EVALUATION OF FOLLICLE DEVELOPMENT AND PREGNANCY RATES IN YEARLING HEIFERS AND LACTATING COWS OF BOS INDICUS × BOS TAURUS AND BOS TAURUS BREEDING THAT WERE SYNCHRONIZED WITH PROGESTOGEN-BASED PROTOCOLS

By

REGINA ESTERMAN

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To my family for providing support and encouragement all my life and to my friends for providing insight and enjoyment through my years at the University of Florida
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A series of experiments were conducted to investigate follicular development and pregnancy rates following several progestogen-based estrous synchronization programs in *Bos indicus* × *Bos taurus* and *Bos taurus* suckled cows and yearling heifers. In the first experiment, suckled *Bos taurus* (Angus) and *Bos indicus* × *Bos taurus* (Brangus) cows were synchronized with a 7-11 protocol involving a 7 d melengestrol acetate (MGA) treatment, followed by prostaglandin F2α (PGF) on d 7, gonadotropin releasing hormone (GnRH) on d 11, and a second PGF treatment on d 18. Breed differences were observed in luteal regression and interval from PG2 to the onset of estrus, but overall estrous response and pregnancy rates were similar between Angus and Brangus cows. In the second experiment, anestrous and estrous cycling Angus and Brangus cows were synchronized with GnRH and a progesterone insert (CIDR) on d 0, followed by CIDR removal and PGF on d 7 (7 d CIDR). Following PGF estrus was detected and cows were artificially inseminated (AI) 8 to 12 h after estrus and cows not exhibiting estrus were timed-AI at 73 to 80 h. Follicle development was tracked through the synchronization treatment by ultrasound exams. Anestrous cows
were evaluated based on whether they ovulated or did not ovulate to GnRH on d 0 and
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indicating stage of follicle developments influence on the effectiveness of the
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*Bos indicus* × *Bos taurus* cows comparing a 7 d CIDR protocol to a modified protocol
with GnRH and CIDR insertion on d 0, followed by CIDR removal and PGF on d 7.5 with
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providing a less labor intensive option with the fixed-time-AI protocol to achieve similar
pregnancy rates in *Bos indicus* × *Bos taurus* cows. In the final experiment *Bos indicus*
× *Bos taurus* heifers were synchronized with one of three treatments: a 7 d CIDR as
described in the second experiment, a 5 d CIDR, or a modified 7 d CIDR protocol with
PGF on d 0 and GnRH on d 2. Heifers in the modified 7 d CIDR protocol had the
greater pregnancy rates compared to the 7 d CIDR and 5 d CIDR treatments. Heifers
with greater reproductive tract scores also had greater pregnancy rates. In the final
experiment, Angus and Brangus cows were also synchronized with the 5 d CIDR and
modified 7 d CIDR protocols in two replications. Differences were observed between
the two groups, but overall pregnancy rates were similar for Angus and Brangus, as well
as the modified 7 d CIDR and 5 d CIDR treatments.
Cattle of *Bos indicus* influence play an important role in beef production systems in the United States. Approximately 30% of cattle in the United States contain some percentage of *Bos indicus* genetics (Chase et al., 2005). Their suitability for the gulf coast and southern United States is unmatched by *Bos taurus* cattle. With approximately 42% of beef cows and 50% of the country’s cow-calf producers located in the southern United States, *Bos indicus* cattle and their crosses dominate these areas (Morrison, 2005). *Bos indicus* cattle have greatly improved tolerance to heat, humidity, and internal and external parasites (Koger, 1963). They also have the ability to utilize lower quality, high-fiber forages frequently found in the gulf coast. Crosses of *Bos indicus* cows are known for increased longevity and maternal calving ease compared to straight *Bos taurus* cows (Turner, 1980). However, *Bos indicus* cows and their crosses have decreased carcass quality (Butler et al., 1956; Koch et al., 1982) and have lower meat tenderness scores (Damon et al., 1960; Koch et al., 1982). Despite decreased carcass attributes, the positive influence of *Bos indicus* breeding in beef production systems is well documented (Rhoad, 1955; Koger, 1973).

The implementation of estrous synchronization and artificial insemination (AI) programs in beef cattle herds has great potential to improve the efficiency and productivity of reproduction. The use of AI programs has a vast array of benefits to producers. By using AI, producers have access to superior genetics compared to what they could typically attain by purchasing bulls. Bulls used for AI can also be selected specifically for calving ease in heifers with high accuracy EPDs to decrease dystocia and labor in calving. By synchronizing estrus and breeding herds over a short period of
time, synchrony of calving is increased. Less labor is required for calving season over a shorter time period and less calf losses are often reported in herds using AI. Cows bred on a synchronized estrus wean older and heavier calves (Schafer et al., 1990) and provide a more uniform calf crop, resulting in greater income to the producer. Despite the benefits, it is estimated that less than 5% of beef herds in the United States use AI programs (Lamb et al., 2010). This is due to a number of reasons, including unpredictability of estrous synchronization protocols and the increased labor required to implement them, primarily for estrous detection. Development of a less labor intensive protocol that achieves consistent and acceptable pregnancy rates could increase the use of AI in United States beef herds. Estrous synchronization and AI are the most important and widely applicable reproductive technologies available (Seidel, 1995).

Implementing AI programs in cattle of Bos indicus influence can be a challenge. In heifers, Bos indicus and their crosses reach puberty at older ages than Bos taurus heifers (Warnick et al., 1956; Temple et al., 1961; Reynolds, 1967). Differences in concentrations of reproductive hormones and altered sensitivities of their release have been noted for LH (Griffen and Randel, 1978), estradiol (Segerson et al., 1984), and progesterone (Rhodes et al., 1982; Segerson et al., 1984) between Bos indicus and Bos taurus cattle. Cattle of Bos indicus influence also have an increased percentage of three-wave follicle growth patterns (Rhodes et al., 1995; Zeitoun et al., 1996; Martinez et al., 2003). Characteristics associated with estrus are also different between Bos indicus and Bos taurus cattle. Estrus is more difficult to detect in cattle of Bos indicus breeding (Galina et al., 1982; Orihuela et al., 1983), primarily due to decreased estrus duration (Rae et al., 1999) and increased incidence of silent heats (Dawuda et al., 1989;
Lamothe-Zavaleta et al., 1991). For these reasons, using estrous synchronization protocols developed in cows of *Bos indicus* breeding result in decreased estrous response and pregnancy rates, often to unacceptable levels. With decreased estrous response in *Bos indicus* cattle, incorporation of a timed-AI to a synchronization program is critical; however, precise timing of when to implement the timed-AI in *Bos indicus* cattle has not been refined.

The goals of the experiments to follow are to develop a greater understanding of the reproductive physiology of *Bos indicus × Bos taurus* cows compared to *Bos taurus* and why they respond differently to estrous synchronization protocols. In addition, through multiple experiments we hope to further understand how cattle of *Bos indicus* influence respond to gonadotropin releasing hormone (GnRH) at different stages of the estrous cycle to refine timing of GnRH treatment. With this information, existing protocols can be modified to provide greater pregnancy rates in cattle of *Bos indicus* influence. Collectively, the goal is to develop a low-cost effective synchronization protocol for cattle of *Bos indicus* breeding that is easy to implement and results in synchronized AI pregnancy rates of at least 50%.
Hypothalamic-Pituitary-Ovarian Axis

Hormones produced in the brain drive reproductive function in cattle. Pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus regulates the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary through positive feedback.

Gonadotropin releasing hormone, a decapeptide, is produced in the hypothalamus and released through tonic and surge centers. The ventromedial and arcuate nuclei comprise the tonic center, which mediates the basal release of GnRH. The tonic center yields high frequency, low amplitude pulses observed during the luteal phase of the estrous cycle. In contrast, the suprachiasmatic and preoptic nuclei comprise the surge center, which mediates the low frequency, high amplitude pulses of GnRH associated with ovulation. The surge is preceded by estradiol release from the dominant follicle, enhancing the LH surge required for ovulation (Wettemann et al., 1972). In a study by Vizcarra et al. (1999), GnRH intravenously infused every hour resulted in more frequent LH pulses compared to infusion every 4 h. However, FSH secretion tended to be increased with infusion of GnRH every 4 h. Molter-Gerard et al. (1999) demonstrated that in ewes immunized against GnRH and provided exogenous GnRH in set frequencies, low GnRH pulse frequency enhanced the storage of FSH such that basal concentrations are consistently available for follicular recruitment. They also demonstrated that higher frequency pulses of GnRH increased storage of LH in preparation for the LH surge preceding ovulation. These studies indicate that LH and
FSH secretion are differentially regulated based upon the frequency and duration of GnRH stimulation.

The transfer of GnRH from the hypothalamus to the anterior pituitary, where it acts to release LH and FSH, utilizes a unique system called the hypothalamo-hypophyseal portal system. Axons from nerve cells in the tonic and surge centers of the hypothalamus extend into the pituitary stalk and terminate on a capillary plexus where GnRH is released into the hypothalamo-hypophyseal portal system. This allows for transfer of minute amounts of GnRH to the anterior pituitary. Without a functional hypothalamo-hypophyseal porta system present, GnRH cannot effectively stimulate release of LH or FSH (Anderson et al., 1981).

Follicle stimulating hormone stimulates follicular maturation and production of the steroid hormone estradiol, through aromatization of androgens. Receptors for FSH are located on granulosa cells of growing follicles, acting to promote follicular recruitment, growth, and continued maturation. Follicles can grow to a diameter of 4 mm without the influence of gonadotropins and FSH stimulation is required for continued follicle growth to 9 mm. In addition, LH pulses are required for the follicle to reach ovulatory size (Gong et al., 1996). Both FSH and LH are secreted together during the mid-luteal phase of the estrous cycle. However, FSH is also secreted in separate pulses, and the frequency of pulses declines from the early- to mid-luteal phase of the estrous cycle (Walters et al., 1984; Adams et al., 1992).

Luteinizing hormone is the dominant luteotrophic factor in the bovine, and without LH, CL function ceases (Simmons and Hansel, 1964; Armstrong and Black, 1966; Hoffmann et al., 1974). Luteinizing hormone has a very short half-life in circulation, and
therefore it must be secreted in a pulsatile manner in order to effectively reach its target. The release of LH is dependent upon pulsatile release of GnRH from the hypothalamus (Anderson et al., 1981). The release of GnRH and subsequent release of LH varies throughout the estrous cycle (Walters et al., 1984). The amount of LH in a pulse and the amplitude of the pulse do not change from the early- to mid-luteal phases of the estrous cycle; however there is a 2 fold increase in frequency of pulses in the early-luteal phase (Walters et al., 1984). Rahe et al. (1980) reported a pulse frequency of 20 to 30 pulses/24 h in the early luteal phase and 6 to 8 pulses/24 h in the mid-luteal phase.

A reduced LH secretion has been reported in ovariectomized Brahman cows compared to Hereford cows in response to a GnRH challenge (Griffen and Randel, 1978). Similarly, Rhodes et al. (1978) observed a decrease in peak concentration of the LH surge after estradiol treatment in Brahman cows when compared to Hereford cows. Brahman cows also exhibited a longer interval from exogenous estradiol treatment to LH peak when compared to Hereford cows. Furthermore, Portillo et al. (2008) reported that as the percentage of Bos indicus breeding increased in the crossbred animal, the amount of LH released to a GnRH challenge on day 6 of the estrous cycle decreased. It appears that the Brahman pituitary has a decreased responsiveness to gonadotropin stimulation compared to Bos taurus breeds and it is dependent on the amount of Bos indicus breeding in the animal.

Hormones produced by the ovary, including estradiol and progesterone, also play key regulatory roles throughout the estrous cycle. The pattern of estradiol release is similar to that of LH, and pulses of estradiol may be caused by pulses of LH (Walters et
al., 1984). As concentrations of progesterone decline during the late-luteal phase of the
estrous cycle, estrogen secretion begins to increase (Wettemann et al., 1972). The rise
in estrogen first reduces, but then increases the ability of the pituitary to release LH,
causing the LH surge that leads to ovulation (Kesner et al., 1981).

**Estrous Cycle**

The bovine estrous cycle consists of a series of events including the growth and
development of a follicle, its subsequent ovulation, and preparation of the uterus for
conception, all to allow repeated attempts for pregnancy. The estrous cycle is
comprised of two major parts, the follicular and luteal phases. The follicular phase
starts at the regression of the corpus luteum (CL) and ends with ovulation, and is sub-
divided into proestrus and estrus. The luteal phase starts with ovulation and ends with
the regression of the CL and is sub-divided into metestrus and diestrus.

Estrus is the most easily distinguished phase of the estrous cycles in the bovine,
as it is the time when the female is sexually receptive to the male. This behavior is
caused by high concentrations of estradiol, the predominate hormone of the follicular
phase. Estradiol increases about 3 d prior to estrus with concentrations of
approximately 3.6 pg/ml during the luteal phase, to a peak concentration of 9.7 pg/ml
the day before estrus (Wettemann et al., 1972). Estradiol concentrations have been
reported to be greater in *Bos taurus* (Angus) cows compared to *Bos indicus* (Brahman)
cows for days 7 to 17 of the estrous cycle (Segerson et al., 1984). However, Alvarez et
al. (2000) reported no differences in basal or maximum estradiol concentrations for
Angus and Brahman cows. Elevated estradiol concentrations regulate the surge of LH
that leads to ovulation (Walters and Schallenberger, 1984). Ovulation occurs
approximately 26 h after the onset of behavioral estrus in both *Bos taurus* (Looper et al.,
The expression of estrus allows for copulation to occur and the opportunity for pregnancy.

The primary and most reliable indicator of estrus is for a cow to allow herself to be mounted by another cow (Glencross et al., 1981). Secondary signs of estrus include mounting other cows, clear mucous discharge from the vulva, swelling of the vulva, restlessness, following other cows, chin resting, and lip curling. These signs can occur before, during, or after estrus and do not relate to the time of ovulation, but should instead be used as clues that a cow should be observed more closely (Nebel, 2003).

The duration of estrus varies between *Bos taurus* and *Bos indicus* cattle, and may also vary for cows undergoing a spontaneous naturally occurring estrus versus a synchronized estrus. Richardson et al. (2002) observed that *Bos taurus* heifers that underwent either a synchronized and (or) a spontaneous estrus had similar durations of estrus. Rae et al. (1999) reported that the duration of a synchronized estrus to be 8.5 h for Angus (*Bos taurus*) and 6.7 h for Brahman (*Bos indicus*) heifers, while crossbred (*Bos taurus x Bos indicus*) heifers had an duration of 11.9 h. Landaeta-Hernandez et al. (2002) did not observe differences in estrus duration for Angus, Brahman, and Senepol cows undergoing both a synchronized and spontaneous estrus. Multiple studies have observed shorter estrus durations in *Bos indicus* heifers (Plasse et al., 1970) and cows (Randel, 1984; Pinheiro et al., 1998), compared to *Bos taurus* heifers (Richardson et al., 2002) and cows (Schams et al., 1977).

Metestrus is the 3 to 5 d stage from ovulation until the CL is fully functional. In *Bos taurus* beef cows, ovulation occurs 24 to 36 h from the onset of estrus (Looper et
al., 1998; Rorie et al., 1999). Plasse et al. (1970) reported that ovulation occurred 25.6 h after the beginning of estrus and 18.9 h after the end of estrus in Brahman heifers. Likewise, Pinheiro et al. (1998) reported that ovulation occurred 26.6 h after the onset of estrus in Nelore (Bos indicus) cows. After the LH surge induces ovulation, the ovulated follicle undergoes cellular and tissue remodeling changes to form the CL. During metestrus, the predominate hormone switches from estradiol to progesterone and progesterone concentrations remain < 1 ng/mL as the CL begins to develop. As the CL grows in size and progesterone output increases, the cow enters diestrus. Diestrus is the longest phase of the estrous cycle, lasting 10 to 14 d. Concentrations of progesterone from 1.0 to 13.0 ng/ml are observed when a CL is present in Bos indicus cows (Ruiz-Cortes and Olivera-Angel, 1999), while concentrations of progesterone from 4.7 to 12.0 ng/mL have been reported for Bos taurus cows with a CL (Henricks et al., 1971). Regardless of whether or not a pregnancy occurs, the CL forms into a fully functional, progesterone-producing structure. If an embryo is present in the uterus, the CL and high concentrations of progesterone are maintained throughout gestation. If pregnancy does not occur, the CL remains active until d 17 to 18 of the estrous cycle, at which time it undergoes luteolysis, stimulated by prostaglandin F$_{2\alpha}$ (PGF) secretion from the uterus. Upon luteolysis, concentrations of progesterone decline rapidly allowing for another estrous cycle.

Proestrus occurs 3 to 4 d prior to estrus and is characterized by increased follicular growth. Following regression of the CL, the predominate hormone transitions to progesterone to estradiol (Schams et al., 1998). During proestrus, a single dominate
follicle emerges from a pool and is selected to grow into an ovulatory follicle which produces estradiol.

The length of the estrous cycle is approximately 21 d in *Bos taurus* breeds (Hansel et al., 1973). Alvarez et al. (2000) reported no difference in estrous cycle length of Senepol (tropical *Bos taurus*; 20.4 d), Angus (temperate *Bos taurus*; 19.5 d), or Brahman (tropical *Bos indicus*; 19.7 d) cows. However, Plasse et al. (1970) observed an estrous cycle length of 27.7 d in Brahman heifers evaluated over a full year, suggesting that *Bos indicus* animals may have a longer estrous cycle. Length of the estrous cycle may also differ between heifers and cows, as Plasse et al. (1970) reported an average length of 27.7 d in Brahman heifers, compared to Llewelyn et al. (1987), who observed a 23 d estrous cycle in Boran cows. However, in *Bos taurus* beef cows (Zollers et al., 1989) and heifers (Mihm et al., 2000), estrous cycle length has little variation from 21 d.

**Follicular Development**

Cattle are born with a finite amount of oogonia available during their lifetime. Oogonia are developed during mid-gestation when the primordial germ cells migrate to the gonadal ridge and are arrested in prophase I of meiosis and stored in primordial follicles (Hirshfield et al., 1991). It has been hypothesized that granulosa cells modulate the transcriptional activity of the oocyte genome (De La Fuente and Eppig, 2001). Around the time of puberty, these inhibitory factors are removed by the LH surge, and just prior to ovulation, meiotic divisions resume (Senger, 1999).

Oogonia stored in the ovary awaiting signals to grow are maintained in a quiescent stage and are known as primordial follicles. The primordial follicle is characterized by a single layer of granulosa cells surrounding the oocyte, and from this
stage follicles gradually and continually leave the resting pool to begin growth (Fortune, 1994). A quiescent, primordial follicle will have 5 to 8 flattened granulosa cells; however, when the follicle is activated, the number of flattened cells declines rapidly as they become increasingly cuboidal with greater than 8 granulosa cells (Braw-Tal, 2002). At this stage of follicular growth, the cells are still transcriptionally inactive (Fair et al., 1997b). The mechanism by which these follicles are gradually released and signaled to grow is unknown (Fortune, 1994).

Once the oocyte reaches approximately 18 granulosa cell stage, it is completely surrounded by cuboidal granulosa cells and is termed a primary follicle (Braw-Tal, 2002). As the number of cuboidal granulosa cells grow, they begin producing mRNA for the protein follistatin (Braw-Tal, 1994). Follistatin acts to inhibit activin A, thereby protecting the growing follicle from the growth-inhibiting effects of activin A (Braw-Tal, 1994). Additionally, the oocyte begins to produce growth differentiation factor 9 (GDF9) and bone morphogenic protein 15 (BMP15), both of which are structurally similar to transforming growth factor-β (TGFβ) and expressed exclusively in the oocyte (McGrath et al., 1995; Dube et al., 1998) to increase granulosa proliferation (Braw-Tal, 2002). At about the same time, the surrounding granulosa cells begin to produce kit ligand to promote oocyte growth (Braw-Tal, 2002).

The oocyte continues to grow a second layer of cuboidal granulosa cells, and is termed a secondary follicle (Fair et al., 1997b; Braw-Tal, 2002). At this stage, the oocyte becomes transcriptionally active (Fair et al., 1997b), develops the zona pellucida, and forms gap junctions between the oocyte and granulosa cells (Fair et al., 1997a). Hence, the follicle moves from being dependent on intraovarian signals to
being an autonomous unit where its growth is regulated only by follicle-produced factors (Braw-Tal, 2002). More layers of granulosa cells are added and a cavity or antrum begins to surround the oocyte, at which point it is termed a tertiary follicle. The antrum begins to accumulate follicular fluid and the size of the follicle begins to increase. Granulosa cells differentiate into inner cumulus cells and outer mural cells (Vanderhyden, 2002). The tertiary follicle continues to grow into a mature graafian follicle capable of ovulation. From this stage, the follicle will grow continuously until it reaches one of two fates, atresia or ovulation (Fortune, 1994).

Once follicles have developed to a graafian stage, they begin to progress through stages of recruitment, selection, and dominance. An FSH surge results in the recruitment of a number of small tertiary follicles. A few follicles will be selected to grow further and will eventually culminate in a single dominant follicle. As follicles increase in size they can be categorized into three size classes: class 1 follicles are ≤ 5 mm, class 2 follicles are 6 to 9 mm, and class 3 follicles are ≥ 10 mm.

In cattle, follicles grow in a wave-like pattern to ensure that at any given time there is an eligible follicle available for ovulation (Sirois and Fortune, 1988). Typically, there are two to three follicular waves per estrous cycle, with some studies showing more two-wave cycles (Ginther et al., 1989), and others showing more three-wave cycles (Savio et al., 1988; Sirois and Fortune, 1988). In two-wave cycles, the waves begin around days 2 and 11 of the estrous cycle, whereas in cows with three-wave cycles, the waves begin around days 2, 9, and 16 of the estrous cycle (Sirois and Fortune, 1988). The number of follicular waves a cow has may be related to the level of dominance of the CL, with a three-wave cow having a more dominant CL than a two-
wave cow. Townson et al. (2002) observed that cattle experiencing two follicular waves ovulated larger, older, and potentially less fertile follicles based on pregnancy rates than cattle ovulating after three follicular waves. This study confirmed the trend of higher pregnancy rates in three follicular wave cows compared to two follicular wave cows reported by Ahmad et al. (1997). Moreover, Mihm et al. (1994) theorized that additional days of growth prior to ovulation of the dominant follicle compromised pregnancy rates.

Atresia is a form of apoptosis, or programmed cell death. Atresia is the most common fate of follicles, as only a select number will actually achieve dominance and ovulate. While atresia can occur at any stage of follicular growth, it is not evenly distributed across follicular development and is greatest just before the final stages of follicular dominance (Fortune, 1994).

For a follicle to grow from a 300 µm antral follicle to a 3 to 5 mm follicle detectable via ultrasound, it is calculated to take more than 30 d (Lussier et al., 1987). Up to this point in development, follicles can grow independently of gonadotropin stimulation (Scaramuzzi et al., 1993). Recruitment begins with a rise in FSH, which stimulates the growth of a cohort of small follicles beyond 4 mm in diameter (Adams et al., 1992; Ginther et al., 1997). As FSH declines, fewer follicles continue to grow, while others undergo atresia (Austin et al., 2001). In a study by Austin et al. (2001), by about 33 h post-FSH peak, there were two follicles still growing, and by 53 h a dominant follicle could be identified and measured at 8.5 mm. This dominant follicle grows between 12 to 20 mm and has enhanced estradiol and inhibin production to prevent growth of another cohort (Ginther et al., 1999). The dominant follicle continues to grow for an additional 3 to 4 d before it regresses from the negative feedback of progesterone.
on LH (Sunderland et al., 1994). The decline in estradiol from the former dominant follicle causes another FSH rise and another wave begins (Sunderland et al., 1994). If luteolysis occurs during the time of dominance of the second dominant follicle, it will ovulate; however, if luteolysis does not occur during dominance, the follicle regresses and a third follicular wave is initiated (Cooke et al., 1997).

Breed may also effect follicular growth and development in cattle. Alvarez et al. (2000) demonstrated that non-lactating Brahman cows had significantly greater numbers of small (2-5 mm), medium (6-8 mm) and large (≥ 9 mm) follicles when compared to non-lactating Angus and Senepol cows. However, there was no difference in the occurrence of two vs. three follicular waves or estrous cycle length among the breeds. *Bos indicus* can have estrous cycles with up to four follicular waves in both heifers (Rhodes et al., 1995) and cows (Zeitoun et al., 1996; Martinez et al., 2003). Viana et al. (2000) showed that Gir (*Bos indicus*) cattle experienced mostly three (60%) and four (26.7%) follicular waves. However, the number of follicular waves did not affect the estrous cycle length. Numerous studies have also noted a smaller dominant follicle size of 10 to 12 mm in *Bos indicus* cows on the last follicular wave (Bo et al., 1993a,b; Figueiredo et al., 1997; Rhodes et al., 1995), compared to 14 to 20 mm in *Bos taurus* cows (Ginther et al., 1989; Kastelic et al., 1990a; Bo et al., 1993b).

**Corpus Luteum Function and Luteolysis**

**Overview**

The CL is a heterogeneous structure on the ovary with a unique population of cells with different morphological, endocrine, and biochemical properties (Niswender and Nett, 1988). It is comprised of endothelial cells, small luteal cells, large luteal cells, fibroblasts, smooth muscle cells, immune cells, and pericytes (O’Shea et al., 1989;
Farin et al., 1986; Hansel et al., 1991). There are two types of steroidogenic cells in the CL, small and large luteal cells, both of which produce progesterone when the animal is cycling or pregnant (O'Shea, 1987).

The CL is formed from the granulosa and theca cells of the antral follicle after ovulation (O'Shea, 1987). Preparation for the transformation of these cells to progesterone producing luteal cells is initiated prior to ovulation (McNatty and Sawers, 1975), but can occur independently of ovulation (Kesler et al., 1981). This transformation is termed luteinization and is characterized by the change from production of estradiol to progesterone (Juengel and Niswender, 1999). The granulosa cells of the follicle differentiate into large luteal cells (O'Shea, 1987), and are stimulated to produce progesterone primarily by growth hormone (Liebermann and Schams, 1994). Growth hormone receptors on the CL are located primarily on large luteal cells (Lucy et al., 1993). The theca cells of the follicle differentiate into small luteal cells (Donaldson and Hansel, 1965), and are stimulated to produce progesterone primarily by LH (Niswender and Nett, 1988). Luteinizing hormone receptors on the CL are primarily located in small luteal cells. The mechanism by which LH stimulates progesterone production involves formation of cAMP and activation of protein kinase A (Schams and Berisha, 2004). It has also been suggested that large luteal cells may differentiate into small luteal cells (Fisch et al., 1989) and small luteal cells may differentiate into large luteal cells (Cran, 1983), as luteal structures that appear to be in an intermediate stage between the two have been identified (Priedkalns et al., 1968). Small luteal cells are primarily responsible for higher magnitude, LH-stimulated progesterone production; whereas, large luteal cells are responsible for basal progesterone secretion (Hansel et
The actions of PGF are primarily mediated by the large luteal cells, as they contain most of the PGF receptors (Pate, 1994). After the LH surge and ovulation, the walls of the follicle fold in (O'Shea et al., 1980), granulosa cells hypertrophy (Fawcett et al., 1969), and gap junctions between granulosa cells are reduced (Murdoch, 1985) to form the CL. Pulsatile secretion of LH is required for proper formation of the CL from days 2 to 7 of the estrous cycle and for full functionality of the CL from days 7 to 12 of the estrous cycle; however, LH is not necessary for the CL to be maintained and produce normal circulating concentrations of progesterone past d 12 (Peters et al., 1994).

The CL is one of the few tissues capable of angiogenesis, or the generation of new blood vessels from existing blood vessels by migration and proliferation of endothelial cells (Schams and Berisha, 2004). The development of this microcirculatory system involves the breakdown of the follicular basement membrane, endothelial cell proliferation, and development of capillary lumina (Smith et al., 1994). The precise control of angiogenesis is essential for normal luteal function, and once complete, the CL becomes one of the most highly vascularized organs in the body with the greatest rate of blood flow (Wiltbank et al., 1988).

Differences between breed types have been observed for CL function, weight, and progesterone output. Rhodes et al. (1982) reported that Hereford × Holstein heifers displayed heavier CL weights and had greater progesterone output than Brahman heifers. However in the same study, despite having a larger CL size and having greater progesterone content, systemic concentrations of progesterone did not differ between the breeds. In contrast, Segerson et al. (1984) reported greater mean concentrations of
progesterone from d 7 to d 17 of the estrous cycle for Angus (5.3 ng/mL) than Brahman (4.1 ng/mL) cows. Corpus luteum size has been reported to be 17 to 21 mm in diameter in *Bos indicus* cows (Bo et al., 1993a,b; Figueiredo et al., 1997; Rhodes et al., 1995), compared to 20 to 30 mm in *Bos taurus* cows (Ginther et al., 1989; Kastelic et al., 1990a; Bo et al., 1993b). Rhodes et al. (1982) also indicated that Brahman heifers may show a more seasonal effect of CL progesterone content than Hereford × Holstein heifers, with progesterone content of the CL being lower in the summer than in the winter; however, systemic concentrations of progesterone did not differ for season or breed. In contrast, Zeitoun et al. (1996) reported higher circulating concentrations of progesterone for Brahman cows in the spring (6.3 ng/mL) than in the fall (4.8 ng/mL). It has also been reported that CL of Brahman cattle are more deeply imbedded in the ovarian stroma and can be more difficult to detect by rectal palpation (Rhodes et al., 1982; Plasse et al., 1968).

**Luteolysis**

Prostaglandin F$_{2a}$ (PGF) is the main luteolytic agent in the bovine and initiates CL regression (Gooding et al., 1972; Inskeep, 1973). At the end of the estrous cycle, the CL is regressed by episodic release of PGF from the uterus. The PGF is transferred from the uterus to the ovary where it acts on the CL by a unique counter-current exchange system between the uterine vein and ovarian artery (McCracken et al., 1972). This subsequently causes a marked decrease in concentrations of progesterone between d 16 and 19 of the estrous cycle (Hansel et al., 1973) and leads to behavioral estrus and ovulation (Thatcher and Chenault, 1976). Prostaglandin F$_{2a}$ has a very short half-life and is rapidly inactivated by oxidation in the bloodstream from a single pass
through the lungs (Kindahl, 1980). For this reason, a counter-current exchange system evolved to transport PGF from the uterus, by the uterine artery, to the ovary and return by the ovarian vein, thus preventing the effective amount of PGF from being lost in systemic circulation (McCracken et al., 1972). McCracken et al. (1972) also discovered that infusion of as little as 25 µg/hr of PGF into the artery leading to the ovary resulted in a rapid decrease in progesterone secretion; however, the small concentrations of PGF were ineffective at inducing luteolysis when administered into peripheral circulation.

Exposure to or inhibition of progesterone regulates the timing of release of PGF, and subsequent CL regression (Ottobre et al., 1980; Schams et al., 1998). In vivo studies demonstrated that prior to d 12 of the estrous cycle progesterone inhibits PGF release (Schams and Berisha, 2004). Additionally, progesterone exposure to the endometrium enhances synthesis of PGF and has a priming effect on luteolysis (Boshier et al., 1981). After d 12 the luteal tissue begins to lose its sensitivity for progesterone and PGF is no longer inhibited. Progesterone down-regulates its own receptors within the endometrium, thereby decreasing its action, and causing an increase in the actions of estradiol (Robinson et al., 2001). The decrease in progesterone receptors, and increase in estrogen receptors leads to an increase in oxytocin receptors (Vallet et al., 1990). The increase in estradiol causes high frequency, low amplitude oxytocin release from the hypothalamic oxytocin pulse generator (McCracken et al., 1999) and enhances oxytocin receptors in the uterus (Meyer et al., 1988). Oxytocin produced from both the posterior pituitary and CL (Wathes and Swann 1982; Flint and Sheldrick 1982; Wathes et al., 1983) activates sub-luteolytic PGF secretions from the uterus (Hooper et al., 1986). The release of uterine
PGF acts on PGF receptors in luteal cells to further stimulate the release of oxytocin (Hooper et al., 1986). These events culminate to cause secretion of large amounts of PGF from the uterus and increases in oxytocin to induce luteolysis. All of these events continue until the PGF receptor response system is desensitized and luteal oxytocin release ceases (McCracken et al., 1999).

Regression of the CL involves both functional and structural luteolysis. Functional luteolysis involves the reduction in progesterone production and structural luteolysis involves the degradation and eventual removal of the physical structure of the CL. The progesterone producing capability of the CL is decreased rapidly at luteolysis by several mechanisms including the decrease in gene expression for steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase (3β-HSD), and the LH-receptor, all of which are important in maintaining luteal function (Tsai et al., 2001). The StAR protein is an essential component in the regulation of steroid biosynthesis, 3β-HSD is a key enzyme in the production of steroid hormones, and LH-receptor numbers are reduced, inhibiting steroidogenesis. Other stimulatory factors for functional degradation include the up-regulation of gene expression for c-fos, prostaglandin G/H synthase-2 (PGHS-2), and monocyte chemoattractant protein-1 (MCP-1), all of which are induced by PGF (Tsai et al., 2001). The protein c-fos, is a primary response gene which regulates protein synthesis and release, PGHS-2 is an enzyme which metabolizes arachidonic acid to form important intermediates to prostaglandin production, and MCP-1 is an inflammatory mediator which increases macrophages within the CL during luteal regression. Physical degradation includes a decrease in cytoplasmic granulation, rounding of the cell outline, peripheral vacuolation
of the large luteal cells, condensation of the cytoplasm, loss of prominent nuclei, and an increase in prominence of connective tissue (Hansel et al., 1973). As the CL ages, artery walls thicken and begin to deteriorate, which contributes to declining concentrations of progesterone (Donaldson and Hansel, 1965). The vasoconstrictive properties of PGF may also cause decreased blood flow through the CL to contribute to luteal regression (Schams and Berisha, 2004). Collectively, these processes cause the sharp decline of concentrations of progesterone, allowing for an increase LH pulse amplitude and frequency in response to rising estradiol concentrations (Chenault et al., 1975).

The actions of exogenous PGF are not effective for luteolysis during all stages of the estrous cycle (Lauderdale, 1972; Henricks et al., 1974). Early (d 7) in the estrous cycle, PGF is less effective at inducing luteolysis than later in the estrous cycle (d 15) (Tanabe and Hann, 1984). It was hypothesized that this was due to a lack of luteal PGF receptors, however this was disproved by Wiltbank et al. (1995) who reported that PGF receptor concentration and affinity for both developing (days 2 to 4 of the estrous cycle) and active CL (days 6 to 10 of the estrous cycle) were similar. Therefore, further studies by Silva et al. (2000) investigated the ability of PGF to be converted to its inactive form, 13,14-dihydro-15-keto PGF (PGFM), by the enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH). The amount of PGDH increased during the estrous cycle and pregnancy when PGF was not an effective luteolysin, but did not increase during the times when PGF was an effective luteolysin. Furthermore, Silva et al. (2000) reported that the amount of mRNA for PGDH was greater on days PGF was not effective and significantly lower when PGF was effective. They also observed that
concentrations of cyclooxygenase (COX-2), which catalyzes the conversion of arachidonic acid to PGF, increased on similar days, but were undetectable when PGF was effective.

Another proposed reason for the ineffectiveness of PGF to induce luteolysis early in the estrous cycle is the lack of endothelin-1 (ET-1), a promoter of luteolysis that appears in large quantities in the CL just after PGF exposure (Wright et al., 2001). Levy et al. (2000) reported low concentrations of ET- converting enzyme (ETE-1), the enzyme which converts ET-1 to its active state, in the luteal tissue early in the estrous cycle. They subsequently observed a four-fold increase in ETE-1 during the transition from the early to mid-luteal phase of the estrous cycle, indicating the lack of active ET-1 early in the estrous cycle may contribute to why the early developing CL is not susceptible to PGF-induced luteolysis.

**Estrous Expression**

Estrous detection is the process of determining if an animal is in estrus and is primarily conducted by visual observation. Cows display estrus when they have a large follicle producing adequate estradiol concentrations to induce estrous behavior. Estrus can be spontaneous and naturally occurring, or result from initiation of luteolysis by administration of exogenous PGF.

Detection of estrus can be one of the most challenging aspects of an artificial insemination (AI) program. The primary underlying changes in the cow that allow for the detection of estrus include increased physical activity and locomotion (Farris et al., 1954), swelling of the vulva (Foote et al., 1979), and most importantly standing to be mounted by another cow.
There are numerous aids that can be used to detect estrus. One of the most common and inexpensive products is the Estrotect Heat Detector (Rockway, Inc., Spring Valley, WI). The Estrotect Heat Detector is a small scratch-off patch in the form of a sticker that is placed on the tail-head of the cow or heifer. The product features a silver, scratch-off surface, similar to that of a lottery ticket, which when scratched away reveals a brightly colored signal layer. The Estrotect allows a producer to determine if a cow has received only a few mounts compared to a large number of mounts based on the degree to which the patch is scratched off.

The HeatWatch (DDx, Inc., Denver, CO) system was the first remote sensing system to allow 24 hour monitoring of standing events associated with estrus (Nebel 2003). This system utilizes a computer transponder tucked into a mesh patch, which is affixed to the tail-head of the cow. When the cow that is in estrus is mounted, the transponder is activated and the information is transmitted to a computer where the HeatWatch software records the mounting activity. The computer records data for each individual cow, including the date, time, and duration of each standing event, which are used to determine the precise time of the start and end of estrus as well as the intensity of estrus. The computer software also allows for the development of individual cow reports (Nebel 2003).

Breed differences are also apparent in the onset of estrus, ability to detect estrus, duration of estrus, time of day estrus is initiated, and the intensity of estrus. In 2 year-old heifers where estrus was synchronized, Rae et al. (1999) observed mean estrus durations of 8.5 hours for Angus (\textit{Bos taurus}), 6.7 hours for Brahman (\textit{Bos indicus}), and 11.9 hours for crossbred (\textit{Bos taurus} × \textit{Bos indicus}) heifers. Other studies have
observed estrus durations of 11 to 12 h in synchronized *Bos taurus* heifers (Richardson et al., 2002). Pinheiro et al. (1998) observed a 10.9 h estrus duration in Nelore (*Bos indicus*) cows. Rae et al. (1999) reported that breed had a significant effect on total mounts received during a synchronized estrus in Angus, Brahman, and Angus × Brahman 2 year-old heifers. Additionally, estrus may be more difficult to detect in *Bos indicus* cattle. Behavioral estrus in *Bos indicus* breeds may be expressed more by secondary signs of estrus, such as head butting (Galina et al., 1982; Orihuela et al., 1983). *Bos indicus* cattle may also have an increased occurrence of silent heats and absence of visual estrus (Dawuda et al., 1989; Lamothe-Zavaleta et al., 1991).

Synchronization of estrus promotes the formation of sexually-active groups, which in turn increases the mounting activities of both *Bos taurus* and *Bos indicus* cows (Hurnick et al., 1975; Landaeta-Hernandez et al., 2002) compared to a spontaneous, naturally occurring estrus. Social status in the herd can also affect behavioral estrus. Landaeta-Hernandez et al. (2004) observed that dominate cows tended to receive less mounts than subordinate and intermediate cows, due to the dominate cows avoiding being mounted by subordinate cows. For this reason, dominate cows may take longer to be detected in estrus.

The time of day which cows display estrus can also be influenced by breed. It has been shown that the onset of estrus occurs more frequently during the nighttime hours than daytime hours in both *Bos taurus* (Stevenson et al., 1996) and *Bos indicus* (Pinheiro et al., 1998) cows. Landaeta-Hernandez (2002) observed more cows initiate estrus during night hours (60%) than day hours (40%), they observed more Senepol (*Bos taurus*) cows initiate estrus during the daytime hours than Brahman cows.
Decreased estrus duration and total number of mounts received during estrus were influenced by greater temperature-humidity index (Landaeta-Hernandez 2002).

**Estrous Synchronization**

**Overview**

Estrous synchronization is a powerful management tool that allows producers to have a large number of cattle in estrus during a short period of time to make utilization of artificial insemination more practical. Through the use of hormone treatments, the estrous cycle can be manipulated in several ways. Prostaglandin F$_2\alpha$ can be used to shorten the luteal phase and induce estrus in cows with a functional CL. Progestins can lengthen the luteal phase and prevent estrus/ovulation during the treatment period, which is generally 7 to 14 d. And finally, GnRH can induce ovulation, either to synchronize follicular waves, or to induce ovulation for a timed-AI protocol.

Synchronization of the estrous cycle can be achieved in several ways including synchronization of estrus, synchronization of follicle wave growth, and (or) a combination of both. New protocols are also investigating ways to produce the most developmentally competent follicle at ovulation, which will provide an oocyte with the best chance of developing into a successful pregnancy. The combination of these synchronization schemes can yield a tight synchrony of estrus that allows producers to either inseminate after an observed estrus or at a predetermined time known as timed-AI. To synchronize estrus or follicle development, exogenous administration of GnRH, PGF, and progestins can be used together to develop an effective estrous synchronization protocol. The different protocols must be matched to an individual producer’s cattle, needs, facilities, and labor resources.
**Prostaglandin F$_{2\alpha}$**

Prostaglandin F$_{2\alpha}$ regresses the CL, causing a sharp decline in progesterone production, which allows for estradiol to increase and the cow to subsequently exhibit estrus 3 to 5 d later. In order for PGF to exert its luteolytic effects, the cow must have a functional CL (Rowson et al., 1972). Prostaglandin F$_{2\alpha}$ and its analogs are less effective at causing luteolysis during the early luteal phase (d 1 to 4) of the cycle (Rowson et al., 1972; Lauderdale et al., 1974). In a study by Chenault et al. (1976), concentrations of progesterone were decreased to < 0.5 ng/mL within 24 h after PGF administration. Exogenous PGF administration can be used to initiate luteolysis without compromising the fertility of the subsequent estrus (Lauderdale et al., 1974).

Stage of the estrous cycle also has a significant effect on the both the ability of PGF to induce estrus and the interval from PGF administration to the onset of estrus. Kiracofe et al. (1985) observed that when PGF was administered on days 1 to 4 of the estrous cycle very few displayed estrus and when inseminated had significantly decreased fertility. Tanabe and Hann (1984) observed estrus in 86.0, 90.0, and 98.0% of dairy heifers administered PGF on d 7, 11, and 15 of the estrous cycle, respectively. They also identified a significant difference in the interval to onset of estrus, with d 7 heifers displaying estrus earliest (43.9 h), d 11 heifers the latest (71.5 h), and d 15 heifers intermediately (53.0 h). However, the synchrony of estrus over the 24 h interval from 32 to 56 h was 88.4, 13.3, and 73.5% for d 7, 11, and 15, respectively. Similarly, Watts and Fuquay (1985) reported estrous responses of 43.0, 83.6 and 100% for cows administered PGF on days 5-7, 8-11 and 12-15, respectively. Within the same treatment groups, they also observed an average interval to estrus of 59, 70, and 72 h. King et al. (1982) observed that the stage of cycle when PGF is administered influences
the interval to estrus in both heifers and cows with cows early in the estrous cycle (d 5 to 9) showing estrus earlier following PGF than cows late in the estrous cycle (d 10 to 15). They also indicated that the interval to estrus after PGF is shorter for heifers than cows.

In a large New Zealand study of dairy herds using a single dose of PGF, cows treated on either d 7 or d 15/16 of the estrous cycle demonstrated the most precise synchrony of estrus (Macmillan and Henderson, 1984). In the same study, cows which received PGF on days 12 or 13 showed the most variable estrous response. These studies demonstrate the significant effects that stage of the estrous cycle has on the subsequent interval to estrus in *Bos taurus* (Macmillan and Henderson, 1984; Stevenson et al., 1984). Differences in the synchrony of estrus following PGF administered at different stages of the estrous cycle result from different stages of follicular development. If the cow has a dominant follicle in its growing phase at the time of CL regression by PGF, the interval to estrus will be shorter compared to a cow which has a regressing follicle and will, therefore, have to recruit and mature a new follicle before ovulation can occur.

Conception rates following PGF appear to be affected by stage of the estrous cycle, although the data is somewhat conflicting. Watts and Fuquay (1985) observed conception rates of 56.8, 62.1, and 78.3% for heifers administered PGF on d 5 to 7, d 8 to 11, and d 12 to 15 of the estrous cycle, respectively. In contrast, other studies have observed no difference in pregnancy rates when PGF was administered during the early or late luteal phase of the estrous cycle (Stevenson et al., 1984). Diskin et al. (2001)
suggested that the stage of cycle effects may be less pronounced in dairy heifers because they have a shorter interval to estrus with less variation than dairy cows.

There is some evidence to suggest that breed type may also affect the efficacy of PGF. Initial work reported a considerable difference in estrous response after a PGF induced estrus. In *Bos taurus* cattle, estrous responses of 70 to 95% have been reported after a single dose of PGF (Tanabe and Hann, 1984; Watts and Fuquay, 1985). However, estrous responses of 46 to 62% (Landivar et al., 1985; Orihuela et al., 1983) were observed after a single dose of PGF in *Bos indicus* cows. In a study by Pinheiro et al. (1998) with a group of Nelore (*Bos indicus*) cows and heifers, they reported an estrous response of only 46.4 and 33.3% respectively, following two doses of PGF 11 d apart. In *Bos indicus* cattle, the decreased estrous response may be due to inhibition of estrus expression caused by incomplete regression of the CL, and therefore elevated concentrations of progesterone (Pinheiro et al., 1998; Rekwot et al., 1999). Cornwell et al. (1985) reported estrous responses of only 50 and 67% for Brahman heifers treated with PGF on days 7 and 10 of the estrous cycle, respectively. However, in heifers that did not display estrus, concentrations of progesterone decreased initially, but began to increase to pre-treatment concentrations within 48 h after PGF. This effect has also been observed in *Bos taurus* cattle (Chenault et al., 1976; Copelin et al., 1988).

To prevent the partial luteolysis in *Bos indicus* cows, the administration of PGF has been split into two consecutive doses administered 24 h apart (Cornwell et al., 1985). Administration of the second dose sustains the luteolytic actions of PGF and prevents the rebounding of concentrations of progesterone caused by incomplete CL
regression. In two recent studies in *Bos indicus* type females, the dose of PGF was split into two half doses (12.5 mg/dose) administered 24 h apart in heifers (Bridges et al., 2005) and cows (Portillo et al., 2003). The split-dose of PGF increased estrous response, timed-AI pregnancy rate, and synchronized pregnancy rates for heifers following MGA treatment in yearling heifers by increasing luteolysis, but not two year old *Bos indicus × Bos taurus* heifers (Bridges et al., 2005). In mature cows of *Bos indicus* breeding synchronized with a GnRH on d 0 of a 7-d melengestrol acetate (MGA) feeding and PGF on d 7, luteolysis was increased with the split dose administration but there was no difference in estrous response or synchronized pregnancy rates (Portillo et al., 2003).

**Progestins**

Exogenous progestins mimic the luteal phase of the estrous cycle and prevent the expression of estrus and ovulation during the duration of their use. Progestins are commonly used to artificially extend the estrous cycle during an estrous synchronization protocol. Several types of progestins are utilized including natural progesterone and synthetic progestins, such as norgestomet and MGA. The two most commonly used progestins for estrous synchronization include the controlled intravaginal progesterone-release device (CIDR), which contains natural progesterone, and MGA, an orally active synthetic progesterone.

The CIDR is a T-shaped intravaginal insert which consists of a nylon spine covered with an elastic, silicone coating. The CIDR available for commercial use in the United States contains 1.38 g of progesterone and has been successfully used to synchronize estrus in both beef and dairy heifers and suckled beef cows and lactating dairy cows. The typical duration of use of the CIDR is 7 d with PGF administered either
the day before CIDR removal (Lucy et al., 2001) or the day of CIDR removal (Larsen et al., 2006).

Melengestrol acetate is an orally-administered progestin, and when administered at a rate of 0.5 mg/head/d it effectively suppresses estrus (Zimbelman and Smith, 1966). Treatments of progestins generally range from 7 to 14 d in length, and lead to a reduction in fertility of the subsequent ovulation when administered for periods greater than 7 d (Hill et al., 1971; Patterson et al., 1986). This reduction in fertility is caused by the development of a persistent dominant follicle (Guthrie et al., 1970). Melengestrol acetate is most commonly used in heifers, and is administered for 14 d, followed by an injection of PGF either 17 (Brown et al., 1988) or 19 d (Lamb et al., 2000) after MGA withdrawal.

Ireland and Roche (1982) observed that the concentrations of exogenously administered progesterone were negatively correlated with an animal’s ability to secrete LH. Cows receiving sub-luteal concentrations of progesterone have an increased frequency of LH pulses during the course of treatment (Roberson et al., 1989). This insufficient concentration of circulating progesterone allows LH frequency to increase, estradiol to rise, and follicles to experience a prolonged period of dominance (Kojima et al., 1992). This pattern of LH secretion prevents the wave-like growth seen in a normally cycling cow with elevated progesterone. Since there is no follicle turnover, a single follicle continues to grow and remains dominant with no further follicle recruitment for the duration of progestin treatment (Sirois and Fortune, 1990). Depending on the length of progestin treatment, the follicle may be aged and less fertile upon ovulation,
with the period of dominance being directly correlated to the reduction in fertility (Mihm et al., 1994).

When low concentrations of progesterone are administered to inhibit estrus over extended (> 9 d) periods of time in the absence of a functional CL, follicles grow to a larger maximum size (Zimbelman and Smith, 1966), persist on the ovary (Trimberger and Hansel, 1955), and lead to a temporary reduction in fertility of the ovulated oocyte (Hill et al., 1971). Low doses of progesterone also increase LH pulse frequency and prolong pre-ovulatory secretion of estradiol (Sirois and Fortune, 1990). Hill et al. (1971) observed decreased pregnancy rates caused by ovulation failure, fertilization failure, and abnormal oocytes following a 14 d MGA treatment. Chow et al. (1982) also observed greater LH and estradiol concentrations during the estrus following exogenous progesterone treatment compared to non-treated heifers. Guthrie et al. (1970) and Kinder et al. (1996) concluded that the development of a persistent dominant follicle was the cause of reduced fertility following a long term exogenous progesterone treatment. In a study by Ahmad et al. (1995), cows were pre-synchronized to ovulate either normal, growing follicles or larger persistent follicles. Estradiol concentrations were greater in cows with persistent follicles than cows with normal growing follicles. In the same study, fewer oocytes and embryos were recovered from cows that ovulated persistent follicles than growing follicles. Additionally, fewer embryos (14%) from persistent follicles reached the 16 cell stage or greater compared to embryos from growing follicles (86%).

Episodic release of LH is controlled by the circulating concentrations of progesterone (Roberson et al., 1989). To more closely mimic a cow's natural luteal
phase, Stock and Fortune (1993) completed a study in heifers with three treatments including a blank CIDR, one CIDR, or two CIDRs, over a period of 14 d. The blank CIDR served as a control, one CIDR mimicked a synchronization program, and two CIDRs mimicked the greater concentrations of progesterone in a normal luteal phase. The single CIDR treatment resulted in ovulatory follicles that were larger in size and remained dominant for three times longer compared to controls. The two CIDR group had more follicular waves per cycle than the control and one CIDR treatments. This study demonstrated that the progesterone provided in a normal, one CIDR protocol was not sufficient to fully suppress LH pulses resulting in a single follicle that maintained dominance, and led to a decrease in fertility (Stock and Fortune, 1993).

In a study by Wehrman et al. (1996), embryos were transferred into recipient cows that had ovulated a persistent dominant follicle 7 d prior. Pregnancy rates were similar between recipients that ovulated normal follicles and those that ovulated a persistent dominant follicle. These results combined with the findings of Mihm et al. (1994) where progesterone concentrations were similar after ovulation of either a persistent dominant follicle or a normal follicle, suggests that the reduction in fertility of the persistent dominant follicle is a result of problems with the follicle and probably not alterations in the uterine environment. These effects do not carry over to the second estrus following withdrawal of progesterone treatment (Roberson et al., 1989).

Follicles which undergo a prolonged period of dominance can also have reduced fertility. Austin et al. (1999) observed no differences in the subsequent fertility of heifers which ovulated follicles after a period of 2, 4, 6, or 8 d of dominance, with pregnancy rates of 89, 68, 78 and 71% respectively. However, in heifers with dominant follicle
durations of 10 or 12 d had decreased pregnancy rates of 52 and 12%, respectively. This indicates that follicles on the ovary only one to two days longer can result significantly less fertile ovulations.

Progestins have been shown to initiate estrus and ovulation in some anestrous cows (Fike et al., 1997; Lucy et al., 2001) and prepubertal heifers (Imwalle et al., 1998; Fike et al., 1999; Hall et al., 1997). Follicle growth can be accelerated in both anestrous cows (Garcia-Winder et al., 1987) and prepubertal heifers (Anderson et al., 1996) by stimulating LH secretion during and after progesterone treatment. After a 7 d CIDR treatment, Fike et al. (1997) initiated estrus or CL formation in 70% of anestrous cows and Lucy et al. (2001) observed approximately 40% of anestrous cows to resume cycling. Similarly, Fike et al. (1997) observed 66.1 of anestrous cows and 81.4% of prepubertal heifers in estrus within 180 h following MGA treatment. Hall et al. (1997) implanted heifers with norgestomet implants for 10 d, and observed that while puberty was induced, it was dependent on age, with a greater number of older heifers achieving puberty than younger heifers. Within 10 d after a 7 d MGA treatment, Imwalle et al. (1998) induced puberty in 8/8 MGA treated heifers, compared to only 4/9 control heifers, due to enhanced LH secretion after withdrawal of MGA.

Gonadotropin Releasing Hormone

In an estrous cycling animal, when progesterone begins to decrease and estradiol from the dominant follicle rises, GnRH is triggered and LH is released, leading to ovulation of the eligible follicle (Fortune et al., 1988). Exogenous GnRH agonists work indirectly through the anterior pituitary to initiate ovulation. The effects of GnRH are observed within 2 to 4 h of injection through an increase in peripheral concentrations of both LH and FSH (Chenault et al., 1990; Stevenson et al., 1993).
This induced LH surge causes ovulation within 24 to 32 h after treatment (Thatcher et al., 1989; Macmillan and Thatcher, 1991; Pursley et al., 1994) of large (> 9 mm; Sirois and Fortune, 1988), dominant (Guilbault et al., 1993) ovarian follicles in their growth phase of development. However, GnRH will not initiate ovulation of all large follicles, particularly follicles which are regressing or in a state of atresia (Guilbault et al., 1993) and have a declining number of LH receptors (Rollosson et al., 1994).

Following ovulation, estradiol concentrations are dramatically decreased in peripheral circulation both after a GnRH treatment induced LH surge (Twagiramungu et al., 1994b) and LH surge caused by removal of a large follicle (Hughes et al., 1987). If a follicle is, in fact, ovulated to GnRH, the rapid decrease in estrogen output will result in an FSH surge, causing recruitment and development of a new wave of follicular growth 1 to 2 d later (Ko et al., 1991; Adams et al., 1992). Additionally, the FSH peak provided by the GnRH injection 2 to 4 h after treatment will stimulate the recruitment of a new follicular wave (Chenault et al., 1990; Rettmer et al., 1992).

One of the reasons that GnRH does not initiate ovulation of all large follicles is due to stage of cycle effects. In a study by Moreira et al. (2000) in dairy heifers, an Ovsynch protocol initiated on days 2, 5, 10, 15, and 18 of the estrous cycle resulted in ovulation rates of 0, 100, 25, 60, and 100%, respectively, to the initial GnRH injection. These results are expected, as on d 2 of the estrous cycle a dominant follicle is not yet present to ovulate to GnRH. On d 5, a first wave dominant follicle is present to ovulate to GnRH. By d 10 most of the first wave dominant follicles have already become atretic, and will not ovulate to GnRH; however, some animals may already have a second wave dominant follicle eligible for ovulation. On d 15, most cows will have a second wave
dominant follicle capable of ovulation, but is more variable than the first wave. On d 18, cows are in the proestrus phase with a dominant follicle present, which yields high ovulation rates. A similar study by Vasconcelos (1999) in lactating dairy cows observed lowest ovulation rates for cows administered GnRH on days 1 to 4 (23%), moderate ovulation rates on days 10 to 16 (54%), and greatest ovulation rates on days 5 to 9 (96%) and 17 to 21 (77%).

The use of GnRH agonists has been shown to synchronize follicular growth and yield precise ovulation times without compromising fertility (Twagiramungu et al., 1995). It has been suggested with mixed results that treatment with GnRH agonists may alter the subsequent CL's ability to secrete progesterone. There have been reports of both increased (Macmillan et al., 1985a,b; Stevenson et al., 1993) and decreased (Ford and Stormshak, 1978, Rodger and Stormshak, 1986) progesterone production following treatment with a GnRH agonist, as well as numerous studies which showed no changes (Macmillan and Thatcher, 1991; Prescott et al., 1992). However, studies by Twagiramungu et al. (1992b; 1994a) demonstrated that both the CL existing at the time of GnRH treatment, and the CL formed from the GnRH-induced ovulation, equally respond to an exogenous PGF induced luteolysis. Therefore, it appears that GnRH-induced CL are similar to a naturally formed CL, at least in regard to luteolysis.

Another use of GnRH is to induce ovulation during the AI period to perform a TAI as a method to reduce or eliminate the need for estrous detection. This is possible, because a great degree of synchrony is achieved, with 56 to 76% of cows displaying estrus between 24 and 72 h after PGF in cattle that had previously received GnRH 7 d before PGF (Twagiramungu et al., 1992b,c). With the addition of a second GnRH
injection, given 48 hrs after PGF, Pursley et al. (1994) observed that dairy cows and heifers ovulated between 24 and 32 h after GnRH.

It also appears that breed may have an effect on response to GnRH. Cattle of *Bos indicus* breeding may have altered sensitivities to GnRH treatment and subsequent gonadotropin concentrations. In a study by Griffin and Randel (1978), ovariectomized Brahman and Hereford cows were treated with GnRH and the resulting LH response was significantly decreased in Brahman cows, indicating that they may be less responsive to a GnRH-induced LH release. Portillo et al. (2008) also reported that the amount of LH released to a GnRH challenge on day 6 of the estrous cycle decreased as the percentage of *Bos indicus* breeding increased in the crossbred animal; however, there were no differences in ovulation rates between Angus and the *Bos indicus × Bos taurus* cattle.

**Select Synch/CIDR + TAI**

The Select Synch/CIDR protocol consists of administration of a CIDR plus GnRH on d 0 with CIDR removal and PGF on d 7. Following PGF, estrus is typically detected for 5 d and cows are AI 8 to 12 h after exhibiting estrus. In the initial development of the GnRH + PGF estrous synchronization program (Thatcher et al., 1989; Macmillan and Thatcher, 1991; Pursley et al., 1994), which is now called the Select Synch program, GnRH initiates ovulation of large follicles (≥ 10 mm diameter) that serves to synchronize of the subsequent follicle wave. Administration of PGF 7 d after GnRH initiates luteolysis resulting in the expression of estrus. One of the problems with the Select Synch system is the premature expression of estrus several days before PGF (DeJarnette et al., 2001) resulting in the need for additional estrous detection. The base Select Synch protocol involves estrous detection and subsequent AI over a set number
of days, typically 2 d before PGF and 5 d after PGF (Thompson et al., 1999; Stevenson et al., 2000). This results in a group of cattle with synchronized follicular growth, and an improved synchrony of estrus following a PGF–induced luteolysis. Estrous rates of 70 to 83% (Thatcher et al., 1989; Coleman et al., 1991; Twagiramungu et al., 1992b,c) and pregnancy rates of 65 to 85% (Coleman et al., 1991; Twagiramungu et al., 1992b,c), both over a 4 d estrus detection period, have been reported in beef and dairy cattle.

The effectiveness of Select Synch systems in *Bos taurus* cattle have been well established. Following PGF, 59 to 88% of *Bos taurus* cows display estrus (Geary et al., 2000; Stevenson et al. 2000). Conception rates of 46 to 55% have been achieved in lactating dairy cows (Pursley et al., 1995), 26 to 63% in dairy heifers (Schmitt et al., 1996), and 33 to 69% in *Bos taurus* beef cows (Geary et al., 1998; Stevenson et al., 2000). Overall synchronized pregnancy rates in *Bos taurus* cows following the Select Synch system range from 38 to 71% (Geary et al., 2000; Stevenson et al. 2000).

Lemaster et al. (2001) evaluated the Select Synch protocol in cows of *Bos indicus* breeding and reported pregnancy rates of only 20.8%. This unacceptable pregnancy rate was attributed to the difficultly in detecting estrus in *Bos indicus* type cows, which resulted in a decreased number of cows inseminated when they did not display estrus.

As indicated previously, the premature expression of estrus several days before PGF (DeJarnette et al., 2001) in the Select/Sync system results in the need for additional estrous detection and limits the overall effectiveness of the system. The addition of progestins like MGA or a CIDR between GnRH and PGF treatments prevents the need for the extra estrous detection (Thompson et al., 1999; DeJarnette
Another benefit of the progestin is that it can induce estrous cyclicity in some anestrous cows (Fike et al., 1997; Lucy et al., 2001), and may be particularly beneficial in cows that calve late in the breeding season or are in poor body condition (Stevenson et al., 2000; Lamb et al., 2001).

Although the data is limited, the CIDR may be more effective in inducing estrus than an MGA treatment. Perry et al. (2004) induced 25% more estrous cycles and 30% greater ovulation rates in early postpartum Bos taurus beef cows using a 7 d CIDR treatment vs. normal (0.55 mg) or high (4.41 mg) levels of MGA over the same treatment period. Inclusion of a progesterone source also aids in reinitiating estrous cycles in cows deemed noncycling at the start of the experiment. This occurs by progesterone enhancing pulsatile release of LH after progestogen withdrawal, encouraging further follicular growth and ovulation (Anderson et al., 1996).

A timed-AI (TAI) can also be combined with the Select Synch/CIDR system. Following PGF, estrus is detected for 72 h and cows exhibiting estrus are AI 8 to 12 h later. Cows not detected in estrus at 73 to 80 h are administered GnRH and TAI. The addition of a clean-up TAI to the Select Synch/CIDR has resulted in greater pregnancy rates in Bos taurus (Larson et al., 2006) and Bos indicus × Bos taurus cows (Lemaster et al., 2001; Esterman et al., 2006). By detecting estrous for the first 3 d following PGF and TAI remaining cows at 73 to 80 h, all cows are inseminated and therefore given an opportunity to become pregnant on the synchronized breeding.

Recent studies have investigated modifying a traditional Select Synch/CIDR protocol to promote development and ovulation of a competent dominant follicle that will have the best chance of developing into a successful pregnancy. Sa Filho et al. (2009)
demonstrated that synchronization protocols which reduce concentrations of progesterone and increase LH stimulation, including protocols with estradiol benzoate (EB) concurrent with CIDR insertion, may stimulate increased pregnancy rates in these cattle. Increased LH secretion may promote the ovulation of a more viable oocyte (Savio et al., 1993; Gong et al., 1995), increase estradiol production by the dominant follicle during proestrus (Bridges et al., 2007), and enhance the secretion of progesterone by the subsequent CL. Collectively, the advanced development encouraged by the reduced concentrations of progesterone and increased LH stimulation has the potential to increase fertility in cattle. Numerous studies have also shown the importance of follicle diameter at ovulation and the capacity of the follicle to produce estradiol on subsequent fertility (Mussard et al., 2003, 2007; Bridges et al., 2004; Perry et al., 2005). Other methods of increasing LH stimulation on the ovulatory follicle have been investigated, including administering PGF on the seventh day of a nine-day CIDR protocol, use of multiple-use CIDRs, and administering equine chorionic gonadotropin (eCG) at CIDR removal (Meneghetti et al., 2009; Sa Filho et al., 2009).

Collectively, the Select Synch/CIDR + TAI protocol is an effective synchronization protocol for use in Bos taurus cows (Larson et al., 2006); however, additional work in Bos indicus × Bos taurus cows needs to be done to achieve optimal pregnancy rates following this protocol (Esterman et al., 2006). Additionally, the protocol is somewhat labor intensive and some producers may not have the resources to detect estrous and properly implement this program.

**CO-Synch + CIDR**

The CO-Synch + CIDR synchronization protocol utilizes the same initial setup of a Select Synch system with GnRH and CIDR insertion on d 0 followed by CIDR removal
and PGF on d 7. However, following PGF, all cows are TAI regardless of whether or not they exhibit estrus. In the initial studies with the CO-Synch protocol, all cows were TAI 48 h after PGF (Stevenson et al., 2000; Geary et al., 2001). The addition of the TAI eliminates the labor involved in estrous detection and minimizes the number of times each cow must be worked. Similar to the Select Synch/CIDR + TAI protocol, all cows are inseminated and therefore, given the opportunity to become pregnant.

CO-Synch protocols have been used in *Bos taurus* cows with TAI administered at varying intervals following PGF (from 48 to 72 h) with similar success (Stevenson et al., 2003; Bremer et al., 2004; Larson et al., 2006). Similar to Select Synch protocols, the inclusion of a CIDR to the CO-Synch protocol is important in cattle of *Bos indicus* influence. Martinez et al. (2002b) reported that the addition of a CIDR to a CO-Synch protocol in lactating *Bos taurus* beef cows yielded similar results to CO-Synch without a CIDR, with pregnancy rates of 42.9 and 45.1%, respectively. The Martinez et al. (2002b) study agrees with Johnson et al. (2000), who observed similar pregnancy rates in CO-Synch + CIDR (45%) and CO-Synch (47%) treatments for postpartum *Bos taurus* beef cows. However, Martinez et al. (2002a), reported an increase in pregnancy rates from 39.1 to 68.0% with the addition of a CIDR to the CO-Synch system in *Bos taurus* beef heifers. Similarly, Lamb et al. (2001) reported increased pregnancy rates for CO-Synch + CIDR (58.6%) over CO-Synch (48.1%) treatments with an even greater increase in pregnancy rate among anestrous *Bos taurus* beef cows, with 59% for CO-Synch + CIDR compared with 39% for CO-Synch treated cows.

Limited work has been conducted with the CO-Synch programs for cattle of *Bos indicus* breeding. Lemaster et al. (2001) reported pregnancy rates of only 31.0% in *Bos
*Bos indicus* × *Bos taurus* cows synchronized with a CO-Synch with TAI at 48 h after PGF. With the inclusion of a CIDR and TAI 48 h post PGF, Saldarriaga et al. (2007) reported pregnancy rates of 33 to 39% and Yelich (2002) reported pregnancy rates of 33%. While the CO-Synch programs achieve acceptable pregnancy rates in *Bos taurus* cows with TAI ranging from 48 to 60 h (Stevenson et al., 2003; Bremer et al., 2004; Larson et al., 2006), it may be necessary for the TAI to be later than 48 h in cattle of *Bos indicus* influence. When a Select Synch/CIDR protocol is used with no TAI in *Bos indicus* × *Bos taurus* cows, it is common for no cows to display estrus prior to 48 h after PGF, with the average interval from PGF to estrus 70 h and the interval from PGF to ovulation 99 h (Saldarriaga et al., 2007). Compared to the average interval from PGF to estrus of *Bos taurus* cows at 55 h (Geary and Whittier, 1998; Martinez et al., 2003; Lamb et al., 2004), this further suggests that delaying the TAI in *Bos indicus* × *Bos taurus* cows may be necessary to achieve acceptable pregnancy rates. One study evaluating a delayed TAI to 66 h after PGF in *Bos indicus* × *Bos taurus* cows achieved pregnancy rates of about 45% (Zaluaga et al., 2010). Further research is needed to determine what the appropriate interval is to perform TAI after PGF in cattle of *Bos indicus* influence.

**Five Day CIDR**

The concept of reducing CIDR treatment from the traditional 7 d to 5 d was initially investigated by Bridges et al. (2007). In traditional 7 d CIDR synchronization programs, the second GnRH injection can lead to ovulation of follicles with smaller than typical diameter, leading to reduced TAI pregnancy rates (Lamb et al., 2001; Perry et al., 2005). It was suggested by several investigators (Mussard et al., 2003a,b, 2007; Bridges et al., 2004) that conception rates following TAI were related to the length of proestrus and the ovulatory follicle’s ability to produce elevated concentrations of
estradiol prior to TAI. To increase the length of proestrus and increase secretion of estradiol, it was hypothesized to reduce CIDR treatment from 7 d to 5 d prior to a TAI breeding. In several experiments, Bridges et al. (2007) demonstrated that a 5 d CIDR protocol with a TAI at 72 h after PGF had 10.5% greater pregnancy rates compared to a 7 d CIDR protocol with a TAI at 60 h, in *Bos taurus* suckled beef cows. Additionally, the 5 d CIDR treatment had no interactions with parity or estrous cycling status, suggesting increased pregnancy rates could be expected across all subgroups of cows. Additional experimentation is needed to determine if the 5 d CIDR protocol will be effective in heifers and cows of *Bos indicus* influence.

**7-11 Synch**

The 7-11 synchronization protocol was developed by Kojima et al. (2000) as a program to utilize short-term MGA feeding without the decreased fertility often observed following long-term (> 10 d) MGA treatment. Use of MGA is advantageous due to its decreased cost and ease of administration as it can be added to the feed. The 7-11 program involves administering MGA for 7 d at a rate of 0.5 mg/head/d with PGF given on the last day of MGA (d 7). Four days following the end of MGA (d 11), GnRH is administered to initiate ovulation of large follicles developed during the term of MGA feeding. Eleven days after MGA withdrawal, PGF is administered to induce luteolysis and initiate the onset of estrus. Estrus is typically detected for 5 to 7 d after PGF with cows AI 8 to 12 h after observed in estrus. This program results in a highly synchronized group of cows ovulating a follicle from their first follicular wave, which yields a fertile ovulation from both estrous cycling and anestrous cows. In the initial study by Kojima et al. (2000), pregnancy rates to the protocol were similar to those of a Select Synch protocol, but a greater synchrony of estrus following PGF over a 24-hour
peak time interval (42 to 66 h) was observed for 7-11 synchronized cows. In addition, the 7-11 protocol had greater AI pregnancy rates than the Select Synch protocol. The 7-11 program has also been used in conjunction with fixed TAI with some success (Hixon et al., 2001; Kojima et al., 2002). No studies have investigated the effectiveness of the 7-11 protocol in cattle of Bos indicus influence.

**Lactating Cows**

**Lactation and Nutrition**

The primary reason for cows to be anestrus at the start of the breeding season is less than adequate nutrition during the postpartum period, which does not allow for the occurrence of estrous cycles (Short et al., 1990). Nutritional status has a direct influence on reproductive function and estrous cycling status of suckled beef cows (Oyedipe et al., 1982) and body condition score (BCS; scale 1 = emaciated; 5 = moderate; 9 = very fat) can be used as an indirect measure of nutritional status (Richards et al., 1986). At the start of the breeding season, cows of BCS > 5 have an increased chance of becoming pregnant compared to cows with a BCS < 5. In a study by Lamb et al. (2001), for each unit increase in BCS there was a 23% increase in pregnancy rates following a synchronized breeding. Many reports stress the importance of BCS on response to a synchronized AI breeding and subsequent pregnancy rates (Stevenson et al., 2000; DeJarnette et al., 2004; Larson et al., 2006).

Suckled cows have a longer interval from parturition to their first estrus compared to nonsuckled cows (Graves et al., 1968; Short et al., 1972). Weaning of calves prior to the start of the breeding season can shorten the postpartum interval (Bellows et al., 1974; Lusby et al., 1981); however, this is not generally practical since weaning the calves early can significantly reduce weaning weights that a producer would achieve at
a normal weaning age around 205 d. Alternatively, studies have demonstrated that removal of calves for 48 h prior to breeding can accelerate the onset of estrus. Temporary calf removal for 48 h has been shown to increase conception rates of beef cows to a timed insemination when used in conjunction with synchronization protocols (Smith et al., 1979; Kiser et al., 1980; Yelich et al., 1995; Geary et al., 2001). The positive effects of 48 h calf removal in combination with estrous synchronization are also observed in greater 25-d pregnancy rates with cows establishing pregnancy earlier in the breeding season (Yelich et al., 1995). Temporary calf removal has no significant effect on calf weaning weight (Odde et al., 1986), but can cause a greater frequency of short estrous cycles (Odde et al., 1980). When implementing synchronization programs which use a progestogen, the incidence of short estrous cycles is reduced (Odde et al., 1986; Ramirez-Godinez et al., 1981).

Cow Age

For a cow to achieve maximum productivity in her lifetime, she must be bred to calve as a two year old and produce a calf on a yearly interval. Rebreeding of a two year old first calf heifer is one of the most challenging aspects of a breeding program. Heifers bred to calve at two years of age take 20 to 40 d longer to resume ovarian function compared to mature cows (Wiltbank, 1970). Decreased pregnancy rates are frequently observed in first calf heifers compared to their mature cow herdmates. However, outside of breeding a first calf heifer, cow age does not generally influence pregnancy rates. In a multi-year study by Renquist et al. (2006), cow age had no effect on pregnancy rate in multiparous Bos taurus cows; however, the study did not include primiparous cows.
**Postpartum Interval**

Postpartum interval, measured as days from calving to breeding, can have a strong influence on the number of estrous cycling cows within a herd at the start of the breeding season (Stevenson et al., 2000; Lamb et al., 2001). Decreased nutritional intake is the primary reason for cows to be anestrous at the start of the breeding season. Uterine involution is a period of time in cows following calving when conception is not possible (about 3 weeks post calving), followed by an additional 2 to 3 weeks where pregnancy is possible, but not optimal (Kiracofe, 1980). Uterine involution can take 40 to 60 days to complete and involves reduction in uterine size, loss of tissue, and repair (Gier and Marion, 1968), suggesting that many cows \( \leq 50 \text{ d postpartum} \) may not have completely involuted. Uterine involution is complete when the uterus has returned to non-pregnant size and position, with both horns similar in diameter with consistent tone (Casida et al., 1968).

Stevenson et al. (2000) observed greater reproductive performance for cows with longer postpartum intervals in suckled *Bos taurus* beef cows and they reported a 5.7% increase in estrous cycling status for each 10 d increase in postpartum length. Other studies have demonstrated increased estrous response in estrous cycling versus noncycling cows in both *Bos indicus \times Bos taurus* (Lemaster et al., 2001) and *Bos taurus* cows (Geary et al., 2000; Stevenson et al., 2000). Many reports also indicate estrous cycling status and postpartum interval influence synchronized pregnancy rates in *Bos indicus \times Bos taurus* (Lemaster et al., 2001) and *Bos taurus* cows (Larson et al., 2006). Collectively, these studies stress the importance of knowing where cows are in relation to calving when initiating a synchronization program in suckled cows to ensure
they are far enough from calving to achieve acceptable pregnancy rates following the program.

**Replacement Heifers**

**Puberty**

Puberty is defined as the first behavioral estrus followed by the development of a CL (Kinder et al., 1987). It involves the maturation of both the endocrine and reproductive systems. The “gonadostat” theory proposed by Ramirez and McCann (1963) is the mechanism regulating pubertal development in heifers, suggesting that the prepubertal increase in LH secretion is caused by the declining negative feedback of estradiol on hypothalamic centers which control LH secretion. Reduced estradiol receptors in the hypothalamus and pituitary may increase LH secretion by reducing the efficacy of estradiol to exert its negative feedback effects (Day et al., 1987), and the decline in estradiol receptors results in increased LH secretion. Estradiol’s induction of the pre-ovulatory LH surge is essential for puberty to occur (Kinder et al., 1987). Increases in LH pulse frequency (Schams et al., 1981; Day et al., 1987), increases in LH concentration (Day et al., 1987), and reduced LH pulse amplitude (Schams et al., 1981) have been recorded in the time from 1 month of age to puberty.

When a heifer reaches puberty is dependent on many factors, including body weight, genotype, age, nutrition, and environment. *Bos indicus* cattle reach puberty at greater ages and heavier BW compared to *Bos taurus* cattle (Wiltbank et al., 1966; Plasse et al., 1968; Gregory et al., 1979; Nelson et al., 1982), with *Bos taurus × Bos indicus* cattle reaching puberty intermediately between the two breed types. Nutritional status is an important regulator of age at onset of puberty in beef heifers. The age at which a heifer reaches puberty is inversely related to growth rate (Wiltbank et al., 1969;
Short and Bellows, 1971). In short, as yearling heifer nutrition increased, the age at which heifers’ attained puberty decreased. Heifers must achieve approximately 60 to 65% of their mature BW to become pubertal (Dale et al., 1959; Lamond et al., 1970) and at low BCS they will likely have a delay in the onset of puberty. Greatest conception rates are achieved on the second and third estrus following puberty (Byerly et al., 1987; Perry et al., 1991), and therefore, the sooner a heifer becomes pubertal, the sooner she will be eligible to become pregnant during the breeding season.

The use of exogenous progestogens, such as MGA or CIDR, can hasten the onset of puberty in some heifers (Short et al., 1976; Anderson et al., 1996; Imwalle et al., 1998). Treatment with progestogens enhances pulsatile release of LH after progestogen withdrawal, encouraging further follicular growth and ovulation (Anderson et al., 1996).

It is important that heifers reach puberty before the breeding season begins and get pregnant early in the breeding season in order to maintain a yearly calving interval and optimize productivity throughout their lifetimes. Heifers calving first at 2 yr of age produce more calves during their lifetime compared to heifers calve first at 3 yr of age or older (Pope, 1967; Chapman et al., 1978). Heifers calving early in their first calving season have a greater lifetime calf production compared to those that calve late (Lemeister et al., 1973), due to the additional time they have to become pregnant the following season when they are rebred as a first calf heifer.

Reproductive Tract Scoring

Reproductive tract scores (RTS; scale 1 to 5) as described by Anderson et al. (1981) is a subjective evaluation of the heifers reproductive tract that is used to predict the pubertal status and breeding potential of the heifer. The RTS is an accurate and repeatable test (Rosenkrans and Hardin 2002) when performed by a trained technician.
When performed approximately 30 d before the start of the breeding season, heifers with RTS 1 to 3 are considered pre-pubertal; whereas, heifers with RTS 4 are peripubertal and heifers with a RTS 5 are pubertal. A heifer with RTS 1 has an immature uterus with < 20 mm horn diameter with no uterine tone, ovaries are approximately 15 × 10 × 8 mm with follicles < 8 mm. A RTS 2 is characterized by 20 to 25 mm uterine horn diameter with no tone, 18 × 12 × 10 mm ovaries and follicles of about 8 mm, and is expected to take > 30 d to reach puberty. A RTS 3 has 25 to 30 mm uterine horns with good tone, ovaries 30 × 16 × 10 mm and follicles ranging from 9 to 10 mm, and is expected to reach puberty within 30 d. A RTS 4 is nearing puberty, with 32 to 35 mm uterine horns with good tone, 32 × 18 × 12 mm ovaries with > 10 mm follicles. A RTS 5 is a pubertal heifer that has > 35 mm uterine horns, > 32 × 20 × 12 mm ovaries with a CL present. Heifers with higher RTS generally have higher synchronized pregnancy rates and breeding season pregnancy rates. Heifers with RTS 1 and 2 will often conceive later in the breeding season compared to those with higher RTS. In a large study by Patterson and Bullock (1995), estrous response over 144 h following a MGA-PGF estrous synchronization protocol was 54, 66, 76, 83, and 86% for beef heifers with RTS 1, 2, 3, 4, and 5, respectively. It has been suggested that a synchronization protocol should be initiated when 50% of heifers are RTS 4 or 5 to achieve acceptable pregnancy rates (Patterson et al., 2000).

By evaluating RTS, heifers with a decreased potential to become pregnant to a synchronized breeding can be eliminated before the costs of synchronization are incurred (LeFever and Odde 1986). Differences between *Bos taurus* and *Bos indicus × Bos taurus* have been observed, as *Bos indicus* females often have smaller, less
prominent, and more difficult to detect ovarian structures compared to *Bos taurus* females (Segerson et al., 1984), which may lead to a categorizing heifers into the wrong RTS classification. Stevenson et al. (1996) observed that RTS was a significant predictor of conception rates in yearling *Bos indicus × Bos taurus* heifers; however, Rae et al. (1999) did not observe a strong correlation between RTS and first-service conception rates in two year old *Bos indicus* and *Bos indicus × Bos taurus* heifers.
CHAPTER 3
COMPARISON OF THE 7-11 SYNCHRONIZATION PROTOCOL BETWEEN SUCKLED ANGUS AND BRANGUS COWS

Many estrous synchronization systems have been developed in suckled beef cows but, most of these systems have been developed in *Bos taurus* cattle and the knowledge of their efficacy in cattle of *Bos indicus* influence is limited. The exact reason(s) for the compromised response to synchronization protocols in cattle of *Bos indicus* breeding are unclear but could be due to the subtle differences in their reproductive physiology compared to *Bos taurus* cattle. Differences in concentrations of reproductive hormones and altered sensitivities of their release have been noted for LH (Griffen and Randel, 1978), estradiol (Segerson et al., 1984), and progesterone (Rhodes et al., 1982; Segerson et al., 1984) between *Bos indicus* and *Bos taurus* cattle. Cattle of *Bos indicus* breeding also have an increased percentage of three-wave follicle growth patterns (Rhodes et al., 1995; Zeitoun et al., 1996; Martinez et al., 2003) than *Bos taurus* cattle, which could affect the ability of GnRH to initiate ovulation and synchronize follicle development. Characteristics associated with estrus are also different between *Bos indicus* and *Bos taurus* cattle. Estrus is more difficult to detect in cattle of *Bos indicus* breeding (Galina et al., 1982; Orihuela et al., 1983) due to decreased estrus duration (Rae et al., 1999) and increased incidence of silent heats (Dawuda et al., 1989; Lamothe-Zavaleta et al., 1991).

The 7-11 synchronization protocol was developed by Kojima et al. (2000) and involves a 7 d melengestrol acetate (MGA) treatment with PGF$_{2\alpha}$ on the last day of MGA. Four days after the last day of MGA, GnRH is administered to induce ovulation, and 7 d later second PGF$_{2\alpha}$ treatment is administered and cattle are inseminated after the subsequent estrus. This protocol is appealing to producers, as it utilizes a low-cost
progestogen source and is relatively easy to implement. However, it is important that cows consume the necessary amount of MGA supplement to prevent estrus behavior and adequate bunk space is available to administer the supplement. Acceptable AI pregnancy rates have been achieved with this protocol in *Bos taurus* cattle, but it has not yet been evaluated in suckled cattle of *Bos indicus* breeding.

Therefore, objectives of this experiment were to compare the response of suckled Angus (*Bos taurus*) and Brangus (*Bos indicus* × *Bos taurus*) cows to the 7-11 synchronization protocol for the following: 1) evaluate the effectiveness of GnRH to initiate ovulation when given 4 d after a 7-d MGA treatment, 2) evaluate follicle size and luteal regression following PGF$_{2\alpha}$, 3) evaluate the estrous characteristics after the second PGF$_{2\alpha}$ induced estrus, and 4) evaluate the AI and breeding season pregnancy rates following administration of the 7-11 program.

**Materials & Methods**

**Animals**

The experiment was conducted from March to June, 2006 at the University of Florida, Department of Animal Sciences Santa Fe Beef Unit. Suckled two-and three-year old Angus (n = 44) and Brangus (n = 38) cows were utilized. At the start of synchronization, Angus and Brangus cows were 2.5 ± 0.8 years of age, BW was 488 ± 10 kg for Angus and 514 ± 11 kg for Brangus, body condition score (BCS: 1 = severely emaciated, 5 = moderate, 9 = very obese; Wagner et al., 1988) was 5.5 ± 0.8 for Angus and 5.6 ± 0.8 for Brangus, hip height (HH) was 129 ± 1 cm for Angus and 134 ± 1 cm Brangus, and cows were 63.1 ± 3.7 and 57.4 ± 4.0 days postpartum (DPP) for Angus and Brangus cows, respectively. Throughout the experiment, cows were housed in similar sized pastures by breed, and fed stored Bermuda grass hay *ad libitum* and a
protein/energy supplement to meet nutrient requirements. Calves remained with cows throughout the experiment and were separated briefly when cows were worked through the chute. For two weeks prior to the start of experiment, cows were moved through the working facilities two to three times per week to acclimate them to frequent handling.

**Experimental Protocol**

Day 1 was designated as the start of the synchronization protocol. On d -9 and -2, blood samples were collected by jugular venipuncture into evacuated tubes with an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ) for the evaluation of concentrations of progesterone to determine estrous cycling status. After collection, blood samples were immediately placed on ice, centrifuged (3000 × g for 15 min), and plasma was separated and stored at -20°C until further analysis. A cow was deemed to be estrous cycling (cycling) if either sample had concentrations of progesterone ≥ 1 ng/mL at either blood sample, and anestrous (noncycling) if concentrations of progesterone were < 1 ng/mL at both samples.

On d 1 of the experiment, a 7 d melengestrol acetate (MGA; 0.5 mg/(cow • d) treatment was initiated (Figure 3-1). Cows were administered MGA in a carrier supplement (1 kg/(cow • d), which was fed in bunks. Additional bunk space was made available to allow for proper MGA consumption and 0.2 kg/(cow • d) additional supplement was provided to allow for consumption by calves and excess supplement consumed by dominant cows. On d 7, cows received PGF$_{2α}$ (PG1; 25 mg, i.m., Prostamate, Agrilabs, St. Joseph, MO), followed by GnRH (100 µg; i.m., Cystorelin, Merial, Athens, GA) on d 11. A second PGF$_{2α}$ (PG2) was administered on d 18. On d
1, 7, 11, and 18, plasma samples were collected by jugular venipuncture for evaluation of progesterone and blood samples were processed as previously described.

From the concentrations of progesterone on d -9 and -2, a subgroup of cows (scan) were selected for the intense ultrasonography portion of the experiment. Based on estrous cycling status, cows were designated to be anestrous (ANEST; concentrations of progesterone < 1 ng/mL on both d -9 and -2), cycling with high progesterone (CYCH; concentrations of progesterone < 1 ng/mL on d -9 and > 1 ng/mL on d -2), or cycling with low progesterone (CYCL; cows with concentrations of progesterone > 1 ng/mL on d -2 and were administered PGF$_{2\alpha}$ (25 mg) on d 0 to mimic a low progesterone environment during MGA). Each scan group (ANEST, CYCH, and CYCL) consisted of 6 Angus and 6 Brangus cows, for a total of 36 cows in the scan group. On d 7, 11, 13, and 18 scan cows were evaluated by transrectal ultrasonography using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 7.5 MHz transducer. At each ultrasonography evaluation, height and width of all luteal structures, luteal cavities, and follicles ≥ 5 mm in diameter were measured with the internal calipers of the ultrasonography machine and their locations on the ovaries were recorded. Volume of the corpus luteum (CL) was calculated using the formula for volume of a sphere ($\pi d^3/6$). When a luteal cavity was present, its volume was subtracted from the volume of the outer sphere resulting in net luteal volume (CL volume) represented by luteal tissue. On d 13 of the experiment, ultrasonography was conducted to determine if GnRH initiated ovulation, with ovulation defined as disappearance of the largest follicle from the previous ultrasonography examination.
Following PG2, estrus was monitored using electronic heat detection monitors (HeatWatch, DDx, Boulder, CO) for 5 d. Mounts received during estrus were recorded when a cow stood to be mounted by another cow, applying pressure to the HeatWatch transponder. The transponder recorded the time and duration of each mounting event that was ≥ 1 sec. Initiation of estrus was defined as three or more mounts within a 4-h period and the end of estrus defined was the last mount recorded prior to a period of extended inactivity of at least 8 h (Landaeta et al., 1999). Duration of estrus was calculated by subtracting the time of the initial mount from the time of the last mount. Interval from PG2 to the onset of estrus was calculated as the interval from PG2 to first mount as detected by HeatWatch. Total number of mounts received during estrus was recorded. Interval from estrus to AI was calculated by subtracting the time of AI from the time of the initial mount recorded by HeatWatch.

Cows were inseminated by two AI technicians (equally distributed across breeds) 8 to 12 h after declared in estrus by HeatWatch. Cows were inseminated using frozen-thawed semen from 16 sires, which were pre-assigned to cows before the start of the experiment. At 120 h after PG2 and again 10 d following PG2, blood samples were taken on any cow that had not displayed estrus for evaluation of concentrations of progesterone. Estrous detection as determined by HeatWatch and AI continued for an additional 25 d after the synchronized breeding. Following the AI period, Angus and Brangus cows were placed in individual breeding pastures with single bulls of good fertility for an additional 40 d breeding period. Pregnancy was diagnosed approximately 30 d after each AI, at the end of the breeding season, and 30 d after the bulls were removed using a real-time B-mode ultrasound with a 5.0 MHz transducer. Due to the
time differences between breeding periods, differences in fetal size (Curran et al., 1986) were used to determine whether a pregnancy resulted from the synchronized breeding, additional AI period, or clean-up bull.

**Definitions**

Corpus luteum regression was defined as the number of cows which had a CL as determined by ultrasonography evaluation or concentrations of progesterone $\geq 1$ ng/mL on d 18 of the experiment and either exhibited estrus or had concentrations of progesterone of $< 1$ ng/mL by 120 h following PG2. Estrous response was the number of cows displaying estrus as determined by HeatWatch during the 5 d after PG2 divided by the total number of cows treated. Conception rate was the number of cows that displayed estrus, were inseminated, and became pregnant, divided by the number of cows that displayed estrus and were inseminated. Synchronized pregnancy rate was the number of cows pregnant to the AI divided by the total number of cows treated. Breeding season pregnancy rate was the number of cows pregnant at the end of a 65 d breeding season, which included the 25 d AI breeding and a 40 d bull breeding, divided by the total number treated.

**Radioimmunoassay**

Concentrations of progesterone were determined in multiple assays by direct solid-phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA) without extraction as described by Seals et al. (1998) with intra- and interassay CV of 6.9 and 12.8%, respectively. Sensitivity of the assay was 0.01 ng/mL.

**Statistical Analysis**

The GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the statistical analysis of categorical data. The effect of breed, estrous cycling status,
ovulation or no ovulation to GnRH, scan groups (CYCH, CYCL, and ANEST), and all appropriate interactions were evaluated for ovulation rate to GnRH, estrous response, CL regression to PG2, conception rate, synchronized pregnancy and breeding season pregnancy rates, while cow age, DPP, and BCS were included as covariates. When covariates were significant (P < 0.05) they were treated as independent variables, with cow age divided into two categories (2 yr and 3 yr). The effect of cows involved in the intensive ultrasonography group vs. those not ultrasounded was evaluated for estrous response, conception rate, synchronized pregnancy rate, and breeding season pregnancy rate. The effect of AI technician was evaluated for conception rate and synchronized pregnancy rate. The effects of interval from PG2 to the onset of estrus along with the two-way interaction with breed and cycling status was evaluated for conception rate. The effect of breed, scan group, and the interaction on follicle diameters and luteal tissue volumes on d 0, 11, and 18 were analyzed using GLM procedure of SAS. The effect of breed, cycling status, and the interaction on concentrations of progesterone on d 0, 7, 11, and 18 were analyzed using the GLM procedure of SAS.

**Results**

At the start of synchronization, a similar (P > 0.05) number of Angus (45.5%; 20/44) and Brangus (31.6%; 12/38) were cycling. On d 1 (first day of MGA treatment), concentrations of progesterone were similar (P > 0.05) for Angus and Brangus (2.02 ± 0.4 and 1.35 ± 0.5 ng/mL, respectively). However, CYCH and CYCL cows had greater (P < 0.05) concentrations of progesterone on d 1 (4.0 ± 0.7 and 4.5 ± 0.7 ng/mL, respectively) compared to ANEST cows (0.4 ± 0.7 ng/mL). There were no (P > 0.05) effects of breed × cycling status on concentrations of progesterone on d 0.
On the last day of MGA (d 7), the largest follicle in scan cows was similar (P > 0.05) for Angus (17.6 ± 0.8 mm) and Brangus cows (16.4 ± 0.8 mm). The largest follicle was also greater (P < 0.05) for CYCL (17.9 ± 0.9 mm) and ANEST (17.5 ± 0.8 mm) compared to CYCH (14.7 ± 0.8 mm). There were no (P > 0.05) effects of breed × scan group on the largest follicle on d 7. As expected, concentrations of progesterone were less (P < 0.05) for CYCL (0.6 ± 0.6 ng/mL) and ANEST (0.7 ± 0.6 ng/mL) compared to CYCH (4.7 ± 0.6 ng/mL). However, concentrations of progesterone were similar (P > 0.05) for Angus and Brangus, and there were no (P > 0.05) effects of breed × cycling status. In scan cows with a CL, Brangus had a greater (P < 0.05) luteolysis (100.0%; 9/9) compared to Angus (77.8%; 7/9). Following PG1, a similar (P > 0.05) number of Angus (45.5%; 20/44) and Brangus (44.7%; 17/38) displayed estrus. Estrous response following PG1 was also similar (P > 0.05) for CYCH (58.3%; 7/12), CYCL (58.3%; 7/12), and ANEST (50.0%; 6/12) scan groups. Cycling cows (56.3%; 18/32) had a similar (P > 0.05) estrous response following PG1 than noncycling cows (38.0%; 19/50).

Ovulation rate following PG1 from either estrus or GnRH was similar (P > 0.05) for scan group Angus and Brangus cows, but tended (P = 0.09) to be greater for CYCH and CYCL compared to ANEST (Table 3-1). Size of the follicle ovulated following PG1 was similar (P > 0.05) for Angus and Brangus, but tended (P = 0.09) to be greater for CYCL and ANEST compared to CYCH (Table 3-1). There were no effects (P > 0.05) of breed × scan group on ovulation rate or ovulatory follicle size following PG1.

At PG2, concentrations of progesterone for all cows were similar (P > 0.05) for Angus and Brangus at 3.16 ± 0.4 and 4.00 ± 0.4 ng/mL, respectively. Concentrations of progesterone tended (P = 0.07) to be greater for CYCL (4.8 ± 0.7 ng/mL) compared to
CYCH (4.2 ± 0.7 ng/mL) and ANEST (4.2 ± 0.7 ng/mL). There were no (P > 0.05) effects of breed × cycling status on concentrations of progesterone at PG2. Size of the largest follicle and luteal volume were similar (P > 0.05) for Angus (13.4 ± 0.5 mm; 4925 ± 632 mm³) and Brangus (13.2 ± 0.5 mm; 4281 ± 632 mm³), as well as for CYCH (13.0 ± 0.6 mm; 4474 ± 766 mm³), CYCL (13.7 ± 0.6 mm; 4962 ± 766 mm³), and ANEST (13.3 ± 0.6 mm; 4327 ± 839 mm³). There were no (P > 0.05) effects of breed × scan group on largest follicle and luteal volumes at PG2. For all cows, Angus tended (P = 0.09) to have greater luteolysis following PG2 compared to Brangus (95.1%; 39/41 and 83.8%; 31/37, respectively). Luteolysis was similar (P > 0.05) for CYCH (75.0%; 9/12), CYCL (100.0%; 12/12), and ANEST (90.0%; 9/10), as well as for cows determined to be cycling (90.6%; 29/32) and noncycling (89.1%; 41/46) at the start of synchronization. There were no (P > 0.05) effects of breed × cycling status on luteolysis.

Distribution of estrus was similar (P > 0.05) for CYCH, CYCL, and ANEST cows, as well as for cycling and noncycling cows (data not shown). However, estrus distribution differed (P < 0.05) between breeds (Figure 3-2). Estrous response was similar (P > 0.05) for Angus and Brangus cows (Table 3-2). Estrous response was also similar (P > 0.05) for CYCH (66.7%; 8/12), CYCL (83.3%; 10/12), and ANEST (66.7%; 8/12), as well as for cycling (71.9%; 23/32) and noncycling (66.0%; 33/50) cows. There were no (P > 0.05) effects of breed × scan group or breed × cycling status on estrous response. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence estrous response. Additionally, the intensive ultrasonography cows (72.2%; 26/36) had a similar (P > 0.05) estrous response to cows that were not ultrasounded (65.2%; 30/46). Interval from PG2 to the onset of estrus was greater (P < 0.05) for
Angus compared to Brangus cows (Table 3-2). However, duration of estrus and total mounts received during estrus were similar (P > 0.05) for Angus and Brangus (Table 3-2). Interval from PG2 to the onset of estrus and estrous duration were similar (P > 0.05) for CYCH (55 h 55 m ± 4 h 44 m; 10 h 21 m ± 1 h 23 m), CYCL (65 h 17 m ± 4 h 14 m; 10 h 23 m ± 1 h 15 m), and ANEST (53 h 34 m ± 4 h 44 m; 9 h 9m ± 1 h 23 m) cows, respectively. Total mounts received during estrus were also similar (P > 0.05) for CYCH (55.3 ± 10.4), CYCL (38.5 ± 9.3), and ANEST (38.1 ± 10.4). Interval from PG2 to the onset of estrus, estrous duration, and total mounts received during estrus were similar (P > 0.05) for cycling and noncycling cows (data not shown).

Conception rate was similar (P > 0.05) for Angus and Brangus (Table 3-3). Conception rate was similar (P > 0.05) for CYCH (87.5%; 7/8), CYCL (60.0%; 6/10), and ANEST (75.0%; 6/8), as well as for cycling (73.9%; 17/23) and noncycling (69.7%; 23/33) cows. There were no (P > 0.05) effects of breed × scan group or breed × cycling status on conception rate. Interval from PG2 to the onset of estrus, and its interaction with breed and cycling status, did not (P > 0.05) influence conception rates. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence conception rate. Neither interval from PG2 to the onset of estrus nor interval from onset of estrus to time of AI influenced (P > 0.05) conception rate. There were no (P > 0.05) effects of AI technician on conception rates. Cows in the intensive ultrasonography group (73.1%; 19/26) had a similar (P > 0.05) conception rate to cows that were not ultrasounded (70.0%; 21/30).

Synchronized pregnancy rate was similar (P > 0.05) for Angus and Brangus (Table 3-3). Synchronized pregnancy rate was also similar (P > 0.05) for CYCH
(58.3%; 7/12), CYCL (50.0%; 6/12), and ANEST (50.0%; 6/12), and cycling (53.1%; 17/32) and noncycling (46.0%; 23/50) cows. There were no (P > 0.05) effects of breed × scan group or breed × cycling status on synchronized pregnancy rate. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence synchronized pregnancy rate. There were no (P > 0.05) effects of AI technician on synchronized pregnancy rates. Cows in the intensive ultrasonography group (52.8%; 19/36) had a similar (P > 0.05) synchronized pregnancy rate to cows that were not ultrasounded (45.7%; 21/46).

Breeding season pregnancy rate was similar (P > 0.05) for Angus and Brangus (Table 3-3). Breeding season pregnancy rate was also similar (P > 0.05) for CYCH (91.7%; 11/12), CYCL (75.0%; 9/12), and ANEST (91.7%; 11/12). There were no (P > 0.05) effects of breed × scan group on breeding season pregnancy rate. Cows that were cycling at the start of synchronization (84.4%; 27/32) had similar (P > 0.05) breeding season pregnancy rates to noncycling cows (84.0%; 42/50). Age of the cow influenced (P < 0.05) breeding season pregnancy rate. Three year old cows had a greater (P < 0.05) breeding season pregnancy rate (92.5%; 37/40) compared to two year old cows (76.2%; 32/42). Cows in the intensive ultrasonography group (86.1%; 31/36) had a similar (P > 0.05) breeding season pregnancy rate to cows that were not ultrasounded (82.6%; 38/46).

**Discussion**

On d 7 of MGA feeding, the largest follicle present on the ovary was similar between Angus Brangus. Previous work in cattle of *Bos indicus* influence indicates that they have smaller dominant follicle diameters (Bo et al., 1993a,b; Figueiredo et al.,
1997; Rhodes et al., 1995) compared to *Bos taurus* cows (Ginther et al., 1989; Kastelic et al., 1990; Bo et al., 1993b). The largest follicle on d 7 was greater for CYCL and ANEST cows compared to CYCH cows. This is likely due to the low circulating progesterone in CYCL and ANEST cows, resulting in increased LH pulse frequency (Roberson et al., 1989), leading to stimulated follicular growth and an extended period of dominance (Kojima et al., 1992). Cows in the CYCH group have a CL present and thus higher circulating concentrations of progesterone, which moderates follicle size by inhibiting the frequency of LH pulses.

A similar estrous response was observed in Angus and Brangus cows, as well as for ANEST, CYCL, and CYCH groups and cycling and noncycling cows. The MGA treatment induced estrus in only 38.0% (19/50) of anestrous cows, compared to 56.3% (18/32) of estrous cycling cows.

Ovulation rates after PG1, following either estrus or GnRH, were similar (94.4%) for Angus and Brangus cows. Ovulation rates tended to be greater for CYCL and CYCH compared to ANEST cows; however, this difference resulted from one ANEST cow failing to ovulate compared to 100% ovulation rates in CYCL and CYCH groups. Size of the follicle ovulated after PG1 was similar for Angus and Brangus cows, but tended to be greater for CYCL and ANEST compared to CYCH. Differences in ovulatory follicle sizes after PG1 between scan groups resulted from low circulating progesterone in CYCL and ANEST cows, leading to increased LH pulses (Roberson et al., 1989) and increased follicle size (Kojima et al., 1992) compared to CYCH with greater circulating progesterone.
At PG2, concentrations of progesterone were similar for Angus and Brangus, but tended to be greater for CYCL compared to CYCH and ANEST. This is interesting, as we expected to see greater concentrations of progesterone in CYCL and CYCH due to increased ovulation rates to GnRH in these groups. The CYCL cows ovulated larger follicles to GnRH, which may have yielded a more active CL. The CYCH cows ovulated smaller follicles and ANEST cows ovulated at a lower rate to GnRH, which could explain their reduced concentrations of progesterone. However, CL volumes and size of the largest follicle present on the ovary at PG2 were similar for Angus and Brangus cows, as well as for CYCL, CYCH, and ANEST groups. Angus cows tended to have greater luteolysis following PG2 compared to Brangus cows. This is different than observed following PG1 on d 7, but similar to previous studies (Lemaster et al., 2001). Reduced luteolysis from PG1 to PG2 in Brangus cows is likely due to the young age of the CL at PG2 and refractoriness often observed in the CL early in the luteal phase (Levy et al., 2000) compared to mostly older and more mature CLs at PG1.

Estrous response following PG2 was very good and was similar for Angus and Brangus, as well as for ANEST, CYCL, and CYCH, and cycling and noncycling cows. This suggests that the 7-11 protocol effectively synchronizes estrus in both cycling and noncycling Angus and Brangus cows. Distribution of estrus following PG2 was different for Angus and Brangus cows and Angus cows had an increased interval from PG2 to the onset of estrus compared to Brangus cows. A greater number of Brangus cows displayed estrus earlier (36 to 48 h) compared to Angus cows. A similar number of Angus and Brangus displayed estrus at 60 h, more Angus cows displayed estrus at 72 h, and a similar number of Angus and Brangus cows displayed estrus at 84 to 120 h. In
summary, Brangus cows exhibited estrus earlier following PG2 compared to Angus cows, which exhibited estrus later following PG2. Angus and Brangus cows also had similar estrus duration and received a similar number of mounts during estrus. Previous studies have shown mixed results for cows of *Bos indicus* influence having similar (Esterman et al., 2008) or decreased (Plasse et al., 1970; Randel, 1984; Pinheiro et al., 1998) estrus durations compared to *Bos taurus* cows. Past work has suggested cows of *Bos indicus* breeding have decreased estrus intensity (Rae et al., 1999; Esterman et al., 2007), but more recent work in our laboratory demonstrated similar, if not greater (Esterman et al., 2008), estrous intensity in Brangus cows. Interval from PG2 to the onset of estrus, duration of estrus, and mounts received during estrus were all similar for ANEST, CYCL, and CYCH, suggesting a synchronized wave of follicle development between the groups after MGA treatment followed by PG1 and GnRH to setup synchronization 7 d prior to PG2.

Conception rates were excellent in this experiment and were similar for Angus and Brangus cows, as well as ANEST, CYCL, and CYCH. Cycling and noncycling cows also had similar conception rates, collectively indicating that if cows exhibit estrus, conception rates are similar among experimental variables. Synchronized pregnancy rates were also similar for Angus and Brangus, ANEST, CYCL, and CYCH, and cycling and noncycling cows. This suggests that the 7-11 protocol can be effectively used in both cycling and anestrous Angus and Brangus cows, at different stages of their estrous cycle with success. Synchronized pregnancy rates in the current study were 20% less for Angus cows and 18% less for Brangus cows compared to the initial study by Kojima et al. (2000) using the 7-11 protocol; however, synchronized pregnancy rates in the
current study are about 7% greater compared to a Select Synch/CIDR protocol in *Bos indicus* × *Bos taurus* cows without a timed-AI (Lucy et al., 2001). To maximize pregnancy rates in cattle of *Bos indicus* influence, incorporation of a timed-AI is important to ensure all cows are inseminated and have the opportunity to become pregnant. The 7-11 protocol in the current study was limited by not all cows having the opportunity to be inseminated. Therefore, additional work should be done to evaluate the addition of a fixed-time AI to the 7-11 protocol in cattle of *Bos indicus* influence. Based on the distribution of estrus following PG2, a fixed-time AI should be successful at 60 to 66 h after PG2.

Breeding season pregnancy rates were similar for Angus and Brangus, ANEST, CYCL, and CYCH, and cycling and noncycling cows. Age of the cow influenced (P < 0.05) breeding season pregnancy rate. Three year old cows had greater breeding season pregnancy rates (92.5%) compared to two year old cows (76.2%). Two year old cows having their first calf are the most difficult subset of a herd to rebreed. Heifers bred to calve at two years of age take 20 to 40 d longer to resume ovarian function compared to mature cows (Wiltbank, 1970). Decreased pregnancy rates are frequently observed in first calf heifers compared to their older herdmates.

In summary, differences were observed in scan groups for d 7 largest follicle size, concentrations of progesterone, and ovulatory follicle sizes to GnRH, but at the time of PG2, cows appeared to be well synchronized and prepared to ovulate following PG2. Breed differences were observed in d 7 largest follicle sizes, luteal regression, and interval from PG2 to the onset of estrus, but overall estrous response and pregnancy rates were similar between Angus and Brangus cows. The low-cost
(approximately $10/cow) 7-11 synchronization protocol effectively synchronized estrus in both Angus and Brangus cows with overall synchronized pregnancy rates of 47.0 to 50.0%. This protocol was limited by not all cows having the opportunity to be inseminated. To optimize pregnancy rates to the 7-11 program, additional studies should address the incorporation of a timed-AI to allow more cows the opportunity to become pregnant to the synchronization.
Table 3-1. Effect of breed and scan group (cycling with high progesterone (CYCH), cycling with low progesterone (CYCL), and anestrous (ANEST)) on ovulation rates to GnRH and ovulatory follicle size (LS mean ± SE) in Angus and Brangus cows synchronized with a 7-11 synchronization protocol.a

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Follicles ovulating to GnRH, %b</th>
<th>Ovulatory follicle size following PG1, mm, (range)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>18</td>
<td>94.4 (18)</td>
<td>16.9 ± 0.8 (10 to 23)</td>
</tr>
<tr>
<td>CYCH</td>
<td>6</td>
<td>100.0 (6)</td>
<td>14.5 ± 1.2 (10 to 18)</td>
</tr>
<tr>
<td>CYCL</td>
<td>6</td>
<td>100.0 (6)</td>
<td>18.8 ± 1.4 (17 to 21)</td>
</tr>
<tr>
<td>ANEST</td>
<td>6</td>
<td>83.3 (6)</td>
<td>18 ± 1.4 (13 to 23)</td>
</tr>
<tr>
<td>Brangus</td>
<td>18</td>
<td>94.4 (18)</td>
<td>17.7 ± 0.8 (13 to 24)</td>
</tr>
<tr>
<td>CYCH</td>
<td>6</td>
<td>100.0 (6)</td>
<td>17.2 ± 1.3 (14 to 21)</td>
</tr>
<tr>
<td>CYCL</td>
<td>6</td>
<td>100.0 (6)</td>
<td>18.5 ± 1.3 (13 to 24)</td>
</tr>
<tr>
<td>ANEST</td>
<td>6</td>
<td>83.3 (6)</td>
<td>17.4 ± 1.4 (16 to 19)</td>
</tr>
</tbody>
</table>

P values

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>P = 0.09</td>
<td>P = 0.09</td>
<td></td>
</tr>
<tr>
<td>Breed × Group</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

a All cows were fed melengestrol acetate (MGA; 0.5 mg/head/d) for 7 d and administered prostaglandin F₂α (PGF₂α) on the last day of MGA. Cows received GnRH 4 d later and a second PGF₂α 7 d after GnRH. Estrus was detected for 5 d and cows that exhibited estrus were AI approximately 8 to 12 h later.

b Percentage of cows that ovulated to GnRH on d 11 divided by the total treated.

c Size of the largest follicle ovulating to GnRH after the first PGF₂α treatment.
Table 3-2. Estrous characteristics as determined by HeatWatch of Angus and Brangus cows synchronized with a 7-11 synchronization protocol. With the exception of estrous response, estrous characteristics are presented as LS means ± SE.\(^a\)

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>Estrous response (%)(^b)</th>
<th>Interval from PGF(_{2α}) to onset of estrus (hr, min)(^c)</th>
<th>Duration of estrus (hr, min)(^d)</th>
<th>Total mounts during estrus(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>44</td>
<td>70.5</td>
<td>61 h 5 m ± 2 h 24 m</td>
<td>9 h 19 m ± 41 m</td>
<td>38.2 ± 5.2</td>
</tr>
<tr>
<td>Brangus</td>
<td>38</td>
<td>65.8</td>
<td>52 h 12 m ± 2 h 41 m</td>
<td>9 h 17 m ± 46 m</td>
<td>46.6 ± 5.8</td>
</tr>
</tbody>
</table>

\(^a\) All cows were fed melengestrol acetate (MGA; 0.5 mg/head/d) for 7 d and administered prostaglandin F\(_{2α}\) (PGF\(_{2α}\)) on the last day of MGA. Cows received GnRH 4 d later and a second PGF\(_{2α}\) 7 d after GnRH. Estrus was detected for 5 d and cows that exhibited estrus were AI approximately 8 to 12 h later.

\(^b\) Percentage of cows displaying estrus 5 d after the second PGF\(_{2α}\) of the total treated.

\(^c\) Time from second PGF\(_{2α}\) administration to the first mount of estrus, as determined by HeatWatch.

\(^d\) Time from the first mount of estrus to the last mount of estrus, as determined by HeatWatch.

\(^e\) Total mounting events which occurred during estrus, as determined by HeatWatch.
Table 3-3. Estrous response and pregnancy rates of Angus and Brangus cows following synchronization with a 7-11 synchronization protocol.\(^a\)

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>Estrous response (%)(^b)</th>
<th>Conception rate (%)(^c)</th>
<th>Synchronized pregnancy rate (%)(^d)</th>
<th>Breeding season pregnancy rate (%)(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>44</td>
<td>70.5 (44)</td>
<td>67.7 (31)</td>
<td>47.7 (44)</td>
<td>81.8 (44)</td>
</tr>
<tr>
<td>Brangus</td>
<td>38</td>
<td>65.8 (38)</td>
<td>76.0 (25)</td>
<td>50.0 (38)</td>
<td>86.6 (38)</td>
</tr>
</tbody>
</table>

\(^a\) All cows were fed melengestrol acetate (MGA; 0.5 mg/head/d) for 7 d and administered prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) on the last day of MGA. Cows received GnRH 4 d later and a second PGF\(_{2\alpha}\) 7 d after GnRH. Estrus was detected for 5 d and cows that exhibited estrus were AI approximately 8 to 12 h later.

\(^b\) Percentage of cows displaying estrus 5 d after the second PGF\(_{2\alpha}\) of the total treated.

\(^c\) Percentage of cows pregnant to AI of the total that exhibited estrus and were AI.

\(^d\) Percentage of cows pregnant during the synchronized breeding of the total treated.

\(^e\) Percentage of cows pregnant during the 65 d breeding season of the total treated.
Figure 3-1. Description of 7-11 Synch protocol for all cows and cows within scan groups (cycling with high progesterone (CYCH), cycling with low progesterone (CYCL), and anestrous cows (ANEST)).
Figure 3-2. Distribution of estrus after the second PGF\(_{2\alpha}\) in Angus and Brangus cows following synchronization with a 7-11 synchronization protocol. No cows exhibited estrus during the first 36 h after CIDR removal. Distribution of estrus differed (P < 0.05) between breeds.
Inclusion of GnRH at the start of a synchronization protocol causes ovulation of dominant follicles greater than 9 mm in diameter (Sirois and Fortune, 1988; Guilbault et al., 1993). After GnRH induction of the LH surge and ovulation, estradiol concentrations are dramatically decreased in the peripheral circulation (Twagiramunga et al., 1994). The rapid decrease in estrogen results in an FSH surge, causing recruitment of a new wave of follicular growth 1 to 2 d later (Ko et al., 1991; Adams et al., 1992). Additionally, the FSH peak provided by the GnRH injection 2 to 4 h after treatment will stimulate the recruitment of a new follicular wave (Chenault et al., 1990; Rettmer et al., 1992). With a new wave of follicle growth, follicle development is more effectively synchronized. By synchronizing both the follicle development and luteal lifespan, a more synchronous estrus can be achieved (Thatcher et al., 1989; Coleman et al., 1991; Twagiramungu et al., 1992a,b).

Due to differences in the length of cow’s estrous cycle and number of follicular waves, predicting how effectively GnRH will ovulate follicles can be a challenge (Moreira, 2000). Treatment with GnRH is effective once a dominant follicle reaches 10 mm in diameter and continues to be effective until the follicle either ovulates on its own, becomes atretic (Guilbault et al., 1993), or has a declining number of LH receptors (Rollosson et al., 1994). Across all stages of the estrous cycle, it is estimated that about 50\% of Bos taurus cows will ovulate to GnRH (Martinez et al., 1999; Colazo et al., 2005). Cattle of Bos indicus breeding are known to have a greater an increased percentage of three and four follicular waves (Zeitoun et al., 1996; Viana et al., 2000; Martinez et al., 2003). With a greater number of multiple follicular waves, the windows
of opportunity for GnRH to ovulate a follicle are reduced. There have been no formative studies that have evaluated the effectiveness of GnRH to induce ovulation across several stages of the estrous cycle in cattle of *Bos indicus* breeding.

Therefore, the objectives of this experiment are to: 1) evaluate follicle development following ovulation or no ovulation to GnRH in anestrous Angus and Brangus cows, 2) evaluate luteolysis, estrous characteristics, and pregnancy rates following ovulation or no ovulation to GnRH in anestrous Angus and Brangus cows, 3) determine ovulation rates to GnRH in estrous cycling Angus and Brangus cows on days 2, 6, 10, 14, and 18 of their estrous cycles, and 4) evaluate luteolysis, estrous characteristics, and pregnancy rates following the Select Synch/CIDR + TAI protocol in cycling Angus and Brangus cows on days 2, 6, 10, 14, and 18 of their estrous cycles.

**Materials & Methods**

**Animals**

The experiment was conducted from March to June of 2007 at the University of Florida, Department of Animal Sciences, Santa Fe Beef Unit in two phases (phase 1 = anestrous cows; phase 2 = estrous cycling cows). Suckled Angus (n = 37) and Brangus (n = 37) cows were utilized for the experiment. Throughout the experiment, cows were housed in similar sized pastures by breed, and fed stored Bermuda grass hay *ad libitum* and a protein/energy supplement to meet their nutrient requirements. Calves remained with cows throughout the experiment and were separated briefly when cows were worked through the chute. In phase 1, blood samples were collected on d -12 and -2 by jugular venipuncture into evacuated tubes with an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ) for the evaluation of concentrations of progesterone to determine estrous cycling status. After collection, blood samples were
placed immediately on ice, transported back to the laboratory, centrifuged (3000 × g for 15 min), and plasma was separated and stored at -20°C until further analysis. A cow was deemed to have estrous cycles (cycling) if either sample had concentrations of progesterone ≥ 1 ng/mL and anestrous (noncycling) if concentrations of progesterone were < 1 ng/mL at both samples. Only cows determined to be anestrous were utilized for phase I of the experiment.

**Experimental Protocol**

In phase 1 (anestrous cows), day 0 was designated as the start of the synchronization protocol (Figure 4-1). At the start of synchronization, suckled Angus and Brangus cows were 2 years of age, BW was 472 ± 14 kg for Angus and 466 ± 14 kg for Brangus, body condition score (BCS: 1 = severely emaciated, 5 = moderate, 9 = very obese; Wagner et al., 1988) was 5.0 ± 0.1 for Angus and 5.2 ± 0.1 for Brangus, and Angus cows were 72.0 ± 4.6 days postpartum (DPP) while Brangus cows were 61.5 ± 4.6 DPP. Cow BW, BCS, and DPP were similar (P > 0.05) between Angus and Brangus cows.

On d 0 of the experiment, all cows received GnRH (100 µg; i.m., Cystorelin, Merial, Athens, GA) and a new CIDR (1.38 g progesterone; Eazi-Breed CIDR, Pfizer Animal Health, New York, NY). On d 2, ovulation status to GnRH was evaluated and six cows that ovulated to GnRH and six cows that failed to ovulate to GnRH within each breed group were selected to continue on the experiment. On d 7, CIDR were removed and cows received PGF (25 mg, i.m., Lutalyse, Pfizer Animal Health, New York, NY).

In phase 2 (estrous cycling cows), cows were pre-synchronized using a 7 d CIDR with PGF on d 7 so the cows would be on either day 2, 6, 10, 14, or 18 of their estrous
cycle (DOC) at the start of synchronization. During pre-synchronization, estrus was detected visually with the aid of estrous detection patches (Estrotect, Rockway, Inc., Spring Valley, WI), and the day each cow displayed estrus was determined to be day 0 of that cow’s estrous cycle. Pre-synchronization groups were staggered over several weeks prior to the synchronization in order for all cows to begin the synchronization on the same day. At the start of synchronization, suckled Angus cows were (mean ± SD) 5.3 ± 2.4 years of age and Brangus cows were 3.6 ± 1.1 years of age, BW was 542 ± 43 kg for Angus and 541 ± 63 kg for Brangus, BCS was 5.3 ± 0.4 for Angus and 5.4 ± 0.5 for Brangus, and Angus cows were 59.2 ± 7.0 DPP while Brangus cows were 54.7 ± 11.1 DPP. On day 0 of synchronization, all cows received GnRH and a CIDR with CIDR removal 7 d later concomitant with PGF (Figure 4-1). Following PGF, estrus was monitored using electronic heat detection monitors (HeatWatch, DDx, Boulder, CO) for 3 d. Cows were inseminated by a single AI technician 8 to 12 h after the onset of estrus. Cows not exhibiting estrus by 73 h post PGF were TAI and received GnRH at 73 to 75 h.

In both phases of the experiment, from d 0 to 10, and 7 d after estrus or administration of GnRH, plasma samples were collected by jugular venipuncture for evaluation of progesterone, and blood samples were treated as previously described. From d 0 to d 10 and 7 d after estrus, cows were also evaluated by transrectal ultrasonography using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 7.5 MHz transducer. At each ultrasonography evaluation, height and width of all luteal structures, luteal cavities, and follicles ≥ 5 mm in diameter were measured with the internal calipers of the
ultrasonography machine and their locations on the ovaries were recorded. Follicles were classified based on size into three categories by diameter: class I follicles (3 to 5 mm), class 2 follicles (6 to 9 mm), and class 3 follicles (≥ 10 mm). Volume of the corpus luteum (CL) was calculated using the formula for volume of a sphere ($\pi d^3/6$). When a luteal cavity was present, its volume was subtracted from the volume of the outer sphere resulting in net luteal volume (CL volume) represented by luteal tissue. On d 2 of the experiment, ultrasonography was conducted to determine if GnRH initiated ovulation, with ovulation defined as disappearance of the largest follicle from the previous ultrasonography examination.

Estrous detection data was recorded by Heatwatch, mounts received during estrus were recorded when a cow stood to be mounted by another cow, applying pressure to the Heatwatch transponder. The transponder recorded the time and duration of each mounting event that was ≥ 1 sec. Initiation of estrus was defined as three or more mounts within a 4-h period and the end of estrus defined was the last mount recorded prior to a period of extended inactivity of at least 8 h (Landaeta et al., 1999). Duration of estrus was calculated by subtracting the time of the initial mount from the time of the last mount. Interval from PGF to the onset of estrus was calculated as the interval from PGF to first mount as detected by HeatWatch. Total number of mounts received during estrus was also determined. Interval from estrus to AI was calculated by subtracting the time of AI from the time of the initial mount recorded by HeatWatch.

Cows were inseminated using frozen-thawed semen from 8 sires in Phase 1 and 22 sires in Phase 2, which were pre-assigned to cows before the start of the
experiment. Estrous detection as determined by HeatWatch and AI continued for an additional 25 d after the synchronized breeding. Angus and Brangus cows were then placed in individual breeding pastures with single bulls of good fertility for an additional 40 d breeding period. Pregnancy was diagnosed approximately 30 d after AI, at the end of the breeding season, and again 30 d later using a real-time B-mode ultrasound with a 5.0 MHz transducer.

**Definitions**

Corpus luteum regression was defined as the number of cows which had a CL as determined by ultrasonography evaluation or concentrations of progesterone ≥ 1 ng/mL on d 7 of the experiment and either exhibited estrus or had concentrations of progesterone of < 1 ng/mL by 72 h following PGF.

Estrous response was the number of cows displaying estrus as determined by HeatWatch during the 3 d after PGF divided by the total number of cows treated. Conception rate was the number of cows that displayed estrus, were inseminated, and became pregnant, divided by the number of cows that displayed estrus and were inseminated. Timed-AI pregnancy rate was the number of cows that did not display estrus, were timed-AI, and became pregnant, divided by the total number of cows that did not display estrus and were timed-AI. Synchronized pregnancy rate was the number of cows pregnant to the total AI divided by the total number of cows treated. Breeding season pregnancy rate was the number of cows pregnant at the end of a 65 d breeding season, which included the 25 d AI breeding and a 40 d clean-up bull breeding divided by the total number treated.
Radioimmunoassay

Concentrations of progesterone were determined in multiple assays by direct solid-phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA) without extraction as described by Seals et al. (1998) with intra- and interassay CV of 13.6 and 24.3%, respectively. Sensitivity of the assay was 0.01 ng/mL.

Statistical Analysis

The GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the statistical analysis of categorical data. In phase 1, the effects of breed and ovulation response to GnRH (+/-) were evaluated for estrous response, CL regression to PGF, conception rate, timed-AI pregnancy and synchronized pregnancy rate, while cow age, DPP, and BCS were included as covariates. In phase 2, the effect of breed, ovulation status to GnRH, and DOC were evaluated for estrous response, CL regression to PGF, conception rate, timed-AI pregnancy and synchronized pregnancy rate, while cow age, DPP, and BCS were included as covariates. For both phases, the effect of interval from PGF to the onset of estrus along with the two-way interaction with breed was evaluated for conception rate. For phase 1, the effect of breed, ovulation status to GnRH on d 0, day of synchronization, and appropriate interactions were evaluated for concentrations of progesterone, follicle numbers, and luteal tissue volumes from d 0 to d 9 using the GLIMMIX procedure of SAS with a poisson distribution. The random variable was cow (breed × DOC). For phase 2, the effect of breed, DOC, day of synchronization, and their appropriate interactions were evaluated for concentrations of progesterone, follicle numbers, and luteal tissue volumes from d 0 to d 9 using the GLIMMIX procedure of SAS with a poisson distribution. The random variable was cow (breed × ovulation status to GnRH on d 0). For both phases, the distribution of estrus after CIDR removal...
for breed, ovulation status to GnRH on d 0, and DOC (phase 2) were analyzed using
the LIFETEST procedure (survival analysis) of SAS.

Results

In anestrous cows (phase 1), ovulatory follicle size to GnRH on d 0 for cows in
the OVGnRH group was 13.2 ± 0.5 (10 to 17) mm. Ovulatory follicle size to GnRH on d
0 was similar (P > 0.05) for Angus (12.7 ± 0.7 mm) and Brangus (13.7 ± 0.7 mm) cows.

The day of dominant follicle emergence (the day of synchronization when the
ovulatory follicle was first identified) was similar (P > 0.05) for Angus and Brangus cows,
as well as cows that ovulated and did not ovulate to GnRH on d 0 (P > 0.05; Table 4-1).
There were no breed × OVGnRH effects on day of dominant follicle emergence.

From d 0 to d 9, the mean number of class I follicles (3 to 5 mm) was greater (P <
0.05) for Brangus (20.9 ± 1.6 follicles) compared to Angus (15.7 ± 1.6 follicles). There
was also a day of synchronization effect (P < 0.05) on the number of class I follicles. As
day of synchronization increased, the number of class I follicles increased (P < 0.05;
Figure 4-2). Whether cows ovulated (OVGnRH) or did not ovulate to GnRH (No
OVGNRH) on d 0 did not (P > 0.05) influence number of class I follicles. There were no
(P > 0.05) effects of breed × day, OVGnRH × day, breed × OVGnRH, or breed × day ×
OVGnRH on class I follicle numbers. From d 0 to d 9, the mean number of class 2
follicles (6 to 9 mm) was similar (P > 0.05) for Angus (0.80 ± 0.12) and Brangus (0.77 ±
0.12), as well as for OVGnRH and No OVGnRH. Day of synchronization affected (P <
0.05) class 2 follicle numbers and there was a day of synchronization × OVGnRH effect
(P < 0.05; Figure 4-3). There were no (P > 0.05) effects of breed × day, breed ×
OVGnRH, or breed × day × OVGnRH on class 2 follicle numbers. From d 0 to d 9, the
number of class 3 follicles (≥ 10 mm) was similar (P > 0.05) for Angus (1.57 ± 0.15) and
Brangus (1.38 ± 0.15). Mean number of class 3 follicles did not (P > 0.05) differ due to OVGnRH. There was a tendency (P = 0.08) for a breed × OVGnRH effect. Angus cows that did not ovulate to GnRH had a greater (P < 0.05) number of class 3 follicles (1.90 ± 0.20) compared to Angus that ovulated to GnRH (1.23 ± 0.20), but Brangus cows did not differ for number of class 3 follicles that ovulated to GnRH (1.41 ± 0.20), or did not ovulate to GnRH (1.33 ± 0.20). Day of synchronization affected (P < 0.05) class 3 follicle numbers and there was a day of synchronization × OVGnRH effect (P < 0.05; Figure 4-4). There were no (P > 0.05) effects of breed × day or breed × day × OVGnRH on class 3 follicle numbers.

Change in size of the eventual ovulatory follicle from d 0 to d 9 was affected (P < 0.05) by breed and there was (P < 0.05) a day of synchronization effect, but there was no effect of breed × day of synchronization (P > 0.05; Figure 4-5). Angus cows had greater (P < 0.05) ovulatory follicle size (17.6 ± 0.82 mm) compared to Brangus cows (14.9 ± 0.79 mm; Table 4-1). Cows that ovulated to GnRH on d 0 had similar (P > 0.05) ovulatory follicle sizes (15.8 ± 0.89 mm) to cows that did not ovulate to GnRH on d 0 (16.6 ± 0.93 mm) and there was no breed × OVGnRH effect on ovulatory follicle size. There was no effect of OVGnRH on change in size of the eventual ovulatory follicle (P > 0.05; data not shown). There were no (P > 0.05) effects of breed × day, breed × OVGnRH, day × OVGnRH or breed × day × OVGnRH on change in size of the eventual ovulatory follicle.

Following CIDR removal, the eventual ovulatory follicle growth rate was similar (P > 0.05) for Angus and Brangus cows, as well as for cows that ovulated and did not
ovulate to GnRH on d 0 (P > 0.05; Table 4-1). There were no breed × OVGnRH effects on eventual ovulatory follicle growth rate following CIDR removal.

Concentrations of progesterone from d 0 to the time of breeding were greater (P < 0.05) for Brangus (3.74 ± 0.17 ng/mL) compared to Angus (2.78 ± 0.17 ng/mL) cows. Progesterone profiles differed (P < 0.05) for day of the synchronization, but there was no (P > 0.05) day of synchronization × breed effect (Figure 4-6). Whether a cow ovulated to GnRH or not did not (P > 0.05) influence mean progesterone concentrations, but there was a day × OVGnRH effect (Figure 4-7). There were no effects (P > 0.05) of breed × day, breed × OVGnRH, or breed × day × OVGnRH on concentrations of progesterone.

Luteal volumes from d 0 to d 9 were similar (P > 0.05) for Angus and Brangus cows. Luteal volumes differed (P < 0.05) for day of synchronization (P < 0.05; data not shown). There were no effects (P > 0.05) of breed × day on luteal volume.

Luteal regression was 100.0% for both Angus and Brangus cows, as well as 100.0% for cows that ovulated to GnRH on d 0. Estrous response was similar (P > 0.05) for Angus and Brangus cows (Table 4-2). Estrous response was also similar (P > 0.05) for cows that ovulated to GnRH on d 0 and cows that did not ovulate to GnRH on d 0 (Table 4-2). There were no breed × ovulation to GnRH on d 0 effects on estrous response. When included as covariates, DPP and BCS did not (P > 0.05) influence estrous response. Interval from PGF to the onset of estrus was similar (P > 0.05) for Angus and Brangus cows (Table 4-2). However, cows that ovulated to GnRH on d 0 had a greater (P < 0.05) interval from PGF to the onset of estrus compared to cows that did not ovulate to GnRH on d 0 (Table 4-2). Duration of estrus tended (P = 0.06) to be
longer for Angus cows compared to Brangus cows, but was similar (P > 0.05) whether cows ovulated or did not ovulate to GnRH on d 0 (Table 4-2). There was an effect of breed × ovulation status to GnRH on d 0 on duration of estrus, but numbers were limited in this interaction (Table 4-2). The number of mounts received during estrus was similar (P > 0.05) for Angus and Brangus cows (Table 4-2). Cows that ovulated to GnRH on d 0 had a similar (P > 0.05) number of mounts (34.2 ± 3.8) compared to cows that did not ovulate to GnRH on d 0 (21.5 ± 6.0). The distribution of estrus following CIDR removal was similar for Angus and Brangus cows (P > 0.05; data not shown). Distribution of estrus following CIDR removal tended (P = 0.07) to be different for cows that ovulated and did not ovulate to GnRH on d 0, but this was a caused by only a single cow that did not ovulate to GnRH on d 0 displaying estrus.

Conception rate was similar (P > 0.05) for Angus and Brangus cows (Table 4-3). Cows that ovulated to GnRH on d 0 tended (P = 0.09) to have a greater conception rate (60.0%; 3/5) compared to cows that did not ovulate to GnRH on d 0 (0%; 2/2). There were no effects of breed × ovulation to GnRH on d 0 on conception rate. Timed-AI pregnancy rate was similar (P > 0.05) for Angus and Brangus cows (Table 4-3), as well as for cows that ovulated to GnRH on d 0 and cows that did not ovulate to GnRH on d 0 (14.3%; 1/7 and 50.0%; 5/10, respectively). There tended (P = 0.06) to be a breed × ovulation status to GnRH on d 0 effect on timed-AI pregnancy. Synchronized pregnancy rate was similar (P > 0.05) for Angus and Brangus cows, as well as for cows that ovulated or failed to ovulate to GnRH on d 0 (Table 4-3). There was no (P > 0.05) breed × ovulation status to GnRH on d 0 effect on synchronized pregnancy rate.
included as covariates, DPP and BCS did not (P > 0.05) influence conception rate, timed-AI pregnancy rate, or synchronized pregnancy rate.

In cows that were estrous cycling (phase 2), ovulation rate to GnRH on d 0 was similar (P > 0.05) between Angus and Brangus cows (Table 4-4). Day of the estrous cycle affected (P < 0.05) ovulation rate to GnRH. No cows (0/10) that were on d 2 of their estrous cycle at the start of synchronization ovulated to GnRH on d 0. Cows on d 6, 10, 14, and 18 of their estrous cycles had ovulation rates to GnRH of 100.0, 30.0, 70.0, and 70.0%, respectively. Average size of the follicle ovulated to GnRH was similar (P > 0.05) for Angus (13.9 ± 0.6 mm) and Brangus (14.1 ± 0.6 mm) cows. However, cows at DOC 6 and 14 ovulated smaller (P < 0.05) follicles to GnRH compared to cows at DOC 10 or 18 (Table 4-4).

The day of dominant follicle emergence was similar (P > 0.05) for Angus and Brangus cows (Table 4-5). Day of dominant follicle emergence differed for DOC groups (P < 0.05; Table 4-5). Cows at DOC 2 had the earliest emergence (0.24 ± 0.76). Cows at DOC 10 and 18 had a similar (P > 0.05) day of follicle emergence (3.04 ± 0.67 and 2.20 ± 0.63, respectively), which were earlier (P < 0.05) compared to cows at DOC 6 and 14, which had the latest day of follicle emergence (4.48 ± 0.67 and 4.10 ± 0.63, respectively). Whether a cow ovulated (3.4 ± 0.5) or failed to ovulate to GnRH (2.4 ± 0.5) did not (P > 0.05) affect day of dominant follicle emergence.

From d 0 to d 9, the number of class I follicles (≤ 5 mm) was greater (P < 0.05) for Brangus (33.5 ± 1.2 follicles/d) compared to Angus (27.5 ± 1.1 follicles/d). There was also a day of synchronization effect (P < 0.05) on the number of class I follicles. As day of synchronization increased, the number of class I follicles increased (P < 0.05;
Cow DOC and ovulation status to GnRH on d 0 did not influence class I follicle numbers. There were no effects of breed × day, DOC × day, breed × DOC, or breed × day × DOC on class I follicle numbers. From d 0 to d 9, the number of class II follicles (6 to 9 mm) was similar (P > 0.05) for Angus and Brangus, as well as for all DOC groups. Day of synchronization affected (P < 0.05) class II follicle numbers and there was a day of synchronization × breed effect (P < 0.05; Figure 4-9), suggesting a difference in follicle recruitment between the breeds. Ovulation status to GnRH on d 0 affected (P < 0.05) class II follicle numbers and there was an ovulation status to GnRH on d 0 × day effect (P < 0.05; Figure 4-10); however, there were no (P > 0.05) interactions with DOC or breed. There were no (P > 0.05) effects of DOC × day, breed × DOC, or breed × day × DOC on class II follicle numbers. From d 0 to d 9, the number of class III follicles (≥ 10 mm) was similar (P > 0.05) for Angus and Brangus. Cow DOC (P < 0.05) and day of synchronization (P < 0.05) affected class III follicle numbers and there was a DOC × day of synchronization effect (P < 0.05; Figure 4-11). Ovulation status to GnRH on d 0 affected (P < 0.05) class II follicle numbers and there was an ovulation status to GnRH on d 0 × day effect (P < 0.05; Figure 4-12); however, there were no (P > 0.05) interactions with DOC or breed.

Overall mean change in size of the eventual ovulatory follicle from d 0 to d 9 did not differ (P > 0.05) due to breed; however, there was a day of synchronization effect (P < 0.05; Figure 4-13). There tended (P = 0.10) to be an effect of breed × day of synchronization (Figure 4-13). Cow DOC also affected (P < 0.05) change in ovulatory follicle size and there tended (P = 0.10) to be an effect of DOC × day of synchronization.
(Figure 4-14). There were no (P > 0.05) effects of breed × DOC or breed × DOC × day of synchronization on change in size of the eventual ovulatory follicle.

Following CIDR removal, the eventual ovulatory follicle growth rate was similar (P > 0.05) for Angus and Brangus cows (Table 4-5). Ovulatory follicle growth rate differed for DOC groups (P < 0.05; Table 4-5). Follicle growth rate was slowest (P < 0.05) for cows at DOC 2 and 18, which were similar (P > 0.05) to each other. Cows at DOC 18 had similar (P > 0.05) follicle growth rates to cows at DOC 10. Follicle growth rates were similar (P > 0.05) for cows at DOC 6, 10, and 14. Whether a cow ovulated (0.95 ± 0.15 mm/d) or failed to ovulate to GnRH (0.96 ± 0.17 mm/d) did not (P > 0.05) affect follicle growth rate following CIDR removal.

Concentrations of progesterone from d 0 to the time of breeding were greater (P < 0.05) for Angus (5.48 ± 0.27 ng/mL) compared to Brangus (4.61 ± 0.27 ng/mL) cows. Progesterone profiles differed (P < 0.05) for DOC groups, as well as for day of the synchronization (P < 0.05), and there was an effect of DOC × day (P < 0.05), as described in Figure 4-15. There was an effect of breed × DOC (P < 0.05). This interaction was due to Angus having overall greater concentrations of progesterone than Brangus, but the magnitude of the difference varied from day to day, suggesting that physiologically, Angus cows may be more responsive to progesterone following GnRH. Ovulation status to GnRH on d 0 and it’s interaction with day of synchronization did not (P > 0.05) influence concentrations of progesterone. There were no effects (P > 0.05) of breed × DOC, breed × day, or breed × DOC × day on concentrations of progesterone.
Luteal volumes from d 0 to the time of breeding were similar (P > 0.05) for Angus and Brangus cows. Luteal volumes differed (P < 0.05) for each DOC group, as well as for each day of the synchronization (P < 0.05), and there was an effect of DOC × day (P < 0.05), as described in Figure 4-16. Luteal volume was affected (P < 0.05) by ovulation status to GnRH on d 0, but there was no (P > 0.05) ovulation status to GnRH on d 0 × day effect (Figure 4-17). There were no effects (P > 0.05) of breed × DOC, breed × day, or breed × DOC × day on luteal volume.

Luteal regression following PGF was similar (P > 0.05) for both Angus (92.0%; 23/25) and Brangus (92.0%; 23/25) cows. Luteolysis was similar (P > 0.05) for DOC groups, with 100.0% for cows at DOC 10, 14, and 18 and 80.0% for cows at DOC 2 and 6. Luteolysis was also similar (P > 0.05) for cows that ovulated (92.6%; 25/27) and did not ovulate (91.3%; 21/23) to GnRH on d 0. Ovulation rate following PGF was similar (P > 0.05) for Angus (88.0%; 22/25) and Brangus (92.0%; 23/25). Ovulation rate following PGF did not (P > 0.05) differ between DOC groups with ovulation rates of 70.0, 90.0, 90.0, 100.0, and 100.0% for cows at DOC 2, 6, 10, 14, and 18, respectively. Ovulation rate following PGF was similar (P > 0.05) for cows that ovulated (92.6%; 25/27) and did not ovulate (87.0%; 20/23) to GnRH on d 0. Ovulatory follicle size following PGF was similar (P > 0.05) for Angus (14.9 ± 0.6 mm) and Brangus (15.2 ± 0.6 mm) cows (Table 4-5). Ovulatory follicle size following PGF differed (P < 0.05) for DOC 2 (13.4 ± 0.9 mm), 6 (14.2 ± 0.8 mm), 10 (15.0 ± 0.8 mm), 14 (15.4 ± 0.8 mm), and 18 (16.9 ± 0.8 mm) and their tended (P = 0.06) to be a breed × DOC effect (Table 4-5). Ovulatory follicle size was also similar (P > 0.05) for cows that ovulated (15.4 ± 0.5 mm) and failed to ovulate to GnRH (14.7 ± 0.6 mm) on d 0.
Estrous response was greater (P < 0.05) for Brangus compared to Angus cows (Table 4-6). Estrous response differed (P < 0.05) for DOC groups (Table 4-6). Estrous response was greater (P < 0.05) for cows that ovulated to GnRH on d 0 (48.7%; 19/39) compared to cows that failed to ovulate to GnRH on d 0 (20.0%; 7/35). There were no (P > 0.05) effects of breed × ovulation status to GnRH on estrous response. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence estrous response. Interval from PGF to the onset of estrus was similar (P > 0.05) for Angus and Brangus cows, as well as for DOC groups (Table 4-6). Duration of estrus was similar (P > 0.05) for Angus and Brangus cows, as well as DOC groups (Table 4-6). Brangus cows received a greater (P < 0.05) number of mounts during estrus (53.9 ± 8.0 mounts) compared to Angus cows (20.3 ± 10.4 mounts). Number of mounts received during estrus was similar (P > 0.05) for DOC groups (Table 4-6). There were no (P > 0.05) breed × DOC effects on interval from PGF to the onset of estrus, estrus duration, or total mounts received during estrus. Distribution of estrus was similar (P > 0.05) for Angus and Brangus cows (Figure 4-18), cows that ovulated and failed to ovulate to GnRH on d 0, as well as DOC groups (data not shown).

Conception rates were similar (P > 0.05) for Angus compared to Brangus cows (Table 4-7). Conception rate did not differ due to DOC (Table 4-7). Cows that ovulated to GnRH on d 0 (47.4%; 9/19) had similar (P > 0.05) conception rates compared to cows that failed to ovulate to GnRH on d 0 (57.1%; 4/7). There were no (P > 0.05) effects of breed × ovulation status to GnRH on conception rates. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence conception rate.
Interval from PGF to the onset of estrus did not (P > 0.05) affect conception rates, and there was no effect of breed × interval PGF to the onset of estrus.

Timed-AI pregnancy rates were similar (P > 0.05) for Angus and Brangus cows (Table 4-7). Timed-AI pregnancy rate was affected (P < 0.05) by DOC (Table 4-7). Cows that failed to ovulate to GnRH on d 0 (42.9%; 12/28) tended (P = 0.09) to have a greater timed-AI pregnancy rate compared to cows that ovulated to GnRH on d 0 (20.0; 4/20). There were no (P > 0.05) effects of breed × ovulation status to GnRH on d 0 on timed-AI pregnancy rates. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence timed-AI pregnancy rate.

Synchronized pregnancy rate was similar (P > 0.05) for Angus and Brangus cows (Table 4-7). Cows 2, 6, and 18 DOC had similar (P > 0.05) synchronized pregnancy rates, but were lower (P < 0.05) compared to cows 10 and 14 DOC (Table 4-7). However, cows 10, 14, and 18 DOC were similar (P > 0.05) to each other. There were no (P > 0.05) effects of breed × DOC on synchronized pregnancy rates (Table 4-7). Synchronized pregnancy rates were similar (P > 0.05) for cows that ovulated to GnRH on d 0 (33.3%; 13/39) and cows that failed to ovulate to GnRH on d 0 (45.7%; 16/35). There were no (P > 0.05) effects of breed × ovulation status to GnRH on d 0 on synchronized pregnancy rates. When included as covariates, cow age, DPP, and BCS did not (P > 0.05) influence synchronized pregnancy rate.

**Discussion**

In phase 1, anestrous cows ovulated follicles to GnRH on d 0 that were of similar size to previous studies in both Angus and Brangus cows (Esterman et al., 2007), all of which were greater than 10 mm in diameter (Sirois and Fortune, 1988). The day of eventual ovulatory follicle emergence was similar for Angus and Brangus, as well as for
cows that ovulated to GnRH on d 0 and those that did not. However, a difference in day of follicle emergence would have been expected for cows that ovulated to GnRH on d 0 and those that did not. This suggests that anestrous cows that did not ovulate to GnRH on d 0 may be developing a follicle in response to the CIDR’s progesterone and GnRH induced LH and FSH at the same time as those that did ovulate. This implies that the developing follicle was not already present on the ovary at the time of CIDR insertion and that some anestrous cows have a potential ovulatory follicle that is non-responsive to GnRH.

A greater number of class I follicles were observed in Brangus compared to Angus cows. This increase of follicle numbers in cows of *Bos indicus* breeding has been observed in another study (Alvarez et al., 2000). No differences were observed in numbers of class 2 or 3 follicles between Angus and Brangus, which is different than Alvarez et al. (2000), who observed increased class 2 and 3 follicle numbers in Brahman cows. Alvarez et al. (2000) also observed a greater number of class 2 follicles compared to the current study. The number of class 1, 2, and 3 follicles increased as the day of synchronization increased. Subluteal levels of progesterone provided by the CIDR induce increased LH pulse frequency (Roberson et al., 1989), leading to stimulated follicular growth and an extended period of dominance (Kojima et al., 1992). Ovulation status to GnRH on d 0 did not influence the number of class 1, 2, or 3 follicles. There was also a day of synchronization × ovulation status to GnRH on d 0 effect on class 2 and 3 follicle numbers. This was caused by a consistent number of class 2 follicle numbers in anestrous cows that did not ovulate to GnRH, whereas, cows that ovulated to GnRH on d 0 had a reduced number of class 2 follicles from d 0 to d 3, then
a rise in class 2 follicles from d 4 to d 5 as a new dominant follicle grew up, and a
decline from d 6 to d 9 as the class 2 follicle became a class 3 follicle, and eventually
the ovulatory follicle. The interaction effect on class 3 follicle numbers was a result of
similar class 3 follicle numbers on d 0, reduced follicle numbers following ovulation to
GnRH from d 1 to d 2, and an increase over d 3 to d 5 as new class 3 follicles were
developed.

Brangus cows had a reduced change in the size of the eventual ovulatory follicle
compared to Angus cows. This agrees with previous literature that cows of *Bos indicus*
influence ovulate smaller follicles (Bo et al., 1993a,b; Figueiredo et al., 1997; Rhodes et
al., 1995) compared to *Bos taurus* (Ginther et al., 1989; Kastelic et al., 1990; Bo et al.,
1993b) cows, but different than other work in our laboratory suggesting similar ovulatory
follicle sizes between Angus and Brangus cows (Esterman et al., 2007). Interestingly,
there was no effect of ovulation status to GnRH on d 0 on change in size of the eventual
ovulatory follicle or post-PGF ovulatory follicle growth rate. We would have expected
differences in eventual ovulatory follicle sizes and an advanced growth rate in newly
developed follicles from cows that ovulated to GnRH on d 0, but this was not the case.
This suggests that LH pulses induced by subluteal levels of progesterone from the
CIDR may have played a bigger role in synchronizing follicle growth than the induction
of ovulation by GnRH on d 0.

Greater concentrations of progesterone were observed in anestrous Brangus
cows compared to anestrous Angus cows throughout the synchronization. This is
particularly interesting, as differences were apparent even when CL induced by
ovulation by GnRH on d 0 were not yet developed and functional. Brangus cows may
be metabolize less progesterone and maintain greater circulating concentrations of progesterone in response to the CIDR compared to Angus cows. With equal numbers of Angus and Brangus cows ovulating to GnRH on d 0 and no differences observed in luteal volumes, Brangus still maintained concentrations of progesterone of about 1 ng/ml greater than Angus throughout the synchronization treatment. Day of synchronization also affected circulating concentrations of progesterone. As expected, progesterone values were low on d 0 and increased with the addition of the CIDR treatment. Interestingly, despite 50% of the cows ovulating to GnRH on d 0 and developing a CL that was confirmed by ultrasound exams, there was no ovulation status to GnRH on d 0 effect on progesterone profiles. This suggests that progesterone production by the anestrous cow’s first CL may be reduced. However, there was an ovulation status to GnRH × day effect on progesterone profiles (Figure 4-3). Anestrous cows that did not ovulate to GnRH on d 0 had a peak progesterone concentration from d 2 to d 3, which then declined slowly until CIDR removal. Anestrous cows that ovulated to GnRH also observed a peak in progesterone concentration following CIDR insertion, which declined slowly until d 5, when the newly formed CL became functional and provided an additional rise in circulating concentrations of progesterone.

Luteolysis was 100% in anestrous Angus and Brangus cows in the current study. This is very good, especially considering all CL eligible for luteolysis were formed by ovulation to GnRH on d 0. Several studies have suggested reduced or incomplete luteolysis in cattle of Bos indicus breeding due to decreased responsiveness to PGF (Lemaster et al., 2001), but that was not observed in this experiment for anestrous cows induced to ovulate. Estrous response was similar in anestrous Angus cows compared
to anestrous Brangus cows. The estrous response was much lower than other reports in anestrous *Bos taurus* cows (Esterman et al., 2007) and may have been due to stress in this particular group of cows. Angus cows in both phases of this experiment were difficult to work through the facilities and were more anxious in the chute compared to the Brangus cows. The estrous response observed in anestrous Brangus cows in this experiment was at or slightly below average for *Bos indicus × Bos taurus* cows (Esterman et al., 2007). Cows that ovulated to GnRH on d 0 had similar estrous response compared to cows that did not ovulate to GnRH on d 0.

The interval from PGF to the onset of estrus was similar for Angus and Brangus cows. Cows that ovulated to GnRH on d 0 had a longer interval from PGF to the onset of estrus compared to cows that did not ovulate to GnRH on d 0. This is a result of the largest follicle being turned over on d 0, resulting in a new follicle having to grow and develop during the synchronization treatment. This new follicle may have taken longer to reach its steroidogenic capacity. However, it is interesting that estrus was delayed, while eventual ovulatory follicle size and growth rates were similar to cows that did not ovulate to GnRH on d 0. Estrus duration was shorter for Brangus compared to Angus cows (Table 4-2). Reduced estrus duration in cows of *Bos indicus* influence has been observed in several other studies (Plasse et al., 1970; Randel, 1984; Pinheiro et al., 1998). This short duration of estrus emphasizes the importance of using estrous detection aids in *Bos indicus* influenced cows, as short estruses may occur entirely during the night hours (Pinheiro et al., 1998). However, due to using the HeatWatch estrous detection system in the current study, Brangus cows were detected in estrus and had an overall greater estrous response compared to Angus cows. Estrus duration
in Angus cows in the current study was similar to other studies in *Bos taurus* cows (Esterman et al., 2007). Despite reduced duration of estrus in Brangus cows, a similar number of mounts were recorded for Angus and Brangus. This is in contrast to other studies demonstrating a decreased estrous intensity in cows of *Bos indicus* breeding (Rae et al., 1999). Cows that ovulated to GnRH on d 0 received a similar number of mounts during estrus compared to cows that did not ovulate to GnRH on d 0.

Similar conception rates were observed for Angus and Brangus cows. Cows that ovulated to GnRH on d 0 had greater conception rates compared to cows that did not ovulate to GnRH on d 0, but estrous response was reduced in this study, so the numbers for this measure were low. Reduced timed-AI pregnancy rates were observed for cows that ovulated to GnRH on d 0 compared to cows that did not ovulate to GnRH on d 0. This is a combination of reduced timed-AI pregnancy rates (14.3%) from what would be expected in anestrous cows (18 to 25%; Lemaster et al., 2001; Esterman et al., 2009) and excellent timed-AI pregnancy rates (50.0%) in cows that did not ovulate to GnRH on d 0. There was a breed × ovulation status to GnRH on d 0 effect on timed-AI pregnancy rate. Angus cows that ovulated to GnRH on d 0 had no cows become pregnant to the timed-AI, compared to 60.0% of Angus cows that did not ovulate to GnRH, 50.0% of Brangus cows that ovulated to GnRH, and 40.0% of Brangus that did not ovulate to GnRH becoming pregnant. The reason for the poor performance in anestrous Angus cows that ovulated to GnRH on d 0 is unknown. The reduced timed-AI pregnancy rates in these cows carried over to synchronized pregnancy rates, inducing a breed × ovulation status to GnRH effect on synchronized pregnancy rate for the same reasons. Overall, similar synchronized pregnancy rates were observed in
anestrous Angus and Brangus cows, as well as cows that ovulated and did not ovulate to GnRH on d 0.

In phase 2, estrous cycling Angus and Brangus cows had similar ovulation rates to GnRH on d 0. This is similar to other reports in our laboratory suggesting similar ovulation rates to GnRH in cows of *Bos indicus* breeding (Esterman et al., 2007). Day of the estrous cycle at initiation of the Select Synch/CIDR + TAI protocol affected ovulation rates to GnRH on d 0. As expected, no cows at DOC 2 ovulated to GnRH, as they had ovulated 2 d prior and have not had time to develop another dominant follicle ≥ 10 mm that would be eligible for ovulation. Cows at DOC 6 had 100% ovulation to GnRH on d 0. On d 6 of their cycle, these cows should all be in the peak of their first follicular wave and have a dominant follicle eligible for ovulation. Cows at DOC 10 and 14 had ovulation rates to GnRH of 30% and 70%. This indicates the difficulty in predicting when GnRH will be effective mid-cycle due to differences in the number of follicle waves, particularly in *Bos indicus* influenced cattle that are known to have increased incidence of estrous cycles with three to four follicular waves (Zeitoun et al., 1996; Alvarez et al., 2000; Viana et al., 2000; Martinez et al., 2003). Cows at DOC 18 had a 70% ovulation rate to GnRH. Size of the follicle ovulated to GnRH was similar for Angus and Brangus cows, which is similar to the follicle sizes observed in phase 1 with anestrous cows (Table 4-4). Ovulatory follicle sizes to GnRH differed for DOC groups. Cows at DOC 6 and 14 ovulated smaller follicles to GnRH compared to cows on DOC 10 and 18. Smaller follicles ovulated by DOC 6 and 14 are likely the result of GnRH inducing ovulation of follicles in an earlier stage of growth that had just deviated from their subordinate follicle that had not reached their maximum diameter yet. Larger
follicles ovulated by DOC 10 and 18 are most likely the result of GnRH inducing ovulation of follicles that are in a later stage of dominance that were present as large follicles for 1 to 3 d prior to GnRH.

Eventual ovulatory follicle emergence was similar for Angus and Brangus cows, similar to results in phase 1 with anestrous cows (Table 4-5). Day of emergence differed for DOC groups. Cows at DOC 2 emerged earliest, as no cows ovulated to GnRH on d 0 and many of these cows eventually ovulated a follicle that was present on the ovary at the time of CIDR insertion. Cows at DOC 6 and 14 had the latest emergence. Most DOC 6 and 14 cows ovulated to GnRH on d 0 and had to recruit and develop a new follicle, delaying their emergence compared to cows that had not ovulated to GnRH and may have already had a small follicle ready to develop. Cows at DOC 10 and 18 emerged intermediate to DOC 2 and DOC 6/14 due to having a mixed population of cows that ovulated and did not ovulate to GnRH on d 0. Within DOC 10 and 18, there were cows that had follicles present on the ovary to develop, as well as cows that ovulated to GnRH and had to recruit and develop a new follicle.

The number of class 1 follicles was greater for estrous cycling Brangus compared to estrous cycling Angus cows. This is in agreement with the results of phase 1 in anestrous cows. Also similar to phase 1 anestrous cows, estrous cycling cows had a greater number of class 1 follicles as the day of synchronization increased. Cow DOC did not influence class 1 follicle numbers. The number of class 2 follicles was similar for Angus and Brangus cows, similar to phase 1 in anestrous cows, and there was no effect of DOC. Interestingly, there was a day of synchronization × breed effect on class 2 follicle numbers, suggesting a difference in follicle recruitment between
breeds. Class 3 follicle numbers were not affected by breed. Cow DOC (P < 0.05) and DOC × day of synchronization (P < 0.05) effects were observed for class 3 follicle numbers. Cow DOC effects were particularly apparent in DOC 6, where all class 3 follicles were eliminated by ovulation to GnRH between d 1 and d 2, taking several days to develop another follicle to reach a class 3 size. Cow DOC effects were also apparent in DOC 2, as they had no class 3 follicles present on d 0 and their class 3 follicle numbers steadily grew as the day of synchronization progressed. Cows at DOC 10, 14, and 18 were not as predictable, as the class 3 follicle numbers varied with the mixed stages of follicle wave growth they were in.

Size of the eventual ovulatory follicle was similar for Angus and Brangus cows. This is unlike previous reports of smaller follicles ovulated by Bos indicus type cows compared to Bos taurus cows, and unlike phase 1 anestrous cows, where Angus cows ovulated larger follicles than Brangus cows. Day of synchronization affected change in size of the eventual ovulatory follicle, with the follicle growing larger with each day of synchronization, as expected. There tended to be a day of synchronization × breed effect on the change in size of the eventual ovulatory follicle, due to Brangus cows having larger follicles d 1 to 5 compared to Angus cows. By d 6, eventual ovulatory follicle sizes were similar for each breed on each day. Cow DOC affected the change in size of the eventual ovulatory follicle and follicle growth rate from PGF to time of breeding. The slowest growth rate was observed for DOC 2 and 18, in which most of the cows developed a follicle that was already present on the ovary at CIDR insertion. Faster growth rates were observed for DOC 6, 10, and 14 due to later emergence and the need to compensate on growth rate to reach an optimal ovulatory follicle size by the
time of breeding. Follicle growth rates were similar for Angus and Brangus cows, similar to phase 1 in anestrous cows.

Progesterone profiles were greater for estrous cycling Angus compared to Brangus cows. Angus cows averaged 0.87 ng/ml greater concentrations of progesterone, which is the opposite of anestrous cows in phase 1. This suggests that in estrous cycling cows, Angus may produce greater progesterone. Luteal volumes were similar for Angus and Brangus, so the increased progesterone is not a function of increased CL volume, but possibly a more steroidogenically active CL or subtle differences in how the different breeds metabolize progesterone. Previous work has shown increased progesterone output from a smaller CL in Bos indicus cows (Rhodes et al., 1982). Cow DOC affected progesterone profiles and luteal volumes. Cows at DOC 2 had low progesterone and small luteal volume at CIDR insertion and rose steadily until PGF. Cows at DOC 6 started with high progesterone and large luteal volumes, maintained these for a few days, then had an additional rise in progesterone and luteal volume with the addition of a secondary CL from ovulation to GnRH on d 0. Cows at DOC 10 and 14 started with high progesterone and large luteal volumes and maintained these levels until PGF. DOC 14 cows began to decline progesterone and luteal volumes around d 6, as this is when the CL would have been regressing if the cow had continued on a natural cycle. Similarly, DOC 18 cows started with moderate progesterone and luteal volumes at CIDR insertion and steadily declined through each day of synchronization, due to the CL’s natural lifespan ending and only 70% of these cows developing secondary CLs. There was an effect of breed × DOC due to Angus having overall greater concentrations of progesterone than Brangus, but the magnitude
of the difference varied from day to day, suggesting that physiologically, Angus cows may be more responsive to progesterone following GnRH.

Luteolysis was 92% for both Angus and Brangus cows, with no difference between breeds, similar to phase 1 in anestrous cows. Luteolysis was also similar for DOC groups. Ovulation rate after PGF was similar for Angus and Brangus cows. Cows at DOC 2 had reduced ovulation rates after PGF, indicating that cows early in their estrous cycle are not as effectively synchronized using this protocol. Ovulatory follicle sizes after PGF were similar for DOC 2, 6, 10, and 14, but greater for DOC 18. This supports the earlier ovulatory follicle emergence in DOC 18 cows, in additional to the longer period of follicle growth.

Estrous response was greater for Brangus compared to Angus cows. This is similar to phase 1 anestrous cows, but different than previous reports of similar estrous response between the two breed types (Esterman et al., 2007). Cows that ovulated to GnRH on d 0 had a greater estrous response compared to cows that did not ovulate to GnRH. This is what we would expect, as the effectiveness of GnRH on d 0 increases the synchrony of follicle development and should yield a greater response. However, this increase in estrous response for cows that ovulated to GnRH on d 0 is different than was observed in anestrous cows. The interval from PGF to the onset of estrus was similar for Angus and Brangus cows, as observed in phase 1. Estrus duration was similar for Angus and Brangus cows. Previous studies (Rae et al., 1999) have observed differences in estrus duration between the two breed types. Brangus cows in this experiment had similar estrus durations to those normally observed in Bos taurus cows (Esterman et al., 2007) and the Angus cows in this experiment had shorter estrus
durations compared to previous studies (Esterman et al., 2007) and anestrous cows in phase 1. Brangus cows also had a greater estrous intensity (measured by number of mounts) compared to Angus, which is different than the literature (Rae et al., 1999) and the estrus mounts received in phase 1 anestrous cows. This could be due in part to the extended estrus duration in Brangus cows in the current study. Estrous intensity was similar for DOC groups.

Conception rates were similar for Angus and Brangus cows, as observed in anestrous cows in phase 1. Conception rates were different for DOC groups. Cows at DOC 2 had no cows display estrus, so no cows were bred following estrus. Cows at DOC 6 had only 10% estrus response (one cow), but that single cow conceived. Cows at DOC 10 had a 30% estrous response with 100% conception rates, DOC 14 had a 60% estrous response and 33% conception rates, and DOC 18 had a 90% estrous response with 44% conception rates. Therefore, with low numbers in each category, it is difficult to compare conception rate data in this experiment. Cows that ovulated to GnRH and cows that did not ovulate to GnRH on d 0 had similar conception rates, suggesting that if a cow displays estrus following PGF that conception rates were similar in this experiment.

Timed-AI pregnancy rates were also similar for Angus and Brangus cows, similar to anestrous cows in phase 1. Cows that did not ovulate to GnRH on d 0 tended to have greater timed-AI pregnancy rates compared to cows that ovulated to GnRH on d 0. Interestingly, this same phenomenon was observed in phase 1, suggesting that if cows ovulate to GnRH on d 0, but fail to exhibit estrus following PGF, their fertility may be compromised. There was a DOC effect on timed-AI pregnancy rates; however, similar
to conception rates in the current study, limited numbers of cows submitted to the timed-AI make the results difficult to interpret.

Synchronized pregnancy rates were similar for estrous cycling Angus and Brangus cows. Cows initiating the Select Synch/CIDR + TAI protocol early in the estrous cycle (DOC 2 and 6) had reduced synchronized pregnancy rates compared to cows initiating the protocol later in the estrous cycle (DOC 10, 14, and 18). Synchronized pregnancy rates were similar for cows that ovulated to GnRH and cows that failed to ovulate to GnRH on d 0, which is similar to anestrous cows in phase 1.

Throughout both phases of the experiment, no differences were observed for cow age, DPP, and BCS for estrous response, conception rate, timed-AI pregnancy, or synchronized pregnancy rate. This is most likely due to the tight window of calving dates, similar BCS, and comparable ages represented by the cows in both phases of this experiment, or too few cows to accommodate the loss in degrees of freedom with additional covariation.
Table 4-1. Effect of breed and day of estrous cycle on day of ovulatory follicle emergence and ovulatory follicle growth rate from CIDR removal to AI (LS mean ± SE) in anestrous Angus and Brangus cows synchronized with a Select Synch + CIDR and TAI synchronization protocol (Phase 1).\(^a\)

<table>
<thead>
<tr>
<th>Variable(^b)</th>
<th>n</th>
<th>Day of ovulatory follicle emergence(^c)</th>
<th>Growth rate of ovulatory follicle from CIDR removal to AI, mm/d(^d)</th>
<th>Ovulatory follicle size following CIDR removal, mm(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>12</td>
<td>2.36 ± 0.45</td>
<td>0.75 ± 0.18</td>
<td>17.6 ± 0.82(^f)</td>
</tr>
<tr>
<td>Brangus</td>
<td>12</td>
<td>2.33 ± 0.43</td>
<td>0.54 ± 0.17</td>
<td>14.9 ± 0.79(^g)</td>
</tr>
<tr>
<td>OVGnRH</td>
<td>12</td>
<td>2.45 ± 0.45</td>
<td>0.64 ± 0.18</td>
<td>15.8 ± 0.89</td>
</tr>
<tr>
<td>No OVGnRH</td>
<td>12</td>
<td>2.25 ± 0.43</td>
<td>0.65 ± 0.17</td>
<td>16.6 ± 0.93</td>
</tr>
</tbody>
</table>

P values

<table>
<thead>
<tr>
<th>Breed</th>
<th>P &gt; 0.05</th>
<th>P &gt; 0.05</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVGnRH</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Breed × OVGnRH</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

\(^a\) All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF\(_{2\alpha}\) administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

\(^b\) OVGnRH = cows that ovulated to GnRH on d 0, No OVGnRH = cows that failed to ovulate to GnRH on d 0.

\(^c\) Day of synchronization when ovulatory follicle is first identified.

\(^d\) Growth rate (mm/day) of ovulatory follicle from CIDR removal to the time of AI.

\(^e\) Size of the ovulatory follicle after CIDR removal.
Table 4-2. Effect of breed and ovulation status to GnRH on d 0 on estrous characteristics following PGF$_{2\alpha}$ in anestrous Angus and Brangus cows synchronized with a Select Synch/CIDR + TAI synchronization protocol (Phase 1).\textsuperscript{a}

<table>
<thead>
<tr>
<th>Variable$^{b}$</th>
<th>n</th>
<th>Estrous response, %$^{c}$</th>
<th>Interval from PGF$_{2\alpha}$ to onset of estrus (hr, min)$^{d}$</th>
<th>Duration of estrus (hr, min)$^{e}$</th>
<th>Total mounts during estrus$^{f}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>12</td>
<td>16.7 (12)</td>
<td>49 h 5 min ± 3 h 44 min</td>
<td>16 h 20 min ± 3 h 53 min</td>
<td>32 ± 7.6</td>
</tr>
<tr>
<td>Brangus</td>
<td>12</td>
<td>41.7 (12)</td>
<td>49 h 6 min ± 2 h 21 min</td>
<td>5 h 21 min ± 2 h 27 min</td>
<td>30 ± 4.8</td>
</tr>
<tr>
<td>OVGnRH</td>
<td>12</td>
<td>41.7 (12)</td>
<td>51 h 16 min ± 1 h 30 min</td>
<td>6 h 8 min ± 3 h 1 min</td>
<td>34.2 ± 3.8</td>
</tr>
<tr>
<td>No OVGnRH</td>
<td>12</td>
<td>16.7 (12)</td>
<td>43 h 40 min ± 2 h 22 min</td>
<td>14 h 20 min ± 4 h 46 min</td>
<td>21.5 ± 6.0</td>
</tr>
</tbody>
</table>

P values

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P = 0.06</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>OVGnRH</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P = 0.04</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Breed $\times$ OVGnRH</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P = 0.04</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF$_{2\alpha}$ administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

\textsuperscript{b} OVGnRH = cows that ovulated to GnRH on d 0, No OVGnRH = cows that failed to ovulate to GnRH on d 0.

\textsuperscript{c} Percentage of cows displaying estrus 3 d after PGF$_{2\alpha}$ of the total treated.

\textsuperscript{d} Time from PGF$_{2\alpha}$ administration to the first mount of estrus, as determined by HeatWatch.

\textsuperscript{e} Time from the first mount of estrus to the last mount of estrus, as determined by HeatWatch.

\textsuperscript{f} Total mounting events which occurred during estrus, as determined by HeatWatch.
Table 4-3. Effect of breed and ovulation status to GnRH on d 0 on estrous response, conception, timed-AI pregnancy, and synchronized pregnancy rates following PGF$_{2\alpha}$ in anestrous Angus and Brangus cows synchronized with a Select Synch/ CIDR + TAI synchronization protocol (Phase 1).\(^a\)

<table>
<thead>
<tr>
<th>Variable(^b)</th>
<th>n</th>
<th>Estrous response, %(^c)</th>
<th>Conception rate, %(^d)</th>
<th>Timed-AI pregnancy rate, %(^e)</th>
<th>Synchronized pregnancy rate, %(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Angus</strong></td>
<td>12</td>
<td>16.7 (12)</td>
<td>50.0 (12)</td>
<td>30.0 (10)</td>
<td>33.3 (12)</td>
</tr>
<tr>
<td>OVGnRH</td>
<td>6</td>
<td>16.7 (6)</td>
<td>100.0 (1)</td>
<td>0.0 (5)</td>
<td>16.7 (6)</td>
</tr>
<tr>
<td>No OVGnRH</td>
<td>6</td>
<td>16.7 (6)</td>
<td>0.0 (1)</td>
<td>60.0 (5)</td>
<td>50.0 (6)</td>
</tr>
<tr>
<td><strong>Brangus</strong></td>
<td>12</td>
<td>41.7 (12)</td>
<td>40.0 (5)</td>
<td>42.9 (7)</td>
<td>41.7 (12)</td>
</tr>
<tr>
<td>OVGnRH</td>
<td>6</td>
<td>66.7 (6)</td>
<td>50.0 (4)</td>
<td>50.0 (2)</td>
<td>50.0 (6)</td>
</tr>
<tr>
<td>No OVGnRH</td>
<td>6</td>
<td>16.7 (6)</td>
<td>0.0 (1)</td>
<td>40.0 (5)</td>
<td>33.3 (6)</td>
</tr>
<tr>
<td><strong>P values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>OVGnRH</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P = 0.09</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Breed × OVGnRH</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P = 0.06</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

\(^a\) All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF$_{2\alpha}$ administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

\(^b\) OVGnRH = cows that ovulated to GnRH on d 0, No OVGnRH = cows that failed to ovulate to GnRH on d 0

\(^c\) Percentage of cows displaying estrus 3 d after PGF$_{2\alpha}$ of total treated.

\(^d\) Percentage of cows pregnant to AI of the total that exhibited estrus and were AI.

\(^e\) Percentage of cows pregnant to timed-AI of the total that were timed-AI.

\(^f\) Percentage of cows pregnant during the synchronized breeding of the total treated.
Table 4-4. Effect of breed and day of estrous cycle (DOC) on ovulation rates to GnRH and ovulatory follicle size (LS mean ± SE) in estrous cycling Angus and Brangus cows synchronized with a Select Synch/CIDR + TAI synchronization protocol (Phase 2). \(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Follicles ovulating to GnRH on d 0, %(^b)</th>
<th>Ovulatory follicle size to GnRH, mm, (range)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>25</td>
<td>56.0 (25)</td>
<td>13.9 ± 0.6 (11 to 17)</td>
</tr>
<tr>
<td>Brangus</td>
<td>25</td>
<td>52.0 (25)</td>
<td>14.1 ± 0.6 (10 to 18)</td>
</tr>
<tr>
<td>Day 2</td>
<td>10</td>
<td>0.0 (10)</td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>10</td>
<td>100.0 (10)</td>
<td>13.2 ± 0.6 (11 to 17)(^d)</td>
</tr>
<tr>
<td>Day 10</td>
<td>10</td>
<td>30.0 (10)</td>
<td>15.7 ± 1.0 (14 to 17)(^e)</td>
</tr>
<tr>
<td>Day 14</td>
<td>10</td>
<td>70.0 (10)</td>
<td>12.7 ± 0.7 (10 to 14)(^d)</td>
</tr>
<tr>
<td>Day 18</td>
<td>10</td>
<td>70.0 (10)</td>
<td>15.6 ± 0.7 (14 to 18)(^e)</td>
</tr>
</tbody>
</table>

P values

<table>
<thead>
<tr>
<th>Breed</th>
<th>P &gt; 0.05</th>
<th>P &gt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Breed × DOC</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

\(^a\) All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF\(_{2\alpha}\) administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

\(^b\) Percentage of cows that ovulated to GnRH on d 0 divided by the total treated.

\(^c\) Size of the largest follicle on d 0 that ovulated by 48 h later.

\(^d,e\) Means without a common superscript within a column differ (P < 0.05).
Table 4-5. Effect of breed and day of estrous cycle (DOC) on day of ovulatory follicle emergence and ovulatory follicle growth rate from CIDR removal to AI (LS mean ± SE) in estrous cycling Angus and Brangus cows synchronized with a Select Synch + CIDR and TAI synchronization protocol.\(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Day of ovulatory follicle emergence(^b)</th>
<th>Ovulatory follicle size growth rate from CIDR removal to AI, (\text{mm/d})(^c)</th>
<th>Ovulatory follicle size following CIDR removal, (\text{mm})(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>25</td>
<td>3.15 ± 0.43</td>
<td>1.03 ± 0.15</td>
<td>14.9 ± 0.58</td>
</tr>
<tr>
<td>Brangus</td>
<td>25</td>
<td>2.47 ± 0.42</td>
<td>0.84 ± 0.15</td>
<td>15.2 ± 0.57</td>
</tr>
<tr>
<td>Day 2</td>
<td>10</td>
<td>0.24 ± 0.76(^e)</td>
<td>0.29 ± 0.26(^e)</td>
<td>13.4 ± 0.90(^e,f)</td>
</tr>
<tr>
<td>Day 6</td>
<td>10</td>
<td>4.48 ± 0.67(^f)</td>
<td>1.21 ± 0.23(^f)</td>
<td>14.2 ± 0.78(^e,f)</td>
</tr>
<tr>
<td>Day 10</td>
<td>10</td>
<td>3.04 ± 0.67(^f,g)</td>
<td>1.20 ± 0.23(^f,g)</td>
<td>15.0 ± 0.78(^f,g)</td>
</tr>
<tr>
<td>Day 14</td>
<td>10</td>
<td>4.10 ± 0.63(^f)</td>
<td>1.23 ± 0.22(^f)</td>
<td>15.4 ± 0.74(^f,g)</td>
</tr>
<tr>
<td>Day 18</td>
<td>10</td>
<td>2.20 ± 0.63(^g)</td>
<td>0.68 ± 0.22(^e,g)</td>
<td>16.9 ± 0.74(^g,h)</td>
</tr>
</tbody>
</table>

**P values**

- Breed: \(P > 0.05\)
- DOC: \(P > 0.05\)
- Breed × DOC: \(P = 0.06\)

\(^a\) All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF\(_{2\alpha}\) administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

\(^b\) Day of synchronization when ovulatory follicle is first identified.

\(^c\) Growth rate (mm/day) of ovulatory follicle from CIDR removal to the time of AI.

\(^d\) Size of the ovulatory follicle after CIDR removal.

\(^e,f,g,h\) Means without a common superscript within a column differ (\(P < 0.05\)).
Table 4-6. Effect of breed and day of estrous cycle (DOC) on estrous characteristics as determined by HeatWatch of estrous cycling Angus and Brangus cows synchronized with a Select Synch/CIDR + TAI synchronization protocol. With the exception of estrous response estrous characteristics are presented as LS means ± SE.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Estrous response, %&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Interval from PGF&lt;sub&gt;2α&lt;/sub&gt; to onset of estrus (hr, min)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Duration of estrus (hr, min)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Total mounts during estrus&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>25</td>
<td>28.0 (25)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>49 h 2 m ± 3 h 39 m</td>
<td>8 h 46 m ± 1 h 14 m</td>
<td>20.3 ± 10.4&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brangus</td>
<td>25</td>
<td>48.0 (25)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>54 h 26 m ± 2 h 47 m</td>
<td>11 h 15 m ± 57 m</td>
<td>53.9 ± 8.0&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 2</td>
<td>10</td>
<td>0.0 (10)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Day 6</td>
<td>10</td>
<td>10.0 (10)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>64 h 24 m ± 8 h 48 m</td>
<td>12 h 15 m ± 3 h 28 m</td>
<td>30.0 ± 31.8</td>
</tr>
<tr>
<td>Day 10</td>
<td>10</td>
<td>30.0 (10)&lt;sup&gt;g,h&lt;/sup&gt;</td>
<td>62 h 20 m ± 5 h 5 m</td>
<td>11 h 38 m ± 2 h 0 m</td>
<td>61.7 ± 18.3</td>
</tr>
<tr>
<td>Day 14</td>
<td>10</td>
<td>60.0 (10)&lt;sup&gt;h,i&lt;/sup&gt;</td>
<td>50 h 2 m ± 3 h 35 m</td>
<td>11 h 18 m ± 1 h 25 m</td>
<td>49.7 ± 13.0</td>
</tr>
<tr>
<td>Day 18</td>
<td>10</td>
<td>90.0 (10)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>49 h 26 m ± 2 h 56 m</td>
<td>9 h 3 m ± 1 h 9 m</td>
<td>30.7 ± 10.6</td>
</tr>
</tbody>
</table>

**P values**

- **Breed**: P < 0.05   P > 0.05   P > 0.05   P < 0.05
- **DOC**: P < 0.05   P > 0.05   P > 0.05   P > 0.05
- **Breed × DOC**: P > 0.05   P > 0.05   P > 0.05   P > 0.05

<sup>a</sup> All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF<sub>2α</sub> administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

<sup>b</sup> Percentage of cows displaying estrus 3 d after PGF<sub>2α</sub> of the total treated.

<sup>c</sup> Time from PGF<sub>2α</sub> administration to the first mount, as determined by HeatWatch.

<sup>d</sup> Time from the first mount to the last mount, as determined by HeatWatch.

<sup>e</sup> Total mounting events which occurred during estrus, as determined by HeatWatch.

<sup>f,g,h,i</sup> Means without a common superscript within a column differ (P < 0.05).
Table 4-7. Effect of breed and day of estrous cycle (DOC) on estrous response and pregnancy rates in cycling Angus and Brangus cows synchronized with a Select Synch/CIDR + TAI synchronization protocol.\(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Estrous response, %(^b)</th>
<th>Conception rate, %(^c)</th>
<th>Timed-AI pregnancy rate, %(^d)</th>
<th>Synchronized pregnancy rate, %(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angus</td>
<td>25</td>
<td>28.0 (25)</td>
<td>57.1 (7)</td>
<td>38.9 (18)</td>
<td>44.0 (25)</td>
</tr>
<tr>
<td>Brangus</td>
<td>25</td>
<td>48.0 (25)</td>
<td>50.0 (12)</td>
<td>23.1 (13)</td>
<td>36.0 (25)</td>
</tr>
<tr>
<td>Day 2</td>
<td>10</td>
<td>0.0 (10)</td>
<td>0.0 (0)</td>
<td>10.0 (10)</td>
<td>10.0 (10)(^f)</td>
</tr>
<tr>
<td>Day 6</td>
<td>10</td>
<td>10.0 (10)</td>
<td>100.0 (1)</td>
<td>11.1 (9)</td>
<td>20.0 (10)(^f)</td>
</tr>
<tr>
<td>Day 10</td>
<td>10</td>
<td>30.0 (10)</td>
<td>100.0 (3)</td>
<td>57.1 (7)</td>
<td>70.0 (10)(^g)</td>
</tr>
<tr>
<td>Day 14</td>
<td>10</td>
<td>60.0 (10)</td>
<td>33.3 (6)</td>
<td>100.0 (4)</td>
<td>60.0 (10)(^g)</td>
</tr>
<tr>
<td>Day 18</td>
<td>10</td>
<td>90.0 (10)</td>
<td>44.4 (9)</td>
<td>0.0 (1)</td>
<td>40.0 (10)(^f,g)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P values</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>DOC</td>
<td></td>
<td>P &lt; 0.01</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Breed × DOC</td>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

\(^a\) All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF\(_{2\alpha}\) administered at the time of CIDR removal. Estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 73 to 75 h and given GnRH.

\(^b\) Percentage of cows displaying estrus 3 d after PGF\(_{2\alpha}\) of total treated.

\(^c\) Percentage of cows pregnant to AI of the total that exhibited estrus and were AI.

\(^d\) Percentage of cows pregnant to timed-AI of the total that were timed-AI.

\(^e\) Percentage of cows pregnant during the synchronized breeding of the total treated.

\(^f,g\) Means without a common superscript within a column differ (P < 0.05).
Figure 4-1. Description of experimental protocol for phase 1 (anestrous cows) and phase 2 (estrous cycling cows). In phase 1, n = 6 Angus (AN) that ovulated to GnRH (OVGnRH) on d 0, n = 6 Brangus (BN) that failed to ovulate to GnRH on d 0 (No OvGNRH), n = 6 AN No OVGnRH, and n = 6 BN No OVGnRH were selected for this experiment. In phase 2, n = 5 AN and n = 5 BN cows were selected for each of 2, 6, 10, 14, and 18 day of the cow’s estrous cycle (DOC) for the experiment. All cows were synchronized with a Select Synch/CIDR + TAI protocol.
Class I follicle numbers (3 to 5 mm diameter) of anestrous Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 1). Breed of cow (P > 0.05) and day of synchronization (P < 0.05) influenced the number of class I follicles present on the ovary. There was no (P > 0.05) effect of breed × day of synchronization.
Figure 4-3. Class 2 follicle numbers (6 to 9 mm diameter) of anestrous Angus and Brangus cows during a Select Synch/CIDR + TAI protocol (Phase 1). Day of synchronization (P < 0.05) and influenced the number of class 2 follicles present on the ovary. There was no (P > 0.05) effect of breed or whether a cow ovulated to GnRH (OVGnRH) or did not ovulate to GnRH (No OVGnRH), but there was a day of synchronization × OVGnRH effect (P < 0.05).
Figure 4-4. Class 3 follicle numbers (≥ 10 mm diameter) of anestrous Angus and Brangus cows during a Select Synch/CIDR + TAI protocol (Phase 1). Day of synchronization (P < 0.05) influenced the number of class 3 follicles present on the ovary. There was no (P > 0.05) effect of breed or whether a cow ovulated to GnRH (OVGnRH) or did not ovulate to GnRH (No OVGnRH) on class 3 follicle numbers. There was a day of synchronization × OVGnRH effect (P < 0.05).
Figure 4-5. Change in size of the eventual ovulatory follicle of anestrous Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 1). Day of synchronization (P < 0.05) and breed (P < 0.05) influenced the eventual ovulatory follicle size, but there was no (P > 0.05) day of synchronization × breed effect. Whether a cow ovulated to GnRH (OVGnRH) or did not ovulate to GnRH (No OVGnRH) did not (P > 0.05) affect eventual ovulatory follicle size.
Figure 4-6. Progesterone profiles of anestrous Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 1). Breed (P < 0.05) and day of synchronization (P < 0.05) influenced progesterone profiles, but there was no (P > 0.05) breed × day of synchronization effect.
Figure 4-7. Progesterone profiles of anestrous Angus and Brangus cows during a Select Synch/CIDR + TAI protocol (Phase 1). Day of synchronization influenced (P < 0.05) progesterone profiles. There was no (P > 0.05) effect of ovulation to GnRH on d 0 (OVGnRH) or no ovulation to GnRH on d 0 (No OVGnRH), but there was (P < 0.05) a day of synchronization × OVGnRH effect.
Figure 4-8. Class I follicle numbers (3 to 5 mm diameter) of estrous cycling Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 2). Breed of cow (P > 0.05) and day of synchronization (P < 0.05) influenced the number of Class I follicles present on the ovary. There was no (P > 0.05) effect of breed x day of synchronization.
Figure 4-9. Class 2 follicle numbers (6 to 9 mm diameter) of estrous cycling Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 2). Breed and day of cycle (DOC) that cows initiated the synchronization protocol on did not (P > 0.05) influence the number of class 2 follicles present on the ovary. Day of synchronization affected (P < 0.05) the number of class 2 follicles and there was (P > 0.05) a breed × day of synchronization effect.
Figure 4-10. Class 2 follicle numbers (6 to 9 mm diameter) of estrous cycling Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 2). Breed and day of cycle (DOC) that cows initiated the synchronization protocol on did not (P > 0.05) influence the number of class 2 follicles present on the ovary. Day of synchronization (P < 0.05) and ovulation status to GnRH (OVGNRH) affected (P < 0.05) the number of class 2 follicles.
Figure 4-11. Class 3 follicle numbers (≥ 10 mm diameter) of estrous cycling Angus (AN) and Brangus (BN) cows initiating a Select Synch/CIDR + TAI protocol on different days of the estrous cycle (DOC; Phase 2). Breed did not (P > 0.05) influence the number of class 3 follicles present on the ovary. Day of synchronization (P < 0.05), DOC (P < 0.05) and day of synchronization × DOC (P < 0.05) affected the number of class 3 follicles.
Figure 4-12. Class 3 follicle numbers (≥ 10 mm diameter) of estrous cycling Angus (AN) and Brangus (BN) cows initiating a Select Synch/CIDR + TAI protocol on different days of the estrous cycle (DOC; Phase 2). Breed did not (P > 0.05) influence the number of class 3 follicles present on the ovary. Day of synchronization (P < 0.05), DOC (P < 0.05), ovulation status to GnRH on d 0 (OVGnRH; P < 0.05), day of synchronization × OVGnRH (P < 0.05), and day of synchronization × DOC (P < 0.05) affected the number of class 3 follicles.
Figure 4-13. Change in size of the eventual ovulatory follicle of estrous cycling Angus (AN) and Brangus (BN) cows during a Select Synch/CIDR + TAI protocol (Phase 2). Breed did not (P > 0.05) influence eventual ovulatory follicle size. Day of synchronization (P < 0.05), DOC (P < 0.05), day of synchronization × DOC (P < 0.05), and day of synchronization × breed (P < 0.05) affected eventual ovulatory follicle size.
Figure 4-14. Change in size of the eventual ovulatory follicle of estrous cycling Angus (AN) and Brangus (BN) cows initiating a Select Synch/CIDR + TAI protocol on different days of the estrous cycle (DOC; Phase 2). Breed did not (P > 0.05) influence eventual ovulatory follicle size. Day of synchronization (P < 0.05), DOC (P < 0.05), day of synchronization × DOC (P < 0.05), and day of synchronization × breed (P < 0.05) affected eventual ovulatory follicle size.
Figure 4-15. Progesterone profiles of estrous cycling Angus (AN) and Brangus (BN) cows initiating a Select Synch/CIDR + TAI protocol on different days of the estrous cycle (DOC; Phase 2). Day of cycle that cows initiated the synchronization protocol on influenced (P < 0.05) their progesterone profiles on each day of synchronization.
Figure 4-16. Luteal volumes of estrous cycling Angus (AN) and Brangus (BN) cows initiating a Select Synch/CIDR + TAI protocol on different days of the estrous cycle (DOC; Phase 2). Day of cycle that cows initiated the synchronization protocol on influenced (P < 0.05) their luteal volumes on each day of synchronization.
Figure 4-17. Luteal volumes of estrous cycling Angus (AN) and Brangus (BN) cows initiating a Select Synch/CIDR + TAI protocol on different days of the estrous cycle (Phase 2). Ovulation status to GnRH on d 0 (OVGnRH) influenced ($P < 0.05$) their luteal volumes on each day of synchronization. There was no ($P > 0.05$) OVGnRH × day of synchronization effect.
Figure 4-18. Distribution of estrus after PGF$_{2\alpha}$ in estrous cycling Angus and Brangus cows following synchronization with a Select Synch/CIDR + TAI synchronization protocol. No cows exhibited estrus during the first 36 h after CIDR removal. Distribution of estrus was similar (P > 0.05) between breeds.
CHAPTER 5
COMPARISON OF A SELECT SYNCH/CIDR + TAI VS. A CO-SYNCH + CIDR ESTROUS SYNCHRONIZATION PROTOCOL IN SUCKLED BOS INDICUS × BOS TAURUS COWS

Development of an estrous synchronization protocol that achieves acceptable and repeatable synchronized pregnancy rates in cattle of *Bos indicus* breeding is particularly important to producers in subtropical regions of the United States and the world. Many synchronization protocols exist; however, nearly all of them were developed for *Bos taurus* cattle. A frequently used and effective synchronization protocol in *Bos taurus* cattle is administration of GnRH followed 7 d later with PGF$_{2\alpha}$ (Thatcher et al., 1989; Pursley et al., 1995). Limited research has employed the GnRH + PGF$_{2\alpha}$ protocol either with (Heirs et al., 2003) or without a progestogen (Lemaster et al., 2001) in *Bos indicus* × *Bos taurus* cattle with limited success. The unpredictability of these synchronization protocols in cattle of *Bos indicus* breeding make it difficult to utilize a fixed-time AI (TAI). Previous work in *Bos indicus* type cattle has demonstrated pregnancy rates of approximately 30% in a straight timed-AI protocol (Lemaster et al., 2001). Addition of progestogen can also increase the percentage of anestrous cows that exhibit estrus (Stevenson et al., 2000; Larson et al., 2006).

The intravaginal progesterone-releasing insert (CIDR) is an effective synchronization product that also induces estrus in some anestrous cattle (Lucy et al., 2001; Larsen et al. 2006). Synchronization using GnRH with a CIDR on d 0, then PGF$_{2\alpha}$ at CIDR removal on d 7, followed by estrous detection and AI is termed the Select Synch/CIDR protocol. Shortening of estrous detection to 3 d followed by a clean-up TAI of cows failing to exhibit estrus is termed the Select Synch/CIDR + TAI protocol (Larson et al., 2006). Pregnancy rates of 40 to 50% can be achieved in *Bos indicus* ×
Bos taurus cattle using this protocol (Esterman et al., 2006). However, this protocol is labor intensive and many producers are not willing to detect estrus in their cattle. Development of a TAI protocol in cattle of Bos indicus breeding that results in pregnancy rates comparable to the Select Synch/CIDR + TAI protocol would make utilization of AI a more feasible option.

Recent work with the CO-Synch + CIDR protocol with TAI at 48 h in Bos indicus × Bos taurus cattle yielded timed-AI pregnancy rates of only 33-39% (Saldarriaga et al., 2007). Often Bos indicus × Bos taurus cattle do not display estrus until ≥ 48 h post CIDR removal, and Saldarriaga et al. (2007) observed an average interval to estrus of 70 h and average interval to ovulation of 99 h in following a Select Synch/CIDR protocol, which is considerably greater than the interval to estrus of 55 h observed in Bos taurus cattle (Geary and Whittier, 1998; Martinez et al., 2000). This suggests that the TAI in a CO-Synch + CIDR program should probably be delayed in cattle of Bos indicus influence to optimize pregnancy rates. Hence, leaving the CIDR in the cows for an additional 12 h may allow further follicular development, which could allow for development of larger follicles prior to CIDR removal and possible to increase estrous response and improve the degree of estrous synchrony in, and fertility may be achieved. One study has been conducted in Bos indicus × Bos taurus cattle using a modified CO-Synch with TAI at 66 h, achieving about 47% timed-AI pregnancy rates (Zalauga et al., 2010), however cow numbers were limited.

The objective of this experiment was to evaluate the effectiveness of a Select Synch/CIDR + TAI protocol compared to a CO-Synch + CIDR protocol with TAI at performed at 60 to 66 h in suckled Bos indicus × Bos taurus cows.
**Materials & Methods**

**Animals**

This experiment was conducted from January to May of 2008 at the Bar L Ranch, Marianna, Florida and the University of Florida Beef Research Unit, Gainesville, Florida. Five groups of primiparous and multiparous suckled *Bos indicus* × *Bos taurus* cows (n = 659) were used. Cow age, estrous cycling status, BW, BCS, and days postpartum (DPP) for each group is described in Table 5-1. Across the five groups, mean (± SD) cow age was 5.3 ± 2.4 yr, DPP was 69.2 ± 15.0 d, BW was 537 ± 70 kg, and BCS was 5.3 ± 0.6 (BCS: 1 = severely emaciated, 5 = moderate, 9 = very obese; Wagner et al., 1988). Groups 1 (n = 173) and 3 (n = 193) contained 100% multiparous cows. Group 2 (n = 152) contained 61.2% primiparous cows (first calf at 2 yr old) with the remainder multiparous, group 4 (n = 94) contained 27.7% primiparous cows (first calf at 3 yr old) with the remainder multiparous, and group 5 (n = 47) contained 25.5% primiparous cows (first calf at 3 yr old) with the remainder multiparous. The degree of *Bos indicus* (Brahman) breeding was approximately 10 to 40% for groups 1, 2 and 3 with the remainder being *Bos taurus* (Angus, Charolais) breeding. For groups 4 and 5, breed composition was known and it was 25 to 75% *Bos indicus* with the remainder being *Bos taurus*. Groups 1, 2, and 3 were started on the experimental protocol in three consecutive weeks and groups 4 and 5 were started on the experimental protocol three weeks after group 3 and there was 3 weeks between starting each group. Calves remained with cows throughout the experiment and were separated briefly when cows were worked through the chute.
Experimental Protocol

On d -10, BW and BCS were recorded and blood samples were collected by jugular venipuncture into evacuated tubes with an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ) for the determination of concentrations of progesterone to evaluate estrous cycling status. After collection, blood samples were immediately placed on ice and transported to the laboratory where they were centrifuged (3000 × g for 15 min) and plasma was separated and stored at -20°C until further analysis. A cow was determined to have an estrous cycle (cycling) if either sample had concentrations of progesterone ≥ 1 ng/mL, and not have an estrous cycle (noncycling) if concentrations of progesterone were < 1 ng/mL at both samples. Plasma concentrations of progesterone were determined in multiple assays by direct solid-phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA) without extraction as described by Seals et al. (1998) with intra- and interassay CV of 8.3 and 21.5%, respectively. Sensitivity of the assay was 0.01 ng/mL.

At the start of the synchronization (d 0), cows were equally distributed by cow age, DPP, BCS, and breed when known to one of two treatments, which included Select Synch/CIDR + TAI (SSC) and a modified CO-Synch + CIDR (COS) protocol (Figure 5-1). Cows in both treatments received GnRH (100 µg i.m.; Cystorelin, Merial, Athens, GA) at CIDR (1.38 g progesterone; Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) insertion on d 0. On d 7, SSC cows CIDR were removed and cows received PGF$_2$α (25 mg i.m.; Lutalyse, Pfizer Animal Health, New York, NY). On d 7.5, COS cows CIDR were removed and cows received PGF$_2$α. All cows received an estrous detection patch.
(Estrotect, Rockway, Inc., Spring Valley, WI) at CIDR removal to aid in estrous detection.

Estrus was visually detected in both treatments two times daily at 0700 and 1700 h for approximately 1 h for 3 d following PGF$_{2\alpha}$. To aid in the detection of estrus, Estrotect detection patches were used (Estrotect, Rockway, Inc., Spring Valley, WI). Estrus was defined as a cow standing to be mounted by another cow and/or a half to fully activated Estrotect patch. Cows in the SSC treatment were inseminated 8 to 12 h after observed in estrus through 72 h after PGF$_{2\alpha}$. Cows in the SSC treatment that had not displayed estrus by 0800 h, 73 h after PGF$_{2\alpha}$ were exposed to fixed-time AI and administered GnRH (100 µg i.m.; Cystorelin) between 76 and 80 h after PGF$_{2\alpha}$. Cows in estrus at the 72 h observation were inseminated 8 to 12 h later, and not included in the timed-AI. Cows in the COS treatment were TAI between 60 to 66 h after PGF$_{2\alpha}$ and administered GnRH (100 µg i.m.; Cystorelin).

Frozen-thawed semen was used in all groups. In groups 1 and 2, all cows were inseminated by a single AI technician and bred to a single AI sire. In group 3, all cows were inseminated by two AI technicians and bred to a single AI sire. In groups 4 and 5, cows were bred to 20 pre-assigned AI sires dictated by the farm’s breeding program. In group 4 cows were inseminated by two AI technicians and in group 5 all cows were inseminated by a single AI technician. In groups 1, 2, 3, and 5, 7 d after the last cow was inseminated, clean-up bulls were placed with cows. In group 4, estrous detection continued for 30 d and cows displaying estrus were inseminated a second time. Pregnancy was diagnosed approximately 55 d after AI using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT).
with a 5.0 MHz transducer. Due to the 7 d period in which no cows were inseminated or bred by the clean-up bull, differences in fetal size (Curran et al., 1986) were used to determine whether a pregnancy resulted from the synchronized breeding or clean-up bull.

**Definitions**

Estrous response was the number of cows displaying estrus for 3 d after PGF$_{2\alpha}$ and inseminated divided by the total number of cows treated. Conception rate was the number of cows that displayed estrus, were AI and became pregnant, divided by the number of cows that displayed estrus and were AI. Timed-AI pregnancy rate was the number of cows that were timed-AI and became pregnant, divided by the total number of cows that were timed-AI. Synchronized pregnancy rate was the total number of cows that were inseminated and became pregnant, divided by the total number of cows treated. Thirty-day pregnancy rate was the number of cows pregnant during the first 30 d of the breeding season divided by the total number of cows treated.

**Statistical Analysis**

The GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the statistical analysis of this experiment. The effects of treatment, group, DPP, and all appropriate interactions, were evaluated for synchronized pregnancy, and thirty-day pregnancy rates using a logit link. Group was treated as a fixed variable. Cow age, DPP, BCS, and interval from PGF$_{2\alpha}$ to the onset of estrus were included as covariates. When covariates were significant (P < 0.05) they were treated as independent variables with BCS divided into three categories (≤ 4, 4.5, 5.0, ≥ 5.5). All BCS were represented in each group. The effect of interval from PGF$_{2\alpha}$ to the onset of estrus and its interaction with treatment, were evaluated for conception rate. The effects of AI sire
and AI technician were nested within groups and evaluated for synchronized pregnancy rates. Adjusted odds ratios and 95% confidence intervals were calculated for synchronized pregnancy rates between treatments.

**Results**

Synchronized pregnancy rates were similar (P > 0.05) for SSC (49.4%) and COS (47.1%) treatments (Table 5-2). Groups of replication differed (P < 0.05) in synchronized pregnancy rates (Figure 5-2). Cycling status did not (P > 0.05) influence synchronized pregnancy rates and there was no (P > 0.05) treatment × cycling status effect (SSC cycling 51.9%, n = 96/185; COS cycling 45.1%, n = 82/182; SSC non-cycling 46.3%, n = 68/147; COS non-cycling 49.7%, 72/145). Days postpartum did not influence (P > 0.05) synchronized pregnancy rates and there were no (P > 0.05) treatment × DPP effects. Body condition score influenced (P < 0.05) synchronized pregnancy rates (Table 5-3), but there were no (P > 0.05) treatment × BCS effects. Synchronized pregnancy rate was not (P > 0.05) influenced by cow age and there were no (P > 0.05) treatment × age effects. When nested within groups, there were no (P > 0.05) effects of AI sire or AI technician on synchronized pregnancy rates. For both treatments combined, interval from CIDR removal to the onset of estrus affected (P < 0.05) synchronized pregnancy rates. Cows exhibiting estrus at 36 h (73.5%; 25/34) and 48 h (69.4%; 86/124) had greater synchronized pregnancy rates compared to cows exhibiting estrus at 60 h (55.7%; 73/131). Cows exhibiting estrus at 72 h (56.9%; 29/51) had similar (P > 0.05) synchronized pregnancy rates to cows at 48 and 60 h, but were lower (P < 0.05) compared to cows exhibiting estrus at 36 h. Timed-AI pregnancy rate for SSC cows was 32.3% (53/164). There was no (P > 0.05) effect of interval from CIDR removal to the onset of estrus × treatment on synchronized pregnancy rates.
Estrous response over 3 d following PGF$_{2\alpha}$ for the SSC cows was 50.6% (168/332) and estrous response for the 2.5 d following PGF$_{2\alpha}$ for the COS cows was 52.6% (172/327). Estrous response was different (P < 0.05) between groups, but there was no treatment \times group effect. Estrous response for SSC cows was influenced (P < 0.05) by cycling status at the start of synchronization, with a greater number of noncycling cows (59.2%; 87/147) displaying estrus compared to cycling cows (43.8%; 81/185). A similar (P > 0.05) number of noncycling (57.2%; 83/145) and cycling (48.9%; 89/182) COS cows displayed estrus over the 2.5 d following PGF$_{2\alpha}$. Cow age, DPP, and BCS did not (P > 0.05) influence estrous response in SSC cows. In SSC cows that displayed estrus, the interval from PGF$_{2\alpha}$ to the onset of estrus was 59.1 ± 0.7 h. In COS cows that displayed estrus, the interval from PGF$_{2\alpha}$ to the onset of estrus was 51.0 ± 0.7 h.

The conception rate for SSC cows was 66.1% (111/168). Conception rate was not (P > 0.05) affected by group (data not shown) or cycling status (cycling 66.7%, 54/81; non-cycling 65.5%, 57/87). Interval from PGF$_{2\alpha}$ to the onset of estrus did not (P > 0.05) affect conception rates. Age, BCS, and DPP did not (P > 0.05) affect conception rate in SSC cows. In SSC cows that did not display estrous and were timed-AI, timed-AI pregnancy rate averaged 32.3% (53/164) across all groups. Timed-AI pregnancy rates differed between groups (P < 0.05; 44.2% (19/43), 34.8% (16/46), 27.9% (12/43), 29.4% (5/17), and 6.7% (1/15) for groups 1, 2, 3, 4, and 5, respectively) and cycling status (P < 0.05; cycling 40.4%, 42/104; non-cycling 18.3%, 11/60). Timed-AI pregnancy rate was not (P > 0.05) affected by age, DPP, or BCS.
Cows in the COS treatment which displayed estrus prior to the timed-AI had pregnancy rates of 59.3% (102/172). Pregnancy rates of COS cows which displayed estrus prior to the timed-AI was not (P > 0.05) influenced by cow age, DPP, or BCS. Interval from PGF$_{2\alpha}$ to the onset of estrus affected (P < 0.05) pregnancy rates in COS cows that displayed estrus prior to the timed-AI. Pregnancy rates were similar (P > 0.05) for cows displaying estrus at 36 h (73.5%; 25/34) and 48 h (63.9%; 39/61), which were greater (P < 0.05) compared to cows that displayed estrus at 60 h (49.4%; 38/77). Cows in the COS treatment that failed to display estrus prior to the timed-AI had pregnancy rates of 33.6% (52/155). Pregnancy rates of COS cows were not (P > 0.05) influenced by cow age, BCS, or DPP.

Thirty-day pregnancy rates were similar (P > 0.05) for SSC (80.1%; 209/261) and COS (81.7%; 210/257) treatments. There were also no group (P > 0.05; data not shown) or treatment × group (P > 0.05) effect on thirty-day pregnancy rate. Cycling status did not (P > 0.05) influence thirty-day pregnancy rates and there were no (P > 0.05) treatment × cycling status effects. Cow age, DPP, and BCS did not (P > 0.05) affect thirty-day pregnancy rate.

**Discussion**

Results of this experiment suggest that comparable synchronized pregnancy rates can be achieved using the COS synchronization protocol compared to the more labor-intensive SSC synchronization protocol in suckled cows of *Bos indicus* breeding.

Overall synchronized pregnancy rates of SSC cows in the current study were 13.9% greater than previous reports in *Bos indicus × Bos taurus* cows synchronized with a SSC system without a CIDR (Lemaster et al., 2001), but were 8.6% less than reports in *Bos taurus* cows synchronized with a SSC protocol with a CIDR (Larson et
The overall synchronized pregnancy rate of SSC cows in this study is comparable to other studies in *Bos indicus* × *Bos taurus* cows synchronized with a SSC system with a CIDR (Esterman et al., 2006). This emphasizes the importance of the CIDR in synchronizing *Bos indicus* × *Bos taurus* cows. Inclusion of a CIDR in a synchronization protocol prevents premature expression of estrus prior to PGF$_{2\alpha}$ (DeJarnette et al., 2001) and induces estrus in some anestrous cattle (Stevenson et al., 2000; Lucy et al., 2001; Larsen et al. 2006).

Previous studies using a traditional COS synchronization program have yielded reduced pregnancy rates in *Bos indicus* × *Bos taurus* cows. Lemaster et al. (2001) observed pregnancy rates of only 31.0% in *Bos indicus* × *Bos taurus* cows synchronized with a COS protocol without a CIDR with the timed-AI at 48 h post-PGF$_{2\alpha}$. Greater pregnancy rates of 33 to 39% were achieved by Saldarriaga et al. (2007) with the inclusion of a CIDR to the COS protocol with the timed-AI at 48 h post-PGF$_{2\alpha}$. While the COS programs achieve acceptable pregnancy rates in *Bos taurus* cows with timed-AI ranging from 48 to 60 h (Stevenson et al., 2003; Bremer et al., 2004; Larson et al., 2006), we believe that the timing of the timed-AI needs to be later than 48 h in cattle of *Bos indicus* influence. When a Select Synch/CIDR protocol is used with no timed-AI, it is common for no cows to display estrus prior to 48 h after PGF$_{2\alpha}$, with the average interval from PGF$_{2\alpha}$ to estrus 70 h and the interval from PGF$_{2\alpha}$ to ovulation 99 h (Saldarriaga et al., 2007). Compared to the average interval from PGF$_{2\alpha}$ to estrus of *Bos taurus* cows at 55 h (Geary and Whittier, 1998; Martinez et al., 2000; Lamb et al., 2004), this suggests that delaying the timed-AI in *Bos indicus* × *Bos taurus* cows is necessary to achieve optimal pregnancy rates. One study evaluating a delayed timed-
AI to 66 h after PGF$_{2\alpha}$ in Bos indicus × Bos taurus cows achieved pregnancy rates of about 45% (Zaluaga et al., 2010), but further research and greater cow numbers are needed to confirm these findings.

One reason the modified COS protocol may have been successful in this experiment (synchronized pregnancy rates of 44.0%) is the possible timing of GnRH treatment and the addition of all cows receiving GnRH treatment regardless of whether or not they displayed estrus. Additional GnRH causes a LH surge and a dominant follicle exposed to greater LH pulse frequency may have greater final maturation (Perry et al., 2007) and improved estradiol production prior to ovulation. Preovulatory estradiol is essential for preovulatory LH secretion (Baratta et al., 2001), and preparation of an ideal oviductal and uterine environment for the embryo to develop in (Miller et al., 1977; Geisert et al., 1988).

Another reason for the success of the modified COS may have been the 12 h delay in removing the CIDR to d 7.5 as opposed to the traditional d 7 CIDR removal. Recent work from our laboratory (Esterman et al., 2008) demonstrated slightly slower dominant follicle growth rates in Bos indicus × Bos taurus (0.84 mm/d) compared to Bos taurus (1.03 mm/d) cows. By extending the CIDR for an additional 12 h, further time is allowed for follicular growth and development. Work by Zaluaga et al. (2010) demonstrated that Bos indicus × Bos taurus cows synchronized with a COS protocol that had greater follicle sizes at timed-AI had greater pregnancy rates. This suggests that by allowing these cattle to develop larger follicles prior to CIDR removal, greater estrous response, estrous synchrony, and fertility may be achieved. While we did not measure follicle diameters in this experiment, extending the CIDR by 12 h to allow
further follicle growth and maturation may have allowed for growth of larger follicles with greater steroidic capacity at timed-AI.

One concern with delaying the timed-AI to 60 to 66 h was the potential reduction in pregnancy rates of the cows displaying estrus early (≤ 48 h) following PGF2α. From the time of insemination, it takes 4 to 8 h for the sperm to capacitate and reach the site of fertilization (Hunter and Wilmut, 1984; Hawk, 1987), which results in sperm arriving to the site of fertilization 20 to 28 h after estrus in cows which exhibited estrus early. Comprehensive studies using the HeatWatch system indicate that in beef cows ovulation occurs at 30 to 31 h after the onset of estrus or 13 to 16 h after the end of estrus (White et al., 2002). However, in traditional estrus detection systems, it is difficult to determine the precise timing of the start and end of estrus, as cows are only observed twice daily. In COS cows in current study, 10.4% exhibited estrus at 36 h and 29.1% at 48 h after PGF2α. While we expected decreased pregnancy rates in these cows, particularly those exhibiting estrus at 36 h, timed-AI pregnancy rate of 73.5% and 64.9% were observed for cows in estrus at 36 and 48 h, respectively. These timed-AI pregnancy rates were greater than for cows exhibiting estrus at 60 h, which were 49.4%. It was suggested by Saacke et al. (2000) that late insemination (> 12 h after onset of estrus) was preferable to early insemination (< 12 h after onset of estrus), as late insemination resulted in greater fertilization rates, but overall poorer embryo quality and early insemination resulted in decreased fertilization rates, but greater embryo quality (Saacke et al., 2000); however, we did not characterize this in the current study.

At the start of the experiment, a similar number of cows were cycling in four out of the five groups. The group with the lower percent estrous cycling (group 2, 38.2%;
58/152) had the greatest average DPP (75.4 ± 1.2 d), but were all two year old cows with their first calf, which is likely the reason for the reduced estrous cyclicity at the start of synchronization. With all groups combined, 55.7% (367/659) of cows were estrous cycling at the start of synchronization, which is greater than other reports in *Bos indicus* × *Bos taurus* cows (Lemaster et al., 2001), but lower than reports in *Bos taurus* cows (Larson et al., 2006). Cycling status and postpartum interval did not influence synchronized pregnancy rates, which is different than other reports in *Bos indicus* × *Bos taurus* (Lemaster et al., 2001) and *Bos taurus* cows (Larson et al., 2006). The primary reasons for cows to be noncycling at the start of the breeding season are nutritional status, as measured by BCS (Short et al., 1990) and DPP (Williams, 1990). In this experiment, the average BCS at the start of synchronization was 5.1 to 5.5, indicating that these cows were receiving adequate nutrition. Cows also averaged 53 to 75 DPP and we would expect that by this interval after calving that uterine involution would be complete and the cow would be physiologically prepared to initiate cyclicity. The addition of a CIDR in both the SSC and COS protocols likely aided in reinitiating estrous cycles in cows deemed noncycling at the start of the experiment. We expect we did not observe cycling status and postpartum interval effects in the current study for these reasons, and previous work in our laboratory (Esterman et al., 2008) has demonstrated similar results in cows with adequate BCS and DPP when synchronized with a CIDR protocol.

Cow BCS influenced synchronized pregnancy rates in the current study. Nutritional status has a direct influence on reproductive function and cycling status of suckled beef cows (Oyedipe et al., 1982) and BCS can be used as an indirect measure
of nutritional status (Richards et al., 1986). As expected, cows with lower BCS had lower synchronized pregnancy rates compared to cows with higher BCS. These findings are consistent with other reports stressing the importance of BCS on response to a synchronized AI breeding and subsequent pregnancy rates (Stevenson et al., 2000; DeJarnette et al., 2004; Larson et al., 2006).

In the current study, 50.6% of SSC cows exhibited estrus in the first 72 h after PGF$_{2\alpha}$. This is greater than $Bos$ $indicus \times Bos$ $taurus$ cows synchronized with the same protocol without a CIDR by Lemaster et al., 2001 (33.0%), but slightly less than $Bos$ $taurus$ cows synchronized with the same protocol including a CIDR (57.0%; Larson et al., 2006). The ability of a CIDR to induce estrous cycles in anestrous cows has been previously reported (Stevenson et al., 2000; Lucy et al., 2001; Larsen et al. 2006), and may have been the reason for increased estrous response in this study compared to the estrous response observed by Lemaster et al. (2001). Estrus is difficult to detect in cattle of $Bos$ $indicus$ breeding (Randel, 1984; Galina et al., 1996). Cows of $Bos$ $indicus$ breeding exhibit a shorter and less intense estrus (Plasse et al., 1970; Randel, 1984; Pinheiro et al., 1998), may show more subtle signs of estrus (Galina et al., 1982; Orihuela et al., 1983), have fewer mounts during estrus (Rae et al., 1999), and often initiate estrus during the evening hours (Pinheiro et al., 1998; Landaeta-Hernandez et al., 2002). It does not appear that estrous detection was a problem in this trial, because cows were intensely monitored by visual detection during the morning and early evening hours. Furthermore, Estrotect patches aided in detecting any estrus, which either began in, or occurred entirely during the evening hours.
Following estrus, the conception rate of SSC cows in the current study was 66.1%, which is greater than the 57.6% reported by Lemaster et al. (2001) in *Bos indicus × Bos taurus* cows synchronized using the same protocol without a CIDR. Inclusion of a CIDR and cows in greater BCS in the current study are likely the reasons for the 8.5% increase in conception rate. Larson et al. (2006) reported conception rates of 67.0% in *Bos taurus* cows with a CIDR and 70.0% without a CIDR, however cows were estrous detected and AI through 84 h after PGF$_{2\alpha}$, compared to 72 h after PGF$_{2\alpha}$ in the current study. Timed-AI pregnancy rate in SSC cows (32.3%) was greater than previous studies in both *Bos taurus* (Larson et al., 2006) and *Bos indicus × Bos taurus* (Lemaster et al., 2001) cows.

The goal of this experiment was to determine if AI pregnancy rates were comparable between the SSC system and the fixed TAI COS synchronization protocol. In this experiment, there was no significant difference in the pregnancy rates achieved with the SSC and COS systems. Cows synchronized with the SSC system were only 1.05% more likely to become pregnant to the synchronized breeding compared to cows in the COS treatment. This suggests that the less labor intensive COS protocol may be a useful alternative to the SSC protocol in cattle of *Bos indicus* breeding.
Table 5-1. General description of *Bos indicus* × *Bos taurus* cows synchronized with either a Select Synch/CIDR + TAI or CO-Synch + CIDR synchronization protocol by group. Mean ± SD (range).a

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cycling status (%)</th>
<th>Cow age, (range)</th>
<th>Body weight, kg (range)</th>
<th>Body condition score, 1-9, (range)</th>
<th>Days postpartum, (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>173</td>
<td>59.0 (173)c</td>
<td>6.5 ± 1.9 (3 - 10)b</td>
<td>557 ± 55 (418 - 705)b</td>
<td>5.4 ± 0.7 (3.5 - 7.0)b,c,d</td>
<td>74.0 ± 17.5 (48 - 128)b,c,d</td>
</tr>
<tr>
<td>2</td>
<td>152</td>
<td>38.2 (152)b</td>
<td>2.4 ± 0.6 (2-7)c</td>
<td>463 ± 49 (367 - 630)c</td>
<td>5.2 ± 0.5 (4.0 - 7.0)c</td>
<td>75.4 ± 16.3 (47 - 102)b,c,d</td>
</tr>
<tr>
<td>3</td>
<td>193</td>
<td>59.6 (193)c</td>
<td>6.6 ± 2.0 (4 - 10)b</td>
<td>562 ± 57 (432 - 730)b</td>
<td>5.5 ± 0.6 (4.0 - 7.0)d</td>
<td>62.8 ± 5.9 (54 - 99)c</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>64.9 (94)c</td>
<td>5.2 ± 2.2 (3 - 12)d</td>
<td>561 ± 61 (427 - 736)b</td>
<td>5.1 ± 0.6 (3.5 - 6.5)c</td>
<td>71.3 ± 13.2 (50 - 99)d</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>66.0 (47)c</td>
<td>5.1 ± 1.8 (3 - 8)d</td>
<td>557 ± 67 (397 - 736)b</td>
<td>5.1 ± 0.7 (3.5 - 6.5)c</td>
<td>53.8 ± 8.4 (41 - 69)e</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>659</td>
<td>55.7 (659)</td>
<td>5.3 ± 0.6 (2 - 12)</td>
<td>537 ± 70 (367 - 736)</td>
<td>5.3 ± 0.6 (3.5 - 7.0)</td>
<td>69.2 ± 15.0 (41 - 128)</td>
</tr>
</tbody>
</table>

P Value | P < 0.01 | P < 0.01 | P < 0.01 | P < 0.01 | P < 0.01 |

a All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF$_{2\alpha}$ administered at the time of CIDR removal. For Select Synch/CIDR + TAI cows, estrus was detected for 3 d and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 76 to 80 h and given GnRH. All CO-Synch + CIDR cows were fixed TAI at 60 to 66 h after PGF$_{2\alpha}$.

b,c,d,e Means without a common superscript within a column differ (P < 0.05).
Table 5-2. Synchronized pregnancy rates following PGF$_{2\alpha}$ in *Bos indicus* × *Bos taurus* cows synchronized with either a Select Synch/CIDR + TAI (SSC) or CO-Synch + CIDR (COS) synchronization protocol.$^{a}$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Pregnancy LS means ± SE</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC</td>
<td>332</td>
<td>0.46 ± 0.03</td>
<td>1.10</td>
<td>0.80 - 1.50</td>
<td>0.56</td>
</tr>
<tr>
<td>COS</td>
<td>327</td>
<td>0.44 ± 0.03</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF$_{2\alpha}$ administered at the time of CIDR removal. For Select Synch/CIDR + TAI cows, estrus was detected for 3 d, and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 76 to 80 h and given GnRH. All CO-Synch + CIDR cows were fixed TAI at 60 to 66 h after PGF$_{2\alpha}$. 
Table 5-3. Synchronized pregnancy rates following PGF$_{2\alpha}$ in Bos indicus × Bos taurus cows synchronized with either a Select Synch/CIDR + TAI (SSC) or CO-Synch + CIDR (COS) synchronization protocol by BCS.$^a$

<table>
<thead>
<tr>
<th>BCS</th>
<th>n</th>
<th>Pregnancy LS means ± SE$^b$</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 4</td>
<td>24</td>
<td>0.25 ± 0.03</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>94</td>
<td>0.43 ± 0.04</td>
<td>2.22</td>
<td>0.81 - 6.12</td>
<td>0.12</td>
</tr>
<tr>
<td>5.0</td>
<td>186</td>
<td>0.47 ± 0.05</td>
<td>2.64</td>
<td>1.00 - 6.95</td>
<td>0.05</td>
</tr>
<tr>
<td>≥ 5.5</td>
<td>355</td>
<td>0.52 ± 0.09</td>
<td>3.27</td>
<td>1.26 - 8.43</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$ All cows received GnRH at initiation of the 7 d CIDR treatment, with PGF$_{2\alpha}$ administered at the time of CIDR removal. For Select Synch/CIDR + TAI cows, estrus was detected for 3 d and cows that exhibited estrus were AI approximately 8 to 12 h later. Cows which had not displayed estrus were timed-AI at 76 to 80 h and given GnRH. All CO-Synch + CIDR cows were fixed TAI at 60 to 66 h after PGF$_{2\alpha}$.  

$^b$ Effects of treatment and group were included in the model. All BCS were represented within each group.
Figure 5-1. Description of Select Synch/CIDR + TAI (SSC) and CO-Synch + CIDR (COS) protocols.
Figure 5-2. Synchronized pregnancy rates of suckled *Bos indicus* × *Bos taurus* cows synchronized with a Select Synch/CIDR + TAI (SSC) or CO-Synch + CIDR (COS) protocol. Synchronized pregnancy rates were similar (P > 0.05) for SSC and COS treatments, but differed (P < 0.05) for group of replication. There were no (P > 0.05) treatment × group effects.
CHAPTER 6
DEVELOPMENT OF AN IMPROVED TIMED ARTIFICIAL INSEMINATION PROTOCOL IN BEEF HEIFERS AND COWS OF BOS INDICUS × BOS TAURUS BREEDING

Development of an effective timed-AI protocol in *Bos indicus* × *Bos taurus* cows and heifers which achieves consistent acceptable pregnancy rates is a challenge. While many synchronization protocols are successfully used in *Bos taurus* cattle, when implemented on *Bos indicus* × *Bos taurus* cattle results are inconsistent and often do not provide satisfactory pregnancy rates. Traditional synchronization protocols are based on control of follicle dynamics, regulation of luteal regression, and timing of ovulation. However, to optimize pregnancy rates from these protocols, steps should also be taken to promote development and ovulation of a competent dominant follicle that will have the best chance of developing into a successful pregnancy. Recent work by Sa Filho et al. (2009) suggests that synchronization protocols which reduce progesterone exposure and increase LH stimulation may enhance pregnancy rates in these cattle. Increased LH secretion may promote the ovulation of a more viable oocyte (Savio et al., 1993; Gong et al., 1995), increase estradiol production by the dominant follicle during proestrus (Bridges et al., 2007), and enhance the secretion of progesterone by the subsequent CL, all of which have the potential to increase fertility in cattle.

Therefore, the objective of Exp. 1 was to compare three synchronization treatments: 1) 5 d Select Synch/CIDR + TAI (5dCIDR), 2) 7 d Select Synch/CIDR + TAI (7dCIDR), and 3) modified 7 d CIDR protocol with PGF$_{2\alpha}$ at the start of synchronization to induce luteolysis, provide increased LH pulses, and stimulate follicle development (7dMOD) on subsequent pregnancy rates in yearling *Bos indicus* × *Bos taurus* heifers.
The objective of Exp. 2 was to compare the 5dCIDR and 7dMOD treatments and evaluate their pregnancy rates in suckled *Bos taurus* and *Bos indicus × Bos taurus* cows.

**Materials & Methods**

**Exp. 1 Animals**

Experiment 1 was conducted from December 2009 to April 2010 at the Rollins Ranches, Okeechobee, Florida. Yearling replacement heifers of *Bos indicus × Bos taurus* breeding (n = 343) were used. Mean (± SD) BW was 307.9 ±32.8 kg and BCS was 5.0 ± 0.6 (1 = severely emaciated, 5 = moderate, 9 = very obese; Wagner et al., 1988). On d -7, heifers were rectally palpated to assess reproductive tract score (RTS) to determine pubertal status (Anderson et al., 1991). Heifer RTS averaged 3.0 ± 1.3 and heifers were randomly distributed by RTS to three treatment groups.

**Exp. 1 Experimental Protocol**

Treatments included a 7 d Select Synch/CIDR + TAI (7dCIDR), 5 d Select Synch/CIDR + TAI (5dCIDR) or modified 7 d CIDR (7dMOD) estrous synchronization program (Figure 1). On d -7 of the experiment, heifers in the 7dCIDR treatment received a controlled intravaginal drug releasing device (CIDR, Pfizer Animal Health, New York, NY) and GnRH (100 µg i.m.; Cystorelin, Merial, Athens, GA). On d -7, heifers in the 7dMOD treatment received a CIDR and PGF$_{2α}$ (PGF; 25 mg i.m.; Lutalyse Sterile Solution, Pfizer Animal Health, New York, NY) to induce luteolysis of the CL. Two days following CIDR insertion (d -5) heifers in the 7dMOD treatment received GnRH. Heifers assigned to the 5dCIDR protocol received GnRH and a CIDR on d -5. On d 0, all treatments were administered PGF (25 mg i.m.; Lutalyse Sterile Solution)
and CIDRs were removed. All heifers also received a second injection of PGF (25 mg) approximately 8 h after the initial injection to ensure luteal regression.

Estrus was detected visually in all treatments two times daily at 0700 and 1700 h for 1 h at each observation over the 60 h following PGF. To assist with estrous detection, heifers were tail painted at the time of PGF. Estrus was defined as a heifer standing to be mounted by another heifer and/or rubbed tail paint. Heifers detected in estrus were inseminated approximately 8 to 12 h later. Heifers not detected in estrus by 60 h post CIDR removal were exposed to fixed-time AI and administered GnRH 100 µg i.m.; Cystorelin) at 72 h.

Frozen-thawed semen from three pre-assigned AI sires were used and heifers were inseminated by four AI technicians. Ten days following timed-AI, bulls were placed with the heifers for the remainder of the 120 d breeding season. Pregnancy was diagnosed approximately 55 d after AI using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 5.0 MHz transducer. Due to the 10 d period in which no heifers were inseminated or bred by the clean-up bull, differences in fetal size (Curran et al., 1986) were used to determine whether a pregnancy resulted from the synchronized breeding or clean-up bull.

Breeding season pregnancy rates were determined approximately 30 days after the end of the 120 d breeding season via ultrasonography.

**Exp. 1 Definitions**

Estrous response was the number of heifers displaying estrus for 60 h after CIDR removal and inseminated divided by the total number of heifers treated. Conception rate was the total number of heifers that displayed estrus, were inseminated, and became pregnant, divided by the total number of heifers that displayed estrus and were
inseminated. Timed-Al pregnancy rate was the total number of heifers that failed to display estrus, were timed-inseminated and became pregnant, divided by the total number of heifers failed to display estrus and were timed-inseminated. Synchronized pregnancy rate was the number of heifers that became pregnant to the synchronized breeding, divided by the total number of heifers treated. Thirty-day pregnancy rate was the number of heifers pregnant during the first 30 d of the breeding season divided by the total number of heifers treated. Breeding season pregnancy rate was the number of heifers pregnant at the end of the 120 d breeding season, divided by the total number of heifers treated.

Exp. 1 Statistical Analysis

The GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the statistical analysis of this experiment. The effects of treatment, RTS, and interaction, were evaluated for estrous response, conception rate, timed-Al pregnancy, thirty-day pregnancy, and breeding season pregnancy rates. Heifer BW, BCS, and interval from PGF to the onset of estrus were included as covariates. When covariates were significant (P < 0.05) they were treated as independent variables with BCS divided into three categories (≤ 4.5, 5.0, ≥ 5.5). The effect of Al sire and Al technician were also evaluated for synchronized pregnancy rates. The effect of interval from PGF to the onset of estrus and its interaction with treatment, were evaluated for conception rate.

Exp. 2 Animals

Experiment 2 was conducted from April to June 2010 at the University of Florida Santa Fe Beef Unit, Gainesville, Florida. Suckled cows of Bos taurus (Angus) and Bos indicus × Bos taurus (Brangus) breeding (n = 216) in two groups were used. The two groups consisted of early (n = 160) and late (n = 44) calving cows, which were started
on the synchronization program 35 d apart. Mean (± SD) cow age was 4.4 ± 2.2 yr, days postpartum (DPP) was 63.8 ± 19.1 d, BW was 517.3 ± 79.1 kg and BCS was 4.5 ± 0.5 at the start for the experiment for both groups. Estrous cycling status was similar (P > 0.05) between groups, but cow BW, BCS, DPP, and age differed (P < 0.05) between groups (Table 6-3). Throughout the experiment, cows were housed in pastures and fed stored Bermuda grass hay ad libitum and a protein/energy supplement to meet nutrient requirements. Calves remained with cows throughout the experiment and were separated briefly when cows were worked through the chute.

**Exp. 2 Experimental Protocol**

On d -14 and d -7, blood samples were collected by jugular venipuncture into evacuated tubes with an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ) for the determination of concentrations of progesterone to evaluate estrous cycle status. After collection, blood samples were immediately placed on ice, centrifuged (3000 × g for 15 min) and plasma was separated and stored at -20°C until further analysis. A cow was determined to have estrous cycles (cycling) if either sample had concentrations of progesterone ≥ 1 ng/mL, and no estrous cycles (noncycling) if concentrations of progesterone were < 1 ng/mL at both samples. Concentrations of progesterone were determined in multiple assays using direct solid-phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA) without extraction as described by Seals et al. (1998) with intra- and interassay CV of 9.3% and 11.7%, respectively. Sensitivity of the assay was 0.01 ng/mL. Cows were randomly distributed by DPP and BCS to the two treatment groups 5dCIDR or 7dMOD as described above. The GnRH used in this experiment was 100 µg i.m. Factrel, Ayerst, Canada.
Estrus was detected visually in both treatments two times daily at 0700 and 1700 h for 1 h at each observation during the 72 h following PGF. All cows received an estrous detection patch (Estrotect, Rockway, Inc., Spring Valley, WI) at CIDR removal to aid in estrous detection. Estrus was defined as a cow standing to be mounted by another cow and/or a half to fully rubbed Estrotect patch. Cows detected in estrus were inseminated approximately 8 to 12 h later. Cows not detected in estrus by 72 h post CIDR removal were timed-AI and administered GnRH at 72 h.

Frozen-thawed semen from 19 pre-assigned AI sires was used and cows were inseminated by two AI technicians (one AI technician for group 1 and one AI technician for group 2). In the first group, estrous detection continued for 30 d and cows displaying estrus were inseminated a second time. In group 2, 7 d after the last cow was inseminated, clean-up bulls were placed with cows. Pregnancy was diagnosed approximately 30 d after AI using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 5.0 MHz transducer. Due to the 7 d period in which no cows were inseminated or bred by the clean-up bull, differences in fetal size (Curran et al., 1986) were used to determine whether a pregnancy resulted from the synchronized breeding or clean-up bull. Breeding season pregnancy rates were determined approximately 30 days after the end of the breeding season via ultrasonography.

**Exp. 2 Definitions**

Estrous response was the number of cows displaying estrus for 3 d after PGF and inseminated divided by the total number of cows treated. Conception rate was the number of cows that displayed estrus, were AI and became pregnant, divided by the number of cows that displayed estrus and were AI. Timed-AI pregnancy rate was the
number of cows that were timed-AI and became pregnant, divided by the total number of cows that were timed-AI. Synchronized pregnancy rate was the total number of cows that were inseminated and became pregnant, divided by the total number of cows treated. Thirty-day pregnancy rate was the number of cows pregnant during the first 30 d of the breeding season divided by the total number of cows treated.

**Exp. 2 Statistical Analysis**

The GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the statistical analysis of this experiment. The effects of treatment, breed, group, cycling status, and all appropriate interactions, were evaluated for estrous response, conception rate, timed-AI pregnancy, and synchronized pregnancy rates. Cow DPP, BCS, and interval from PGF to the onset of estrus were included as covariates. When covariates were significant (P < 0.05) they were treated as independent variables. The effect of interval from PGF to the onset of estrus and its interaction with treatment, were evaluated for timed-AI pregnancy rate.

**Results**

In Exp. 1, the interval from PGF to the onset of estrus was similar (P > 0.05) for 7dCIDR (51.4 ± 1.1 h) and 7dMOD (49.0 ± 1.0 h). The 5dCIDR heifers had a greater (P < 0.05) interval from PGF to the onset of estrus (54.7 ± 1.4 h) compared to 7dMOD CIDR heifers and tended (P = 0.07) to have a greater interval from PGF to the onset of estrus compared to 7dCIDR heifers. Heifers in the 5dCIDR treatment had reduced estrous response (21.2%; 24/113) compared to 7dMOD (42.7%; 50/117) and 7dCIDR (34.5%; 39/113) treatments, which were similar (P > 0.05; Table 6-1) to each other. Estrous response was influenced (P < 0.05) by RTS (Table 6-2) with reduced estrous response for heifers at RTS 1 and 2 compared to heifers at RTS 3, 4, and 5. There
were no (P > 0.05) effects of treatment × RTS on estrous response. Heifer BW and BCS at the start of synchronization did not (P > 0.05) influence estrous response and there were no (P > 0.05) interactions of BW and BCS with treatment or RTS on estrous response.

Heifers in the 7dMOD treatment had a greater (P < 0.05) conception rate (62.0%; 31/50) compared to 7dCIDR (38.5%; 15/39) and 5dCIDR (33.3%; 8/24) treatments, which were similar (P > 0.05; Table 6-1). There were no (P > 0.05) effects of RTS (Table 6-2) or treatment × RTS on conception rate (data not shown). Heifer BW and BCS at the start of synchronization did not (P > 0.05) influence conception rates and there were no (P > 0.05) interactions of BW and BCS with treatment or RTS. Interval from PGF to the onset of estrus tended (P = 0.11) to affect conception rates. Heifers displaying estrus at 36 h (45.5%; 5/11) and 48 h (56.5%; 35/62) had similar (P > 0.05) conception rates. Heifers displaying estrus at 48 h achieved greater (P < 0.05) conception rates compared to heifers which displayed estrus at 60 h (35.0%; 14/40), but heifers in estrus at 60 h were similar (P > 0.05) to heifers in estrus at 36 h.

Timed-AI pregnancy rates were not (P > 0.05) influenced by treatment, RTS or treatment × RTS (Table 6-1, 6-2). Heifer BW and BCS at the start of synchronization did not (P > 0.05) influence timed-AI pregnancy rates and there were no (P > 0.05) interactions of BW and BCS with treatment or RTS.

Synchronized pregnancy rates were (P < 0.05) influenced by treatment (Table 6-1). Heifers in the 7dMOD treatment had a greater (P < 0.05) synchronized pregnancy rate (37.6%; 44/117) compared to 7dCIDR (23.0%; 26/113) and 5dCIDR (19.5%; 22/113) treatments, which were similar (P > 0.05) to each other. Synchronized
pregnancy rate was influenced (P < 0.05) by RTS (Table 6-2) with reduced synchronized pregnancy rates for heifers at RTS 1 and 2 compared to heifers at RTS 3, 4, and 5, but there was no (P > 0.05) effect of treatment × RTS (data not shown). Heifer BCS did not influence (P > 0.05) synchronized pregnancy rates and there were no (P > 0.05) interactions with RTS, but there was a BCS × treatment effect (P < 0.05). Heifers in 7dCIDR had synchronized pregnancy rates of 17.5% (7/40), 29.6% (13/44), and 20.7% (6/29), 7dMOD of 23.4% (11/47), 41.9% (18/43), and 55.6% (15/27), and 5dCIDR of 11.4% (5/44), 16.7% (6/36), and 33.3% (11/33), for BCS ≤ 4.5, 5, and ≥ 5.5, respectively. Heifer BW at the start of synchronization did not (P > 0.05) influence synchronized pregnancy rates and there were no (P > 0.05) interactions with treatment or RTS. There were effects (P < 0.05) of AI sire and AI technician on synchronized pregnancy rates, but there were no (P > 0.05) effects of AI sire × AI technician. The three AI sires used in this experiment had synchronized pregnancy rates of 19.4% (6/31), 26.0% (76/292), and 50.0% (10/20). The four AI technicians in this experiment had synchronized pregnancy rates of 21.8% (31/142), 22.3% (23/103), 27.8% (15/54), and 52.3% (23/44).

Thirty-day pregnancy rates were not (P > 0.05) influenced by 7dCIDR (56.5%; 64/113), 7dMOD (56.4%; 66/117), or 5dCIDR (55.8%; 63/113) treatments. Heifer RTS affected (P < 0.05) thirty-day pregnancy rates (Table 6-2) with reduced thirty-day pregnancy rates for heifers at RTS 1 and 2 compared to heifers at RTS 3, 4, and 5. There was no (P > 0.05) effect of treatment × RTS on thirty-day pregnancy rates (data not shown). Heifer BW at the start of synchronization did not (P > 0.05) influence thirty-day pregnancy rates and there were no (P > 0.05) interactions with treatment or RTS.
Heifer BCS at the start of synchronization affected (P < 0.05) thirty-day pregnancy rates, but there were no (P > 0.05) interactions with treatment or RTS. As BCS at the start of synchronization increased, thirty-day pregnancy rates increased (P < 0.05). Heifers BCS ≥ 5.5 achieved the greatest thirty-day pregnancy rates (74.2%; 66/89), followed by heifers BCS 5 (56.9%; 70/123), which were greater (P < 0.05) than heifers ≤ 4.5 (43.5%; 57/131).

Overall breeding season pregnancy rates were not (P > 0.05) affected by 7dCIDR (82.3%; 93/113), 7dMOD (82.1%; 96/117), or 5dCIDR (79.7%; 90/113) treatments. Heifer RTS affected (P < 0.05) breeding season pregnancy rates. Heifers with RTS 1 (54.0%; 28/51) and 2 (70.3%; 52/74) achieved similar (P > 0.05) breeding season pregnancy rates. Heifers with RTS 3 (84.2%; 64/76) were greater (P < 0.05) compared to RTS 1 and 2, but less (P < 0.05) than RTS 4 (94.9%; 93/98) and 5 (95.5%; 42/44), which were similar (P > 0.05). There was no (P > 0.05) effect of treatment × RTS on breeding season pregnancy rates (data not shown). Heifer BW at the start of synchronization did not (P > 0.05) influence breeding season pregnancy rates and there were no (P > 0.05) interactions of BW with treatment or RTS. Heifer BCS at the start of synchronization affected (P < 0.05) breeding season pregnancy rates, but there were no (P > 0.05) interactions with treatment or RTS. As BCS at the start of synchronization increased, breeding season pregnancy rates increased (P < 0.05). Heifers BCS ≥ 5.5 achieved the greatest breeding season pregnancy rates (94.4%; 84/89), followed by heifers BCS 5 (86.2%; 106/123), which were greater (P < 0.05) than heifers ≤ 4.5 (67.9%; 89/131).
In summary for Exp. 1, treatment affected (P < 0.05) estrous response, conception rate, and synchronized pregnancy rate, but not (P > 0.05) thirty-day pregnancy or breeding season pregnancy rates. Heifer RTS influenced estrous response, synchronized pregnancy, thirty-day pregnancy, and breeding season pregnancy rates. There were no (P > 0.05) treatment × RTS effects observed in this experiment. Heifer BCS at the start of synchronization affected (P < 0.05) thirty-day and breeding season pregnancy rates, but not (P > 0.05) estrous response, conception, timed-AI pregnancy, or synchronized pregnancy rates.

In Exp. 2 across both groups, estrous response was similar (P > 0.05) with 65.0% (65/100) of 5dCIDR cows and 73.8% (76/103) of 7dMOD cows displaying estrus over the 3 d following PGF (Table 6-4). Estrous response was influenced (P < 0.05) by estrous cycling status. Estrous cycling cows had a 77.2% (71/92) estrous response compared to 62.7% (69/110) for non-cycling cows (Table 6-5). There was no (P > 0.05) effect of breed or group of replication on estrous response in this experiment (Table 6-4). There were no (P > 0.05) significant two, three, or four way interactions of treatment, breed, group, and cycling status for estrous response. Cow DPP and BCS did not (P > 0.05) influence estrous response when included as covariates. The interval from PGF to the onset of estrus was similar (P > 0.05) between treatments at 62.0 ± 8.9 h for 5dCIDR cows and 61.7 ± 8.6 h for 7dMOD cows.

The conception rates for the 5dCIDR (63.1%; 41/65) and 7dMOD (60.5%; 46/76) were similar (P > 0.05). Conception rate was not (P > 0.05) affected by breed or cycling status (Table 6-4, 6-5). Conception rates differed (P< 0.05) between groups (Table 6-4). There were no (P > 0.05) significant two, three, or four way interactions of
treatment, breed, group, and cycling status on conception rates. Cow DPP and BCS did not (P > 0.05) influence conception rates when included as covariates. Interval from PGF to the onset of estrus did not (P > 0.05) affect conception rates. There was no (P > 0.05) effect of AI sire on conception rates.

For cows that did not display estrous and were timed-AI, timed-AI pregnancy rates were similar (P > 0.05) for treatment, group, and cycling status (Table 6-4, 6-5). Timed-AI pregnancy rates were greater (P < 0.05) for Angus (48.5%; 16/33) compared to Brangus (13.8%; 4/29) cows. There were no (P > 0.05) significant two, three, or four way interactions of treatment, breed, group, and cycling status on timed-AI pregnancy rates. Timed-AI pregnancy rate was not (P > 0.05) affected by DPP or BCS when included as covariates. There was no (P > 0.05) effect of AI sire on timed-AI pregnancy rates.

Synchronized pregnancy rates were similar (P > 0.05) for 5dCIDR (52.0%; 52/100) and 7dMOD (53.4%; 55/103) treatments as well as for estrous cycling (54.4%; 50/92) and non-cycling (50.9%; 56/110) cows. Groups of replication differed (P < 0.05) in synchronized pregnancy rates (Table 6-4). Synchronized pregnancy rates were greater (P < 0.05) for Angus (60.7%; 68/112) compared to Brangus (42.9%; 39/91) cows. There were no (P > 0.05) significant two, three, or four way interactions of treatment, breed, group, and cycling status on synchronized pregnancy rates. Synchronized pregnancy rate was not (P > 0.05) affected by DPP or BCS when included as covariates. There was no (P > 0.05) effect of AI sire on synchronized pregnancy rates.
In summary for Exp. 2, similar (P > 0.05) synchronized pregnancy rates were achieved using the 5dCIDR and 7dMOD synchronization protocols. Breed differences were observed (P < 0.05) in timed-AI and synchronized pregnancy rates. Differences (P < 0.05) in conception and synchronized pregnancy rates were observed between groups of replication. Estrous cycling cows displayed a greater (P < 0.05) estrous response, but were similar (P > 0.05) to non-cycling cows for conception, timed-AI pregnancy, and synchronized pregnancy rates.

**Discussion**

In Exp. 1, heifers in the 7dMOD treatment had greater (P < 0.05) synchronized pregnancy rates compared to heifers in the 5dCIDR or 7dCIDR treatments. Traditional synchronization protocols are based on control of follicle dynamics, regulation of luteal regression, and timing of ovulation. We hypothesized that steps should also be taken to promote development and ovulation of a competent dominant follicle that will have the greatest chance of developing into a successful pregnancy. By administering PGF to regress any existing CL, the ability of GnRH to ovulate follicles is enhanced. The advanced development encouraged by the reduced concentrations of progesterone and increased LH stimulation has the potential to increase fertility in cattle. Numerous studies have also shown the importance of follicle diameter at ovulation and the capacity of the follicle to produce estradiol on subsequent fertility (Mussard et al., 2003, 2007; Bridges et al., 2004; Perry et al., 2005). The results of this experiment support our hypothesis that providing a low progesterone environment during follicle development in the 7dMOD treatment enhances pregnancy rates in *Bos indicus × Bos taurus* heifers. The mechanisms by which this manipulation improves fertility is unknown and will require additional investigations; however, we believe the increased
fertility is a combination of increased ovulation rates, ovulation of a more viable oocyte, increased estradiol production by the dominant follicle, and enhanced progesterone production by the subsequent CL. Other methods of increasing LH stimulation on the ovulatory follicle have been investigated, including administering PGF on the seventh day of a nine-day CIDR protocol, use of multiple used CIDRs, and administering equine chorionic gonadotropin (eCG) at CIDR removal (Meneghetti et al., 2009; Sa Filho et al., 2009).

Heifers in the 7dMOD treatment had greater estrous response, conception rates, and synchronized pregnancy rates in Exp. 1 compared to 7dCIDR and 5dCIDR. The increased estrous response is likely due to the presence of a more mature follicle with greater steroidogenic capacity that is producing greater quantities of estradiol (Staigmiller et al., 1982). This carries over to increased conception rates, which we hypothesize are resulting from greater ovulation rates and the ovulation of a more viable oocyte. While treatment did not influence timed-AI pregnancy rates in heifers, the greater estrous response and conception rates culminated in greater synchronized pregnancy rates for heifers in the 7dMOD treatment compared to the 7dCIDR and 5dCIDR treatments. No treatment effects were observed for thirty-day or breeding season pregnancy rates. Other studies have demonstrated that use of exogenous progesterone may induce puberty in non-pubertal heifers, leading to greater pregnancy rates following the synchronization (Stevenson et al., 1996).

Heifer RTS affected estrous response, synchronized pregnancy, thirty-day pregnancy, and breeding season pregnancy rates. Reproductive tract scores are indicative of pubertal status and breeding potential of the heifer as described by
Anderson et al. (1991) and is an accurate and repeatable test (Rosenkrans and Hardin 2002). By evaluating RTS, heifers with poor potential to become pregnant to a synchronized breeding can be eliminated before the costs are incurred (LeFever and Odde 1986). Differences between *Bos taurus* and *Bos indicus × Bos taurus* have been observed, as *Bos indicus* females often have smaller, less prominent, and more difficult to detect ovarian structures compared to *Bos taurus* females (Segerson et al., 1984), which may lead to an overall lower detected RTS. Estrous response was greater in the current study for heifers with RTS 3, 4 and 5 compared to 1 and 2. This is similar to results from LeFever and Odde (1986) who observed > 90% of heifers with RTS 4 and 5 in estrus 4 d after synchronization compared to < 80% of heifers RTS 1, 2, and 3. Conception rates were not affected by RTS in the current study. Stevenson et al. (1996) observed that RTS was a significant predictor of conception rates in yearling *Bos indicus × Bos taurus* heifers; however, Rae et al. (1999) did not observe a strong correlation between RTS and first-service conception rates in two year old *Bos indicus* and *Bos indicus × Bos taurus* heifers. The effect of heifer RTS at the start of the breeding season also affected thirty-day and breeding season pregnancy rates in Exp. 1. Greatest conception rates are achieved on the second and third estrus following puberty (Byerly et al., 1987; Perry et al., 1991), and therefore, the sooner a heifer becomes pubertal, the sooner she will be eligible to become pregnant within the breeding season.

Heifer BCS at the start of synchronization did not affect estrous response, conception rate, or synchronized pregnancy rate. However, BCS influenced thirty-day and breeding season pregnancy rates, indicating that heifers of lower BCS at the start
of the breeding season were unable to catch up to heifers of greater BCS throughout the breeding season. Heifers of low BCS may not be receiving adequate nutrition to meet their growth requirements. Heifers must achieve approximately 60% of their mature BW to become pubertal (Dale et al., 1959) and at low BCS they will likely have a delay in the onset of puberty.

In Exp. 2, similar synchronized pregnancy rates were achieved using the 5dCIDR and 7dMOD synchronization protocols. Limited cow numbers and differences in cow age, BCS, DPP, and BW between the two groups may have prevented any underlying differences in synchronized pregnancy rate from reaching significance. Greatly reduced synchronized pregnancy rates were observed between groups. It is unclear why synchronized pregnancy rates were dramatically reduced in group 2, as these cows were greater in BW and BCS compared to group 1. Group 2 cows averaged 12 d shorter postpartum compared to group 1, but averaged 9.7% greater estrous cyclicity, indicating that despite being shorter postpartum, more cows in group 2 had initiated estrous cycles at the start of synchronization. Decreased synchronized pregnancy rates in group 2 may have resulted from problems with the semen or AI technician.

Estrous response was similar for cows in the 7dMOD and 5dCIDR treatment, unlike the results observed in Exp. 1. Conception rates were not affected by treatment, but differences were observed between groups. Greater conception rates were observed for group 1 compared to group 2. Similar to the group difference in synchronized pregnancy rates, it is unclear why conception rates were dramatically reduced in group 2.
Breed differences were observed in timed-AI and synchronized pregnancy rates, but not in estrous response or conception rates. Cattle of *Bos indicus* influence commonly display a decreased estrous response compared to *Bos taurus* cows due to exhibiting a shorter and less intense estrus (Plasse et al., 1970; Randel, 1984; Pinheiro et al., 1998), showing more subtle signs of estrus (Galina et al., 1982; Orihuela et al., 1983), having fewer mounts during estrus (Rae et al., 1999), and often initiating estrus during the evening hours (Pinheiro et al., 1998; Landaeta-Hernandez et al., 2002). It does not appear that estrous detection was a problem in this experiment, as cows were intensely monitored by visual detection during the morning and early evening hours and Estrotect patches aided in detecting any estrus, which either began in, or occurred entirely during the evening hours. However, recent studies from our laboratory have demonstrated similar estrous response between Angus and Brangus cows (Esterman et al., 2007; 2008). In cows that display estrus following PGF, conception rates are generally similar between Angus and Brangus cows (Esterman et al., 2007, 2008). Reduced timed-AI pregnancy rates were observed in Brangus cows (13.8%) compared to other studies (Lemaster et al., 2001). Timed-AI pregnancy rates observed in this experiment for Angus cows were greater than commonly observed in *Bos taurus* cows synchronized with similar protocols (Larson et al., 2006). Synchronized pregnancy rates were also reduced (P < 0.05) for Brangus cows (42.9%) compared to Angus cows (60.7%). Angus cows in the current study had synchronized pregnancy rates comparable to or greater than other *Bos taurus* cows synchronized with similar protocols (Larson et al., 2006; Esterman et al., 2008). Synchronized pregnancy rates in
Brangus were also similar to or greater than other studies in *Bos indicus × Bos taurus* cows (Lemaster et al., 2001; Esterman et al., 2008).

Estrous cycling cows displayed a greater estrous response, but were similar to non-cycling cows for conception, timed-AI pregnancy, and synchronized pregnancy rates. Additionally, no effects of BCS or DPP were observed for estrous response, conception, timed-AI pregnancy, or synchronized pregnancy rates. Other studies have demonstrated increased estrous response in estrous cycling versus noncycling cows in both *Bos indicus × Bos taurus* (Lemaster et al., 2001) and *Bos taurus* cows (Geary et al., 2000; Stevenson et al., 2000). However, no estrous cycling effects were observed for conception, timed-AI pregnancy, or synchronized pregnancy rates, which is different than that of other reports in *Bos indicus × Bos taurus* (Lemaster et al., 2001) and *Bos taurus* cows (Larson et al., 2006). Nutritional status is the primary reason for cows to be noncycling at the start of the breeding season and is generally measured by BCS (Short et al., 1990) and DPP (Williams, 1990). The average BCS at the start of synchronization in Exp. 2 was 4.2 to 5.1, indicating that these cows were receiving low to adequate nutrition. Cows also averaged 54 to 64 DPP and we would expect that by this interval after calving uterine involution would be complete and the cow would be physiologically prepared to initiate cyclicity. The addition of a CIDR in both protocols may have also aided in reinitiating estrous cycles in cows deemed noncycling at the start of the experiment. We expect we did not observe cycling status and postpartum interval effects in the current study for these reasons, and previous work in our laboratory (Esterman et al., 2008) has demonstrated similar results in cows of adequate DPP when synchronized with a CIDR protocol.
In summary, inclusion of PGF at the start of synchronization creates a low progesterone environment with potentially greater LH secretion during follicle development, which may yield a follicle with greater steroidogenic capacity and potentially ovulate a more viable oocyte. Further work will be required to determine LH secretion patterns and steroid output from the dominant follicle. The 7dMOD protocol displayed greater synchronized pregnancy rates in heifers compared to the 7dCIDR or 5dCIDR protocols. In cows, similar synchronized pregnancy rates were observed in the 7dMOD and 5dCIDR protocols.
Table 6-1. Treatment effects on estrous, conception and pregnancy rates of *Bos indicus* × *Bos taurus* heifers synchronized with a 7 d CIDR, modified 7 d CIDR, or 5 d CIDR protocol.

<table>
<thead>
<tr>
<th>Treatments&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N</th>
<th>Estrous response (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Conception rate (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Timed-AI pregnancy rate (%)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Synchronized pregnancy rate (%)&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>7dCIDR</td>
<td>113</td>
<td>34.5 (113)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>38.5 (39)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.9 (74)</td>
<td>23.0 (113)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>7dMOD</td>
<td>117</td>
<td>42.7 (117)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>62.0 (50)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>19.4 (67)</td>
<td>37.6 (117)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>5dCIDR</td>
<td>113</td>
<td>21.2 (113)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>33.3 (24)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>15.7 (89)</td>
<td>19.5 (113)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**P-value**

<table>
<thead>
<tr>
<th>N</th>
<th>Estrous response (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Conception rate (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Timed-AI pregnancy rate (%)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Synchronized pregnancy rate (%)&lt;sup&gt;e&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> 7dCIDR = 7 d CIDR + GnRH (100 µg) at CIDR insertion.  
7dMOD = 7 d CIDR + PGF<sub>2α</sub> at CIDR insertion and GnRH (100 µg) on d 2 of CIDR treatment.  
5dCIDR = 5 d CIDR + GnRH (100 µg) at CIDR insertion.

All treatments had two PGF treatments, one at CIDR removal and the other 8 h later. Estrus was detected for 60 h after CIDR removal and all heifers were AI approximately 8 to 12 h after being detected in estrus. All heifers not detected in estrus by 60 h were timed-AI and received GnRH at 72 h.

<sup>b</sup> Percentage of heifers displaying estrus 60 h after PGF<sub>2α</sub> of total treated.
<sup>c</sup> Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.
<sup>d</sup> Percentage of heifers pregnant to timed-AI of the total that were timed-AI.
<sup>e</sup> Percentage of heifers pregnant during the synchronized breeding of the total treated.
<sup>f,g</sup> Means without a common superscript within a column differ (P < 0.05).
Table 6-2. Reproductive tract score (RTS) effects on estrous, conception and pregnancy rates of *Bos indicus* × *Bos taurus* heifers synchronized with a 7 d CIDR, modified 7 d CIDR, or 5 d CIDR protocola.

<table>
<thead>
<tr>
<th>RTS b</th>
<th>N</th>
<th>Estrous response (%)c</th>
<th>Conception rate (%)d</th>
<th>Timed-AI pregnancy rate (%)e</th>
<th>Synchronized pregnancy rate (%)f</th>
<th>Thirty-day pregnancy rate (%)g</th>
</tr>
</thead>
<tbody>
<tr>
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<td>13.7 (51)h</td>
<td>14.3 (7)</td>
<td>9.1 (44)</td>
<td>9.8 (51)h</td>
<td>31.4 (51)h</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>10.8 (74)h</td>
<td>50.0 (8)</td>
<td>12.1 (66)</td>
<td>16.2 (74)h</td>
<td>44.6 (74)h</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>39.5 (76)i</td>
<td>50.0 (30)</td>
<td>23.9 (46)</td>
<td>34.2 (76)i</td>
<td>59.2 (76)i</td>
</tr>
<tr>
<td>4</td>
<td>98</td>
<td>49.0 (98)i</td>
<td>54.2 (48)</td>
<td>18.0 (50)</td>
<td>35.7 (98)i</td>
<td>68.4 (98)i</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>45.5 (44)i</td>
<td>40.0 (20)</td>
<td>25.0 (24)</td>
<td>31.8 (44)i</td>
<td>72.7 (44)i</td>
</tr>
</tbody>
</table>

P-value

<table>
<thead>
<tr>
<th></th>
<th>P &lt; 0.05</th>
<th>P &gt; 0.05</th>
<th>P &gt; 0.05</th>
<th>P &lt; 0.05</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
</table>

a 7dCIDR = 7 d CIDR + GnRH (100 µg) at CIDR insertion.
7dMOD = 7 d CIDR + PGF₂α at CIDR insertion and GnRH (100 µg) on d 2 of CIDR treatment.
5dCIDR = 5 d CIDR + GnRH (100 µg) at CIDR insertion.

All treatments had two PGF treatments, one at CIDR removal and the other 8 h later. Estrus was detected for 60 h after CIDR removal and all heifers were AI approximately 8 to 12 h after being detected in estrus. All heifers not detected in estrus by 60 h were timed-AI and received GnRH at 72 h.

b Reproductive tract score (RTS) 1 = immature, prepubertal, 5 = pubertal with corpus luteum present.

c Percentage of heifers displaying estrus 60 h after PGF₂α of total treated.
d Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.
e Percentage of heifers pregnant to timed-AI of the total that were timed-AI.
f Percentage of heifers pregnant during the synchronized breeding of the total treated.
g Percentage of heifers pregnant during the first 30 days of the breeding season of the total treated.
h,i Means without a common superscript within a column differ (P < 0.05).
Table 6-3. General description of Angus and Brangus cows in two groups or replication synchronized with either a 5 day CIDR (5dCIDR) or 7 day modified CIDR (7dMOD) synchronization protocol\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cycling Status (%)</th>
<th>Body weight, kgs, range</th>
<th>Cow age, yr, range</th>
<th>Body condition score, 1-9, range</th>
<th>Days postpartum, d, range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160</td>
<td>43.8 (160)</td>
<td>510.5 ± 82.3, 352 to 682</td>
<td>4.2 ± 2.2, 2 to 10</td>
<td>4.4 ± 0.5, 3.5 to 6.0</td>
<td>66.4 ± 20.2, 33 to 109</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>53.5% (44)</td>
<td>543.2 ± 59.5, 412 to 670</td>
<td>5.1 ± 2.1, 3 to 10</td>
<td>4.7 ± 0.7, 3.5 to 6.5</td>
<td>54.2 ± 10.2, 29 to 68</td>
</tr>
</tbody>
</table>

P-value \textsuperscript{a}  

\textsuperscript{a} 7dMOD = 7 d CIDR + PGF\textsubscript{2α} at CIDR insertion and GnRH (100 µg) on d 2 of CIDR treatment.  
5dCIDR = 5 d CIDR + GnRH (100 µg) at CIDR insertion.

All treatments had two PGF treatments, one at CIDR removal and the other 8 h later. Estrus was detected for 60 h after CIDR removal and all cows were AI approximately 8 to 12 h after being detected in estrus. All cows not detected in estrus by 60 h were timed-AI and received GnRH at 72 h.
Table 6-4. Estrous response, conception, timed-AI pregnancy, and synchronized pregnancy rates following PGF$_{2\alpha}$ in Angus and Brangus cows synchronized with either a 5 day CIDR (5 d) or 7 day modified CIDR (7 d) synchronization protocol.$^a$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Estrous response, %$^b$</th>
<th>Conception rate, %$^c$</th>
<th>Timed-AI pregnancy rate, %$^d$</th>
<th>Synchronized pregnancy rate, %$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN 5 d</td>
<td>42</td>
<td>69.1 (42)</td>
<td>79.3 (29)</td>
<td>53.9 (13)</td>
<td>71.4 (42)</td>
</tr>
<tr>
<td>7 d</td>
<td>47</td>
<td>70.2 (47)</td>
<td>72.7 (33)</td>
<td>50.0 (14)</td>
<td>66.0 (47)</td>
</tr>
<tr>
<td>BN 5 d</td>
<td>36</td>
<td>61.6 (36)</td>
<td>63.6 (22)</td>
<td>7.1 (14)</td>
<td>41.7 (36)</td>
</tr>
<tr>
<td>7 d</td>
<td>34</td>
<td>73.5 (34)</td>
<td>60.0 (25)</td>
<td>22.2 (9)</td>
<td>50.0 (34)</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN 5 d</td>
<td>12</td>
<td>66.7 (12)</td>
<td>25.0 (8)</td>
<td>50.0 (4)</td>
<td>33.3 (12)</td>
</tr>
<tr>
<td>7 d</td>
<td>11</td>
<td>81.8 (11)</td>
<td>33.3 (9)</td>
<td>0.0 (2)</td>
<td>27.3 (11)</td>
</tr>
<tr>
<td>BN 5 d</td>
<td>10</td>
<td>60.0 (10)</td>
<td>33.3 (6)</td>
<td>25.0 (4)</td>
<td>30.0 (10)</td>
</tr>
<tr>
<td>7 d</td>
<td>11</td>
<td>81.8 (11)</td>
<td>44.4 (9)</td>
<td>0.0 (2)</td>
<td>36.4 (11)</td>
</tr>
</tbody>
</table>

P-values

| Treatment | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| Breed     | P > 0.05 | P = 0.11 | P < 0.05 | P < 0.05 |
| Treatment x Breed | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |

$^a$ 7dMOD = 7 d CIDR + PGF$_{2\alpha}$ at CIDR insertion and GnRH (100 µg) on d 2 of CIDR treatment.
5dCIDR = 5 d CIDR + GnRH (100 µg) at CIDR insertion.

All treatments had two PGF treatments, one at CIDR removal and the other 8 h later. Estrus was detected for 60 h after CIDR removal and all cows were AI approximately 8 to 12 h after being detected in estrus. All cows not detected in estrus by 60 h were timed-AI and received GnRH at 72 h.

$^b$ Percentage of cows displaying estrus 3 d after PGF$_{2\alpha}$ of total treated.
$^c$ Percentage of cows pregnant to AI of the total that exhibited estrus and were AI.
$^d$ Percentage of cows pregnant to timed-AI of the total that were timed-AI.
$^e$ Percentage of cows pregnant during the synchronized breeding of the total treated.
Table 6-5. Estrous response, conception, timed-AI pregnancy, and synchronized pregnancy rates following PGF$_{2\alpha}$ in Angus and Brangus cows synchronized with either a 5 day CIDR (5dCIDR) or 7 day modified CIDR (7dMOD) synchronization protocol by treatment and cycling status.$^a$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Estrous response, %$^b$</th>
<th>Conception rate, %$^c$</th>
<th>Timed-AI pregnancy rate, %$^d$</th>
<th>Synchronized pregnancy rate, %$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5dCIDR Cycling</td>
<td>51</td>
<td>68.6 (51)</td>
<td>60.0 (35)</td>
<td>37.5 (16)</td>
<td>52.9 (51)</td>
</tr>
<tr>
<td>5dCIDR Non-cycling</td>
<td>49</td>
<td>61.2 (49)</td>
<td>66.7 (30)</td>
<td>26.3 (19)</td>
<td>51.0 (49)</td>
</tr>
<tr>
<td>7dMOD Cycling</td>
<td>41</td>
<td>87.8 (41)</td>
<td>58.3 (36)</td>
<td>40.0 (5)</td>
<td>56.1 (41)</td>
</tr>
<tr>
<td>7dMOD Non-cycling</td>
<td>61</td>
<td>63.9 (61)</td>
<td>61.5 (39)</td>
<td>31.8 (22)</td>
<td>50.8 (61)</td>
</tr>
</tbody>
</table>

P-values

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Cycling status</th>
<th>Treatment × Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrous response</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Conception rate</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Timed-AI</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Synchronized</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

$^a$ 7dMOD = 7 d CIDR + PGF$_{2\alpha}$ at CIDR insertion and GnRH (100 µg) on d 2 of CIDR treatment.
5dCIDR = 5 d CIDR + GnRH (100 µg) at CIDR insertion.

All treatments had two PGF treatments, one at CIDR removal and the other 8 h later. Estrus was detected for 60 h after CIDR removal and all cows were AI approximately 8 to 12 h after being detected in estrus. All cows not detected in estrus by 60 h were timed-AI and received GnRH at 72 h.

$^b$ Percentage of cows displaying estrus 3 d after PGF$_{2\alpha}$ of total treated.

$^c$ Percentage of cows pregnant to AI of the total that exhibited estrus and were AI.

$^d$ Percentage of cows pregnant to timed-AI of the total that were timed-AI.

$^e$ Percentage of cows pregnant during the synchronized breeding of the total treated.
Figure 6-1. Description of 7 day Select Synch/CIDR + TAI, modified 7 day CIDR, and 5 day Select Synch/CIDR + TAI protocols.
Development and refining of an estrous synchronization protocol that achieves acceptable pregnancy rates in *Bos indicus* × *Bos taurus* cows is a challenge. While many synchronization protocols have been developed in *Bos taurus* cattle, their effectiveness in *Bos indicus* × *Bos taurus* cows is limited and often do not achieve acceptable pregnancy rates. The objective of the experiments completed was to develop a greater understanding of follicle development and the effectiveness of GnRH in *Bos indicus* × *Bos taurus* cows, further investigate differences in estrus characteristics between *Bos taurus* and *Bos indicus* × *Bos taurus* cows, and evaluate pregnancy rates to several synchronization protocols that have been demonstrated to be successful in *Bos taurus* cows, in *Bos indicus* × *Bos taurus* cows.

In Chapter 3, we investigated the response of Angus and Brangus cows to a 7-11 Synch protocol developed by Kojima et al. (2000). The protocol involved a 7 d MGA feeding with PGF (PG1) on d 7. On d 11, GnRH was given to induce ovulation, and on d 18 the cow received a second PGF (PG2), and was bred on the following estrus. This protocol was of particular interest in *Bos indicus* × *Bos taurus* cows as it is inexpensive, relatively easy to implement, and had achieved good pregnancy rates in *Bos taurus* beef cows. We hypothesized that the pre-synchronization effects of the MGA and GnRH would improve pregnancy rates following the second PGF in *Bos indicus* × *Bos taurus* cows compared to traditional CIDR protocols. Results from Chapter 3, suggest that the 7-11 protocol can be used effectively in both cycling and anestrous Angus and Brangus cows, at different stages of their estrous cycle with success. Synchronized pregnancy rates were 20% less for Angus cows and 18% less for Brangus cows.
compared to the initial study by Kojima et al. (2000) using the 7-11 protocol; however, synchronized pregnancy rates in Chapter 3 are about 7% greater compared to a Select Synch/CIDR protocol in *Bos indicus* × *Bos taurus* cows without a timed-AI (Lucy et al., 2001). To maximize pregnancy rates in cattle of *Bos indicus* influence using the 7-11 protocol, incorporation of a timed-AI is important to ensure all cows are inseminated and have the opportunity to become pregnant. However, even without a timed-AI, the low-cost 7-11 synchronization protocol effectively synchronized estrus in both Angus and Brangus cows with overall synchronized pregnancy rates of 47.7 to 50.0%.

In Chapter 4, postpartum Angus and Brangus cows were used to evaluate cycling status and day of estrous cycle (DOC) effects at initiation of a Select Synch/CIDR + TAI protocol on ovulation rate, follicle development, and pregnancy rates. The experiment was conducted in two phases (phase 1 = anestrous, phase 2 = estrous cycling). Anestrous cows were selected to have either ovulated or not ovulated to GnRH on d 0. Estrous cycling cows were pre-synchronized to be 2, 6, 10, 14, and 18 DOC on d 0 (start of synchronization). On d 0, all cows received GnRH and a CIDR followed by PGF with CIDR removal on d 7. Estrus was detected for 3 d and cows were AI 8 to 12 h after detected in estrus. Cows not exhibiting estrus by 73 h post PGF were timed-AI and received GnRH at 73 to 75 h. In summary for Chapter 4, anestrous cows respond similarly to the Select Synch/CIDR + TAI protocol whether they ovulate to GnRH on d 0 or not. Breed differences were observed in estrous response and other estrus characteristics. Similar pregnancy rates were observed in anestrous Angus and Brangus cows that both ovulated and did not ovulate to GnRH on d 0. In estrous cycling cows, ovulation rates to GnRH on d 0 and the size of the follicles ovulated varied
for different days of the estrous cycle that the protocol was initiated on. Breed
differences in estrous response and estrous characteristics were also observed in
estrous cycling cows. Estrous response differed for DOC groups. Overall, greater
synchronized pregnancy rates were achieved when cows initiated the Select
Synch/CIDR + TAI protocol at later stages of the estrous cycle (DOC 10, 14, and 18)
compared to early stages of the estrous cycle (DOC 2 and 6).

In Chapter 5, five groups (n = 659) suckled *Bos indicus* × *Bos taurus* cows were
used to compare a Select Synch/CIDR + TAI protocol (SSC) to a modified CO-Synch +
CIDR protocol (COS). Cows received GnRH and a CIDR on d 0. On d 7, SSC cows
had their CIDR removed and received PGF, whereas COS cows had their CIDR
removed and received PGF on d 7.5. Estrus was detected for 3 d following PGF and
SSC cows were AI 8 to 12 h after observed in estrus. Cows on the SSC protocol not
exhibiting estrus by 72 h after PGF were timed-AI between 76 to 80 h and received
GnRH. All COS cows were timed-AI at 60 to 66 h after PGF and received GnRH. In
summary, Chapter 5 demonstrated that the less labor intensive COS synchronization
protocol achieved comparable pregnancy rates to the SSC synchronization protocol of
47.1% and 49.4%, respectively. This may provide producers with a more economical
and less labor intensive option for estrous synchronization in cattle of *Bos indicus*
influence.

In Chapter 6, we hypothesized that steps should be taken beyond control of
follicle dynamics, regulation of luteal regression, and timing of ovulation to promote
development and ovulation of a competent dominant follicle that will have the best
chance of developing into a successful pregnancy. Two experiments were conducted to
compare a 5 d CIDR protocol to a modified 7 d CIDR protocol in yearling *Bos indicus × Bos taurus* heifers and suckled *Bos taurus* and *Bos indicus × Bos taurus* cows. In Exp. 1 three synchronization treatments, 7 d CO-Synch + CIDR (7dCIDR), 5 d CO-Synch + CIDR (5dCIDR) or modified 7 d CIDR (7dMOD), were evaluated for subsequent pregnancy rates in yearling *Bos indicus × Bos taurus* heifers. In Exp. 2 the two treatments, 5dCIDR and 7dMOD, were evaluated for subsequent pregnancy rates in suckled *Bos taurus* and *Bos indicus × Bos taurus* cows. In summary for Chapter 6, inclusion of PGF at the start of synchronization creates a low progesterone environment with greater LH secretion during follicle development, which yields a follicle with greater steroidogenic capacity and potentially ovulates a more viable oocyte. The modified 7 d CIDR protocol achieved greater synchronized pregnancy rates in *Bos indicus × Bos taurus* heifers and similar synchronized pregnancy to a 5 day CIDR protocol in *Bos indicus × Bos taurus* cows.

In summary for all chapters, several conclusions can be drawn from follicle data. *Bos indicus × Bos taurus* cows have greater numbers of small (≤ 5 mm) follicles compared to *Bos taurus* cows, but no differences were observed in numbers of medium (6 to 9 mm) or large (≥ 10 mm) follicles. *Bos indicus × Bos taurus* and *Bos taurus* cows seem to ovulate similar size follicles in response to GnRH at similar rates; however, the stage of the estrous cycle the synchronization treatments are initiated on play major roles in ovulation rates and ovulatory follicle sizes to GnRH. Day of eventual ovulatory follicle emergence and follicle growth rates seem to be similar for *Bos indicus × Bos taurus* and *Bos taurus*, but are also influenced by stage of the estrous cycle. *Bos taurus*
cows also may ovulate larger follicles following synchronization compared to *Bos indicus × Bos taurus*, but more data is needed to confirm this finding.

Conclusions regarding progesterone, luteolysis, and estrus characteristics were not as well defined in these experiments. Concentrations of progesterone were greater in anestrous *Bos indicus × Bos taurus* compared to *Bos taurus* cows in Chapter 4. However, in the same chapter in estrous cycling cows, *Bos taurus* cows had elevated concentrations of progesterone compared to *Bos indicus × Bos taurus* cows. Similar luteolysis was observed in all experiments of suckled *Bos taurus* and *Bos indicus × Bos taurus* cows. Characteristics of estrus certainly differ between *Bos taurus* and *Bos indicus × Bos taurus* cows, but the results are mixed. Differences in interval from PGF to the onset of estrus, estrus duration, and/or the number of mounts received were observed throughout the experiments; however, the results were not consistent. This is in agreement with the literature that often indicates mixed results for estrus characteristics between the breed types.

Overall estrous response and pregnancy data from all chapters indicated varied responses. Estrous response between *Bos taurus* and *Bos indicus × Bos taurus* cows was similar in Chapters 3 and 6. *Bos indicus × Bos taurus* cows had greater estrous response compared to *Bos taurus* in both phases of Chapter 4, which is interesting and the opposite of the literature. Estrous response may have been compromised in this particular group of *Bos taurus* cows due to the stress they displayed during the experiments. Synchronized pregnancy rates were similar between breed types in Chapters 3 and 4. Greater synchronized pregnancy rates were observed in *Bos taurus*...
compared to *Bos indicus* × *Bos taurus* in Chapter 6, due primarily to the reduced pregnancy rates of *Bos indicus* × *Bos taurus* cows in the second replication.

Effects of performance responses, including RTS, estrous cycling status, days postpartum, cow age, and BCS can be concluded from the chapters. In Chapter 6, heifers with greater RTS had increased estrous response, synchronized pregnancy, and breeding season pregnancy rates. This reiterates previous studies indicating the importance of pubertal status or proximity to puberty when initiating a synchronization protocol in yearling heifers. Surprisingly, limited effects of cycling status and no effects of postpartum interval were observed in these experiments. This is likely due to cows being of adequate days postpartum and in good BCS at the start of the breeding season, as well as the cows calving in synchronized groups. Limited effects of BCS were observed in Chapter 5, but no effects of BCS were observed in Chapters 3, 4, or 6. Cows in these experiments were in good BCS and this may have been the reason no BCS effects were observed. Adequate nutrition, as measured by BCS, is an important factor in the reproductive success of any program, regardless of whether or not AI programs are being implemented. Finally, effects of cow age were very limited in these experiments. First calf heifers displayed reduced breeding season pregnancy rates in Chapter 3 compared to 3 year old cows, but no differences were observed on the synchronized breeding. Besides the cow age effect in Chapter 3, no effects of cow age were observed in any other experiment. This suggests that while pregnancy rates may be reduced in primiparous cows, cow age does not influence pregnancy rates.
LIST OF REFERENCES


De La Fuente, R., and J.J. Eppig. 2001. Transcriptional activity of the mouse oocyte


BIOGRAPHICAL SKETCH

Regina D. Esterman was born in Salem, Virginia. Regina is the oldest of two children of Jim and Lavonne Esterman, of West Chesterfield, New Hampshire. During her childhood, Regina lived in Virginia, Tennessee, Louisiana, and Alabama, before finally settling in Ohio. She attended Northwest High School in Canal Fulton, Ohio, and was an active member of Summit County 4-H, United States Pony Club, and the local horse show circuits. In addition to these activities, Regina participated in the post-secondary option her junior and senior years of high school, attending The Ohio State University Agricultural Technical Institute full-time. While attending Ohio State, Regina was awarded 2002 Outstanding Student of the Year. In 2002, Regina received her A.S. from Ohio State concurrently with graduation from high school. Regina then enrolled at Colorado State University. During her studies at Colorado State, Regina was a member of Kappa Alpha Theta sorority, Colorado State Polo Team, and the Collegiate Horseman's Association. Upon graduation in 2004, Regina moved to Gainesville, Florida to pursue a Master of Science degree at the University of Florida, under the mentorship of Dr. Joel Yelich. Following completion of her Master of Science degree, Regina continued onto her Ph.D. program as a USDA fellow, again under the mentorship of Dr. Joel Yelich. During her time at Florida, Regina was active in the Animal Science Graduate Student Association, holding several offices, including President.