COMPARISON OF TWO SHORT-TERM PROGESTOGEN BASED ESTROUS SYNCHRONIZATION PROTOCOLS IN YEARLING HEIFERS AND SUCKLED POSTPARTUM COWS OF *Bos indicus* × *Bos taurus* BREEDING

By

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To my family and friends for always giving the support and encouragement I needed.

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COMPARISON OF TWO SHORT-TERM PROGESTOGEN BASED ESTROUS SYNCHRONIZATION PROTOCOLS IN YEARLING HEIFERS AND SUCKLED POSTPARTUM COWS OF *Bos indicus* × *Bos taurus* BREEDING

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A series of experiments were conducted with suckled cows and yearling heifers of *Bos indicus* × *Bos taurus* breeding to compare two synchronization protocols including the Select Synch/CIDR+timed-AI (SSC+TAI) and a modified 7-11 protocol. In Experiment 1, suckled *Bos indicus* × *Bos taurus* cows (n = 324) were used in a 2 × 2 factorial design comparing SSC+TAI to a modified 7-11 (7-10) protocol and within each synchronization treatment there were two prostaglandins (PG) treatments including cloprostenol sodium (CPG) and dinoprost tromethamine (DPG). Cows were equally distributed to treatments by body condition score (BCS) and days postpartum (DPP). On day 0, the 7-10 treatment received an autoclaved once-used-CIDR that was removed on day 7 concomitant with DPG followed by GnRH on day 10. On day 17, 7-10 cows received either DPG or CPG. Also on day 10, SSC+TAI treatment received a new CIDR concomitant with GnRH with CIDR removal on day 17 where cows received either DPG or CPG. Estrus was visually detected twice daily (0700 and 1600 h) for 72 h after $PGF_{2\alpha}$ and cows were AI 6 to 12 h after a detected estrus. Non-responders were timed-AI + GnRH 72 to 76 h post PG. In experiment 2, yearling heifers over two breeding seasons (n = 218 and 137) were randomly assigned to the same treatments as Experiment 1, estrous detection

and AI were administered in the same manner as Experiment 1. In experiment 1, estrous response (ER), conception (CR), and synchronized pregnancy rate (SPR) were affected ($P <$ 0.05) by synchronization treatment but not ($P > 0.05$) PG treatment. The SPR were 45.5% and 31.2 for the SSC+TAI and 7-10 treatments, respectively. As BCS increased from ≤ 4.5 to ≥ 5.5, ER, CR, SPR, and thirty day pregnancy rates (TDPR) increased ($P < 0.05$). In experiment 2, ER, CR, TAIPR, SPR, and TDPR were similar ($P > 0.05$) between the synchronization and PG treatments, with the mean ER, CR, TAIPR, SPR and TDPR being 66.8, 65.0, 16.1, 48.7, and 76.3%, respectively.

In Experiment 3, yearling heifers (n = 407) were synchronized with either the SSC +TAI treatment from Experiment 1 or 7-11 treatment that included melengestrol acetate (MGA; 0.5 mg/head/d) for 7 d with GnRH 4 d after the last day of MGA followed by DPG 7 d later. Estrus was detected and heifers were inseminated similar to Experiment 1. Heifers were distributed to treatment by reproductive tract score (RTS; Scale: 1=immature to 5= estrous cycling) and BCS. The 7-11 heifers had greater ($P < 0.05$) ER (55.2 vs. 41.9%), CR (47.0 vs. 31.3%), and SPR (33.5 vs. 24.8%) compared to SSC+TAI heifers, respectively. Heifers exhibiting estrus at 60 h after PG (61.7%) had a greater ($P < 0.05$) CR compared to heifers that had exhibited estrus at ≤ 36 (35.3%), 48 (31.6%), and 72 h (36.2%), which were all similar ($P > 0.05$) to each other. As RTS increased from ≤ 2 to a ≥ 3, ER, CR, SPR, and TDPR all increased ($P < 0.05$). In summary, there was a differential response to synchronization treatments as cows treated with the SSC+TAI had greater SPR compared to the 7-10 treatment; whereas, heifers synchronized with the 7-11 treatment had greater SPR compared to the SSC+TAI treatment.

CHAPTER 1
INTRODUCTION

Recent advances in reproductive technologies have made a significant impact on the beef cattle herd on both a national and global scale. Most notably is the use of artificial insemination (AI) to expedite genetic gains and to decrease costs associated with housing and purchasing bulls of superior genetic quality. However, broad dissemination and utilization of AI in the United States beef cattle herd is limited, primarily due to the time and labor costs associated with detecting estrous during the 21 day estrous cycle. Incorporation of estrous synchronization into an AI program decreases the amount of time needed to detect estrus and (or) can eliminate estrous detection when a fixed breeding time is used. Additional benefits of using estrous synchronization includes promoting estrous cyclicity in some non-cycling animals, increasing the percentage of cattle pregnant early in the breeding season, shortening the breeding and subsequent calving seasons, and increasing uniformity of the calf crop. A more uniform calf crop is important since calves are worth more at weaning.

Numerous factors can influence the effectiveness of estrous synchronization protocols including nutritional status, estrous cycling status, stage of the estrous cycle, days postpartum at the start of the synchronization protocol, and animal genotype. There are essentially three genotypes used in the beef cattle production systems in the United States including *Bos taurus*, *Bos indicus*, and *Bos indicus* × *Bos taurus*. The majority of cattle in the Gulf Coast region of United States, including other tropical and subtropical climates of the world, are of *Bos indicus* × *Bos taurus* breeding. Cattle with *Bos indicus* breeding are vital to efficient production in these environments because the cattle can withstand the hot and humid environment, utilize lower quality forages common in these environments, and are more resistant to parasites compared to cattle of *Bos taurus* breeding. With that said, the majority of the research and development of estrous synchronization protocols is based on the *Bos taurus* genotype. Furthermore, limited research indicates that *Bos indicus* and *Bos indicus* × *Bos taurus* cattle have subtle physiological and behavioral differences that elicit different responses to estrous synchronization pharmaceuticals and protocols compared to *Bos taurus* cattle. Consequently, the primary objective of the research reported in this thesis will focus on evaluating the effectiveness of two estrous synchronization protocols in cattle of *Bos indicus* × *Bos taurus* breeding.

There are several pharmaceuticals available to beef cattle producers that can be used in estrous synchronization protocols. Whether used alone or together, the primary goal when using these products is to manipulate the estrous cycle so that estrus and (or) ovulation is more predictable and uniform across the group of cattle being synchronized. There are three classes of pharmaceuticals that are primarily used in estrous synchronization protocols including prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), gonadotropin-releasing hormone (GnRH), and progestogens. The primary function of $PGF_{2\alpha}$ is to induce lysis of the corpus luteum, which results in the expression of estrus in 3 to 5 d. In contrast, GnRH is used to manage follicle development by stimulating a pre-ovulatory surge of luteinizing hormone (LH) to initiate ovulation of growing follicles $\geq$ 10 mm in diameter and stimulation of follicle stimulating hormone (FSH) which will assist follicular recruitment into the next follicular wave. Treatment with GnRH can be used to either stimulate follicle turnover to initiate a new follicle wave or to synchronize ovulation in conjunction with AI. The final pharmaceutical that can be used are progestogens. The primary functions of progestogens are to prevent expression of estrus and ovulation by preventing the pre-ovulatory surge of LH. There are two progestogens available for use as estrous synchronization agents and they are melengestrol acetate (MGA) and the intra-vaginal progesterone insert known as the CIDR. Progestogens can also have secondary effects by priming the hypothalamic-pituitary-ovarian-uterine axis, which can serve to initiate estrus in some anestrous cattle and possibly enhance pregnancy rates in synchronized cattle.

One of the most common estrous synchronization protocols used by beef cattle producers is the Select Synch protocol. This protocol utilizes GnRH followed by a $PGF_{2\alpha}$ treatment 7 d later. Estrus is detected for 5 to 7 d post $PGF_{2\alpha}$ with AI occurring 6 to 12 h after an observed estrus. The down side to the protocol is that some cattle exhibit estrus 1 to 2 d prior to $PGF_{2\alpha}$, which increases the amount of estrous detection required. Subsequent research demonstrated that administration of a progestogen between GnRH and $PGF_{2\alpha}$ eliminated the need for extra estrous detection before $PGF_{2\alpha}$ without compromising fertility. The progestogen used in the Select Synch protocol can be either MGA or a CIDR, but the CIDR appears to be the preferred method for several reasons. First, addition of the CIDR does not increase labor requirements because the cattle are handled to administer the GnRH and $PGF_{2\alpha}$. Second, since MGA is administered in the feed, inadequate MGA consumption can result in cattle that "break through" and express estrus between the GnRH and $PGF_{2\alpha}$ treatments. And third, the CIDR may provide more "progesterone like" effects compared to MGA. The "progesterone like" effects include priming the hypothalamic-pituitary-ovarian-uterine axis, which could induce estrous cyclicity in some anestrous cattle and quite possibly enhance pregnancy rates. Although, the research is not definitive on this issue, additional research is needed to validate whether the CIDR is more effective compared to MGA as a synchronization agent. When a CIDR is used with the Select Synch system it is called the Select Synch/CIDR synchronization system. Additional research in cattle of *Bos taurus* breeding demonstrated that three days of estrous detection and AI combined with a timed-AI plus GnRH 72 to 80 h after $PGF_{2\alpha}$ for cattle not exhibiting estrus resulted in similar and more consistent AI pregnancy rates compared to 5 d of estrous detection and AI. The aforementioned synchronization protocol is called the Select Synch/CIDR+timed-AI protocol, which has been shown to be an effective protocol in both suckled cows and yearling heifers of *Bos taurus* breeding. In contrast, limited research has been conducted on the effectiveness of the Select Synch/CIDR+timed-AI system in suckled postpartum cows and yearling heifers of *Bos indicus* × *Bos taurus* breeding. Therefore, the first objective of my research was to evaluate the effectiveness of the Select Synch/CIDR+timed-AI synchronization protocol in suckled postpartum cows and yearling heifers of *Bos indicus* × *Bos taurus* breeding.

One of the problems with utilizing progestogens, particularly long term progestogen treatments greater than 10 d, is a decrease in fertility of the estrus immediately after progestogen removal. With short term (≤ 7 d) progestogen treatments there can be a slight decrease in fertility, but that decrease can be overcome by administering GnRH at the initiation of the progestogen treatment in the GnRH + $PGF_{2\alpha}$ protocols. Another way to utilize a progestogen and avoid the depression in fertility is to use the 7-11 estrous synchronization protocol. The 7-11 protocol consists of 7 d of MGA with $PGF_{2\alpha}$ on the last day of MGA followed by GnRH 4 d later. This pre-synchronization portion of the 7-11 protocol is designed specifically to synchronize follicle development in a majority of cattle. Seven days after GnRH, another $PGF_{2\alpha}$ treatment is administered followed by 5 to 7 d of estrous detection. Research in suckled postpartum cows and yearling heifers of *Bos taurus* breeding has demonstrated that the synchrony of estrus was excellent and a combination of estrous detection and timed-AI could be incorporated into the 7-11 protocol resulting in acceptable synchronized pregnancy rates. However, no such research has been conducted with the 7-11 protocol in either suckled postpartum cows or yearling heifers of *Bos indicus* × *Bos taurus* breeding.

The cost of the 7-11 protocol is approximately $10.00/animal to implement compared to $15.00/animal for the Select Synch/CIDR+timed-AI protocol. The increased cost of the Select Synch/CIDR+timed-AI is primarily due to the cost of the CIDR, which are approximately $10 each. A cost efficient alternative to using a new CIDR is utilization of a once used CIDR in the Select Synch+timed-AI protocol. However, limited research has evaluated this alternative and additional research is needed to determine if the once-used CIDR used in the Select Synch protocol results in similar responses compared to a new CIDR in both estrous cycling and anestrous cows. The once used CIDR could also be included in the 7-11 protocol as an alternative to MGA. This is particularly true in programs where MGA consumption is of concern or producers do not want to have to provide additional supplements to administer the MGA. Therefore, a second objective of the research was to evaluate the effectiveness of the 7-11 estrous synchronization protocol in cattle of *Bos indicus* × *Bos taurus* breeding and to evaluate the effectiveness of incorporating the once-used CIDR into the 7-11 estrous synchronization protocol.

CHAPTER 2
REVIEW OF LITERATURE

Assisted Reproduction in Beef Cattle

One of the most important goals in beef cattle production is to improve the genetic quality of the cow herd from year to year in an effective and economic manner. Many recent improvements in biotechnology and beef cattle management have made this more accessible to the average producer, namely development of artificial insemination (AI), and estrous synchronization protocols. Historically, reports of AI date back hundreds of years; however, it has only been used commercially in the later part of this century (Foote, 2002). Development of effective semen collection and storage methods, improved methods of delivery of frozen/thawed semen to females, development of enhanced estrous detection and estrous synchronization methods as well as a more complete understanding of the reproductive physiology of the male and female have all been a vital part in developing successful assisted reproduction programs.

Artificial insemination allows producers an unlimited availability to superior sires without expending costs associated with purchasing and housing a bull. Utilization of AI also allows for rapid genetic improvement of the cowherd by using specific genetic indicators such as expected progeny differences (EPD), which serve as indicators of the bull's ability to pass on his traits to his progeny. However, the costs associated with implementing an AI program including labor and supplies, frozen/thawed semen, and natural service bull costs have to be weighed with success of pregnancy and profits (Pace, 1985).

Estrous synchronization used in conjunction with AI is a great way to capitalize on this technology. Estrous synchronization uses exogenous hormones to manipulate the life span of the corpus luteum (CL) and (or) to synchronize follicle development. Synchronization of the CL and (or) follicle development, allows for a synchronization of estrus in most females of the herd allowing for a shorter window for AI breeding. Treatment with an estrous synchronization protocol can also increase the number of females pregnant early in the breeding season, which results in a shorter calving season and a more uniform calf crop at weaning. All of these variables drive profits and increase an operation's production efficiency. With that said, additional costs are associated with implementing an estrous synchronization and AI program including increased labor, synchronization pharmaceuticals, frozen semen, and hiring an AI technician. Consequently, beef producers want estrous synchronization protocols that require minimal cattle handling and labor, cost effective, and consistently yield acceptable AI pregnancy rates. There are numerous factors that influence the effectiveness of an estrous synchronization protocols including but not limited to onset of puberty, estrous cycling status, parity, lactation status, nutrition, environment, management techniques, and cattle genotype. These factors can act either alone or in concert to influence the outcome to an estrous synchronization protocol. Therefore, the purpose of this literature review is to discuss and summarize many of these factors as they relate to the implementation of estrous synchronization protocols in cattle of *Bos indicus* × *Bos taurus* breeding

The Estrous Cycle

The bovine estrous cycle is an accretion of hormonal and physiological events which provides repeated opportunities for mating and quite possibly pregnancy. These reproductive events will begin at puberty and continue through adult life, only to be interrupted by pregnancy and the subsequent lactation. The estrous cycle is delineated into two phases, follicular and luteal, which are recognized by the ovarian structures present. The follicular phase is defined as the period from regression of the CL to ovulation of a newly developed follicle. The follicular phase can be further separated into proestrus and estrus. Estradiol secreted from selected dominant follicles during the follicular phase will drive female receptivity, estrous behavior, and the surge of luteinizing hormone (LH) which induces ovulation. The longer of the two phases, the luteal phase, is defined as the period from ovulation until CL regression. The luteal phase can also be further separated into two stages, metestrus and diestrus. The erupted follicle from the follicular phase eventually luteinizes and forms a CL, which secretes progesterone and begins the process of developing the uterus into an environment acceptable for pregnancy. If pregnancy does not occur, the uterus secretes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and luteolysis occurs and the female returns to estrus.

The length of the estrous cycle in cattle of *Bos taurus* breeding is on average 21 d (Hansel et al., 1973). However, variations in estrous cycle length exist between *Bos indicus* and *Bos taurus* type cattle, with *Bos indicus* breeds having longer estrous cycles. Mattoni et al. (1988) reported a mean estrous cycle length of 22.6 d in Zebu cattle and Plasse et al. (1970) reported 27.7 d in Brahman cattle. Gir cows have a mean estrous cycle length of 21.7 d (Moreira-Viana et al., 2000). Alternatively, Alvarez et al. (2000) reported similar estrous cycle lengths for Angus (19.5 d), Senepol (20.4 d), and Brahman (19.7 d) cows. Variation in estrous cycle length has also been observed between heifers and cows of *Bos indicus* breeding, as Boran cows had a 23 d estrous cycle (Llewelyn et al., 1987), compared 27.7 d in to Brahman heifers (Plasse et al., 1970). Conversely, *Bos taurus* cows (Zollers et al., 1989) and heifers (Mihm et al., 2000) exhibit minimal variation from the 21 d estrous cycle length.

Estrus demarcates the beginning of the estrous cycle. It is the most recognizable stage as it is diagnosed by behavioral characteristics where the female is sexually receptive to the male. Estradiol concentrations peak during estrus, causing the behavioral expression of estrus and initiation of the preovulatory surge of LH (Wettemann et al., 1972; Short et al., 1973). Approximately 6 to 8 h after the peak estradiol concentrations, the preovulatory surge of LH occurs (Walters and Schallenberger, 1984) resulting in ovulation 26 to 48 h after the beginning of behavioral estrus (Plasse et al., 1970; Looper et al., 1998). Circulating concentrations of estradiol have been reported to be less in *Bos indicus* compared to *Bos taurus* cows during day 7 to 17 of the estrous cycle (Segerson et al., 1984). Visual signs of estrus include but are not limited to the female standing to be mounted by another animal, vulvular mucous discharge, Flehmen response, restlessness, sniffing other cow's genitals, and chin resting (Van Eerdenburg et al., 2002). These signs can occur before, during, or after estrus and should be used as visual keys in diagnosing the best time for breeding, but should not necessarily be used to determine exact time of ovulation (Nebel, 2003; Forster et al., 2006).

The duration (length of estrus) and intensity of estrus (number of mounts received during the duration of estrus) can be influenced by several factors including genotype, estradiol concentrations, social status, temperature and humidity, and the number of animals in estrus at one time. *Bos indicus* breeds tend to have a shorter and less intense estrus (Plasse et al., 1970; Galina et al., 1982; Mattoni et al., 1988; Pinheiro et al., 1998) compared to *Bos taurus* breeds (Stevenson et al., 1996; Richardson et al., 2002). The duration of estrus in *Bos taurus* breeds ranges from 3 to 26 h with a mean of 14 h (Schams et al., 1977). Conversely, the duration of estrus in *Bos indicus* and *Bos indicus* × *Bos taurus* breeds ranges from 1 to 24 h with a mean of 7 h (Mattoni et al., 1988; Rae et al., 1999). Landaeta-Hernandez (2002) also reported that the duration of a synchronized estrus tended to be shorter in Senepol compared to Angus or Brahman cows. However, breed had no effect on the duration and total number of mounts received during either a synchronized or spontaneous estrus; however, duration of estrus and total number of mounts received during estrus were greater for the synchronized compared to the spontaneous estrus. The variation in duration and intensity of estrus within and between breed types may be attributed to circulating estradiol concentrations (Rhodes and Randal, 1978; Lyimo et al., 2000). Lyimo et al. (2000) reported that increased estradiol concentrations were indicative of increased behavioral estrus activity. Since circulating estradiol concentrations have been reported to be greater in *Bos taurus* compared to *Bos indicus* cows (Segerson et al., 1984), it could explain why cattle of *Bos indicus* breeding have a decreased duration of estrus and estrous intensity. In contrast, Alvarez (2000) reported similar estradiol concentrations between Angus and Brahman cows, but the authors did not report on any behavioral estrus data. Social status within herd can also influence behavioral estrus. Landaeta-Hernandez et al. (2004) reported that dominant and intermediate cows had a longer duration of estrus compared to subordinate cows but dominant cows tended to have less total mounts during estrus compared to subordinate and intermediate cows. The later response suggests that dominant cows probably avoided being mounted as frequently during estrus compared to subordinate cows.

Increasing the number of cows in estrus at a single point in time increased mounting activity in both *Bos taurus* and *Bos indicus* females (Galina et al., 1994), which can lead to formation of sexually active groups and make it easier to detect estrus. Hurnik (1975) reported that increasing the number of cows in estrus from 1 to ≥ 4 cows increased the number of mounts received from 11 to 49 in *Bos taurus* cattle. One of the problems with detecting estrus in cattle of *Bos indicus* breeding is the increased incidence of the silent estrus (Plasse et al., 1970; Dawuda et al., 1989; Lamothe-Zavaleta et al., 1991), high incidence of estrus expression at night (Pinheiro et al., 1998), increased secondary estrus activities other than mounting such as head butting (Orihuela et al., 1983), and decreased incidence of mounting activity (Galina et al., 1982). Therefore, it is imperative for producers that want to AI cattle of *Bos indicus* breeding that the cattle be synchronized to increase the opportunity for the cattle to be detected in estrus.

Numerous other factors influence behavioral estrus including environmental conditions such as temperature and humidity (Landaeta-Hernandez et al., 2002), time of day (Pinheiro et al., 1998), parity (Flores et al., 2006), and frequency with which cattle are handled through working facilities (Lemaster et al., 1999). It is unclear whether these variables act alone or in concert to affect estrous behavior, but it demonstrates the multitude of factors that influence behavioral estrus.

Ovulation typically occurs between 24 to 36 h after the onset of estrus in *Bos taurus* (Looper et al., 1998; Rorie et al., 1999) and *Bos indicus* cattle (Plasse et al., 1970; Mattoni et al., 1988; Pinheiro et al., 1998). Estrus and ovulation are followed by metestrus, which lasts from 3 to 5 d and is characterized by formation of a functional CL and synthesis and secretion of progesterone. Progesterone concentrations remain low until the CL is fully functional. During metestrus there is a transition from estradiol dominance to progesterone dominance and increased concentrations of circulating progesterone initiate the next stage, which is diestrus.

Diestrus is the longest stage of the estrous cycle lasting 10 to 14 d and is characterized by increased progesterone concentrations that are great enough to block the activity of estradiol. Progesterone also primes the uterus to develop a suitable environment for a potential conceptus. If pregnancy occurs, the CL will be maintained throughout gestation and continue to synthesize and secrete progesterone. If pregnancy does not occur, the CL will remain functional until day 17 to 18 of the estrous cycle, at which time luteolysis is initiated by $PGF_{2\alpha}$ secreted from the uterus. At this point, degeneration of the luteal tissue occurs and resumption of a new estrous cycle begins. The length of diestrus is directly related to life of the CL.

Progesterone concentrations during the luteal phase of the estrous cycle are also different between *Bos taurus* and *Bos indicus* cattle. Adeyemo and Heath (1980) observed higher peak concentrations of progesterone during the luteal phase in *Bos taurus* heifers compared to *Bos indicus* heifers. Progesterone concentrations were also greater in *Bos indicus* heifers 1 or 2 d before estrus. Progesterone concentrations increased from early to mid and late luteal phases, with the increase being more discernible in the Angus compared to Brahman cows (Segerson et al., 1984). Mean progesterone concentrations were also greater in Angus compared to Brahman cows that were either pregnant or not pregnant (Segerson et al., 1984).

Proestrus is the interval from luteolysis until the onset of estrus and is initiated 3 to 4 d before estrus. Proestrus is characterized by increased follicular growth and is the transition from progesterone dominance to estrogen dominance (Chenault et al., 1975; Kesner et al., 1982). During proestrus, a subordinate follicle is selected from a pool of follicles and becomes a dominant follicle, which will eventually be the ovulatory follicle.

Hormonal Regulation: The Hypothalamic-Pituitary-Ovarian Axis

Estrous cyclicity and overall reproductive function in cattle is highly dependent upon the complex interactions of hypothalamic and pituitary hormones and the hormone secreting structures of the reproductive tract. Release of gonadotropin releasing hormone (GnRH) from the hypothalamus mediates the secretion of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), from the anterior pituitary. Gonadotropins act on specific target tissues and (or) cells of the ovary that synthesize and secrete progesterone and estrogen. Both positive and negative feedback effects induced by either estradiol or progesterone will further control synthesis and secretion of gonadotropins from the hypothalamus and pituitary.

Gonadotropin releasing hormone is a neuropeptide (10 amino acids) produced in two sets of paired nerve cell bodies in the hypothalamus. The GnRH is synthesized and secreted from the nerve cells located in the tonic and surge centers of the hypothalamus. Nerve axons extend from the hypothalamic nerve cell bodies to the capillary plexus at the anterior pituitary where GnRH is released. This unique system is called the hypothalamic-hypophyseal portal system. The portal system is essential as it allows transfer of GnRH directly to the anterior pituitary instead of the general circulatory system where it would be quickly degraded. Basal secretion of GnRH is controlled by the ventromedial and arcuate nuclei or the tonic center of the hypothalamus and is characterized with low amplitude and high frequency LH pulses during the luteal phase of the estrous cycle when progesterone concentrations are increased. The tonic center continues to release basal concentrations of LH until the appropriate positive stimulus are received, which is increased estrogen preceded by a rapid decline in progesterone. The suprachiasmatic and preoptic nuclei are part of the surge center which is responsible for the release of GnRH during the preovulatory phase of the estrous cycle. The surge center mediates high amplitude and low frequency GnRH pulses, which enhance the LH surge necessary for ovulation. Estradiol secreted from the dominant follicle precedes this event to enhance the surge of LH (Wettemann et al., 1972). Each GnRH pulse precedes each LH pulse (Schams et al., 1974).

Luteinizing hormone, a glycoprotein, is the dominant luteotrophic hormone in the bovine. It is vital to CL function (Simmons and Hansel, 1964; Hoffmann et al., 1974) and is necessary for progesterone synthesis (Armstrong and Black, 1966). Decreasing LH concentrations surrounding the LH surge actually inhibits progesterone secretion and the vitality of CL development (Quintal-Franco et al., 1999). During the early luteal period, LH pulses are characterized by low amplitude and high frequency, whereas during the midluteal period LH pulses are characterized by high amplitude and low frequency (Rahe et al., 1980). Changes in circulating concentrations of estradiol and progesterone can effect LH secretion. Ginther et al. (1996) reported that decreasing the circulating concentrations of progesterone increased LH pulse frequency and estradiol concentrations within 6 h. Development of large and persistent dominant follicles occurs during low progesterone treatment due to an increase in LH pulse frequency (Roberson et al., 1989; Savio et al., 1990). Although, LH pulses are necessary for follicle development beyond 9 mm (Gong et al., 1996), decreased LH pulse frequency can result in atresia of large follicles (Savio et al., 1990).

The physiology of LH secretion is different between *Bos indicus* and *Bos taurus* cattle. Randel (1976) reported a longer interval from an exogenous estradiol treatment to LH peak and decreased peak LH concentrations in Brahman compared to Hereford cows. Additionally, the interval from the LH surge to ovulation and interval from the initiation of estrus to ovulation was decreased in Brahman compared to Angus cows (Randel, 1976). Brahman cows also have a decreased responsiveness to estradiol and or gonadotropins (Rhodes et al., 1978). Portillo et al. (2006) reported that as the percentage of *Bos indicus* breeding increased, the amount of LH released in response to a GnRH challenge (500 μg) decreased when administered on day 6 of the estrous cycle.

The other glycoprotein hormone synthesized and secreted from the anterior pituitary is FSH. The primary function of FSH is to promote follicular development and production of estradiol and an FSH surge precedes the initiation of each follicular wave during the estrous cycle (Adams et al., 1992). Follicle stimulating hormone has receptors located on the granulosa cells of the growing follicle. Gibbons et al. (1997) demonstrated that the number of follicles that advanced into a follicular wave was increased by increasing the FSH surge at an exaggerated amount. As FSH peaks, follicles approach 4 mm in diameter (Ginther et al., 1996) and FSH must be present in order for a follicle to grow past 9 mm (Gong et al., 1996). The final decline in FSH is part of the deviation mechanism or selection of a dominant follicle (Ginther et al., 1997). Growth of the dominant follicle further suppresses FSH secretion (Ginther et al., 1997).

The ovarian steroid hormones, estradiol and progesterone, are also intricately involved in the regulation of the estrous cycle. The negative effect of progesterone on gonadotropin secretion decreases as circulating progesterone decreases, which allows for an increase in LH pulse frequency and a decrease in LH amplitude. This reduction occurs quickly in the three days approaching estrus (Wettemann et al., 1972) and the change in LH secretion allows for the dominant follicle to secrete increasing amounts of estradiol (Schallenberger et al., 1984). Estradiol increases GnRH production as well as GnRH receptors in the anterior pituitary having a positive effect on LH secretion (Vizcarra et al., 1997; Nett et al., 2002), which allows for decreased LH pulse frequency, increased LH pulse amplitude, which results in an LH surge and ovulation of the dominant follicle (Kesner et al., 1981).

Follicular Development, Selection, and Ovulation

Folliculogenesis is the process by which potential ovulatory follicles are formed from a pool of naïve, or primordial follicles (Spicer and Echternkamp, 1986). Folliculogenesis begins prior to puberty and continues in cycles of development and death throughout the life span of the animal. Primordial germ cells migrate to the gonadal ridge and are stored in primordial follicles, and form oogonia during mid gestation (Hirshfield et al., 1991; McGee and Hsueh, 2000). Lifetime supplies of oogonia within the ovary are established during gestation and the oogonia replicate by mitosis. The fetal ovary contains growing, preantral, and early antral follicles due to follicles leaving the resting pool while others are still being formed (Fortune, 2003). At birth, oogonia are suspended at the first meiotic division and are deemed primary oocytes (Fortune, 1994). In ruminants, the oocytes are arrested at this stage until the LH surge initiates ovulation and meiosis resumes (Vanderhyden, 2002). Initiation of follicle growth and its subsequent development are characterized by proliferation of granulosa cells, change in shape of granulosa cells, enlargement of the oocyte, and formation of the zona pellucida (Braw–Tal, 2002). There are three stages of follicle development based primarily on the layers of granulosa cells that comprise the follicle and they include primary, secondary, and tertiary follicles. Primary oocytes are housed in primordial follicles and are characterized by a single layer of granulosa cells consisting of 5 to 8 flattened cells, and are often referred to as quiescent follicles (Braw–Tal, 2002). As the follicles leave the resting pool they become primary follicles and the granulosa cells multiply and become cuboidal and express markers of cell proliferation (Fair et al., 1997; Fortune, 2003). Development continues to the secondary follicle stage with the addition of a second layer of granulosa cells, which progresses through the addition of 6 to 7 layers (Fortune, 2003). Additionally, the zona pellucida appears and gap junctions are formed between the oocyte and granulosa cells (Fair et al., 1997a). Stromal cells are recruited to form the thecal layer and appear at the mid or late preantral stage (Gougeon, 1996). Folliculogenesis is independent of gonadotropins (Roche, 2006) until the formation of thecal cells at the secondary follicle stage (Scaramuzzi et al., 1993; Mihm et al., 2002). The secondary follicle transitions into a tertiary follicle with the development of an antral cavity. The granulosa cells differentiate into cumulus and mural cells, the latter of which are associated with steroidogenic functions (Vanderhyden, 2002). As the follicle continues to develop, the antrum continues to accumulate follicular fluid increasing the follicle size and is designated as a Graafian follicle.

Follicular development is a cyclic process, and progresses through stages or waves of recruitment, selection, and dominance (Hodgen, 1982). The aforementioned stages are the basis for the wave-like phenomenon of follicle development that occurs during the estrous cycle in cattle. From an evolutionary standpoint, wave-like follicle development provides for an eligible ovulatory follicle to always be available (Sirois and Fortune, 1988). Cattle have between 1 to 5 follicular waves during one estrous cycle (Viana et al., 2000), although, two to three wave patterns are more commonly observed (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989).

Recruitment is a process where a cohort of small follicles (2 to 4 mm diameter) begins to mature with support of sufficient pituitary gonadotropic stimulation (Hodgen, 1982; Adams et al., 1992a). Stimulation from FSH promotes the growth of the cohort of follicles resulting in development of 5 to 10 follicles (Lucy et al., 1992) between 4 to 5 mm (Adams et al., 1992a). Selection is the process whereby a follicle is chosen, avoids atresia, and has the potential to become an ovulatory follicle (Hodgen, 1982). Atresia is programmed cell death and it is the most common fate of most follicles as only a few follicles reach dominance and ovulate. Greater than 99% of ovarian follicles undergo atresia during the lifetime of the cow (Hsueh et al., 1994). Fortune (1994) reported that atresia most commonly occurred before the final stages of follicular dominance in cattle, but it can occur at any stage of follicular growth. As FSH concentrations decline, fewer follicles continue to grow and are committed to atresia (Austin et al., 2001). Deviation, a part of selection, is when the second largest follicle decreases in size or stops growing in parallel with the dominant follicle, while the dominant follicle continues to grow (Ginther et al., 1996; Ginther et al., 1997).

Dominance occurs when the selected follicle dominates through inhibition of recruitment of a new cohort of follicles (Hodgen, 1982). The dominant follicle has increased estradiol and inhibin production, the latter of which inhibits the recruitment of follicles of the next follicular wave (Lucy et al., 1992; Ginther et al., 1999) and there is 3 to 4 d of additional growth of the dominant follicle. In a majority of estrous cycles the first wave dominant follicle regresses (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a) and a second wave evolves through the recruitment and selection stages. The regression of the first-wave dominant follicle is initiated by elevated circulating progesterone concentrations that have a negative effect on LH secretion, which causes regression of the dominant follicle (Sunderland et al., 1994). In contrast, if luteolysis occurs, progesterone concentrations decrease and estradiol production from the dominant follicle initiates an increase in LH secretion and eventually causes an LH surge resulting in ovulation (Wettemann et al., 1972). Maturation of the second dominant follicle in two-wave cycles coincides with regression of the CL and this follicle ovulates after luteolysis (Savio et al., 1988; Sirois and Fortune, 1988; Taylor and Rajamahendran, 1991). In three-wave follicle patterns the second wave dominant follicle becomes atretic and a third wave is recruited, selected, and becomes the dominant follicle that eventually ovulates.

Follicular dynamics is defined as the continual growth and regression of antral follicles that lead to the development of the preovulatory follicles (Lucy et al., 1992). Savio et al. (1990) described follicular dynamics beginning early in the estrous cycle as a cohort of follicles is recruited out of a pool of smaller antral follicles (2 to 4 mm). After 2 to 4 d of recruitment, medium-sized follicles (6 to 9 mm) can be detected. After recruitment, selection begins for a single follicle and this selected follicle continues to grow and the other recruited follicles decrease in size. The dominant follicle (> 10 mm) of the first wave remains active until day 8 to 11 of the estrous cycle (Ginther et al., 1989a). The first wave dominant follicle prevents other follicles from reaching a diameter > 5 mm. The first wave dominant follicle will ovulate if luteolysis is initiated by day 5 to 8 of the estrous cycle (Kastelic et al., 1990); however, in most cases the first wave dominant follicle regresses (Savio et al., 1988) and a second follicular wave begins around day 12 to 14 of the estrous cycle (Rajakoski, 1960). Follicular dynamics can be affected by breed and follicle number and size are most notably affected. The number of small follicles (2 to 5 mm) is greater in Brahman compared to Angus or Senepol cows (Alvarez et al.,

2000) and Brahman cows have more medium and total number of follicles compared to Angus cows (Simpson et al., 1994). Dominant follicle size is also different between breeds, as Figueiredo et al. (1997) reported that Nelore cattle had a smaller dominant follicle compared to *Bos taurus* counterparts.

The number of follicular waves during the estrous cycle can be influenced by many factors such as length of luteal dominance (Ginther et al., 1989b), parity and (or) age (Figueiredo et al., 1997), plane of nutrition (Rhodes et al., 1995; Mackey et al., 1999), lactation status (Lucy et al., 1992), and breed (Savio et al., 1988; Ginther et al., 1989b; Rhodes et al., 1995; Viana et al., 2000; Martinez et al., 2003). In general, *Bos indicus* cattle have been observed in numerous studies to have mostly three- and four-wave follicle wave patterns (Rhodes et al., 1995; Zeitoun et al., 1996; Viana et al., 2000; Martinez et al., 2003) compared to *Bos taurus* cattle that have primarily two- and three-wave follicle wave patterns (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989b). Follicular dynamics in Gir cattle is characterized by an increased incidence of estrous cycles with three or four follicle waves, associated with a low persistence of the dominant follicle (Viana et al., 2000). However, breed has also been shown to have no effect on the number of follicular waves between *Bos indicus* and *Bos taurus* cattle (Figueiredo et al., 1997; Alvarez et al., 2000).

The length of the estrous cycle can be influenced by the number of follicle waves that occur during the estrous cycle. The average length of the estrous cycle for cattle with two-wave follicle patterns is 20 d (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989). Whereas, cattle with three-wave follicle patterns have estrous cycles that range from 21 to 23 d (Savio et al., 1988; Ginther et al., 1989; Viana et al., 2000). Taylor and Rajamahendran (1991) observed that cattle with three follicular waves had a longer estrous cycle due to a longer luteal phase. The reason(s) for the difference in number of follicle wave patterns between breeds is unclear. One theory suggests it may be due to luteal dominance as Ginther et al. (1989) observed that luteolysis began later in three-wave estrous cycles compared to two-wave estrous cycles. Another explanation could be the smaller diameter of the intermediate wave dominant follicles compared to the diameter of ovulatory follicles, which is associated with a shorter plateau phase that may contribute to greater number of waves observed in Zebu cattle (Viana et al., 2000).

The number of follicular waves during the estrous cycle could also affect fertility of the subsequent estrus. Townson et al. (2002) reported that dairy cows had increased fertility after ovulation of a third wave dominant follicle compared to ovulation of a second wave dominant follicle. The reason for this could be that two wave patterns have a longer dominance of the ovulatory follicle, which is associated with decreased fertility and integrity of the follicle. Cattle that ovulated a dominant follicle from the third wave had follicles that were smaller in diameter, which is indicative of young growing follicles. Sirois and Fortune, (1990) and Stock and Fortune, (1993) demonstrated that ovulatory follicles with prolonged dominance, induced by exogenous progesterone in the absence of luteal progesterone, had significantly lower pregnancy rates compared to females that ovulated a normal follicle. Austin et al. (1999) reported that restricting the duration of dominance of pre-ovulatory follicle to 4 d yielded pregnancy rates > 70% in beef heifers.

Corpus Luteum Function and Luteolysis

The preovulatory surge of gonadotropins responsible for ovulation also causes differentiation of residual follicular cells that form the CL and initiates synthesis and secretion of progesterone (Niswender et al., 2000). Luteinizing hormone is the major luteotropin responsible for the development of the CL and production of progesterone (Armstrong and Black, 1966) and

LH pulses are needed for the development of a fully functional CL (McCracken et al., 1999; Niswender et al., 1994) in cattle. Although, pulsatile LH secretion is not needed for the maintenance of the CL after day 12 of the estrous cycle (Peters et al., 1994). Initial development of the CL takes approximately 3 d post ovulation in cattle. Fields and Fields (1996) reported that the weight of the CL was 640 mg by day 3 of the estrous cycle and increased to 5.1 g by day 14 of the estrous cycle. This growth is primarily due to hypertrophy of the granulosa and thecal cells. The CL develops two distinct populations of cells consisting of large and small luteal cells (Hansel et al., 1991), which differ morphologically and physiologically. Large luteal cells are derived from granulosa cells and small luteal cells from thecal cells (Niswender et al., 2000). Unlike small luteal cells, large luteal cells do not respond to LH because they do not express LH receptors (Mamluk et al., 1998). However, large luteal cells are responsible for the majority of basal progesterone (Fields and Fields, 1996), and are the target of the luteolytic effects of $PGF_{2\alpha}$ during luteolysis (Pate, 1996). Small luteal cells are primarily responsible for higher magnitude, LH-stimulated progesterone production (Hansel et al., 1991).

During ovulation, the follicle wall collapses and the basement membrane breaks down which gives rise to development of an extensive vascular network that invades the antral space of the follicle. Angiogenesis or formation of new blood vessels plays a key role in formation and growth of the CL. The mature CL has a dense system of blood vessels and has one of the highest rates of blood flow per unit of tissue compared to any other organ (Reynolds and Redmer, 1999).

Luteal progesterone has multiple functions including regulating gonadotropin secretion, preventing ovulation, and maintenance of pregnancy. Progesterone acts on the hypothalamus to reduce GnRH pulse frequency and preventing the LH surge (Evans et al., 1997). With a reduction in LH pulse frequency; there is an increase in pulse amplitude which is ideal for luteal function. Progesterone is also essential to the maintenance of pregnancy by preventing myometrial contractions to keep the uterus in a "quiescent state". Progesterone also stimulates the endometrium to produce nutrients and other factors vital for conceptus development (Geisert et al., 1992).

Breed also appears to effect luteal function but the reports are conflicting. Castilho et al. (2000) and Moreira-Viana et al. (2000) reported that *Bos indicus* cattle had a decreased luteal tissue area compared to *Bos taurus* cattle. Rhodes et al. (1995) and Figueiredo et al. (1997) observed that the maximum CL diameter in *Bos indicus* heifers was 17 to 18 mm compared to 25 to 39 mm in *Bos taurus* heifers (Adams et al., 1993). Similar findings have been reported by Castilho et al. (2000), Perea et al. (1998), Ruiz-Cortes and Olivera-Anger, (1999). In contrast, Alvarez et al. (2000) reported that the average and maximal CL diameters of Brahman and Senepol cows were greater compared to Angus cows. Breed differences have also been reported for progesterone concentrations. Segerson et al. (1984) observed greater progesterone concentrations in Angus compared to Brahman cows from d 7 to 17 of the estrous cycle. Randel et al. (1977) made similar observations, where Brahman and Brahman × Hereford heifers had circulating progesterone concentrations that were less compared to Hereford heifers. In contrast, Alvarez et al. (2000) reported similar progesterone concentrations for Brahman, Angus, and Senepol cows before day 14 of the estrous cycle.

Luteolysis includes the structural and functional demise of the CL, which causes a decline of progesterone synthesis and secretion (McCracken et al., 1999). Luteolysis allows a new reproductive cycle to begin and another opportunity for mating and a subsequent pregnancy. Elevated blood progesterone concentrations during most of the estrous cycle block the uterus from generating the luteolytic signal, which is $PGF_{2\alpha}$ (Gooding et al., 1972; Inskeep, 1973). The progesterone block is diminished on approximately day 16 of the estrous cycle and is due to the loss of progesterone receptors in the luminal and glandular epithelium of the uterus in cattle (Niswender et al., 2000), which allows the endometrium to produce $PGF_{2\alpha}$. Progesterone also promotes the accumulation of phospholipids in the luminal and glandular epithelia of the endometrium, which serves as a substrate to generate arachidonic acid a precursor for $PGF_{2\alpha}$ synthesis (Niswender et al., 1994; Spencer, 2004).

The neuroendocrine control of luteolysis has been thoroughly described in the model proposed by McCracken et al. (1999). Late in the luteal phase there is a down regulation of progesterone receptors in the hypothalamus and endometrium that causes a decrease in progesterone action. With the decrease in progesterone dominance, there is a return to the dominance of estrogen action on these tissues. Estrogen acts to stimulate the hypothalamic oxytocin pulse generator to secrete oxytocin at high frequency, low amplitude pulses. This initiates small pulses of $PGF_{2\alpha}$ to be released from the endometrium, which is sufficient to initiate release of luteal oxytocin. Luteal release of oxytocin assists in amplifying the release of endometrial $PGF_{2\alpha}$. With the increased pulses and concentrations of $PGF_{2\alpha}$, they are high enough to inhibit progesterone secretion and cause the release secretion of luteal oxytocin, further reinforcing endometrial $PGF_{2\alpha}$ synthesis. This cascade of events continues until $PGF_{2\alpha}$ receptor response becomes desensitized and the CL is fully degraded and luteolysis is complete (Flint et al., 1990).

Prostaglandin $F_{2\alpha}$ travels through a unique countercurrent mechanism which exists in the utero-ovarian pedicle of the cow (Hixon and Hansel, 1974). In this system, $PGF_{2\alpha}$ travels from the uterine vein directly to the ovarian artery, thereby avoiding the systemic circulation, which prevents it from being metabolized systemically. Kawakami et al. (1995) reported the concentration of $PGF_{2\alpha}$ in ovarian arterial blood increased to greater than 300 times the jugular venous blood within one minute after the start of $PGF_{2\alpha}$ infusion into the uterine vein. Prostaglandin $F_{2\alpha}$ has a very short half life (Kindahl, 1980), and will be broken down quickly by enzymes found in pulmonary tissue.

Many agents have been identified as mediators in structural and functional luteolysis of the CL. Evidence suggests that tumor necrosis factor-α (TNF-α) induced apoptosis plays a role in structural degradation in ruminants (Sawyer et al., 1990; Juengel et al., 1993; Zheng et al., 1994). Ji et al. (1991) and Shaw and Britt, (1995) demonstrated that $PGF_{2\alpha}$ elevates endogenous concentrations of luteal TNF-α and in vitro. Additionally, the endothelial cells express a TNF-α receptor and are sensitive to TNF-α induced apoptosis. Although, the exact mechanisms have not been fully identified for functional luteolysis, it is hypothesized that $PGF_{2\alpha}$ is mediated by endothelial cell-derived vasoconstrictive peptide, also known as endothelin-1 (ET-1), which alters normal patterns of progesterone synthesis (Milvae, 2000). Prostaglandin $F_{2\alpha}$ activates the ET-1 gene in luteal cells thereby stimulating its production. Secretion of ET-1 further stimulates production of $PGF_{2\alpha}$ from luteal cells, which acts back on the CL to further enhance ET-1 synthesis and secretion (Ohtani et al., 1998; McCracken et al., 1999; Milvae, 2000). Endothelin-1 binds to specific receptors in both small and large luteal cells and activates a decrease in basal and LH-induced production of progesterone (Milvae, 2000). This action could be responsible for the inability of $PGF_{2\alpha}$ to induce luteolysis in the early part of the estrous cycle since there is a lack of ET-1 and ET-1 only appears in large quantities in the CL just after $PGF_{2\alpha}$ exposure (Wright et al., 2001). A recent study by Friedman et al. (2000) outlined a positive link of $PGF_{2\alpha}$ induced ET-1 and TNF-α in functional and structural luteolysis. At luteolysis, ET-1 secretion from luteal cells and TNF-α production by local macrophages up-regulate one another's production and they work together to inhibit progesterone production. The low concentrations of progesterone along with increased expression of the TNF-α receptor facilitate TNF-α apoptosis of the endothelial cells in the CL. This leads to the functional and structural demise of the CL.

Puberty

Puberty can be defined as the first behavioral estrus accompanied by the development of a CL that is maintained for a period characteristic of a particular species (Kinder et al., 1987). The process which culminates in puberty actually starts before birth and continues throughout prepubertal development. Puberty is a result of the maturation of the endocrine and reproductive systems of the young animal so it eventually functions similar to an adult. It is proposed that the primary mechanism regulating pubertal development in heifers is the "gonadostat" theory, proposed by Ramirez and McCann (1963). The theory suggests that the prepubertal increase in LH secretion is the result of a decline in the negative feedback of estradiol on hypothalamic centers that control LH secretion. Day et al. (1987) proposed that the reason for the changes in LH secretion could be due to a decline in estradiol receptors in the hypothalamus and pituitary, which results in a decline in the efficacy of estradiol to exert negative feedback effects on LH secretion. The decline in estradiol receptors permits an increase in pulsatile secretion of LH. The peripubertal increase in mean LH may result from either a decreased ability of estradiol to inhibit GnRH secretion and (or) a decrease in negative feedback of estradiol at the pituitary, thereby increasing pituitary responsiveness to GnRH. Circulating estradiol concentrations will eventually reach concentrations sufficient to cause the pre-ovulatory surge of LH. Schams et al. (1981) reported an increase in LH pulse frequency and a decrease in LH pulse amplitude from 1 month of age until the onset of puberty. Day et al. (1984) also observed an increase in LH pulse frequency and an increase in mean LH concentration during the 126 d preceding puberty.

Finally, induction of the pre-ovulatory LH surge by estradiol is an essential component for puberty to occur (Kinder et al., 1987).

Age at puberty is influenced by many variables including genotype, age, nutrition, and environment. The data are conclusive that *Bos indicus* breeds reach puberty at older ages and heavier weights compared to *Bos taurus* cattle (Wiltbank et al., 1966; Plasse et al., 1968; Laster et al., 1978; Gregory et al., 1979; Nelson et al., 1982; Dow et al., 1982). Nelsen et al. (1982) reported Brahman heifers were 428 d old and weighed 287 kg at the onset of puberty compared to Angus heifers that were 343 d old and weighed 227 kg. Dow et al. (1982) observed that only 17% of Brahman × Hereford heifers reached puberty by 15 mo of age compared to 92% of Hereford × Red Poll heifers. Heterosis derived from crossbreeding *Bos indicus* with *Bos taurus* cattle decreases the age at puberty compared to the age at puberty in the straight bred *Bos indicus* heifers.

Nutrition is a key factor that can dictate the onset of puberty in beef heifers. Early work by Wiltbank et al. (1969) and Short and Bellows (1971) demonstrated that age-at-puberty was inversely related to growth rate. In short, as yearling heifer nutrition increased, the age at which heifers' attained puberty decreased. With this information, the "target weight" principal was conceptualized by Lamond (1970). This principal dictated that heifers needed to be approximately 65% of their dam's mature weight to attain puberty. Therefore, a "target weight" could be estimated and heifers could be placed on a nutritional plan allowing for a specified rate of gain to attain their "target weight" at the start of the breeding season. Subsequent studies demonstrated that plane of nutrition was associated with LH secretion. Pre-pubertal heifers maintained on a low gain diet failed to exhibit an increase in LH pulse frequency compared to contemporaries fed a growth diet, which resulted in increased LH pulse frequency and decreased age at puberty (Day et al., 1986). Recent research by Gasser et al. (2006), demonstrated that precocious puberty can be induced in early weaned heifers fed a high-concentrate diet by increasing LH pulse frequency. Treatment of heifers with exogenous progestogens has also been documented to enhance the onset of puberty in some pre-pubertal heifers (Short et al., 1976; Anderson et al., 1996; Imwalle et al., 1998). The mode of action appears to be associated with enhancing the pulsatile release of LH after progestogen withdrawal allowing for increased follicular growth and eventually ovulation and development of a CL (Anderson et al., 1996).

The age at which heifers attain puberty and get pregnant can affect the subsequent reproductive performance throughout a female's lifetime. Heifers that have their first calf at 2 yr of age produce more calves during their lifetime compared to heifers that have their first calf at 3 yr of age or greater (Pope, 1967; Donaldson, 1968, Chapman et al., 1978). Furthermore, heifers that calve early in their first calving season have a greater lifetime calf production compared to those that calve late (Lemeister et al., 1973). Consequently, cows that calve late in the calving season tend to calve late or not at all in subsequent years (Burris and Priode, 1958). The Lemeister et al. (1973) study also demonstrated that calves born late in the calving season usually weigh less and are worth less economically, which tends to decrease the total productivity of the dam compared to calves born early in the calving season. In summary, it is important that heifers attain puberty before the start of the breeding season, get pregnant early in the breeding season, and calve early the next calving season in order to maintain a yearly calving interval and optimize a cow's productivity throughout her lifetime in the cowherd. .

Maturation of the endocrine system plays a key role in determining when the onset of puberty occurs in the yearling heifer. Yet, the remainder of the reproductive axis including the uterus and ovaries also pay a significant role in the onset of puberty. During the 50 d preceding puberty, uterine weights increased rapidly and major morphological changes in the ovary occurred (Day et al., 1987). The reproductive tract score (RTS), which uses rectal palpation to quantify uterine size and presence of ovarian structures, was developed in yearling heifers of *Bos taurus* breeding by Anderson et al. (1991) to predict when and if puberty occurred in yearling heifers prior to the start of the breeding season. The RTS was developed on a scale of 1 to 5. A RTS of 1 is characterized with uterine horns less than 20 mm in diameter and no palpable structures on the ovary and is considered an immature tract. A RTS of 2 is characterized with uterine horns 20 to 25 mm in diameter with no tone, follicles no greater than 8 mm in diameter, and is identified as being greater than 30 d from puberty. A RTS of 3 is characterized with uterine horns of 20 to 25 mm in diameter with slight tone, follicles that are 8 to 10 mm in diameter, and is identified as being within 30 d of puberty. A RTS of 4 has uterine horns of at least 30 mm in diameter with good tone, follicles that are greater than 10 mm in diameter with a possible CL, and is identified as potentially estrous cycling. A RTS of 5 has uterine horns greater than 30 mm in diameter with good tone, a CL present, and is identified as estrous cycling. A common management practice used in yearling heifers of *Bos taurus* breeding is to conduct a RTS approximately 1 mo prior to the start of the breeding season. The RTS can be used to predict the response to an estrous synchronization protocol, predict the potential reproductive performance during the breeding season, and (or) to make culling decisions. In general, as RTS increases from a 1 to a 5, both the response to a synchronization treatment increases (Patterson et al., 2000) and breeding season pregnancy rates increase (Randle et al., 2000). For example, Randle et al. (2000) reported pregnancy rates of 58% for heifers with a RTS of 1 compared to 89% for heifers with a RTS of 5. However, essentially no research has been conducted in yearling heifers of *Bos indicus* × *Bos taurus* breeding utilizing the RTS as a management tool to either predict the reproductive performance of yearling heifers or as a culling tool. Therefore, additional research needs to be conducted to determine if the RTS developed in *Bos taurus* heifers has the same potential to predict reproductive performance in yearling heifers of *Bos indicus* × *Bos taurus* breeding.

Post Partum Interval and Return to Estrous

There are numerous factors that can influence the return to estrus after calving including dystocia problems encountered during calving, days since calving, time of weaning, parity, suckling and lactational status, body condition at calving, and pre and postpartum nutrition. The interval from calving to first ovulation in suckled cows ranges from 35 to 100 d (Casida, 1971; Foote, 1974; Wettemann et al., 1986; Hoffman et al., 1996); whereas average intervals to first estrus ranges from 65 to 104 d (Graves et al., 1968; Wiltbank, 1970; Casida, 1971). Young cows have a longer interval from calving to first estrus compared to older cows. For example, Wiltbank (1970) reported that the interval from calving to first estrus was 53.4 d in cows' ≥5-years-old, 60.2 d in 4-year-old cows, 66.8 d in 3-year-old cows, and 91.6 d in 2-year-old cows (Wiltbank, 1970). Body condition score (BCS) and nutritional status at calving tends to be the most influential factor on the resumption of estrous. Body condition can be used as in indirect indicator of nutritional status as it estimates the amount of fat that an animal contains and cows on an increased nutrient intake have greater BCS (Richards et al., 1986). Additionally, body energy reserves are related to reproductive function of postpartum cows (Dziuk and Bellows, 1983). Most studies suggests a minimum BCS of ≥ 5 at calving is needed to ensure adequate body stores so peak reproductive performance can be attained during the subsequent breeding season (Dziuk and Bellows, 1983; Richards et al., 1986). Cows calving at a BCS of 7 to 9 (Scale 1-9) were capable of returning to estrus within 60 d after calving. Dietary restrictions of cows during the late pre-partum period resulted in weight loss and decreased body fat, which lead to a decrease in the number of cows returning to estrus early in the breeding season (Whitman, 1975; Wettemann et al., 1982). Wiltbank et al. (1964) observed that cows fed the recommended level of TDN averaged 49 d from calving to first estrus as compared with 73 and 72 d for cows receiving 75% and 150% of the recommended level, respectively.

There is also a relationship between nutrient intake and function of the endocrine system. Low-energy diets decrease mean LH concentrations in postpartum cows (Terqui et al., 1980) whereas, cows fed a diet deficient in protein failed to have increased LH pulse frequency as postpartum intervals increase (Nolan et al., 1989). These studies support the idea that suppression or lack of LH pulsatility during the postpartum period leads to longer periods of anestrous. Another factor that affects normal endocrine activity is the suckling action of the calf. Randel (1981) reported once-daily suckled *Bos indicus* × *Bos taurus* heifers had a decreased postpartum interval by an average of 99 d compared to normal suckled first calf heifers. Mean interval from calving to estrus for normal suckled heifers was 168.2 ± 13.8 d, compared to 68.9 ± 6.2 d for once-daily suckled heifers. Suckling releases opioids that have a negative effect on the hypothalamus, which function to decrease GnRH release (Malven et al., 1986). This negative effect on GnRH suppresses LH pulse frequency thereby, preventing an early return to estrus. Walters et al. (1982) noted that by removing calves from cows 3 wk after calving or later resulted in a rapid removal of the negative effects of suckling on the tonic release of LH secretion. Bellows et al. (1974) reported that early weaning calves also decreased the postpartum interval in cows producing multiple or single calves.

Manipulation of the Estrous Cycle

Traditional AI programs were focused on determining when estrus occurred over a 21 d estrous cycle to determine when cattle were ready to be inseminated. This process was very time and labor consuming and limited whether or not producers could implement an AI program.

Development and implementation of estrous synchronization protocols allowed for a large number of cattle to be in estrus during a short time period compared to the detection of estrus during the 21 d estrous cycle. For most estrous synchronization protocols behavioral estrus will be exhibited during a 3 to 7 d period, which allows for more efficient use of AI. Some protocols have actually eliminated estrous detection and inseminate all cows at a predetermined timed known as timed-AI. Most synchronization protocols utilize exogenous hormones including $PGF_{2\alpha}$, GnRH, and progestogens either alone and (or) in combination to manipulate the estrous cycle. The primary function of a synchronization protocol is to provide for a tightly synchronized estrus and or ovulation by manipulating follicle development and luteolysis.

Prostaglandin $F_{2\alpha}$

It has been well established that prostaglandin$F_{2\alpha}$ is the luteolytic signal in beef cattle (Gooding et al., 1972; Inskeep, 1973) that originates from the uterus and initiates the demise of the CL. Rowson et al. (1972) and Lauderdale et al. (1974) demonstrated that an exogenous administration of $PGF_{2\alpha}$ during the luteal phase of the estrous cycle causes luteolysis of the CL without compromising fertility of the subsequent estrus (Lauderdale et al., 1974). Consequently, $PGF_{2\alpha}$ became the first pharmaceutical used for the purpose of estrous synchronization.

Subsequent research by Watts and Fuquay (1985) demonstrated that stage of the estrous cycle at administration of $PGF_{2\alpha}$ had an effect on estrous response, interval from $PGF_{2\alpha}$ to the onset of estrus, and conception rate. In this study, heifers in the early stages of the estrous cycle (day 5 to 7) had an estrous response of 43% and an interval from $PGF_{2\alpha}$ to the onset of estrus 59 h. In contrast, heifers in the middle (day 8 to 11) and later (day 12 to 15) stages of the estrous cycle had a greater estrous response (84 and 100%; respectively) but longer intervals from $PGF_{2\alpha}$ to the onset of estrous (71 and 72 h; respectively) compared to heifers in the early stages of the estrous cycle. First service conception rate also increased as the estrous cycle progressed. The interval from $PGF_{2\alpha}$ to the onset of estrus was longer for heifers in the mid to late stages of the estrous cycle, which is due to status of the follicular waves and size of the dominant follicle at $PGF_{2\alpha}$ (Sirois and Fortune, 1988). During the early stages of the estrous cycle, a dominant follicle is growing and ready to ovulate if luteolysis is induced compared to mid and late stages of the estrous cycle where follicles could be in the early stages of development and require additional time to reach ovulatory capacity and size.

A single dose of $PGF_{2\alpha}$ initiates luteolysis in 85 to 100% of *Bos taurus* females (King et al., 1982; Tanabe and Hann, 1984; Kiracofe et al., 1985) resulting in estrous responses between 75 and 95% (Tanabe and Hann, 1984; Watts and Fuquay, 1985). However, there has been limited research conducted to determine if genotype influences the effectiveness of $PGF_{2\alpha}$ to initiate luteolysis. Pinheiro et al. (1998) demonstrated that Nelore cattle treated with two injections of $PGF_{2\alpha}$ 11 d apart had a 33.3% estrous response in heifers and 46.4% estrous response in cows. Orihuela et al. (1983) reported estrous responses of 56 and 62% in Zebu cows following $PGF_{2\alpha}$ treatment. It should be noted that in the preceding studies that estrous response was used as an indirect indicator of luteolysis and no blood samples were collected to evaluate blood progesterone concentrations to confirm if luteolysis occurred. Because estrus is difficult to detect in *Bos indicus* cattle and there is an increased incidence of silent estrus in *Bos indicus* type cattle (Plasse et al., 1970), caution should be taken when using only estrous response when evaluating effectiveness of $PGF_{2\alpha}$ to initiate luteolysis. It has been hypothesized that the decreased estrous response in *Bos indicus* cattle was due to decreased effectiveness of the $PGF_{2\alpha}$ to initiate complete regression of the CL (Pinheiro et al., 1998; Rekwot et al., 1999) resulting in rebounding of progesterone production by the CL that block the expression of estrus. Recent research by Bridges et al. (2005) supported this hypothesis by demonstrating that a $PGF_{2\alpha}$

induced luteolysis was decreased in yearling heifers of *Bos indicus* × *Bos taurus* breeding compared to yearling heifers of *Bos taurus* breeding. However, in that same study, they also demonstrated that there may be an age effect because luteolysis was similar between two-year old virgin heifers of *Bos indicus* × *Bos taurus* breeding compared to two-year old virgin heifers of *Bos taurus* breeding.

To combat the inability of $PGF_{2\alpha}$ to initiate luteolysis in a majority of *Bos indicus* cattle, researchers added a second treatment of $PGF_{2\alpha}$ 24 h after the first one (Cornwell et al., 1985; Santos et al., 1988). Administration of the second $PGF_{2\alpha}$ treatment initiated complete luteolysis and prevented the CL from continuing to secrete progesterone. Bridges et al. (2005) administered two consecutive $PGF_{2\alpha}$ treatments 24 h apart to yearling heifers in the later stages of the estrous cycle and observed increased estrous response, timed-AI pregnancy rate, and synchronized pregnancy rate compared to heifers that received a single dose. Portillo et al. (2007) administered two half treatments of $PGF_{2\alpha}$ (12.5 mg) 24 h apart to mature suckled *Bos indicus* × *Bos taurus* cows and observed a significantly enhanced luteolysis, although, estrous response and synchronized pregnancy rates were similar compared to cows that received a single dose of $PGF_{2\alpha}$.

There are two common forms of prostaglandins available for commercial use. The trade names of Lutalyse® Sterile Solution, Prostamate®, and In-Synch® are from the chemical class dinoprost tromethamine, a naturally occurring prostaglandin, which is administered at a rate of 25 mg (5 cc) per dose. Estrumate® and Estroplan® are a synthetic analogous of $PGF_{2\alpha}$ made from cloprostenol sodium. These analogs are slightly more potent and are administered at a rate of 500 µg (2 cc). Both classes of prostaglandins provide for acceptable pregnancy rates when used in synchronization systems (Johnson, 1978; Seguin et al., 1984; Jackson et al., 1979).

Comparisons of both classes of prostaglandins have been made in *Bos taurus* animals (Young and Anderson, 1986; Salverson et al., 2002). Salverson et al. (2002) observed similar results when cloprostenol sodium was compared to dinoprost tromethamine in *Bos taurus* heifers synchronized with the long term MGA + $PGF_{2\alpha}$ protocol. Evaluation of the effectiveness of the two classes of prostaglandins in cattle of *Bos indicus* breeding has not been completely characterized in either suckled cows or yearling heifers. In non-lactating cows of *Bos indicus* breeding synchronized with a GnRH + $PGF_{2\alpha}$ based protocol, Heirs et al. (2003) reported a numerical increase in synchronized pregnancy rates of 5% in cloprostenol sodium treated cows compared to dinoprost tromethamine treated cows. In a recent study in suckled postpartum *Bos indicus* × *Bos taurus* cows synchronized with GnRH + $PGF_{2\alpha}$ based protocol concomitant with a CIDR, estrous response, conception rate, and synchronized pregnancy rates were similar between cows that received either cloprostenol sodium or dinoprost tromethamine (Esterman et al., 2007). There has been no research evaluating the effectiveness of cloprostenol sodium and dinoprost tromethamine in GnRH + $PGF_{2\alpha}$ based protocol concomitant with a CIDR in yearling heifers of to *Bos indicus* × *Bos taurus* breeding.

Progestogens

The primary function of a progestogen is to prevent the expression of estrus by mimicking the actions of the CL. Progestogens act to prevent the ovulatory surge of LH and thereby prevent ovulation from occurring (Patterson et al., 1989). An additional benefit of using progestogens is that they can initiate estrous cycles in some anestrous cows (Miksch et al., 1978; Smith et al., 1987; Fike et al., 1997; Yelich et al., 1997; Yavas and Walton, 2000) and pre-pubertal heifers (Anderson et al., 1996; Hall et al., 1997). Administration of a progestogen in anestrous cows and pre-pubertal heifers stimulates LH secretion both during and after progestogen treatment, which accelerates follicle growth resulting in ovulation after progestogen withdrawal (Garcia-Winder et al., 1987; Anderson et al., 1996). There are two major classes of progestogens including natural progesterone and synthetic analogues including norgestomet and melengestrol acetate (MGA). Progestogens can be administration via injection, implant, intravaginally, or orally. The two most common progestogens used in estrous synchronization protocols include the intravaginal progesterone insert or CIDR and MGA, which is orally active and administered in the feed.

The CIDR is an intravaginal insert made with a progesterone impregnated silicone coating. When inserted into the vagina, the moisture acts to move the progesterone out of the silicone coating. In the United States, the CIDR contains 1.38 g of progesterone, and it is estimated that 0.7 g of progesterone is used in its initial use (Savio et al., 1993), which is typically 7 d. Most estrous synchronization protocols leave the CIDR in the vagina for 7 d with administration of $PGF_{2\alpha}$ either one day before (Lucy et al., 2001) or at CIDR removal (Martinez et al., 2000; Stevenson et al., 2004; Larsen et al., 2006). The primary function of the CIDR is to improve estrous synchrony compared to a single $PGF_{2\alpha}$ treatment or unsynchronized cattle. An additional benefit of the CIDR is that it can initiate estrus in anestrous cows and peripubertal heifers. Lucy et al. (2001) reported that a 7 d CIDR induced estrus in approximately 40% of the anestrous cows and 40% of the prepubertal heifers. Fike et al. (1997) reported that 70% of anestrous cows were initiated into estrus or had formation of a CL after a 7 d CIDR treatment.

Since there is approximately 0.7 g (Rathbone et al., 2002) progesterone left in the CIDR after a single use (Savio et al., 1993), there has been growing interest among producers to use a once-used CIDR. Beal et al. (2003) reported that utilization of a once-used CIDR for 7 d effectively suppressed estrus in beef females. Furthermore, autoclaving the once-used CIDR before re-insertion results in greater serum progesterone concentrations compared to a once-used

CIDR that was only cleaned and not autoclaved (Zulagea and Williams, 2006). Moreover, Colazo et al. (2004) reported similar pregnancy rates after a fixed timed-AI in heifers that received either a new or once used CIDR. Therefore, the potential to use a once-used CIDR exists, but research in this area is limited relative to the effectiveness of a once-used CIDR to synchronize estrus as well as the ability to induce estrus in anestrous cattle.

Melengestrol acetate is capable of promoting endometrial proliferation, maintenance of pregnancy, and preventing the expression of estrus (Duncan et al., 1964; Zimbelman and Smith, 1966; Prichard et al., 1969; Schul et al., 1970). One of the advantages of using MGA is that it can be administered in the feed. The minimal effective dose needed to maintain pregnancy (4 mg/d) is much greater compared to that needed to prevent estrus and ovulation (0.5 mg/d) (Zimbelman, 1963b, Zimbelman and Smith, 1966). Early research demonstrated that administering MGA for more than 9 d lead to decreased fertility of the subsequent estrus and ovulation compared to untreated controls (Hill et al., 1971). Patterson et al. (1989) and Beal et al. (1988) further demonstrated that administration of MGA for 7 d or 9 d; respectively, also resulted in decreased fertility in some animals depending on what day of the estrous cycle they started the MGA treatment. Heifers which began MGA treatment after day 12 of the estrous cycle had decreased fertility compared to heifers where MGA was initiated prior to day 12 of the estrous cycle. The reduction in fertility is temporary, as fertility at the subsequent estrus is not affected (Zimbelman et al., 1970).

The decrease in fertility after a long term MGA treatment is caused by the development of what is termed a “persistent dominant follicle” (Guthrie et al., 1970). A persistent dominant follicle is a follicle that has a longer lifespan, increased maximal diameter, and increased estradiol secretion compared to normal ovulatory follicles (Sirois and Fortune, 1990; Savio et al., 1993b). Development of the persistent dominant follicle typically occurs with long-term progestin treatments (9 to 14 d) in the absence of a CL, which results in increased LH pulse frequency and decreased pulse amplitude (Kojima et al., 1992). The altered LH secretory profile results in continued follicle growth and development due to the absence of luteal progesterone that would normally initiate follicle turnover. After MGA withdrawal, estrus occurs within 3 to 7 d. Initial research indicated that the decreased fertility at the estrus after MGA withdrawal was due to increased estrogen concentrations that altered the uterine environment (Wordinger et al., 1972; Gibbons et al., 1973) and did not allow for conditions conducive to fertilization and (or) proper embryo development. However, recent research has demonstrated that fertilization actually occurs in the majority of cattle and the reduction is fertility is due to decreased embryo viability (Ahmad et al., 2005) and deceased oocyte competence (Stock and Fortune, 1993; Savio et al., 1993; Mihm et al., 1994a). Additionally, Mihm et al. (1992) reported that progesterone concentrations did not vary between ovulation of a normal follicle compared to ovulation of a persistent dominant follicle indicating that luteal function was not altered and probably does not contribute to the decrease in fertility.

There can also be a decreases in fertility associated with short term (≤ 9 d; Beal et al., 1988); Patterson et al., 1989) progestogen treatments. The decrease in fertility with a short term progestogen treatment is due to the duration of dominance of a follicle. Austin et al. (1999) reported that heifers which ovulated follicles between 2 and 8 d of dominance had pregnancy rates between 71 and 89%. In contrast, when follicles were ovulated that had either 10 or 12 d of dominance, pregnancy rates were 52 and 12%, respectively. Animals that ovulated follicles with prolonged dominance had significantly lower pregnancy rates compared to females that ovulated follicles of normal duration (Sirois and Fortune, 1990; Stock and Fortune, 1993).

Gonadotropin Releasing Hormone (GnRH)

Administration of exogenous GnRH alters follicular development by inducing luteinization and (or) ovulation of dominant follicles (Macmillan et al., 1985; Thatcher et al., 1989), which results in synchronization of a new follicular wave (Twagiramungu et al., 1994). Gonadotropin releasing hormone acts on the pituitary to induce release of the gonadotropes LH and FSH (Chenault et al., 1990). Gonadotropin releasing hormone is produced commercially as Fertagyl®, Factrel®, and Cystorelin® and is administered at 2 mL as an either an intravenous or intramuscular injection.

Concentrations of LH and FSH increase within 2 to 4 h of an endogenous GnRH treatment (Stevenson et al., 1993) and ovulation occurs 24 to 32 h after treatment (Thatcher et al., 1989; Pursley et al., 1994b). However, the ability of GnRH to initiate ovulation is stage of estrous cycle dependent. A dominant growing follicle must be present on the ovary in order for a GnRH induced ovulation to occur (Moreira et al., 2000). Kohram et al. (1998) demonstrated that GnRH administered between days 4 to 7 and days 15 to 18 induced ovulation, decreased estrogen concentrations, and initiated a new wave of follicle development. In another study using dairy heifers, GnRH induced ovulation less frequently in heifers that were day 2 and day10 of the estrous cycle compared to day 5, 15 and 18 of the estrous cycle (Moreira et al., 2000). Lack of a dominant follicle on day 2 and d 10 is representative of the initiation of follicular waves in cattle where recruitment and selection is taking place and have not reached at least 10 mm in diameter, which appears to be the minimal size that a follicle needs to be to be ovulated by GnRH (Sartori et al., 2001). There does not appear to be a breed effect on the ability of GnRH to ovulate follicles across several stages of the estrous cycle. In a recent study by Esterman et al. (2008), ovulation rates were similar for Angus and Brangus cows that received GnRH across several days of the estrous cycle at the initiation of a CIDR treatment.

There are essentially two types of CL that result from a GnRH induced ovulation including newly developed CL and an accessory CL. An accessory CL is an additional CL that develops in the presence of an existing CL. The CL that develops from a GnRH induced ovulation appears to be similar to a naturally formed CL in its capacity to be regressed with endogenous $PGF_{2\alpha}$ (Twagiramungu et al., 1992). However, mixed results have been reported about the integrity of progesterone output from an induced ovulation. As would be expected, induced ovulations that result in development of an accessory CL have increased progesterone concentrations (Twagiramungu et al., 1994; Wolfenson et al., 1994). However, progesterone concentrations can also be less following a GnRH treatment compared to concentrations normally observed during the estrous cycle (Ford and Stormshak, 1978; Rodger and Stormshak, 1986).

The purpose of including GnRH in an estrous synchronization and AI program is essentially two fold. First, it is used to induce ovulation and to synchronize the subsequent follicle wave by stimulating FSH to recruit the new follicle wave. In turn, when $PGF_{2\alpha}$ is administered 7 d after GnRH it results in a very synchronous estrus (Thatcher et al., 1989; Macmillan and Thatcher, 1991; Pursley et al., 1994). And second, GnRH administered in conjunction with AI, known as a timed-AI, functions to synchronize ovulation with delivery of semen into the reproductive tract. The later scenario is particularly effective when used in conjunction with GnRH followed seven days later with $PGF_{2\alpha}$ and a timed-AI approximately 48 to 60 h after $PGF_{2\alpha}$ (Pursley et al., 1995, 1997; Burke et al., 1996; Schmitt et al., 1996).

Synchronization Protocols

The primary functions of synchronization protocols is to either synchronize estrus so cattle can be inseminated over a 1 to 5 d period or to synchronize ovulation so cattle can be inseminated at a predetermined timed know as a timed-AI. Initial synchronization protocols focused on the use of either a single $PGF_{2\alpha}$ or two $PGF_{2\alpha}$ treatments 10 to 11 d apart. The major drawback with the $PGF_{2\alpha}$ protocols is that they only work in cattle that are going through estrous cycles and they require considerable estrous detection. The more preferred methods of synchronization include combining one of several pharmaceuticals including GnRH, a progestogen, and $PGF_{2\alpha}$. Synchronization protocols significantly reduce estrous detection and (or) eliminate estrous detection completely by incorporating a timed-AI. Furthermore, they can induce estrus in some anestrous cattle. It appears that some synchronization protocols are more effective in suckled postpartum cows compared to heifers. The numerous synchronization protocols available to producers can best be tailored to fit a producers operation. Although, most producers want synchronization protocols that require minimal animal handling, and result in acceptable and consistent AI pregnancy rates. The general consensus is that acceptable pregnancy rates are 50%.

One of the most widely used estrous synchronization programs for yearling heifers is the 14 d melengestrol acetate (MGA) treatment followed by $PGF_{2\alpha}$ 17 d later (MGA-$PGF_{2\alpha}$; Brown et al., 1977). The MGA-$PGF_{2\alpha}$ protocol was designed to bypass inseminating heifers at the infertile estrus immediately after MGA withdrawal and took advantage of administering $PGF_{2\alpha}$ in the later stages of the estrous cycle when it was more effective in initiating luteolysis. Subsequent research illustrated that administering $PGF_{2\alpha}$ 19 d after the last day of MGA (Lamb et al., 2000) improved the synchrony of the $PGF_{2\alpha}$ induced estrus without reducing fertility. Heifers can be inseminated either after an observed estrus with estrous detection typically lasting 5 to 7 d or estrus can be detected for 3 d with a timed-AI + GnRH on the third day for heifers not exhibiting estrus with each insemination protocol yielding similar AI pregnancy rates . Beef cattle producers like to use the MGA-$PGF_{2\alpha}$ system because it is easy to implement, cost effective, requires minimal animal handling, and yields consistent synchronized pregnancy rates (typically > 50%) in yearling heifers of *Bos taurus* breeding (Brown et al., 1988; Lamb et al., 2000; Salverson et al., 2002). In contrast, Bridges et al. (2005) reported synchronized pregnancy rates of only 34.5% in yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with the MGA-$PGF_{2\alpha}$ system where estrus was detected for 3 d with timed-AI+GnRH on day 3 for heifers not exhibiting estrus. Synchronized pregnancy rate was improved to 42.5% when the $PGF_{2\alpha}$ treatment (25 mg) was split (12.5 mg; split- $PGF_{2\alpha}$) and administered on two consecutive days, which resulted in greater luteolysis for the split-$PGF_{2\alpha}$ compared to the single $PGF_{2\alpha}$. Even with the addition of the split-$PGF_{2\alpha}$ treatment to the MGA-$PGF_{2\alpha}$ system, synchronized pregnancy rates are still less for *Bos indicus* × *Bos taurus* heifers compared to *Bos taurus* heifers. Recent work in our lab reported that follicle development was different between *Bos taurus* (Angus) and *Bos indicus* × *Bos taurus* (Brangus) heifers after a 14 d MGA treatment (Woodall et al., 2006). First, ovulation rate after MGA withdrawal tended to be greater in Angus (100%; n=10/10) compared to Brangus (80%; n=8/10) heifers. Second, Brangus (n=5/10) had more one, three, and four follicle wave patterns compared to Angus (n=2/10), which resulted in asynchronous follicle development at $PGF_{2\alpha}$ for Brangus heifers. In a subsequent experiment, GnRH was given either 3 or 10 d after a 14 d MGA treatment to synchronize follicle development followed by $PGF_{2\alpha}$ 7 d after GnRH. The day 3 GnRH treatment resulted in more heifers in estrus during the 3 d after $PGF_{2\alpha}$ compared to GnRH given 10 d after MGA withdrawal (Woodall et al., 2007a). In subsequent field trials, synchronized pregnancy rates were similar between GnRH given 3 d after a 14 d MGA treatment (34.8%; n=178) compared to the original MGA-$PGF_{2\alpha}$ system (38.5%; n=174; Woodall et al., 2007b). Both treatments received a split-PG with estrous detection and AI for 3 d after $PGF_{2\alpha}$ and heifers not exhibiting estrus by 73 h after $PGF_{2\alpha}$ being timed-AI with GnRH between 73 to 80 h. Since less than acceptable pregnancy rates are obtained when synchronizing *Bos indicus* × *Bos taurus* heifers with a long-term MGA-$PGF_{2\alpha}$ treatment, other synchronization system must be evaluated.

Short-term (7 d) CIDR treatments are another alternative to synchronizing estrus. In a recent set of field trials, Lamb et al., (2006) evaluated the effectiveness of several short term progestogen treatments in yearling *Bos taurus* heifers. Lamb et al. (2006) reported that one of the most consistent synchronization protocols in yearling heifers included administration of GnRH concurrent with a 7 d Eazi-Breed CIDR with $PGF_{2\alpha}$ at CIDR removal followed by a fixed timed-AI + GnRH 60 h after $PGF_{2\alpha}$, which yielded synchronized pregnancy rates of 53%. Equally effective was the same CIDR synchronization protocol but with 3 d of estrous detection combined with a timed-AI plus GnRH for heifers not exhibiting estrus by 84 h after $PGF_{2\alpha}$, which yielded a synchronized pregnancy rate of 57%. There has been limited research conducted in yearling heifers of *Bos indicus* × *Bos taurus* breeding evaluating short-term CIDR treatments. Lucy et al. (2001) reported that CIDR+$PGF_{2\alpha}$ treated heifers had a greater estrous response (84%) during the first 3 d of the breeding period compared to $PGF_{2\alpha}$ treated heifers (57%). We have recently initiated a multiyear study in our lab comparing the effectiveness of cloprostenol sodium (Estrumate) compared to dinoprost tromethamine (Lutalyse) in the Select Synch/CIDR+timed-AI program in Angus, Brahman, and Brahman × Angus 2-year old virgin heifers. The overall estrous, conception, timed-AI pregnancy, and synchronized pregnancy rates for the first two years were 48.5 (n=163), 60.9 (n=79), 30.7 (n=84), and 45.3% (n=163), respectively (Unpublished data). There were no prostaglandin or breed effects on any of the variables measured. Synchronized pregnancy rates ranged from 40.5 to 54.1% across treatments and years suggesting that the Select Synch/CIDR+timed-AI synchronization program could work in yearling heifers of *Bos indicus* × *Bos taurus* breeding but additional research is needed to confirm this.

The 7 d CIDR protocol has also been researched in suckled *Bos taurus* cows. Larsen et al. (2006) observed greater pregnancy rates for the Select Synch/CIDR+timed-AI and CO-Synch+CIDR treatments compared to the CO-Synch without CIDR treatment. Conception rates were greater for the Select Synch+timed-AI and Select Synch/CIDR+timed-AI cows compared to cows synchronized with a CIDR alone. The authors concluded that the Co-Synch+CIDR yielded similar pregnancy rates compared to estrous detection protocols and can be used as an effective timed-AI protocol in suckled *Bos taurus* beef cows. Lucy et al. (2006) reported a tendency for first service conception rate and pregnancy rates to be greater in the CIDR+$PGF_{2\alpha}$ compared to unsynchronized controls in suckled *Bos taurus* and *Bos indicus* × *Bos taurus* beef cows from seven different locations.

Utilization of a once-used-CIDR has been shown to be an option to the new CIDR when used in synchronization protocols. Colazo et al. (2003) conducted three experiments using heifers and suckled beef cows. Experiment one compared efficacy of a new (1.9 g) or once-used CIDR inserted for 9 d and estradiol cypionate with or without progesterone at CIDR insertion in heifers. Pregnancy rate was not affected by number of times a CIDR was used or addition of progesterone. The second experiment in heifers and cows received either a once- or twice-used CIDR, or either estradiol benzoate with or without progesterone at CIDR insertion. Synchronized pregnancy rates were decreased for the twice-used CIDR, but progesterone did not affect pregnancy rates. In the third experiment, heifers received a new, once-used, twice-used, or two twice-used CIDR, pregnancy rates were similar for all treatments and there was no affect of progesterone. These results suggest that incorporation of a once-used CIDR into a synchronization program has potential. Follow up research conducted by Solorzano et al. (2004, 2008), demonstrated that a once-used CIDR could effectively synchronize estrus in non-lactating beef cows and yearling heifers that were eventually used for embryo transfer. It should be noted that the afore mentioned experiments utilized a CIDR containing 1.9 g of progesterone compared to 1.38 g CIDR which is the only type available for purchase in the United States. Consequently, it is recommended that CIDR purchased in the United States only be re-used one time. Recent research also indicates that autoclaving the once-used CIDR actually results in greater circulating blood progesterone concentrations compared to a once-used CIDR that was not autoclaved (Eaton et al., 2007).

The 7-11 estrous synchronization protocol consist of a 7 d MGA treatment with $PGF_{2\alpha}$ on the last day of MGA followed by GnRH 4 d later; seven days after GnRH, $PGF_{2\alpha}$ is administered to synchronize estrus (Kojima et al., 2000). The concept behind the 7-11 protocol is that the 7 d MGA treatment prevents cattle from exhibiting estrus and the first $PGF_{2\alpha}$ treatment initiates luteolysis of any CL present at MGA removal. This pretreatment regime results in a majority of cattle with an ovulatory sized follicle present when GnRH is administered 4 d after MGA withdrawal. The GnRH treatment functions to synchronize follicle development so a majority of cattle will have a healthy growing follicle ready for ovulation at the second $PGF_{2\alpha}$ administered 7 d after GnRH. The 7-11 protocol is an effective protocol for synchronizing estrus for either a detected estrus in *Bos taurus* (Kojima et al., 2000) or a timed-AI in *Bos taurus* cattle (Bader et al., 2005).

In a recent study conducted in our lab, estrous cycling and anestrous postpartum Brangus and Angus cows received GnRH 4 d after a 7 d MGA treatment with $PGF_{2\alpha}$ at MGA withdrawal, GnRH induced ovulation in 94.4% of Brangus (n=17/18) and Angus (n=17/18) cows (Esterman et al., 2007). The resulting five day estrous response (70.5, 65.8%), conception (67.7, 76.0%), and synchronized pregnancy rates (47.7, 50.0%) were similar ($P > 0.05$) for Angus and Brangus, respectively. Because GnRH effectively ovulated follicles in Brangus cows when administered 4 d after a short term (7 d) progestogen treatment, it appears that the 7-11 synchronization protocol is an effective synchronization protocol for use in suckled cows of *Bos indicus* × *Bos taurus* breeding. However, additional research will need to be conducted to evaluate the effectiveness of the 7-11 protocol in yearling heifers of *Bos indicus* × *Bos taurus* breeding.

In summary, the following objectives are being proposed and will constitute the research to be presented in this thesis.

- **Objective 1:** Evaluate the effectiveness of the Select Synch/CIDR+timed-AI synchronization protocol and a modified 7-11 protocol in yearling heifers and suckled cows of *Bos indicus* × *Bos taurus* breeding.

- **Objective 2:** Evaluate the effectiveness of cloprostenol sodium and dinoprost tromethamine when used in conjunction with the synchronization treatments outlined in objective 1.

- **Objective 3:** Evaluate the effectiveness of the 7-11 and Select Synch/CIDR+timed-AI synchronization protocols in yearling heifers of *Bos indicus* × *Bos indicus* breeding.

CHAPTER 3

COMPARISON OF TWO PROGESTOGEN BASED ESTROUS SYNCHRONIZATION PROTOCOLS AND CLOPROSTENOL SODIUM VS. DINOPROST TROMETHAMINE IN SUCKLED POST PARTUM COWS AND YEARLING HEIFERS OF B*os indicus* × *Bos taurus* BREEDING

Introduction

In the Gulf Coast region of the Southeastern US, the majority of cattle contains some *Bos indicus* influence because of their superior ability to deal with heat stress, utilization of low quality forages, and increased parasite tolerance. However, the slight physiological and behavioral differences that exist in cattle of *Bos indicus* breeding could cause differential responses to estrous synchronization protocols that have been developed primarily in cattle of *Bos taurus* breeding. Cattle of *Bos indicus* breeding have an increased sensitivity and respond differently to exogenous hormones (Randel, 1984; Portillo et al., 2007) compared to cattle of *Bos taurus* breeding. Furthermore, estrus is more difficult to detect in cattle of *Bos indicus* breeding due to a decreased expression and duration of estrus (Galina et al., 1982) as well as an increased incidence of silent estruses (Galina et al., 1996).

One of the most consistent synchronization protocols in suckled cows (Larson et al., 2006) and yearling heifers (Lamb et al., 2006) of *Bos taurus* breeding includes administration of a 7 d Eazi-Breed™ CIDR® (CIDR) with GnRH at CIDR insertion and $PGF_{2\alpha}$ at CIDR removal, followed by 3 d of estrous detection and AI with a timed-AI plus GnRH for cattle not exhibiting estrus by 72 h after $PGF_{2\alpha}$. This protocol is known as the Select Synch/CIDR+timed-AI protocol. Lucy and coworkers (2001) conducted a multi-location study in suckled beef cows and yearling heifers that received a 7 d CIDR with $PGF_{2\alpha}$ one day prior to CIDR removal followed by 3 d of estrous detection and AI. The AI pregnancy rates were similar for suckled cows and yearling heifers of *Bos indicus* × *Bos taurus* breeding compared to locations with cattle of *Bos taurus* breeding. It should be noted that GnRH was not administered at CIDR insertion in the

Lucy et al. (2001) study. Therefore, additional experiments are needed in cattle of *Bos indicus* × *Bos taurus* breeding to evaluate CIDR synchronizations treatments when GnRH is administered at CIDR insertion and at timed-AI for induction of ovulation in cows not expressing estrus.

The 7-11 synchronization protocol is another short term progestogen synchronization protocol that is frequently used in cattle of *Bos taurus* breeding (Kojima et al., 2000). The 7-11 protocol consists of a 7 d melengestrol acetate (MGA) treatment with $PGF_{2\alpha}$ on the last day of MGA followed by GnRH 4 d later. Seven days after GnRH, $PGF_{2\alpha}$ is administered to synchronize estrus. The 7-11 synchronization protocol is effective in *Bos taurus* cattle (Kojima et al., 2000; Stegner et al., 2004) but no research has been conducted to evaluate the effectiveness of the 7-11 protocol in yearling heifers of *Bos indicus* × *Bos taurus* breeding and one study has been conducted in suckled cows of *Bos indicus* × *Bos taurus* breeding (Esterman et al., 2007b).

As production costs increase, producers seek to implement new management practices that either reduce their operating costs and (or) increase productivity. Therefore, there has been considerable interest in using a once-used CIDR in estrous synchronization protocols. Utilization of a once-used CIDR for 7 d suppresses estrus in beef females (Beal et al., 2003) and autoclaving a once-used CIDR increases circulating progesterone concentrations compared to a non-autoclaved once-used CIDR (Zuluaga and Williams, 2008). Autoclaving also reduces the risk of disease transmission. Moreover, synchronized pregnancy rates after a fixed timed-AI were similar in heifers receiving either a new or once-used CIDR (Colazo et al., 2004). Therefore, there appears to be potential for incorporating a once-used CIDR into some estrous synchronization protocols. Additionally, a minimal amount of research has been conducted evaluating the effectiveness of prostaglandin type, cloprostenol sodium compared to dinoprost tromethamine, when used in GnRH+$PGF_{2\alpha}$ estrous synchronization protocols in cattle of *Bos indicus* × *Bos taurus* breeding. Heirs et al. (2003) reported similar synchronized AI pregnancy rates for cloprostenol sodium compared to dinoprost tromethamine in non-lactating *Bos indicus* × *Bos taurus* cows synchronized with GnRH+$PGF_{2\alpha}$ protocol combined with MGA and Esterman et al. (2007a) reported similar results between the two prostaglandins in suckled *Bos indicus* × *Bos taurus* cows synchronized with GnRH/CIDR+$PGF_{2\alpha}$ protocol. However, no direct comparisons have been made between cloprostenol sodium and dinoprost tromethamine in yearling *Bos indicus* × *Bos taurus* heifers synchronized with GnRH+$PGF_{2\alpha}$ synchronization protocols.

Therefore, the objectives of these experiments were to evaluate the effectiveness of cloprostenol sodium compared to dinoprost tromethamine when used in a modified 7-11 protocol and a Select Synch/CIDR+timed-AI protocol in yearling heifers and suckled cows of *Bos indicus* × *Bos taurus* breeding.

Materials and Methods

Experiment 1

Experiment 1 (Figure 3-1) was conducted from January to March 2007 at the Bar L Ranch, Marianna, Florida. In Experiment 1, multiparous suckled postpartum *Bos indicus* × *Bos taurus* cows (n = 324) were used. The percentage of *Bos indicus* breeding of cows utilized ranged from approximately 7 to 38% with the remainder being *Bos taurus* breeding. The experiment was a 2 × 2 factorial design. At the start of the experiment (d 0), cows were equally distributed by body condition score (BCS; 1 = emaciated, 5 = moderate, 9 = very fat; Richards et al., 1986) and days post partum (DPP) to one of two progestogen based synchronization treatments and one of two $PGF_{2\alpha}$ treatments. The synchronization treatments included a modified 7-11 treatment, which will be termed the 7-10 treatment, and the Select Synch/CIDR+timed-AI treatment. Within each synchronization treatment, half the cows received one of two $PGF_{2\alpha}$ treatments including dinoprost tromethamine (dinoprost, 25 mg i.m.; Prostamate®, Agrilabs, St. Joseph, MO) and cloprostenol sodium (cloprostenol, 500 µg i.m.; Estrumate®, Schering-Plough Veterinary Corp., Kenilworth, NJ). The original 7-11 synchronization protocol administers GnRH 4 d after MGA withdrawal (Kojima et al., 2000). Since an intravaginal CIDR was used in the present study, it was hypothesized that the disappearance of the negative feedback effect of progesterone on LH secretion should take less time to clear the circulatory system compared to MGA. Therefore, GnRH was administered on day 10 instead of day 11 resulting in the name change to the 7-10 synchronization treatment. On day 0, the 7-10 treatment received an autoclaved once-used CIDR (Eazi-Breed™ CIDR®, 1.38 g progesterone, Pfizer Animal Health, New York, NY) that was removed on day 7 concomitant with dinoprost followed by GnRH (500 µg; Cystorelin®, Merial Animal Health, Duluth, GA) on day 10. The once-used autoclaved CIDR was used in place of MGA to test its effectiveness as a low cost alternative progestogen source in the 7-10 protocol. On day 17, 7-10 cows received either dinoprost or cloprostenol. Also on day 10, the Select Synch/CIDR+timed-AI treatment received a new CIDR concomitant with GnRH followed by CIDR removal on day 17 where cows received either dinoprost or cloprostenol.

To aid in estrous detection, all cows received Estrotect™ estrous detection patches (Rockway, Inc., Spring Valley, WI) on day 18 of the experiment. Estrus was visually detected twice daily (0700 and 1600 h) for 72 h after $PGF_{2\alpha}$ and cows were inseminated 6 to 12 h after detection of estrus. Non-responders were timed-AI + GnRH 72 to 76 h post $PGF_{2\alpha}$. Estrus was defined as a cow standing to be mounted by another cow, showed signs of visible mucous, and/or had a half to full red estrous detection patch. Two AI technicians inseminated cows using frozen-thawed semen from a single sire of known fertility and technicians were equally distributed across treatments for both cows that either exhibited estrus or were timed-AI. Seven days after the timed-AI, natural service sires were placed with cows. Using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 5.0 MHz transducer, pregnancy was diagnosed approximately 55 d after AI. Due to the 7 d period where no cows were exposed to natural service sires, differences in fetal size were used to determine whether a pregnancy resulted from the synchronized AI breeding (54 and 55 d pregnant) or natural service sire (≤ 48 d pregnant). Embryos develop a C shape appearance approximately day 25 of gestation, which transitions to an L shape embryos by day 32. By day 48, the fetus is approximately 35 mm in length with visible limb buds and by day 55 the fetus is approximately 50 mm in length and has identifiable ribs (Curran et al., 1986).

Estrous response was defined as the number of cows displaying estrus for 3 d after prostaglandin and artificially inseminated (AI) divided by the total number of cows treated. Conception rate was defined as the number of cows that became pregnant to AI divided by the number of cows that displayed estrus and were AI. Timed-AI pregnancy rate was the number of cows that failed to display estrus, were timed-AI, and became pregnant divided by the total number of cows that were timed-AI. Synchronized pregnancy rate was the number of cows pregnant to AI 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.

Binomially distributed data including estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and 30 d pregnancy rate, were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). All models included synchronization treatment (7-10 and Select Synch/CIDR+timed-AI), $PGF_{2\alpha}$ treatment (cloprostenol and dinoprost), DPP, cow age, BCS, AI technician, and all appropriate interactions. Days postpartum, cow age, and BCS were analyzed as categorical variables. Days postpartum was evaluated in three categories ≤ 43 d, 44 to 59 d, and ≥ 60 d. Cow age ranged from 3 to 9 yr of age resulting in seven categories. Body condition score was grouped into three categories ≤ 4.5, 5, ≥ 5.5. The effect of interval from $PGF_{2\alpha}$ to the onset of estrus (48, 60, and 72 h) on conception rate also was analyzed with synchronization treatment, $PGF_{2\alpha}$ treatment, interval from $PGF_{2\alpha}$ to the onset of estrus, DPP, cow age, BCS, and all appropriate interactions included in the model. Because of a limited number of cows exhibiting estrus between 0 and 48 h after $PGF_{2\alpha}$, these times periods were combined to one group of cows' ≤ 48 h. The final logistic regression model entered variables by a stepwise selection based on the Wald statistics criterion when $P < 0.20$ and removed variables based on $P < 0.10$. Variables were considered significant at $P \leq 0.05$. Variables that were significant were entered into a linear regression model using GENMOD procedure of SAS to calculate the adjusted odds ratios and 95% confidence intervals. Odds ratios were reported in tables and were used to calculate relative risk, and the relative risk was reported in the results.

Experiment 2

Experiment 2 (Figure 3-1) was conducted with yearling *Bos indicus* × *Bos taurus* heifers (13 to 15 mo old) at the Bar L Ranch, Marianna, Florida, during two consecutive years, 2006 (year 1, n = 218) and 2007 (year 2, n = 137). The percentage of *Bos indicus* breeding ranged from approximately 7 to 38% with the remainder being *Bos taurus* breeding. The experiment was a 2 × 2 factorial design and heifers were equally but randomly assigned to the same treatments used in Experiment 1. Body condition scores were recorded for heifers at CIDR insertion.

For all treatments and years, heifers received Estrotect™ estrous detection patches on day 18 of the experiment to aid in estrous detection. Estrus detection and insemination protocols were similar as described in Experiment 1. Heifers were inseminated with frozen-thawed semen from a single sire in year 1 and two pre-assigned sires in year 2. Heifers were inseminated by the same AI technician in both years. Seven days after the timed-AI, natural service sires were placed with heifers in both years. Pregnancy was diagnosed approximately 54 to 56 d after AI using a real-time B-mode ultrasound (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 5.0 MHz transducer. Due to the 7 d period in which heifers were not exposed to natural service sires, differences in fetal size (Curran et al., 1986) as described for Experiment 1 were used to determine whether a pregnancy resulted from the synchronized breeding or the natural service sire.

Estrous response was defined as the number of heifers displaying estrus for 3 d after prostaglandin and inseminated divided by the total number of heifers treated. Conception rate was defined as the number of heifers that became pregnant to AI divided by the number of heifers that displayed estrus and were AI. Timed-AI pregnancy rate was the number of heifers that failed to display estrus, were timed-AI, and became pregnant divided by the total number of heifers that were timed-AI. Synchronized pregnancy rate was the number of heifers pregnant to AI 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.

Binomially distributed data including estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty-day pregnancy rate were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). All models included synchronization treatment (7-10 and Select Synch/CIDR+timed-AI), $PGF_{2\alpha}$ treatment (cloprostenol and dinoprost), year, BCS, and all appropriate interactions. Body condition score was analyzed as a categorical variable and was grouped into three categories ($\leq$ 5, 5.5, $\geq$ 6). The effect of interval from $PGF_{2\alpha}$ to the onset of estrus ($\leq$ 48, 60, and 72 h) on conception rate was also analyzed with synchronization treatment, $PGF_{2\alpha}$ treatment, interval from $PGF_{2\alpha}$ to the onset of estrus, year, BCS, and all appropriate interactions included in the model. Because of a limited number of heifers exhibiting estrus between 0 and 48 h after $PGF_{2\alpha}$, these time periods were combined into one group of heifers' $\leq$ 48 h. The final logistic regression model entered variables by a stepwise selection based on the Wald statistics criterion when $P <$ 0.20 and removed variables based on $P < 0.10$. Variables were considered significant at $P \leq$ 0.05. Variables that were significant were entered into a linear regression model using GENMOD procedure of SAS to calculate the adjusted odds ratios and 95% confidence intervals. Odds ratios were reported in tables and were used to calculate relative risk, and relative risk was reported in the results.

Results

Experiment 1

Synchronization treatment affected ($P < 0.05$) estrous response with the risk of displaying estrous in the Select Synch/CIDR+timed-AI treated cows being 1.2 times greater compared to 7-10 cows. Body condition score also affected ($P < 0.05$) estrous response. Cows in BCS $\leq$ 4.5 had an estrous response that was 73.7 percent of what cows that were BCS $\geq$ 5.5 expressed. Cows with a BCS 5 tended ($P = 0.1$) to have a decreased estrous response compared to cows in

BCS $\geq$ 5.5 (Table 3-1). There were no effects ($P > 0.05$) of $PGF_{2\alpha}$ treatment, age, DPP, and all appropriate interactions on estrous response.

Synchronization treatment affected ($P < 0.05$) conception rate as the Select Synch/CIDR+timed-AI cows had a 1.4 higher risk of becoming pregnant to AI after an observed estrus compared to 7-10 cows. Body condition also affected ($P < 0.05$) conception rate. Cows with BCS $\geq$ 5.5 had 2 times greater risk of becoming pregnant after an observed estrus compared to cows that were BCS $\leq$ 4.5. Cows in BCS 5 tended ($P = 0.1$) to have decreased conception rate compared to cows in BCS $\geq$ 5.5 (Table 3-2). There was also an AI technician effect ($P < 0.05$) on conception rate (56.8 vs. 45.2%). There were no effects ($P > 0.05$) of $PGF_{2\alpha}$ treatment, age, DPP, interval from $PGF_{2\alpha}$ to estrus, and all appropriate interactions on conception rate.

There were no effects ($P > 0.05$) of synchronization treatment, $PGF_{2\alpha}$ treatment, BCS, age, AI technician, and all appropriate interactions on timed-AI pregnancy rate. Timed-AI pregnancy rates for the 7-10 and Select Synch/CIDR+timed-AI were 17.5 and 20.9%, respectively. However, DPP tended ($P = 0.1$) to effect timed-AI pregnancy rate (Table 3-3). In general, as days from calving increased, timed-AI pregnancy rates also increased.

Synchronization treatment affected ($P < 0.05$) synchronized pregnancy rate with cows treated with Select Synch/CIDR+timed-AI having a 1.5 greater risk of becoming pregnant during the synchronized breeding compared to 7-10 cows (Table 3-4). Body condition score also affected ($P < 0.05$) synchronized pregnancy rate (Table 3-4). Cows with a BCS $\geq$ 5.5 had a 2.1 greater risk of becoming pregnant during the synchronized breeding compared to cows with a BCS $\leq$ 4.5. Cows with a BCS $\geq$ 5.5 also had 1.4 times greater risk of becoming pregnant during the synchronized breeding compared to cows with BCS 5. An increased ($P < 0.05$) percentage of cows with a DPP $\geq$ 60 got pregnant to the synchronized breeding compared to cows that were $\leq$

43 DPP. Cows with a DPP ≥ 60 had a risk of becoming pregnant 1.8 times that of the ≤ 43 DPP cows. Synchronized pregnancy rates were similar ($P > 0.05$) for cows 44-59 DPP compared to cows ≥ 60 DPP (Table 3-4). There were no effects ($P > 0.05$) of $PGF_{2\alpha}$ treatment, age, AI technician, and all appropriate interactions on synchronized pregnancy rate.

There were no significant effects of synchronization treatment, $PGF_{2\alpha}$ treatment, age, DPP, and all appropriate interactions on thirty-day pregnancy rate. However, cows with a BCS 5 and ≤ 4.5 had decreased ($P < 0.05$) thirty-day pregnancy rates compared to cows with a BCS ≥ 5.5 (Table 3-5).

Experiment 2

Synchronization treatment, $PGF_{2\alpha}$ treatment, year, BCS, and all appropriate interactions had no effect ($P > 0.05$) on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty-day pregnancy rate (Table 3-6). Across the synchronization and $PGF_{2\alpha}$ treatments, the mean estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty-day pregnancy rate were 66.8, 65.0, 16.1, 48.7, and 76.3% respectively. The mean synchronized pregnancy rates for the dinoprost and cloprostenol treatments were 45.5 and 52.0%, respectively. However, there was a treatment by interval from $PGF_{2\alpha}$ to the onset of estrus interaction ($P < 0.05$; Figure 3-2) on conception rate. For heifers that exhibited estrus 48 h after $PGF_{2\alpha}$, conception rates were similar ($P > 0.05$) between treatments. Whereas, for heifers that exhibited estrus at 60 h, the Select Synch/CIDR+timed-AI treatment tended ($P = 0.10$) to have a decreased conception rate compared to the 7-10 treatment. Conversely, for heifers that exhibited estrus at 72 h, the Select Synch/CIDR+timed-AI treatment had an increased ($P < 0.05$) conception rate compared to the 7-10 treatment.

Discussion

The true measure of the effectiveness of a synchronization treatment is the synchronized pregnancy rate or the number of cattle pregnant to the synchronized AI breeding. In Experiment 1, 14.3% more suckled *Bos indicus* × *Bos taurus* synchronized with the Select Synch/CIDR+timed-AI treatment became pregnant compared to the 7-10 treatment. The synchronized pregnancy rate of the 7-10 treatment are considerably less compared to reports in suckled *Bos taurus* cows synchronized with 7-11 protocol with either estrous detection and AI (Kojima et al., 2000) or a timed-AI (Bader et al., 2004). Both studies reported synchronized pregnancy rates greater than 60% for estrous cycling and anestrous cows. Likewise, the synchronized pregnancy rate for the Select Synch/CIDR+timed-AI treatment was less (13%) compared to a report by Larson et al. (2006) in estrous cycling and anestrous suckled *Bos taurus* cows synchronized with the same protocol. Larson et al. (2006) observed a range in mean synchronized pregnancy rates between 51 to 61% for cows across different BCS, estrous cycling statuses, DPP, and parities. The decreased response of the 7-10 and Select/Synch+timed-AI treatments in suckled *Bos indicus* × *Bos taurus cows* compared to similarly synchronized suckled *Bos taurus* cows (Kojima et al., 2000; Bader et al., 2004; Larson et al., 2006) is due to slight decreases in estrous response, conception rate, and timed-AI pregnancy rates, which function together to decrease synchronized pregnancy rates in the suckled *Bos indicus* × *Bos taurus* cows. It is difficult to determine the exact reason(s) for the reduced estrous response and conception rates between the suckled *Bos indicus* × *Bos taurus* in the present study compared to what is reported for *Bos taurus* cows. As with this study and research in the literature, there are very few instances where cattle of both *Bos indicus* × *Bos taurus* and *Bos taurus* breeding are at the same location and receiving the same synchronization treatments under similar environmental conditions. Therefore, making general comparisons about responses to estrous synchronization protocols between the two genotypes must be made with caution. In general, it has been well established that estrus is more difficult to detect in cattle of *Bos indicus* breeding due to a decreased expression and duration of estrus (Galina et al., 1982) as well as an increased incidence of silent estrus (Galina et al., 1996), which could play a role in the decreased estrous response for both synchronization treatments. What role if any, the increased incidence of three and four-wave follicle growth patterns, which are more frequent in cattle of *Bos indicus* breeding (Rhodes et al., 1995; Viana et al., 2000; Martinez et al., 2003), had in the estrous response of a synchronized estrous is uncertain but should not be discounted.

As mentioned previously, the primary reasons for the greater synchronized pregnancy rate for the Select Synch/CIDR+timed-AI compared to the 7-10 treatment were significant increases in estrous response and conception rate for the Select Synch/CIDR+timed-AI treatment. The three day estrous response for the 7-10 cows was 10.1% less compared to Select Synch/CIDR+timed-AI cows. Kojima et al. (2000) reported a peak estrous response by 66 h after $PGF_{2\alpha}$ of 92% in suckled *Bos taurus* cows synchronized with the 7-11 protocol, which is 43% greater compared to the 72 h estrous for the 7-10 treated *Bos indicus* × *Bos taurus* cows. Esterman et al. (2007) observed similar five day estrous responses between suckled Angus (*Bos taurus)* and Brangus (*Bos indicus* × *Bos taurus)* cows synchronized with the 7-11 protocol at the same location. In contrast, the 72 h estrous response (59.9%) for the Select Synch/CIDR+timed-AI cows was only slightly less compared to the 72 h estrous response (69.3%) reported by Larson et al. (2006) in suckled *Bos taurus* cows. One reason for the significantly decreased estrous response of the 7-10 compared to the Select Synch/CIDR+timed-AI treatment could be due to pre-synchronization portion of the 7-10 treatment that used a once-used CIDR did not create the same synchrony of follicular wave development compared to cows treated with the traditional 7-11 protocol that uses MGA. In Brangus cows synchronized with the 7-11 protocol, Esterman et al. (2007) reported that GnRH initiated ovulation in 94% of the cows after MGA treatment similar to reports in *Bos taurus* cows (Kojima et al., 2000). Since ovulation rate to GnRH was not determined in the present study, the ability of GnRH to ovulate follicles three days after CIDR removal is not known. Hence, it is unclear if the timing of GnRH relative to CIDR removal was appropriate and if a majority of follicles were of ovulatory size for GnRH to ovulate. It is also possible that the low circulating concentrations of progesterone from the once-used CIDR altered LH secretory patterns (Kojima et al., 1992) and follicle growth and development (Bergfeld et al., 1996) that resulted in abnormal follicle development resulting in development of some persistent dominant follicles that were not ovulated by GnRH (Woodall et al., 2007a). Any of these scenarios could have resulted in asynchronous follicle development at the second $PGF_{2\alpha}$ resulting in a low estrous response during the three day estrous detection period.

The second reason for the decreased synchronized pregnancy rates of the 7-10 treatment compared to the Select Synch/CIDR+timed-AI treatment was a significant reduction in conception rate for the 7-10 treatment. Conception rate of the 7-10 treatment is also considerably less compared to suckled *Bos taurus* (Kojima et al., 2000) and *Bos indicus* × *Bos taurus* (Esterman et al., 2007) cows synchronized with a 7-11 protocol. In both studies, conception rates were greater than 65% compared to 45.5% in the current study. In suckled *Bos indicus* × *Bos taurus* cows synchronized with the Select Synch protocol without a CIDR, Lemaster et al. (2006) reported a conception rate of 57.7%. The conception rates for the Select Synch/CIDR+timed-AI treatment were similar to a report by Larson et al. (2006) in suckled *Bos taurus* cows synchronized with a similar protocol and other reports in *Bos taurus* synchronized cows (Kojima et al., 2000; Stegner et al., 2004). It is unclear why the conception rates were decreased in the 7-10 treatment. Based on the how the 7-10 treatment was designed, the pretreatment phase with the once-used CIDR followed 3 d later by GnRH should have worked to synchronize follicle development by ovulating a majority of the follicles so they are synchronized for the subsequent $PGF_{2\alpha}$ treatment 7 d later, which is essentially the Select Synch protocol. The newly developed follicles should have an increased fertility compared to ovulations of "aged" dominant follicles (Mihm et al., 1994) or persistent dominant follicles (Kinder et al., 1996). Additionally, the hormonal environment that follicles of the Select Synch/CIDR+timed-AI treatment were exposed to was different compared to the GnRH+$PGF_{2\alpha}$ portion of the 7-10 treatment. The Select Synch/CIDR+timed-AI would have been exposed to elevated progesterone concentrations between GnRH and $PGF_{2\alpha}$; whereas, the 7-10 treatment did not receive this treatment. Although, addition of a CIDR to the Select Synch+timed-AI protocol did not improve conception rates to an observed estrus (Larson et al., 2006) in *Bos taurus* cows. Whether the progesterone from the CIDR is enhancing conception rates in the *Bos indicus* × *Bos taurus* cows in the present study remains in question and needs to be evaluated further.

The one variable that had a significant effect on conception rate was BCS across both synchronization treatments. Cows with a BCS 5 had approximately a 25% greater conception rate compared to cows with a BCS ≤ 4.5, regardless of synchronization treatment. In contrast, Larson et al. (2006) reported only a 7% increase in conception rate in cows with a BCS > 5 compared to cows with a BCS < 5 in suckled *Bos taurus* cows synchronized with the Select Sync/CIDR+timed AI. Body condition can be used as an indirect measure of nutritional status (Richards et al., 1986), and nutritional status influences estrous cycling status of suckled beef cows (Smith et al., 1976; Oyedipe et al., 1982) as well as the number of cows in estrus within 60 d after calving (Whitman, 1975; Wettemann et al., 1982). It is possible that a greater percentage of cows with a BCS ≥ 5 were going through estrous cycles at the start of the synchronization treatments resulting in greater fertility compared to cows with a BCS < 5. Both Lucy et al. (2001) and Larsen et al. (2006) observed numerically greater conception rates for estrous cycling cows compared to anestrous cows when inseminated after a synchronized estrus.

Body condition score at the start of the synchronization treatment also had an effect on synchronization response, as cows with a body condition score ≥ 5.5 had significantly greater estrous response, conception rate, synchronized pregnancy rate, and thirty-day pregnancy rate compared to cows with a BCS ≤ 4.5. This agrees with a report by Lamb et al. (2001) in *Bos taurus* cows where for each increase in BCS of one unit, the proportion of cows pregnant to AI after a synchronized ovulation increased by 23%. In contrasts, Larson et al. (2006) reported only a 7% increase in conception rate in cows that had BCS > 5 compared to cows with a BCS < 5 in suckled *Bos taurus* cows synchronized with the Select Synch/CIDR+timed AI. Although in the Larson et al. (2006) study, there were a limited number of cows with BCS < 5 that could have influenced the results. For the *Bos indicus* × *Bos taurus* cows in the current experiment, synchronized pregnancy rates increased by more than 30% for cows with a BCS ≥ 5.5 compared to a BCS of ≤ 4.5. The importance of BCS on the response to a synchronization treatment has been reported by others (Yelich et al., 1988; De Jarnette et al., 2004; Larson et al., 2006) and the common theme among these studies is that cows with BCS ≥ 5, respond well to most synchronization systems for cattle of either *Bos taurus* or *Bos indicus* × *Bos taurus* breeding. Therefore, it appears that suckled *Bos indicus* × *Bos taurus* cows that have a BCS > 5 at the start of a synchronization protocol respond favorably to synchronization protocols and have an excellent opportunity of getting pregnant, similar to what is observed in suckled *Bos taurus* cows.

Treatment had no effect on timed-AI pregnancy rates. However, timed-AI pregnancy rates were numerically less for 7-10 (17%) compared to Select Synch/CIDR+timed-AI (20%). Larson et al. (2006) reported timed-AI pregnancy rates for the Select Synch+timed-AI without a CIDR, Select Synch/CIDR+timed-AI, and CIDR+timed-AI of 39, 26, and 38%, respectively in suckled *Bos taurus* cows. There certainly appears to be a considerable reduction in timed-AI pregnancy rates between cattle of *Bos indicus* × *Bos taurus* compared to *Bos taurus* cattle. This could be due to several reasons ranging from lack of cattle going through estrous cycles and asynchronous follicle development at CIDR removal for the cattle of *Bos indicus* × *Bos taurus*. Furthermore, the increased incidence of three and four wave follicle wave patterns in *Bos indicus* × *Bos taurus* compared to *Bos taurus* cattle could result in asynchronous follicle development at the timed-AI resulting in either aged follicles with reduced fertility (Sirois and Fortune, 1990; Mihm et al., 1994) ovulating to GnRH or follicles that are not of the adequate size ovulating to GnRH (Lamb et al., 2001; Perry et al., 2005; Busch et al., 2008), which also have reduced fertility. A recent study by Esterman et al. (2008) in suckled cows of *Bos indicus* × *Bos taurus* breeding synchronized with the Select Synch/CIDR with estrous detection, reported that conception rate peaked for cattle that exhibited estrus by 60 h after $PGF_{2\alpha}$ and conceptions rates decreased in a linear fashion for cows that exhibited estrus between 72 and 96 h post $PGF_{2\alpha}$. This suggests that maybe the timed-AI needs to be performed at 60 h for the 7-10 protocol to avoid development of follicles with longer durations of dominance, which could be more susceptible to decreased fertility. It should also be noted, the timed-AI groups also include anestrous cows that did not respond to the synchronization treatments and therefore had no opportunity to conceive and get pregnant.

Timed-AI and synchronized pregnancy rates were also significantly influenced by DPP at the start of the synchronization treatment. Timed-AI pregnancy rate increased by 16% and synchronized pregnancy rate increased by 21% in cows ≥ 60 DPP compared to cows ≤ 43 DPP. Both, Esterman et al. (2007) in *Bos indicus* × *Bos taurus* cows synchronized with a Select Synch/CIDR+timed-AI protocol and Stevenson et al. (2000) in *Bos taurus* cows synchronized with Select Synch concurrent with a norgestomet implant observed an increase in synchronized pregnancy rates as the interval from calving increased. The present study and others (Stevenson et al., 2000; Esterman et al., 2007) stress the importance of knowing where cows are in their postpartum period before starting a synchronization program. However, the interval from calving to the initiation of the synchronization treatment did not influence thirty day pregnancy rates, which were similar between the three postpartum interval categories. In contrast, BCS at the start of the synchronization treatment continued to influence the opportunity for cows to get pregnant as the breeding season progressed as observed by others (Spitzer et al., 1995; Kunkle et al., 1998). Many factors affect fertility and response to synchronization protocols and re-breeding of the postpartum cow, most importantly estrous cycling status. Factors that control the resumption of estrous cyclicity in postpartum females include endocrine function, (Erb et al., 1971), nutritional status (Short et al., 1976), BCS (Stevenson et al., 2000), and suckling status (Randel, 1981).

In Experiment 2, there were no synchronization treatment (7-10 vs. Select Synch/CIDR+timed-AI) or $PGF_{2\alpha}$ treatment (dinoprost vs. cloprostenol), affects on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, or thirty day pregnancy rate for *Bos indicus* × *Bos taurus* yearling heifers, which is in stark contrast to the results obtained for the same synchronization treatments for cows in Experiment 1. In *Bos taurus* heifers synchronized with the Select Synch/CIDR+timed-AI protocol, Lamb et al. (2006) reported synchronized pregnancy rates that were approximately 5% greater compared to the Select Synch/CIDR+timed-AI treatment and approximately 12% greater compared to the 7-10 treatment of the present study. The 72 h estrous responses were 5.6% less for the Select Synch+timed-AI and 8.8% less for the 7-10 treatments compared to *Bos taurus* heifers synchronized with the Select Synch/CIDR+timed-AI reported by Lamb et al. (2006). Conception rates were similar between synchronization treatments and were also similar compared to conception rates reported by Lamb et al. (2006) in similarly synchronized *Bos taurus* heifers. Similar to the results observed for cows of Experiment 1, estrous response and timed-AI pregnancy rates were less in the *Bos indicus* × *Bos taurus* synchronized heifers compared to reports in *Bos taurus* synchronized heifers (Lamb et al., 2006). In *Bos indicus* × *Bos taurus* heifers synchronized with a 7 d CIDR with $PGF_{2\alpha}$ provided the day before CIDR removal, Lucy et al. (2001) reported similar conception and synchronized pregnancy rates compared to estrous cycling *Bos taurus* heifers. Furthermore, of the *Bos indicus* × *Bos taurus* heifers that were going through estrous cycles at CIDR insertion, 83% exhibited estrus within 3 d after $PGF_{2\alpha}$, which was similar to *Bos taurus* heifers in the same study. There have been no studies evaluating the 7-11 protocol in yearling heifers of *Bos indicus* × *Bos taurus* breeding. Heifers treated with the 7-10 protocol had slightly decreased conception and synchronized pregnancy rates compared to long term MGA based protocols in *Bos taurus* heifers (Wood et al., 2001; Patterson et al., 1993). Bridges et al. (2005) synchronized yearling heifers of *Bos indicus* × *Bos taurus* breeding with a 14 d MGA treatment followed by two consecutive $PGF_{2\alpha}$

treatments 19 and 20 d after MGA, and heifers were AI after a detected estrus for 72 h and non-responders were timed-AI plus GnRH. They reported similar synchronized pregnancy rates compared to the 7-10 treatment. It should also be mentioned that both estrous response and subsequent conception rates were considerably less in the Bridges et al. (2005) study compared to the 7-10 treatment. However, the timed-AI pregnancy rates were nearly doubled in the Bridges et al. (2005) study, which resulted in similar synchronized pregnancy rates compared to the current experiment. Hence, it appears that a decreased estrous response is one of the limiting factors that decrease the overall effectiveness of synchronization systems in heifers of *Bos indicus* × *Bos taurus* breeding compared to yearling *Bos taurus* heifers. Additional studies need to be conducted in heifers of *Bos indicus* × *Bos taurus* breeding to determine what physiological and management factors influence the onset of behavioral estrus. Additionally, the relationship between follicle development during and after a synchronization treatment and what affect it has on the expression of behavioral estrus after a CIDR treatment needs to be evaluated.

As mentioned previously, conception rates were similar between synchronization and $PGF_{2\alpha}$ treatments. However, there was a treatment by interval from $PGF_{2\alpha}$ to onset of estrus effect on conception rate. For the 7-10 treatment, conception rate peaked for heifers that exhibited estrus at 60 h after $PGF_{2\alpha}$ and decreased at 72 h; whereas, the inverse was observed for the Select Synch/CIDR+timed-AI heifers. Although the numbers of heifers are limited in each estrus category, there were no significant year effects on conception rates. Additionally, the same AI technician inseminated heifers in both years and a single AI sire was used in year 1 and two AI sires were used in year 2, which had similar conception rates. Therefore, it appears that conception rates are influenced not only by synchronization treatment but when heifers exhibited estrus relative to $PGF_{2\alpha}$. However, it should be noted that since estrous detection ceased at 72 h post $PGF_{2\alpha}$ it is not known what the conception rates would have been for the heifers exhibiting estrus after 72 h. Although, it may prove beneficial to move the timed-AI in the 7-10 treatment from 72 h to approximately 60 to 66 h to take advantage of improved fertility of the 60 h estrus.

The interval to estrus following $PGF_{2\alpha}$ is dependent on the stage of ovarian follicular growth when $PGF_{2\alpha}$ is administered (Geary et al., 1999; Hittinger et al., 2004). Kastelic et al. (1990) observed cows with a mature dominant follicle present at $PGF_{2\alpha}$ display estrus earlier after $PGF_{2\alpha}$ compared to cows with a growing and developing follicle at $PGF_{2\alpha}$, which takes longer to exhibit estrus. The 7-10 treated heifers should have a greater synchrony of follicle development compared to the Select Synch/CIDR+timed-AI group since the GnRH after the initial $PGF_{2\alpha}$ in the 7-10 treatment should have initiated ovulation in a majority of animals resulting in a synchronous follicle development compared to GnRH administered at CIDR insertion in the Select Synch/CIDR treatment. The number of follicles ovulating to GnRH at CIDR insertion in the Select Synch/CIDR treatment would probably have been less compared to the 7-10 treatment since there would be follicles at all stages of follicle development at CIDR insertion and stage of follicle development can influence ovulation rates to GnRH (Moreira et al., 2000). Therefore, there could have been a mix of follicles at different stages of development at CIDR removal for the Select Synch/CIDR treatment resulting in ovulation of both newly developed follicles and aged follicles. Heifers with aged follicles and (or) a greater duration of dominance should exhibit estrus earlier after $PGF_{2\alpha}$ and fertility of the aged follicles would be decreased compared to newly developed follicles (Mihm et al., 1994). Bridges et al. (2008) hypothesized that fertility of the ovulatory follicle is a function of the duration of proestrus and capacity of the ovulatory follicle to produced increased estradiol concentrations preceding estrus and AI. They further hypothesized that by reducing the duration of a CIDR treatment from 7 to

5 d could enhance secretion of estradiol by the potential ovulatory follicle even with younger follicles of reduced diameters. They conducted a series of experiments that synchronized suckled *Bos taurus* cows with a 5 d Co-Synch protocol with timed-AI at 72 h compared with a 7 d Co-Synch with timed-AI at 60 h. With the 5 d program, newly developed follicles would be approximately 3 to 4 d from emergence compared to 5 to 6 d from emergence for the 7 d program. The 5 d program significantly increased timed-AI pregnancy rates compared to the 7 d treatment in suckled beef cows. These findings certainly demonstrate the importance of duration of follicle development on fertility of a synchronization system. Whether these findings will translate to beef heifers is unknown but it does provide evidence to explain the significant shifts in fertility observed between synchronization systems based on follicle development.

In Experiments 1 and 2, the response to cloprostenol and dinoprost was similar between treatments for suckled cows and yearling heifers. This agrees with Hiers et al. (2003) who reported similar synchronized pregnancy rates in *Bos indicus* × *Bos taurus* cows synchronized with a modified Co-synch protocol with either cloprostenol or dinoprost treatments. Likewise, Salverson et al. (2002) reported a similar response in *Bos taurus* heifers treated with either cloprostenol or dinoprost treatments. Therefore, it appears that cloprostenol or dinoprost are equally effective when used in either the 7-10 or the Select Synch/CIDR+timed-AI synchronization protocols in cattle of *Bos indicus* × *Bos taurus* breeding.

In summary, the Select Synch/CIDR+timed-AI had increased conception and synchronized pregnancy rates compared to the 7-10 treatment in suckled cows of *Bos indicus* × *Bos taurus* breeding in Experiment 1. The effectiveness of the treatment was influenced by BCS, as BCS increased, the estrous response, conception rate, and synchronized pregnancy rates increased. Days postpartum also influenced effectiveness of the synchronization treatments, as time from calving to start of synchronization increased, synchronized pregnancy rates increased. There was no effect of prostaglandin treatment on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, or thirty day pregnancy rate. In Experiment 2, there were no synchronization or prostaglandin treatment effects on any of the reproductive responses measured in the yearling heifers of *Bos indicus* × *Bos taurus* breeding.

Implications

The Select Synch/CIDR+timed-AI protocol is a practical synchronization method for *Bos indicus* × *Bos taurus* yearling heifers and postpartum cows that are in good body condition and ≥ 60 d postpartum at the start of the synchronization treatment. The 7-10 synchronization protocol yields similar results as the Select Synch/CIDR+timed-AI protocol when used in yearling heifers but decreased results in suckled *Bos indicus* × *Bos taurus* cows. Cloprostenol sodium and dinoprost tromethamine are equally effective and yielded similar synchronized pregnancy rates in both yearling heifers and suckled *Bos indicus* × *Bos taurus* cows.

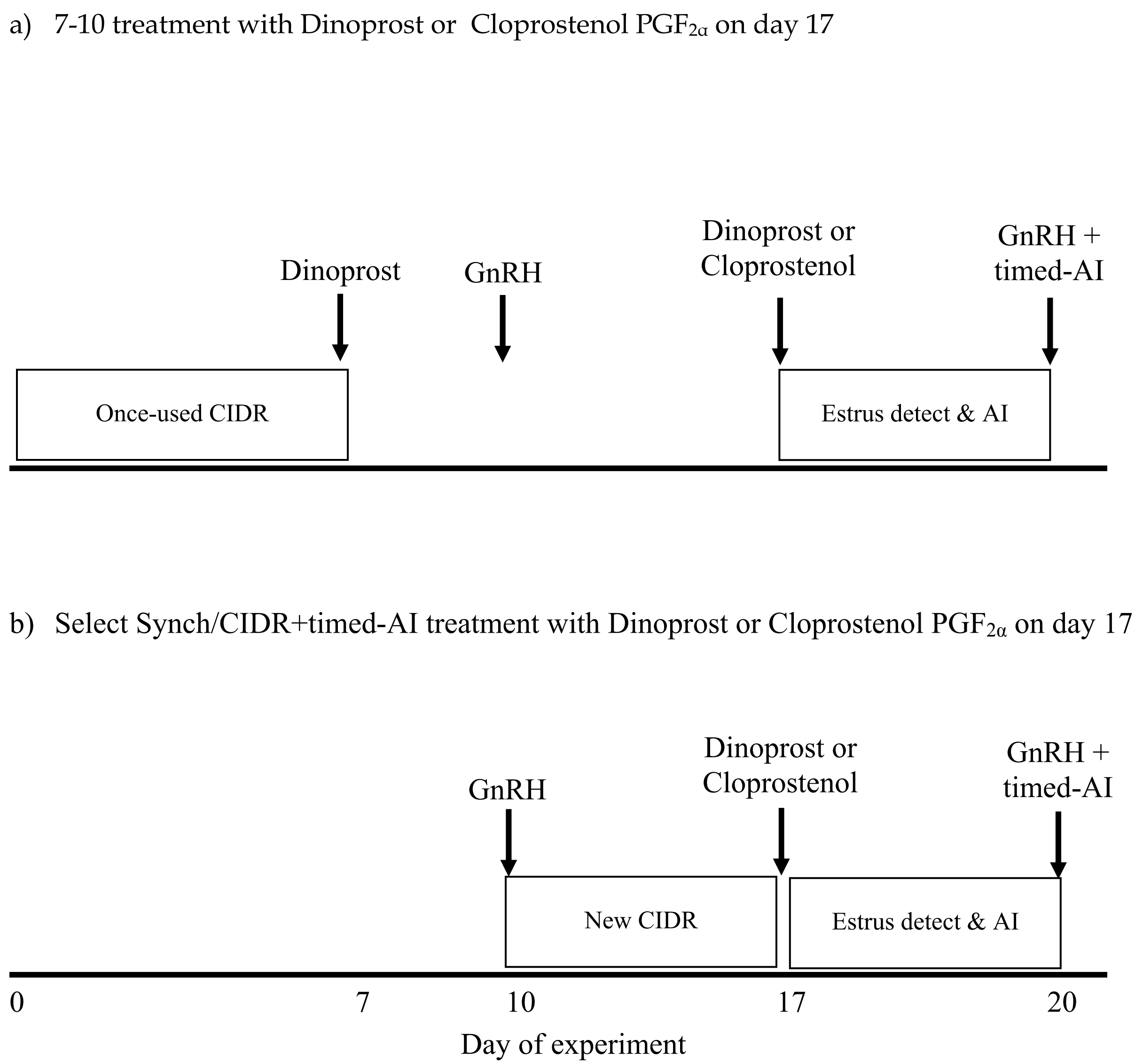

Figure 3-1. Experimental design evaluating the effects of two progestogen and two $PGF_{2\alpha}$ treatments in *Bos indicus* × *Bos taurus* suckled cows (Experiment 1) and yearling heifers (Experiment 2). a) 7-10 treatment: on day 0 received a once-used CIDR that was removed on d 7 concomitant with dinoprost tromethamine (Dinoprost; 25 mg i.m.) followed by GnRH (500 µg i.m.) on day 10. On day 17, females received either dinoprost or cloprostenol sodium (Cloprostenol; 500 µg i.m.). b) Select Synch/CIDR+timed-AI treatment: on day 10 received a new CIDR (1.38 g) concomitant with GnRH. Day 17 CIDR was removed and females received either Dinoprost or Cloprostenol. For all four treatments estrus was detected for 3 d and females were inseminated 6 to 12 h after detected estrus. Females not exhibiting estrus by 72 h were timed-AI and received GnRH.

Table 3-1. Effect of synchronization treatment and body condition score (BCS) on estrous response in suckled *Bos indicus* × *Bos taurus* cows.[a]

Variable	Estrous response, %[c]	Odds Ratio	95% CI[d]	*P-value*
Treatment				
Select Synch/CIDR+timed-AI	59.9 (100/167)	1.59	1.02-2.48	0.05
7-10	49.0 (77/157)	Referent		
BCS[b]				
≤ 4.5	48.8 (59/121)	0.47	0.25-0.89	0.02
5	54.4 (75/138)	0.59	0.32-1.10	0.10
≥ 5.5	66.2 (43/65)	Referent		

[a] Refer to Figure 3-1 for details of treatments.
[b] Body condition score: 1 = emaciated, 5 = moderate; 9 = very fat.
[c] Percentage of cows displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.
[d] 95% confidence interval.

Table 3-2. Effect of synchronization treatment and body condition score (BCS) on conception rate in suckled *Bos indicus* × *Bos taurus* cows.[a]

Variable	Conception rate, %[c]	Odds Ratio	95% CI[d]	*P-value*
Treatment				
Select Synch/CIDR+timed-AI	62.0 (62/100)	2.08	1.10-3.95	0.03
7-10	45.5 (35/77)	Referent		
BCS[b]				
≤ 4.5	35.6 (21/59)	0.20	0.08-0.47	0.0003
5	60.0 (45/75)	0.51	0.22-0.1.17	0.11
≥ 5.5	72.1 (31/43)	Referent		

[a] Refer to Figure 3-1 for details of treatments.
[b] BCS: 1 = emaciated, 5 = moderate; 9 = very fat.
[c] Percentage of cows pregnant to AI of the total that exhibited estrus and were AI.
[d] 95% confidence interval.

Table 3-3. Effect of days postpartum (DPP) on timed-AI pregnancy rate in suckled *Bos indicus* × *Bos taurus* cows.[a]

DPP	Timed-AI pregnancy rate, %[b]	Odds Ratio	95% CI[c]	*P-value*
≤ 43	11.5 (7/61)	0.34	0.12-0.98	0.05
44-59	21.7 (10/46)	0.73	0.27-1.96	0.54
≥ 60	27.5 (11/40)	Referent		

[a] Refer to Figure 3-1 for details of treatments.
[b] Percentage of cows that failed to display estrus, were timed-AI, and became pregnant of the total number of cows that were timed-AI.
[c] 95% confidence interval

Table 3-4. Effect of synchronization treatment, body condition score (BCS), and days postpartum (DPP) on synchronized pregnancy rate in suckled *Bos indicus* × *Bos taurus* cows.[a]

Variable	Synchronized pregnancy rate, %[c]	Odds Ratio	95% CI[d]	*P-value*
Treatment				
Select Synch/CIDR+timed-AI	45.5 (76/167)	2.01	1.25-3.24	0.004
7-10	31.2 (49/157)	Referent		
BCS[b]				
≤ 4.5	26.5 (32/121)	0.29	0.15-0.55	0.0002
5	40.6 (56/138)	0.52	0.28-0.97	0.04
≥ 5.5	56.9 (37/65)	Referent		
DPP				
≤ 43	27.8 (32/115)	0.45	0.25-0.82	0.009
44-59	40.5 (45/111)	0.73	0.42-1.30	0.28
≥ 60	49.0 (48/98)	Referent		

[a] Refer to Figure 3-1 for details of treatments.
[b] BCS: 1 = emaciated, 5 = moderate; 9 = very fat.
[c] Percentage of cows pregnant during the synchronized breeding of the total treated.
[d] 95% confidence interval

Table 3-5. Effect of body condition score (BCS) on thirty day pregnancy rates in suckled *Bos indicus* × *Bos taurus* cows.

Variable	Thirty day pregnancy rate, %[b]	Odds Ratio	95% CI[c]	*P-value*
BCS[a]				
≤ 4.5	72.7 (88/121)	0.32	0.12-0.78	0.01
5	77.5 (107/138)	0.42	0.17-1.00	0.05
≥ 5.5	89.2 (58/65)	Referent		

[a] BCS: 1 = emaciated, 5 = moderate, 9 = very fat.
[b] Percentage of cows pregnant during the first 30 d of breeding season of the total treated.
[c] 95% confidence interval.

Table 3-6. Effectiveness of 7-10 treatment compared to Select Sync/CIDR+timed-AI (SSC+TAI) treatment on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty day pregnancy rate in yearling heifers of *Bos indicus* × *Bos taurus* breeding.[a]

Variable	7-10	SSC+TAI	Odds Ratio[g]	95% CI[h]	*P-value*
Estrous response, %[b]	65.2 (116/178)	68.4 (121/177)	1.26	0.80-1.97	0.32
Conception rate, %[c]	62.1 (72/116)	67.8 (82/121)	1.34	0.78-2.30	0.29
Timed-AI pregnancy rate, %[d]	14.5 (9/62)	17.9 (10/56)	1.30	0.48-3.42	0.62
Synchronized pregnancy rate, %[e]	45.5 (81/178)	52.0 (92/177)	1.30	0.85-1.97	0.22
Thirty day pregnancy rate, %[f]	74.7 (133/178)	78.0 (138/177)	1.20	0.73-1.96	0.47

[a] Refer to Figure 3-1 for details of treatments.
[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.
[c] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.
[d] Percentage of heifers that failed to display estrus, were timed-AI, and became pregnant of the total number of heifers that were timed-AI.
[e] Percentage of heifers pregnant during the synchronized breeding of the total treated.
[f] Percentage of heifers pregnant during the first 30 d of the breeding season of the total number of heifers treated.
[g] 7-10 used as referent value
[h] 95% confidence interval

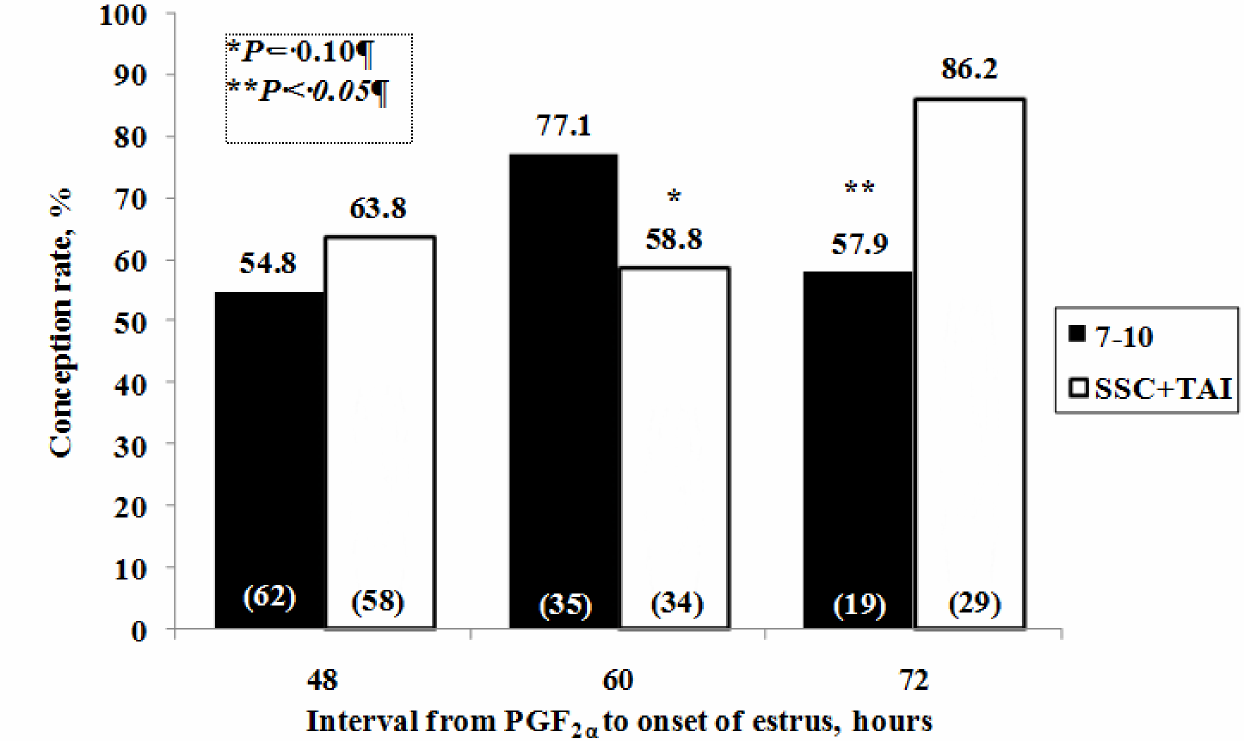

Figure 3-2. Effect of interval from $PGF_{2\alpha}$ to onset of estrus on conception rate in *Bos indicus* × *Bos taurus* yearling heifers synchronized with 7-10 or Select Synch/CIDR+timed-AI (SSC+TAI) treatments. Numbers in parenthesis indicate the number of heifers in each category. Treatment by interval from $PGF_{2\alpha}$ to onset of estrus ($P = 0.02$).

CHAPTER 4
EVALUATION OF TWO PROGESTOGEN BASED ESTROUS SYNCHRONIZATION PROTOCOLS IN YEARLING HEIFERS OF *BOS INDICUS* × *BOS TAURUS* BREEDING

Introduction

Estrous synchronization is a reproductive management tool that allows for an increased number of cattle displaying estrus and ovulating over a 3 to 5 d period, which allows for a minimal number of days of estrous detection or elimination of estrous detection resulting in a timed artificial insemination (timed-AI). A common estrous synchronization protocol used in *Bos taurus* cattle, the Select Synch protocol, utilizes GnRH with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 7 d later (Macmillan and Thatcher, 1991; Pursley et al., 1995), followed by 5 d of estrous detection. However, a common problem with this protocol is premature expression of estrus prior to $PGF_{2\alpha}$, resulting in additional estrous detection (DeJarnette et al., 2001). Addition of an exogenous progestogen between the GnRH and $PGF_{2\alpha}$ eliminates the need for additional estrous detection (Thompson et al., 1999; DeJarnette et al., 2003). Addition of a progesterone insert like the Eazi-Breed™ CIDR® (CIDR) to the Select Synch protocol, combined with estrous detection for 72 h after CIDR removal and timed-AI + GnRH for non-responders (Select Synch/CIDR+timed-AI), is an effective estrous synchronization protocol in yearling *Bos taurus* heifers (Lamb et al., 2006). However, the effectiveness of this protocol in yearling *Bos indicus* × *Bos taurus* heifers has not been thoroughly evaluated.

The 7-11 estrous synchronization protocol consists of a 7 d melengestrol acetate (MGA) treatment with $PGF_{2\alpha}$ on the last day of MGA followed by GnRH 4 d later; 7 d after GnRH, $PGF_{2\alpha}$ is administered to synchronize estrus (Kojima et al., 2000). The 7-11 protocol is an effective synchronization protocol in *Bos taurus* cattle (Kojima et al., 2000; Stegner et al., 2004) but no research has been conducted evaluating the effectiveness of the 7-11 protocol in yearling heifers of *Bos indicus* × *Bos taurus* breeding. The basis behind the 7-11 treatment is that the administration of GnRH after MGA results in ovulation in majority of the animals resulting in very synchronous follicle development pattern. In a recent study conducted by Esterman et al. (2007), estrous cycling and anestrous suckled postpartum Brangus and Angus cows received the 7-11 protocol, and GnRH induced ovulation in 94.4% of both Brangus and Angus cows. The resulting 5 d estrous response, conception, and synchronized pregnancy rates were similar between Angus and Brangus cows. Since GnRH effectively ovulates and synchronizes follicles when administered 4 d after a short term (7 d) progestogen treatment in Brangus cows, the 7-11 protocol could be an effective synchronization protocol in yearling heifers of *Bos indicus* × *Bos taurus* breeding.

Therefore the objective of this experiment was to evaluate the effectiveness of the 7-11 and Select Synch/CIDR+timed-AI synchronization protocols in yearling heifers of *Bos indicus* × *Bos taurus* breeding.

Materials and Methods

The experiment was conducted at three locations in North Central Florida from January to March, 2007. Yearling (12 to 15 mo) *Bos indicus* × *Bos taurus* heifers (n = 410) were used in the experiment and the percentage of *Bos indicus* breeding ranged from approximately 1/4 to 1/2 with the remainder being *Bos taurus* breeding (Table 4-1). At the start of the experiment (d 0) heifers were distributed by reproductive tract score (RTS, Scale 1 to 5; 1 = non-cycling, 5 = estrous cycling; Anderson et al., 1991; See Table B-1 for complete RTS score description) and body condition score (BCS, Scale 1 to 9; 1 = emaciated, 5 = moderate, 9 = very fat; Richards et al., 1986) to one of two progestogen based synchronization protocols including the 7-11 and Select Select Synch/CIDR+timed-AI protocols (Figure 4-1). The 7-11 heifers received MGA (MGA®200 premix, Pfizer Animal Health, New York, NY) in a carrier supplement fed at a rate of 0.9 kg/head/d to deliver 0.5 mg MGA/head/d from day 0 to 7 of the experiment with $PGF_{2\alpha}$

(25 mg i.m.; Lutalyse® Sterile Solution, Pfizer Animal Health, New York, NY) concomitant with the last day of MGA. On day 11, heifers received GnRH (500 µg i.m.; Cystorelin®, Merial, Duluth, GA) followed by $PGF_{2\alpha}$ on day 18. The Select Synch/CIDR+timed-AI heifers received the same carrier supplement without MGA from day 0 to 7. On day 11, a CIDR (Eazi-Breed™ CIDR®, 1.38 g progesterone; Pfizer Animal Health, New York, NY) was inserted concomitant with GnRH. The CIDR was removed on day 18 concurrent with $PGF_{2\alpha}$ (25 mg i.m.; Lutalyse®).

Heifers from both synchronization treatments received Estrotect™ estrous detection patches (Rockway, Inc., Spring Valley, WI) on day 18 of the experiment to aid in estrous detection and estrus was visually detected twice daily (0700 and 1600 h) for 72 h after $PGF_{2\alpha}$. Heifers were artificially inseminated (AI) 6 to 12 h after detection of estrus. Non-responders were timed-AI and treated with GnRH 72 to 76 h post $PGF_{2\alpha}$. Estrus was defined as either a heifer standing to be mounted by another heifer, showed signs of visible mucous, and (or) had a half to full red estrous detection patch. One AI technician within each location inseminated heifers using frozen-thawed semen from multiple sires. Whenever possible, AI sires were equally distributed across treatments and between heifers that either exhibited estrus and (or) were timed-AI. Natural service sires were placed with heifers 10 d after timed-AI. Using a real-time B-mode ultrasonography machine (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 5.0 MHz transducer pregnancy was diagnosed approximately 55 d after AI. Due to the 10 d period where no cows were exposed to natural service sires, differences in fetal size were used to determine whether a pregnancy resulted from the synchronized AI breeding (54 and 55 d pregnant) or natural service sire ($\leq$ 48 d pregnant). Embryos develop a C shape appearance approximately day 25 of gestation, which transitions to an L shape embryos by day 32. By day

48, the fetus is approximately 35 mm in length with visible limb buds and by day 55 the fetus is approximately 50 mm in length and has identifiable ribs (Curran et al., 1986).

Estrous response was defined as the number of heifers displaying estrus for 5 d after $PGF_{2\alpha}$ and inseminated divided by the total number of heifers treated. Conception rate was defined as the number of heifers that became pregnant to AI divided by the number of heifers that displayed estrus and were AI. Timed-AI pregnancy rate was the number of heifers that failed to display estrus, were timed-AI, and became pregnant divided by the total number of heifers that were timed-AI. Synchronized pregnancy rate was the number of heifers pregnant to AI 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.

Binomially distributed data including estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty-day pregnancy rate, were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). All models included synchronization treatment (7-11 and Select Synch/CIDR+timed-AI), location, BCS, and all appropriate interactions. Body condition was analyzed as a categorical variable and was grouped into three categories (≤ 4.5, 5, ≥ 5.5). The effect of interval from $PGF_{2\alpha}$ to the onset of estrus (≤ 36, 48, 60, and 72 h) on conception rate was also analyzed with synchronization treatment, location, BCS, and all appropriate interactions included in the model. Because of a limited number of heifers exhibiting estrus between 0 and 36 h after $PGF_{2\alpha}$, these time periods were combined into ≤ 36 h. The final logistic regression model entered variables by a stepwise selection based on the Wald statistics criterion when $P < 0.20$ and removed variables based on $P < 0.10$. Variables were considered significant at $P \leq 0.05$. Variables that were significant were entered into a linear regression model using GENMOD procedure of SAS to calculate the adjusted odds ratios and 95% confidence intervals. Reproductive tract score (RTS) was not included in the initial model due to confounding effects with BCS; therefore, a second model was conducted evaluating the same binomial variables of estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty-day pregnancy rate and categorical variables including synchronization treatment, location, RTS, and all appropriate interactions. Reproductive tract score was evaluated as a categorical variable and was grouped into three categories (≤ 2, 3, ≥ 4). The initial analysis indicated that all response variables were similar between RTS 4 and 5, so the two RTS categories were combined for the final analysis. The same statistical process as previously described was used to determine the final logistic regression model as well as the linear regression model. Adjusted odds ratios and 95% confidence intervals are also reported. Odds ratios were reported in tables and were used to calculate relative risk, and relative risk was reported in the results.

Results

An increased ($P < 0.05$) percentage of heifers expressed estrus during the 72 h following $PGF_{2\alpha}$ for the 7-11 compared to Select Synch/CIDR+timed-AI treatment (Table 4-2). Estrous response was also affected ($P < 0.05$) by BCS. Heifers with a BCS ≤ 4.5 and 5 had a decreased ($P < 0.05$) estrous response compared to heifers with a BCS ≥ 5.5 (Table 4-3). The relative risk of heifers with a BCS ≤ 4.5 exhibiting estrus was 60% of heifers with a BCS ≥ 5.5 and heifers with a BCS 5 exhibiting estrus was 74% of heifers with a BCS ≥ 5.5. Estrous response was not affected ($P > 0.05$) by location nor were there any significant interactions.

Conception rates were also affected ($P < 0.05$) by synchronization treatment. The risk of heifers being pregnant to an AI after an observed estrus was 1.5 times greater for the 7-11 compared to Select Synch/CIDR+timed-AI heifers (Table 4-2). In addition, conception rate was also affected ($P < 0.05$) by the interval from $PGF_{2\alpha}$ to the onset of estrus (Figure 4-2); however, there was no ($P > 0.05$) treatment by interval from $PGF_{2\alpha}$ to the onset of estrus effects on conception rate. Heifers exhibiting estrus at 60 h had an increased ($P < 0.05$) conception rate compared to heifers that had exhibited estrus at ≤ 36, 48, and 72 h (Figure 4-2), which were similar ($P > 0.05$) to each other. Conception rates were not ($P > 0.05$) affected by BCS and location, nor were there any significant interactions.

Timed-AI pregnancy rates were similar ($P > 0.05$) between the 7-11 and Select Synch/CIDR+timed-AI treatments (Table 4-2) and there were no effects ($P > 0.05$) of BCS and location on timed-AI pregnancy rate nor were there any significant interactions.

Synchronized pregnancy rates were affected ($P < 0.05$) by synchronization treatment. The risk of heifers treated with the 7-11 becoming pregnant to the synchronized breeding was 1.35 times greater compared to the Select Synch/CIDR+timed-AI treatment (Table 4-2). Body condition score had no effect ($P > 0.05$) on synchronized pregnancy rate. Synchronized pregnancy rates were not influenced by ($P > 0.05$) BCS and location, nor were there any significant interactions. Thirty day pregnancy rates were similar ($P > 0.05$) between treatments (Table 4-2) and were not affected ($P > 0.05$) by BCS, location, nor were there any significant interactions.

The effect of RTS on response to synchronization treatments by RTS category are presented in Table (4-4). There were no treatment by RTS effects ($P > 0.05$) but RTS affected ($P < 0.05$) estrous response, conception rate, synchronized pregnancy rate, and thirty-day pregnancy rate. It should be mentioned that heifers with a RTS ≥ 4, had a greater ($P < 0.05$) BCS (5.2 ± 0.02) compared to heifers with a RTS of 3 (5.0 ± 0.02) and ≤ 2 (4.7 ± 0.02). Heifers with a RTS 3 had a greater ($P < 0.05$) BCS compared to heifers with RTS ≤ 2. Heifers with a

RTS ≥ 4, had a 2.0 times greater risk of exhibiting estrus compared to heifers with a RTS ≤ 2 and a 1.3 times greater risk to exhibit estrus compared to heifers with a RTS 3 (Table 4-4). For conception rate, heifers with RTS ≥ 4 had a 6 times greater risk of being pregnant after exhibiting estrus and being AI compared to heifers with RTS ≤ 2. Conception rates were similar ($P > 0.05$) between heifers with a RTS 3 and ≥ 4 (Table 4-4).

Timed-AI pregnancy rates tended ($P = 0.10$) to be greater for heifers with a RTS ≥ 4 compared to heifers with a RTS ≤ 2 (Table 4-4). Synchronized pregnancy rates were similar ($P < 0.05$) for heifers with RTS ≥ 4 and 3. However, heifers with RTS ≥ 4 had a 4 times greater risk of becoming pregnant to the synchronized breeding compared to heifers with RTS 2 (Table 4-4). Heifers with RTS ≥ 4 had a 2.3 greater risk of being pregnant during the first thirty-days of the breeding season compared to heifers with RTS ≤ 2 and a 1.2 greater risk compared to heifers with a RTS 3(Table 4-4).

Discussion

Estrous response was greater for the 7-11 compared to Select Synch/CIDR+timed-AI heifers. A possible reason for the increased estrous response of the 7-11 treatment could be that 7-11 treatment may do a better job of synchronizing follicle development compared to the Select Synch/CIDR treatment. The MGA in combination with $PGF_{2\alpha}$ allows for more follicles to be of ovulatory size at MGA withdrawal resulting in most animals ovulating to GnRH (Kojima et al., 2001), which provides for a very synchronous wave of follicle development at the subsequent $PGF_{2\alpha}$. In contrast, the administration of GnRH at the initiation of the Select Synch/CIDR+timed-AI protocol probably does not do as an effective job of ovulating and synchronizing follicle development since animals are at various stages of the follicle development at CIDR insertion, resulting in GnRH ovulating fewer follicles (Moreira et al., 2000). Consequently, follicle development at CIDR withdrawal may not be as synchronous for the Synch/CIDR+timed-AI compared to the 7-11 treatment, resulting in a decreased 72 h estrous response.

The estrous response for both treatments are considerably less compared to studies in *Bos taurus* heifers synchronized with either the Select Synch/CIDR+timed-AI (Lamb et al., 2006), short term progestogen protocols (Patterson et al., 1989), and CIDR + $PGF_{2\alpha}$ protocol (Lucy et al., 2001). In all of the aforementioned studies, the 3 d estrous responses were approximately 20 to 30% greater compared to those observed in the present study in yearling *Bos indicus* × *Bos taurus* heifers. However, the 3 d estrous responses for both treatments are similar to that reported by Bridges et al. (2005) in yearling *Bos indicus* × *Bos taurus* heifers synchronized with a long term (14 d) MGA + $PGF_{2\alpha}$ synchronization protocol. The decreased estrous response is probably due to heifer genotype and estrous cycling status of heifers at the start of the synchronization treatment. Patterson et al. (1989) demonstrated that yearling *Bos indicus* × *Bos taurus* heifers going through estrous cycles had a significant reduction in estrous response (22%) compared to contemporary *Bos taurus* heifers synchronized with a short term progestogen treatment. Estrus is more difficult to detect in cattle of *Bos indicus* breeding due to a decreased expression and duration of estrus (Galina et al., 1982) as well as an increased incidence of silent estrus (Galina et al., 1996). However, utilization of the estrous detection patches should have assisted identifying heifers that did not exhibit a “good” standing estrous during the two times that estrus was visually detected (0700 and 1600 h). Therefore, the method of estrous detection is probably not the major factor responsible for the decreased estrous response. Since blood samples were not taken on the heifers, it is not known how many heifers were going through estrous cycles at the start of the synchronization treatment. *Bos indicus* × *Bos taurus* and *Bos* *taurus* heifers that are going through estrous cycles at the start of a CIDR+$PGF_{2\alpha}$ treatment (Lucy et al., 2001) had significantly increased estrous responses compared to pre-pubertal heifers. Additionally, the ability of the synchronization treatments to induce estrous cycles in the pre-pubertal heifers is unclear. Lucy et al. (2001) reported that a CIDR+$PGF_{2\alpha}$ treatment induced estrous cycles in prepubertal *Bos indicus* × *Bos taurus* and *Bos taurus* heifers over several locations ranging from 40 to 100%.

Onset of puberty is related inversely to growth rate (Wiltbank et al., 1969; Short and Bellows, 1971) and heifers with higher growth rates are typically going to have greater BCS. Body condition score can be used as an indirect indicator of nutritional status (Herd and Sprott, 1986; Wettemann and Lusby, 1987) and estrous cycling status (Schillo et al., 1992; Hall et al., 1995) or as an indirect indicator of when heifers attain puberty. Heifers with a BCS ≥ 5.5 had the greatest estrous response (62.3%), which was significantly greater compared to heifers with a BCS ≤ 5. The estrous response for heifers with a BCS ≥ 5.5 is comparable to values observed for *Bos taurus* heifers synchronized with the Select Synch/CIDR+timed-AI (Lamb et al., 2006) protocol. Moreover, Lucy et al. (2001) reported a 3 d estrous response of 80% in yearling *Bos indicus* × *Bos taurus* heifers that were going through estrous cycles when synchronized with a CIDR+$PGF_{2\alpha}$ protocol. Based on reports in the literature and the estrous response of heifers with a BCS ≥ 5.5 in the present experiment, acceptable estrous responses can be achieved in synchronized yearling *Bos indicus* × *Bos taurus* heifers but they are probably influenced by pubertal status more than anything else. The onset of puberty is influenced by many factors including age, genotype, and nutrition. Heifers of *Bos indicus* breeding are known to reach puberty at older ages compared to *Bos taurus* heifers (Dow et al., 1982; Nelsen et al., 1982). Therefore, the low number of heifers going through estrous cycles at the start of the experiment was probably the primary reason for the decreased estrous response for the two synchronization treatments, which was probably due to heifers not being the appropriate weight and age to reach puberty.

There could also be environmental effects that are not easily quantifiable that could have influenced estrous response. In estrous cycling *Bos indicus* × *Bos taurus* heifers and estrous cycling and prepubertal *Bos taurus* heifers synchronized with a long term (14 d) MGA + $PGF_{2\alpha}$ protocol, Bridges et al. (2005) reported similar 3 d estrous responses (< 50%) for both genotypes similar to heifers in the current study. Both the current study and Bridges et al. (2005) study were conducted in sub-tropical environment within a 50 mile radius of each other during a similar time of the year (January to March). Reproductive function has been observed to decrease in *Bos indicus* × *Bos taurus* cattle in the winter months (Randel, 1984), which could have negatively affected estrous response. Frequent handling of heifers of *Bos indicus* × *Bos taurus* breeding can also have a negative effect on the intensity of estrus (Lemaster et al., 1999) and could have altered the behavioral and endocrine physiology associated with estrus (Hardin and Randel, 1982). The increased frequency of cattle handling could have resulted in increased cortisol concentrations that may have had a negative effect on LH secretion (Dunlap et al., 1981) and the preovulatory LH surge (Stoebal and Moberg, 1979). Another indication of effect of stress on reproductive function has recently been observed by Cooke et al. (2008) in prepubertal *Bos indicus* × *Bos taurus* heifers. They observed that by acclimating heifers to frequent animal handling for three times a week for a 30 d period approximately 100 d before the start of the breeding season enhanced the attainment of puberty by the start of the breeding season compared to non-acclimated contemporaries.

There was a marked increase in conception rates for heifers synchronized with the 7-11 (47.0%) compared to the Select Synch/CIDR+timed-AI treatment (31.3%). Thus the 7-11 treatment may be doing a better job of synchronizing follicle development compared to the Select Synch/CIDR+timed-AI treatment resulting in follicles that are more fertile. The 7-11 protocol is designed to maximize follicle development at $PGF_{2\alpha}$ treatment (Kojima et al., 2001). In contrast, the administration of GnRH at the initiation of the Select Synch/CIDR+timed-AI protocol probably is not as effective at ovulating and synchronizing follicle development since animals are at various stages of follicle development at CIDR insertion and GnRH will ovulate fewer follicles (Moreira et al., 2000). Consequently, there is the probability of an increased population of non-ovulated follicles that have extended durations of dominance at CIDR removal resulting in ovulation of aged oocytes of decreased fertility (Mihm et al., 1994). Follicles with prolonged dominance under the influence of exogenous progesterone had significantly lower pregnancy rates compared with females with a normal ovulatory follicle (Sirois and Fortune, 1990; Stock and Fortune, 1993). Austin et al. (1999) reported that restricting the duration of dominance of a pre-ovulatory follicle to 4 d consistently yielded pregnancy rates > 70% in estrous cycling beef heifers. Furthermore, Bridges et al. (2008) reported that reducing the duration of a CIDR treatment from 7 to 5 d in postpartum *Bos taurus* beef cows resulted in significant increases in timed-AI pregnancy rates of a Co-Synch protocol. They attributed the increased fertility to a decreased duration of dominance of follicles induced to ovulate after CIDR removal.

Conception rates for the 7-11 treatment were similar to a report by Bridges et al. (2005) in peripubertal yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with a long term 14 d MGA + PGF protocol. In contrasts, conception rate for the Select Synch/CIDR+timed-AI treatment was approximately 20% less compared to Bridges et al. (2005) study. Moreover, both treatments are considerably less compared to *Bos taurus* heifers synchronized with variations of the CIDR + PGF protocols where conception rates ranged from 60 to 80% (Lamb et al., 2006; Lucy et al., 2001). The conception rates for the 7-11 heifers (47%) were similar to a report by Patterson et al. (1989) in estrous cycling *Bos indicus* × *Bos taurus* heifers synchronized with either a short term progestogen treatment or unsynchronized controls. Additionally, Patterson et al. (1989) demonstrated that there was a breed by energy level interaction on conception rates in estrous cycling *Bos indicus* × *Bos taurus* heifers but not similarly treated *Bos taurus* heifers. They observed a 30% increase in fertility of the *Bos indicus* × *Bos taurus* heifers that were on an increasing plane of nutrition at breeding compared to *Bos indicus* × *Bos taurus* heifers on a low plane of nutrition. Several studies with synchronized *Bos taurus* heifers have reported that fertility to an observed estrus and AI are similar between pre-pubertal and estrous cycling heifers (Lucy et al., 2001; Lamb et al., 2006). It could be possible that the level of nutrition was not adequate enough to enhance the fertility of the *Bos indicus* × *Bos taurus* heifers in our study. Body condition had no effect on conception rates across the 7-11 or Select Synch/CIDR+timed-AI treatments. Although, BCS can be used as an indicator of both pubertal status and nutrition level, a single record taken at the start of the synchronization treatment is probably not an accurate indicator of whether any significant changes in the plane of nutrition occurred in the weeks preceding the onset of treatment. Therefore, it is not clear what if any effects plane of nutrition and (or) estrous cycling status at the start of treatment had on conception rates.

Timed-AI pregnancy rates were similar between the 7-11 and Select Synch/CIDR+timed-AI treatments but they were nearly half the values observed for *Bos taurus* heifers synchronized with the Select Synch/CIDR+timed-AI protocol (Lamb et al., 2006). It should be noted that there was an effect of interval from $PGF_{2\alpha}$ to the onset of estrus on conception rate and heifers that exhibited estrus at 60 h after $PGF_{2\alpha}$ had conception rates that were nearly twice that of heifers that exhibited estrus < 36, 48 and 72 h. Therefore, the timing of the timed-AI needs to be re-evaluated and it is possible that it should be conducted at 60 h after $PGF_{2\alpha}$. However, the primary reason that the timed-AI pregnancy rates were so low is probably due to the limited number of heifers that were going through estrous cycles at the start of the experiment. Furthermore, neither synchronization treatment probably induced estrous cycles in the prepubertal heifers, which resulted in a substantial number of heifers that were still prepubertal at the timed-AI.

The 7-11 treatment had a greater synchronized pregnancy rate compared to the Select Synch/CIDR+timed-AI protocol, which was due to the greater estrous response and conception rate of the 7-11 compared to the Select Synch/CIDR+timed-AI. As mentioned previously, the 7-11 treatment probably was more effective at synchronizing follicle waves allowing a new growing follicle ready to ovulate after $PGF_{2\alpha}$, which resulted in more heifers exhibiting estrus compared to the Select Synch/CIDR+timed-AI. The synchronized pregnancy rates of the 7-11 (33.5%) are similar to those reported by Bridges et al. (2005; 34.5%) in yearling *Bos indicus* × *Bos taurus* heifers synchronized with the 14 d MGA+$PGF_{2\alpha}$ protocol with a single $PGF_{2\alpha}$ treatment. Bridges et al. (2005) significantly improved the synchronized pregnancy rate (42.5%) by adding an additional $PGF_{2\alpha}$ to the treatment, which enhanced the $PGF_{2\alpha}$ induced luteolysis resulting in greater estrous response and timed-AI pregnancy rates. It should be noted that heifers in the Bridges et al. (2005) study were in later stages of the estrous cycle (day 12 to 15) where $PGF_{2\alpha}$ is supposed to be more effective in initiating luteolysis (Tanabe and Hahn, 1984; Watts and Fuquay, 1985). A limited amount of research indicates that response to $PGF_{2\alpha}$ is decreased when administered early in the estrous cycle compared to the middle and late stages of the estrous cycle in *Bos taurus* (Tanabe and Hahn, 1984; Watts and Fuquay, 1985) and *Bos indicus* cattle (Hardin et al., 1980; Santos et al., 1988). Incomplete luteolysis can lead to decreased estrous response, which directly affects synchronized pregnancy rates. Whether incomplete luteolysis occurred in both synchronization treatments is not known since no blood samples were taken at $PGF_{2\alpha}$ and during the days after $PGF_{2\alpha}$ to confirm if luteolysis occurred. The synchronized pregnancy rates for both treatments are considerably less compared to 58% reported by Lamb et al. (2006) in *Bos taurus* heifers synchronized with Select Synch/CIDR+timed-AI protocol and 49% reported by Lucy et al. (2001) in *Bos taurus* and *Bos indicus* × *Bos taurus* heifers synchronized with a CIDR + $PGF_{2\alpha}$ protocol. The primary reason for the decreased synchronized pregnancy rates was due to the low estrous response, conception rate, and timed-AI pregnancy rate for the 7-11 and Select Synch/CIDR+timed-AI treatments. All three of the response variables were probably influenced by estrous cycling status at the start of the synchronization treatment.

Reproductive tract score (Anderson et al., 1991; Scale 1 to 5) can also be used as an indirect indicator of pubertal status in yearling heifers. Heifers with a RTS of 1 and 2 are considered pre-pubertal, while heifers with a RTS of 3 are approximately 1 mo from initiating estrous cycles, and heifers with a RTS of 4 or 5 are considered to be estrous cycling. Therefore, conducting a RTS at the initiation of the experiment was used to provide an estimate of the pubertal status of the heifers before the start of the synchronization treatment. Although, RTS had an effect on estrous response, conception rate, synchronized pregnancy rate, and thirty-day pregnancy rate regardless of synchronization treatment, the magnitude in differences between response variables across the different RTS categories was less than anticipated. Patterson et al.

(2000) reported significant increases in estrous response in *Bos taurus* heifers as RTS increased from a 1 to 3 and reaching a plateau for RTS 4 and 5, which is similar to our observations. It is not surprising that the conception rate for the RTS 1 and 2 was only 6.7%, which was significantly less compared to heifers with RTS $\geq$ 3. However, what was unexpected was that conception rates did not increase as RTS increased from a 3 to either a 4 or 5 as observed in synchronized yearling *Bos taurus* heifers (Herd and Sprott, 1986). Additionally, it was unexpected that synchronized pregnancy rates were similar for heifers with a RTS of 3 compared to RTS of 4 and 5 as synchronized pregnancy rates typically increase as RTS increase from a 3 to a RTS of 4 or 5 (Brown et al., 1988). However, thirty day pregnancy rates tended to be greater for heifers with a RTS of 4 and 5 compared to RTS of 3, which were all greater compared to RTS 1 and 2. The results from this study suggest that RTS may not be an effective indictor of pubertal status in yearling heifers of *Bos indicus* × *Bos taurus* breeding compared to using it to estimate pubertal status in yearling *Bos taurus* heifers. Additional research needs to be conducted to determine if RTS can be used as an accurate predictor of pubertal status based on both blood progesterone and transrectal ultrasonography data to confirm the activity and presence of luteal tissue in yearling heifers of *Bos indicus* × *Bos taurus* breeding.

In summary, the 7-11 treatment provided increased estrous response, conception rates, and synchronized pregnancy rates compared to the Select Synch/CIDR+timed-AI treatment. Synchronization treatments had similar timed-AI pregnancy rates. Estrous response was directly affected by BCS at start of synchronization but BCS had no effect on any of the other response variables. Although, RTS had significant affects on estrous response, conception rate, synchronized pregnancy rate and thirty day pregnancy rate, the difference was primarily between heifers with RTS of 1 and 2 compared to heifers with a RTS of 3, 4, and 5.

Implications

The 7-11 synchronization treatment resulted in more yearling heifers of *Bos taurus* × *Bos indicus* breeding pregnant to the synchronized breeding compared to the Select Synch/CIDR+timed-AI protocol; however, the synchronized pregnancy rates are less compared to *Bos taurus* heifers. The role that RTS and BCS play as predictors of pubertal status in *Bos indicus* × *Bos taurus* heifers when synchronizing remains unclear. However, considerations must be made for timing of initiation of synchronization in heifers especially for *Bos taurus* × *Bos indicus* heifers as they reach puberty at later ages.

Table 4-1. Physical description of *Bos indicus* × *Bos taurus* yearling heifers including reproductive tract score (RTS), body condition score (BCS), and breed composition for heifers synchronized with two progestogen based estrous synchronization protocols (LSMeans ± SE).

Location	n	RTS[a]	BCS[b]	Breed composition[c]
1	61	3.8 ± 0.14	5.5 ± 0.05	Brangus (n=61)
2	119	3.6 ± 0.10	5.0 ± 0.04	Brangus (n=9) *Bos indicus* crossbred (n=110)
3	230	3.7 ± 0.07	5.0 ± 0.03	*Bos indicus* crossbred (n=230)

[a] Reproductive tract score:1 = non-cycling, 5 = estrous cycling.

[b] Body condition score: 1 = emaciated, 5 = moderate; 9 = very fat.

[c] Brangus = 3/8 Brahman 5/8 Angus; *Bos indicus* crossbred approximately 1/4 to 1/2 *Bos indicus* breeding with remainder being *Bos taurus* breeding.

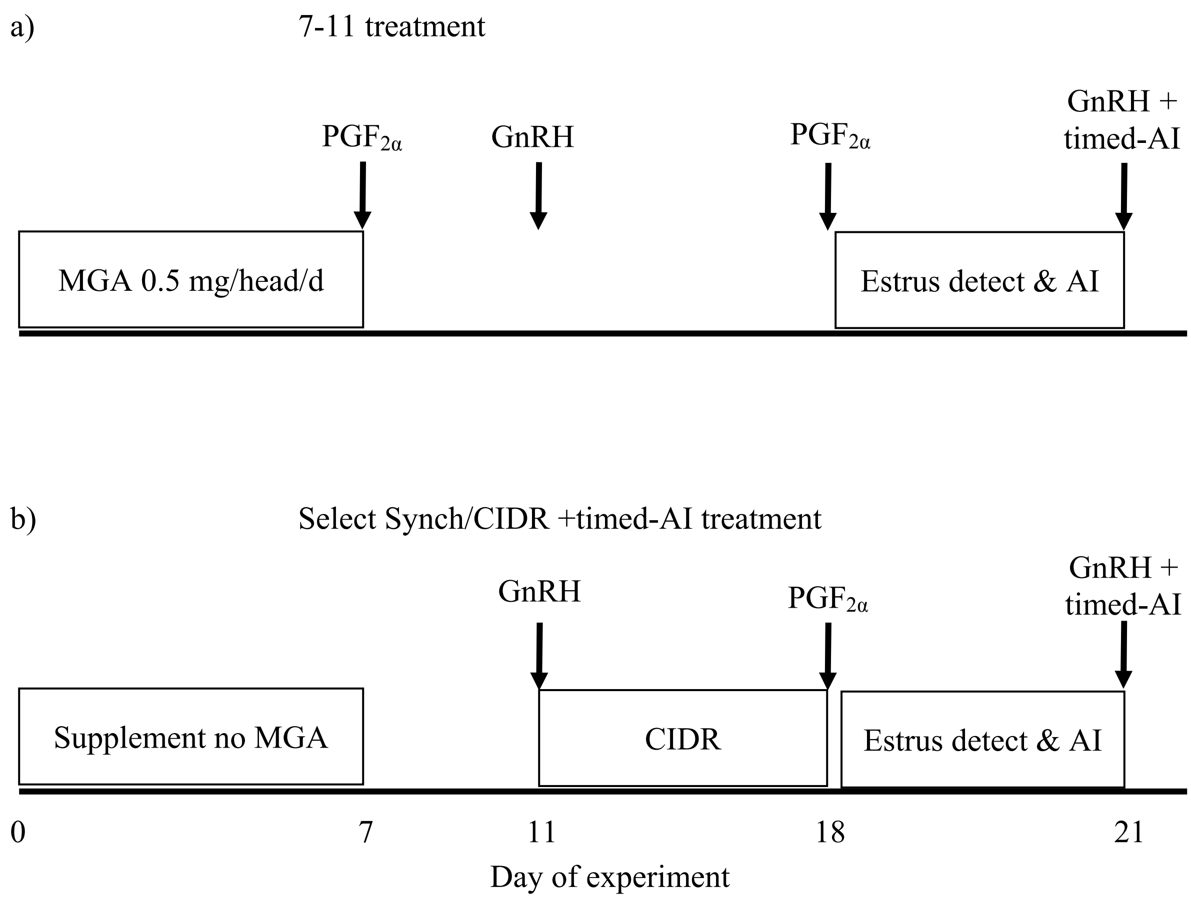

Figure 4-1. Experimental design evaluating the effects of two progestogen based synchronization treatments in yearling *Bos indicus* × *Bos taurus* heifers. a) 7-11 treatment was administered MGA (0.5 mg/hd/d) for 7 d with $PGF_{2\alpha}$ (25 mg, i.m.) on last day of MGA. GnRH (500 µg, i.m.) was administered on day 11 and $PGF_{2\alpha}$ was administered on day 18. b) Select Synch/CIDR+timed-AI treatment received the same carrier supplement without MGA from day 0 to 7. On day 11 a CIDR (Eazi-Breed™ CIDR®) was inserted concomitant with GnRH. The CIDR was removed on day 18 concurrent with $PGF_{2\alpha}$. For both treatments, estrus was detected for 3 d and heifers were inseminated 6 to 12 h after a detected estrus. Heifers not exhibiting estrus by 72 h were timed-AI and received GnRH between 72 to 76 h.

Table 4-2. Effectiveness of the 7-11 treatment compared to Select Sync/CIDR+timed-AI (SSC+TAI) synchronization treatment on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty day pregnancy rate in yearling heifers of *Bos indicus* × *Bos taurus* breeding.[a]

Variable	7-11	SSC+TAI	Odds Ratio[g]	95% CI[h]	*P-value*
Estrous response, %[b]	55.2 (117/212)	41.9 (83/198)	0.60	0.40-0.89	0.01
Conception rate, %[c]	47.0 (55/117)	31.3 (26/83)	0.51	0.29-0.93	0.03
Timed-AI pregnancy rate, %[d]	16.8 (16/95)	20.0 (23/115)	1.23	0.61-2.5	0.56
Synchronized pregnancy rate, %[e]	33.5 (71/212)	24.8 (49/198)	0.65	0.43-1.00	0.05
Thirty day pregnancy rate, %[f]	65.7 (138/210)	60.4 (119/197)	0.80	0.53-1.19	0.27

[a] Refer to Figure 4-1 for details of treatments.
[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.
[c] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.
[d] Percentage of heifers that failed to display estrus, were timed-AI, and became pregnant of the total number of heifers that were timed-AI.
[e] Percentage of heifers pregnant during the synchronized breeding of the total treated.
[f] Percentage of heifers pregnant during the first 30 d of the breeding season of the total number of heifers treated.
[g] 7-11 treatment used as referent value
[h] 95% confidence interval

Table 4-3. Effect of body condition score (BCS) on estrous response in *Bos indicus* × *Bos taurus* yearling heifers.

BCS[a]	Estrous response, %[b]	Odds Ratio	95% CI[c]	*P-value*
≤ 4.5	37.5 (30/80)	0.37	0.20-0.67	0.001
5	46.4 (104/224)	0.54	0.33-0.87	0.01
≥ 5.5	62.3 (66/106)	Referent		

[a] Body condition score: 1 = emaciated, 5 = moderate; 9 = very fat.
[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.
[c] 95% confidence interval

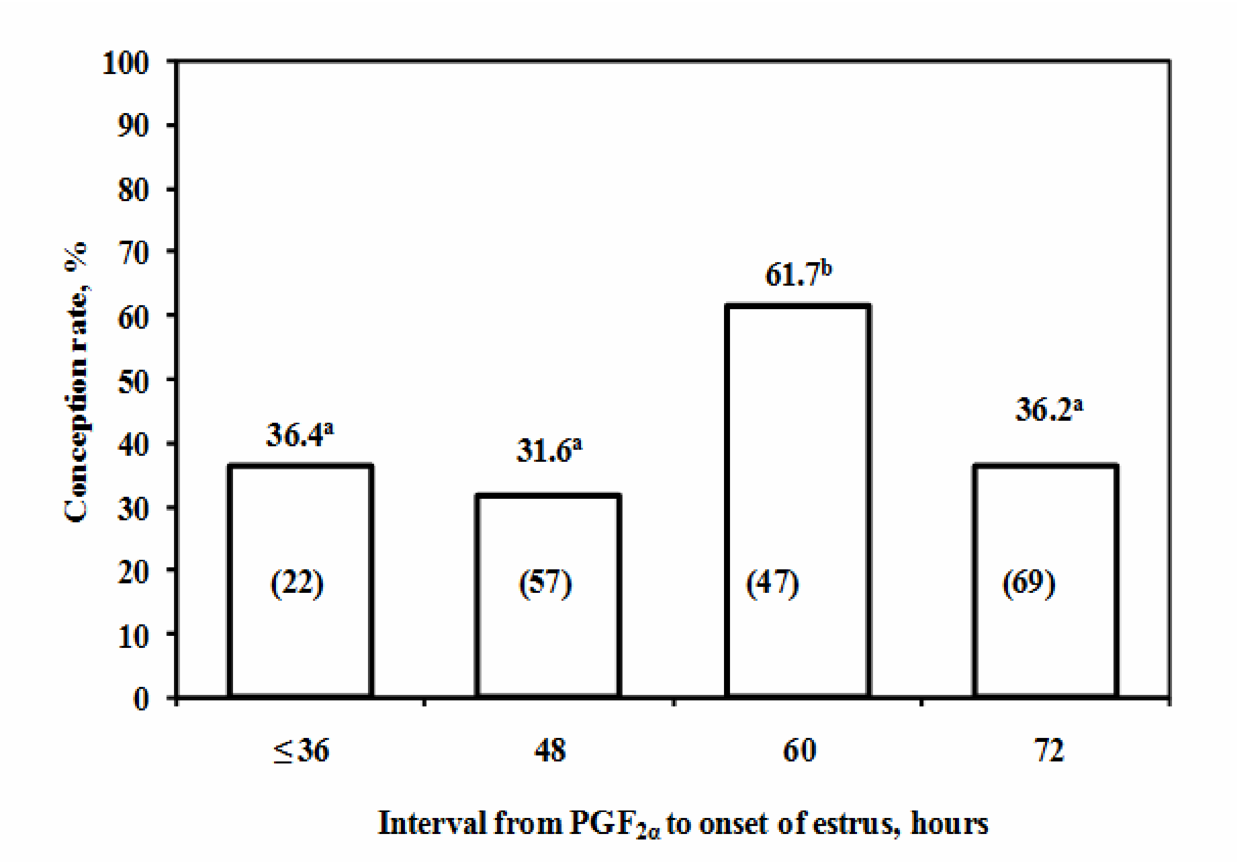

Figure 4-2. Conception rates for the different interval from $PGF_{2\alpha}$ to onset of estrus categories in yearling heifers of *Bos indicus* × *Bos taurus* breeding. Means are expressed as a percentage of total that exhibited estrus and became pregnant. Means without a common superscript differ ($P < 0.05$). Interval from $PGF_{2\alpha}$ to onset of estrus ($P < 0.05$) and synchronization treatment × interval from $PGF_{2\alpha}$ to onset of estrus ($P > 0.05$).

Table 4-4. Effect of reproductive tract score (RTS) on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate and thirty day pregnancy rate in yearling heifers of *Bos indicus* × *Bos taurus* breeding.[a]

Variable	RTS[b]	Means	Odds Ratio	95% CI[c]	*P-value*
Estrous response, %[d]	≤ 2	28.3 (15/53)	0.30	0.16-0.58	0.0004
	3	44.1 (56/127)	0.60	0.39-0.94	0.02
	≥ 4	56.1 (129/230)	Referent		
Conception rate, %[e]	≤ 2	6.7 (1/15)	0.07	0.10-0.60	0.01
	3	44.6 (25/56)	1.15	0.59-2.20	0.68
	≥ 4	42.6 (55/129)	Referent		
Timed-AI pregnancy rate, %[f]	≤ 2	7.9 (3/38)	0.34	0.09-1.24	0.10
	3	22.5 (16/71)	1.22	0.57-2.58	0.61
	≥ 4	19.8 (20/101)	Referent		
Synchronized pregnancy rate, %[g]	≤ 2	7.6 (4/53)	0.17	0.06-0.48	0.001
	3	32.3 (41/127)	0.97	0.61-1.55	0.91
	≥ 4	32.6 (75/230)	Referent		
Thirty day pregnancy rate, %[h]	≤ 2	32.1 (17/53)	0.22	0.07-0.68	0.008
	3	59.1 (75/127)	0.49	0.24-1.0	0.06
	≥ 4	72.6 (167/230)	Referent		

[a] Refer to Figure 4-1 for details of treatments.
[b] Reproductive tract score (Scale 1-5)
[c] 95% confidence interval
[d] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.
[e] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.
[f] Percentage of heifers that failed to display estrus, were timed-AI, and became pregnant of the total number of heifers that were timed-AI.
[g] Percentage of heifers pregnant during the synchronized breeding of the total treated.
[h] Percentage of heifers pregnant during the first 30 d of the breeding season of the total number of heifers treated.

CHAPTER 5
CONCLUSIONS AND IMPLICATIONS

Incorporation of estrous synchronization into an AI program decreases the amount of time spent detecting estrus and can eliminate estrous detection when a fixed insemination time is used. Additional benefits of using estrous synchronization includes promoting estrous cyclicity in some non-cycling cattle, increasing the percentage of cattle pregnant early in the breeding season, shortening the breeding season, shortening the subsequent calving season, and increasing the uniformity of the calf crop. A majority of the research and development of estrous synchronization protocols is based on the *Bos taurus* genotype. Furthermore, limited research indicates that *Bos indicus* and *Bos indicus* × *Bos taurus* cattle have subtle physiological and behavioral differences that can elicit different responses to estrous synchronization products compared to *Bos taurus* cattle. Consequently, the primary objective of the research reported in this thesis focused on evaluating the effectiveness of two estrous synchronization protocols in cattle of *Bos indicus* × *Bos taurus* breeding.

In chapter 3, two experiments were conducted to evaluate the effectiveness of two prostaglandins, cloprostenol sodium (cloprostenol) compared to dinoprost tromethamine (dinoprost), when used in either the Select Synch/CIDR+timed-AI or the 7-10 synchronization programs. In Experiment 1, suckled postpartum *Bos indicus* × *Bos taurus* cows were synchronized with the protocols and yearling heifers of *Bos indicus* × *Bos taurus* breeding were used in Experiment 2.

For both the suckled postpartum cows and the yearling heifers, prostaglandin treatment had no effect on any of the response variables evaluated. This is similar to reports in cattle of *Bos indicus* breeding as well as *Bos taurus* breeding. It was originally hypothesized that the cloprostenol, which is a prostaglandin analog, may have been more effective compared to the natural prostaglandin, dinoprost. However, this does not appear to be the case based on the results of our experiment. Therefore, based on the limited number of animals tested in these experiments as well as the reports in the literature, it appears that any subtle difference in dynamics of CL regression between the two luteolytic drugs is not sufficient to alter either estrous response or pregnancy rates. Hence, producers can use either type of prostaglandin without any reduction in reproductive performance.

For the suckled cows in Experiment 1, estrous response, conception rate, and synchronized pregnancy rate were affected ($P < 0.05$) by synchronization treatment as the Select Synch/CIDR+timed-AI treatment had greater ($P < 0.05$) estrous response, conception rate, and synchronized pregnancy rate compared to the 7-10 treatment.

Additionally, the synchronized pregnancy rates of both the 7-10 and Select Synch/CIDR +timed-AI treatments are considerably less compared to reports in suckled *Bos taurus* cows synchronized with either the 7-11 or Select Synch/CIDR+timed-AI protocols. The primary reasons for the decreased response of the 7-10 compared to the Select Synch/CIDR+timed-AI treatment as well as the decreased response of both treatments compared to similarly synchronized suckled *Bos taurus* cows is due to a decrease in the estrous response, conception rates, and timed-AI pregnancy rates. All of the aforementioned factors acted in concert and resulted in less than acceptable synchronized pregnancy rates in the suckled *Bos indicus* × *Bos taurus* cows. The first question that needs to be addressed is why the estrous response and conception rates were significantly reduced in the 7-10 compared to the Select Synch/CIDR+timed-AI treatment. One reason for the decreased estrous response for the 7-10 cows could be the pre-synchronization portion of the 7-10 treatment is not creating the same synchrony of follicle development of the follicles being ovulated to GnRH compared to the traditional 7-11 protocol, where MGA is used instead of a once-used CIDR. Since ovulation rate to GnRH was not determined in the present study, it is not known how effective GnRH was in ovulating follicles after removal of the once-used CIDR or if the timing of the GnRH treatment relative to CIDR removal was appropriate. Type of progestogen treatment and concentration of circulating progestogen can alter follicle growth and development, which could have also resulted in asynchronous follicle development at GnRH. Therefore, if ovulation did not occur in a majority of cows at GnRH in the 7-10 treatment, follicle development would have been asynchronous at $PGF_{2\alpha}$ resulting in a decreased estrous response compared to the Select Synch CIDR+timed-AI treatment. Because cattle of *Bos indicus* influence have an increased incidence of three and four wave follicle development patterns, additional research needs to be conducted to determine if follicle wave patterns influence how cattle of *Bos indicus* breeding respond to synchronization systems.

The second question that needs to be investigated is why cattle of *Bos indicus* influence consistently have decreased estrous response to synchronization treatments compared to *Bos taurus* cattle synchronized with similar protocols. One factor that had a significant effect on estrous response in Experiment 1 was body condition score (BCS) of the cattle at the start of the synchronization treatments. In summary, as BCS increased from ≤ 4.5 to a ≥ 5, there was a significant increase in estrous response. Although estrous cycling status was not determined in the present study, suckled cows with a BCS > 5 are typically cattle that are either close to or are going through estrous cycles. Therefore, it is imperative for producers that want to use estrous synchronization and AI that their cattle are in good BCS at the start of the breeding season in order to get cattle to exhibit estrus and achieve acceptable pregnancy rates. Additionally, it is not clear what effect environment has on estrous response, since there are a limited number of studies that actually evaluate the effect of environment on response to synchronization protocols in both cattle of *Bos indicus* and *Bos taurus* breeding under similar environmental conditions. This would include investigating not only how environmental factors effect an animal's physiology but also their behavior in response to environmental stressors.

Conception rates were also influenced by synchronization treatment as the 7-10 had decreased conception rates compared to the Select Synch/CIDR+timed-AI treatment. It is not clear why conception rates were decreased in the 7-10 treatment. Based on how the 7-10 treatment is designed, pretreatment with the once-used CIDR followed 3 d later by GnRH should have worked to synchronize follicle development for the subsequent $PGF_{2\alpha}$ treatment. The newly developed follicles should have had an increased fertility compared to "aged" dominant follicles or persistent dominant follicles, which could occur in some Select Synch/CIDR+timed-AI treated cows. Additionally, the hormonal environment that follicles were exposed to during the GnRH + PGF portion of the 7-10 treatment were certainly different compared to the Select Synch/CIDR+timed-AI treatment. Whether progesterone from the CIDR affected the follicle development pattern of the 7-10 treatment enough to decrease the subsequent conception rates is unclear. Conception rates of the 7-10 treatment were considerably less compared to those observed in suckled *Bos taurus* and *Bos indicus* × *Bos taurus* cows synchronized with a 7-11 protocol (Esterman et al., 2007b). It is important to note that acceptable conception rates can be achieved in suckled *Bos indicus* × *Bos taurus* that exhibit estrus and inseminated as reflected by the conception rates of the Select Synch/CIDR+timed-AI treatment, which are comparable to reports in *Bos taurus* cows synchronized with the same protocol. As with estrous response, BCS had a significant effect on conception rates, as cows with a BCS ≥ 5, had approximately a 30% greater conception rate compared to cows with a BCS ≤ 4.5. Body condition score is an indirect measure of nutritional status and has a direct influence on reproductive function and estrous cycling status of suckled beef cows. Cows that receive enough pre-partum nutrition have an excellent opportunity of returning to estrus within 60 d after calving and get pregnant to a synchronized AI. Therefore, if producers want to achieve acceptable pregnancy rates to a synchronized AI, they must make sure that their cattle are in adequate body condition at the start of the synchronization treatments. However, producers will have to determine if the extra cost required for the increased nutrition needed to keep cows in good body condition at the start of the breeding season can be offset by the price obtained for the AI calves that will be produced by the synchronized breeding.

Treatment had no effect on timed-AI pregnancy rates across the two synchronization treatments. However, timed-AI pregnancy rates for both treatments are approximately 6 to 20% less compared to reports for *Bos taurus* cattle synchronized with similar synchronization protocols. As previously discussed with estrous response and conception rate, asynchrony of follicle development at the time of $PGF_{2\alpha}$ could have been part of the reason for the decreased timed-AI pregnancy rate. It is possible that follicles were not of the correct size to ovulate to GnRH, were infertile follicles when ovulating to GnRH, or cows were still anestrous and had follicles of inadequate size to ovulate to GnRH. Timed-AI pregnancy rates across the two treatments were also influenced by DPP at the start of the synchronization treatment. Timed-AI pregnancy rate increased by 16% in cows' $\geq$ 60 DPP compared to cows at or below 43 DPP. In general, as the interval from calving to start of the breeding season increases, the number of cows going through estrous cycles also increases. Therefore, some of the decrease in timed-AI pregnancy rates could be attributed to increased incidence of anestrous compared to estrous cycling cows. Another possible reason for the decreased timed-AI pregnancy rates could be that the timing of the timed-AI + GnRH treatment is not synchronized correctly with the follicle development after CIDR removal. A recent study in our lab with suckled *Bos indicus* × *Bos taurus* cows synchronized with a Select Synch/CIDR protocol demonstrated that conception rate peaked for cows that exhibited estrus at 60 h post $PGF_{2\alpha}$ and conception rates decreased significantly for cows that exhibited estrus after 60 h. Therefore, further research is needed to evaluate if the timed-AI should be conducted at 60 h instead of 72 h after $PGF_{2\alpha}$.

Synchronization treatment had a significant effect on synchronized pregnancy rate as a greater number of Select Synch/CIDR+timed-AI cows became pregnant to the synchronized breeding compared to the 7-10 treatment. Additionally, the synchronized pregnancy rates for both treatments were less compared to similarly synchronized *Bos taurus* cows. As already discussed, a reduction in estrous response and conception rate of the 7-10 treatment were the primary reasons for the decreased synchronized pregnancy rates for the 7-10 compared to the Select Synch/CIDR+timed-AI treatment in the present experiment as well as the decreased response compared to similarly synchronized *Bos taurus* cows. Nevertheless, acceptable synchronized pregnancy rates can be achieved in suckled *Bos indicus* × *Bos taurus* cows synchronized with the Select Synch/CIDR+timed-AI protocol if cows are of adequate BCS and DPP at the start of the breeding season. The reason(s) for the less than acceptable synchronized pregnancy rates for the 7-10 treatment are not clear, and its practicality as a low cost synchronization protocol is questionable. Future studies need to evaluate what effect the once-used CIDR has on follicle development and if an asynchrony in follicle development after CIDR removal effects the response to GnRH administered 3 d after CIDR removal. In conclusion, it appears that the major factors contributing to the success of the synchronization treatments used in the present study are BCS and DPP. Therefore, producers need to pay particular attention to managing body condition prior to the start of the synchronization treatment and wait longer after calving before cows are started on a synchronization treatment to take advantage of the DPP effect. Future studies need to fully characterize the effectiveness of the two treatments ability to induce estrous in anestrous suckled *Bos indicus* × *Bos taurus* cows, with an emphasis on the Select Synch/CIDR+timed-AI protocol. Furthermore, intensive studies need to be conducted in *Bos indicus* × *Bos taurus* and *Bos taurus* cows in similar environments to completely characterize follicle wave patterns between the genotypes and to determine if they have any effect on response to the Select Synch/CIDR+timed-AI protocol.

In Chapter 3, Experiment 2, yearling heifers over two consecutive years were synchronized with the same estrous synchronization protocols as Experiment 1. The estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and thirty day pregnancy rate were similar ($P > 0.05$) between the 7-10 and Select Synch/CIDR+timed-AI treatment and between cloprostenol and dinoprost treatment. However, there was a treatment by interval from $PGF_{2\alpha}$ to the onset of estrus ($P < 0.05$; Figure 3-2) effect on conception rate. Conception rates were similar ($P > 0.05$) between treatments for heifers that exhibited estrus 48 h after $PGF_{2\alpha}$. However, the Select Synch/CIDR+timed-AI treatment tended ($P = 0.10$) to have a decreased conception rate compared to the 7-10 heifers that exhibited estrus at 60 h after $PGF_{2\alpha.}$ Whereas, the Select Synch/CIDR+timed-AI treatment had an increased ($P < 0.05$) conception rate compared to the 7-10 treatment for heifers that exhibited estrus 72 h after $PGF_{2\alpha}$.

In Experiment 2, there were no synchronization (7-10 vs. Select Synch/CIDR+timed-AI) or $PGF_{2\alpha}$ treatments (dinoprost vs. cloprostenol), affects on estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate or thirty day pregnancy rate for *Bos indicus* × *Bos taurus* yearling heifers. This is in stark contrast to the results obtained for the same synchronization treatments used on suckled cows in Experiment 1. Similar to results observed for cows of Experiment 1, estrous response and timed-AI pregnancy rates were slightly less in the *Bos indicus* × *Bos taurus* heifers compared to reports in *Bos taurus and Bos indicus* × *Bos taurus* heifers synchronized with a 7 d CIDR with $PGF_{2\alpha}$ provided the day before CIDR removal. However, conception and synchronized pregnancy rates were similar compared to what has been observed in estrous cycling *Bos taurus* heifers. Although, estrous cycling status was not determined in the present study, the final results suggest that most of the heifers were pubertal and responded favorably to either synchronization treatment and producers have the option to use either synchronization system. There have been no studies evaluating the 7-11 protocol in yearling heifers of *Bos indicus* × *Bos taurus* breeding. However, heifers treated with the 7-10 protocol had only slightly decreased conception and pregnancy rates compared to long term MGA based protocols in *Bos taurus* heifers. As observed with the cows in Experiment 1, it appears that a decreased estrous response is one of the limiting factors that decrease the overall effectiveness of synchronization systems in heifers of *Bos indicus* × *Bos taurus* breeding compared to yearling *Bos taurus* heifers. Additional studies need to be conducted in heifers of *Bos indicus* × *Bos taurus* breeding to determine the relationship between follicle development both during and after a synchronization treatment on the expression of estrus and subsequent conception rates. Because there was a treatment by interval from $PGF_{2\alpha}$ to onset of estrus effect on conception rate, it may be beneficial to determine when the timed-AI should be performed relative to the $PGF_{2\alpha}$ treatment for both the 7-10 and Select Synch/CIDR+timed-AI treatments. It will also be of interest to determine if estrous detection can be eliminated, and only a fixed timed-AI can be used in yearling heifers of *Bos indicus* × *Bos taurus* breeding.

In Chapter 4, yearling *Bos indicus* × *Bos taurus* heifers were synchronized with either the Select Synch/CIDR+timed-AI or 7-11 treatment. Heifers were distributed to treatment by reproductive tract score (RTS) and BCS. The 7-11 heifers had greater ($P < 0.05$) estrous response, conception rate, and synchronized pregnancy rate compared to Select Synch/CIDR+timed-AI heifers. Heifers exhibiting estrus at 60 h had a greater ($P < 0.05$) conception rate compared to heifers that had exhibited estrus at ≤ 36, 48, and 72 h, which were similar ($P > 0.05$) to each other. As RTS increased from ≤ 2 to a ≥ 3, estrous response, conception rate, synchronized pregnancy rate, and thirty day pregnancy rate all increased ($P < 0.05$).

Estrous response was greater for 7-11 heifers compared to Select Synch/CIDR+timed-AI heifers. The difference observed between treatments could be due to the 7-11 treatment synchronizing follicular wave development more effectively compared to the Select Synch/CIDR+timed-AI treatment. The MGA in combination with $PGF_{2\alpha}$ allows most heifers to have a large dominant follicle present at MGA withdrawal that is ready to be ovulated by GnRH; thereby, synchronizing follicle development for the subsequent $PGF_{2\alpha}$, and thereby improving the estrous response. The Select Synch/CIDR+timed-AI protocol is probably not as effectively synchronizing follicle development since heifers are at all stages of follicle development at GnRH and CIDR insertion, which probably results in asynchronous follicle development and a less synchronous estrus. The estrous response for both treatments are considerably less compared to studies in yearling *Bos taurus* and *Bos indicus* × *Bos taurus* heifers synchronized with either the Select Synch/CIDR+timed-AI, short term progestogen protocols, and CIDR + $PGF_{2\alpha}$ protocol. The decreased estrous response is probably due to a combination of heifer genotype and the numbers of heifers that are pubertal at the start of the synchronization treatment, which was probably influenced by level of nutrition. Although, estrus is more difficult to detect in cattle of *Bos indicus* breeding due to a decreased expression and duration of estrus as well as an increased incidence of silent estrus, the use of estrous detection aids probably made up for any deficiencies that occurred in estrous detection efficiency. Since blood samples were not taken, it is not known how many heifers were pubertal at the start of the synchronization treatment. Additionally, it is not known how effective the synchronization treatments were in inducing estrous cycles in the pre-pubertal heifers. Body condition score, an indirect indicator of nutritional status and estrous cycling status, had a significant effect on estrous response as estrous response increased to acceptable levels in heifers that had a BCS $\geq$ 5.5 at the start of the treatment. Consequently, the low number of estrous cycling heifers was probably the primary reason for the decreased estrous response for the two synchronization treatments in the present study.

There was a marked increase in conception rates for heifers synchronized with the 7-11 compared to the Select Synch/CIDR+timed-AI treatment. This suggest that the 7-11 treatment may be doing a better job of synchronizing follicle development compared to the Select Synch/CIDR+timed-AI treatment resulting in follicles that are more fertile. The duration of follicle dominance is negatively associated with follicle fertility as follicles with prolonged durations of dominance have significantly decreased fertility compared to follicles that are ovulated with a normal duration of dominance. Therefore, it is possible that a greater number of 7-11 heifers had newly developed follicles present at CIDR removal compared to the Select Synch/CIDR+timed-AI treatment, which resulted in greater fertility for the 7-11 treatment. Additionally, conception rates for both treatments are considerably less compared to *Bos taurus* heifers synchronized with variations of the CIDR + PGF protocols were the conception rates ranged from 60 to 80%. For that reason, additional research needs to be conducted to fully characterize the effects of prolonged follicle dominance on fertility in yearling heifers of *Bos indicus* × *Bos taurus* breeding and to determine why there is such a significant reduction in conception rates in yearling heifers of *Bos indicus* × *Bos taurus* compared to *Bos taurus* heifers. Timed-AI pregnancy rates were similar between the 7-11 and Select Synch/CIDR+timed-AI treatments but they were nearly half the values observed for *Bos taurus* heifers synchronized with the Select Synch/CIDR+timed-AI protocol and yearling *Bos indicus* × *Bos taurus* heifers synchronized with the 14 d MGA + $PGF_{2\alpha}$ protocol. The primary reason for decreased timed-AI pregnancy rates is probably due to the decreased number of heifers going through estrous cycles at the start of the synchronization treatment resulting in a significant number of pre-pubertal heifers at the timed-AI. The low timed-AI pregnancy rates also suggest that the synchronization treatments did not induce estrous cycles in many of the pre-pubertal heifers. It should also be noted that there was an effect of interval from $PGF_{2\alpha}$ to the onset of estrus on conception rate and heifers that exhibited estrus at 60 h had conception rates that were nearly twice heifers that exhibited estrus < 36, 48 and 72 h. Therefore, when the timed-AI is performed needs to be re-evaluated and it may be beneficial to conduct it at 60 h after $PGF_{2\alpha}$ instead of 72 h.

The 7-11 treatment had greater synchronized pregnancy rates compared to the Select Synch/CIDR+timed-AI treatment and the primary reasons were that the 7-11 treatment had a greater estrous response and conception rate compared to the Select Synch/CIDR+timed-AI. The 7-11 treatment was probably more effective at synchronizing follicle waves allowing a new follicle to be ready to ovulate after $PGF_{2\alpha}$ compared to the Select Synch/CIDR+timed-AI treatment. With that said, synchronized pregnancy rates for both treatments were considerably less compared to what has been observed in *Bos taurus* heifers synchronized with similar synchronization protocols. The primary reason for the decreased synchronized pregnancy rates is probably due to the small percentage of heifers that had reached puberty at the start of the treatment. Therefore, additional experiments need to be conducted to determine how effective the 7-11 and Select Synch/CIDR+timed-AI treatments are synchronizing follicle development as well as how effective the treatments are for inducing estrous cycles in pre-pubertal heifers.

Utilization of the RTS at the start of the experiment was used to try and predict the estrous cycling status of the heifers without having to work the heifers an additional time to take blood sample 10 d before the start of the experiment. In general, as RTS increased from a 1 or 2 to ≥ 3, the estrous response, conception rate, and synchronized pregnancy rate increased. However, what is interesting is that the reproductive performance did not increase significantly as RTS increased from a 3 to ≥ 4, with the exception of estrous response, although this increase was only 12%. One would have expected a more significant increase in estrous response and synchronized pregnancy rate for heifers with an RTS of 4 and 5, which should be pubertal heifers compared to prepubertal heifers with RTS 3. Utilization of RTS is an often used management practice in yearling heifers of *Bos taurus* breeding to predict future reproductive performance and to cull heifers (i.e., RTS 1 and 2) that will have substandard reproductive performance. Consequently, it appears that we need to re-evaluate the manner in which RTS is used to predict potential response to a synchronization treatment in cattle of *Bos indicus* × *Bos taurus* breeding.

In summary, the 7-11 treatment provided increased estrous response, conception rates, and synchronized pregnancy rates compared to the Select Synch/CIDR+timed-AI treatment. Estrous response was directly affected by BCS at start of synchronization treatment, but interestingly enough, BCS did not affect the overall reproductive performance of the heifers. Although RTS had significant affects on estrous response, conception rate, and synchronized pregnancy rate, using RTS to predict the potential response to a synchronization treatment was questionable. Results from this experiment demonstrate the importance of having heifers either going through estrous cycles or near the onset of puberty before the start of the breeding season in order to achieve acceptable synchronized pregnancy rates. The synchronization treatments used in the current study will need to be further tested in a population of heifers of known estrous cycling status to determine the true response to treatment.

Relative to yearling heifers of *Bos indicus* × *Bos taurus* breeding, future research needs to investigate the reasons for the less than acceptable estrous response and conception rates observed in heifers synchronized with either the 7-11 or Select Synch/CIDR+timed-AI treatments. This includes a complete characterization of the effectiveness of synchronization treatments in peri-pubertal heifers as determined by blood progesterone concentrations. Because nutrition can have a significant effect on reproductive performance, it will be also be important to determine what effect nutritional status and different nutritional management strategies have on conception rates in synchronized heifers. It will also be important to characterize the utilization of RTS as a reproductive management tool and to determine its use as an indicator of a heifer's breeding ability based on the heifer's weight and age at the start of the breeding season. Environmental factors such as temperature, humidity, plane of nutrition, and animal handling and management can all influence the endocrine, reproductive, and behavioral factors associated with the expression of estrus as well as the overall treatment response. Future research must focus on identifying the significance of these variables and determine which one(s) can be controlled and (or) modified to enhance the onset of puberty and response to a synchronization treatment.

APPENDIX A
SUPPLEMENTAL TABLES AND FIGURES FOR CHAPTER 3

Table A-1. Effect of synchronization treatment and prostaglandin treatment on estrous response, conception rates and pregnancy rates in suckled cows of *Bos indicus* × *Bos taurus* breeding synchronized with either a modified 7-11 (7-10) or Select Synch/CIDR+timed-AI (SSC+TAI) treatment with either Cloprostenol sodium (Cloprostenol) or Dinoprost tromethamine (Dinoprost).[a]

Variable	Estrous response, %[b]	Conception rate, %[c]	Timed-AI pregnancy rate, %[d]	Synchronized pregnancy rate, %[e]
7-10	49.0 (77/157)	45.5 (35/77)	17.5 (14/80)	31.2 (49/157)
SSC+TAI	59.9 (100/167)	62.0 (62/100)	20.9 (14/67)	45.5 (76/167)
P-value	< 0.05	< 0.05	> 0.05	< 0.05
Cloprostenol	57.0 (94/165)	48.9 (46/94)	23.9 (17/71)	38.2 (63/165)
Dinoprost	52.2 (83/159)	61.5 (51/83)	14.5 (11/76)	39.0 (62/159)
P-value	> 0.05	> 0.05	> 0.05	> 0.05

[a] See Figure 3-1 for details of treatments.

[b] Percentage of cows displaying estrus 72 h after $PGF_{2\alpha}$ 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 to timed-AI of the total that were timed-AI.

[e] Percentage of cows pregnant during the synchronized breeding of the total treated

Table A-2. Estrous, conception and pregnancy rates by prostaglandin treatment of suckled cows of *Bos indicus* × *Bos taurus* breeding synchronized with either a modified 7-11 (7-10) or Select Synch/CIDR+timed-AI (SSC+TAI) treatment with either Cloprostenol sodium (Cloprostenol) or Dinoprost tromethamine (Dinoprost).[a]

Variable	n	Estrous response, %[b]	Conception rate, %[c]	Timed-AI pregnancy rate, %[d]	Synchronized pregnancy rate, %[e]
7-10 Cloprostenol	81	58.0 (47/81)	40.4 (19/47)	20.6 (7/34)	32.1 (26/81)
7-10 Dinoprost	76	39.5 (30/76)	53.3 (16/30)	15.2 (7/46)	30.3 (23/76)
SSC+TAI Cloprostenol	84	56.0 (47/84)	57.5 (27/47)	27.0 (10/37)	44.1 (37/84)
SSC+TAI Dinoprost	83	63.9 (53/83)	66.0 (35/53)	13.3 (4/30)	47.0 (39/83)
P-value		< 0.05	> 0.05	> 0.05	0.06

[a] See Figure 3-1 for details of treatments.

[b] Percentage of cows displaying estrus 72 h after $PGF_{2\alpha}$ 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 to timed-AI of the total that were timed-AI.

[e] Percentage of cows pregnant during the synchronized breeding of the total treated.

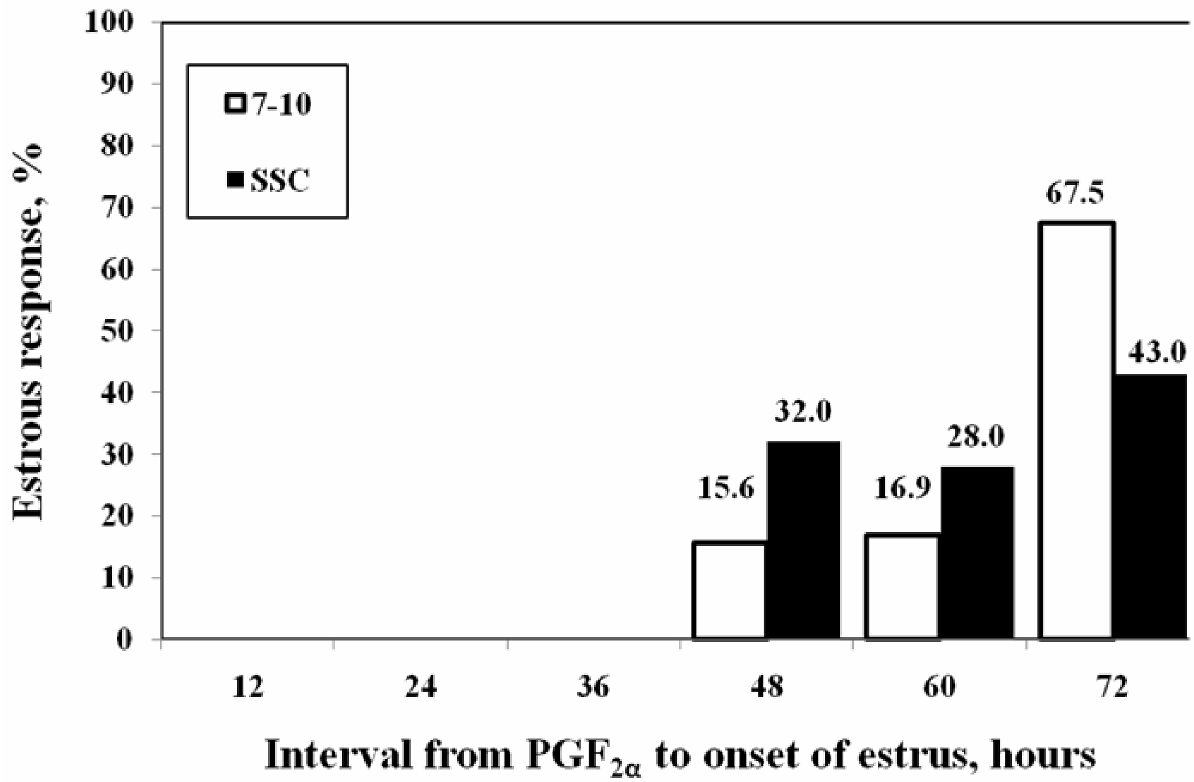

Figure A-1. Effect of 7-10 or Select Synch/CIDR+timed-AI (SSC) treatment on estrous response in suckled postpartum *Bos indicus* ×*Bos taurus* cows. Means are expressed as a percentage of total that exhibited estrus.

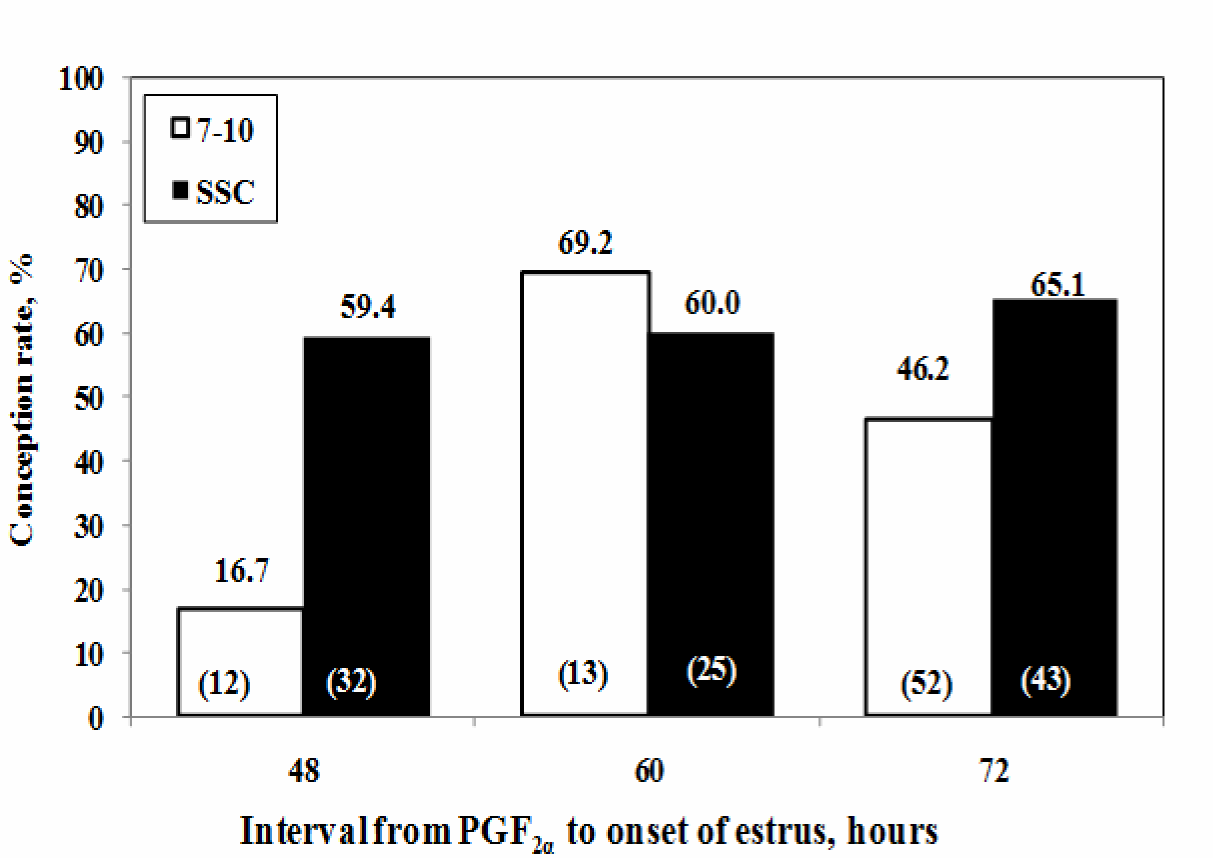

Figure A-2. Effect of 7-10 or Select Synch/CIDR+timed-AI (SSC) synchronization treatment and interval from $PGF_{2\alpha}$ to onset of estrus on conception rate in suckled postpartum *Bos indicus* ×*Bos taurus* cows. Means are expressed as a percentage of total that exhibited estrus and became pregnant.

Table A-3. Estrous response, conception and pregnancy rates by synchronization treatment of yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with either a modified 7-11 (7-10) or Select Synch/CIDR+timed-AI (SSC+TAI) treatment with either Cloprostenol sodium (Cloprostenol) or Dinoprost tromethamine (Dinoprost) for years 1 and 2 Experiment2.[a]

Variable	Estrous response, %[b]	Conception rate, %[c]	Timed-AI pregnancy rate %[d]	Synchronized pregnancy rate, %[e]
7-10	65.2 (116/178)	62.1 (72/116)	14.5 (9/62)	45.5 (81/178)
Year 1	71.6	65.4	9.68	49.5
Year 2	55.1	55.3	19.4	39.1
SSC +TAI	68.4 (121/177)	67.8 (82/121)	17.9 (10/56)	52.0 (92/177)
Year 1	64.2	71.4	20.5	53.2
Year 2	75.0	62.7	11.8	50.0
Sync	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Year	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Sync ×Year	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

[a] See Figure 3-1 for details of treatments.

[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.

[c] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.

[d] Percentage of heifers pregnant to timed-AI of the total that were timed-AI.

[e] Percentage of heifers pregnant during the synchronized breeding of the total treated.

Table A-4. Estrous response, conception and pregnancy rates by prostaglandin treatment of yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with either a modified 7-11 (7-10) or Select Synch/CIDR + timed-AI (SSC+TAI) treatment with either Cloprostenol sodium (Cloprostenol) or Dinoprost tromethamine (Dinoprost) for years 1 and 2 Experiment 2.[a]

Variable	Estrous response, %[b]	Conception rate, %[c]	Timed-AI pregnancy rate, %[d]	Synchronized pregnancy rate, %[e]
Cloprostenol	68.7 (123/179)	67.5 (83/123)	17.9 (10/56)	52.0 (93/179)
Year 1	70.6	71.4	18.8	56.0
Year 2	65.7	60.9	16.7	45.7
Dinoprost	64.8 (114/176)	62.3(71/114)	14.5 (9/62)	45.4 (80/176)
Year 1	65.1	64.8	13.2	46.8
Year 2	64.2	58.1	16.7	43.3
Prostaglandin	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Year	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Prostaglandin × Year	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

[a] See Figure 3-1 for details of treatments.

[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.

[c] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.

[d] Percentage of heifers pregnant to timed-AI of the total that were timed-AI.

[e] Percentage of heifers pregnant during the synchronized breeding of the total treated.

Table A-5. Estrous response, conception and pregnancy rates of yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with either a modified 7-11 (7-10) or Select Synch/CIDR + timed-AI (SSC+TAI) treatment with either Cloprostenol sodium (Cloprostenol) or Dinoprost tromethamine (Dinoprost) for years 1 and 2 Experiment 2.

Variable	n	Estrous response, %[b]	Conception rate, %[c]	Timed-AI pregnancy rate, %[d]	Synchronized pregnancy rate, %[e]
7-10 Cloprostenol	90	71.1(64/90)	62.5 (40/64)	15.4 (4/26)	48.9 (44/90)
Year 1	55	78.2	69.8	8.33	56.4
Year 2	35	60.0	47.6	21.4	37.1
7-10 Dinoprost	88	59.1 (52/88)	61.5 (32/52)	13.9 (5/36)	42.0 (37/88)
Year 1	54	64.8	60.0	10.5	42.6
Year 2	34	50.0	64.7	17.6	41.2
SSC+TAI Cloprostenol	89	66.3 (59/89)	72.9 (43/59)	20.0 (6/30)	55.1 (49/89)
Year 1	54	63.0	73.5	25.0	55.6
Year 2	35	71.4	72.0	10.0	54.3
SSC+TAI Dinoprost	88	70.5 (62/88)	62.9 (39/62)	15.4 (4/26)	48.9 (43/88)
Year 1	55	65.5	69.4	15.8	50.9
Year 2	33	78.8	53.8	14.3	45.5
TRT		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Year		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
TRT × Year		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

[a] Figure 3-1 gives details of treatments.

[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.

[c] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.

[d] Percentage of heifers pregnant to timed-AI of the total that were timed-AI.

[e] Percentage of heifers pregnant during the synchronized breeding of the total treated

APPENDIX B
SUPPLEMENTAL TABLES AND FIGURES FOR CHAPTER 4

Table B-1. Description of the reproductive tract score (RTS) used in Chapter 4

		Ovarian Measurement (mm)				
RTS	Uterine horns	Length	Height	Width	Ovarian structures	Description
1	Immature <20mm diameter No tone	15	10	8	No palpable follicles	Infantile
2	20-25 mm diameter No tone	18	12	10	8 mm follicles	Prepubertal (more than 30 d prepubertal)
3	20-25 mm diameter Slight tone	22	15	10	8-10 mm follicles	Peripubertal (within 30 d of puberty)
4	30 mm diameter Good tone	30	16	12	> 10 mm follicles CL possible	Cycling (Follicular Phase)
5	> 30 mm diameter	>32	20	15	CL present	Cycling (Luteal phase)

Adapted from Anderson et al., 1991.

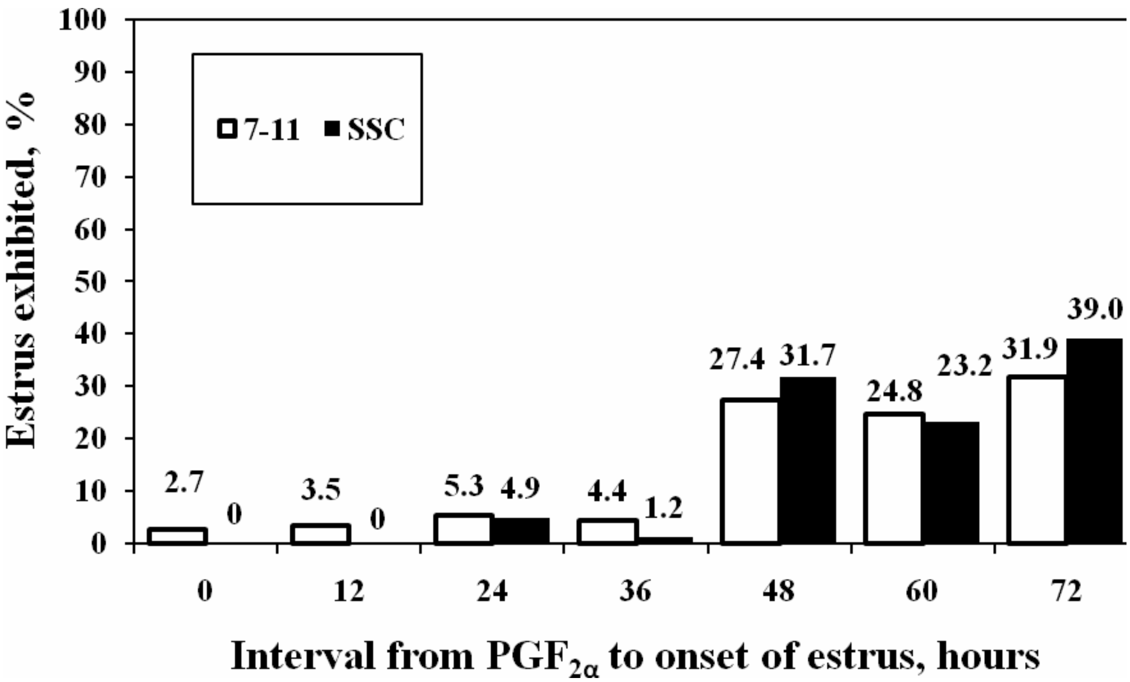

Figure B-1. Effect of estrous synchronization treatment on estrous response in yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with 7-11 or Select Synch/CIDR+timed-AI (SSC). Means are expresses as a percentage of total that exhibited estrus.

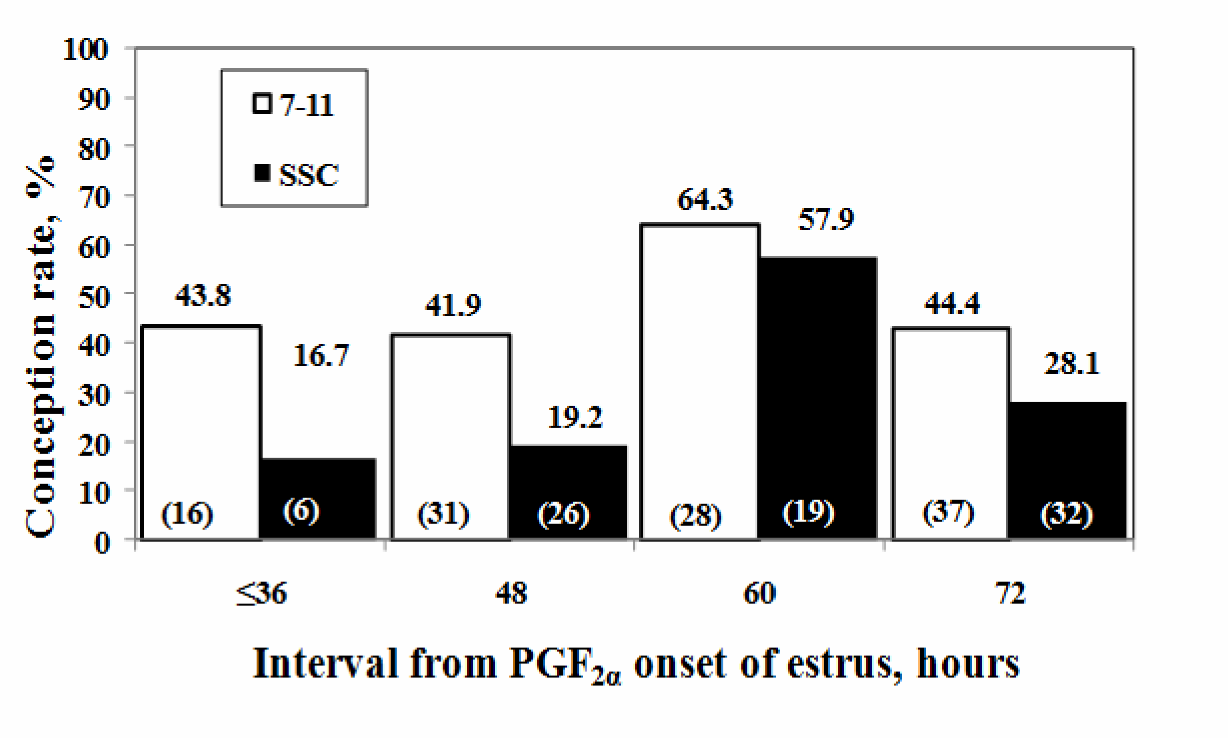

Figure B-2. Effect of estrous synchronization treatment on conception rate in yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with 7-11 or Select Synch/CIDR+timed-AI (SSC). Means are expressed as a percentage of total that exhibited estrus and became pregnant.

Table B-2 Estrous response, conception and pregnancy rates of yearling heifers of *Bos indicus* × *Bos taurus* breeding synchronized with either a 7-11 or Select Synch/CIDR+timed-AI treatment by location.[a]

Location	Estrous response, %[b]	Conception rate, %[c]	Timed-AI pregnancy, rate, %[d]	Synchronized pregnancy rate, %[e]
1	57.4 (35/61)	40.0 (14/35)	7.7 (2/26)	26.2 (16/61)
2	42.9 (51/119)	49.2 (25/51)	14.7 (10/68)	29.4 (35/119)
3	49.6 (114/230)	36.8 (42/114)	23.3 (27/116)	30.0 (69/230)
P-value	> 0.05	> 0.05	>0.05	> 0.05

[a] See Figure 4-1 for details of treatments.

[b] Percentage of heifers displaying estrus 72 h after $PGF_{2\alpha}$ of the total treated.

[c] Percentage of heifers pregnant to AI of the total that exhibited estrus and were AI.

[d] Percentage of heifers pregnant to timed-AI of the total that were timed-AI.

[e] Percentage of heifers pregnant during the synchronized breeding of the total treated.

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BIOGRAPHICAL SKETCH

Erin Nicole McKinniss was born in 1984, in Athens, Ohio. Erin, the oldest daughter of Michael and Lynn McKinniss, was raised in western Ohio on a small purebred Simmental farm with her two younger brothers, Matthew and Stephen. She attended Northmont High School and was active in the varsity softball team, marching and concert bands, the American Junior Simmental Association, and 4-H. In 2002, she was honored with the title of Ohio Beef Ambassador and she represented her state at the national contest. That year she traveled to the Minnesota State Fair as a representative of the program to promote beef to consumers. In 2004 she was elected to the junior board of trustees of the American Junior Simmental Association, where she represented the eastern region and was later elected to First Vice President of the board. Erin earned a bachelor’s degree in animal sciences from The Ohio State University, during which time she was an active member of Sigma Alpha, Saddle and Sirloin, and the Ag council. As part of the animal sciences department, she also was awarded the opportunity to study abroad in Australia for a quarter learning about agriculture production. Upon graduation from Ohio State in 2006, she began a Master of Science degree under Dr. Joel Yelich, at the University of Florida, studying reproductive physiology. Under this appointment Erin held many offices for the Animal Science Graduate Student Association and also taught an undergraduate reproduction lab. Erin’s future plans are to pursue her Ph.D. at the University of Florida, under the tutelage of Dr. Joel Yelich and Dr. Matt Hersom.