning for diagnosis of pregnancy was performed transrectally using an Aloka 500 console (Corometrics Medical Systems, Inc., Wallingford, CT) and 5 MHz probe on d 29. Heifers diagnosed pregnant were returned to their lot with no further treatment.

Open heifers were resynchronized using the CO Synch + CIDR (5 d) synchronization protocol. On d 29 open heifers received GnRH (100 µg i.m.) and

an intravaginal-progesterone releasing insert (CIDR). At d 34 CIDRs were removed and a single dose of 25 mg dinoprost tromethamine administered i.m. All heifers were administered a dose of GnRH (100 µg i.m.) 72 h following CIDR removal (d 37) and were reinseminated by a single trained technician (Figure 2.2).

Figure 2.2. Return Service treatments for resynchronization (RS) and natural service (NS)[a].



[a] Treatment schedule for heifers assigned to RS (resynchronization) and NS (No resynchronization). Heifers assigned to RS were checked for pregnancy on d 29 Open Heifers were given an injection of GnRH and had a CIDR inserted. On d 34, CIDRs were removed and heifers received an injection of PGF2α , followed 72 h later by an injection of GnRH and TAI. Heifers diagnosed pregnant on d 29 in RS received no further treatment. In NS were exposed to cleanup bulls beginning 14 d to d 66 after the initial TAI.

Heifers in NS (n=85) were fitted with a Heat Watch Estrus Detections System transmitter from the time of initial CIDR insertion (d-8) until d 37 for

continuous estrus detection. From d 14 to d 66 clean up bulls were placed with NS heifers. All heifers were continually monitored for estrus using the Heat Watch Estrus Detection System from CIDR insertion until d 37.

Twice daily monitoring of the HeatWatch Estrus Alert System (CowChips, LLC., Denver, CO) allowed for the return to estrus distribution data to be collected from day -8 to 37. Estrus was defined as a minimum of three mounts with a minimum duration of two seconds within a 4-h period.

Blood Samples

A 10 mL blood sample from all heifers was collected on d -18 (10 d before CIDR insertion) and at CIDR insertion (d -8). Progesterone concentrations in these samples were used to determine reproductive status of heifers at the beginning of the study. Blood samples were collected via either the jugular or tail vein in 10 mL tubes containing EDTA (BD Vacutainer™; BD, Franklin Lakes, NJ).

Blood plasma was recovered and stored at -20°C until analyzed for progesterone concentration. Serum was assayed for progesterone concentration with the Coat-A-Count Progesterone Kit (Siemens Healthcare Diagnosotics, Inc., Los Angeles, CA), as described and validated by Srikandakumar et al. (1986). The assay was sensitive to 0.1 ng/mL and the intra-assay coefficient of variation was 13.2%. Animals that had progesterone concentrations greater than 1 ng/mL at either sampling time were considered to be cycling (Perry et al., 1991).

Pregnancy Diagnosis

Pregnancy to initial artificial insemination was determined by ultrasonography at 66 d after initial AI. Any heifers diagnosed open or with a

pregnancy < 30 d were checked for pregnancy using ultrasonagraphy 31 d after the breeding season ended to confirm end of season pregnancy rates. Two heifers in the RS treatment were removed from the study. They were diagnosed pregnant on d 29 to the first TAI. However, they were later diagnosed with pregnancies to natural service.

Economics.

To compare the costs of each treatment, standardized costs were assigned. The drug cost to resynchronize the heifers was $18.48 per heifer ($PGF_{2\alpha}$ -$2.54/dose, GnRH-$3.21/dose, CIDR $9.02/dose, supplies $0.50/head; Johnson and Jones, 2005). The labor cost per head was $1.44/hd/each time they were worked (assuming 100 head of cattle can be worked in 4.5 hours using four hired workers at an $8/hour wage rate; Anderson et al., 2005). A semen cost was assigned at $14/straw (Johnson and Jones, 2005) and an AI technician cost of $7.50/hd (Anderson et al., 2005). Finally, the cost of ultrasound pregnancy diagnosis was assigned at $5/hd.

The cost of using a natural service cleanup bull was established at $1485/bull/year. This annual ownership cost was established from the purchase price of the bull $2,500 less salvage value ($850) and dividing by the useful period of 3 y (Table 2.1; Anderson et al., 2005). This value was then added to costs for maintenance and risk of loss (Table 2.1).

Table 2.1. Annual bull ownership/maintenance costs[1].

	$/bull
Annual ownership cost[2]	$550
Annual maintenance cost	$600
Risk of bull loss[3]	$335
Total cost per bull	*$1,485*

[1] Adapted from Anderson et al., 2005.
[2] Annual ownership cost represents the average annual decline in the bull's value. It is calculated by the difference between the bull's original value and his salvage value divided by his useful life [($2,500 - $850)/3].
[3]Risk of bull loss represents total financial loss due to a bull becoming incapacitated through death, injury, infertility, etc. It is calculated as the difference between the bull's average value and his salvage value, multiplied by the probability of such a loss occurring; [0.2 ($2,500 + $850)/2)].

To evaluate returns for the calves the heifers produced, a calving date was approximated for each heifer using date of conception estimated by ultrasound and a standardized gestation length of 283 d. Typically, calves are marketed on the same day, regardless of calving date. A standard weaning age of 186 d (ISU, 2008) was used, and marketing weight calculated using weight per day of age (WDA) of 2.667 lbs. (ISU, 2008). Ten-year price averages for steers and heifers from Virginia Department of Agriculture and Consumer services (B. McKinnon, Virginia Cattlemen's Association, Daleville, VA, personal communication) were used to assign a price for each calf based on weight, and total calf value calculated.

Statistical Analysis

Age, body weight, days pregnant, and day of return to estrus were analyzed by analysis of variance using the general linear models procedure of

SAS (PROC GLM; SAS Inst. Inc., Cary, NC). Treatment was included as the main effect. Pregnancy rate to initial AI was analyzed using X^2 analysis (PROC FREQ of SAS) with the model of treatment, AI technician and treatment x AI technician interaction. The interaction was removed because it did not reach a significance level of $P > 0.10$. Pretreatment estrous cyclicity status, total AI pregnancy rate, and end of breeding season pregnancy rate were also analyzed using X^2 analysis with the model treatment. The model did not include AI technician for analysis of total AI pregnancy rate and end of breeding season pregnancy rate since a single technician inseminated all heifers for the resynchronization treatment.

RESULTS

The number, age, and body weight of heifers before the initiation of treatments are shown in Table (2.2). There was no difference (P > 0.6) between treatments for age or BW of heifers at the beginning of the trial.

Table 2.2 Number, Age, and BW of heifers prior to treatment initiation (LS mean ± SE)

	Treatment[1]		P Value
	RS	NS	
No. of Heifers[2]	89	85	
Age[3], (d)	407 ± 1.3	408 ± 1.3	0.7
BW[4], (kg)	343 ± 2.6	343 ± 2.7	0.98

[1] Treatment schedule for heifers assigned to RS (resynchronization) and NS (No resynchronization). Heifers assigned to RS were checked for pregnancy on d 29 Open Heifers were given an injection of GnRH and had a CIDR inserted. On d 34, CIDRs were removed and heifers received an injection of PGF2α , followed 72 h later by an injection of GnRH and TAI. Heifers diagnosed pregnant on d 29 in RS received no further treatment. In NS were exposed to cleanup bulls beginning 14 d to d 66 after the initial TAI.
[2] Total number of heifers per treatment
[3] Age (d) of heifers at the initiation of treatments
[4] Body weight (kg) of heifers at initiation of treatments

Cyclic Status.

The percentage of heifers cycling at the beginning of the experiment was greater (P < 0.01) for heifers with RTS 4 (91.7%) or 5 (92.0%) than RTS 3 (74.0%). However, there was no effect of cyclic status on initial AI pregnancy rates (P > 0.4). Cyclic heifers exhibited a 51.7% pregnancy rate compared to 43.5% for non-cyclic heifers.

Return to Estrus

The average day of return to estrus after initial AI was 17.3 (RS) and 19.6 (NS), and did not differ between treatments (P = 0.2). Figures 2.3, 2.4, and 2.5

illustrate the distribution of the return to estrus after the initial synchronized period for all heifers, by treatment, and by cyclic status, respectively. Return to estrus exhibited a biphasic pattern with 21 heifers in estrus d 5-11 and 41 heifers in estrus from d 14-25.

Figure 2.3. Return to estrus distribution for all heifers.

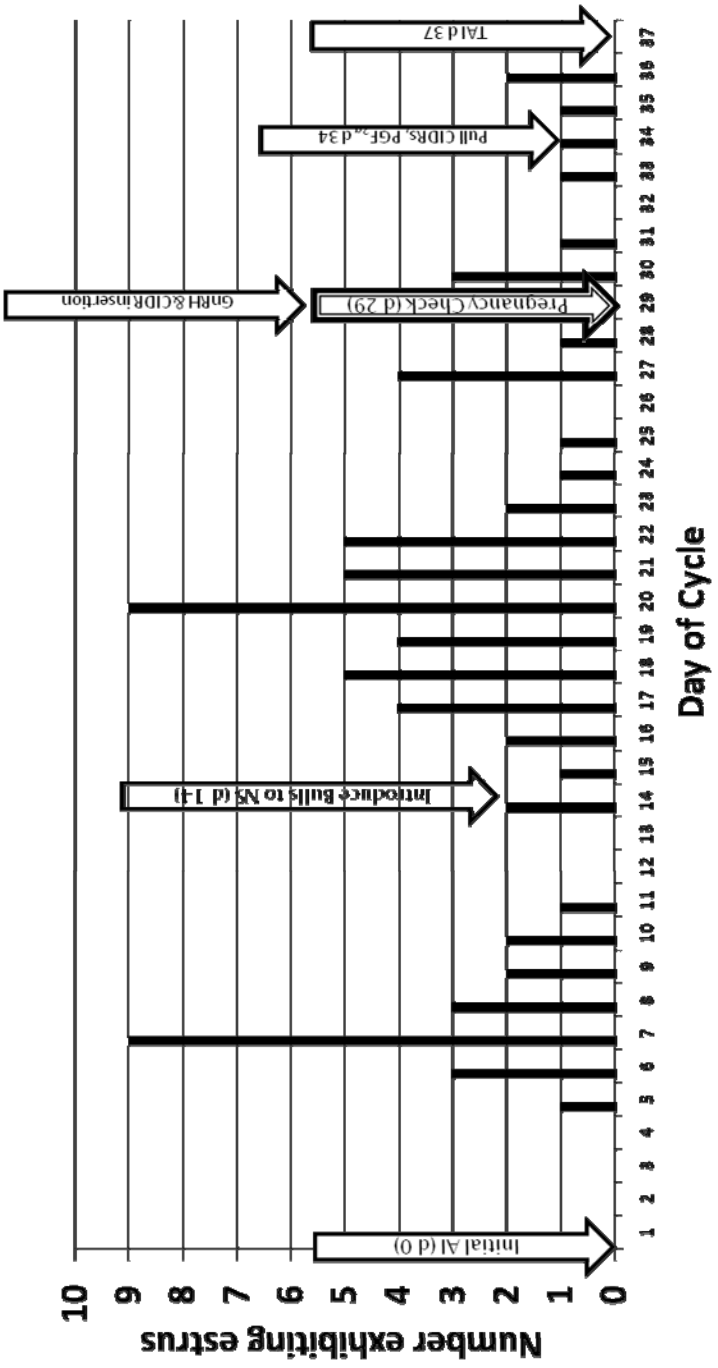
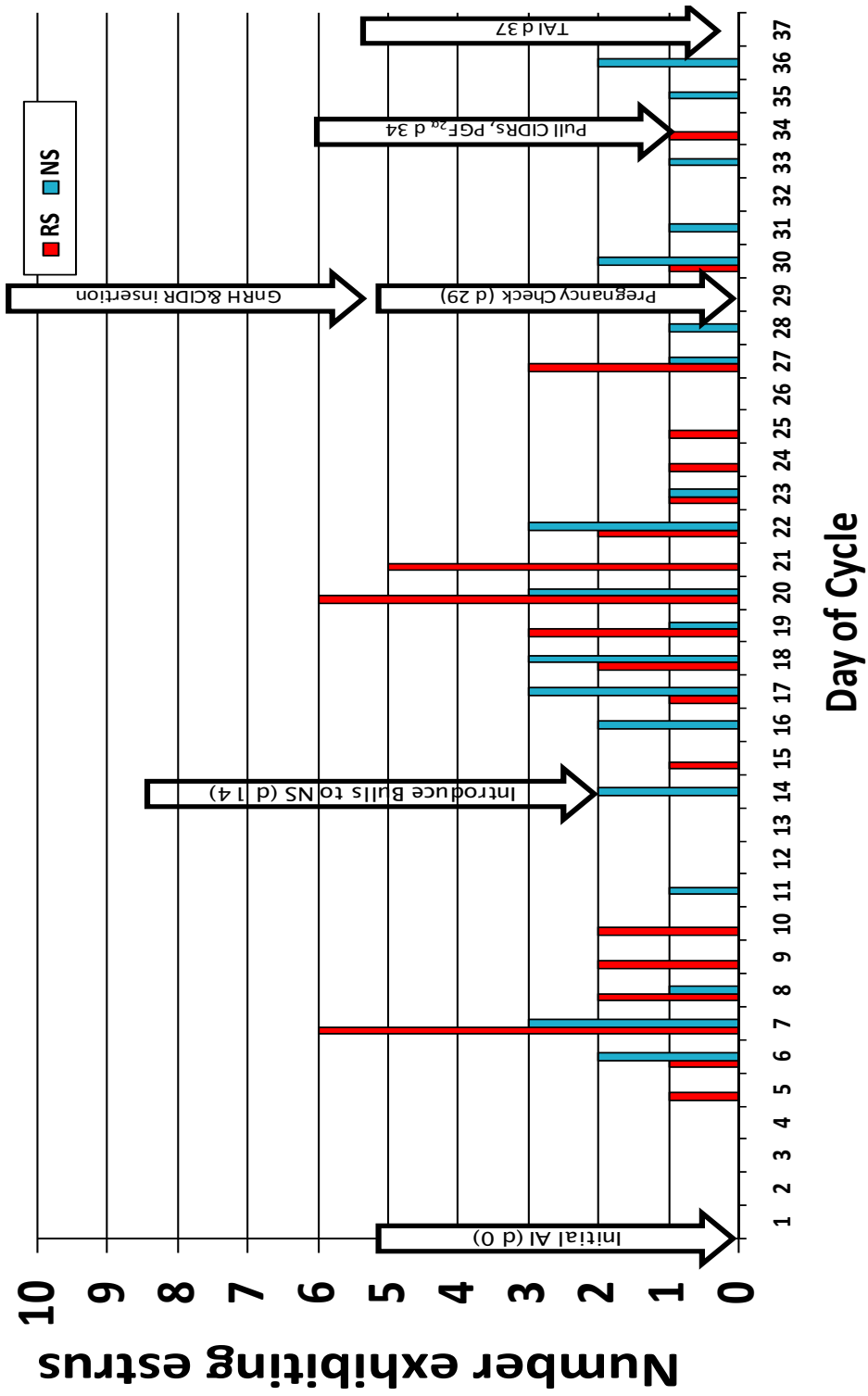
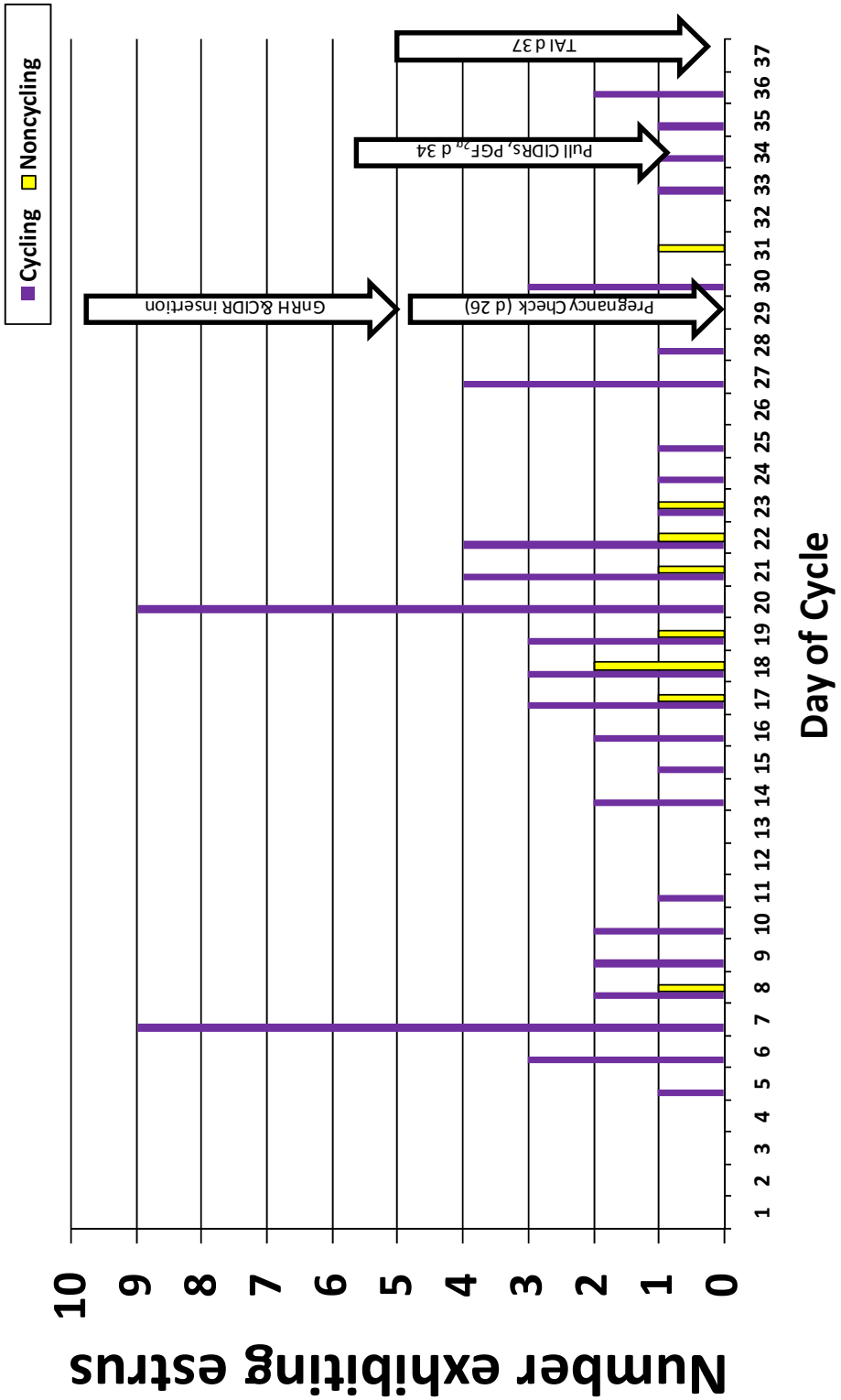


Figure 2.4. Return to estrus distribution by treatments[1].



[1] See figure 2.2 for a description of treatment protocols

Figure 2.5. Return to estrus distribution by pretreatment cyclic status.



Conception and Pregnancy Rates.

The pregnancy rates to the initial synchronized AI for RS and NS are shown in Table 2.3.

Table 2.3 Pregnancy rates by treatment

	Treatments [1]		P Value
	RS	NS	
Pregnancy Rate to initial TAI[2]			
Proportion	41/89	48/85	
%	46.1	56.5	0.17
Pregnancy Rate to AI[3]			
Proportion	62/89	48/85	
%	69.7	56.5	0.07
Pregnancy rate at end of the breeding season[4]			
Proportion	62/89	76/85	
%	69.7	89.4	0.001

[1] See figure 2.2 for a description of treatment protocols.

[2] Conception rate to initial AI determined by ultrasound

[3] Pregnancy rate to AI (RS=initial AI + resynchronized AI; NS= initial AI)

[4] Pregnancy rate at the final pregnancy diagnosis.

Total AI pregnancies tended to be greater for RS (initial AI + resynchronized AI) than for NS (initial AI only) (RS=69.7%, NS=56.5%; P = 0.07). The pregnancy rate was greater for NS (89.4%) than RS (69.7%) at the end of the breeding season (P < 0.01). When compared to the second synchronized AI pregnancy rates in RS (43.8%), the pregnancy rate for heifers exposed to bulls in NS (75.7%) was higher (P < 0.01). There was no effect of treatment (P > 0.10), technician (Table 2.4; P > 0.5), or cyclic status before treatments (Table 2.5; P >0.4).

Table 2.4 Pregnancy rate to initial AI by technician

	Technician		P Value
	A	B	
Pregnancy Rate			
Proportion	51/97	37/77	
%	52.6	48.1	0.55

Table 2.5 Pregnancy rate to initial AI by cyclic status

	Cyclic Status [1]		P Value
	Cycling	Prepubertal	
Pregnancy Rate to initial TAI			
Proportion	78/151	10/23	
%	51.7	43.5	0.47

[1] Cyclic status before initiation of treatments (One or both samples having a progesterone concentration of > 1ng/mL).

Analysis of Days Pregnant

When open heifers were removed from the treatments, average d pregnant were not different for the groups (RS=83.5, NS=86.6; Table 2.6; P > 0.27).

Table 2.6 Average days pregnant at final pregnancy diagnosis by treatment (LS mean ± SE)

	Treatment[1]		P Value
	Resynchronization	Natural Service	
Days Pregnant[2]	84.5 ± 2.1	86.6 ± 1.9	0.4512

[1] Treatment schedule for heifers assigned to RS (resynchronization) and NS (No resynchronization). Heifers assigned to RS were checked for pregnancy on d 29 Open Heifers were given an injection of GnRH and had a CIDR inserted. On d 34, CIDRs were removed and heifers received an injection of PGF2α, followed 72 h later by an injection of GnRH and TAI. Heifers diagnosed pregnant on d 29 in RS received no further treatment. In NS were exposed to cleanup bulls beginning 14 d to d 66 after the initial TAI.
[2] Days pregnant at final pregnancy diagnosis

Economics.

To compare the costs of resynchronization and natural service cleanup a standard herd size of 100 hd was assumed and costs were assigned for RS and NS following initial timed insemination. For RS, a 100 hd herd with a 52% pregnancy rate to initial AI would leave 48 heifers to be resynchronized. The cost of pregnancy check would be $5/hd for a total of $500. Table 2.7 shows the costs of resynchronization, with a total cost for the resynchronization system of $2,701.28. The cost of resynchronization per resynchronized pregnancy ($2,701.28 / 21 resynchronized pregnancies) would be $128.63.

Table 2.7. Cost to use resynchronization protocol on 48 heifers.

	Cost	Total
No. of heifers	100	
Pregnancy rate to 1st AI	52%	
No. of heifers to rebreed	48	
Cost of Pregnancy Check	$5/hd	$500.00
Cost of Drugs[1]	$18.48	$887.04
Labor Cost[2]	$1.44/hd/working	$282.24
Semen Cost[3]	$14/straw	$672.00
AI technician cost[4]	$7.50/hd	$360.00
Total cost to resynchronize 48 heifers		*$2,701.28*

[1] $PGF_{2\alpha}$ -$2.54/dose, GnRH-$3.21/dose, CIDR $9.02/dose, supplies $0.50/head (Johnson and Jones, 2005).

[2]For budgeting labor costs it is assumed 100 head of cattle can be worked in 4.5 hours using four hired workers at an $8/hour wage rate--does not include AI technician (Anderson et al., 2005).

[3] Semen cost per straw (Johnson and Jones, 2005).

[4] Assumed Technician cost/head (Anderson et al., 2005).

To determine the cost of natural service, a 52% pregnancy rate to initial AI was assumed with a bull to heifer ratio of 1:24. The total bull cost to rebreed 48 females was $2,970 ($1485 x 2). Assuming a 76% pregnancy rate for heifers exposed to bulls (NS conception rate for this study), the cost for natural service clean up per pregnancy was $82.50 ($2,970 / 36).

For comparison of financial returns for each treatment, predicted average calf values at weaning were used. The average calf value (weaning weight x price) for RS was $447.63 and $472.57 for NS. The average calf value per heifer exposed was $195.84 for RS and $357.62 for NS (Table 2.8).

Table 2.8. Mean calf age, weight, price, and value[1] at weaning by treatments[2].

	Treatments	
	Resynchronization	Natural Service
Mean calf age at weaning (d)	183.5	185.6
Mean calf weight at weaning (lbs.)	489.3	495.1
Mean price (cwt)	$100.78	$100.68
Mean calf value	$447.63	$472.57
Calf value per exposed female	$195.94	$357.62

[1] Values based on assumed pregnancy rates.
[2] Treatment schedule for heifers assigned to RS (resynchronization) and NS (No resynchronization). Heifers assigned to RS were checked for pregnancy on d 29 Open Heifers were given an injection of GnRH and had a CIDR inserted. On d 34, CIDRs were removed and heifers received an injection of PGF2α, followed 72 h later by an injection of GnRH and TAI. Heifers diagnosed pregnant on d 29 in RS received no further treatment. In NS were exposed to cleanup bulls beginning 14 d to d 66 after the initial TAI.

DISCUSSION

The purpose of this study was to evaluate the efficacy and costs of the resynchronization protocol. In order to complete the objectives, we evaluated the return to estrus distribution after the initial AI, compared the resynchronized AI to the cleanup bulls, and evaluated the costs of resynchronization versus natural service. We chose the CO-Synch + CIDR (5d) protocol because it has been shown to have increased pregnancy rates in 2 year old cows (Bridges et.al., 2005) and heifers (Wilson et al., 2007) when compared to the 7 day CO Synch + CIDR protocol.

There was an increase in the percentage of heifers cycling as RTS increased from 3 to 4 or 5. This is expected because heifers with a RTS 3 are thought to be on the verge of cycling whereas heifers with a RTS of 4 or 5 are presumed to be cycling (Patterson et al., 1999). However, there was no effect of cyclic status or RTS score on pregnancy rate to initial AI. The 5 d CIDR protocol may have induced a fertile estrus (Bridges et al., 2008) in acyclic or low RTS heifers which resulted in similar AI pregnancy rate to cyclic heifers. Alternatively, a low number of heifers were classified as acyclic or RTS 3. Despite an 8% decrease in pregnancy rates in acyclic heifers, there may have been insufficient power to detect a significant difference. Other studies using progestin based synchronization systems reported similar (Lamb et al., 2006) or decreased (Lucy et al., 2001) pregnancy rates in acyclic heifers compared to cyclic heifers.

The average number of days pregnant was greater for NS than RS. This difference may be attributed to the fact that heifers in NS were exposed to fertile

bulls 23 days before the RS resynchronized group was reinseminated. . Regardless of the system used, heifers that returned to estrus prior to d 14 after the initial AI did not have the opportunity to conceive at that estrus. This is because bulls were not turned in until d 14.

Days pregnant is an important early indicator of reproductive efficiency. In addition, date of conception impacts overall profitability because an early conception date corresponds with a calving date early in the season. When calves are born earlier in the calving season they are older and tend to weigh more at weaning (Lesmeister et al., 1973). This is important because most calves are sold on a per pound basis, thus heavier calves would bring more at market.

The average day of return to estrus following initial AI did not differ between groups ($P > 0.10$) which was expected because the treatments did not differ to that point. Figure 2.3, shows two distinct curves. The initial curve represents heifers that either did not respond to the initial synchronization or heifers that short cycled. The heifers that showed return to estrus after d 27 may be heifers that were pregnant, but lost that early pregnancy and are returning to estrus. Figure 2.5 shows the return to estrus distribution by cyclic status. It is important to note that 9 of the 23 heifers that were noncycling at the beginning of the treatments return to estrus by d 31. When added to the 10 heifers that conceived to the initial AI, there are 19 heifers either pregnant or cycling that were prepubertal at the beginning of the treatments. This is expected because all heifers had a RTS of 3 or higher and were treated with a progestin.

The pregnancy rates to the initial AI did not differ between treatment groups despite a 10% difference in pregnancy rate. Due to drought conditions fewer animals were available for this project than as originally designed. This may have lessened our ability to detect a difference due to the smaller number of animals. In contrast, a significant difference in pregnancy rate to initial AI would not be expected as the treatments were the same until after initial AI. Wilson and colleagues (2007) reported a pregnancy rate to FTAI in heifers synchronized with CO-Synch + CIDR (5d) of 57.8%. Our initial pregnancy rates are lower compared to the reports of Wilson and colleagues, but within the range of 25.8 to 71.5 % for other FTAI studies (Schmitt et al., 1996; Johnson and Day, 2004; Martinez et al., 2004; Lamb et al., 2006; Busch et al., 2007).

There was a tendency for RS to have a higher AI pregnancy rate overall due to approximately half of RS being resynchronized and inseminated a second time. The total AI pregnancy rate of 69.7% for RS is comparable to other reported pregnancy rates after resynchronization ranging from 60.0 to 84.9% (Stevenson et al., 2003; Colazo et al., 2006). Heifers that short cycled after the initial synchronization would have been at d 19 of their estrous cycle when they were diagnosed open, had a CIDR inserted and were administered GnRH. Heifers that had normal cycles would have been on approximately d 6 of their cycle when they were administered GnRH and had a CIDR inserted. Hence, a resynchronization protocol must be able to synchronize heifers regardless of the stage of the estrous cycle.

The pregnancy rates to the fertile bulls in NS (75.7%) were greater than the conception rates to the second resynchronized AI for RS (43.8%). The end of the breeding season pregnancy rate approached 20% greater for NS versus RS. The lower end of the season pregnancy rate for RS is most likely due to the limited opportunities to conceive (2) in the entire breeding season compared to approximately 3 opportunities for NS heifers. The end of season pregnancy rate for NS heifers (89.4%) reported here agrees with other studies in beef heifers reporting 86 to 97% (Lamb et al., 2006; Busch et al., 2007). Natural service (NS) heifers were inseminated to the initial AI and then exposed to fertile bulls 14 d later until the end of the 66 d breeding season, allowing 3 estrous cycles for these heifers to become pregnant. Therefore, NS heifers had twice the opportunities to conceive, and are therefore more likely to conceive when compared to the RS treatment.

The return to estrus distribution was bimodal (Figure 2.3) and would therefore make estrus detection and reinsemination very labor intensive and time consuming, because the return to estrus is not synchronized very tightly. This indicates that any resynchronization protocol not utilizing pregnancy diagnosis would have to be able to synchronize animals at all stages of their cycle without compromising conception to the initial AI.

Future studies that investigate potential resynchronization protocols without pregnancy diagnosis could allow heifers more opportunities to conceive during the breeding season and could take advantage of the first estrus after initial AI. If GnRH were administered and a CIDR was inserted on d 12 after the

initial AI, the short cycle heifers would be on d 5 of their cycle (d 0 being estrus). Removal of CIDR on d 19 after the initial AI should allow a majority of animals to exhibit estrus in a 3 d period and be re-inseminated. Another option, would be to utilize FTAI after the CIDR was removed, thus decreasing the amount of labor required for estrus detection. However, this could be counteracted with a decrease in pregnancy rates expected in timed insemination protocols.

The RS treatment had 27 open heifers at the end of the breeding season, with a cost per resynchronized pregnancy $46 higher than NS. For a commercial producer, the higher costs, increased labor, and lower number of pregnancies will likely limit the adoption of this protocol. An opportunity to offset the lower pregnancy rate and costs of RS may exist for certain producers who realize substantial value for AI pregnancies. For example, producers in the replacement female business may be able to offset increased pregnancy costs utilizing RS if an AI bred replacement heifer brings $100 more than her natural service bred counterpart. The high number of open heifers in RS will require strategies to maximize the value of open heifers. These may include retained ownership to harvest or embryo transfer. However, failing to conceive after two opportunities to be synchronized and bred may compromise the value of these animals as embryo transfer recipients.

The difference in average calf value is due to the differences between treatments in expected calving dates and thus the differences in calf age and weight at weaning. The treatment difference in calf value per cow exposed is due to the difference in pregnancy rates and calving dates for the treatments.

Any program using resynchronization after FTAI needs to be cost effective, work in heifers that are at various stages of the estrous cycle, and result in an acceptable conception rate.

CONCLUSION

The purpose of this study was to determine the effectiveness of a resynchronization protocol for beef heifers (5 d CO-Synch + CIDR & FTAI at 72 h). This was done through evaluation of return to estrus distribution, comparison of resynchronization to natural service clean up, and evaluation of costs for both.

During this study, the animals were handled multiple times and pregnancy diagnosis by trained professionals was required. The labor, knowledge, and financial requirements are all reasons why the use of AI (and therefore the adoption of resynchronization) is not widely adopted in the beef industry.

While resynchronization may yield more AI pregnancies in a breeding season, it is important for producers to evaluate the economic impact for their herd. The costs of resynchronization could be reduced if the pregnancy rate to resynchronization was increased. Further research for resynchronization protocols is warranted.

In the present study, the resynchronization of estrus after the initial FTAI yielded a limited number of pregnancies in the breeding season. The resynchronization program also cost more per pregnancy. Further investigation into resynchronization should focus on both biological and economic impacts.

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