

**SYNCHRONIZATION OF FOLLICULAR WAVE EMERGENCE, LUTEAL
REGRESSION, AND OVULATION FOR FIXED-TIME ARTIFICIAL
INSEMINATION IN BEEF COWS AND HEIFERS**

A Dissertation

by

KYLE JEFFREY STUTTS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Physiology of Reproduction

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ABSTRACT

Synchronization of Follicular Wave Emergence, Luteal Regression, and
Ovulation for Fixed-time Artificial Insemination in Beef
Cows and Heifers. (August 2005)

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Two experiments tested the efficacy of gonadotropin-releasing hormone (GnRH) or estradiol cypionate (ECP) administration in combination with controlled internal drug release (CIDR) inserts on pregnancy rate to fixed-time artificial insemination (FTAI) in beef heifers. In the first experiment, heifers in the two ECP groups received ECP in conjunction with the CIDR insert. The CIDR insert was removed and PGF was administered on either d 7 or 9. Heifers received ECP 24 h after CIDR removal and were inseminated 30 h after ECP. Heifers in the two GnRH groups received GnRH in conjunction with the CIDR insert. Heifers received PGF on either d 6 or 7, and the CIDR insert was removed on d 7. Heifers were inseminated and received GnRH 48 h after CIDR removal. Pregnancy rates to FTAI were higher for heifers treated with GnRH. Pregnancy rate to FTAI was highest for heifers in the GnRH group that received PGF on d 6 and was lowest for the group that received ECP in combination with a 7-d CIDR insert.

In the second experiment, heifers received GnRH in conjunction with the CIDR insert. CIDR inserts were removed and PGF was administered on d 7. Heifers were inseminated 60 h later, with one group receiving GnRH at insemination. Pregnancy rate to FTAI was higher for heifers that received GnRH at insemination.

The final experiment tested the efficacy of estradiol 17_β (E17) or GnRH in combination with a CIDR insert on pregnancy rate to FTAI in Brahman cows. Either E17 or GnRH was administered in conjunction with the CIDR insert. CIDR inserts were removed on d 7. Cows in the GnRH group received PGF on d 6 and GnRH at insemination which occurred 48 h after CIDR removal. Cows in the E17 group received PGF on d 7, E17 24 h later, and were inseminated 30 h after E17 administration. Pregnancy rate to FTAI was higher for cows treated with E17.

These data indicate acceptable pregnancy rates were obtained when the CIDR insert was combined with either GnRH in beef heifers or with E17 in Brahman cows.

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CHAPTER I

INTRODUCTION

One of the most underutilized tools for beef cattle production is artificial insemination. Artificial insemination (AI) is utilized in less than 5% of the beef cow population in the United States. The advantages of incorporating AI into beef production operations are well documented and numerous. The major advantage of AI is the genetic improvement that can be made in a herd with the ability to breed females to virtually any breed of bull from anywhere in the world. AI provides an opportunity to increase productivity over a wide range of economically important traits. Other advantages include a reduction in sexually transmitted diseases, elimination of danger to humans as well as other animals by keeping a bull on site, increased accuracy in breeding records, and the ability to use multiple sires in a breeding system without the need to own multiple bulls. Finally, utilizing AI in combination with estrous synchronization in a beef production setting allows for shortened breeding and calving seasons which result in older, heavier calves at weaning and a longer postpartum period for rebreeding.

The major reasons that AI is not used to a greater extent are that most beef cows are maintained in range environments and AI programs require extensive

This dissertation follows the style of Journal of Animal Science.

labor and management in order to achieve satisfactory results. Most producers in the beef cattle industry rely on their beef cattle operations as a secondary income and do not have the time or the labor to incorporate an AI program into their production practices. Other producers have tried using AI in the past, but no longer do so because poor results associated with improper management technique caused a decrease in profits.

Another limiting factor to the widespread use of AI in the U.S. beef cattle industry is the investment of time and labor in detecting estrus and breeding cows in a timely manner. Estrous synchronization protocols that will consistently result in a precise and fertile ovulation and allow the use of timed breeding are essential to the expansion of the utilization of AI in beef cattle.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Estrus synchronization, as the name implies, is the manipulation of the estrous cycle in order to bring a group of females, at random stages of the estrous cycle, into estrus at a precise time. Females may then be inseminated according to estrus, usually 12 h after observed estrus, or cows may be inseminated at a predetermined time without reference to estrus. The latter method is referred to as schedule breeding or fixed-time insemination. Methods to control the estrous cycle in beef cattle have been studied for over 60 yr. The following is a discussion of select hormones and products that have been used in the past or are currently being used to control the estrous cycle in cattle.

Progestins

Progesterone is the dominant ovarian hormone present in the circulation during metestrus and diestrus of the estrous cycle and is secreted from the corpus luteum (CL). This period of the estrous cycle is also referred to as the luteal phase and lasts from the development of a functional CL at 7 d after estrus until regression or luteolysis of the CL at d 16-17 of the cycle. Progestins suppress estrus in cattle and have been used extensively to alter the estrous

cycle. Studies during the 1940s revealed that estrus could be delayed and therefore synchronized by administration of exogenous progesterone to cattle or sheep. This led to many studies in which progestins were administered by injection, released by an intravaginal sponge, or fed for a period of up to and exceeding the length of the estrous cycle to synchronize estrus following the cessation of administration. It was determined that an increased duration of progestin administration resulted in an increased rate of estrus synchronization (Odde, 1990). However, fertility was compromised following administration of progestins for 14 d or longer and pregnancy rates were unacceptable (Zimbelman and Smith, 1966).

One of the first methods used to synchronize estrus in cattle was the long-term feeding of melengestrol acetate (MGA; Zimbelman and Smith, 1966). MGA is a synthetic progestin that suppresses estrus when fed at the rate of 0.5 mg/hd/d. MGA is still utilized extensively today by feedlots to suppress estrus in beef heifers that are being fed for harvest. Although long-term feeding of MGA effectively synchronized estrus, fertility was compromised (Zimbelman and Smith, 1966). A similar percentage of heifers fed MGA for 10 to 18 d exhibited estrus within 6 d following cessation of treatment as did heifers in the control group over a 20-d period. However, conception rates were 14 percentage points lower for heifers fed MGA vs controls (Odde, 1990).

More recent research utilizing ultrasonography to monitor follicular development has demonstrated that follicles under the influence of exogenous

progesterone continued to develop and persist on the ovary in cyclic females (Anderson and Day, 1994). Although these follicles were capable of ovulating and developing corpora lutea, fertility was depressed because the capability of the fertilized oocytes to develop to the 16-cell stage was compromised (Ahmad et al., 1995).

The precise reason for reduced fertility when MGA is used to synchronize estrous has yet to be determined. Several possible or contributing factors include an increase in size of the dominant follicle that does not ovulate during the treatment, an increase in concentrations of estradiol as compared to the concentrations during the normal estrous cycle, or a reduction in size and weight of the CL formed after treatment with MGA (Kojima et al., 1992). Another possibility is that a subluteal level of progesterone alters the secretory pattern of luteinizing hormone (LH). Administration of a low level of progesterone results in a high frequency of LH pulses. Wehrman et al. (1993) reported that estradiol concentration is higher prior to estrus, and onset of the preovulatory LH surge occurs sooner after removal of the source of progesterone in cows receiving a low dose compared to those receiving a high dose of exogenous progesterone. Increased LH support in cows receiving a subluteal level of progesterone allowed for the dominant follicle to escape atresia and persist on the ovary for an extended period of time. The presence of the dominant follicle allowed for an increased concentration of estrogen for a prolonged period of time. The authors concluded that exposure to an elevated level of estrogen or a subluteal level of

progesterone for an abnormally long period may result in abnormal oocyte maturation, abnormal gamete/embryo transport, or inadequate preparation of the uterus for pregnancy leading to decreased conception rates or increased embryonic mortality (Wehrman et al., 1993).

Another method to administer exogenous progesterone is the progesterone-releasing intravaginal device (PRID). This device was evaluated in several trials during the 1970s. Bulman et al. (1978) applied the PRID to lactating dairy cows (that were at least 50 d post-parturition) for a 14-d period. The PRID effectively synchronized estrus, and conception rate to a double insemination at 48 and 72 hr after PRID removal was 50%. It was also reported that ovulation occurred in 8 of 9 cows whose ovaries had been inactive for at least 50 d after calving.

It is well established that administration of exogenous progesterone can hasten the attainment of puberty in heifers and cause postpartum anestrous cows to become estrous cycling. Rasby et al. (1998) conducted a study on peripubertal crossbred Angus heifers. When approximately 40% of the heifers in the study had attained puberty (as evidenced by a functioning CL), heifers were treated with either progesterone for 7 d or progesterone for 7 d plus an injection of estradiol benzoate (EB) at 24 to 30 hr after cessation of progesterone treatment. In comparison to control heifers that received no treatment, treated heifers had an increase in proportion that developed a normal CL, a decrease in proportion that had a short estrous cycle, and an increase in proportion that exhibited an estrus following treatment. In this study, treatment induced

formation of a normal CL in 114 of 203 prepubertal heifers. These results indicate that the components for puberty are in place relatively early in life, but heifers remain prepubertal until a specific sequence of endocrine events take place and allow for initiation of estrous cycles.

Lucy et al. (2001) studied the effects of progesterone treatment on primiparous and multiparous suckled beef cows. Treatment with progesterone resulted in a greater incidence of estrus within the first 3 d of the breeding period compared to controls. This improved estrous response led to an increase in pregnancy rate during that 3-d period with treated cows having a pregnancy rate of 36% compared to a pregnancy rate of 7% for untreated cows.

Several experiments were conducted by Stevenson et al. (2003) to determine the effect of synchronizing estrus in postpartum suckled beef cows with progesterone, norgestomet, and MGA. The authors concluded that progestin treatment improved pregnancy rates in all trials and that most of the improvement in pregnancy rates was due to increased pregnancy rates in anestrous beef cows.

The ability of exogenous progestins to induce estrus in anestrous cattle has been attributed to, in part, increased LH secretion both during and after treatment. It has been reported that progestin treatment increased LH secretion in postpartum beef (Garcia-Winder et al., 1986) as well as seasonal dairy cows (Rhodes et al., 2002). In addition, LH secretion following weaning was increased in cows with prior exposure to progestin (Breuel et al., 1993). This

induced increase in LH is important because it mimics the proestrus increase in LH leading to the preovulatory LH surge (Day, 2004).

Prepubertal heifers treated with progestins either at 11 or 12.5 mo of age had similar increases in LH concentration following removal of the exogenous progestin; however, puberty was induced only in the older heifers (Hall et al., 1997). These results call into question the importance of increased LH concentrations to induce puberty in prepubertal heifers. In another study, prepubertal heifers were subjected to different levels of exogenous progestin (Anderson et al., 1996). In heifers receiving the high level of progestin, LH was suppressed; however, puberty was induced in 80% of these heifers. These results indicate that increased levels of LH are not obligatory to induce puberty in prepubertal heifers and exposure to exogenous progestin can induce puberty independently in peripubertal heifers (Day, 2004).

Prostaglandin F₂

Prostaglandins are lipids consisting of 20-carbon unsaturated hydroxy fatty acids derived from arachidonic acid. Prostaglandin F₂ (PGF) is produced by the uterine endometrium and is responsible for luteolysis, or degradation of the CL, in cattle. The bovine estrous cycle can be divided into two phases, the follicular phase and the luteal phase. The follicular phase is characterized by follicle growth culminating in selection of a dominant follicle and subsequent ovulation. The luteal phase is the longest phase of the cycle (approximately d 6

to d 16 of the estrous cycle). The luteal phase is characterized by the functioning CL secreting progesterone. During the late luteal phase (d 16-18 of the cycle) PGF is released from the uterus and binds to the CL causing luteal regression.

During the 1970s, it was discovered that PGF was luteolytic in cattle and could be used to synchronize estrus (Lauderdale et al., 1974). Thatcher and Chenault (1976) reported that an injection (i.m.) of PGF caused rapid regression of the CL which initiated normal transitory hormonal patterns resulting in ovulation in estrous cycling dairy heifers. Luteal function was affected by treatment of heifers with PGF (25 mg) as evidenced by a decrease in estrous cycle length, a decrease in CL size, and a significant decrease in plasma progesterone level (Kimball and Lauderdale, 1975).

It was later determined that PGF had limited utility in synchronizing estrus because it was only effective in cattle that were cycling and had a CL (d 5 to 17 of the cycle). Therefore, prepubertal heifers, anestrous females, females on d 0 to 4 of the estrous cycle, and females in the final days of the estrous cycle subsequent to luteolysis were not responsive. Strategies were devised to overcome some of these obstacles, such as two injections of PGF administered 10 to 12 d apart, but there was still great variability in the time from treatment to estrus. Treatment with two injections of PGF still required 5 to 7 d of estrus detection and AI.

Another strategy incorporated an estrus detection period for 4 or 5 d prior to injection of PGF followed by another period of estrus detection after the injection. This method decreased the amount of PGF used to synchronize estrous, but it doubled the time required for estrus detection. These methods were effective in cycling females, but still were not of utility in anestrus or prepubertal females.

Single timed inseminations have been attempted using the two-injection PGF system. Although acceptable conception rates have been reported in some experiments, there has been significant variation and a greater incidence of low conception rates when timed insemination has been used. Fogwell et al. (1986) conducted a large trial incorporating 45 herds. Conception rates to a single timed insemination at 80 hr following PGF injection were 22 percentage points lower compared to heifers bred at 12 hr after detection of estrus. The low fertility and the wide range in conception rate (6.7 to 85.7%) in those herds are most likely due to the timing of estrus following administration of PGF. It was later determined that the interval from treatment with PGF to estrus was dependent upon the stage of the follicular wave at treatment (Lucy et al., 1992). Larger, more mature follicles ovulated sooner than their smaller, less mature counterparts.

Exogenous Progestins and Prostaglandin F₂

During the 1980s, numerous studies evaluated the concurrent use of MGA and PGF to synchronize estrus. Initially, MGA was fed daily for 7 d and PGF was administered on the last day of MGA feeding. The rationale behind this protocol was to reduce the period of time that exogenous progestins were administered in an attempt to overcome the reduction in fertility observed with long-term feeding of MGA (Beal et al., 1988). This procedure effectively synchronized estrus, but fertility was suppressed (Chenault et al., 1990). Conception rates to AI during a 6-d synchronization period were 68% for heifers administered a single injection of PGF compared to 52% for heifers administered the short-term MGA-PGF protocol.

Later, a modification to this synchronization protocol consisted of feeding MGA for 14 d followed by an injection of PGF 17 d after cessation of MGA. Heifers were monitored for estrus and inseminated over a 5-d period following administration of PGF. Results from this study were most encouraging because this protocol was effective in heifers that were either prepubertal (40% pregnancy rate) or estrous cycling (68% pregnancy rate) at the initiation of treatment (Brown et al., 1988). This system effectively synchronized estrus within 7 d following the last feeding of MGA (Patterson et al., 1989); however, heifers were not inseminated at this time because of the suppressive effects of long-term administration of progestins on fertility. The administration of PGF 17 d after the last feeding of MGA causes the timing of PGF administration to occur

after d 10 of the estrous cycle in the majority of cattle and maximum response to PGF is observed at this time (Watts and Fuquay, 1985).

As mentioned earlier, the later PGF is administered in the estrous cycle, the higher the response in terms of CL regression and subsequent estrus. Heifers that received PGF after d 12 of the estrous cycle had higher estrus response rates (91%) compared to heifers that received PGF between d 9 and 12 (77%) of the cycle (Beal, 1998). In several studies the interval between the last MGA feeding and the administration of PGF was increased from 17 d to 19 d. Deutscher (2000) reported that a greater percentage of heifers in the 19-d than in the 17-d group (92.4% vs 86.7%) exhibited estrus within the 5-d breeding period following administration of PGF. First service conception rate was also 5.1 percentage points higher for the heifers in the 19-d group compared to those in the 17-d group. Lamb et al. (2000) reported similar results in a study using heifers from 12 different sources. Heifers that received PGF on d 17 after MGA feeding had first service conception rates of 75.9% compared to 81.4 % for heifers that received PGF 19 d after MGA feeding. Average interval to estrus following administration of PGF was shorter for heifers in the 19-d group (56.2 hr) compared to heifers in the 17-d group (73.1 hr). In general, heifers in the later stages of their estrous cycles had higher conception rates. Heifers in the late CL group (d 15 to 17) had a conception rate of 67%, whereas heifers in the early CL group (d 7 to 11) had a conception rate of 43% (Deutscher, 2000).

The most recent combination of progestin and PGF became popular during the 1990s. During this time exogenous progesterone was administered by a Controlled Internal Drug Release (CIDR) insert which was placed intravaginally for 7 d followed by an injection of PGF on d 6 or 7 of treatment. During the 7 d in the vagina, the CIDR releases progesterone at a relatively constant rate suppressing estrus and ovulation. By the end of the treatment period, there are two populations of females: 1) those that do not have a functional CL, and 2) those that have a functional CL greater than d 6 of the cycle which is susceptible to the luteolytic effect of PGF. Therefore, approximately 95% of the treated females should exhibit estrus within 5 d of treatment. Lucy et al. (2001) compared the use of a CIDR in combination with PGF to PGF alone for estrus synchronization. The CIDR+PGF treatment improved estrus synchronization compared to PGF alone (59% vs 33%), and the improved estrus synchrony led to greater pregnancy rates (36% vs 22%) in postpartum beef cows. The CIDR+PGF treatment improved the rate of synchronized estrus (3-d period) and pregnancy rate compared to PGF alone or control females in both cyclic and acyclic beef and dairy cows as well as in prepubertal and pubertal dairy heifers.

Norgestomet and Estradiol

In the 1980s and 1990s, the most popular method to synchronize estrus was the use of norgestomet (NOR) in combination with estradiol valerate (EV) marketed under the name of Syncro-Mate-B (SMB). SMB consisted of a

subcutaneous ear implant of norgestomet inserted for 9 d and an injection of NOR and EV at the time of implantation. The NOR diffused from the implant at a rate adequate to suppress estrus during the 9-d implantation period (Kesler et al., 1995). The injection of NOR and EV caused regression of an early developing CL, and by the end of the implantation period, estrus was being suppressed by the NOR from the implant (Peterson et al., 2000). This treatment resulted in 77 to 100% of cattle exhibiting estrus soon after treatment; however, fertility was variable with first service conception rates ranging from 33 to 68% (Odde, 1990).

Spitzer et al. (1981) conducted five trials with a total of 958 beef heifers. First service pregnancy rate for heifers treated with the SMB regimen was 55% compared to 67% for non-treated control heifers. However, pregnancy rate during the 5-day breeding period following treatment was significantly higher for treated heifers (54%) than for control heifers (21%). In addition, of the heifers treated with the SMB regimen, first service pregnancy rate did not differ among heifers inseminated 12 hr after detected estrus, heifers inseminated once from 45 to 55 hr after implant removal, or heifers inseminated twice at 48 and 60 hr after implant removal. These results indicate that this treatment regimen may be utilized for scheduled breeding.

Later, It was determined that stage of the cycle at initiation of treatment with the SMB protocol was the primary factor leading to the variability in conception rates. Mathis et al. (2001) reported that pregnancy rate for heifers in diestrus

(53.6%) at the initiation of treatment was higher than for heifers in metestrus (43.7%) or heifers in proestrus (44.4%) when insemination occurred at 12 hr after the onset of estrus. The authors also conducted a study in which heifers were inseminated at 48 to 50 hr after NOR implant removal. Pregnancy rate was higher for heifers that had a plasma progesterone level > 1 ng/mL (37.6%) than for heifers that had a plasma progesterone level < 1 ng/mL (18.5%) at the initiation of treatment. These results supported the authors' hypothesis that fertility is enhanced when a progestin-based estrus synchronization protocol is initiated during diestrus.

Controlling Follicular Waves

Follicle growth during the bovine estrous cycle occurs in waves. During a follicular wave, a group or cohort of follicles begins growing until a single follicle becomes significantly larger than the subordinate follicles. The dominant follicle then inhibits growth of the subordinate follicles which become atretic and regress (Kastelic, 1994). In the presence of a CL, the dominant follicle proceeds through the growth phase, then a static phase, then finally regresses and is no longer capable of inhibiting folliculogenesis and a new follicular wave is initiated. In the absence of a CL, increased pulsatile secretion of gonadotropins will cause the dominant follicle to mature and secrete an increasing amount of estradiol which stimulates a surge in LH and results in ovulation. The estrous cycle of most cattle is characterized by having two or three follicular waves with the last wave

giving rise to the ovulatory follicle. Folliculogenesis is a complex process involving pituitary gonadotropins, ovarian steroids, and other non-steroidal factors (Garcia et al., 1999). The mechanisms that promote the selection of the dominant follicle have not yet been determined, but much progress has been made in understanding follicular development and its regulation. Most treatments that have been developed to control emergence of a new follicular wave have been based on the physical or hormonal removal of the suppressive effect of the dominant follicle. Hormonal treatments that allow control over follicular wave emergence and ovulation facilitate the use of fixed-time insemination in cattle and eliminate the need for time-consuming estrus detection.

The three most common methods of controlling follicular wave emergence are treatment with an injection of estrogen or gonadotropin-releasing hormone (GnRH) or ultrasound-guided follicle ablation. Estradiol is a steroid hormone and is the biologically active estrogen produced by developing follicles on the ovary. Estradiol has a wide range of physiological functions which include: induction of behavioral estrus in the female, development of female secondary sex characteristics, stimulation of duct growth and development of the mammary gland, and exertion of both positive and negative feedback controls on LH and FSH release through the hypothalamus. GnRH is a decapeptide synthesized and stored in the hypothalamus and provides a link between the neural and endocrine systems. GnRH is released into the hypophyseal portal system

where it is transported to the anterior pituitary and causes release of LH and FSH in response to neural stimuli.

Estradiol has been shown to induce follicular wave turnover, or emergence of a new follicular wave, in the majority of treated cattle regardless of the stage of the follicular wave when treatment is administered (Colazo et al., 2004a). The mechanism responsible for estradiol-induced synchronization of follicular growth appears to involve the suppression of plasma FSH concentrations followed by synchronous resurgence of FSH after atresia or removal of the dominant follicle.

In contrast, GnRH appears to have a direct effect on the dominant follicle at the time of treatment. Treatment with GnRH causes an acute release of LH and FSH. If the dominant follicle present at the time of treatment has expressed LH receptors, it will ovulate in response to the acute increase in plasma LH (Peeler et al., 2004). Atresia or ovulation of the dominant follicle depends on the status of the dominant follicle at the time of GnRH treatment. Ovulation of a growing dominant follicle occurred 100% of the time; however, ovulation of static or regressing dominant follicles occurred 33% and 0% of the time, respectively (Beal, 1998). GnRH treatment only synchronized emergence of a new follicular wave if ovulation occurred (Colazo et al., 2004a).

Follicle ablation is a surgical method of removing follicles from the ovary which allows for emergence and growth of a new group of follicles. Follicle ablation is carried out by transvaginal ultrasound-guided follicle aspiration of all follicles ≥ 5 mm in diameter.

Synchronization of Estrus and Ovulation

In more recent years, research on controlling the length of the estrus cycle has led to a greater understanding of follicular development. With this clearer understanding of folliculogenesis, methods to control or manipulate follicular development have been developed. These methods have been combined with traditional methods of controlling estrous cycle length in order to not only synchronize the time of estrus but to synchronize the time of ovulation as well. The primary goal of controlling cycle length (or CL function) and follicular development (that will result in a precise, tightly synchronized ovulation) is to devise a treatment that will facilitate the use of a single, timed insemination without the need for detection of estrus (Beal, 1998). It was determined that in order to maximize fertility and pregnancy rates in cattle, hormone treatments used to synchronize estrus should result in the presence of a dominant follicle of short (2 to 4 d) duration at estrus (Austin et al., 1999).

GnRH+PGF

One of the first treatments used to control the time of estrus and ovulation consisted of an injection of GnRH followed by an injection of PGF 6 or 7 d later. Forbes et al. (1997) reported that the synchrony of estrus is more precise for cows treated with the GnRH-PGF protocol compared to cows treated with two injections of PGF 14 d apart. In addition, conception rates between treatments were similar in beef cows inseminated 12 hr after estrus detection, however,

pregnancy rates were higher for cows treated with the GnRH-PGF protocol (39% vs 28%).

Pursley et al. (1995a) reported that timing of ovulation following the PGF injection in the GnRH-PGF treatment ranged from 84 to 120 hr. In order to increase the synchrony of ovulation they added a second injection of GnRH 48 hr after the PGF injection. Ovulation was synchronized within an 8-hr period in all lactating cows and in all heifers in which a CL had regressed from the PGF injection. This treatment (GnRH-PGF-GnRH) was termed Ovsynch because it effectively synchronized follicular development, estrus, and ovulation.

The tight synchrony of ovulation when using the Ovsynch protocol facilitated the use of timed insemination. Pregnancy rate following timed insemination when lactating dairy cows were treated with the Ovsynch protocol was similar to that reported for cows treated with the double injection PGF protocol and inseminated 12 hr after detection of estrus (38.9% vs 37.8%; Pursley et al., 1997). Pursley et al. (1995b) also conducted a trial to evaluate the timing of insemination after the second GnRH injection. Pregnancy rates varied when cows were inseminated 0, 8, 16, 24, and 32 hr after the second injection of GnRH in the Ovsynch protocol. Pregnancy rate was highest when cows were inseminated at 16 hr (44%) after the second GnRH injection, but the small reduction in pregnancy rate when cows were inseminated at the time of the second GnRH injection made this procedure attractive especially when inseminating a large number of cows at one time because it reduced the number

of times each cow had to be handled. The treatment regimen in which the second injection of GnRH is administered at the time of the scheduled insemination has been termed Cosynch.

GnRH+PGF+MGA

Perry et al. (2002) combined the Ovsynch protocol with the MGA-PGF protocol in an attempt to increase conception rates to timed insemination. Postpartum beef cows were fed MGA for 14 d followed by an injection of GnRH on d 12 after MGA withdrawal. An injection of PGF was administered 19 d following MGA withdrawal, and cows received a second injection of GnRH at the time of insemination 72 hr after PGF. Pregnancy rate to fixed-time insemination was higher when MGA was included (61% vs 47%) in the treatment regimen, and there was no difference between treatment regimens with or without MGA in final pregnancy rate.

There are several other combinations of the GnRH+PGF+MGA estrous synchronization protocol. Stegner et al. (2004) conducted a study incorporating two protocols referred to as MGA Select and 7-11 Synch. Cows treated with the MGA Select protocol were fed MGA for 14 d, received an injection of GnRH 12 d after MGA withdrawal, and an injection of PGF 7 d after GnRH. Cows treated with the 7-11 Synch protocol were fed MGA for 7d and received an injection of PGF on the last day of MGA feeding. An injection of GnRH was administered 4 d after PGF and a second injection of PGF was administered 7 d after GnRH. All cows were inseminated approximately 12 hr after the onset of estrus.

Synchronized conception rate (61%, MGA Select vs 70%, 7-11Synch) and synchronized pregnancy rate (56% vs 64%) were not significantly different between the two treatments, however a tighter synchrony of estrus was observed in cows treated with the 7-11 Synch protocol.

In summary, the GnRH+PGF protocol was successful in inducing ovulation and follicular maturation in combination with luteal regression in anestrous and cycling postpartum cows. With the addition of MGA feeding to the Select Synch, 7-11 Synch, or Cosynch protocols, improved pregnancy rates were recorded.

CIDR+PGF+GnRH or Estradiol

In an attempt to further control estrus and ovulation, it has been proposed that ovulation can be synchronized by inducing synchronous emergence of a new follicular wave in concert with luteal phase control (Martinez et al., 2000a). Synchronous follicle growth should result in synchronous ovulation when the suppressive effect of progesterone is removed. Synchronous ovulation would allow for fixed-time insemination of females and eliminate the time and labor needed for estrus detection. Maximum conception rates to fixed-time insemination can only be achieved if AI occurs at the appropriate interval before ovulation.

Current approaches to fixed-time AI in cattle involve a source of progestin, and synchronization of follicular wave emergence and ovulation. Progesterone has been included in synchronization systems to suppress estradiol-induced LH release, therefore preventing ovulation and estrus (Bo et al., 1994). Several

forms of estrogen as well as GnRH have been used in estrous synchronization protocols to control follicular dynamics of the estrous cycle. In general, these methods cause ovulation or regression of the dominant follicle and allow a new group of follicles to enter the growth phase of development. In progestagen-treated heifers that received 5 mg of estradiol 17_β (E17) or EB, a new follicular wave emerged in an average of 4.1 d regardless of the stage of development of the dominant follicle at the time of treatment (Martinez et al., 2005). Less variability in time from treatment to follicular wave emergence was observed in heifers treated with E17. Females treated with estradiol cypionate (ECP) initiated development of a new follicular wave 4.1 d after treatment (Colazo et al., 2003), and GnRH induced ovulation of large antral follicles with a new follicular wave emerging approximately 2.0 d later (Martinez et al., 2002). However, synchronous emergence of a new follicular wave occurs only when GnRH treatment causes ovulation.

In an attempt to shorten the treatment period of the Cosynch + MGA protocol, Stevenson et al. (2003) treated lactating beef cows with the Cosynch protocol and an intravaginal progesterone-releasing insert (CIDR) concurrently. Cows treated with the Cosynch+CIDR protocol received an injection of GnRH and a CIDR insert at the initiation of treatment. CIDR inserts were removed and cows were injected with PGF 7 d later followed by a second injection of GnRH and a timed insemination 48 hr after CIDR removal. Addition of the CIDR to the Cosynch protocol increased pregnancy rate by 5% overall (66% vs 61%). In

addition, pregnancy rate was higher in anestrous cows (60.0% vs 42.9%) and cows that were less than 60 d postpartum (63.0% vs 41.7%) when treated with the Cosynch+CIDR protocol.

Another method of synchronizing follicular development and estrus has been reported in which EB or E17 is administered to regress the dominant follicle and allow for emergence of a new follicular wave. Day et al. (2000) described a treatment in which cyclic dairy cows received an injection of EB and a CIDR insert at the initiation of treatment. The CIDR was removed and an injection of PGF was administered 7 d later. Each cow then received a second injection of EB 48 hr after CIDR removal. Cows were inseminated 12 hr after detection of estrus, and the conception rate of treated animals (62%) was similar to that of controls (57%). Although timed insemination was not utilized in this trial, the high degree of estrus synchrony (>72% in estrus within a 24-hr interval) indicates that timed insemination would be successful when used with this treatment.

Colazo et al. (2004b) conducted several experiments incorporating timed insemination in heifers and lactating cows. In the first experiment, heifers were treated with a CIDR containing 1.9 g of P4 for 9 d and an injection of ECP with or without progesterone (P4) at CIDR insertion. Heifers received an injection of PGF at CIDR removal, a second injection of ECP 24 hr later, and were inseminated 55 to 60 hr after CIDR removal. Pregnancy rate was not affected by the addition of P4 to the treatment (45.6% ECP vs 48.8% ECP+P4). In the

second experiment, cows were treated the same as in the first experiment with the exception that ECP was replaced with EB and animals were inseminated 52 to 56 hr after CIDR removal. Pregnancy rate was affected by parity (67.9% for heifers vs 53.1% for cows), but progesterone had no effect. In the final experiment, heifers were treated the same as in the second experiment, with the exception heifers were treated with either one new, one once-used, one twice-used, or two twice-used CIDR. Pregnancy rate to fixed-time insemination was not significantly affected by treatment. Pregnancy rates for one new, one once-used, one twice-used, or two twice-used CIDR were 57.5, 63.8, 47.9, and 47.9%, respectively.

Peeler et al. (2004) evaluated two estrous synchronization protocols in dairy heifers. One group was synchronized using a CIDR plus an injection of ECP on d 0, CIDR removal and a PGF injection on d 7, and a second injection of ECP on d 8. The other group received the same treatment except the second injection of ECP was replaced with an injection of GnRH on d 9. All heifers were inseminated at 48, 56, or 72 hr after CIDR removal. Heifers synchronized with the CIDR-ECP treatment had a pregnancy rate of 63% which was similar to heifers treated with the CIDR-GnRH protocol (57.1%). Pregnancy rate was affected by the time of insemination in the heifers treated with the CIDR-ECP protocol, but not for those synchronized with the CIDR-GnRH protocol. Pregnancy rate was higher in heifers treated with the CIDR-ECP protocol when

insemination occurred at 56 hr (81%) after CIDR removal compared to those inseminated at 48 (66.7%) or 72 hr (50.0%).

In another study, Martinez et al. (2000b) compared the use of EB, E17, or GnRH in combination with a CIDR for fixed-time insemination in beef cows and heifers. In the first experiment, heifers received a CIDR insert and an injection of EB+P4, an injection of GnRH, or no further treatment on d 0. On d 7, heifers received an injection of PGF and CIDR were removed. Heifers in the EB+P4 group received an injection of EB 24 hr after PGF and were inseminated 30 hr later. Heifers in the GnRH group received a second injection of GnRH 54 hr after PGF and were inseminated at that time. Heifers in the control group were inseminated 12 hr after detection of estrus. Pregnancy rate was higher in the EB+P4 group (76%) than in the GnRH (48%) or control (38%) groups. In the second experiment, cows were treated the same as in the EB+P4 group in the first experiment except that EB was replaced with E17 in one group of cows. Conception rates to fixed-time insemination were similar between these two treatments (67% E17 vs 71% EB).

The use of GnRH or estradiol in combination with a CIDR apparently synchronizes ovulation and follicular wave emergence which facilitates fixed-time insemination in beef cows and heifers (Martinez et al., 2002). Pregnancy rates to a single fixed-time insemination were highly acceptable with an overall average of 58%.

Summary

Many changes have been made over the years to control the estrous cycle of and facilitate the use of artificial insemination in cattle. In the beginning, estrous was synchronized with the administration of a single hormone in most cases. More recent estrous synchronization protocols are much more complex and incorporate several hormones resulting in a higher rate of estrus synchrony. The current methods of estrous cycle control not only control the time of estrus, but control follicular development as well allowing for a highly synchronized time of ovulation in treated animals. Synchronization protocols that control both the time of estrus and follicular development appear to be the best methods to achieve acceptable pregnancy rates to a single fixed-time insemination.

CHAPTER III

SYNCHRONIZATION OF FOLLICULAR WAVE EMERGENCE, LUTEAL REGRESSION, AND OVULATION FOR FIXED-TIME ARTIFICIAL INSEMINATION OF BEEF HEIFERS

Introduction

The effectiveness of any estrous synchronization system for scheduled breeding in beef cattle is determined by its ability to elicit a precise and tightly synchronized estrus and/or ovulation so that females can be artificially inseminated at a predetermined time. However, maximum conception rates can only be achieved if the ovulation and the time of AI coincide. In an attempt to further control estrus and ovulation, it has been proposed that ovulation can be synchronized by inducing synchronous emergence of a new follicular wave in concert with luteal phase control (Martinez et al., 2000a). Synchronous follicle growth should result in synchronous ovulation when the suppressive effect of progesterone is removed.

Current approaches to fixed-time AI in cattle involve a source of progestin, and synchronization of follicular wave emergence and ovulation. Progesterone has been included in synchronization systems to suppress estradiol-induced LH release (Bo et al., 1994). In progestogen-treated heifers that received 5 mg of estradiol 17 β , a new follicular wave emerged in an average of 4.1 d regardless

of the stage of development of the dominant follicle at the time of treatment (Martinez et al., 2005). Several studies have substituted estradiol 17 β with estradiol cypionate. Average time to follicular wave emergence was 4.1 d after treatment with estradiol cypionate (Colazo et al., 2003). Gonadotropin-releasing hormone (GnRH) or its analogs have also been used in several studies to synchronize follicular wave emergence and ovulation. GnRH induces ovulation of large antral follicles with a new follicular wave emerging approximately 2.0 d later (Martinez et al., 2002). However, synchronous emergence of a new follicular wave occurs only when treatment causes ovulation.

Previously, the primary sources of progesterone for such synchronization systems were either a norgestomet ear implant or feeding of melengestrol acetate. More recently, another alternative is the controlled internal drug release (CIDR) insert. Lucy et al. (2001) reported CIDR inserts increased the percentage of cattle in estrus and pregnant during the initial days of the breeding season while using a protocol that included a 7-d CIDR treatment with PGF $_{2\alpha}$ injected on d 6 of the CIDR treatment. This treatment was effective in both

cyclic and acyclic females. The use of GnRH or estradiol benzoate in combination with a CIDR apparently synchronizes ovulation and follicular wave emergence facilitating fixed-time insemination in beef heifers (Martinez et al., 2002). Pregnancy rates to a single fixed-time insemination were highly acceptable with an overall average of 58%.

The objectives of this study in beef heifers were to: (1) compare the effects of ECP or GnRH in combination with a CIDR insert on pregnancy rate to fixed-time AI, (2) determine whether the pregnancy rate to fixed-time AI was affected by duration of CIDR insert in combination with ECP or by timing of administration of PGF when the CIDR insert was used in combination with GnRH, (3) compare pregnancy rate to fixed-time AI with or without GnRH administration after removal of a CIDR insert, (4) determine retention rate of the CIDR insert, and (5) compare the cost per pregnancy among synchronization protocols that combined either ECP or GnRH with a CIDR insert and PGF.

Materials and Methods

Trial 1

Experimental Design

Peripubertal Angus and Angus crossbred heifers (n=1,906) that were approximately 12 to 14 mo of age and ranged in weight from 307 to 340 kg were used in this study. The heifers were maintained primarily in a feedlot environment on a grain-based diet, but improved winter pasture was also utilized when available.

Heifers were randomly assigned to one of four treatment groups. The treatments included two synchronization protocols utilizing gonadotropin-releasing hormone (GnRH; OvaCyst, Phoenix Scientific, St. Joseph, MO), prostaglandin F₂ (PGF; Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI), and controlled internal drug release (CIDR; Eazi-Breed CIDR, Pharmacia & Upjohn Co., Kalamazoo, MI) inserts, and two synchronization protocols utilizing estradiol cypionate (ECP; ECP, Pharmacia & Upjohn Co., Kalamazoo, MI), PGF, and CIDR inserts.

Estrous synchronization protocols incorporating ECP are illustrated in Figure 1. Heifers in the ECP9 treatment group received a CIDR insert placed intravaginally in addition to an injection (i.m.) of ECP (1 mg) at the initiation of treatment (d 0). On d 9, the CIDR insert was removed and an injection (i.m.) of PGF (25 mg) was administered. A second injection (i.m.) of ECP (0.5 mg) was

administered on d 10, and the heifers were inseminated 30 hr later on d 11. Heifers in the ECP7 treatment group were subjected to the same hormonal regimen as heifers in the ECP9 group with the exception of timing of the administration of hormones. For heifers in the ECP7 group, CIDR inserts were removed and the PGF injection was administered on d 7, the second injection of ECP was administered on d 8, and heifers were inseminated on d 9.

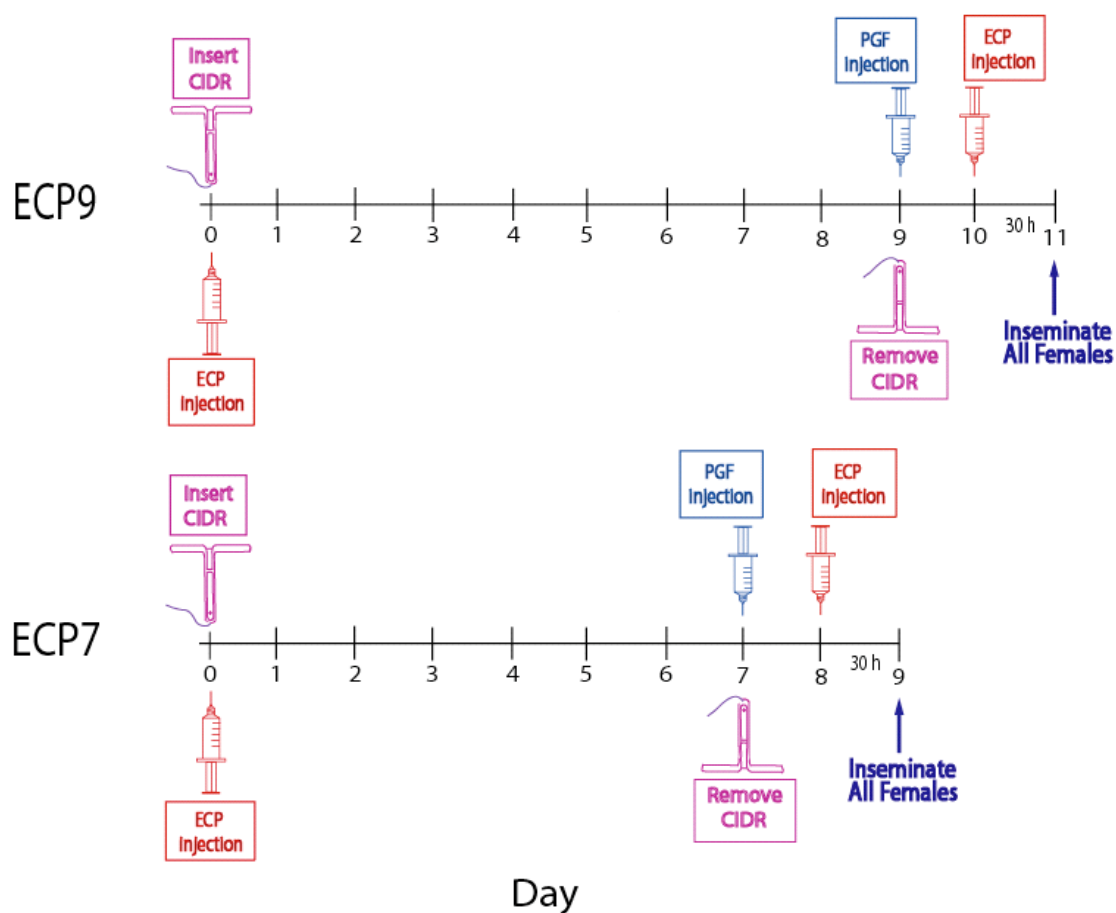


Figure 1. Schematic diagram of estrous synchronization protocols for fixed-time insemination utilized in the ECP9 and ECP7 treatment groups of Trial 1.

Estrous synchronization protocols incorporating GnRH are illustrated in Figure 2. Heifers in the GnRH7 treatment group received a CIDR insert and an injection (i.m.) of GnRH (50 μ g) at the initiation of treatment (d 0). An injection (i.m.) of PGF (25 mg) was administered and CIDR inserts were removed on d 7. Heifers received a second injection (i.m.) of GnRH (50 μ g) at the time of insemination on d 9. Heifers in the GnRH6 treatment group received the

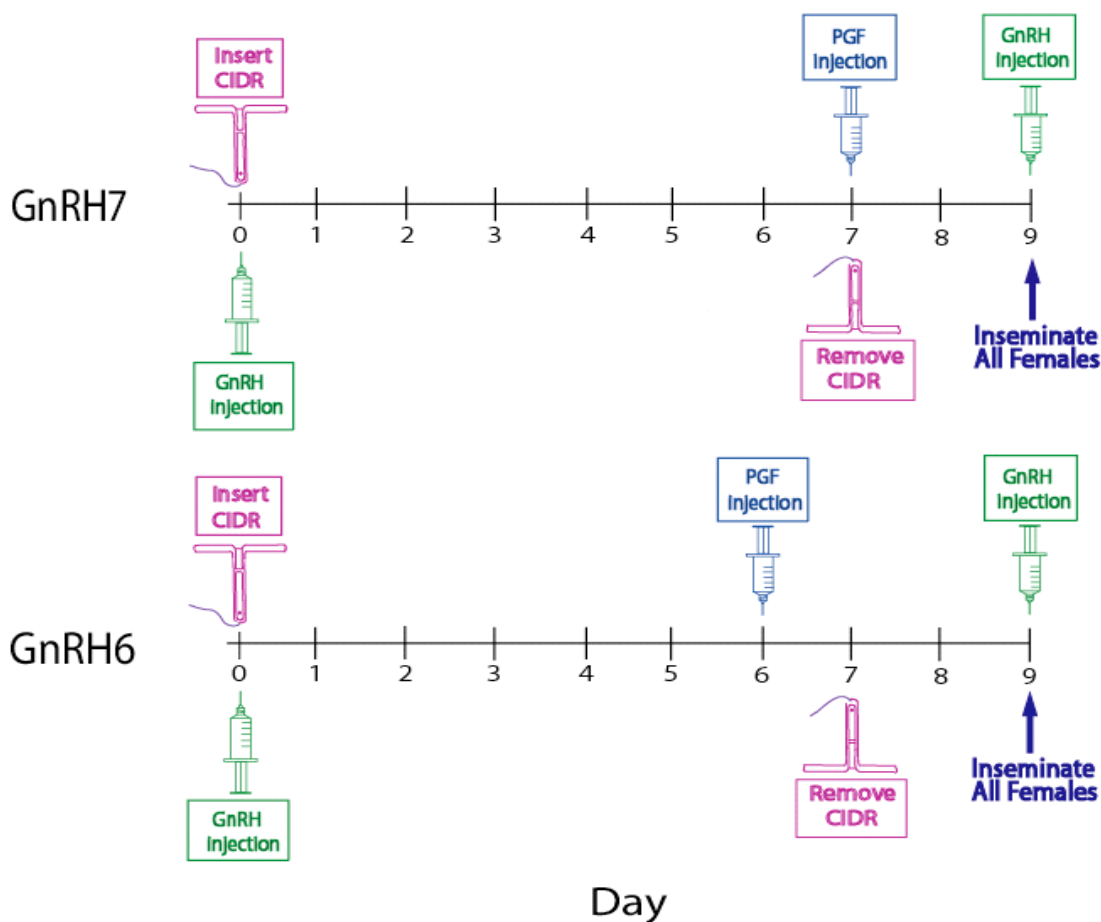


Figure 2. Schematic diagram of estrous synchronization protocols for fixed-time insemination utilized in the GnRH7 and GnRH6 treatment groups of Trial 1.

same hormonal regimen as heifers in the GnRH7 treatment group except the injection of PGF was administered on d 6 rather than d 7.

Heifers were maintained in groups of approximately 250 heifers. All heifers were inseminated to one of four sires (sires were distributed evenly across treatment groups) by six technicians (technicians performed an equal number of inseminations per group). Inseminations took place on eight dates over a 2.5-week period. Heifers remained in their respective treatment groups and were exposed to fertile bulls for the purpose of natural mating for 45 d beginning 14 d after insemination. Pregnancy was determined 30 d after bull removal by transrectal ultrasonography.

Trial 2

Experimental Design

Peripubertal Angus and Angus crossbred heifers (n=999) that were approximately 14 mo of age and had an average weight of 400 kg were used in this study. Heifers were maintained primarily in a feedlot environment on a grain-based diet, but improved winter pasture was also utilized when available.

Heifers were randomly assigned to one of two treatment groups. The treatments included two synchronization protocols utilizing GnRH (OvaCyst, Phoenix Scientific, St. Joseph, MO), PGF (Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI), and CIDR (Eazi-Breed CIDR, Pharmacia & Upjohn Co., Kalamazoo, MI) inserts and are illustrated in Figure 3. Heifers in the GnRH2

treatment group received a CIDR insert and an injection (i.m.) of GnRH (100 μ g) at the initiation of treatment (d 0). An injection (i.m.) of PGF (25 mg) was administered and CIDR inserts were removed on d 7. Sixty hours after CIDR removal, heifers received a second injection (i.m.) of GnRH (100 μ g) and were inseminated at that time. Heifers in the GnRH1 treatment group received the same hormonal regimen as heifers in the GnRH2 group except that they did not receive a second injection of GnRH at the time of insemination.

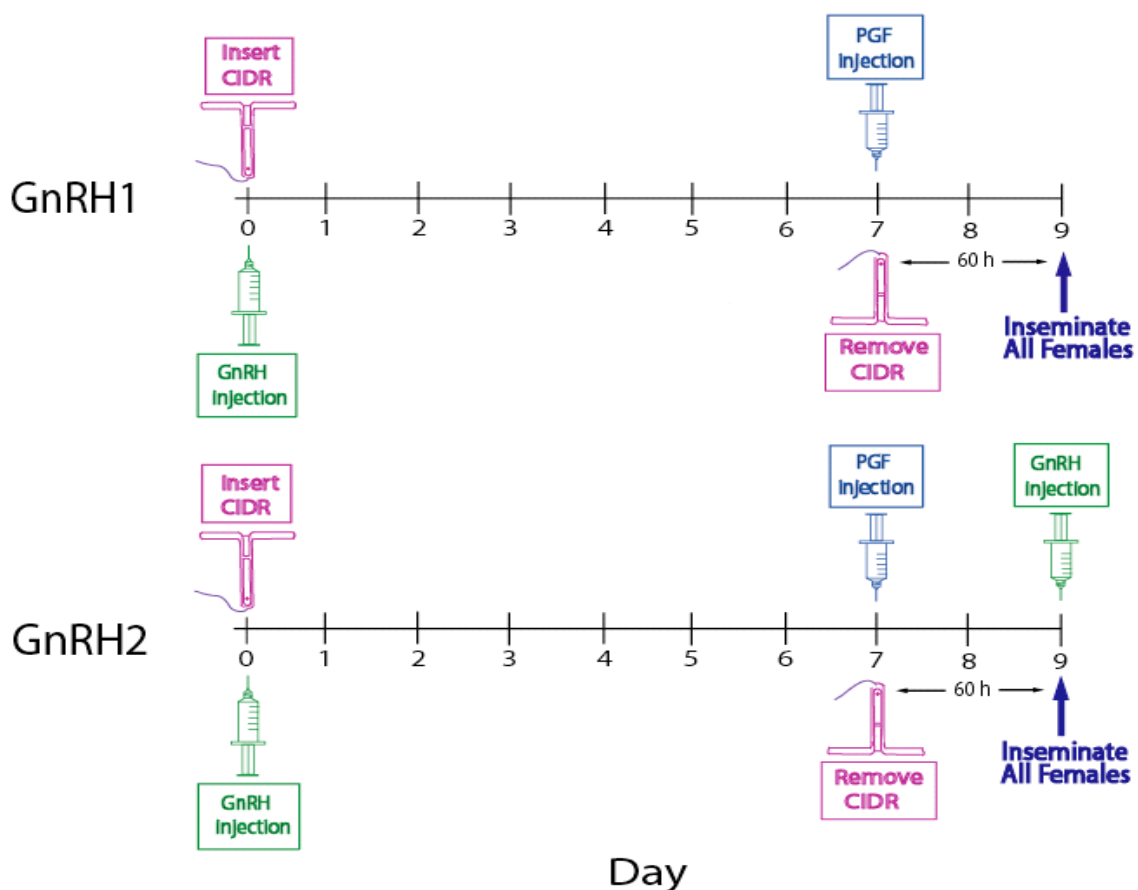


Figure 3. Schematic diagram of estrous synchronization protocols for fixed-time insemination utilized in the GnRH1 and GnRH2 treatment groups in Trial 2.

Heifers were maintained in groups of approximately 250 heifers per group. All heifers were inseminated to one of four sires (sires were distributed evenly across treatment groups) by two technicians that each inseminated one-half of the heifers in each treatment group. Inseminations took place over a 4-d period. Heifers remained in their respective treatment groups and were exposed to fertile bulls in pasture for the purpose of natural mating for 60 d beginning 14 d after insemination. Transrectal ultrasonography was utilized to determine pregnancy 30 d after bull removal.

Statistical Analysis

Data for Trials 1 and 2 were analyzed using the Statistical Analysis System (SAS Inst. Inc., Cary, NC). Pregnancy rates to AI, pregnancy rates to natural service, and final pregnancy rates were analyzed by chi-squared analysis using the frequency procedure of SAS. Pregnancy rate to AI was

calculated by dividing the number of heifers pregnant to FTAI by the number of heifers inseminated. Pregnancy rate to natural service was calculated by dividing the number of heifers pregnant to natural service by the number of heifers inseminated that did not conceive to AI. Final pregnancy rate was calculated by dividing the number of heifers pregnant to AI and natural service by the number of heifers inseminated. Cost per pregnancy to AI was calculated by multiplying the cost of the products used in each estrous synchronization protocol by the number of heifers treated with that protocol and dividing the product by the number of heifers pregnant to the single, timed insemination. Values used to determine cost of each synchronization protocol were: \$8 per CIDR insert, \$2.50 per 100 μ g dose of GnRH, \$1.00 per 1 mg dose of ECP, and \$2.40 per 25 mg dose of PGF. CIDR retention rate was calculated by dividing the number of CIDR inserts manually removed at the end of treatment by the number inserted at the initiation of treatment.

Results

Trial 1

Overall pregnancy rate to fixed-time insemination (FTAI) was 35.83%. Pregnancy rate to FTAI differed ($P<0.03$) across all treatments (Table 1). Pregnancy rate to FTAI was highest ($P<0.03$) for the GnRH 6 (50.40%) treatment group among all four treatments. Pregnancy rate to FTAI was higher ($P<0.01$) for the ECP9 than for the ECP7 group (32.35% vs 19.54%, respectively). Pregnancy rate to FTAI was also higher ($P<0.01$) for the GnRH7 group (43.17%) than either of the groups treated with ECP.

Table 1. Fixed-time AI, natural service, and final pregnancy rate for heifers in Trial 1 by treatment

Item	ECP9	ECP7	GnRH7	GnRH6
Number of heifers	473	527	410	496
Pregnancy rate to FTAI (%)	32.35 ^a	19.54 ^b	43.17 ^c	50.40 ^d
Pregnancy rate to natural service (%)	77.19 ^a	62.50 ^b	80.69 ^a	75.20 ^a
Final pregnancy rate (%)	84.57 ^a	69.83 ^b	89.02 ^a	87.70 ^a

^{a, b, c, d}Percentages within a row without a common superscript differ ($P<0.05$)

Pregnancy rates to natural service are presented in Table 1. Overall pregnancy rate to natural service was 72.60%. Heifers in the ECP7 treatment

group (62.50%) had the lowest ($P<0.01$) pregnancy rate to natural service. The three remaining treatment groups had similar pregnancy rates to natural service (75.20, 77.19, and 80.69% for treatment groups GnRH6, ECP9, and GnRH7, respectively).

The overall final pregnancy rate was 82.27%. Final pregnancy rates are presented in Table 1. The ECP7 treatment group had the lowest ($P<0.01$) final pregnancy rate of all four treatments at 69.83%. All other treatment groups had similar final pregnancy rates (84.57, 87.70, and 89.02% for treatment groups ECP9, GnRH6, and GnRH7, respectively).

CIDR retention rate ranged from 95 to 97% for all treatment groups in this study. Cost to implement each synchronization protocol and cost per pregnancy to FTAI were calculated for each treatment group (Table 2). Cost to implement the GnRH7 treatment was \$12.90/heifer and cost per pregnancy to FTAI in this study was \$29.88. Cost to implement the GnRH6 was also \$12.90/heifer, but due to a higher conception rate to FTAI, the cost per pregnancy to FTAI was lower at \$25.59.

Table 2. Cost to implement each estrous synchronization protocol on a per heifer basis and cost per pregnancy to FTAI for each protocol utilized in Trial 1

Item	ECP9	ECP7	GnRH7	GnRH6
Cost per heifer (\$)	11.15	11.15	12.90	12.90
Cost per pregnancy to FTAI (\$)	34.47	57.05	29.88	25.59

Cost to implement both of the protocols that incorporated ECP was lower than that of either of the GnRH protocols. Cost to implement these protocols was \$11.15/heifer; however, cost per pregnancy to FTAI was higher for both of these protocols because of the reduced pregnancy rates compared to the GnRH protocols (\$57.05 for ECP7 and \$34.47 for ECP9).

Trial 2

Pregnancy rates for heifers in Trial 2 are presented in Table 3. Overall pregnancy rate to FTAI for both treatments combined was 41.34%. Pregnancy rate to FTAI was higher ($P<0.01$) for the GnRH2 treatment group (46.06%) than for the GnRH1 treatment group (36.36%).

Table 3. Fixed-time AI, natural service, and final pregnancy rates for heifers in Trial 2 by treatment

Item	GnRH1	GnRH2
Number of heifers	491	508
Pregnancy rate to FTAI (%)	36.46 ^a	46.06 ^b
Pregnancy rate to natural service (%)	82.05 ^a	58.39 ^b
Final pregnancy rate (%)	88.59 ^a	77.56 ^b

^{a, b}Percentages within a row without a common superscript differ ($P<0.05$)

Pregnancy rates to natural service also differed between the two groups (Table 3). Overall pregnancy rate to natural service was 71.0%. Pregnancy rate

to natural service was higher ($P<0.01$) for the GnRH1 treatment group (82.05%) than for the GnRH2 treatment group (58.39%).

Final overall pregnancy rate to FTAI and natural service for both treatments combined was 82.98%. Final pregnancy rates are presented in Table 2. Final pregnancy rate for the GnRH1 treatment group (88.59%) was higher ($P<0.01$) than that of the GnRH2 treatment group (77.56%).

CIDR retention rate across both treatments was 98%. Cost per pregnancy to FTAI and cost to implement each protocol was calculated for both treatments (Table 4). Cost to implement the GnRH1 protocol was \$12.90/heifer and cost per pregnancy to FTAI in this study was \$35.38. Cost to implement the GnRH2 protocol was higher at \$15.40/heifer, but cost per pregnancy to FTAI in this study was lower at \$33.43.

Table 4. Cost to implement each estrous synchronization protocol on a per heifer basis and cost per pregnancy to FTAI for each protocol utilized in Trial 2

Item	GnRH1	GnRH2
Cost per heifer (\$)	12.90	15.40
Cost per pregnancy to FTAI (\$)	35.38	33.43

Discussion

All synchronization protocols used in both trials were designed to facilitate the use of a single, fixed-time insemination of all heifers. Estradiol and GnRH have been used successfully in many trials to control follicular wave emergence at the initiation of treatment and to induce ovulation following the removal of the suppressive effects of progesterone (Colazo et al., 2003; Martinez et al., 2002; Colazo et al., 2004a). In addition, a source of progesterone (CIDR) was used in this study to facilitate the induction of puberty in heifers that were peripubertal (Day, 2004). Estrous synchronization protocols incorporating GnRH or estradiol with CIDR inserts resulted in pregnancy rates to FTAI of approximately 60% in beef heifers (Martinez et al., 2002).

The estrous synchronization protocols in Trial 1 that incorporated GnRH resulted in acceptable pregnancy rates to FTAI with an average of 47.13%. Colazo et al. (2004a) reported similar results in peripubertal beef heifers. Heifers treated with a similar protocol as the heifers in the GnRH7 group had a pregnancy rate to FTAI of 53.9%. The highest pregnancy rate to FTAI occurred in the GnRH6 treatment group in which PGF was administered on d 6 rather than d 7 as compared to the GnRH7 group. Kastelic et al. (2001) reported that cloprostenol treatment on d 6 compared to d 7 of a 7-d CIDR treatment affected the interval from CIDR removal to the onset of synchronous estrus (40.0 h vs 44.6 h, respectively), but did not affect the rate of synchronous estrus or

pregnancy rate to FTAI. However, pregnancy rate to FTAI for the heifers treated with cloprostenol on d 6 (61.2%) was numerically higher compared to heifers treated on d 7 (54.0%) at the time of CIDR removal. The difference in conception rate between heifers that received PGF on d 6 compared to those that received PGF on d 7 may be due to the timing of insemination in relation to onset of synchronous estrus. However, it has been reported that timing of the FTAI did not influence pregnancy rate in heifers treated with a CIDR/GnRH protocol when PGF was administered on d 7 (Peeler et al., 2004).

Higher pregnancy rates to FTAI were achieved in heifers following treatment with a CIDR in combination with GnRH than in combination with ECP. Heifers treated with ECP had a combined pregnancy rate to FTAI of 25.6% which was lower than expected. Pregnancy rate to FTAI was lower for the ECP7 group (19.54%) which was treated with a CIDR for 7 d compared to the ECP9 group (32.35%) that was treated with a CIDR for a 9-d period. These results are lower than those reported by Colazo et al. (2004a) in which heifers of the same age and BW treated with ECP and CIDR inserts for 8.5 d, rather than 9 d as in this study, had a pregnancy rate to FTAI of 64.8%. The higher pregnancy rate in the ECP9 group was expected because ECP has been reported to have a longer and more variable time to emergence of a new follicular wave after treatment compared to E17 or estradiol benzoate (Colazo et al., 2003). The longer treatment period with the CIDR allowed more time for emergence of a new follicular wave and development of a dominant follicle that

would be more apt to ovulate following removal of the progesterone and the second injection of ECP.

Final pregnancy rate was also lowest for the ECP7 group which was 69.83% compared to 87.02% for the other three treatment groups combined. Wehrman et al. (1993) reported final pregnancy rates in heifers treated with progesterone-releasing intravaginal devices and PGF of 88.0 and 85.4% after a 7-d period of AI and exposure to bulls for 63 d. The difference in final pregnancy rate is most likely due to the low pregnancy rate to FTAI in the ECP7 group rather than fertility of the heifers in this group. Numerically, more heifers in the ECP7 group (n=265) conceived to natural service than in the other three treatment groups (mean n=207).

Cost to implement these synchronization protocols was higher on a per heifer basis for the protocols incorporating GnRH; however, these protocols were also more cost effective on a pregnancy to FTAI basis. The most cost effective protocol was the GnRH6 protocol which cost \$25.59 per pregnancy to FTAI. The least cost effective protocol was the ECP7 protocol. Due to the very low pregnancy rate to FTAI, this protocol cost \$57.05 per pregnancy to FTAI.

In Trial 2, pregnancy rate to FTAI was acceptable for the heifers treated with the GnRH2 protocol. Pregnancy rates to FTAI were higher for the GnRH2 protocol (46.06%) which is similar to Cosynch+CIDR compared to the GnRH1 protocol (36.46%) which is similar to Select Synch+CIDR. Again, these results are lower than those previously reported by Martinez et al. (2002) and Colazo et

al. (2004a). The difference between these two protocols is the presence or absence of a second injection of GnRH at the time of insemination. Heifers in the GnRH2 group received the second injection at insemination which aided in synchronization of ovulation and resulted in a higher pregnancy rate to FTAI.

There was a difference between pregnancy rates to natural service in this study as well. Pregnancy rate to natural service for the GnRH1 group was higher than that previously reported (69%; Wehrman et al., 1993). However, pregnancy rate to natural service was lower for the GnRH2 group compared to previous reports. The lower pregnancy rate to natural service for heifers in the GnRH2 group resulted in a lower final pregnancy rate as well. Final pregnancy rate for the GnRH1 group was similar to that previously reported by Wehrman et al. (1993).

One possibility for the lower pregnancy rate to natural service in the GnRH2 group is a difference in fertility of the bulls used since heifers remained in their respective treatment groups while exposed to the bulls. In the GnRH1 group, 256/312 heifers conceived to natural service compared to only 160/274 in the GnRH 2 treatment group. Another possibility is that the second injection of GnRH administered in the GnRH2 group had some negative effect on

subsequent fertility of those heifers. However, pregnancy rate to natural service for the GnRH7 group in Trial 1 was higher than all but the GnRH1 group in Trial 2 and the hormonal regimen for the GnRH7 and GnRH2 groups are identical except for the timing of insemination.

Retention rate of CIDR inserts was high for all treatment groups in both trials. Retention rate for Trial 1 ranged from 95 to 97% across all treatment groups and retention rate for Trial 2 was 98% for both treatments combined. These results are similar to previous reports. Chenault et al. (2003) reported a retention rate of CIDR inserts of 97% and Rathbone et al. (2002) reported a retention rate across three trials of 97% with a range from 95 to 100%.

Cost to implement each of these protocols was calculated and was lower for the GnRH1 group (\$12.90) compared to the GnRH2 group (\$15.40) due to the additional injection of GnRH. Still, cost per pregnancy to FTAI was lower for the GnRH2 group (\$33.43) compared to the GnRH1 group (\$35.38) due to a higher pregnancy rate to FTAI.

Implications

The results of these studies indicate that the use of two injections of GnRH in combination with a CIDR insert can be used to achieve acceptable pregnancy rates to FTAI in beef heifers. Administration of 50 μg of GnRH produced results similar to injections of 100 μg when used in combination with CIDR inserts. When implementing a 7-d GnRH/CIDR protocol, administration of PGF on d 6 rather than d 7 resulted in a higher pregnancy rate to FTAI. Pregnancy rate to FTAI and final pregnancy rate may be reduced in heifers treated with ECP in combination with a 7-d CIDR protocol. Implementation of the GnRH/CIDR protocols is also more cost effective compared to the ECP/CIDR protocols on a cost per pregnancy to FTAI basis due to the higher pregnancy rate to FTAI for heifers treated with a GnRH/CIDR estrous synchronization protocol. CIDR inserts are an effective method to deliver progesterone in estrous synchronization protocols for fixed-time insemination with a high retention rate of at least 95%.

CHAPTER IV

SYNCHRONIZATION OF FOLLICULAR WAVE EMERGENCE, LUTEAL REGRESSION, AND OVULATION FOR FIXED-TIME ARTIFICIAL INSEMINATION OF BRAHMAN COWS

Introduction

Bos indicus cattle have made significant contributions and are an integral part of beef industries in tropical and subtropical parts of the world. The use of AI represents the most economical and viable tool to advance genetic progress in cattle in these environments. AI is most efficient when used in combination with estrous synchronization; however the lack of effective estrus detection limits the success of AI in *Bos indicus* cattle.

Duration of estrus in *Bos indicus* cattle has been reported to be shorter than in *Bos taurus* cattle with an average duration of about 10 h (Galina and Arthur, 1990). *Bos indicus* cattle also have a particular behavior and temperament that makes estrus detection even more difficult. "Silent" heats have been reported in addition to a lower number of mounts in Brahman crossbred cows compared to Charolais cows (Galina et al., 1982). Social hierarchy appears to play a role in the expression of estrus as the interval from PGF treatment to behavioral estrus was longer in dominant Brahman cows compared to subordinates (Landaeta-

Hernandez, et al., 2002). Complicating the situation is the fact that *Bos indicus* cattle tend to display signs of estrus during the night. Pinheiro et al. (1998) reported that 54% of Nelore cows initiated estrus at night and 30% started and finished behavioral estrus during the night. All these factors help to explain the poor estrus detection rates in AI programs of *Bos indicus* cattle.

Another factor that limits the efficiency of AI programs in not only *Bos indicus* cattle, but all cattle, is the number of cyclic animals at the start of the breeding season. Only 30 to 50% of cows may be estrous cycling at the start of breeding in many herds (Yelich et al., 1995). Recent research suggests that the addition of progesterone to estrous synchronization protocols induces cyclicity in postpartum cows. Lucy et al. (2001) reported that controlled internal drug release (CIDR) inserts increased the percentage of cattle in estrus and pregnant during the initial days of the breeding season while using a protocol that included a 7-d CIDR treatment with PGF₂ α injected on d 6 of the CIDR treatment. This treatment was effective in both cyclic and acyclic females and pubertal and prepubertal heifers.

In order to address the problems of non-cycling females at the initiation of the breeding season and the low rate of estrus detection in *Bos indicus* cattle, it appears that the addition of progesterone to an estrous synchronization protocol that also controls the time of ovulation and allows for fixed-time insemination is necessary. It has been proposed that ovulation can be synchronized by inducing synchronous emergence of a new follicular wave in concert with control

of the luteal phase (Martinez et al., 2000a). Synchronous follicle growth should result in synchronous ovulation when the suppressive effect of progesterone is removed.

Progesterone has been included in synchronization systems to suppress estradiol-induced LH release (Bo et al., 1994). In progestogen-treated heifers that received 5 mg of estradiol 17 β , a new follicular wave emerged in an average of 4.3 d regardless of the stage of development of the dominant follicle at the time of treatment. Gonadotropin-releasing hormone (GnRH) or its analogs have also been used in several studies to synchronize follicular wave emergence and ovulation. GnRH induces ovulation of large antral follicles with a new follicular wave emerging approximately 2 d later (Martinez et al., 2002). However, synchronous emergence of a new follicular wave occurs only when treatment causes ovulation.

The objectives of this study of this study in Brahman cows were to: (1) compare the effects of E17 or GnRH in combination with a CIDR insert on pregnancy rate to fixed-time AI, (2) determine the retention rate of the CIDR insert, and (3) compare the cost per pregnancy between synchronization protocols that combined either E17+P4 or GnRH with a CIDR insert and PGF.

Materials and Methods

Experimental Design

Multiparous, lactating and non-lactating Brahman cows (n=138) maintained on coastal bermudagrass pasture were used in this study. Calves at side ranged in age from 1 to 5 mo. Calving records were not available, so cows were allotted into two postpartum groups by visual appraisal of the age of the calf at side. Body condition scores of cows were recorded at the time of treatment.

Cows were randomly allotted within postpartum group to one of two treatments. The treatment groups consisted of one synchronization protocol incorporating estradiol 17 β (E17), progesterone (P4; E17+P4, Med-Shop Pharmacy, Longview, TX), prostaglandin F₂ α (PGF; ProstaMate, RXVeterinary Products, Grapevine, TX), and controlled internal drug release (CIDR; Pharmacia & Upjohn, Kalamazoo, MI) inserts and one synchronization protocol incorporating gonadotropin-releasing hormone (GnRH; OvaCyst, Phoenix Scientific, St. Joseph, MO), PGF, and CIDR inserts. Estrous synchronization protocols utilized for fixed-time insemination are illustrated in Figure 4. The first treatment group (E17) received a CIDR insert in addition to an injection (i.m.) of E17 (2.5 mg) and P4 (50 mg) at the initiation of treatment (d 0). CIDR inserts were removed and an injection (i.m.) of PGF (25 mg) was administered on d 7. A second injection (i.m.) of E17 (1 mg) was administered on d 8, and cows were inseminated 30 hr later on d 9. Cows in the second treatment group (GnRH)

received a CIDR insert in addition to an injection (i.m.) of GnRH (100 μ g) at the initiation of treatment (d 0). An injection (i.m.) of PGF (25 mg) was administered on d 6 and CIDR inserts were removed on d 7. Cows received a second injection (i.m.) of GnRH (100 μ g) at the time of insemination 48 hr after CIDR removal on d 9.

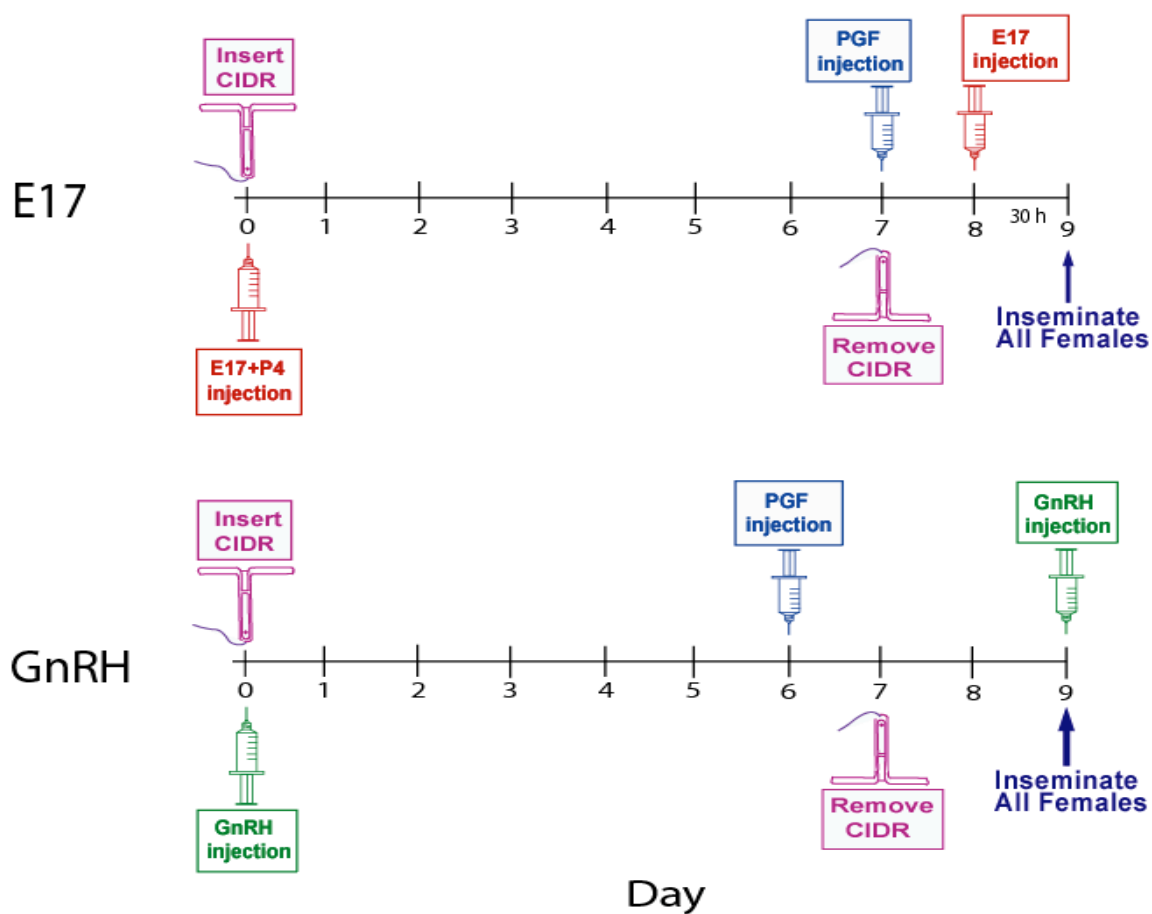


Figure 4. Schematic diagram of estrous synchronization protocols for fixed-time insemination utilized in the E17 and GnRH treatment groups.

All cows were inseminated with two 0.5 mL units of frozen-thawed semen from a single sire by a single technician. Detection of estrus was conducted by application of paint to the tailhead using a paint stick at the time CIDR inserts were removed. Absence of the paint at the time of insemination indicated the cow had been mounted associated with estrus.

Cows remained in their respective treatment groups and were placed with fertile bulls in pasture for the purpose of natural mating for 75 d beginning 14 d after insemination. The bull to cow ratio was approximately 1:17 for both groups. Transrectal ultrasonography was utilized to determine pregnancy 30 d after bull removal.

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS Inst. Inc., Cary, NC). Synchronized estrus rate, pregnancy rates, percentage of non-pregnant estrous cycling cows, body condition, days postpartum, and percentage of non-lactating cows were analyzed by chi-squared analysis using the frequency procedure of SAS. Pregnancy rate to FTAI was calculated by dividing the number of cows pregnant to AI by the number of cows inseminated. Pregnancy rate to natural service was calculated by dividing the number of cows pregnant to natural service by the number of cows inseminated but did not conceive to AI. Final pregnancy rate was calculated by dividing the number of

cows pregnant to AI and natural service combined by the number of cows inseminated.

Estrous cyclicity of cows that were not pregnant at the pregnancy examination was determined by ovarian examination using transrectal ultrasonography. Cost per pregnancy to AI was calculated by multiplying the cost of the products used in each estrous synchronization protocol by the number of cows treated with that protocol and dividing the product by the number of cows pregnant to the single, timed insemination. Values used to determine cost of each synchronization protocol were: \$8 per CIDR insert, \$2.50 per 100 μ g dose of GnRH, \$3.00 per 2.5 mg dose of E17 + 50 mg dose of P4, \$1.00 per 1 mg dose of E17, and \$1.70 per 25 mg dose of PGF. CIDR retention rate was calculated by dividing the number of CIDR inserts manually removed at the end of treatment by the number inserted at the initiation of treatment.

Results

Overall pregnancy rate to fixed-time insemination (FTAI) was 35.51%. Pregnancy and estrus rates are presented in Table 5. Pregnancy rate to FTAI was higher ($P<0.01$) for cows treated with E17 (47.22%) than for cows treated with GnRH (22.73%). Percentage of cows detected in estrus prior to insemination did not differ between groups (80.56% E17 vs 69.70% GnRH).

Table 5. Fixed-time AI, natural service, and final pregnancy rate and synchronized estrus rate for cows by treatment

Item	E17	GnRH
Number of cows	72	66
Pregnancy rate to FTAI (%)	47.22 ^a	22.73 ^b
Detected in estrus (%)	80.56	69.70
Pregnancy rate to natural service (%)	57.89 ^a	13.73 ^b
Final pregnancy rate (%)	77.78 ^a	33.33 ^b
Non-pregnant cows, % estrous cycling	56.25	77.27

^{a, b}Percentages within a row without a common superscript differ ($P<0.05$)

Pregnancy rate to natural service was 32.58%. Pregnancy rate to natural service was higher ($P<0.01$) for the group treated with E17 (57.89%) than for the group treated with GnRH (13.73%). Final pregnancy rate to FTAI and natural

service for both treatments combined was 56.52%. Again, final pregnancy rate was higher ($P < 0.01$) for the E17 group (77.78%) than for the GnRH group (33.33%). Of the cows that were not pregnant after the breeding season, the percentage that was estrous cycling did not differ between the two treatments (56.25% E17 vs 77.27% GnRH).

Table 6. Days postpartum and average body condition score of cows at initiation of estrous synchronization by treatment

Item	E17	GnRH
Number of cows	72	66
Average body condition score	5.44	5.20
Cows > 60 d postpartum (%)	44.12	49.21
Cows < 60 d postpartum (%)	16.18	12.70
Non-lactating cows (%)	39.70	38.09

Production status and body condition score were recorded for each cow at the initiation of treatment and are presented in Table 6. Average body condition score for each group did not differ (5.44 E17 vs 5.20 GnRH, on a 1-9 scale). In addition, between the E17 group and the GnRH group, the percentages of cows < 60 d postpartum (15.28% vs 12.12%), > 60 d postpartum (41.67% vs 46.97%), and non-lactating cows (37.50% vs 36.36%) were similar. Pregnancy rate to FTAI tended ($P < 0.17$) to be higher for the E17 group for cows

< 60 d postpartum, but there were no significant differences between treatments within any of the aforementioned categories.

There was no difference ($P=0.28$) in pregnancy rate to FTAI between lactating and non-lactating cows with 32.2% of lactating cows and 41.2% of non-lactating cows pregnant to FTAI. Of the cows pregnant to FTAI, 57.1% were lactating and 42.9% were not lactating. Within treatment groups, 55.9% of the cows pregnant to FTAI in the E17 group were lactating and 60.0% of the cows pregnant to FTAI in the GnRH group were lactating at the time of treatment.

CIDR retention rate across both treatments was 96%. Cost to implement each treatment and cost per pregnancy to FTAI were calculated for both groups (Table 7). Cost to implement the synchronization protocol used in the GnRH group was \$14.70/cow and cost per pregnancy to FTAI in this study was \$64.68. Cost to implement the synchronization protocol in the E17 group was lower at \$13.70/cow, and cost per pregnancy to FTAI in this study was much lower at \$29.01.

Table 7. Cost to implement each estrous synchronization protocol on a per cow basis and cost per pregnancy to FTAI for each protocol

Item	E17	GnRH
Cost per cow (\$)	13.70	14.70
Cost per pregnancy to FTAI (\$)	29.01	64.68

Discussion

The synchronization protocols used in this trial were designed to facilitate the use of a single fixed-time insemination of all cows. Estradiol and GnRH have been used successfully in many trials to control follicular wave emergence at the initiation of treatment and to induce ovulation following the removal of the suppressive effects of progesterone (Colazo et al., 2003; Martinez et al., 2002; Colazo et al., 2004a). A source of exogenous progesterone (CIDR) was used in this study to facilitate induction of cyclicity in postpartum cows (Day, 2004). Estrous synchronization protocols incorporating GnRH or estradiol with CIDR inserts resulted in pregnancy rates to FTAI of approximately 50 to 60% in *Bos taurus* cows (Bo et al., 1994; Lamb et al., 2001); however, limited information is available in cattle with *Bos indicus* influence.

In this study, an acceptable pregnancy rate to FTAI was achieved in Brahman cows treated with E17 (47.22%), however pregnancy rate to FTAI in the GnRH-treated group was significantly lower (22.73%). Final pregnancy rate was also much lower in the GnRH-treated cows, but this was a result of a poor pregnancy rate to natural service in this group (13.73%). Pregnancy rate to natural service was lower for both groups compared to previous reports. Riley et al. (2001) reported a pregnancy rate to natural service during a 75-d breeding season over 14 yr of 96.4% for Brahman x Hereford cows, and Molina et al.

(2003) reported a pregnancy rate of 88.67% in Zebu cows subjected to a 9-wk rotational breeding season.

The most likely explanation for the difference in pregnancy rates to natural service between the two groups is that the treatment with E17+P4 was more effective at initiating cyclicity compared to the GnRH treatment. If this was the case, the cows in the E17 group had a greater chance of conceiving to FTAI and a greater chance of conceiving to natural service with induction of cyclicity earlier in the breeding season. Both treatment groups were similar in body condition, number of cows < 60 d postpartum, number of cows > 60 d postpartum, and number of non-lactating cows at the initiation of treatment. With these factors held constant between the two groups, another possible explanation for the difference in conception rate to natural service is a difference in fertility of the bulls used for each group as the cows remained in their respective treatment groups during the natural mating period. This study took place during the summer months in central Texas and the heat stress may have resulted in subfertility of the bulls used for natural mating (bulls associated with the GnRH group may have been affected more than bulls associated with the E17 group). A decreased libido during this season of the year could have contributed as well. In addition, among cows that were not pregnant at the end of the breeding season, a greater percentage of cows in the GnRH group were estrous cycling compared to cows in the E17 group at the time of pregnancy determination.

The pregnancy rate to FTAI was lower than expected for the GnRH-treated cows, but similar to previous results for the E17-treated cows. Yelich et al. (2001) conducted a FTAI study in lactating cows containing some *Bos indicus* breeding. Cows were subjected to an estrous synchronization protocol similar to the E17 group in this study except that E17 was replaced with EB and no progesterone was included with the initial injection of EB. Cows were FTAI at 48 h after CIDR removal and this resulted in a pregnancy rate to FTAI of 50.6% which was slightly higher than the results in this study. A recent review reported an overall pregnancy rate to FTAI of 51.3% when cows or heifers were treated with estradiol in combination with a CIDR insert (Bo et al., 2003). Lamb et al. (2001) reported a higher pregnancy rate to FTAI of 58% in *Bos taurus* cows using the same synchronization protocol utilized in the GnRH treatment group. This protocol was also effective in non-cycling cows resulting in a pregnancy rate of 59%. Williams et al. (2002) conducted a study on Brahman-influenced cows and heifers and reported a higher pregnancy rate to FTAI (42.4%) using the Ovsynch protocol alone without a source of exogenous progesterone. Another study using Brahman-influenced cows reported a pregnancy rate of 31.0% when cows were subjected to the Cosynch regimen (Lemaster et al., 2001).

Generally, the addition of progesterone to the Ovsynch or Cosynch protocols results in similar or moderately higher pregnancy rates in beef cows. Hiers et al. (2003) conducted a study with melengestrol acetate in non-lactating

Bos indicus x *Bos taurus* cows using a modified Cosynch protocol. Melengestrol acetate was fed for 6 d between the first injection of GnRH and PGF beginning 1 d after administration of GnRH and FTAI occurred 72-80 h after administration of PGF. Pregnancy rate to the timed insemination was 39%.

In a recent review by Bo et al. (2003), it was stated that pregnancy rates to FTAI reported in *Bos indicus* cattle were not different from those reported in *Bos taurus* cattle when treated with the Ovsynch protocol, with pregnancy rates ranging from 42 to 48%. As expected, pregnancy rates in anestrous cows were significantly lower than in estrous-cycling cows.

Estrous cyclicity was not evaluated prior to initiation of treatment in the present study; however it is not unlikely that a large percentage of cows in this study were anestrous at the initiation of treatment and this may have led to the low pregnancy rate to FTAI in the cows treated with GnRH. Cows in this study were in average body condition, most were lactating, some were early in the postpartum period, and they were grazing low quality native pasture.

Of the two synchronization protocols used in this study, the E17 protocol was more cost effective. The cost to implement the E17 protocol was only \$1/cow less than the GnRH protocol, but cost per pregnancy to FTAI was much lower at \$30.49 compared to \$67.76 for the GnRH group due to the very low pregnancy rate to FTAI in cows treated with GnRH.

Implications

Results of this study indicate that acceptable pregnancy rates to FTAI can be achieved in postpartum, suckled and non-lactating Brahman cows using CIDR inserts in combination with E17. In addition to higher pregnancy rates, the E17/CIDR protocol was also the most cost effective based on pregnancy rate to FTAI. Pregnancy rate to FTAI may be reduced when injections of GnRH are used in combination with a CIDR insert to synchronize estrus in postpartum, Brahman cows. Retention rate of CIDR inserts was high, and the inserts proved to be an effective method of delivering progesterone in an estrous synchronization protocol for FTAI.

CHAPTER V

SUMMARY

Results of these studies indicate that fixed-time insemination can be successful in lactating and non-lactating Brahman cows and in beef heifers. These studies also verified the utility of CIDR inserts in estrous synchronization protocols for FTAI in Brahman cows and beef heifers. When synchronizing estrous in beef heifers for FTAI, a protocol that incorporates two injections of GnRH with a CIDR insert and an injection of PGF one day prior to CIDR removal resulted in the highest pregnancy rate. Protocols incorporating CIDR inserts and ECP resulted in reduced pregnancy rates in beef heifers. Conversely, a protocol that incorporates E17 with a CIDR insert resulted in a higher pregnancy rate to FTAI in lactating and non-lactating Brahman cows, and lower pregnancy rates were observed when CIDR inserts were used in combination with GnRH. The more efficacious protocols were also more cost effective in their respective studies.

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