EFFECT OF LONG-TERM MELENGESTROL ACETATE TREATMENTS ON FOLLICLE DYNAMICS AND RESPONSE TO GONADOTROPIN-RELEASING HORMONE AND PROSTAGLANDIN $F_{2\alpha}$ SYNCHRONIZATION TREATMENTS IN Bos indicus × Bos taurus HEIFERS

By

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To my loving parents and sister. For their support, encouragement, and love.

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In experiment 1, yearling Angus (n = 40) and Brangus (n = 26) heifers received melengestrol acetate (MGA; 0.5 mg/hd/d) for 14 d with prostaglandin $F_{2\alpha}$ (PG) administered either 19 d or 19 and 20d after MGA withdrawal for Angus and Brangus, respectively. A subgroup of Angus (n=11) and Brangus (n=10) heifers had transrectal ultrasonography conducted daily after MGA withdrawal until 7 d after PG to evaluate follicle development. There tended (P = 0.07) to be more Angus (100%; 11/11) compared to Brangus (80%; 8/10) heifers ovulating within 7 d after MGA withdrawal. Follicle wave patterns between MGA withdrawal and PG consisted of one (0/11; 1/10), two (9/11; 5/10), three (2/11; 3/10) or four (0/11; 1/10) waves for Angus and Brangus, respectively. The number of heifers with follicle ≥ 10 mm on 9 (54.5, 80.0 %), 10 (81.8 %, 70.0 %), and 11 d (90.9, 80.0%) after MGA were similar between Angus and Brangus respectively; but greater ($P < 0.05$) on 12 (100, 70.0 %) and 13 d (100, 50 %) for Angus compared to Brangus, respectively. Because of the asynchrony of follicle wave patterns from MGA withdrawal to PG for Brangus compared to Angus, the best time to administer GnRH to synchronize follicle development in Brangus heifers may be immediately after MGA withdrawal. In Experiment 2 cycling Bos indicus x Bos taurus (BI × BT) heifers

were pre-synchronized to start a 14 d MGA (0.5 mg/hd/d) treatment on d 2 of the estrous cycle. Heifers were randomly assigned to receive GnRH (100 μg) either 3 (G3; n = 25) or 10 d (G10; n = 23) after MGA withdrawal with PG (12.5 mg) 7 and 8 d after GnRH. During MGA, heifers within each treatment received no PG or two consecutive PG treatments on d 4 and 5, 8 and 9, or 12 and 13, to simulate different periods of low-level progestogen exposure (SOF). Ovulation to GnRH was 76.0 and 47.8% for the G3 and G10, respectively. For G3 and G10 treatments, heifers in the d 14 SOF group did not respond as effectively as the other SOF groups. Following PG, more ($P < 0.05$) G3 (76%) heifers exhibited estrus during the first 72 h after PG compared to G10 (43.5%) heifers. In Experiment 3, yearling BI×BT (n=295) heifers at two locations were synchronized with two MGA + PG treatments. Treatment 1 was the same as in Experiment 1 (MGA-PG; n=174) while treatment 2 was the same as the G3 treatment in Experiment 2 (MGA-G-P; n=178). Heifers were AI 8 to 12 h after an observed estrus. Heifers not detected in estrus by 72 h after PG were timed -AI concomitant with GnRH. Estrous response, conception, timed-AI, and synchronized pregnancy rates were similar ($P > 0.05$) between MGA-PG (48.3, 54.9, 22.4, 38.1%) and MGA-G-PG (56.7, 52.4, 18.8, 37.8%), respectively. In summary follicle dynamics during the 19 d after a long term MGA treatment are different between Angus and Brangus heifers. Although, incorporation of a GnRH treatment 3 d after a 14 d MGA treatment effectively induced ovulation and resulted in a very synchronous estrus when PG was administered 7 d later, it did not improve the AI pregnancy rates compared to the MGA-PG estrous synchronization system.

CHAPTER 1
INTRODUCTION

Artificial insemination (AI) provides producers with the opportunity to improve their herd through the use of superior genetics. Additionally, a successful AI program benefits the producer economically by decreasing the number of bulls needed while potentially increasing the performance and uniformity of the calf crop. However, the implementation of a successful AI program requires significant labor, which offset the economic benefits and limits the practicality of AI. Therefore, a major requirement of a successful AI program requires estrous synchronization systems that result in a large number of cattle that can be AI in a short period of time.

Numerous estrous synchronization systems have been developed to meet the needs of each production scenario. Products available for estrous synchronization systems include progestins, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and gonadotropin-releasing hormone (GnRH). Progestins can be used to lengthen the estrous cycle by preventing the LH surge, estrus, and ovulation. Prostaglandin $F_{2\alpha}$ acts to artificially shorten the estrous cycle by initiating luteolysis. Finally, GnRH can be administered to control follicle wave emergence or to initiate ovulation. Furthermore, these products can be combined to prevent estrus and ovulation, shorten the estrous cycle, and to control follicle development. The success of an estrous synchronization system is dependant on its ability to bring a high percentage (> 75%) of cattle into estrus in a short time period (< 7 d). Conversely, the effectiveness of these products in synchronizing estrus depend on the genetics of the herd, body condition, reproductive status (i.e., estrous cycle vs anestrous), stage of the estrous cycle, environment, and breed-type (*Bos taurus* vs. *Bos indicus*). Breed is an important contributing factor in synchronization systems where most systems in use today have been designed for cattle of *Bos taurus* breeding. Therefore, these systems need to be evaluated or new

systems need to be developed to account for the physiological and behavioral differences in cattle of *Bos indicus* breeding.

Throughout Florida, the most common form of cattle production is cow/calf operations. However, the subtropical environment of Florida presents cattle producers with a problem where elevated temperatures and decreased nutrient availability are not suitable for most breeds of cattle. Therefore, cattle normally found in Florida contain some degree of *Bos indicus* breeding. Cattle of *Bos indicus* breeding provide the Florida cattlemen many advantages in that they are adapted to the hot, humid environment, able to survive on low quality forages, and are more resistant to parasites than cattle of *Bos taurus* breeding. Conversely, several behavioral and physiological differences are observed in *Bos indicus* cattle, resulting in reduced reproductive performance and decreased effectiveness of commonly used estrous synchronization systems.

In cow/calf operations, the greatest opportunity to implement an estrous synchronization system is in first service breeding of heifers. Heifers offer many benefits that make them best suited to for the implementation of an estrous synchronization system. First, heifers are usually managed in groups supplemented to reach targeted weights and condition scores. Second, heifers do not have the negative effects of lactation and suckling calf. Third, heifers are usually cycling prior to the breeding season. Finally, since heifers are managed in groups and do not have calves, they are easily handled. Estrous synchronization and AI of heifers benefit the producer by reducing labor required for detecting estrus. Producers can choose to inseminate to calving-ease sires, therefore, reducing the number of calving-ease bulls needed for natural service. Moreover, an effective estrous synchronization system allows more heifers the opportunity to become pregnant early in the first 30 days of the breeding season. More heifers being exposed early in the breeding season results in more heifers calving early, reducing labor

required during calving season. Also, time of first calving affects lifetime performance of the cow, where cattle calving as two-year olds will have a greater lifetime production than those calving at a later date.

One of the most common estrous synchronization systems for heifers utilizes a long term (14 d) melengestrol acetate (MGA) treatment and $PGF_{2\alpha}$. administered 19 d after MGA withdrawal. This estrous synchronization system was developed in *Bos taurus* heifers and results in excellent AI pregnancy rates. Conversely, this system is less effective in heifers of *Bos indicus* breeding. Recent research has increased the effectiveness of this system in *Bos indicus* heifers by altering the delivery of $PGF_{2\alpha}$, but it does still not result in AI pregnancy rates observed in *Bos taurus* heifers. Therefore, this review will focus on the physiological and behavioral characteristics of reproductive function in cattle of *Bos indicus* breeding and to review the estrous synchronization literature in an attempt to identify why there is a reduced reproductive performance to estrous synchronization systems in cattle of *Bos indicus* breeding.

CHAPTER 2
REVIEW OF LITERATURE

Endocrine Control of the Estrous Cycle

Regulation of mammalian reproduction is primarily controlled at the level of the hypothalamus and pituitary. The main hypothalamic hormone involved in regulating the hypothalamic-pituitary-gonadal axis and reproduction is gonadotropin-releasing- hormone (GnRH). Gonadotropin-releasing-hormone, a decapeptide consisting of ten amino acids, is released from the hypothalamus and signals the release of the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary (Schally et al., 1971). These pituitary derived gonadotropins act on ovarian cells to signal changes in ovarian function and secretion of hormones. Furthermore, either positive or negative feedback of steroid hormones on the hypothalamus acts to regulate the release of GnRH and gonadotropins.

Neurons responsible for the secretion of GnRH are loosely dispersed throughout the hypothalamus and GnRH is secreted from two distinct areas of the hypothalamus in either a "tonic" fashion or as a "surge". Tonic secretion, as observed during the luteal phase of the estrous cycle, is characterized by high amplitude, low frequency pulses under the negative feedback effect of progesterone and it is driven by neurons in the ventromedial and arcuate nuclei. Whereas, the surge-like secretion of GnRH, as observed during estrus and driven by the positive feedback of estradiol secretion, is responsible for the LH surge and it is controlled by the preoptic and suprachiasmatic nuclei (Smith and Jennes, 2001). The GnRH secreted from the hypothalamus is released from the median eminence where it enters the hypothalamo-hypophyseal portal system through fenestrations in the capillary walls to be carried to the anterior pituitary. At the anterior pituitary, GnRH acts through a seven transmembrane, G-protein coupled receptor, which stimulates the release of gonadotropins (Kakar et al., 1993).

Luteinizing hormone is necessary for the development of many ovarian events such as corpus luteum (CL) development (Snook et al., 1969), secretion of the gonadal steroid progesterone (Alila et al., 1988), follicle maturation (Ginther et al., 2001), and ovulation (Wettemann et al., 1972; Fortune, 1994). Pulses of GnRH stimulate the release of LH in a pulsatile fashion (Schams et al., 1974) where pulses are characterized by a rapid increase followed by a gradual decline in LH concentrations (Forrest et al., 1980). Anderson et al. (1981) reported 3.4 pulses of LH over an 8 h period in prepubertal beef calves. However, LH secretory patterns are dependent upon the stage of the estrous cycle. During the early luteal phase, LH secretion is characterized by high frequency, low amplitude pulses; whereas during the mid luteal phase LH secretion is characterized by high amplitude, low frequency pulses (Rahe et al., 1980). Walters et al. (1984) observed that pulses of estradiol are observed within 60 min following pulses of LH, with greater estradiol pulses during the early luteal compared to the mid luteal phase. Furthermore, estradiol enhances the release of LH from the anterior pituitary (Cupp et al., 1995) by increasing GnRH receptors in the pituitary (Gregg et al., 1990).

Follicle-stimulating hormone, as its name implies, functions to stimulate the recruitment and growth of a new follicle wave (Sunderland et al., 1994; Evans et al., 1997). Follicle development remains dependent on FSH until follicle deviation (Ginther et al., 2000a); and stimulation of thecal estrogen production requires FSH beyond this point (Mihm et al., 1997). Following hourly infusion of exogenous GnRH, concentrations of LH increased, however no increases in the concentration of FSH were observed (Vizcarra et al., 1997) indicating that FSH secretion is not controlled exclusively by GnRH. Furthermore, Cupp et al. (1995) reported that concentrations of FSH were greater in ovariectomized and ovariectomized + estradiol treated cows than in intact controls, demonstrating that the regulation of FSH secretion could be

controlled by the ovaries. Unlike LH, repeated treatment of GnRH did not result in the reduction of FSH secretion (Schams et al., 1974).

Progesterone, secreted by the corpus luteum (CL), and estradiol, secreted by the dominant follicle, feedback onto the hypothalamus to regulate the secretion of gonadotropins. Bergfeld et al. (1995) reported that cows with high progesterone concentrations had fewer LH pulses as well as lower concentrations of estradiol; whereas, cows with low progesterone concentrations had a greater frequency of LH pulses. During the mid luteal phase, when progesterone concentrations are at peak concentrations, LH pulses are high amplitude and low frequency (6-8 pulses/24 h; Rahe et al., 1980). However, high estradiol concentrations, as observed during the follicular phase, lead to increased LH pulse frequency (Stumpf et al., 1993). Conversely, progesterone and estradiol act to regulate FSH secretion differently compared to LH. Ireland and Roche (1982) and Price and Webb (1988) reported no significant effect of progesterone on FSH secretion. However, treatment of intact (Ireland and Roche, 1982) and ovariectomized (Price and Webb, 1988) heifers with estradiol significantly decreased FSH secretion. Following follicle ablation, FSH concentrations were greater in heifers treated with 0 mg estradiol than those treated with 0.5 mg estradiol (Ginther et al., 2000b).

Reproductive function is similar between cattle of *Bos taurus* and *Bos indicus* breeding, however differences have been noted in the secretory patterns of reproductive hormones between the breeds. Both *Bos taurus* and *Bos indicus* cattle exhibit a pulsatile secretion of LH, but a greater number of LH peaks (3.33 vs. 3.00), magnitude of peaks (overall LH peak height; 10.45 vs. 7.85 ng/mL) and LH pulse heights (the highest LH value minus the lowest LH value; 6.50 vs. 4.28 ng/mL) are observed in *Bos taurus* compared to *Bos indicus* cows, respectively (Griffen and Randel, 1978). Griffen and Randel, (1978) observed that ovariectomized Hereford (*Bos taurus*)

and Brahman (*Bos indicus*) cows responded to exogenous GnRH with increased concentrations of LH, but the increases in LH released were significantly less in Brahman cows. In response to exogenous estradiol, Brahman heifers have decreased LH secretion compared to Hereford and Brahman × Hereford heifers (Randel, 1976). In addition, Brahman cows were less responsive and Brahman × Hereford cows tended to be less responsive compared to Hereford cows treated with exogenous estradiol as determined by subsequent LH secretion (Rhodes and Randel 1978). Furthermore, the interval from estradiol treatment to LH response was longer in Brahman compared to Hereford and Brahman × Hereford heifers. These results are supported by Rhodes et al. (1978) who reported that Brahman cows secrete less LH in response to exogenous estradiol and take longer to respond compared to Hereford and Brahman × Hereford cows. Therefore, decreased secretion of LH in response to estradiol in *Bos indicus* cattle may be due to a decreased sensitivity of the hypothalamus to the positive feedback effects of estradiol.

Puberty

Throughout fetal development, the female reproductive tract forms and the ovaries are populated with gametes. Shortly after birth, ovarian function begins with follicular growth and development followed by steroid production, but the female does not ovulate. Puberty is defined as the time when the female first expresses estrus and ovulates. During the peripubertal period, the secretion of gonadotropins and the feedback effects of steroids on the hypothalamus change prior to and after the first ovulation. Factors such as body weight gains from weaning to puberty (Plasse et al., 1968) and age (Nelsen et al., 1985) have been shown to play major roles in the timing of the onset of puberty.

At approximately 3 - 5 months-of-age, the hypothalamic-pituitary axis of the heifer becomes functional, as LH secretion can be regulated by the actions of estradiol on the hypothalamus (Staigmiller et al., 1979). Furthermore, Barnes et al. (1980) reported that heifers

(approximately 3 to 9 months-of-age) were capable of releasing LH in response to exogenous GnRH, but not in sufficient quantities to cause an increase in follicle development and high enough estradiol production to stimulate an LH surge. Gonzalez-Padilla et al. (1975) reported that pituitary and hypothalamic hormones were released in bursts, with LH bursts being of high amplitude and low frequency in 14.5 mo old prepubertal Angus heifers. As the onset of puberty approaches, circulating concentrations of LH steadily increase (Swanson et al., 1972; Day et al., 1984) and the continual increase in LH secretion becomes the primary endocrine factor regulating the onset of puberty (Kinder et al., 1995). Day et al. (1984) reported that increases in pulsatile secretion of LH during the peripubertal period was due to a decrease in the negative feedback effects of estradiol and the decline in sensitivity of the hypothalamus to estradiol was due to a decrease in the concentration in estradiol receptors in the hypothalamus and anterior pituitary (Day et al., 1987). Removal of the negative effects of estradiol by ovariectomy results in an acute increase in LH concentrations (Day et al., 1984; Anderson et al., 1985). Immediately following ovariectomy, increased circulating LH concentrations were due to an increase in LH pulse frequency; whereas, later increases in circulating LH concentrations were associated with an increase in LH pulse amplitude (Anderson et al., 1985). Treatment of prepubertal, ovariectomized heifers with exogenous estradiol decreased circulating LH concentrations and termination of the episodic release of LH (Schillo et al., 1982), which was dependent on the amount of estradiol administered. However, the suppression of LH secretion, by estradiol, decreases, as the heifer gets older (Schillo et al., 1982). Gonzalez-Padilla et al. (1975) reported a priming peak of LH approximately -11 to -9 d prior to the pubertal LH peak and the priming peak was associated with a slight increase in progesterone concentrations. Berardinelli et al. (1979) subsequently reported that a non-palpable CL accompanied the prepubertal increase in

progesterone concentrations and ovariectomy resulted in decreased progesterone concentrations, indicating that the increase in progesterone concentrations probably originated from the ovaries. The authors suggested that the priming peak of LH served as a transition from pre- to postpubertal LH concentrations and progesterone exposure played a key role in the establishment of puberty. Consequently, the heifer becomes less responsive to the negative feedback effects of estradiol as she matures, resulting in increased LH pulse frequency, which ultimately reaches a threshold to initiate estrus and ovulation followed by secretion of luteal progesterone resulting in attainment of puberty. Thus a short estrous cycle, accompanied by an increase in progesterone concentrations, is followed by the first ovulation, and progesterone concentrations increase to concentrations ≥ 1 ng/mL, resulting from the newly formed CL. At this point the heifer will continue with regular estrous cycles and ovulations (Schillo et al., 1992).

In addition to maturation of the endocrine system as the female approaches puberty, the reproductive tissues including the ovaries and uterus also undergo maturational changes. Following birth, diameters of ovarian follicles increase from 2 to 34 wk of age, with the greatest increases occurring between 2 to 8 wk of age (Evans et al., 1994). Day et al. (1987) reported that as puberty approached, there was no change in ovarian weight or in the numbers of small (<3 mm), medium (3 to 6 mm), or large follicles (7 to 12 mm) but there was an increase in the numbers of follicles >12 mm. However, a follicle >12 mm was only observed in heifers that were close to reaching puberty. Growth and development of follicles occurs in a wave-like fashion in prepubertal heifers (Adams et al., 1994) similar to postpubertal heifers (Sirois and Fortune, 1988). Uterine weight also increases as the heifer nears puberty with the most rapid increase in the 50 d preceding puberty (Day et al., 1987). The increase in uterine weight is likely

due to increased estradiol secretion from the ovaries, which is associated with the onset of puberty (Day et al., 1987).

Breed plays a major role in the age at which puberty is attained in cattle. *Bos indicus* and *Bos indicus* × *Bos taurus* cattle reach puberty at older ages and heavier weights than cattle of *Bos taurus* breeding (Reynolds et al., 1963; Plasse et al., 1968; Gregory et al., 1979; Baker et al., 1989; Rodrigues et al., 2002). The range in age at puberty is approximately 14 to 24 mo for *Bos indicus* and 15 to 20 mo for Bos indicus × *Bos taurus* crossbred heifers (Plasse et al., 1968), and 9 to 15 mo for *Bos taurus* (Wiltbank et al., 1966). Baker et al. (1989) reported that Jersey (255 d) and Holstein (282 d) dairy heifers reached puberty at younger ages compared to Angus (418 d) and Hereford (466 d) heifers, whereas, Brahman heifers were the oldest at puberty (537 d). Conversely, crossbred Angus × Brahman (442 d) and Hereford × Brahman (472 d) reached puberty at a younger age than Brahman heifers. In support of the crossbred data, Gregory et al. (1979) noted that Pinzgaur crossed with *Bos taurus* heifers attained puberty at 303 d while Brahman crossed with *Bos taurus* heifers attained puberty at 398 d. The late attainment of puberty of *Bos indicus* heifers is also reflected in the 9% pubertal by 22 months of age in *Bos indicus* compared to 62% of Hereford heifers (Hearnshaw et al. 1994). In contrast, 82% of the Brahman x Hereford heifers reached puberty by 22 mo emphasizing the importance of cross breeding on decreasing age of puberty in *Bos indicus* based cattle, where reproductive traits are enhanced through heterosis. Rodrigues et al. (2002) reported that both *Bos indicus* and *Bos taurus* heifers underwent a cessation of the negative feedback effects of estradiol on LH secretion but, Bos taurus heifers undergo this cessation at a younger age. However, the extent of the negative feedback effect of estradiol on LH secretion was not amplified in *Bos indicus* heifers.

Ovarian Function

Follicle Growth and Selection

All of the oogonia a female has available during her lifetime are developed during fetal development where primordial germ cells migrate from the margin of the hindgut to the paired somatic gonadal primordia where they become oogonium (McGee and Hsueh, 2000). Oogonia undergo mitosis and the first stages of meiosis before being arrested at prophase of meiosis-1 (Wartenberg et al., 2001; McGee and Hsueh, 2000). After the attainment of puberty, the preovulatory surge of LH initiates resumption of meiosis and maturation of the oogonia (Hyttel, et al., 1997).

The first stage of follicle growth involves a change in shape and increased numbers of granulosa cells; whereas, the second stage of development is associated with an increase in oocyte diameter and granulosa cell numbers (Braw-Tal, 2002). In the activated primordial follicle, an assortment of 5 to 14 flattened and cuboidal granulosa cells form a single layer surrounding the oocyte (Fair et al., 1997a). As development progresses to the primary follicle stage, a single layer of 8 to 20 cuboidal granulosa cells encompass the oocyte and the first stages of zona pellucida formation are observed (Fair et al., 1997a; Braw-Tal and Yossefi, 1997). At this stage, granulosa cells begin to secrete follistatin, which acts to block the effects of the growth inhibitor activin A (Braw-Tal, 1994). Also, the oocyte secretes factors such as growth differentiation factor-9 (GDF-9) and bone morphogenic protein-15 (BMP-15), both of which play roles in granulosa cell proliferation (McGrath et al., 1995; Dube et al., 1998; Braw-Tal, 2002), whereas oocyte growth is promoted by granulosa secretions such as kit ligand (Braw-Tal, 2002). As the follicle progresses to the secondary follicle stage, the oocyte is surrounded by a partial or complete bilayer of granulosa cells and oocyte transcription is enabled (Fair et al., 1997b). Transcriptional activity of oocytes remains inactive until stimulated by FSH, at which

time primordial follicles are activated and RNA synthesis is increased (Fair et al., 1997b). Advancing from the secondary to tertiary stage of development is characterized by the completion of the zona pellucida as well as the formation of a multi-layered granulosa cell population and a small antral cavity (Fair et al., 1997b). In addition, granulosa cells differentiate to form cumulus granulosa cells and mural granulosa cells. Cumulus granulosa cells surround and are in close contact with the oocyte, while mural granulosa cells line the follicle wall and come into contact with the basal lamina (Gilchrist et al., 2004). At the tertiary follicle stage, increasing amounts of follicular fluid collect in the antral cavity and the follicle achieves ovulatory capacity.

In order for the graafian follicle to reach ovulatory status, it must undergo three distinct periods of development. The first period is recruitment, where a cohort of follicles is stimulated to grow under the influence of FSH. The second period is selection, the process of one follicle continuing to grow while the others become atretic. And the third period is dominance, where one follicle continues to grow while suppressing the growth of its subordinates (Sirois and Fortune, 1988; Fortune, 1994; Ginther et al., 2001). Beginning on approximately day 1 to 2 of the estrous cycle, a pool of 5 to 10 follicles < 4 mm in diameter, are recruited in response to a surge in FSH (Sirois and Fortune, 1988; Driancourt, 2001; Sunderland et al., 1994; Evans et al., 1997). The recruited follicles grow beyond a stage that usually results in atresia for other follicles (Fortune, 1994). Ginther et al. (1997) reported that the future dominant follicle emerges 6 to 7 h earlier than it's subordinates, providing a size advantage for the future dominant follicle over the other emerging follicles (Kulick et al., 1999). At this point, the future dominant follicle and subordinate follicles enter a common growth phase until the beginning of deviation (Ginther et al., 1997; Kulick et al., 1999). Deviation is the continued growth of one follicle with a

cessation of growth and regression (termed atresia) of other ovarian follicles (Kulick et al., 1999). After the initial surge in FSH, FSH concentrations decline with the simultaneous growth of follicles from 4 to 8.5 mm in diameter (Ginther et al., 1997; Ginther et al., 1999). Gibbons et al. (1999) observed that 3 mm follicles did not have any detectable capacity to suppress FSH secretion, while follicles reaching 5 mm gain the capacity to suppress FSH secretions. Conversely, growth beyond 5mm in diameter did not result in an increase in FSH suppressing capacity. The first follicle to reach 8.5 mm becomes the dominant follicle (Ginther et al., 1999; Kulick et al. 1999), which is coincident with a decrease in circulating FSH concentrations (Adams et al., 1993; Kulick et al., 1999) and increases in circulating LH concentrations (Kulick et al., 1999). After follicle deviation, circulating estradiol concentrations increase (Kulick et al., 1999) while follicles not selected for dominance become atretic.

The ability of one of the recruited follicles to continue growing while others undergo atresia is still an area of question. A major characteristic of the future dominant follicle is its ability to secrete greater amounts of estrogen (Badinga et al., 1992) around day 5 of the estrous cycle. This obseravtion supports early work of Ireland and Roche (1983) who reported that estrogen-active follicles had a lower incidence of atresia than estrogen-inactive follicles. Compared to subordinate follicles, dominant follicles contain lower amounts of insulin-like binding protein (IGFBP)-2 (Stewart et al., 1996), IGFBP-4, and follistatin (Austin et al., 2001), which support the continued growth of the dominant follicle by maintaining the availability of IGF-1 and activin-A. This is supported by Mihm et al. (2000) who reported that in a pool of recruited follicles, the future dominant follicle had the highest concentrations of estradiol and the lowest concentrations of IGFBP-4. Ireland and Roche (1983) observed that granulosa cells of the selected follicle have a greater ability to bind hCG compared to non-selected follicles on days

5 and 7 of the estrous cycle. The selected follicle could also bind more hCG on day 7 compared to day 3. Xu et al. (1995) reported that mRNA for LH receptors was present in day 4 follicles compared to day 2 follicles. These findings suggest that a follicles ability to achieve estrogenic activity is crucial for follicle selection. Ginther et al. (2001) observed that suppression of LH secretion did not affect the largest follicle prior to deviation but reduced follicle diameter and follicular fluid concentrations of IGF-1 and estradiol concentrations following deviation. The findings of Gong et al. (1995) support this by showing that suppression of LH secretion to basal concentrations and the abolishment of the pulsatile secretion of LH inhibited follicle growth beyond 7-9 mm. Furthermore, LH-receptor mRNA was only found in healthy dominant follicles > 9 mm (Xu et al., 1995). These findings suggest that there is a divergence from dependency from FSH to LH, but not until after deviation. Therefore, LH plays a major role in the growth and function of the dominant follicle following deviation.

In order for one follicle to establish and maintain dominance over its subordinates, it must suppress FSH secretion to prevent recruitment of smaller follicles. Administering recombinant bovine FSH to heifers before selection of the dominant follicle delayed the time for divergence between dominant and subordinate follicles (Adams et al., 1993). Furthermore, cauterization of the dominant follicle resulted in a surge of FSH and recruitment of a new pool of growing follicles soon after ablation (Adams et al., 1992). Treatment of animals with estradiol when the largest follicle reached 6 mm, around the time that endogenous FSH concentrations are normally declining, resulted in the suppression of FSH secretion and follicle diameter within 8 h (Ginther et al., 2000a). Ginther et al. (2000b) also reported that exogenous estradiol given to cattle after dominant follicle ablation caused a 2 to 3 h delay in the FSH surge. Nett et al. (2002) suggested that estradiol suppressed FSH secretion by altering the production of activin β_B in pituitary cells.

Bleach et al. (2001) reported that as FSH concentrations decline, estradiol and inhibin A concentrations increase coincident with the growth of a new dominant follicle. Inhibin originates from granulosa cells and functions to suppress secretion and release of FSH from the anterior pituitary (Good et al., 1995). Sheep immunized against inhibin showed an increase in FSH concentration as well as ovulation rate (Wheaton et al., 1992). Treatment of cattle with antiserum for inhibin and estradiol resulted in increased circulating FSH concentrations for a longer period of time than giving antiserum for inhibin alone, suggesting a synergistic role of suppressing FSH by inhibin and estradiol (Kaneko et al., 1995). Suppressing the synthesis and secretion of FSH with estradiol and inhibin resulted in atresia of subordinate follicles due to their inability to utilize low concentrations of circulating FSH, which is an environment that the dominant follicle can survive in (Ginther et al., 2000b; Austin et al., 2001).

Following the establishment of dominance, follicles must achieve ovulatory competence in order to respond to a pre-ovulatory surge of LH. The dominant follicle becomes more responsive to LH and gains ovulatory capacity when it reaches approximately 10 mm in diameter (Sartori et al., 2001), coincident with LH receptor mRNA in granulosa cells of follicles > 9 mm (Xu et al., 1995). Once the dominant follicle achieves ovulatory competence, it can either ovulate or become atretic, depending on the stage of the estrous cycle. For the dominant follicle to ovulate, luteolysis must occur followed by a decline in progesterone secretion followed by subsequent increases in estradiol secretion, which drives the preovulatory surge of LH resulting in ovulation (Wettemann et al., 1972; Fortune, 1994). When luteolysis does not occur, progesterone concentrations remain elevated, which suppress LH pulses resulting in decreased estradiol secretion (Fortune, 1994; Badinga et al. 1992). In response to decreased estradiol secretion, the dominant follicle becomes atretic, thereby removing the negative feedback effect

of ovarian progesterone, which allows for an increase in FSH concentrations and recruitment of a new follicle wave (Fortune, 1994).

Follicle development in cattle occurs in a wave-like pattern, which allows for a steady supply of ovulatory follicles (Sirois and Fortune, 1988). Each wave is characterized as having one large dominant follicle with ovulatory capacity and several smaller follicles termed subordinates (Sirois and Fortune, 1988). During an estrous cycle, the number of follicular waves varies between animals. Two and three-wave cycles are the most common although one, four, and five wave cycles have been observed (Sirois and Fortune, 1988; Savio et al., 1988; Viana et al., 2000). Estrous cycle length is reflected in the number of waves that occur during the estrous cycle. Estrous cycles with two follicle waves are approximately 20 d in duration; whereas, estrous cycles with three waves last from 21 to 23 d (Ginther et al., 1989; Viana et al., 2000; Sirois and Fortune, 1988; Savio et al., 1988).

In cattle with two follicular waves, wave emergence is approximately days 2 and 11 of the estrous cycle for the first and second wave, respectively; whereas, cattle with three follicular waves, wave emergence is approximately days 2, 9, and 16 of the estrous cycle for the three waves, respectively (Sirois and Fortune, 1988). In two wave cycles, the first wave reaches a maximum diameter about day 6 with regression by day 10 while the second dominant follicle reaches a maximal diameter by day 19 (Savio et al., 1988). For three wave cycles, the first and second wave dominant follicles reach a maximum diameter on day 6 and 16, respectively, followed by regression, while the third wave dominant follicle achieves maximal diameter on day 21 (Savio et al., 1988).

Differences in the number of waves results in different sizes and ages of dominant follicles in a wave. In cycling Holstein heifers exhibiting two wave cycles, the first-wave dominant

follicle (17.1 mm) and ovulatory (16.5 mm) dominant follicle reached a similar average maximal diameter, while the duration between emergence of waves was shorter for the first (9.7 d) than the ovulatory wave (10.4 d; Ginther et al., 1989). Conversely, Savio et al. (1988) noted that the maximal diameter of the first wave dominant follicle (14.3 mm) was smaller than the ovulatory dominant follicle (20.3 mm) in cycling beef heifers over two consecutive estrous cycles. In cycling Holstein heifers exhibiting three follicle waves, Ginther et al. (1989) observed that average follicle diameter was smaller for second (12.9 mm) and ovulatory (13.9 mm) wave dominant follicles compared to the first wave dominant follicle (16.0 mm). The duration between emergence of waves was similar for the first (9.0 d), second (7.2 d), and ovulatory (6.7 d) waves. Sirois and Fortune (1988) reported that in Holstein heifers displaying normal estrous cycles, the second wave dominant follicle (10.2 mm) had the smallest maximal diameter of the three follicles with no differences between the first (12.3 mm) and third (12.8 mm) wave follicles. Contrary to their findings, Savio et al. (1988) demonstrated that the dominant follicles of the first two waves were smaller than the ovulatory follicle of the third wave. Townson et al. (2002) reported that cattle with two follicle waves had larger (17.2 vs. 16.0 mm) and older (6.7 vs. 5.2 d) ovulatory follicles that were less fertile than cattle with three waves, respectively. Furthermore, differences in the length of the luteal phase between two and three wave estrous cycles were reported by Ginther et al. (1989) where luteal regression occurred on day 16 and 19, respectively. Also, the interval from emergence to ovulation was shorter in cows with three compared to two wave cycles, resulting in a shorter period of dominance for the third wave ovulatory follicle (Ginther et al., 1989).

The number of follicle waves within an estrous cycle has been shown to vary according to environmental conditions, nutritional management, and lactation status. Heat stress increased the

proportion of three wave follicular cycles (Wilson et al., 1998), resulted in earlier regression of the first wave dominant follicle followed by earlier recruitment of the second wave in two wave estrous cycles (Wolfenson et al., 1995), decreased the first wave dominant follicle diameter (Badinga et al., 1993), and resulted in earlier emergence of ovulatory follicle and a longer period of dominance (Wolfenson et al., 1995). Nutritional restriction reduced the growth rate and diameter of dominant follicles during an estrous cycle in beef heifers (Mackey et al., 1999) as well as decreased dominant follicle diameter and persistence of the first wave dominant follicle in Brahman heifers (Rhodes et al., 1995). In contrast, supplemented grazing *Bos indicus* × *Bos taurus* heifers had more large follicles than non-supplemented heifers (Maquivar et al., 2005) and feeding calcium salts of long chain fatty acids increased the diameter of the dominant follicle in multiparous Holstein cows (Lucy et al., 1991). Lactational status in dairy cows also effects follicle development, which appears to be driven by the level of nutrition as well as the resulting hormone profiles. Lactating dairy cows have decreased concentrations of glucose, IGF-1, and insulin, which is reflected in fewer class two (6-9 mm) and three (10-15 mm) follicles but more class four (> 15 mm) follicles that are less estrogenic compared to non-lactating dairy cows (De La Sota et al., 1993)

Characteristics of follicular growth are also different between *Bos taurus* and *Bos indicus* cattle. Early work by Segerson et al. (1984) before the advent of ultrasonography, reported more follicles < 5 mm in Brahman cows while Angus cows had more follicles > 5 mm in diameter. Recent work using ultrasonography during an entire estrous cycle revealed that the numbers of small (2-5 mm), medium (6-8 mm), and large (≥ 9 mm) follicles were greater in non-lactating Brahman (39.0, 5.0, and 1.6) compared to Angus (21, 2.3, and 0.9) cows, respectively (Alvarez et al., 2000). Alvarez et al. (2000) also observed that Angus cows had a greater FSH surge and

circulating plasma FSH concentrations compared to Brahman cows indicating that Brahman cows produce more follicles even though they have a smaller FSH surge and lower FSH concentrations. Alvarez et al. (2000) hypothesized that the greater follicle numbers may be due to higher concentrations of IGF-1 in Brahman cows. This finding is supported by Simpson et al. (1994), who reported that Brahman cows had greater circulating IGF-1 concentrations and IGFBP compared to Angus cows.

Alvarez et al. (2002) also indicated that Brahman cows had dominant follicles with a greater maximum diameter compared to Angus cows during the first (15.3 vs. 11.4 mm) and ovulatory (15.6 vs. 12.8 mm) follicle wave, respectively. Growth rate of the first wave dominant follicle tended to be greater in Brahman (1.6 mm/d) compared to Angus cows (1.2 mm/d), whereas growth rate was similar for the ovulatory dominant follicle between Brahman (1.4 vs. 1.4 mm/d) and Angus (1.4 mm/d). Aside from these differences, length of the estrous cycle (19.5 vs. 19.7 d), number of two follicular wave cycles (72.7 vs. 55.6%) and three follicular wave cycles (27.3 vs. 44.4%) was similar between Angus and Brangus cows, respectively (Alvarez et al., 2000). Viana et al. (2000) reported maximal diameters for first (11.8 mm) and ovulatory (12.4 mm) wave follicles in Gir (*Bos indicus*) cows, which were considerably less than the Brahman cows in the Alvarez et al. (2002) study. Other studies in *Bos indicus* cattle reported three follicular waves during the estrous cycle approximately 66.7% (Rhodes et al., 1995) and 60% (Viana et al., 2000) of the time as well as incidences of four follicle waves approximately 7 to 27% of the estrous cycles (Rhodes et al., 1995; Viana et al., 2000). Of interest, Figueiredo et al. (1997) reported that Nelore cows commonly have two follicle waves (83.3%), whereas Nelore heifers had a greater incidence of three follicle waves (64.7%).

Corpus Luteum (CL) Function and Luteolysis

After ovulation, the theca interna and granulosa cells of the ovulatory follicle undergo morphological and biochemical changes to become the CL. The main function of the CL is to synthesize and secrete progesterone, which is required for the maintenance of pregnancy and regulation of the estrous cycle. Corpora lutea are mainly comprised of two cell types, large and small luteal cells. Alila and Hansel (1984) reported that small luteal cells of the early developing CL were primarily from thecal origin, whereas large luteal cells were primarily granulosa in origin. The small luteal cells eventually develop into large luteal cells with age as the original large luteal cells disappear (Alila and Hansel, 1984). Small luteal cells are highly responsive to LH and secrete progesterone under the influence of low LH secretion; whereas, large luteal cells are less responsive to LH and secrete progesterone under high LH secretion and are subjected to the luteolytic effects of $PGF_{2\alpha}$ (Alila et al., 1988). Furthermore, large luteal cells secrete most of the progesterone (> 80%) but not under the influence of LH in cattle and sheep (Ursley and Leymarie, 1979; Fitz et al., 1982; respectively). Hoyer et al. (1984) observed that progesterone production in large luteal cells is independent of elevated intracellular cAMP levels, suggesting that large luteal cells are secreting progesterone at a maximal rate lending them unresponsive to further stimulation. Binding of LH to its receptor on small luteal cells results in the activation of the second messenger system. Upon activation of the second messenger adenyl cyclase, cyclic adenosine monophosphate (camp) is synthesized (Hoyer and Niswender, 1986), which activates protein kinase A and phosphorylate the enzymes necessary for steroidogenesis (Milvae et al., 1996).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is widely known as the primary luteolytic agent in many species, including cattle (Rowson et al., 1972; Inskeep, 1973; Nancarrow et al., 1973). Early

research demonstrated that hysterectomy of ewes and heifers resulted in maintenance of the CL (Wiltbank and Casida, 1956), suggesting that the luteolytic signal came from the uterus. Ligation of the uterine vein ipsilateral to the ovary with the CL resulted in maintenance of the CL for an extended period (Inskeep and Butcher, 1966). Hixon and Hansel (1974) further reported that $PGF_{2\alpha}$ acted on the ovaries through a countercurrent exchange between the uterine vein and ovarian artery. Upon reaching the ovary with the CL, $PGF_{2\alpha}$ initiates the rapid decline in progesterone secretion resulting in elevated LH concentrations leading to estrus and ovulation (Stellflug et al., 1977).

Further research reported that pulses of $PGF_{2\alpha}$ were observed throughout the estrous cycle without the initiation of luteolysis; however, during luteolysis, pulses of $PGF_{2\alpha}$ were more frequent in their release (Zarco et al., 1988). During a spontaneous luteolysis in the cow, pulses of $PGF_{2\alpha}$ secretion were observed along with pulses of oxytocin (Vighio and Liptrap, 1986), suggesting a positive feedback loop between $PGF_{2\alpha}$ and oxytocin (Milvae and Hansel, 1980; Schallenberger et al., 1984). LaFrance and Goff (1985) demonstrated that an injection of 100 IU of oxytocin had no significant effect on $PGF_{2\alpha}$ production as measured by its metabolite (PGFM) on days 3 and 6 of the estrous cycle but when oxytocin was administered on days 17 to 19 of the estrous cycle there were increased concentrations of PGFM. Treatment with progesterone followed by estradiol increased the numbers of endometrial oxytocin receptors (Vallet et al., 1990) and when oxytocin was administered to these animals, concentrations of PGFM increased. It was further demonstrated that during the late stages of the estrous cycle, progesterone down-regulates its own receptor in the uterine endometrium, reducing its action and stimulating the action of estradiol (Robinson et al., 2001). Coincident with the down regulation of uterine progesterone receptors, uterine oxytocin receptors are increased due to increasing estradiol

concentrations (Vallet et al., 1990). The importance of estradiol had been previously reported LaFrance and Goff, (1988) who demonstrated that PGFM concentrations following either a 14 or 21 d treatment with progesterone were significantly greater in heifers treated with estradiol followed by exogenous oxytocin compared to treatments with just oxytocin. Increases in estradiol concentrations increased the frequency of the pulse generator, driving the release of sub-luteolytic levels of $PGF_{2\alpha}$ from the uterus. Furthermore, $PGF_{2\alpha}$ secreted from the uterus acts on the CL to stimulate the release of luteal oxytocin, which amplifies the secretion of uterine $PGF2_{\alpha}$ to luteolytic levels (McCracken et al., 1999). The luteolytic levels of $PGF_{2\alpha}$ activate the $PGF_{2\alpha}$ receptor, located on both large and small luteal cells, and reduce progesterone concentrations (McCracken et al., 1999). Oxytocin binding to its receptor in the uterus activates the inositol 1, 3, 4-triphosphate second messenger system, resulting in the conversion of diacylglycerol to arachidonic acid (Flint et al., 1986) the precursor to $PGF_{2\alpha}$ synthesis and eventual release of $PGF_{2\alpha}$.

Luteolysis is defined as the structural demise of the CL associated with reduced synthesis and secretion of progesterone, followed by a loss in luteal cells (Niswender et al., 2000). The process of luteolysis can be attributed to a variety of actions of $PGF_{2\alpha}$ at the cellular level as well as changes in gene expression. There appears to be downregulation of receptors for luteotropic hormones, however decreases in LH receptors, determined by hCG binding capacity, are not observed until after a fall in progesterone (Spicer et al., 1981). Also, there are changes in the transport of cholesterol into the cell. Following treatment with $PGF_{2\alpha}$, there is a 50% decrease in steroidogenic acute regulatory protein (StAR; Pescador et al., 1996), the transporter of cholesterol across the mitochondrial membrane. Finally, the activity of steroidogenic enzymes, such as 3β-hydroxysteroid dehydrogenase (3β-HSD), required for progesterone synthesis is

decreased within within an hour of $PGF_{2\alpha}$ treatment (Hawkins et al., 1993). In response to $PGF_{2\alpha}$, changes in gene expression include the inhibition of the LH receptor, StAR, and 3β-HSD genes, whereas genes regulating luteal cell gene trancription, such as c-*fos* and prostaglandin G/H synthase-2 (PGHS-2), and genes involved in recruiting monocytes, macrophages, and monocyte chemoattractant protein-1 (MCP-1) are induced (Tsai et al., 2001). Furthermore, $PGF_{2\alpha}$ stimulates the luteal secretion of high amounts of the vasoconstrictive agent, endothelial cell vasoconstrictive peptide endothelin-1 (ET-1), which inhibits luteal progesterone production (Girsh et al., 1996). All the aforementioned changes precede changes in the cellular makeup of the CL. Braden et al. (1988) reported that by 36 hours after treatment with $PGF_{2\alpha}$ large luteal cell numbers remained the same but their diameter decreased; whereas, the number of small luteal cells decreased by 24 hours after treatment.

The luteolytic actions of exogenous $PGF_{2\alpha}$ on the CL can occur as early as day 5 and as late as day 16 of the estrous cycle. Henricks et al. (1974) reported that treatment with $PGF_{2\alpha}$ on days 3 and 4 of the estrous cycle had no effect on plasma progesterone concentrations. However, the unresponsiveness of the early bovine CL does not appear to be due to the lack of receptors for $PGF_{2\alpha}$ as $PGF_{2\alpha}$ receptors appear as early as 2 d after ovulation while receptor numbers and affinity remaned the same through day 10 after ovulation (Wiltbank et al., 1995). Therefore, the refractory period of the early developing CL to $PGF_{2\alpha}$ is not due to the lack of receptors. Tsai and Wiltbank (1998) reported that circulating $PGF_{2\alpha}$ reached the early developing CL to the same extent as the mid-cycle CL, but did not induce intraluteal $PGF_{2\alpha}$. This could be due to the observation that the early CL has a greater capacity to catabolize $PGF_{2\alpha}$ into PGFM due to increased enzymatic activity of 15-hydroxyprostaglandin dehydrogenase (PGDH; Silva et al., 2000). Inhibitors of the second messenger system for $PGF_{2\alpha}$ receptors are also increased during

the early luteal phase of the estrous cycle (Juengel et al., 1998). Conversely, Rao et al. (1979) reported that specific binding of $PGF_{2\alpha}$ to CL membrane increased from the early luteal phase (day 3 of estrous cycle) to the greatest levels observed during the late luteal phase (day 20 of estrous cycle), a time where the CL was actively regressing. Moreover, the authors reported an increased number of $PGF_{2\alpha}$ receptors during the mid luteal phase (day 13 of estrous cycle) but the affinity of $PGF_{2\alpha}$ to its receptor was 203 times less than during the late luteal phase. Additionally, Sakamoto et al. (1995) noted that mRNA for $PGF_{2\alpha}$ receptors increased from the early luteal phase (days 3-5 of estrous cycle) to the late luteal phase (days 15-18 of estrous cycle) and was reduced for the regressed CL. Following the initiation of luteolysis, intra-luteal progesterone secretion began decreasing immediately while intra-luteal $PGF_{2\alpha}$ slightly increased and dramatically increased from 24 hr to 300% (Shirasuna et al., 2004).

Characteristics of luteal development and function appear to be different between *Bos taurus* and *Bos indicus* cattle but the data is conflicting. In general *Bos indicus* cattle have smaller CL than *Bos taurus* cattle regardless of whether studies included removal of ovaries (Irvin et al., 1978; Segerson et al., 1984) or evaluation via ultrasonography (Rhodes et al., 1995). Although Alvarez et al. (2000) reported larger CL sizes in *Bos indicus* compared to *Bos taurus* cows as determined by ultrasonography. Similarly, there are differences in progesterone production but these data are also conflicting. Segerson et al. (1984) reported that luteal progesterone content and serum progesterone concentrations were greater in *Bos taurus* compared to *Bos indicus* cows while Adeyemo and Heath (1980) observed that *Bos taurus* cows had greater concentrations of progesterone throughout the estrous cycle compared to *Bos indicus* cows. In contrast, Irvin et al. (1978) reported no differences in luteal content or concentrations of progesterone between *Bos taurus* and *Bos indicus* cattle. Likewise, Alvarez et al. (2000)

reported that *Bos indicus* cows had similar progesterone concentrations compared to *Bos taurus* cows even though the *Bos indicus* cows had greater CL sizes. Alvarez et al. (2000) suggested that the reason for increased luteal growth in *Bos indicus* cattle may be a result of increased concentrations of growth hormone or IGF-1.

Although not well documented, there appears to be differences in the luteolytic response to $PGF_{2\alpha}$ between *Bos taurus* and *Bos indicus* cattle. A single study in *Bos indicus* (Cornwell et al., 1985) heifers suggests a decreased response to $PGF_{2\alpha}$ during the early luteal phase compared to early luteal phase in *Bos taurus* (Tanabe and Hann, 1984) heifers. In Brahman heifers that did not undergo luteolysis and exhibit estrus, progesterone concentrations initially declined by 12 hr after $PGF_{2\alpha}$ but progesterone concentrations began to increase within 48 hr after $PGF_{2\alpha}$ treatment (Cornwell et al., 1985). Santos et al. (1988) reported an increased estrous response following two consecutive 12.5 or 25 mg $PGF_{2\alpha}$ treatments administered 24 hr apart in Brahman heifers and Brangus cows. Furthermore, Bridges et al. (2005) noted that the percentage of heifers undergoing luteolysis was increased in yearling *Bos indicus* × *Bos taurus* heifers following two consecutive 12.5 mg $PGF_{2\alpha}$ treatments compared to a single 25 mg $PGF_{2\alpha}$ treatments. In the same report, luteolysis was similar between yearling *Bos taurus* and 2 yr-old *Bos indicus* × *Bos taurus* heifers that received either two consecutive 12.5 mg $PGF_{2\alpha}$ treatments or a single 25 mg $PGF_{2\alpha}$ treatments. Therefore, the rate of a $PGF_{2\alpha}$ induced luteolysis appears to be different between *Bos indicus* and *Bos taurus* cattle, and there may well be an effect of age on luteolytic response in *Bos indicus* cattle.

Bovine Estrous Cycle

Estrous cycles in cattle start with the expression of estrus followed by ovulation, growth and development of luteal tissues and follicles, luteolysis, and eventually the onset of estrus again. Associated with this sequence of events are coordinated exchanges in hormonal and

ovarian events. There are two distinct phases that comprise the estrous cycle: the follicular phase and the luteal phase. The follicular phase is the period from luteolysis through ovulation and is further divided into proestrus and estrus. The luteal phase is the period from ovulation to luteolysis and is comprised of metestrus and diestrus.

The length of the estrous cycle length is approximately 20 to 22 d in *Bos taurus* (Sirois and Fortune, 1988; Ginther et al., 1989) and *Bos indicus* (Rhodes et al., 1995; Figueiredo et al., 1997) cattle. Likewise, Alvarez et al. (2000) observed similar estrous cycle lengths between Angus (19.5 d) and Brahman (19.7 d) cows. In stark contrast, Plasse et al. (1970) reported a mean estrous cycle lengths of 28 d in two-year-old Brahman heifers. Numerous studies have demonstrated that estrous cycle length is dictated by the number of follicle waves during the cycle. In cattle with two wave follicle development patterns, estrous cycle length was similar between Nelore cows and heifers (20.7 d; Figueiredo et al., 1997) compared to Holstein heifers (20.4 d; Ginther et al., 1989), which were significantly less than Nelore cows and heifers (22.0 d) and Holstein heifers (22.8 d) with three wave follicle growth patterns. In contrast, Savio et al. (1988) reported similar estrous cycle lengths between *Bos taurus* beef heifers exhibiting either two- (20.5 d) or three- (21.3 d) wave follicle development patterns.

The beginning of the estrous cycle is marked by estrus, where progesterone concentrations are low (0.33 ng/mL) and estradiol concentrations are increasing, which leads to the LH surge and ovulation (Wettemann et al., 1972). Interval from peak estradiol concentration to the preovulatory surge of LH is approximately 6 to 8 h (Walters et al., 1984; Cavalieri et al., 1997). High estradiol concentrations lead to behavioral changes that are characterized by homosexual activity of females in estrus. The interval from estrus to ovulation has been shown to be

approximately 28-32 hr in *Bos taurus* (Walker et al., 1996; Wettemann et al., 1998) and 26 hr in *Bos indicus* cattle (Lamothe-Zavaleta et al., 1991; Pinheiro et al., 1998).

Cattle of *Bos indicus* breeding are more difficult to detect in estrus (Galina et al., 1994) and exhibit more covert signs of estrus such as head butting and smelling of genitalia (Galina et al., 1982; Lamothe-Zavaleta et al., 1991). *Bos indicus* cattle also have an increased incidence of silent estrus (Plasse et al., 1970; Dawuda et al., 1989), which is one of the reasons why estrus is difficult to detect. The recent advent of radiotelemetric heat detection aids has also provided an insight into characteristics of behavioral estrus of cattle. Radiotelemetric heat detection significantly aids in the efficiency of estrous detection compared to visual observation (Stevenson et al., 1996), it provides a detailed record of the initiation of estrus (night vs. day), end of estrus, duration of estrus, and the intensity of estrus based on the number of mounts received. Several authors have a slightly greater percentage of *Bos indicus* cattle in estrus during the night time hours (Pinheiro et al., 1998; Landaeta-Hernandez et al., 2002) compared to *Bos taurus* cattle. Therefore, a greater number of *Bos indicus* cattle exhibiting estrus during the night time hours may impede the effectiveness of visual estrus detection methods and result in fewer animals being detected in estrus.

The duration of estrus has also been reported to be effected by breed. *Bos indicus* cattle have a shorter duration of estrus (Rhodes and Randel, 1978; Lamothe-Zavaleta et al., 1991; Rae et al., 1999) than *Bos taurus* cattle and this appears to be influenced as to whether it is a synchronized estrus or a spontaneous estrus (Landaeta-Hernandez et al., 2002). For a synchronized estrus, the duration of estrus has been reported to be 12 hr in *Bos taurus* heifers (Richardson et al., 2002) and 6-7 hr in *Bos indicus* heifers (Rae et al., 1999). Landaeta-Hernandez et al. (2002) also reported a similar duration of a synchronized estrus between Angus

(19 h) and Brahman (17 h) cows. However, the duration of a subsequent spontaneous estrus was greater for Angus (11 h) compared to Brahman cows (6 h).

The duration of estrus also appears to be correlated with the number of mounts received during estrus as animals with low mounting activity have shorter durations of estrus (Rae et al., 1999). Reports on mounts received during estrus are conflicting between *Bos indicus* and *Bos taurus* cattle. Galina et al. (1982) reported that *Bos indicus* crossbred cows (1.6 mounts/hr) received fewer mounts compared to *Bos taurus* cows (2.8 mounts/hr). Rae et al. (1999) reported that Brahman (25 mounts) heifers received more total mounts compared to Angus (19 mounts) while Brahman x Angus (37 mounts) heifers received more mounts compared to the Angus and Brahman heifers. It should be noted that in the Rae et al. (1999) study the heifers were managed in a single synchronized group and the heifers were not separated by breed. Landaeta-Hernandez et al. (2002) reported a similar number of mounts for Angus (30 mounts) and Brahman (33 mounts) cows during a synchronized estrus but a greater number of mounts for Angus (11 mounts) than Brahman (7 mounts) cows during a spontaneous estrus when the cows were managed in the same pasture. Therefore, the increased number of mounts observed during a synchronized estrus is probably due to an increased number of animals in estrus at a given time, resulting in more mounts and a longer duration. In summary, both the duration of estrus and the number of mounts received during estrus are greater during a synchronized estrus compared to a spontaneous estrus, which supports the conclusion AI programs in *Bos indicus* influenced cattle should be focused around a synchronized estrus.

Environmental effects have also been reported to play a role in the duration and intensity of estrus. Landaeta-Hernandez et al. (2002) reported that the duration of estrus and number of mounts were reduced when the temperature-humidity index was increased. Also, Lamothe-

Zavaleta et al. (1991) reported that the duration of estrus was shorter when temperatures were above 27°C. Furthermore, Plasse et al. (1970) reported an increased incidence of ovulation without estrus in 2-year-old Brahman heifers during the winter months. In addition to environmental effects, social hierarchy can influence the duration and intensity of estrus between *Bos indicus* and *Bos taurus* cattle. Dominant Brahman cows took longer to exhibit estrus compared to dominant Angus cows (Landaeta-Hernandez et al., 2002). However, subordinate Angus cows had a longer interval from $PGF_{2\alpha}$ treatment to the onset of estrus than subordinate Brahman cows (Landaeta-Hernandez et al., 2002). The Landaeta-Hernandez et al. (2002) report suggests that social dominance could play a major role in the expression of behavioral estrus.

Following estrus is the period known as metestrus, which is the period from the end of estrus (day 0.5) to the formation of a functional CL (day 3 to 5). During metestrus, progesterone secretion is low and increases slowly until complete formation of the CL, which marks the end of metestrus and the beginning of diestrus. Diestrus lasts 10 to 14 d and is characterized by high circulating concentrations of progesterone, which suppress the actions of estradiol by preventing any preovulatory surge of LH. Harms et al. (1969) reported that from day 2 to 9 of the estrous cycle, progesterone concentrations increased from 2.8 to 14.1ng/mL in *Bos taurus* heifers. Additionally, Alvarez et al. (2000) noted similar maximal progesterone concentrations for Angus (4.3 ng/mL) and Brahman (4.4 ng/mL) cows. Henricks et al. (1971) observed that peak progesterone concentrations ranged from 5 to 12 ng/mL in *Bos taurus* cattle, while Ruiz-Cortez and Olivera-Angel (1999) reported that peak progesterone concentrations ranged from 1 to 8 ng/mL in *Bos indicus* cattle.

The initiation of luteolysis (day 16 to 19) marks the beginning of proestrus. During proestrus, circulating progesterone concentrations decline while estradiol increases coincident

with dominant follicle development (Henricks et al., 1971). Increasing estradiol concentrations lead to the onset of estrus.

Estrous Synchronization through Manipulation of the Estrous Cycle

Synchronization of the estrous cycle is a management tool that allows for beef and dairy producers to increase the opportunity for success of an artificial insemination (AI) program. Benefits of estrous synchronization include an increased percentage of cattle pregnant early in the breeding season, shortened AI breeding season, shortened calving season, and increased calf crop uniformity. Labor expenses can also be reduced through estrous synchronization by decreasing the time and labor required for estrous detection and breeding as well during the subsequent calving periods. Estrous synchronization can be achieved through the use of several exogenous hormones including prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to shorten the luteal phase; progestins to prevent estrus and ovulation; and gonadotropin releasing hormone (GnRH) to synchronize follicle wave development or to ovulate the dominant follicle in conjunction with AI. These hormones can be combined for control of follicle dynamics, initiate luteal regression, synchronize estrus, and (or) implementation of a timed AI program, eliminating the need for estrus detection.

Progestogens

As mentioned previously, progesterone secreted form the CL acts to prevent the preovulatory surge of LH and expression of estrus during the estrous cycle. Consequently, exogenously administered progestogens such as melengestrol acetate (MGA), norgestomet (Syncro-mate B; SMB), controlled intravaginal progesterone releasing device (CIDR), and injectable progesterone have been used to mimick the actions of progesterone throughout the duration of their administration.

Melengestrol acetate is an orally active progestogen that is used in feedlot heifers to prevent the expression of estrus and as a synchronization agent in yearling beef heifers. Early research utilizing MGA demonstrated that fertility at the subsequent estrus after MGA withdrawal was reduced with both short (< 9 d; Beal et al., 1988) and long term (> 9 d; Hill et al., 1971) MGA treatments. Although, the reduction in fertility was temporary as fertility returned to normal at the subsequent estrus. Hill et al. (1971) suggested that the reduction in fertility was due to several factors including altered cervical mucus, ovulation of abnormal ova, and (or) fertilization failure. In addition, few normal follicles, some hyperplastic follicles, atretic follicles, and atretic follicles with thickened theca interna were observed on the ovaries of MGA treated heifers lacking a functional CL during MGA treatment (Lamond et al., 1971). More recent research demonstrated that fertility decreased in cows and heifers without the presence of a CL during progestin treatment compared to cows with a CL present (Sanchez et al., 1993).

Subsequent research demonstrated that low level progestogen exposure, supplied by a CIDR, in the absence of a functional CL produced an endocrine environment that permitted the dominant follicle to persist on the ovary while suppressing the development of new dominant follicles (Sirois and Fortune, 1990). Sirois and Fortune (1990) concluded that in the absence of endogenous progesterone from the CL, low levels of progesterone supplied by the CIDR resulted in increased LH pulse frequency, which resulted in enhanced follicle development. However, when two CIDR's were administered to mimic normal luteal phase progesterone concentrations resulting in decreased LH pulse frequency and dominant follicle turnover. The type of progestogen administered as well as the presence of a function CL can also dictate whether there is follicle turnover. Custer et al. (1994) treated cows with a progesterone-releasing intravaginal device (PRID) resulting in regression of the dominant follicle present at the initiation of

treatment and recruitment of a new follicle wave. In contrast, dominant follicle turnover was not observed in cows treated with MGA in the absence of luteal function, which was a result of increased LH pulse frequency and development of large single non-ovulatory follicle. Kojima et al. (1992) treated cows with either a CIDR, MGA, or norgestomet in the absence of a functional CL resulting in high frequency low amplitude pulses of LH, which sustained dominant follicle growth and increased circulating concentrations of estradiol. The large follicles that develop under low endogenous progesterone exposure are larger than normal ovulatory follicles and produce greater concentrations of estradiol (Savio et al., 1993) and are commonly referred to as persistent dominant follicles.

The decreased fertility observed with development of persistent dominant follicles is mediated primarily at the level of the follicle. Mihm et al. (1994) demonstrated that as dominance of follicles is greater than 4 d, fertility is increasingly reduced. Ahmad et al. (1995) observed that ovulation of persistent dominant follicles resulted in fewer embryos reaching the 16-cell stage and fewer total embryos collected. Moreover, Revah and Butler (1996) determined that oocytes ovulated from persistent dominant follicles underwent premature maturation in vivo. In addition to altered follicle development, the increased estrogen production of the persistent dominant follicle results in an altered hormonal milieu. The altered hormonal environment alter the synthesis and secretion of oviductal proteins, which create a less than optimal ovarian microenvironment leading to decreased pregnancy rates by altering oviductal function, fertilization, and early embryonic development (Binelli et al., 1999). Therefore, disparities in embryo development and ovarian microenvironment collectively add to the reduction in fertility during long-term low-level progestogen exposure. Conversely, Wehrman et al. (1997) reported similar pregnancy rates following embryo transfer in cattle ovulating either persistent dominant

follicles or normal follicles. Furthermore, progesterone concentrations 7 and 12 d after ovulation were similar between cattle ovulating a persistent dominant follicle and those ovulating a normal follicle (Mihm et al., 1994). Therefore, oocytes ovulated from a persistent dominant follicle lead to decreased fertility while no detrimental effects on CL function are observed following ovulation of a persistent dominant follicle compared to a normal ovulation.

Persistent dominant follicles developed during long term MGA treatments can be regressed either through treatment with exogenous estrogen (Yelich et al., 1997) or progesterone (Anderson and Day, 1994; Garcia et al., 2004) during the MGA treatment. The subsequent follicle ovulated after MGA withdrawal has normal fertility.

Prostaglandin $F_{2\alpha}$

As previously mentioned, $PGF_{2\alpha}$ is the luteolytic hormone in beef cattle. The luteolytic actions of exogenous $PGF_{2\alpha}$ on the CL are effective from day 5 to 16 of the estrous cycle (Rowson et al., 1972; Inskeep, 1973; Kiracofe et al., 1985). As a result administration of exogenous $PGF_{2\alpha}$ such as alfaprostal, chloprostenol, dinaprost tromethamine, and luprostial have been used to synchronize estrus in beef and dairy cattle. In a review of early studies by Inskeep (1973), both CL size and progesterone concentrations were reduced within 24 hr following treatment with exogenous $PGF_{2\alpha}$. Subsequent research reported that progesterone concentrations were reduced to < 0.5 ng/mL within 24 hr after a 30 mg injection of $PGF_{2\alpha}$ resulting in increased estradiol concentrations (Chenault et al., 1976) and the subsequent expression of estrus. The estrous response in cattle of *Bos taurus* breeding undergoing normal estrous cycles following a single administration of $PGF_{2\alpha}$ ranges from 65 to 91% (Lauderdale et al., 1974; Tanabe and Hann, 1984) with estrus being observed within 2 to 7 d. In contrasts, the estrous response in cycling cattle of *Bos indicus* breeding ranges from 56 to 62% in Zebu cattle (Orihuela et al., 1983) and 46.3 to 54.8% in Nelore cattle (Landivar et al., 1985). The variability in estrous

response and interval to the onset of estrus is due to several factors including estrous cycling status, stage of the estrous cycle at $PGF_{2\alpha}$ administration, and breed.

The actions of $PGF_{2\alpha}$ are only seen in cattle that are undergoing normal estrous cycles with a CL present on the ovary. In estrous cycling cattle, estrous response and interval from $PGF_{2\alpha}$ to the onset of estrus are affected by the stage of estrous cycle at $PGF_{2\alpha}$ administration (Tanabe and Hann, 1984; Watts and Fuquay, 1985). Tanabe and Hann (1984) treated Holstein heifers with $PGF_{2\alpha}$ during the early (d 7), mid (d 11), and late (d 15) luteal phase of the estrous cycle and reported a higher percentage of heifers exhibiting estrus within an 80 hr period for each advancing stage of the estrous cycle (86.0, 90.0, and 98.0%; respectively). Of heifers that exhibited estrus, 100.0% of early, 95.9% of late luteal phase, and only 48.9% of mid luteal phase heifers exhibited estrus within 72 hr following $PGF_{2\alpha}$ treatment. These findings are in agreement with Watts and Fuquay (1985) who treated heifers with $PGF_{2\alpha}$ during the early (days 5-7), mid (days 8-11), and late (days 12-15) luteal phase and observed 72 hr estrous response and interval from $PGF_{2\alpha}$ to the onset of estrus of 43.0% and 59 hr, 83.6% and 70 hr, and 78.3% and 72 hr, respectively. Macmillan and Henderson (1984) also reported a similar trend with over 70% of cows treated with $PGF_{2\alpha}$ on day 7 (early diestrous) and 16 (late diestrous) in estrus within 48-72 hr following $PGF_{2\alpha}$ treatment but only 30% of cows treated on days 11 or 12 (mid diestrous) in estrus within 48-72 hr following treatment.

Sirois and Fortune (1988) reported a negative correlation between size of the preovulatory follicle at luteolysis and the interval to ovulation. Furthermore, Kastelic et al. (1990) observed that the interval from $PGF_{2\alpha}$ to the onset of estrus was shorter for heifers administered $PGF_{2\alpha}$ on day 5 compared to day 12 of the estrous cycle. Consequently, follicle wave development at the time of a $PGF_{2\alpha}$ treatment affects the synchrony of the subsequent estrus. With this in mind, it

points out the importance of synchronizing follicle development in conjunction with either luteal regression or removal of a progestogen.

A $PGF_{2\alpha}$ induced luteolysis does not appear to have any negative effects on fertility of the subsequent estrus. Macmillan and Day (1982) reported pregnancy rates of 69% in $PGF_{2\alpha}$ treated cows compared to 60% for non-treated cows with over 2,000 animals in each group. Lauderdale et al. (1974) reported similar pregnancy rates between cows receiving $PGF_{2\alpha}$ compared to those not receiving $PGF_{2\alpha}$. Moreover, Tanabe and Hann (1984) reported similar pregnancy rates in $PGF_{2\alpha}$ treated (77.4%) and non-treated heifers (76.0%). Additionally, Hardin et al. (1980) noted that pregnancy rates were similar between $PGF_{2\alpha}$ treated (30 and 37%) and non- $PGF_{2\alpha}$ treated (37 and 38%) *Bos indicus* cattle. However, stage of the estrous cycle when cattle receive $PGF_{2\alpha}$ may affect fertility. Pregnancy rates for heifers were lowest (56.8%) for heifers receiving $PGF_{2\alpha}$ early (d 5-7) in the estrous cycle compared to heifers receiving $PGF_{2\alpha}$ in the middle (d 8-11; 62.1%) or late (d 12-15; 78.3%) stages of the estrous cycle (Watts and Fuquay, 1985).

There is a limited amount of research that suggests that a $PGF_{2\alpha}$ induced luteolysis maybe compromised in cattle of *Bos indicus* breeding. Hansen et al. (1987) reported that Brahman heifers required a greater dose of $PGF_{2\alpha}$ to achieve luteolysis compared to Brahman cows. Furthermore, the crossbred Brahman heifers required a greater dose of $PGF_{2\alpha}$ to achieve luteolysis from days 8 to10 of the estrous cycle compared to days 11 to 13 of the estrous cycle. Pinheiro et al. (1998) reported that 48 % (12/25) of Nelore cows with a functional CL, failed to respond to a luteolytic dose of $PGF_{2\alpha}$ between day 6 to 8 of the estrous cycle. Furthermore, the authors hypothesized that administration of $PGF_{2\alpha}$ from days 5 to 9 of the estrous cycle may cause partial luteolysis followed by recovery of luteal activity. Cornwell et al. (1985) reported that Brahman heifers treated with $PGF_{2\alpha}$ on day 7 and 10 of the estrous cycle had estrous

responses of 50 and 67%, respectively; whereas, 100% of the heifers exhibited estrus when treated with $PGF_{2\alpha}$ on days 14 and 18 of the estrous cycle. For heifers not responding to $PGF_{2\alpha}$ on days 7 and 10, progesterone concentrations decreased by 12 hr after $PGF_{2\alpha}$ followed by increased concentrations of progesterone within 48 hr of $PGF_{2\alpha}$ treatment. Therefore, the early developing CL (day 5-10 of the estrous cycle) appears to be somewhat refractory to the actions of a single treatment of $PGF_{2\alpha}$ in cattle on *Bos indicus* breeding.

Several researchers have addressed the issue of incomplete luteolysis in females of *Bos indicus* breeding by changing the administration of $PGF_{2\alpha}$ from a single to two consecutive doses of $PGF_{2\alpha}$. Cornwell et al. (1985) administered $PGF_{2\alpha}$ to Brahman heifers on day 7 and 8 of the estrous cycle and reported a significant increase in estrous response (97%) compared to a single injection (69%). When a single $PGF_{2\alpha}$ treatment was administered during the early luteal phase (day 7 and 8) of the estrous cycle, Santos et al. (1988) reported a similar estrous response and interval from $PGF_{2\alpha}$ to the onset of estrus when either 12.5 mg (84%; 94 hr) or 25 mg (83.1%; 100 hr), respectively. Santos et al. (1988) conducted an additional study to test the effectiveness of either two consecutive 12.5 mg or 25 mg $PGF_{2\alpha}$ injections compared to a single 25 mg injection of $PGF_{2\alpha}$ in Brangus (*Bos taurus* × *Bos indicus*) cows, which had previously received a single 25 mg injection of $PGF_{2\alpha}$ 11 d earlier. Estrous response and conception rates were greater for cows receiving two 12.5 mg (82; 73%) and two 25 mg (73; 59%) injections of $PGF_{2\alpha}$ compared to a single 25 mg injection (55; 32%), respectively. The interval from the initial PGF treatment to the onset of estrus was significantly shorter for groups receiving the two consecutive 12.5 mg (79.0 hr) and 25 mg (73.2 hr) injections compared to a single 25 mg (93.9 hr) injection. A recent study by Bridges et al. (2005) reported increased luteolysis when two consecutive 12.5 mg $PGF_{2\alpha}$ treatments (92.5%) were administered 24 hr apart compared to a single 25 mg $PGF_{2\alpha}$

treatment (79.1%) in *Bos indicus* × *Bos taurus* heifers during the mid and late stages of the estrous cycle. Conversely, no increase in luteolysis was observed in yearling Angus (*Bos taurus*) or 2-year-old *Bos indicus* × *Bos taurus* heifers receiving the same $PGF_{2\alpha}$ treatments. In summary, modifying the administration of $PGF_{2\alpha}$ from a single 25 mg treatment to two consecutive 12.5 mg treatments 24 hr apart appears to increase estrous responses by increasing the rate of luteolysis in yearling heifers of *Bos indicus* breeding.

Melengestrol acetate + $PGF_{2\alpha}$

As early as the 1960's, oral progestogens were used for controlling estrous cycles in cattle (Hansel et al., 1961; Zimbelman, 1963; Wiltbank et al., 1967). An early study by Zimbelman and Smith (1966) reported that the optimal dosage of MGA needed to prevent estrus and ovulation was 0.5 mg/hd/d. However, the negative side of using MGA was the development of a persistent dominant follicle (Sirois and Fortune, 1990; Savio et al., 1993) that resulted in reduced fertility (Hill et al., 1971; Ahmad et al., 1995) at the estrus after MGA withdrawal due to improper embryo development (Ahmad et al., 1995). Furthermore, administration of MGA at levels (1.0 and 1.5 ng/mL) above the optimal level to inhibit the expression of estrus (0.5 mg/hd/d) did not provide a progesterone environment similar to mid-luteal progesterone concentrations that would regulate the pulsatile release of LH and stimulate follicle turnover (Kojima et al., 1995). Administering more MGA did result in a greater interval from MGA to the onset of estrus (Zimbelman and Smith, 1966; Hill et al., 1971). The mean interval to estrus was decreased in heifers treated with 0.2 mg/hd/d (2.7 d) compared to heifers treated with 2.0 mg/hd/d (6.3 d) (Zimbelman and Smith, 1966).

Brown et al. (1988) developed a system utilizing MGA and $PGF_{2\alpha}$ that was designed to circumvent the reduction in fertility following long term (14 d) MGA treatment. The MGA was administered for 14 d (0.5 mg/hd/d) and heifers were allowed to exhibit estrus but not

inseminated at the estrus after MGA withdrawal. Seventeen days after the termination of MGA, which placed heifers in the late luteal phase of their estrous cycle, heifers received 25 mg of $PGF_{2\alpha}$ (MGA + $PGF_{2\alpha}$). The MGA + $PGF_{2\alpha}$ system was compared to Syncro-mate B (SMB), which consisted of a 9 d norgestomet implant with an estradiol valerate injection concurrent with implant insertion. Estrous response was similar between the MGA + $PGF_{2\alpha}$ (83.4%) and SMB (90.2%) treatments but conception (68.7 vs. 40.6%) and synchronized pregnancy rates (57.3 vs. 36.6%) were greater for the MGA + $PGF_{2\alpha}$ compared to SMB heifers, respectively. Additionally, estrous response and synchronized pregnancy rate were significantly greater for cycling (91.6 and 68.4%) than non-cycling (71.0 and 40.3%) MGA + $PGF_{2\alpha}$ treated heifers, but were similar in cycling (92.4 and 44.6%) and non-cycling (85.2 and 26.2%) SMB treated heifers. Patterson and Corah (1992) observed a greater 6 d estrous response (79.0 vs. 32.0%), similar conception rates (64.0 vs. 67.0%), and increased synchronized pregnancy rates (50.0 vs. 21.0%) for MGA + $PGF_{2\alpha}$ treatment compared to untreated controls, respectively. Similarly, Jaeger et al. (1992) reported an increased 6 d estrous response (77.0 vs. 25.0%), similar conception rates (64.0 vs. 50.0%), and increased synchronized pregnancy rates (48.7 vs. 14.0%) for the MGA + $PGF_{2\alpha}$ treatment compared to the untreated controls, respectively. Numerous follow-up studies testing the efficacy of the MGA + $PGF_{2\alpha}$ system compared to untreated control heifers have also been conducted and are summarized in Table 2-1.

Other investigators have varied the original MGA + $PGF_{2\alpha}$ system by increasing the interval from MGA withdrawal to $PGF_{2\alpha}$ administration from 17 to 19 d (Nix et al., 1998; Deutscher et al., 2000; Lamb et al., 2000). In a large field study conducted by Lamb et al. (2000), estrous response was similar for heifers treated with $PGF_{2\alpha}$ either 17 (68.3%) or 19 (68.1%) d following MGA withdrawal. However, the synchrony of estrus was improved with

the 19 d treatment as 99% of the 19 d heifers exhibited estrus within 72 hr of $PGF_{2\alpha}$ compared to 74% of 17 d heifers. Moreover, the interval to estrus was reduced for heifers treated 19 d (56.2 hr) compared to 17 d (73.1 hr) after MGA withdrawal. Conception and pregnancy rates were similar for heifers treated 17 (75.9; 51.8%) or 19 d (81.4; 55.4%) following MGA withdrawal, respectively. Lamb et al. (2000) hypothesized that by increasing the interval from MGA withdrawal to $PGF_{2\alpha}$ from 17 to 19 d resulted in a more mature preovulatory follicle at $PGF_{2\alpha}$, which resulted in an earlier expression of estrus.

The MGA + $PGF_{2\alpha}$ system has also been used in a timed AI system with or without GnRH. In two experiments, Larson et al. (1996) subjected heifers to the MGA + $PGF_{2\alpha}$ (17 d) system and compared two different AI protocols including estrous detection and AI for 72 hr following $PGF_{2\alpha}$ to a single fixed-time AI 72 hr following $PGF_{2\alpha}$ for all heifers. In the first experiment, pregnancy rates were similar for heifers bred to an observed estrus (31.0%) compared to fixed time AI 72 hr after $PGF_{2\alpha}$ treatment (36.4%). A second experiment was performed to determine the effects of estrous detection and AI for 72 hr combined with time AI at 72 hr following $PGF_{2\alpha}$ for heifers not exhibiting estrus. Results from the second experiment suggest that combining estrus detection and timed AI yield increased pregnancy rate (48.4%) where heifers not expressing estrus by the third day after $PGF_{2\alpha}$ reduced estrous detection without a reduction in fertility. Therefore, combining estrous detection with fixed time AI subjects all heifers to AI and the opportunity to become pregnant during the synchronized period.

A recent study by Salverson et al. (2002) evaluated the effectiveness of type of $PGF_{2\alpha}$ (cloprostenol vs. dinoprost tromethamine) in the MGA + $PGF_{2\alpha}$ (19 d) system and observed similar estrous (89 vs. 86%), conception (67 vs. 67%) and pregnancy (61 vs. 57%) rates between the two types of $PGF_{2\alpha}$. Another refinement to the MGA + $PGF_{2\alpha}$ system included

administration of GnRH 12 d following MGA withdrawal followed 7 d later with $PGF_{2\alpha}$ (Wood et al., 2001). The purpose of the GnRH was to ovulate or luteinize most large follicles so as to synchronize follicle development when $PGF_{2\alpha}$ was administered with the theory that the subsequent estrus could be more tightly synchronized and allow for a timed-AI. Estrous response from 48 to 60 hr following $PGF_{2\alpha}$ was significantly increased in GnRH treated (71.0%) compared to non-GnRH treated (35.0%) heifers, while 7 d estrous response (100.0 vs. 94.0 %) and the interval from $PGF_{2\alpha}$ to estrus (67.0 vs. 71.0 hr) was similar between treatments, respectively. However, fertility was not evaluated in this study. DeJarnette et al. (2004) conducted a study where heifers were treated with either the protocol described by Wood et al. (2001; MGA + G + $PGF_{2\alpha}$) or a short term MGA (STMGA) protocol where heifers were administered MGA for six days, with GnRH the day before MGA and $PGF_{2\alpha}$ the day after the last day of MGA. In both treatments, estrus detection and AI were performed for 72 hr, at which time all heifers not detected in estrus were timed-AI and received GnRH. DeJarnette et al. (2004) reported increased synchronized pregnancy rates for MGA + G + $PGF_{2\alpha}$ (65%; 55/85) compared to STMGA (46%; 40/87) treated heifers. Therefore, it appears that fertility is not compromised following the MGA + G + $PGF_{2\alpha}$ system.

Melengestrol acetate has also been implicated in the induction of cyclicity in prepubertal heifers. Patterson et al. (1990) noted that 71% of prepubertal *Bos taurus* and 41% of prepubertal *Bos taurus* × *Bos indicus* crossbred heifers had progesterone concentrations ≥ 1 ng/mL following a 7 d MGA treatment. Jaeger et al. (1992) reported that a significantly greater percentage (72.0%) of prepubertal heifers treated with a 14 d MGA treatment reached puberty prior to $PGF_{2\alpha}$ 19 d after MGA withdrawal compared to un-treated heifers in the same period (45.0%). Following a 14 d MGA feeding, Deutscher et al. (2000) observed a 15 to 20% increase in the

percentage of heifers cycling. Imwalle et al. (1998) suggests that in prepubertal heifers treated with MGA that an increased mean LH concentration and LH pulse frequency result in an increased diameter of the largest follicle during MGA treatment, which results in a follicle large enough to ovulate after MGA withdrawal resulting in the formation of a CL and initiation of estrous cycles.

Minimal research has been conducted on the effectiveness of the MGA + $PGF_{2\alpha}$ system for synchronization of estrus in yearling heifers of *Bos indicus* × *Bos taurus* breeding. Stevenson et al. (1996) synchronized *Bos indicus* × *Bos taurus* heifers with MGA (14 d) + $PGF_{2\alpha}$ (17 d) system where estrus was detected for 72 hr and all heifers that were not in estrus were timed-AI at 72 hr. The synchronized pregnancy rate was 42.9% (21/49).

Bridges et al. (2005) conducted a study in yearling *Bos indicus* × *Bos taurus* heifers using the MGA + $PGF_{2\alpha}$ system comparing the effectiveness of a single (25 mg) $PGF_{2\alpha}$ treatment 19 d after MGA withdrawal compared to two (12.5 mg) consecutive $PGF_{2\alpha}$ treatments 24 hr apart on days19 and 20 d following MGA withdrawal. Estrus was detected for 72 hr followed by timed-AI in conjunction with GnRH for heifers not exhibiting estrus by 72 hr. Conception rates (51.5 vs. 48.8%) were similar while estrus response (50.1 vs. 43.2%), TAI pregnancy rate (33.5 vs. 23.9%), and total pregnancy rate (42.5 vs. 34.5%) were significantly improved by modifying the delivery of $PGF_{2\alpha}$ to two consecutive split treatments compared to a single treatment, respectively. The increase total pregnancy rate was due to an increased rate of luteolysis in the two consecutive $PGF_{2\alpha}$ treatments compared to the single $PGF_{2\alpha}$ treatment. Therefore, the MGA + $PGF_{2\alpha}$ system in yearling *Bos indicus* × *Bos taurus* crossbred heifers can be improved by modifying the delivery of $PGF_{2\alpha}$ from a single to split $PGF_{2\alpha}$ treatment; however, the resulting

synchronized pregnancy rates of the MGA + $PGF_{2\alpha}$ system in *Bos indicus* × *Bos taurus* heifers are still considerably less than those observed in *Bos taurus* heifers.

There could be several reasons for the differences in response to the MGA + $PGF_{2\alpha}$ in yearling *Bos indicus* × *Bos taurus* heifers compared to *Bos taurus* heifers. Cattle of *Bos indicus* breeding have a shorter duration and less intense estrus (Rhodes and Randel, 1978; Rae et al., 1999; Landaeta-Hernandez et al., 2002), have an increased incidence of three and four follicle waves (Rhodes et al., 1995; Viana et al., 2000), and reach puberty at older ages (Plasse et al., 1968; Patterson et al., 1991). One area where research has been limited in cattle of *Bos indicus* breeding is evaluating what effect of follicle wave development has on the effectiveness of estrous synchronization treatments. An asynchrony in follicle development at $PGF_{2\alpha}$ can result in large variations in the interval to estrus, undermining the overall effectiveness of a synchronization system and prevent the use of a fixed timed-AI. One way that that the asynchrony of follicle development can be altered is by the administration of GnRH to synchronize follicle development. Therefore, incorporating GnRH during the period between MGA withdrawal and $PGF_{2\alpha}$ in the MGA- $PGF_{2\alpha}$ system in *Bos indicus* based heifers could be used to improve the synchrony of follicle development at $PGF_{2\alpha}$. By increasing the synchrony of follicle development at $PGF_{2\alpha}$, a greater number of heifers should have dominant follicles ready to ovulate, improve the synchrony of estrus, and allow for a fixed timed-AI. Utilizing a fixed-timed-AI would allow for the elimination of estrous detection and allow all animals an opportunity to be inseminated, particularly cattle that have a silent estrus or exhibit estrus more covertly. Therefore, synchronization systems need to be re-evaluated and tailored to better synchronize follicle development in *Bos indicus* based cattle.

Table 2-1. Summary of studies evaluating the melengestrol acetate (MGA) + PGF2α estrous synchronization system in yearling beef heifers.

Study	Breed-type	Day $PGF_{2\alpha}$ was administered after MGA (14 d) withdrawal	Days of estrus detection	Estrous response (%)	Synchronized Pregnancy rate (%)
Brown et al., 1988	*Bos taurus*	17	5	83.4	57.3
Jaeger et al., 1992	*Bos taurus*	17	6	71.4	54.3
Patterson and Corah 1992	*Bos taurus*	17	6	79.0	50.0
Nix et al., 1998	*Bos taurus*	17	5	64.2	55.4
	Bos taurus	19	5	75.1	51.8
Deutscher et al., 2000	*Bos taurus*	17	5	86.7	49.2
	Bos taurus	17	5	77.6	53.8
	Bos taurus	19	5	92.4	57.1
	Bos taurus	19	5	87.6	61.4
Lamb et al., 2000	*Bos taurus*	17	5	68.3	51.8
	Bos taurus	19	5	68.1	55.4
Funston et al., 2002	*Bos taurus*	17	5	77.0	47.0
Salverson et al., 2002	*Bos taurus*	19 (Estrumate)	5	89.0	61.0
	Bos taurus	19 (Lutalyse)	5	86.0	57.0
Stevenson et al., 1996	*Bos indicus* × *Bos taurus*	17	3 + 72 hr timed-AI	68.2	42.9
Bridges et al., 2005	*Bos indicus* × *Bos taurus*	19 (25 mg)	3 + 72 hr timed-AI/GnRH	43.2	34.5
	Bos indicus × *Bos taurus*	19 (12.5 mg) & 20 (12.5 mg)	3 + 72 hr timed-AI/GnRH	50.1	42.5

CHAPTER 3
EVALUATION OF FOLLICULAR DEVELOPMENT BETWEEN A 14 D MELENGESTROL ACETATE (MGA) TREATMENT WITH $PGF_{2\alpha}$ 19 D AFTER MGA WITHDRAWAL IN ANGUS AND BRANGUS HEIFERS

Introduction

Artificial insemination (AI) provides producers with the opportunity to use genetically superior AI sires and AI is routinely used in conjunction with estrous synchronization. One of the major limitations of an AI and estrous synchronization program is the amount of time and labor required to implement and carry out the program. Therefore, estrous synchronization programs need to be developed that require minimal animal handling and result in a high percentage of cattle in estrus during a 2 to 3 d period. One way to minimize animal handling is to use the orally active progestogen melengestrol acetate in a synchronization program.

Melengestrol acetate (MGA) administered for 14 d, coupled with prostaglandin $F_{2\alpha}$ (PG) 17 d after the last day of MGA is an effective estrous synchronization system (MGA-PG) that was developed in yearling *Bos taurus* heifers (Brown et al., 1988). The interval from MGA withdrawal to PG was increased from 17 to 19 d resulting in a shorter interval to peak estrus and more synchronous estrus (Lamb et al., 2000). The effectiveness of the MGA-PG system as measured by synchronized pregnancy rate is less in yearling *Bos indicus* × *Bos taurus* (Bridges et al., 2005) compared to *Bos taurus* heifers (Brown et al., 1988; (Lamb et al., 2000). Even though synchronized pregnancy rates in *Bos indicus* × *Bos taurus* heifers can be increased slightly by modifying the delivery of PG from a single PG treatment to two consecutive PG treatments 24 hours apart (Bridges et al., 2005), the synchronized pregnancy rates are still considerably less in *Bos indicus* × *Bos taurus* compared to *Bos taurus* heifers.

The reason(s) for the decreased effectiveness of the MGA-PG synchronization program in heifers of *Bos indicus* × *Bos taurus* breeding may be attributed to physiological differences

between *Bos indicus* × *Bos taurus* compared to *Bos taurus* heifers. *Bos indicus* cattle have a greater incidence of 3 and 4 follicle waves (Rhodes et al., 1995; Viana et al., 2000) during the estrous cycle and this could result in asynchronous follicle development when PG is administered in the MGA-PG program. Consequently, asynchronous follicle development could be one of the reasons why the estrous response and subsequent synchronized pregnancy rates after PG in the MGA-PG synchronization program are low in *Bos indicus* × *Bos taurus heifers.* Therefore, the objectives of this study were to evaluate follicular development from withdrawal of a 14 d MGA treatment until PG administration 19 d later in order to evaluate the possibility of administering GnRH to synchronize follicle development, and to evaluate follicle development and the subsequent estrous response after PG in yearling Angus (*Bos taurus*) and Brangus (*Bos indicus* × *Bos taurus*) heifers.

Materials and Methods

Yearling Angus (*Bos taurus*; n = 40) and Brangus (*Bos indicus* × *Bos taurus*; n = 26) heifers, at the University of Florida Santa Fe Beef Research Unit were used in the experiment, which was conducted from February to April of 2004. Average heifer age, BW, body condition score (BCS; Richards et al., 1986), and percentage of heifers having estrous cycles at the initiation of the experiment are summarized in Table 3-1. Blood samples were collected -16, -7, and 0 d before initiation of a 14 d melengestrol acetate (MGA) treatment to determine estrous cycling status. The start of the experiment was designated as day 0. Heifers were deemed to be having estrous cycles (**cycling**) if blood plasma progesterone concentrations were ≥ 1.5 ng/mL at two of the three blood samples while heifers were classified as not having estrous cycles (**non-cycling**) if progesterone concentrations were < 1.5 ng/mL at all three samples. A progesterone concentration of ≥ 1.5 ng/mL was chosen after a retrospective analysis revealed that several heifers had progesterone concentrations between 1.0 – 1.5 ng/mL in the absence of luteal tissue

as determined by ultrasonography on day 0 of the experiment. On day 0 of the experiment, Angus and Brangus heifers were distributed by breed, cycling status, and BW into two groups. One group included cycling Angus and Brangus heifers that were to have daily ultrasonography (**scan**) conducted from MGA withdrawal until 5 d after PG and the other group included the remaining Angus and Brangus heifers (cycling and non-cycling) that would have no daily ultrasonography conducted (**non-scan;** Table 3-1). Also on day 0, the scan and non-scan heifers were started on a 14 d MGA (0.5 mg•hd^{-1}•d^{-1}; MGA® 200 Premix, Pfizer, Inc., New York, NY) treatment, which was administered in a high protein pellet fed at a rate of 2 lbs•hd^{-1}•d^{-1}. The cycling scan and non-scan heifers were at random stages of the estrous cycle at the start of the MGA treatment. All heifers received prostaglandin $F_{2\alpha}$ (PG; Lutalyse® Sterile Solution, Pfizer, Inc. New York, NY) starting on d 19 as described by Bridges et al. (2005). Angus heifers received a single 25 mg i.m. PG treatment 19 d after MGA while Brangus heifers received 12.5 mg i.m. PG on both 19 and 20 d following MGA withdrawal. Bridges et al. (2005) reported that a split PG treatment enhanced luteolysis compared to a single PG treatment in yearling *Bos indicus* × *Bos taurus* heifers and the split PG treatment in yearling *Bos indicus* × *Bos taurus* heifers resulted in a similar rate of luteolysis compared to a single PG treatment in *Bos taurus* heifers. Therefore, the breed specific PG treatment was used in the present experiment to ensure that the PG induced luteolysis would be similar between Angus and Brangus heifers.

The scan group consisted of cycling Angus (n = 11) and Brangus (n = 10) heifers that were used to evaluate daily follicular development using transrectal ultrasonography (equipped with a 7.5 MHz linear array transducer) from MGA withdrawal to 5 d after PG. Scan heifers were handled through the working facilities three times a week during MGA treatment to acclimate them to frequent handling to reduce any stress related affects associated with frequent handling.

At the start of the ultrasonography exams, two Brangus heifers had to be replaced because of physical injuries that prevented daily ultrasonography from being conducted. The two heifers were replaced with two non-cycling Brangus heifers of similar age and BW. Non-cycling Brangus heifers were chosen because there were no cycling Brangus heifers available that had a large enough rectal size to allow for daily ultrasonography exams to be performed The two Brangus heifers that were removed from the scan group were placed in the non-scan group where they recovered from their injuries. At each ultrasonography evaluation, height and width of all luteal structures, luteal cavities, and follicles ≥ 3 mm in diameter were measured using the internal calipers of the ultrasonography machine, and their locations on the ovaries were recorded. All ultrasonography exams were conducted by a single technician. Volume of the corpus luteum (CL) was calculated using the formula for volume of a sphere ($\pi d^3/6$). When a luteal cavity was present, its volume was subtracted from the volume of the outer sphere resulting in net luteal volume (CL volume) represented by luteal tissue. Ovarian maps were evaluated retrospectively to determine follicle growth patterns. Emergence of a follicle wave was defined as the time when the eventual dominant follicle could first be identified by ultrasonography. Maximal diameter of a dominant follicle was defined as the maximum diameter that the dominant follicle reached during the follicle wave. The day of maximal diameter was defined as the day following emergence that the dominant follicle reached a maximal diameter. Growth rate of the dominant follicle was determined by subtracting the diameter of the dominant follicle at emergence from the maximal diameter of the dominant follicle divided by the number of days from emergence to maximal diameter. Additionally, blood samples were collected via jugular veinipuncture to determine plasma progesterone concentrations at each ultrasonography exam. During the ultrasonography exams after MGA

withdrawal, ovulation was defined as disappearance of the largest follicle followed by its absence at two consecutive ultrasonography exams. Ovulation rate after MGA for the scan heifers was defined as the number of heifers ovulating a follicle within 7 d after MGA withdrawal divided by the total number of scan heifers.

The non-scan heifers underwent transrectal ultrasonography (equipped with a 7.5 MHz linear array transducer) at MGA withdrawal and at the initial PG. At each ultrasonography evaluation, height and width of all luteal structures, luteal cavities, and follicles ≥ 5 mm in diameter were measured using the internal calipers of the ultrasonography machine, and their locations on the ovaries were recorded. Additionally, blood samples were collected via jugular veinipuncture to determine plasma progesterone at MGA withdrawal, at the initial PG, and 3 d following the initial PG treatment to determine if luteolysis occurred. Both scan and non-scan heifers were deemed to have a functional CL at PG if plasma progesterone concentrations were > 1.5 ng/mL with the presence of luteal tissue as determined by ultrasonography. The PG induced luteolysis for scan and non-scan heifers was defined as a heifer having a functional CL at PG followed by progesterone concentrations < 1.5 ng/mL 3 d after PG. After the onset of the PG induced estrus for the scan heifers, ovulation was defined as disappearance of the largest follicle at the subsequent ultrasonography exam. Ovulation was confirmed by the presence of a functional CL 8 d later as determined by ultrasonography and a blood plasma sample was also collected to determine plasma progesterone concentrations.

Blood plasma samples were collected into evacuated tubes containing an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ). After collection, blood samples were immediately placed on ice until they were centrifuged (3000 × g for 15 min). Plasma was separated and stored at –20°C until further analysis. Progesterone concentrations were

determined by RIA (Seals et al., 1998) using DPC kits (Diagnostic Products Corp., Los Angeles, CA) in multiple assays with intra- and interassay CV of 3.5 and 4.9%, respectively. Sensitivity of the assay was 0.01 ng/mL of plasma assayed.

Estrus was detected throughout the experiment using radiotelemeric estrous detection devices (HeatWatch®, Cow Chips, Denver, CO; Dransfield et al., 1998), which were fitted to all heifers at the initiation of MGA treatment. Estrus was detected from the initiation of MGA until 5 d after PG. Heifers were artificially inseminated (AI) by one of two AI technicians with frozen-thawed semen 8 to 12 h after the onset of the PG induced estrus. Angus heifers were inseminated to two AI sires that were pre-assigned to heifers prior to insemination and the Brangus heifers were inseminated to a single AI sire. Pregnancy was determined approximately 30 d after AI by transrectal ultrasonography, using a real-time, B-mode ultrasound (Aloka 500v, Corometrics Medical Systems, Wallingford, CT) equipped with a 5.0 MHz transducer.

Interval to estrus, duration of estrus, and number of mounts received during estrus were recorded for the estrus following MGA withdrawal and for the estrus after PG administration. For both the estrus after MGA withdrawal and PG, onset of estrus was defined as the first of 3 mounts in a 3 h period and the end of estrus was defined as the time of the last mount recorded during estrus prior to a period of extended inactivity of at least 8 h (Landaeta et al., 1999). The duration of estrus was calculated by subtracting the date and time of the initial mount of estrus from the last mount of estrus. Interval to estrus following MGA withdrawal was calculated by subtracting the approximate date and time of MGA withdrawal from the date and time of the initial mount of estrus. Estrous response after MGA withdrawal was defined as the total number of heifers that exhibited behavioral estrus in 7 d divided by the total number of heifers treated. Interval from PG to the onset of estrus was calculated by subtracting the date and time of the

initial PG from the date and time of the initial mount of estrus. Estrous response after PG was defined as the total number of heifers that exhibited behavioral estrus in 5 d after the initial PG divided by the total number of heifers treated. Conception rate was defined as the total number of heifers that exhibited estrus after PG that were inseminated and became pregnant, divided by the total number of heifers that exhibited estrus and were inseminated. Synchronized pregnancy rate was defined as the total number of heifers that became pregnant to the AI divided by the total number of heifers treated.

Dependent variables tested using the GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) included cycling status, estrous response after MGA, estrous response after PG, conception rate, synchronized pregnancy rate, occurrence of a functional CL at PG, PG induced luteolysis, and occurrence of heifers with follicles ≥ 10 mm in diameter at PG. Independent variables tested were breed, ultrasound group (scan vs. non-scan), and breed × ultrasound group. Cycling status was also evaluated as an independent variable using the model of breed, cycling status (cycling vs. non-cycling), and breed × cycling status for the aforementioned dependent variables. Additionally, ovulation rate following MGA withdrawal in scan heifers was evaluated with the independent variable tested being breed. Diameter of the largest follicle at MGA withdrawal, diameter of the largest follicle at PG, progesterone concentration at PG, and behavioral estrus data including interval to estrus, duration of estrus, and number of mounts received during estrus after MGA withdrawal and after PG were tested using the GLM procedure in SAS. Independent variables tested were breed, ultrasound group and breed × ultrasound group; additionally, breed, cycling status, and breed × cycling status were evaluated. Diameter of the first, second, and third wave dominant follicles normalized for day of emergence, and diameter of the dominant follicle on days 9-13 after MGA withdrawal (d 0) for scan heifers were evaluated with analysis of

variance for repeated measures using the MIXED procedure in SAS. Independent variables tested were breed, day, and breed × day. Additionally, diameter of the eventual ovulatory follicle normalized to PG for scan heifers exhibiting two or three follicle waves was evaluated with analysis of variance for repeated measures using the MIXED procedure in SAS. Independent variables tested were breed, day, number of waves, and all possible interactions. Day of emergence, maximal diameter, and day of maximal diameter for the first, second, and third wave dominant follicles in scan heifers were evaluated using the GLM procedure in SAS. The independent variable tested was breed. In scan heifers exhibiting either two or three follicle waves, estrous response following PG, conception rate, and synchronized pregnancy rate were evaluated using the GENMOD procedure of SAS, while interval from PG to the onset of estrus was tested in the GLM procedure of SAS. Independent variables tested were breed, number of waves and breed × number of waves. One non-scan Brangus heifer exhibited estrus during the MGA treatment so the data was excluded from the estrous response analysis after MGA withdrawal, but was included in all analyses at and after PG. Two non-scan Angus heifers did not have ultrasonography data collected at MGA withdrawal, so they were removed from the ultrasonography analysis. Four non-scan Angus heifers exhibited estrus before PG and they were excluded from the post PG data analysis. Three non-scan Angus heifers did not have ultrasonography data collected at PG so they were removed from the ultrasonography data analysis at PG.

Results

The physical description of the heifers is presented in Table 3-1. The Angus heifers were older ($P = 0.03$), had a greater ($P = 0.02$) BCS, and had a greater ($P = 0.02$) percentage cycling at

the initiation of the experiment compared to Brangus heifers. There were no breed × ultrasound group effects ($P > 0.05$) on age, BW, BCS, and percentage cycling at the start of the experiment.

With all scan and non-scan heifers included in the analysis, diameter of the largest follicle present at MGA withdrawal was similar ($P = 0.72$) for Angus (n= 38; 13.7 ± 0.5 mm) and Brangus (n= 25; 13.5 ± 0.5 mm) heifers. There were no ($P > 0.05$) effects of cycling status or breed × cycling status on diameter of the largest follicle at MGA withdrawal. The percentage of Angus and Brangus heifers in estrus during the 7 d after MGA withdrawal was similar ($P = .88$; Table 3-2). The interval from MGA withdrawal to the onset of estrus, duration of estrus, and number of mounts received during estrus were also similar ($P > 0.05$) between Angus and Brangus heifers (Table 3-2). The scan heifers had a greater ($P = 0.001$) estrous response compared to non-scan heifers, and scan heifers tended ($P = 0.09$) to have a longer interval from MGA withdrawal to the onset of estrus compared to non-scan heifers (Table 3-2). Whether heifers were scan or non-scan did not influence ($P > 0.05$) the duration of estrus or the number of mounts received during the estrus after MGA withdrawal (Table 3-2). There were no ($P > 0.05$) breed × ultrasound group effects on estrous response, interval from MGA withdrawal to the onset of estrus, duration of estrus, and number of mounts received during estrus (Table 3-2). More ($P = 0.001$) cycling heifers (n = 22/35; 62.9%) exhibited estrus after MGA withdrawal compared to non-cycling heifers (n = 7/30; 23.3%), but there was no ($P = 0.55$) breed × cycling status effect. Interval from MGA withdrawal to the onset of estrus (87:03 ± 09:19; 64:36 ± 17:01 h:m), duration of estrus (10:24 ± 01:25; 11:52 ± 02:35 h:m), and number of mounts received (53 ± 9; 70 ± 17) were similar ($P > 0.05$) between cycling and non-cycling heifers, respectively. There were no ($P > 0.05$) breed × cycling status effects for interval from MGA withdrawal to the onset of estrus and duration of estrus, but there was ($P < 0.05$) a breed ×

cycling status effect on number of mounts received during estrus. The number of mounts received for the cycling Angus, non-cycling Angus, cycling Brangus, and non-cycling Brangus were 68 ± 10; 47 ± 28; 38 ± 15; 94 ± 18, respectively.

For scan heifers that exhibited estrus within 7 d after MGA withdrawal, ovulation rate tended ($P = 0.07$) to be greater in Angus (11/11 = 100.0%) compared to Brangus (8/10 = 80.0%) heifers (Figure 3-1). Diameter of the ovulatory follicle was similar ($P = 0.93$) between Angus (17.0 ± 0.9 mm) and Brangus (17.1 ± 1.0) heifers. Two Brangus heifers did not exhibit estrus within 7 d after MGA withdrawal. One heifer had progesterone < 1.5 ng/mL at MGA withdrawal and had initiated regression of a persistent dominant follicle prior to MGA withdrawal. This heifer developed a new follicle wave, which ovulated 11 d after MGA withdrawal (Figure 3-1). The other Brangus heifer had progesterone > 1.5 ng/mL at MGA withdrawal and had initiated a new follicle wave after MGA withdrawal. The newly developed follicle ovulated 12 d after MGA withdrawal (Figure 3-1). Between MGA withdrawal and administration of PG, 81.8% of Angus (n = 9/11) and 50.0% of Brangus (n = 5/10) heifers displayed two waves of follicle growth, while 18.2% of Angus (n = 2/11) and 30.0% of Brangus (n = 3/10) heifers displayed three waves of follicle growth. Of the two remaining Brangus heifers, one heifer had a single follicle wave and the other heifer had four follicle waves.

One of the objectives of the experiment was to characterize follicle dynamics after MGA withdrawal to determine the optimum timing to administer GnRH to initiate ovulation and synchronize follicle wave development in Brangus heifers. Follicle development patterns for the first follicle wave after MGA withdrawal for Angus and Brangus scan heifers are presented in Figure 3-1. Emergence of the first wave dominant follicle after MGA withdrawal was similar (*P*

= 0.27) for Angus (5.7 ± 0.5 d; range 4 to 8 d) compared to Brangus (4.9 ± 0.6 d; range 3 to 9 d) heifers.

Diameters of the first wave dominant follicle for the Angus and Brangus scan heifers are presented in Figure 3-2. There was an effect ($P = 0.001$) of day on the diameter of the dominant follicle 9 to 13 d after MGA withdrawal but there were no ($P > 0.05$) breed or breed × day effects. The percentage of heifers with follicles ≥ 10 mm was also evaluated for d 9 to 13 after MGA withdrawal. A diameter of 10 mm was chosen since follicles can be ovulated by exogenous GnRH at approximately ≥ 10 mm diameters (Moreira et al., 2000). The percentage of heifers with follicles ≥ 10 mm was similar ($P > 0.05$) for Angus (n=11) and Brangus (n=10) heifers on d 9 (54.5 vs. 80.0 %), 10 (81.8 vs. 70.0 %), and 11 (90.9 vs. 80.0%), respectively. However, there were more ($P = 0.02$) Angus (n=100) with follicles ≥ 10 mm on d 12 compared to Brangus (70.0%) and there were more ($P = 0.002$) Angus (100%) with follicles ≥ 10 mm on d 13 compared to Brangus (50.0%). One Brangus heifer ovulated on d 11 and one on d 12 after MGA withdrawal.

When normalized to the day of emergence for the first follicle wave, there was an effect of day ($P = 0.001$) on diameter of the dominant follicle (Figure 3-3) but there were no ($P > 0.05$) breed or breed × day effects. Dominant follicles reached a maximal diameter on a similar ($P = 0.15$) day following emergence and at a similar ($P = 0.61$) diameter for Angus (7.5 ± 0.7 d; 14.5 ± 0.7 mm) and Brangus (5.9 ± 0.8 d; 14.0 ± 0.8 mm), respectively. From day of emergence to maximal diameter, follicle growth rates were similar ($P = 0.42$) for Angus (1.6 ± 0.2 mm/d) and Brangus (1.8 ± 0.2 mm/d) heifers.

When the second follicle wave was normalized to the day of emergence (Figure 3-3), there was an effect of day ($P = 0.001$) on diameter of the dominant follicle but there were no ($P >$

0.05) breed or breed × day effects. Breed tended ($P = 0.10$) to affect the day of emergence after MGA withdrawal of the second wave dominant follicle where emergence occurred on d 11.7 ± 0.8 (range 8 to 16 d) for Brangus compared to d 13.5 ± 0.7 (range 10 to 16 d) for Angus. Dominant follicles reached a maximal diameter on a similar ($P = 0.68$) day following emergence for Angus (6.5 ± 0.6) compared to Brangus (6.1 ± 0.7 d), but maximal diameter of the dominant follicle tended ($P = 0.06$) to be greater in Angus (15.5 ± 0.7 mm) compared to Brangus (13.3 ± 0.8 mm). There was no ($P = 0.35$) effect of breed on follicle growth rate from day of emergence to maximal diameter of the second wave dominant follicle. Follicle growth rate for the Angus was 1.9 ± 0.2 mm/d and 1.7 ± 0.2 mm/d for the Brangus heifers.

For the third follicle wave, there was an effect of day ($P = 0.001$) on diameter of the dominant follicle when normalized to the day of emergence (Figure 3-3). There were no ($P > 0.05$) breed or breed × day effects on mean diameter of the third wave dominant follicle. Breed had no ($P = 0.24$) effect on day of emergence of the third wave dominant follicle after MGA withdrawal where emergence occurred 17.5 ± 1.6 d (range 17 to 18 d) for Angus and 14.8 ± 1.2 d (range 12 to 17 d) for Brangus. Third wave dominant follicles had a similar ($P = 0.40$) maximal diameter on a similar ($P = 0.76$) day after emergence for Angus (13.0 ± 0.9 mm; 5.5 ± 0.6 d) and Brangus (14.0 ± 0.6 mm; 5.3 ± 0.5 d), respectively. Additionally, follicle growth rate from day of emergence to maximal diameter of the third wave dominant follicle was similar ($P = 0.50$) for Angus (1.8 mm/d) and Brangus (2.1 mm/d) heifers.

On the day of PG administration, diameter of the largest follicle tended ($P = 0.09$) to be greater for Angus compared to Brangus heifers (Table 3-3). Cycling status and breed × cycling status had no ($P > 0.05$) effect (Table 3-2) on diameter of the largest follicle at PG. Likewise, there were no ($P > 0.05$) ultrasound group and breed × ultrasound group effects. A similar ($P =$

0.13) percentage of Angus (30/33; 90.9%) and Brangus (22/26; 84.6%) heifers had follicles ≥ 10 mm in diameter at PG. There were no ($P > 0.05$) ultrasound group, breed × ultrasound group, cycling status, and breed × cycling status effects on percentage of heifers with follicles ≥ 10 mm at PG.

The effect of follicle wave pattern (two-wave vs. three-wave) on follicle development during the 6 d prior to PG was also evaluated with follicle development being normalized retrospectively from day of PG (Figure 3-4). Breed tended ($P = 0.07$) to effect follicle development and day ($P = 0.001$) did effect follicle development. The number of follicle waves between MGA withdrawal and PG also affected ($P = 0.01$) follicle development (Figure 3-4). There were no ($P > 0.05$) breed × day, breed × wave, day × wave, or breed × day × wave effects. Additionally, day of emergence after MGA withdrawal of the eventual ovulatory follicle was similar ($P = 0.97$) for the Angus (14.7 ± 0.7 d) and Brangus (14.7 ± 0.8 d) heifers (Figure 3-5).

A greater ($P = 0.001$) percentage of heifers that were cycling at the start of the MGA treatment had a functional CL at PG compared to non-cycling heifers (Table 3-3). However, there was neither ($P < 0.05$) a breed nor breed x cycling group effect on whether heifers had a functional CL at PG (Table 3-3). A greater ($P = 0.001$) percentage of scan (20/21 = 95.2%) compared to non-scan (14/38 = 55.3%) heifers had a functional CL at PG, but there was no ($P = 0.37$) breed × ultrasound group effect. Progesterone concentrations at PG were similar ($P = 0.50$) for Angus and Brangus heifers (Table 3-3). Whereas, progesterone concentrations at PG tended ($P = 0.07$) to be greater in cycling compared to non-cycling heifers, but there was no ($P = 0.31$) breed × cycling group effect. Furthermore, progesterone concentrations at PG tended ($P = 0.08$) to be greater in scan (6.29 ± 0.9 ng/mL) compared to non-scan (4.44 ± 0.6 ng/mL) heifers but there was no ($P = 0.95$) breed × ultrasound group effect. For both the scan and non-scan

heifers with functional CL at PG, luteolysis rates were similar ($P > 0.05$) for Angus (25/25) and Brangus (17/17) heifers.

The effect of breed and cycling status at the initiation of MGA treatment on estrous, conception and synchronized pregnancy rates are presented in Table 3-4. Estrous response, conception rate, and synchronized pregnancy rate were similar ($P > 0.05$) for Angus and Brangus heifers (Table 3-4). Cycling status affected ($P = 0.001$) estrous response as more cycling heifers exhibited estrus compared to non-cycling heifers (Table 3-4). There was no ($P = 0.93$) breed × cycling status effect on estrous response. When analyzed by ultrasound group, more ($P = 0.001$) scan heifers exhibited estrus (95.2%; n=20/21) compared to non-scan (56.1%; n=23/41) heifers. There tended ($P = 0.10$) to be a breed × ultrasound group effect on estrous response. Estrous responses for Angus scan, Angus non-scan, Brangus scan and Brangus non-scan were 90.9% (n=11), 68.0 (n=25), 100.0 (n=10), and 37.5% (n=16), respectively. Breed, cycling status, and breed ×cycling status had no effect on conception rate ($P > 0.05$), which was also the case for ultrasound group, and breed × ultrasound group. The effect of AI sire ($P = 0.69$) and AI technician ($P = 0.38$) did not affect conception rate. Synchronized pregnancy rates were similar ($P = 0.52$) for Angus and Brangus heifers but more ($P = 0.05$) cycling heifers became pregnant during the synchronized breeding compared to non-cycling heifers. There were no ($P > 0.05$) ultrasound group and breed × cycling status effects on synchronized pregnancy rates. However, there was ($P < 0.05$) a breed × ultrasound group effect on synchronized pregnancy rate. Synchronized pregnancy rates for Angus scan, Angus non-scan, Brangus scan and Brangus non-scan were 60.0% (n=11), 36.0 (n=25), 60.0 (n=10), and 18.8% (n=16), respectively.

Interval from PG to the onset of estrus (61:49 ± 5:30; 63:14 ± 6:19 h:m), duration of estrus (11:44 ± 1:05; 10:08 ± 1:15 h:m), and the number of mounts received during estrus (57 ± 9; 42 ±

10) were similar ($P > 0.05$) for Angus and Brangus heifers, respectively. There were no ($P > 0.05$) cycling status or breed × cycling status effects on interval from PG to the onset of estrus, duration of estrus, or number of mounts received during estrus. Likewise, there were no ($P > 0.05$) ultrasound group and breed × ultrasound group effects for interval from PG to the onset of estrus, duration of estrus, or number of mounts received during estrus. Diameter of the largest follicle at PG was negatively correlated (r = -0.60; $P = 0.01$) with the interval from PG to estrus in Angus heifers; whereas, diameter of the largest follicle at PG only tended to be negatively correlated (r = -0.33; $P = 0.09$) with the interval from PG to the onset of estrus in Brangus heifers.

For the scan heifers, number of follicle waves between MGA withdrawal and PG had no ($P = 0.68$) effect on estrous response after PG. Estrous response was 88.9% (8/9) and 100.0% (2/2) for two- and three-wave Angus heifers, respectively, and 100.0% (5/5) and 100.0% (3/3) for two- and three-wave Brangus heifers, respectively. Neither the number of waves nor breed × number of waves effected ($P > 0.05$) estrous response after PG. The number of waves tended ($P = 0.09$) to affect the interval from PG to the onset of estrus and interval from PG to the onset of estrus was affected by breed ($P = 0.02$) and breed × number of waves ($P = 0.01$). Interval from PG to the onset of estrus was 63:21 ± 5:12 h:m and 101:56 ± 10:24 h:m for two- and three-wave Angus heifers, respectively, and 67:43 ± 6:35 h:m and 57:33 ± 8:30 h:m for two- and three-wave Brangus heifers, respectively. There tended ($P = 0.07$) to be an effect of breed on conception rates but number of waves and breed × number of waves did not affect ($P > 0.05$) conception rate. Conception rates were 25.0% (2/8) and 0% (0/2) for two- and three-wave Angus heifers, respectively, and 40.0% (2/5) and 66.7% (2/3) for two- and three-wave Brangus, respectively. Synchronized pregnancy rate was not affected ($P > 0.05$) by breed, number of waves, or breed ×

number of waves. Synchronized pregnancy rates were 22.2% (2/9) and 50.0% (1/2) for two- and three-wave Angus heifers, respectively and 40.0% (2/5) and 66.7% (2/3) for two- and three-wave Brangus heifers, respectively.

In heifers undergoing daily ultrasound evaluations, 100% of the Angus (n=11) and Brangus (n=10) heifers ovulated after PG. Diameter of the ovulatory follicle before ovulation was similar ($P = 0.86$) for Angus (13.1 ± 0.4 mm) compared to Brangus (13.2 ± 0.5 mm) heifers. Volume of the CL 8 d following ovulation was similar ($P = 0.20$) for Angus (4095.6 ± 410 mm^3) compared to Brangus (4876.3 ± 430 mm^3) heifers; however, progesterone concentrations were ($P = 0.05$) greater for Brangus (7.10 ± 0.53 ng/mL) compared to Angus (5.56 ± 0.51 ng/mL) heifers.

Discussion

In order for the MGA-PG system (Brown et al., 1988) to be effective, heifers need to exhibit estrus and (or) ovulate so they are in the late luteal phase of the estrous cycle at PG, which means a majority of heifers must exhibit estrus with 3 to 7 d after MGA withdrawal as reported by Hill et al. (1971). In the present study, one-hundred percent of the scan Angus heifers, but only 80% of the scan Brangus heifers exhibited estrus and (or) ovulated within 7 d of MGA withdrawal. Wood et al. (2001) reported that 81.5% of *Bos taurus* heifers ovulated within 12 d of MGA withdrawal. The two Brangus heifers not exhibiting estrus within 7 d after MGA withdrawal, eventually exhibited estrus and ovulated 11 and 12 d after MGA withdrawal. One of the two Brangus heifers had a functional CL (≥ 1.5 ng/mL) at MGA withdrawal, which regressed shortly after MGA withdrawal. The heifer was not detected in estrus during MGA; although, cattle of *Bos indicus* breeding are noted for having a "silent estrus" (Galina et al., 1982; Orihuela et al., 1983). The heifer was probably at the beginning of the estrous cycle at the initiation of MGA, which would have placed the heifer in the late luteal phase of the estrous cycle at MGA withdrawal. The other Brangus heifer had apparently started regression the largest follicle at

MGA withdrawal resulting in initiation of a new follicle wave, which ovulated 12 d after MGA withdrawal. The reasons for this pattern of follicle development after a long term MGA treatment are unclear. Kojima et al. (1992) reported that some cows receiving a MGA treatment failed to have a preovulatory LH surge within 100 h of MGA withdrawal, which may be due to luteinization (Guthrie et al., 1970) of persistent follicles capable of secreting enough progesterone to prevent the LH surge. This does not appear to be likely since the Brangus heifer had progesterone concentrations < 1 ng/mL for several days prior to and after MGA withdrawal.

After MGA withdrawal, diameters of the largest follicles present were similar between Angus and Brangus heifers and similar to those reported by Wood et al. (2001) in cycling *Bos taurus* heifers. The estrous response after MGA withdrawal was similar between Angus and Brangus heifers but it was considerably less than another study using a long term MGA treatment (Yelich et al., 1997) in yearling *Bos taurus* heifers. The decreased estrous response was primarily due to the decreased percentage of heifers that were cycling (39.6%) at the start of MGA. More cycling heifers (62.9%) exhibited estrus after MGA withdrawal compared to non-cycling heifers (23.3%). These results and others (Brown et al., 1988; Patterson and Corah, 1992; Wood-Follis et al., 2004) point out the importance of having heifers going through estrous cycles at the start of the MGA treatment. With that said, long-term MGA treatments induce estrous cycles in some non-cycling heifers (Patterson et al., 1989) and the MGA treatment induced estrous cycles in 51.9% of the non-cycling heifers in the present experiment. Additionally, the percentage of non-cycling heifers that were cycling at PG was similar between Angus and Brangus heifers suggesting that the MGA treatment was equally effective at inducing estrus in non-cycling heifers across the two breed types.

The incidence of a "silent estrus" (Galina et al., 1982; Orihuela et al., 1983) after MGA withdrawal also contributed to the decreased estrous response. Of the heifers that were cycling at the start of MGA, 84% had a functional CL at PG; however, only 63.9% of the cycling heifers exhibited estrus after MGA withdrawal. Therefore, several Angus and Brangus heifers did not exhibit estrus after MGA withdrawal. Because heifers were fitted with radiotelemeric estrous detection devices, it is unlikely that the method of estrous detection was the reason for decreased estrous response. The incidence of "silent estrus" is well documented in *Bos indicus* cattle (Galina et al., 1982; Orihuela et al., 1983), but it is unclear why so many heifers did not exhibit estrus after MGA withdrawal since persistent follicles have increased estrogen concentrations (Henricks et al., 1973; Kojima et al., 1992). Increased ambient temperatures have been reported to increase the incidence of ovulation without estrus in dairy heifers (Gwazdauskas et al, 1981). However, it is unlikely that elevated temperatures caused the increased incidence of "silent estrus" since the experiment was conducted from February to April when ambient temperatures were probably to low to initiate heat stress.

One of the primary objectives of the experiment was to determine the follicular wave pattern from MGA withdrawal to PG in Brangus compared to Angus heifers. There was considerable variation between Brangus compared to Angus heifers in the number of follicle waves between MGA withdrawal and PG. Eight-two percent of Angus heifers had two follicle wave patterns with the remaining 18% having three follicle wave patterns. In comparison, only 50% of the Brangus heifers had two follicle waves and the remaining heifers had either one, three, or four follicle waves. Therefore, based on the number of follicle waves, there is considerable variation in follicle development patterns for Brangus compared to Angus heifers. The increased incidence of three and four follicle wave patterns in Brangus heifers is similar to

reports in cattle of *Bos indicus* breeding having more three and four follicular wave patterns (Rhodes et al., 1995; Viana et al., 2000) during a normal estrous cycle compared to two-wave patterns.

Growth and development of the first wave dominant follicle after MGA withdrawal was similar between Angus and Brangus heifers with respect to day of emergence, growth rate, day of maximal diameter, and maximal diameter of the dominant follicle. With respect to the first wave follicle development patterns in the current study, they are in agreement with other observations reported in *Bos taurus* cattle (Sirois and Fortune, 1988; Ginther et al., 1989). Conversely, Viana et al. (2000) reported that emergence of the first follicle wave occurred earlier, had a reduced growth rate and maximal diameter in non-lactating *Bos indicus* cows compared to the Brangus heifers in the present study. Emergence of the second follicle wave tended to occur later in Angus compared to Brangus heifers. Additionally, Angus heifers had a greater maximal diameter of the second wave dominant follicle compared to Brangus heifers. The increased incidence of three and four follicle wave patterns is likely influenced by day of emergence and maximal follicle diameter of the second wave dominant follicle. Second wave dominant follicles emerged later (Sirois and Fortune, 1988) and reached a greater maximal diameter (Ginther et al., 1989) in cattle displaying two compared to three wave follicle growth profiles. No differences were observed between Angus and Brangus heifers with regard to the developmental characteristics of the third wave dominant follicle. Characteristics of follicle development, particularly the second and third waves, should be interpreted with discretion since PG was administered before some of the heifers were allowed to complete a normal estrous cycle.

Another research objective was to determine the best time to incorporate GnRH into the MGA-PG system. Wood et al. (2001) conducted an experiment where *Bos taurus* heifers received GnRH 12 d after the withdrawal of a 14 d MGA treatment with PG 7 d after GnRH compared to the MGA-PG system with PG administered 19 d after MGA withdrawal. They hypothesized that GnRH would induce a new follicle wave, which would increase the synchrony of follicle development at PG resulting in a more synchronous estrus. As presented in Figure 1-2, there was considerable variation in the growth and developmental profiles of the first wave dominant follicle in Brangus compared to Angus heifers. For the eleven scan Angus heifers, 100% of the heifers had first wave dominant follicles that were in the growing phase by d 12 after MGA withdrawal. In contrasts, only 60% of the Brangus heifers had a first wave dominant follicle in the growing phase by d 12 after MGA withdrawal. Furthermore, two Brangus heifers had follicles that began to go through atresia by d 10 and another heifer had follicle emergence on d 9 after MGA withdrawal. The asynchrony of follicle development after MGA withdrawal for Brangus heifers was further reflected in the percentage of heifers with follicles ≥ 10 mm between d 9 to 13 after MGA withdrawal, which is important since GnRH is typically effective in growing follicles ≥ 10 mm in diameter (Moreira et al., 2000). By d 11 after MGA withdrawal, 91% of Angus heifers had follicles ≥ 10 mm and 100% by d 12 and 13. In contrasts, by d 11 after MGA withdrawal, 80% of Brangus heifers had follicles ≥ 10 mm and 70.0% by d 12 and 50% by d 13. The reduction in follicles ≥ 10 mm by d 12 and 13 was a result of heifers exhibiting estrus and ovulating, and several heifers having first wave dominant follicles that began to enter atresia and regress by d 12. Therefore, the optimal time to administer GnRH to Brangus heifers should occur approximately 10 d following MGA withdrawal for a couple of reasons. First, a greater percentage of Brangus heifers would have follicles ≥ 10 mm diameter

and in the growing phase of follicle development. Second, for heifers failing to exhibit estrus and ovulate within 7 d after MGA withdrawal the heifers would develop a new follicle wave that should be in the growing phase by d 10 and responsive to GnRH.

Because of the asynchrony of follicle development by d 12 after MGA withdrawal in Brangus heifers, administering GnRH within 2 to 4 d after MGA withdrawal is an option that should also be investigated. By 4 d after MGA withdrawal, the variation in follicle development appears minimal and most heifers have follicles ≥ 10 mm in diameter. However, the effectiveness of GnRH to ovulate a majority of the large persistent dominant follicles needs to be addressed in further experiments.

At PG, diameter of the eventual ovulatory follicle was similar between Angus and Brangus heifers, which agree with a report by Wood et al. (2001) in *Bos taurus* heifers synchronized with MGA-PG. Although the range in day of emergence of the eventual ovulatory follicle was considerable for Angus and Brangus heifers, diameter and growth rate of the eventual ovulatory follicle during the 6 d prior to PG was similar for Angus and Brangus heifers. It is also interesting to note that even with the asynchronous follicle development that Brangus heifers experienced between MGA withdrawal and PG, follicle development at PG was similar between Angus and Brangus heifers. Additionally, the interval from PG to the onset of estrus was similar between breeds, which can be attributed to the similar diameter of the eventual ovulatory follicle at PG for the Angus and Brangus heifers. With that said, there was a significant interaction between breed and the number of follicle waves at PG on the interval from PG to the onset of estrus. Angus and Brangus heifers that had two follicle waves had a similar interval from PG to the onset of estrus. Growth and development of the eventual ovulatory follicle was similar between two wave Angus and Brangus heifers, resulting in similar diameters of the eventual

ovulatory follicle at PG. However, Angus heifers with three follicle waves had a longer interval from PG to the onset of estrus compared to Brangus heifers with three follicle waves. The greater interval from PG to the onset of estrus for the three wave Angus heifers was due to the fact that the follicles were in the early stages of follicle development and diameter of the eventual ovulatory follicle was considerably less than compared to three wave Brangus heifers, which had a more mature follicle and a shorter interval from PG to the onset of estrus. Similarly, Sirois and Fortune (1988) reported that the size of the eventual ovulatory follicle at luteolysis was negatively correlated with the interval to estrus.

Angus and Brangus heifers were synchronized with the MGA-PG protocol described by Lamb et al. (2000) where PG was administered 19 d after the last day of MGA; although, there was a slight modification as the Brangus heifers received a split-PG 19 (12.5 mg) and 20 (12.5 mg) d following MGA withdrawal. For the Angus and Brangus heifers that had a functional CL at PG, PG initiated luteolysis in 100% of the heifers similar to a report by Bridges et al. (2005). Estrous response, conception, and synchronized pregnancy rates were similar between Angus and Brangus heifers. In contrast, the estrous response and synchronized pregnancy rates for Angus and Brangus heifers are considerably less than those observed by other authors (Nix et al., 1998; Lamb et al, 2000; Salverson et al., 2002) in *Bos taurus* heifers synchronized with the MGA-PG system.

The decreased estrous response and subsequently decreased synchronized pregnancy rates can be attributed to the decreased percentage of heifers that were cycling at the initiation of the MGA treatment. Both Angus and Brangus heifers that were cycling prior to the beginning of MGA had a significantly greater estrous response and synchronized pregnancy rate compared to non-cycling heifers. Furthermore, the cycling Angus and Brangus heifers had estrous responses

and synchronized pregnancy rates that are similar to those reported for experiments in *Bos taurus* synchronized with a similar MGA-PG system (Nix et al., 1998; Lamb et al, 2000; Salverson et al., 2002). The importance of cycling status on the overall effectiveness of the MGA-PG system is supported by this and other studies (Brown et al., 1988; Patterson et al., 1989). Attainment of puberty prior to the beginning of the breeding season is also important since fertility increases as the number of estrous cycles a heifer has prior to the initiation of the breeding season increases (Byerley et al., 1987; Galina et al., 1996). Results from the current study suggest that having heifers of *Bos indicus* × *Bos taurus* breeding cycling prior to the start of the breeding season maybe one of the most important, if not the most important factors, in getting *Bos indicus* × *Bos taurus* heifers pregnant to a synchronized AI breeding. Although, having a high percentage of *Bos indicus* × *Bos taurus* heifers cycling at the start of the breeding season can be difficult since cattle of Bos indicus breeding reach puberty at a later age than *Bos taurus* heifers (Plasse et al., 1968; Baker et al., 1989; Rodrigues et al., 2002).

Cycling status had no effect on conception rate, although, conception rates for the Angus and Brangus heifers was still substantially less than other studies (Brown et al., 1988; Jaeger et al., 1992; Lamb et al., 2000) in *Bos taurus* heifers synchronized with the MGA-PG system. Conversely, conception rates of Angus and Brangus heifers are similar to conception rates reported by Bridges et al. (2005) in *Bos indicus* × *Bos taurus* heifers synchronized with the MGA-PG system. However, within the Bridges et al. (2005) study, there was a significant breed effect on conception rates with cycling Angus heifers having a 28.6% greater conception rate compared to cycling *Bos indicus* × *Bos taurus* heifers. The reason (s) for the considerable variation in conception rates within and between studies is difficult to evaluate and could be due to several factors including fertility of the AI sire (DeJarnette et al., 2004), AI technician

(Garcia-Ispierto et al., 2007), stage of follicle development at PG (Austin et al., 1999; Townson et al., 2002), estrous cycling status at the start of a breeding season (Byerley et al., 1987), and environmental conditions in which the studies are conducted (Wolfenson et al., 1995). What, if any, effects that frequent working of cattle had on fertility are unclear. Conception rate was 7% numerically greater in non-scan compared to scan heifers. Within scan and non-scan heifers, each breed responded differently. Conception rates were nearly 33% greater in non-scan compared to scan Angus heifers but only 10% greater in scan compared to non-scan Brangus heifers. To our knowledge, there are no studies evaluating what effect frequent ultrasonography examinations have on fertility. However, frequent ultrasound examinations can have a negative effect on luteal function after ovulation in *Bos indicus* × *Bos taurus* cattle (Lemaster et al., 1999). Luteal function in the scan heifers does not appear to be compromised as all scan heifers developed a functional CL that secreted progesterone 8 d after the PG induced estrus. However, luteal function was not evaluated in the non-scan heifers so no comparison can be made between heifers that were or were not frequently handled.

In the scan heifers, Brangus heifers had greater progesterone concentrations compared to Angus heifers but CL volume was only numerically greater in Brangus compared to Angus heifers. These results are in agreement with Alvarez et al (2000) but do not agree with a report that *Bos indicus* cattle have a smaller CL (Irving et al., 1978) and decreased progesterone concentrations (Segerson et al., 1984).

In summary, follicle development from MGA withdrawal to PG is different between Angus and Brangus heifers. A decreased percentage of Brangus heifers exhibited estrus and ovulated within 7 d after MGA withdrawal. The increased incidence of three and four follicle wave patterns in Brangus heifers contributed to the altered follicle wave dynamics compared to

Angus heifers. Although, diameter of the first wave follicle wave from d 9 to 13 after MGA withdrawal was similar between Angus and Brangus heifers, the number of follicles ≥ 10 mm and in the growing phase were decreasing in Brangus heifers compared to Angus heifers by d 11 and 12 after MGA withdrawal. These results suggest that addition of GnRH to the MGA-PG system for Brangus heifers may need to occur prior to d 12 after MGA withdrawal. Furthermore, administering, GnRH immediately after MGA withdrawal (i.e., day 3 to 4) may actually work better to synchronize follicle development. Even though follicle development was slightly different between Angus and Brangus heifers, the estrous response, conception rate, and synchronized pregnancy rate were similar between breeds. Angus and Brangus heifers that were cycling prior to the start of the MGA treatment had the greatest synchronized pregnancy rates.

Implications

Follicle development during the period between MGA withdrawal and PG was different for Brangus compared to Angus heifers. The most opportune time to administer GnRH after a 14 d MGA treatment to synchronize follicle development in *Bos indicus* × *Bos taurus* heifers may be within 3 to 4 d after MGA withdrawal. Additional research will need to be conducted in *Bos indicus* × *Bos taurus* heifers to evaluate the effectiveness of adding GnRH to the MGA-PG system. Regardless of breed, heifers that were cycling prior to the start of the MGA treatment had greater synchronized pregnancy rates compared to non-cycling heifers. Therefore, producers need to make sure that a majority of yearling *Bos indicus* × *Bos taurus* heifers are going through estrous cycles at the start of a synchronization treatment to achieve acceptable pregnancy rates to a synchronized breeding.

Table 3-1. Age, body weight (BW), body condition score (BCS), and estrous cycling status (Cycling) at the initiation of the 14 d melengestrol (MGA) treatment for Angus and Brangus heifers by ultrasound group (scan vs., non-scan) (LS means ± SE).[a]

Variable	n	Age, d	BW, kg	BCS[b]	Cycling, %[c]
Angus	40	384 ± 2.5	348 ± 5.4	6.3 ± 0.1	25/40 = 62.5
Brangus	26	376 ± 2.9	356 ± 6.1	6.1 ± 0.1	11/26 = 42.3
Non-scan	45	377 ± 2.2	347 ± 4.7	6.1 ± 0.1	17/45 = 37.8
Scan	21	384 ± 3.1	357 ± 6.6	6.2 ± 0.1	19/21 = 90.5
Angus non-scan	29	383 ± 2.6	342 ± 5.6	6.2 ± 0.1	14/29 = 48.3
Angus scan	11	386 ± 4.3	353 ± 9.2	6.3 ± 0.1	11/11 = 100.0
Brangus non-scan	16	370 ± 3.6	352 ± 7.6	6.1 ± 0.1	3/16 = 18.8
Brangus scan	10	382 ± 4.5	360 ± 9.6	6.1 ± 0.1	8/10 = 80.0
P values					
Breed		0.03	0.32	0.02	0.02
Group		0.07	0.24	0.47	0.001
Breed × Group		0.26	0.91	0.38	0.31

[a]Measurements taken on initial day of MGA feeding, experimental day 0.
[b]BCS: 1 = emaciated, 5 = moderate; 9 = obese.
[c]Cycling status determined by blood samples collected d -16, -7. and 0 of experiment. Heifers were classified as cycling if blood plasma progesterone concentrations were ≥ 1.5 ng/mL at two of three blood samples and classified as non-cycling if blood plasma progesterone concentrations were < 1.5 ng/mL at all three blood samples.

Table 3-2. Estrous response, interval to estrus, duration of estrus, and number of mounts received during a HeatWatch® detected estrus for the 7 d following a 14 d melengestrol (MGA) treatment by breed, ultrasound group (scan vs., no-scan), and breed × ultrasound group.[a]

Variable	n	Estrous response, %[b]	Interval from MGA withdrawal to estrus, h:m	Duration of estrus, h:m	Number of mounts
Angus	40	42.5	91:11 ± 9:37	12:55 ± 1:33	66 ± 11
Brangus	25	48.0	71:25 ± 12:07	10:15 ± 1:57	63 ±13
Scan	21	76.2	94:52 ± 9:54	11:53 ± 1:35	65 ± 11
Non-scan	44	29.6	67:45 ± 11:53	11:17 ± 1:55	64 ± 13
Angus scan	11	72.7	103:37 ± 13:59	14:21 ± 2:15	72 ± 15
Angus non-scan	29	31.0	78:46 ± 13:11	11:29 ± 2:07	60 ± 15
Brangus scan	10	80.0	86:07 ± 13:59	9:24 ± 2:15	58 ± 15
Brangus non-scan	15	26.7	56:43 ± 19:47	11:05 ± 3:11	69 ± 22
P-values					
Breed		0.88	0.21	0.29	0.89
Ultrasound group		0.001	0.09	0.81	0.98
Breed × Ultrasound group		0.62	0.88	0.37	0.53

[a]Ultrasound group included scan heifers, which had daily ultrasonography starting the day of MGA withdrawal and continued for 19 d and non-scan heifers did not receive any daily ultrasonography

[b]Number of heifers exhibiting estrus within 7 d of MGA withdrawal divided by the total number of heifers treated.

Table 3-3. Percentage of heifers with a functional CL, progesterone concentration (LSM ± SE), and diameter of the largest follicle (LSM ± SE) at the initial PG treatment.[a]

Variable	n	Functional CL, %[b]	Progesterone concentration, ng/mL	Diameter of largest follicle, mm
Angus	33	75.8	5.5 ± 0.7	11.8 ± 0.3
Brangus	26	61.5	4.8 ± 0.8	11.1 ± 0.3
Cycling[c]	32	84.4	6.1 ± 0.7	11.5 ± 0.3
Non-cycling	27	51.9	4.2 ± 0.8	11.4 ± 0.3
Angus cycling	21	81.0	5.9 ± 0.8	11.9 ± 0.4
Angus non-cycling	12	66.7	5.1 ± 1.1	11.8 ± 0.5
Brangus cycling	11	90.9	6.2 ± 1.2	11.2 ± 0.5
Brangus non-cycling	15	40.0	3.3 ± 1.0	11.0 ± 0.4
P-values				
Breed		0.87	0.50	0.09
Cycling group		0.01	0.07	0.84
Breed × Cycling group		0.15	0.31	0.88

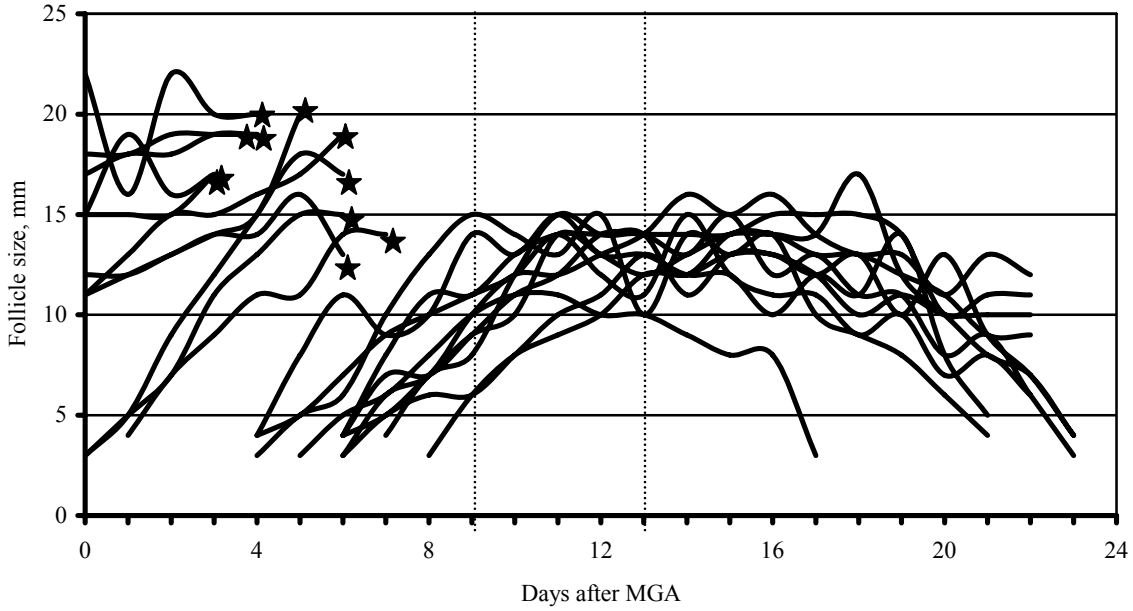
[a]Initial PG treatment was administered 19 d following the withdrawal of a 14 d MGA treatment. Angus heifers received a single (25 mg) PG treatment while Brangus heifers received split (12.5 mg) PG treatments on d 19 and 20.
[b]A heifer was deemed to have a functional CL if progesterone concentrations were ≥ 1.5 ng/mL in the presence of luteal tissue as determined by ultrasonography.
[c] Cycling status determined by blood samples collected d -16, -7. and 0 of experiment with MGA treatment starting on d 0. Heifers were classified as cycling if blood plasma progesterone concentrations were ≥ 1.5 ng/mL at two of three blood samples and classified as non-cycling if blood plasma progesterone concentrations were < 1.5 ng/mL at all three blood samples.

Table 3-4. Effect of breed and cycling status at the initiation of a 14 d melengestrol acetate treatment on estrous response, conception rate and synchronized pregnancy rates of Angus and Brangus heifers synchronized with a 14 d melengestrol acetate treatment followed by either a single (Angus) or split (Brangus) prostaglandin $F_{2\alpha}$ treatment 19 d later.[a]

Variable	Estrous response, %[b]	Conception rate, %[c]	Synchronized pregnancy rate %[d]
Angus	27/36 = 75.0	12/27 = 44.4	12/36 = 33.3
Brangus	16/26 = 61.5	9/16 = 56.3	9/26 = 34.6
Cycling	29/34 = 85.3	15/29 = 51.7	15/34 = 44.1
Non-cycling	14/28 = 50.0	6/14 = 42.9	6/28 = 21.4
Angus cycling	20/23 = 87.0	10/20 = 50.0	10/23 = 43.5
Angus non-cycling	7/13 = 53.8	2/7 = 28.6	2/13 = 15.4
Brangus cycling	9/11 = 81.8	5/9 = 55.6	5/11 = 45.5
Brangus non-cycling	7/15 = 46.7	4/7 = 57.1	4/15 = 26.7
P-values			
Breed	0.59	0.30	0.52
Cycling group	0.001	0.54	0.05
Breed × Cycling group	0.93	0.48	0.61

[a] Cycling status determined by blood samples collected d -16, -7. and 0 of experiment with MGA treatment starting on d 0. Heifers were classified as cycling if blood plasma progesterone concentrations were ≥ 1.5 ng/mL at two of three blood samples and classified as non-cycling if blood plasma progesterone concentrations were < 1.5 ng/mL at all three blood samples.
[b]Percentage of heifers exhibiting estrus during the 5 d following the initial prostaglandin $F_{2\alpha}$ treatment out of the total number treated.
[c]Percentage of heifers that were pregnant to AI of the total number of heifers that exhibited estrous and were AI.
[d]Percentage of the heifers that were pregnant to AI out of the total number treated.

A)



B)

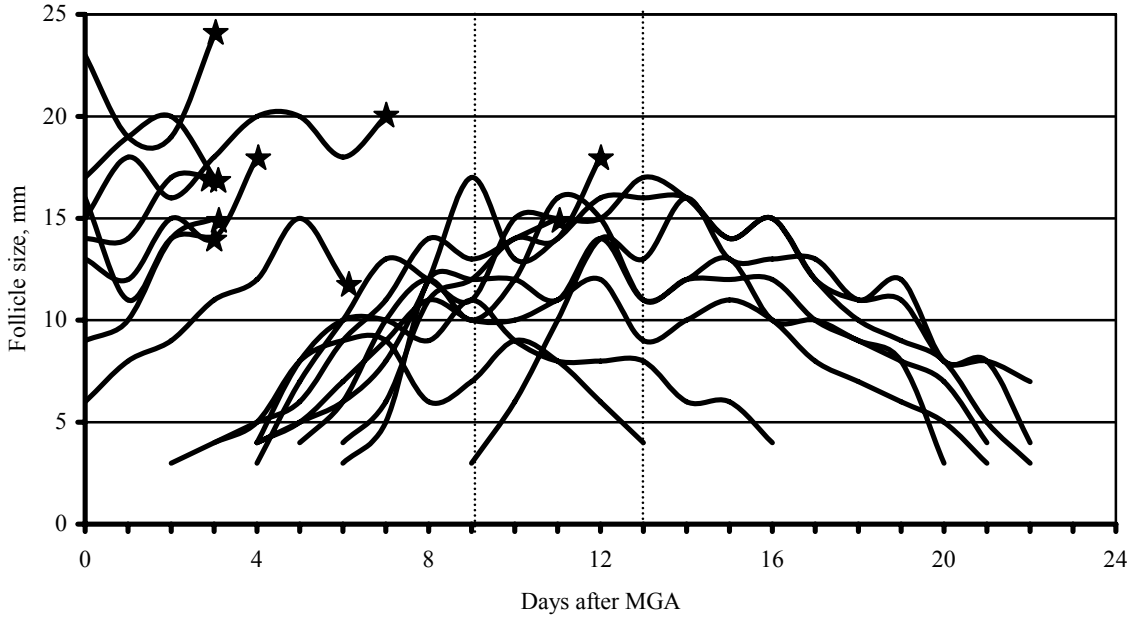


Figure 3-1. Profiles of ovulatory follicles after a 14 d melengestrol acetate (MGA) treatment and the subsequent first wave dominant follicle growth profiles for A) Angus and B) Brangus heifers. Stars indicate day of ovulation and the two dashed lines indicate the potential range when GnRH could be administered to ovulate the first wave follicle after MGA withdrawal to synchronize the next follicle wave.

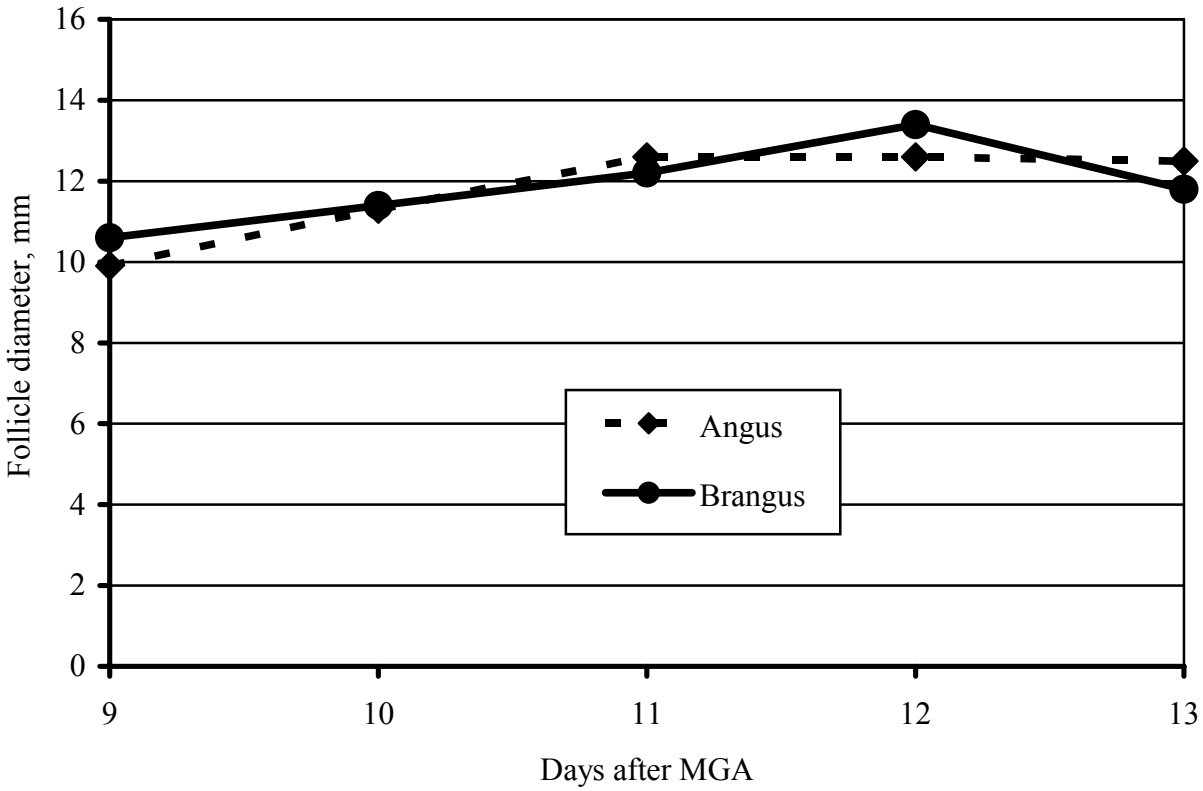


Figure 3-2. Mean first wave dominant follicle diameter during days 9 to 13 following withdrawal of a 14 d melengestrol acetate (MGA) treatment for Angus (n = 11) and Brangus (n = 10) heifers in the scan group. One Brangus heifer ovulated on day 11 and another on day 12. Breed ($P > 0.05$), Day ($P < 0.05$), Breed × Day ($P > 0.05$)

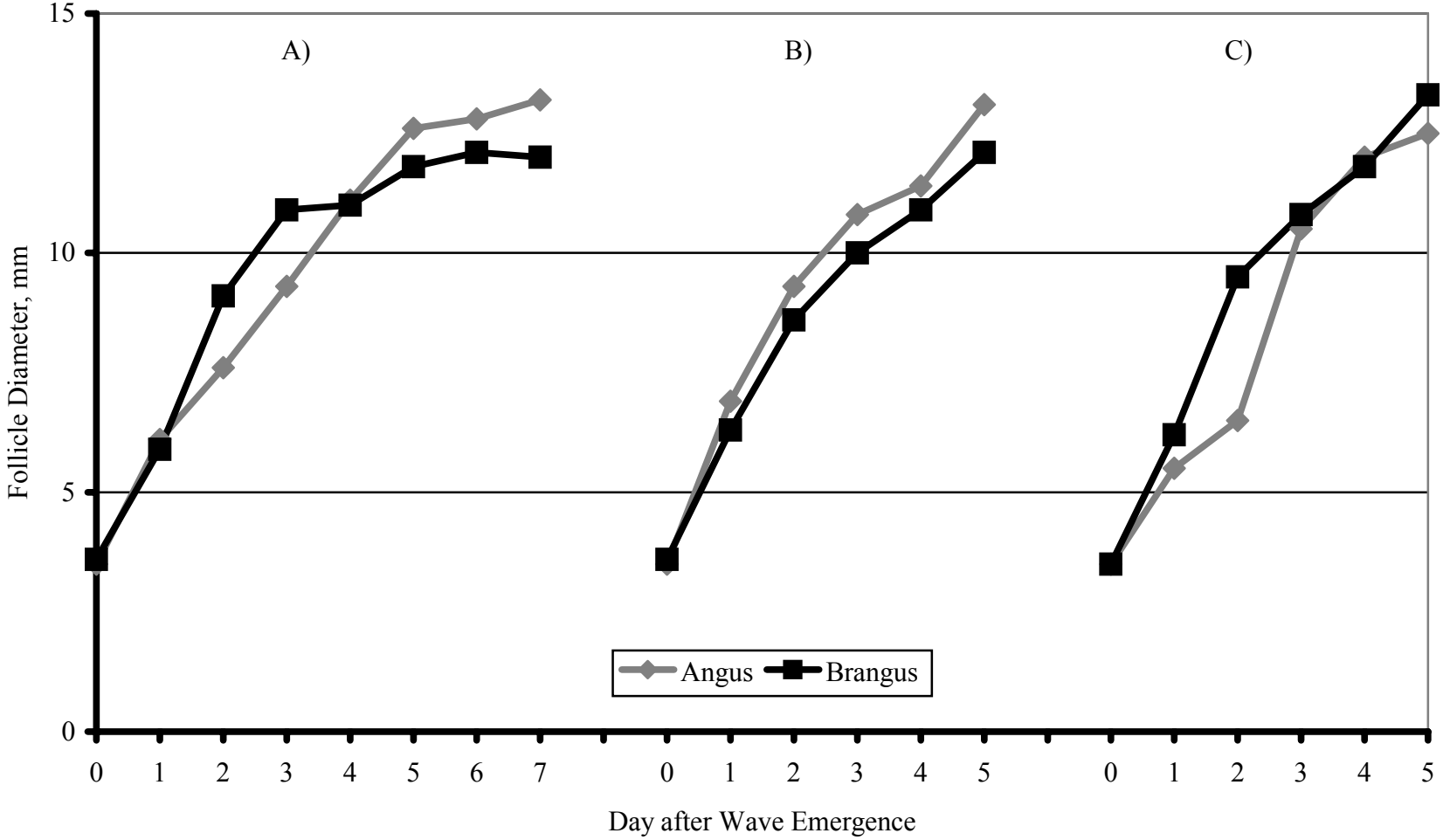


Figure 3-3. Mean diameter of the A) first, B) second, and C) third follicle wave following withdrawal of melengestrol acetate (MGA) for Angus and Brangus heifers. Follicle waves were normalized to the day of wave emergence. For all three follicle wave patterns: Breed ($P > 0.05$), Day ($P < 0.05$), Breed × Day ($P > 0.05$).

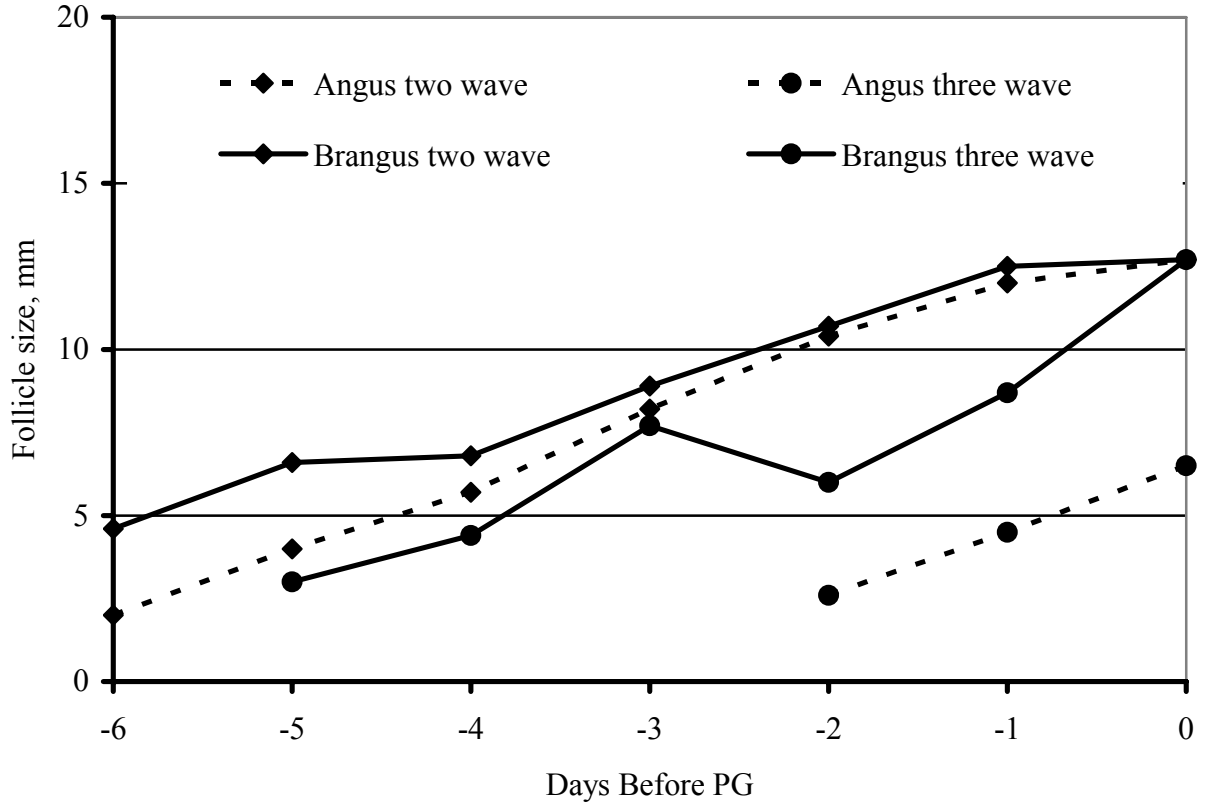


Figure 3-4. Diameter of the eventual ovulatory follicle prior to prostaglandin F2α (PG) treatment for Angus and Brangus heifers based on the number of follicle waves from the last day of a 14 d melengestrol acetate treatment to a PG treatment 19 days later. Breed (P = 0.07), Day (P = 0.001), Number of Waves (P = 0.01), Breed × Day (P = 0.85), Breed × Number of Waves (P = 0.19), Number of Waves × Day (P = 11), Breed × Day × Number of Waves (P = 0.38).

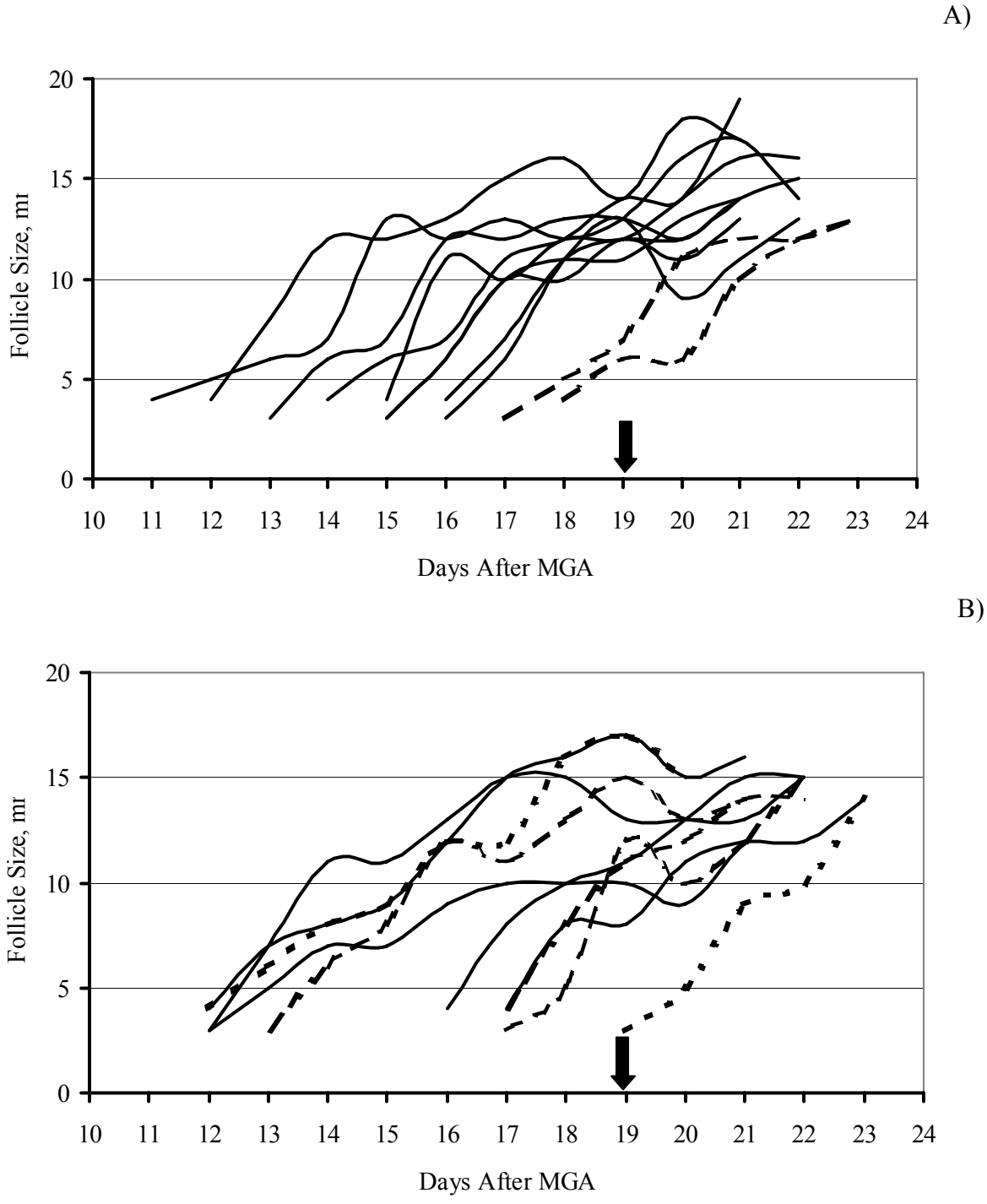


Figure 3-5. Follicle growth patterns for the eventual ovulatory follicle preceding the initial prostaglandin F2α (PG) treatment, which occurred on day 19 (indicated by the arrow) in A) Angus and B) Brangus heifers. Solid lines indicate two-wave follicle growth patterns, long dashed lines indicate three-wave follicle growth patterns and short dashed lines indicate either a single- or four-wave follicle growth pattern.

CHAPTER 4
REFINEMENT OF THE 14 D MELENGESTROL ACETATE (MGA) TREATMENT + PROSTAGLANDIN $F_{2\alpha}$ (PG) 19 D LATER ESTROUS SYNCHRONIZATION SYSTEM IN HEIFERS OF Bos indicus × Bos taurus BREEDING

Introduction

Melengestrol acetate (MGA) administered for 14 d with prostaglandin $F_{2\alpha}$ (PG) administered 17 d after MGA withdrawal is one of the most widely used estrous synchronization systems (MGA-PG) in yearling beef heifers (Brown et al., 1988; Patterson and Corah, 1992). The MGA-PG synchronization system is an effective and predictable system in *Bos taurus* heifers (Brown et al., 1988; Patterson and Corah, 1992; Lamb et al., 2000) but is less effective in *Bos indicus* × *Bos taurus* heifers (Bridges et al., 2005). Modifying the delivery of PG from a single to two consecutive split PG treatments improved the estrous response and synchronized AI pregnancy rates in *Bos indicus* × *Bos taurus* heifers (Bridges et al., 2005), but the synchronized AI pregnancy rates are still less than values observed in *Bos taurus* heifers.

Wood et al. (2000) incorporated gonadotropin-releasing hormone (GnRH) into the MGA-PG system by administering GnRH 12 d after the end of MGA treatment followed by PG 7 d later and reported an increased synchrony of estrus compared to the traditional MGA-PG system (Lamb et al., 2000). Recent research in our lab (See Chapter 3) indicated that follicle development between the MGA withdrawal and PG treatment was not as synchronous in *Bos indicus* × *Bos taurus* (Brangus) heifers compared to *Bos taurus* (Angus) heifers, which resulted from more three wave follicle development patterns in Brangus compared to Angus heifers. Results from Chapter 3 raised the possibility that the most effective time to introduce GnRH into the MGA-PG system may be either a couple of days after MGA withdrawal or 10 d after MGA. Therefore, two experiments were conducted to evaluate: 1) the effect of GnRH administered either 3 or 10 d after the last day of a 14 d MGA treatment with PG 7d after to synchronize

estrus, and 2) evaluate the most effective GnRH treatment from Experiment 1 in a field trial utilizing yearling *Bos indicus* × *Bos taurus* and *Bos taurus* heifers.

Materials and Methods

Experiment 1 was conducted at the University of Florida, Beef Research Unit from October to December, 2004 using 2-year-old *Bos indicus* × *Bos taurus* (n = 58) heifers. Breed composition of the heifers consisted of 3 to 78% Brahman (*Bos indicus*) breeding with the remainder being Angus (*Bos taurus*) breeding. Heifers were equally distributed by percentage Brahman breeding to one of two treatments prior to the start of the experiment. Mean (LSM ± S.E.) age, BW, and body condition score (BCS: Richards et al., 1986) of the heifers were 640 ± 3.7 d, 395 ± 6.3 kg, and 4.8 ± 0.1 for one group designated as the G3 treatment, and 641 ± 3.8 d, 406 ± 6.3 kg, and 4.9 ± 0.1 for the other group designated as the G10 treatment, respectively. Treatment groups were maintained in adjacent pastures throughout the experiment. Prior to the start of the experiment, both treatments were pre-synchronized according to the protocol described by Lemaster et al. (1999), so that heifers would start a 14 d melengestrol acetate (MGA) treatment at d 2 of the estrous cycle. Briefly, heifers received a progesterone insert (EAZI-BREED CIDR®; Pfizer Animal Health, New York, NY) concurrent with 2 mg (i.m.) estradiol benzoate. Seven days later, CIDR were removed and heifers received 25 mg (i.m.) prostaglandin $F_{2\alpha}$ (PG; Lutalyse Sterile Solution, Pfizer Animal Health, New York, NY) followed 24 h later with 0.5 mg (i.m.) estradiol benzoate to synchronize estrus and initiate ovulation. Two days after the expression of estrus, both treatments received MGA (0.5 mg•hd^{-1}•d^{-1}; MGA® Premix, Pfizer Animal Health, New York, NY) in a total mixed ration and the MGA was administered for 14 d. Three days after the last day of MGA, heifers in the G3 (n = 30) treatment received GnRH (100 μg i.m. Cystorelin, Merial, Inc., Duluth, GA); whereas, ten

days after the last day of MGA, heifers in the G10 (n=28) treatment received GnRH. Seven and eight days after GnRH treatment, heifers in each respective treatment received 12.5 mg PG (i.m.; Lutalyse® Sterile Solution) on each day. The experiment was designed so that PG was administered on the same days for both the G3 and G10 treatment.

Heifers were observed for behavioral estrus for approximately 1 h at 0700 and 1700 h daily from MGA withdrawal until GnRH was administered for the G3 and G10 treatments. The same estrous detection protocol was also implemented during the 7 d after the initial PG treatment. All heifers received Kamar detectors (Kamar® Marketing Group, Steamboat Springs, CO) at MGA withdrawal and a new detector at the initial PG treatment to assist in estrous detection. Behavioral estrus was defined as a heifer standing to be mounted by another heifer and (or) signs of vaginal mucous. If a heifer was not detected in estrus by visual observation but had a Kamar that was one-quarter to completely activated, the heifer was considered to have been in behavioral estrus. A three-day estrous response after MGA withdrawal was determined for the G3 and G10 heifers combined and was defined as the total number of heifers exhibiting estrus within 3 d after MGA withdrawal divided by the total number of heifers in the G3 and G10 treatments. A five-day estrous response after MGA withdrawal was also determined for the G10 treatment and was defined as the total number of heifers exhibiting estrus within 5 d after MGA withdrawal divided by the total number of heifers in the G10 treatments. Estrous response after PG was determined as both a three-day estrous response and total estrous response within the G3 and G10 treatments heifers. Three-day estrous response after PG was defined as the total number of heifers exhibiting estrus within 3 d after the initial PG divided by the total treated. Total estrous response was defined as the total number of heifers displaying estrus within 7 d after the initial PG divided by the total number treated.

To evaluate what effect stage of follicle development (SOF) may have had on the effectiveness of GnRH to initiate ovulation, heifers within the G3 and G10 treatments were assigned to receive either no PG (G3, n = 6; G10, n = 6) or PG (12.5 mg i.m.; Lutalyse® Sterile Solution) on d 4 and 5 (G3, n = 5; G10, n = 5), 8 and 9 (G3, n = 8; G10, n = 7), or 12 and 13 (G3, n = 6, G10, n = 5) of MGA treatment to initiate luteolysis. These days were chosen to simulate variable periods of low level progesterone exposure during MGA comparable to heifers being at d 2 (no PG), 6 (PG d 12/13), 10 (PG d 8/9), or 14 (PG d 4/5) of the estrous cycle at the initiation of MGA treatment. The variable lengths of low level progestogen exposure were used to vary the duration of persistence of the dominant preovulatory follicle prior to MGA withdrawal. Blood plasma samples were collected via jugular veinipuncture from all heifers before they received PG during MGA to confirm the presence of a corpus luteum (CL) and at MGA withdrawal to confirm that luteolysis had occurred. Heifers in the d 6, 10, and 12 SOF groups were determined to be at the assigned SOF development if progesterone concentrations were ≥ 1 ng/mL at PG during MGA treatment followed by progesterone concentrations < 1 ng/mL at MGA withdrawal with the absence of a corpus luteum (CL). Heifers in the no PG group would have had progesterone concentrations that were ≥ 1 ng/mL at MGA withdrawal. Presence or absence of a CL was determined by transrectal ultrasonography (Aloka 500v, Corometrics Medical Systems, Wallingford, CT) equipped with a 7.5 MHz linear array transducer.

Ovaries of all heifers were evaluated using transrectal ultrasonography (equipped with a 7.5 MHz linear array transducer) at MGA withdrawal, GnRH, 48 h after GnRH, initial PG after GnRH, and 8 d after the expression of estrus for heifers that were observed in estrus after PG. At each ultrasonography evaluation, height and width of all luteal structures, luteal cavities, and follicles ≥ 3 mm in diameter were measured using the internal calipers of the ultrasonography

machine and their locations on the ovaries were recorded. Volume of the CL was calculated using the formula for volume of a sphere ($\pi d^3/6$). When a luteal cavity was present, its volume was subtracted from the volume of the outer sphere resulting in net luteal volume (CL volume). Additionally, blood plasma samples were collected via jugular veinipuncture at each ultrasonography examination and 48 h after the initial PG to determine plasma progesterone concentrations. Blood plasma samples were collected into evacuated tubes containing an anticoagulant (EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ). After collection, blood plasma samples were immediately placed on ice until they were centrifuged (3000 × g for 15 min) within 3 h after collection. Plasma was separated and stored at -20°C until analysis for progesterone concentrations, in multiple assays, as previously described. Intra- and interassay CV of the assays were 5.4 and 5.6%, respectively, and sensitivity of the assay was 0.1 ng/mL. Ovulation to GnRH was defined as the largest follicle present at GnRH followed by its disappearance at the ultrasonography exam 48 h after GnRH. Seven days after GnRH, the location of the ovulated follicle was verified by presence of a CL as determined by ultrasonography. The CL was deemed functional if progesterone concentrations were ≥ 1 ng/mL. Eight-days after the PG induced estrus, ovulation was confirmed by the presence of a functional CL as previously described. Heifers not exhibiting estrus within 7 d after PG underwent ultrasonography and blood sampling 10 d after the initial PG to assess ovarian function.

Because the G3 and G10 treatments were not started until either 3 or 10 d after MGA withdrawal, a three-day estrous response after MGA withdrawal for the G3 and G10 treatments combined was tested using the GENMOD procedure with SAS (SAS Inst. Inc., Cary, NC) with SOF development being the independent variable. The GENMOD procedure of SAS was also

used to test SOF effects within each treatment. Independent variables tested were treatment, SOF, and treatment × SOF effects. Dependent variables tested were ovulation rate to GnRH, ovulation rate after PG, estrous response after MGA withdrawal, percentage of heifers with follicles ≥ 10mm at GnRH, percentage of heifers with follicles ≥ 10mm at PG, and percentage of heifers with progesterone concentrations ≥ 1 ng/mL at PG. Additionally, three-day estrous response and total estrous response after PG was tested using the GENMOD procedure. Progesterone concentration and diameter of the largest follicle at MGA withdrawal for the G3 and G10 treatments combined were tested using the GLM procedure of SAS, with the independent viable being SOF development. Diameter of the largest follicle present at GnRH, diameter of the largest follicle present at PG, progesterone concentrations at PG, and progesterone concentrations 8 d following behavioral estrus were also tested using the GLM procedure of SAS. Independent variables tested were treatment, SOF, and treatment × SOF effects. Five heifers from the G3 and G10 treatments did not conform to the assigned SOF as mentioned previously and they were removed from all analyses.

Experiment 2 was conducted at two locations from December, 2004 to April, 2005. Yearling *Bos taurus* (Angus; n = 57) and *Bos taurus* × *Bos indicus* crossbred heifers (n = 178) from the Dicks' Brothers Farm, Lake City, FL (Location 1) and yearling *Bos indicus* × *Bos taurus* heifers (n = 117) from the Davis Ranch, Alachua, FL (Location 2) were used in Experiment 2. Prior to the start of the experiment, heifers at Location 1 were randomly distributed to one of two treatments and a BCS was recorded; whereas, heifers at Location 2 were equally distributed to one of two treatments by BCS and reproductive tract score (RTS; Anderson et al., 1991). Mean BCS (LSM ± S.E.) were 5.0 ± 0.05 for the *Bos taurus* and 5.0 ± 0.03 for *Bos indicus* × *Bos taurus* heifers at Location 1 and 5.4 ± 0.04 for *Bos indicus* × *Bos*

taurus heifers at Location 2. Mean RTS (LSM ± S.E.) was 3.8 ± 0.8 for heifers at Location 2. Heifers received MGA (0.5 mg•hd^{-1}•d^{-1}) for 14 d in a total mixed ration at Location 1 and in a high protein pellet fed at a rate of 2 lbs•hd^{-1}•d^{-1} at Location 2. Heifers in treatment 1 (MGA-PG) received 12.5 mg prostaglandin $F_{2\alpha}$ i.m. (PG; Prostamate® Sterile Solution; Agri Laboratories, Ltd. St. Joseph, MO.) 19 and 20 d following MGA withdrawal. Heifers in treatment 2 (MGA-G-PG) received 100 µg GnRH i.m. (Cystorelin, Merial, Inc., Duluth, GA) 3 d following MGA withdrawal with 12.5 mg PG i.m. (Prostamate®) 7 and 8 d after GnRH (MGA-G-PG). The MGA and GnRH treatments were staggered so the PG was delivered on the same days for both treatments. To aid in estrus detection, heifers received an Estrus Alert® heat detection patch (Estrus Alert®, Western Point, Inc, Merrifield, MN) at the second PG.

Estrus was visually detected two times daily at 0700 and 1700 for 3 d after the initial PG. Estrus was defined as a heifer standing to be mounted by another heifer and/or a 1/4 to full red Estrus Alert® patch. Eight to twelve hours after being detected in estrus, heifers were AI with frozen-thawed semen by a single AI technician at both locations. Multiple AI sires were used within each location and equally distributed between treatments. Heifers not detected in estrus by 72 h after the initial PG were timed inseminated (timed-AI) and received 100 µg GnRH i.m. (Cystorelin). Bulls were placed with heifers 10 d after timed-AI for both locations. Pregnancy to AI was determined approximately 55 d following timed-AI for both locations, using a real-time B mode ultrasound (Aloka 500v) equipped with a 5.0 MHz rectal transducer. Due to the 10 d interval where no heifers were bred by AI or the clean-up bull, differences in fetal size (Curran et al., 1986) were used to distinguish between a pregnancy resulting from AI or clean-up bull.

Three-day estrous response was defined as the number of heifers that exhibited estrus during the 3 d between PG and timed-AI, divided by the total number of heifers treated.

Conception rate was defined as the number of heifers observed in estrus, inseminated, and became pregnant, divided by the total number of heifers inseminated. The timed-AI pregnancy rate was defined as the number of heifers that became pregnant to timed-AI, divided by the total number of heifers timed-AI. Synchronized pregnancy rate was defined as the total number of heifers that became pregnant to either AI following estrus detection or timed-AI, divided by the total number of heifers treated. Thirty-day pregnancy rate was defined as the total number of heifers pregnant in the first 30 d of the breeding season divided by the total number of heifers treated.

The GENMOD procedure of SAS was used for the statistical analysis in Experiment 2. Within Location 1 data were initially analyzed with breed in the model to evaluate breed effects. The independent variables included treatment, breed, and treatment × breed, while the dependent variables were estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and 30 d pregnancy rate. If the breed and treatment x breed effects were significant ($P < 0.05$), data for the Angus heifers were analyzed separately from the *Bos indicus* × *Bos taurus* heifers for Location 1. Data from the *Bos indicus* × *Bos taurus* heifers from Locations 1 and 2 were combined and the independent variables included treatment, location, and treatment × location while the dependent variables included estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and 30 d pregnancy rate. Six heifers from Location 1 were removed from analysis. Three were determined to be freemartins at AI, one developed a vaginal infection prior to AI, and two were not present for pregnancy detection. Within Location 2 the effect of RTS was also evaluated. Independent variables included treatment, RTS, and treatment × RTS while the dependent variables included estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, or 30 d pregnancy rates.

Heifers with missing RTS data (n = 5) and those with a RTS of 2 (n = 2) were removed from the RTS analysis for Location 2.

Results

Experiment 1.

Stage of follicle development affected ($P < 0.05$) progesterone concentrations and diameter of the largest follicle at MGA withdrawal across the G3 and G10 treatments (Table 4-1). Heifers that did not receive PG during MGA were the only SOF group that had progesterone concentrations ≥ 1 ng/mL at MGA withdrawal. Progesterone concentrations were greater ($P < 0.001$) for d 2 compared to the d 6, 10, and 14 SOF groups, which were similar ($P > 0.05$) to each other. Diameter of the largest follicle at MGA withdrawal increased ($P = 0.001$) as the length of time that a follicle was exposed to a low-level progestin environment increased (Table 4-1). At MGA withdrawal, the d 14 SOF group had a greater ($P < 0.05$) follicle diameter compared to d 2, 6, and 10 SOF groups, and the d 10 SOF group had a greater ($P < 0.05$) largest follicle diameter compared to d 2 and 6 SOF groups, which were similar ($P > 0.05$) to each other.

Stage of follicle development also affected ($P = 0.02$) the three-day estrous response after MGA withdrawal across the G3 and G10 treatments. The estrous response was similar ($P > 0.05$) for the d 6 and 10 SOF groups, which were both greater ($P < 0.05$) compared to the d 2 and 14 SOF groups. The d 2 and 14 SOF groups had similar ($P > 0.05$) estrous responses. When only the five-day estrous response was evaluated for the G10 treatment, SOF development did not effect ($P = 0.27$) the five-day estrous response. The five-day estrous response was 65.2% (15/23) and the estrous responses for the d 2, 6, 10, and 14 SOF groups were 50.0% (3/6), 80.0% (4/5), 85.7% (6/7), and 40.0% (2/5), respectively.

Diameter of the largest follicle at GnRH was affected ($P < 0.05$) by treatment and SOF development but there was no treatment × SOF effect ($P = 0.21$; Table 4-2). Within the G3

treatment, the d 14 SOF group had a larger ($P < 0.05$) follicle diameter at GnRH compared to the d 2, 6, and 10 SOF groups, which were all similar ($P > 0.05$) to each other. Conversely within the G10 treatment, the d 2 SOF group had a smaller ($P < 0.05$) follicle diameter at GnRH compared to the d 6, 10, and 14 SOF groups, which were all similar ($P > 0.05$) to each other. When diameter of the largest follicle at GnRH was evaluated across the G3 and G10 treatments, diameter of the largest follicle at GnRH was similar ($P > 0.05$) for the d 2, 6, and 10 SOF groups. Whereas, heifers in the G3 treatment from the d 14 SOF group had a greater ($P < 0.05$) follicle diameter compared to the G10 treatment. Diameter of follicle ovulating to GnRH was not affected ($P > 0.05$) by treatment, SOF development, or treatment × SOF effect.

Total ovulation rate was affected ($P = 0.02$; Table 4-2) by treatment but not ($P > 0.05$) by SOF or treatment × SOF. More ($P = 0.02$) G3 (76.0%) heifers ovulated compared to G10 (47.8%) heifers. When treatments were compared across SOF groups, G3 heifers in the d 6 and 10 SOF groups had greater ($P < 0.05$) ovulation rates compared to G10 heifers in the d 6 and 10 SOF groups. Whereas, ovulation rate for the d 2 and 14 SOF groups were similar ($P > 0.05$) for the G3 and G10 treatments. Seventy-six percent (19/25) of G3 heifers had follicles ≥ 10 mm at GnRH but there was no SOF effect ($P = 0.27$) on follicles ≥ 10 mm for heifers in the d 2 (5/6; 83.3%), 6 (4/6; 66.7%), 10 (5/8; 62.5%), and 14 (5/5; 100.0%) SOF groups. For the G10 heifers, 73.9% had follicles ≥ 10 mm in diameter at GnRH and there was no SOF ($P = 0.13$) effect. The percentage of G10 heifers with follicles ≥ 10 mm at GnRH was 50.0 (3/6), 60.0 (3/5), 85.7 (6/7), and 100.0% (5/5) for d 2, 6, 10, and 14 SOF groups, respectively.

The percentage of heifers with a functional CL at PG was similar ($P = 0.59$; Table 4-3) between the G3 (84.0%) and G10 (78.3%) treatments. There was a SOF effect ($P = 0.01$), but no ($P = 0.89$) treatment × SOF effect on the percentage of heifers with a functional CL at PG.

Across SOF groups, more ($P < 0.01$) d 2 (100%), 6 (91%), and 10 (86.7%) heifers had a functional CL at PG compared to d 14 (40%) heifers. Additionally, more ($P = .01$) G10 (43.5%) compared to G3 (12.0%) heifers had two CL on their ovaries at PG. There were no ($P > 0.05$) SOF or treatment × SOF effects on the incidence of heifers with two CL on their ovaries at PG. Progesterone concentrations at PG were greater ($P = 0.01$) for G10 compared to G3 heifers (Table 4-3). Stage of follicle development also affected ($P = 0.01$) progesterone concentrations at PG but there was no ($P = 0.93$) treatment × SOF effect on mean progesterone concentration at PG. Across SOF groups, d 14 (2.4 ± 1.2 ng/mL) had decreased ($P = 0.01$) progesterone concentrations compared to d 2 (6.8 ± 1.1 ng/mL), 6 (7.0 ± 1.2 ng/mL), and 10 (7.7 ± 1.0 ng/mL) of which the later three were similar ($P > 0.05$) to each other. Of the heifers with a functional CL at PG, the PG induced luteolysis occurred in a similar ($P > 0.05$) percentage of G3 (21/21) and G10 (18/18) heifers.

Diameter of the largest follicle at PG was similar ($P = 0.67$; Table 4-3) for G3 and G10 treatments but SOF affected ($P = 0.01$) the diameter of the largest follicle at PG. Diameter of the largest follicle was greater ($P < 0.05$) for the d 14 (15.4 ± 1.0 mm) SOF group compared to d 2 (13.1 ± 0.9 mm), 6 (12.2 ± 0.9 mm), and 10 (11.2 ± 0.8 mm) SOF groups, whereas, diameter of the largest follicle was similar ($P > 0.05$) for the d 2, 6, and 10 SOF groups. There was no ($P = 0.54$) treatment × SOF effect on diameter of the largest follicle at PG.

More heifers (P = 0.01) in the G3 (76.0%) treatment exhibited estrus within 3 d after the initial PG compared to the G10 (43.5%) treatment (Table 4-4; Figure 4-1). In contrast, total estrous response was similar (P = 0.12) between the G3 and G10 treatments. There were no (P > 0.05) SOF or treatment × SOF effects on the 3 d and total estrous responses. Interval from PG to the onset of estrus was not affected ($P > 0.05$) by treatment or treatment × SOF (Table 4-4).

Conversely, there was ($P = 0.04$) a SOF effect on interval from PG to onset of estrus. The d 14 (60.0 ± 7.4 h) SOF group had a shorter ($P < 0.05$) interval from PG to the onset of estrus compared to d 2 (91.2 ± 7.0 h) and d 10 (79.7 ± 6.1 h) SOF groups, but the d 14 interval was ($P > 0.05$) similar compared to the d 6 interval (76.5 ± 7.1 h).

Of heifers that exhibited estrus after PG, a similar ($P = 0.19$) percentage of G3 (91.3%; 21/23) and G10 (100.0%; 19/19) heifers formed a functional CL by d 8 after the onset of estrus. There were two G3 heifers that exhibited estrus but failed to form a functional CL. Neither of these heifers had a functional CL at PG and both were in the d 14 SOF group. There were two G3 heifers that did not exhibit estrus after PG. There was a single heifer in the d 2 group that had a functional CL at PG and had regressed the CL by d 10 after PG. The other G3 heifer was in the d 10 SOF group, and this heifer did not have a functional CL at PG or 10 d following PG. There were four G10 heifers that were not observed in estrus. One heifer from each from the d 2 and 6 SOF groups had a functional CL at PG while the heifers from the d 2 SOF group did not have a functional CL 10 d after PG the heifers from the d 6 SOF group had a functional CL. The remaining two G10 heifers were from the d 10, and 14 SOF groups both of which had a functional CL at PG but did not have a functional CL 10 d after PG.

Progesterone concentrations 8 d after the PG induced estrus were similar ($P = 0.50$) between the G3 (4.8 ± 0.5 ng/mL) and G10 (5.2 ± 0.5 ng/mL) treatments and there were no ($P > 0.05$) SOF or treatment × SOF effects. Progesterone concentrations 8 d following the synchronized estrus for the d 2, 6, 10, and 14 groups were 5.3 ± 1.0, 5.3 ± 1.0, 4.8 ± 0.8, and 3.6 ± 1.0 ng/mL for G3 and 4.9 ± 1.0, 5.7 ± 1.0, 4.7 ± 0.9, and 5.6 ± 1.0 ng/mL for G10, respectively.

Experiment 2.

Within Location 1, there was ($P = 0.02$) a breed effect on estrous response and there were treatment × breed effects ($P < 0.05$) on timed-AI and synchronized pregnancy rates. Therefore, the Angus data was analyzed separately for Location1 and data for the *Bos indicus* × *Bos taurus* heifers in Locations 1 and 2 were analyzed together.

The three-day estrous response was not affected ($P > 0.05$) by treatment or treatment × location for the *Bos indicus* × *Bos taurus* heifers (Table 4-5). However, estrous response was greater ($P = 0.04$) for Location 2 (70/117; 59.8%) compared to Location 1 (85/178; 47.8%). For Angus heifers at Location 1, treatment had no effect ($P = 0.16$) on estrous response (Table 4-6).

There were no ($P > 0.05$) treatment or location effects on conception rate in *Bos indicus* × *Bos taurus* heifers; however, there was a ($P = 0.01$) treatment × location effect (Table 4-5) on conception rates. Conception rates were greater for the MGA-PG treatment at Location 2 and the MGA-G-PG treatment at Location 1 compared to the MGA-PG treatment at Location 1 and MGA-G-PG treatment at Location 2. Treatment had no effect ($P = 0.42$) on conception rates in Angus heifers at Location 2 (Table 4-6). For the *Bos indicus* × *Bos taurus* heifers, there were no ($P > 0.05$) treatment, location, and treatment × location effects on timed-AI pregnancy rates. For the Angus heifers, timed-AI pregnancy rate was greater ($P = 0.01$) for the MGA-PG compared to MGA-G-PG treated heifers (Table 4-6).

There were no ($P > 0.05$) treatment, location, or treatment × location effects on synchronized and 30 d pregnancy rates for *Bos indicus* × *Bos taurus* heifers. For the Angus heifers, synchronized pregnancy rates tended to be greater ($P = 0.08$) and 30 d pregnancy rates were similar ($P = 0.76$) for the MGA-PG compared to the MGA-G-PG treated heifers (Table 4-6).

Within Location 2, reproductive tract scores (RTS) were taken at the initiation of the experiment to evaluate the effect of RTS on response to the synchronization treatments. There were no ($P > 0.05$) treatment, RTS, and treatment × RTS effects on estrous response, timed-AI pregnancy rate, synchronized pregnancy rate, and 30 d pregnancy rate (Table 4-7). In general, as RTS increased from a 3 to either a 4 or 5, estrous response and synchronized pregnancy rate increased numerically. However, conception rate was greater ($P = 0.02$) for the MGA-PG (68.8%) treatment compared to the MGA-G-PG (41.9%) treatment. There were no ($P > 0.05$) RTS or treatment × RTS effects on conception rate.

Discussion

Recent research by Wood and co-workers (2001) indicated that yearling *Bos taurus* heifers treated with MGA for 14 d with GnRH 12 d after the last day of MGA followed by PG 7 d later had an improved synchrony of estrus compared to heifers synchronized with the traditional MGA-PG system. The reason for the increased synchrony of estrus was attributed to the ability of GnRH to synchronize follicle development. Recent research from our lab (See Chapter 3) indicated that follicle wave development during the 19 d after a 14 d MGA treatment was more variable in Brangus compared to Angus heifers. It was concluded that administering GnRH 12 d after MGA withdrawal in Brangus heifers may be too late because some heifers had already initiated a second follicle wave resulting in fewer heifers with follicle in the growing phase capable of ovulating to GnRH (Moreira et al., 2000). Therefore, it was hypothesized that administering GnRH 10 d after MGA withdrawal instead of 12 d may be more effective in Brangus heifers. It was also theorized that administration of GnRH 3 d after MGA withdrawal may actually be more effective since there was less variation in follicle development immediately after MGA withdrawal and a majority of follicles should be large enough to ovulate to GnRH. For that reason, Experiment 1 was designed to determine the effectiveness of

administering GnRH either 3 or 10 d after a 14 d MGA treatment in *Bos indicus* × *Bos taurus* heifers. Because follicle development can be significantly influenced by the stage of luteal development that animals are under the influence of during a long-term MGA treatment (Sirois and Fortune, 1990; Kojima et al., 1992), it was also of interest to evaluate what effect stage of follicle development at the end of the MGA treatment would have on response to the GnRH.

At MGA withdrawal, only the d 2 SOF group had progesterone concentrations indicative of luteal activity compared to d 6, 10, or 14 SOF groups, which all received PG during MGA. Diameters of the largest follicles at MGA withdrawal were smallest in the d 2 and 6 SOF groups compared to the d 10 and 14 SOF groups. The d 2 SOF group had the smallest follicle diameter, as they would have been near the end of the estrous cycle and probably in the middle of a follicle wave (Ginther et al., 1989) due to high luteal progesterone that initiated follicle turnover (Sirois and Fortune, 1990) just before MGA withdrawal. The d 6 SOF group had their CL regressed two days before MGA withdrawal resulting in the presence of newly developing dominant follicle under the influence of a low progesterone environment provided by MGA for approximately 2 d. In contrasts, the d 10 and 14 SOF groups had the largest follicle diameters at MGA withdrawal, which was a result of the dominant follicles being under low progesterone environments for approximately 5 d in the d 10 SOF group and 9 d in the d 14 SOF group. Consequently, the d 10 and 14 SOF groups had dominant follicles that continued to grow, develop, and persist on the ovaries during MGA due to the increased frequency of LH pulses observed during a low progesterone environment (Sirois and Fortune, 1990; Kojima et al., 1992). Therefore, the experimental model was effective in altering follicle development at the end of a 14 d MGA treatment.

The three-day estrous response following MGA withdrawal was approximately 53% across the d 6 and 10 SOF groups. Hill et al. (1971) reported that estrus occurred primarily between 3 to 7 d after treatment with a 14 d MGA withdrawal. It is likely that three days was an inadequate amount of time to allow for a LH surge and the onset of estrus in all animals, which is supported by the observation that approximately 83% of the d 6 and 10 heifers in the G10 treatment exhibited estrus within 5 d after MGA withdrawal. Therefore, heifers with follicles that have been under a low progesterone environment for approximately 2 to 5 d have an excellent opportunity to express estrus and ovulate within 5 d after the end of a 14 d MGA treatment. Consequently, exposure of dominant follicles to a low progesterone environment for approximately 2 to 5 d does not appear to compromise the ability of the follicles to secrete estrogen and ovulate after MGA withdrawal. In contrast, the three-day estrous response for the d 2 SOF group was only 8.3% but it increased to 83.3% by 5 d after MGA withdrawal in the G10 treatment. Heifers in the d 2 SOF group that did not exhibit estrus by d 3 were probably at the start of a new follicle wave at MGA withdrawal due to high luteal progesterone that initiated follicle turnover (Sirois and Fortune, 1990), which would have resulted in a delayed interval to estrus as the new follicle wave developed (Ginther et al., 1989). The d 14 SOF group also had a decreased 3 d estrous response of 20% and the estrous response increased to only 40% within by d 5 d after PG in the G10 treatment. In contrast to the d 2 SOF group, the d 14 SOF group had the largest follicle diameter at MGA withdrawal due to the development of long-term persistent dominant follicles (Sirois and Fortune, 1990; Kojima et al., 1992), which should have been secreting enough estrogen (Kojima et al., 1995) to induce an LH surge and the onset of estrus. However, this was not the case and the reason (s) for the decreased estrous response of the long-

term persistent dominant follicles in the d 14 SOF group could be several fold and will be discussed in subsequent paragraphs.

Across the four SOF groups in the G3 treatment, ovulation rate was a respectable 76%; although, whether ovulation occurred or did not occur tended to be influenced by SOF development at MGA withdrawal. For the G3 treatment, 93% (13/14) of the heifers in the d 6 and 10 SOF groups ovulated to GnRH compared to only 66.7% in the d 2 SOF group. The two heifers in the d 2 SOF group that did not ovulate to GnRH were probably heifers that were in the middle of a follicle wave. This is supported by the observation that they had elevated progesterone concentrations at MGA withdrawal, which probably initiated follicle turnover (Ginther et al., 1989). As a result, they did not have a growing follicle that was capable of ovulating to GnRH (Moriera et al., 2000). In contrasts, the d 14 group had the lowest percentage of heifers ovulating at only 40%, indicating that GnRH was not very effective in ovulating persistent dominant follicles that had been under low progesterone exposure for approximately 9 d. Reasons for the failure of GnRH to initiate ovulation in a majority of the d 14 SOF group is unclear. The increased LH pulse frequency and resulting increased estradiol concentrations observed when no CL is present during an MGA treatment (Kojima et al., 1995) may have depleted the stores of LH in the anterior pituitary. In cows that had previously been treated with MGA for 9 d, Kojima et al. (1992) did not detect a LH surge in 80% of cows after MGA withdrawal. Furthermore, the LH stores may be more easily depleted in cattle of *Bos indicus* breeding since they have less LH released in response to an exogenous GnRH treatment compared to cattle of *Bos taurus* breeding (Griffen and Randel, 1978; Portillo et al., 2007). One could also speculate that the increased LH pulse frequency observed during a low progesterone environment may have lead to down regulation of LH receptors in the granulosa cells of the

persistent dominant follicles leading to ovulation failure. Another explanation may be that long-term persistent follicles may become cystic (Sirois and Fortune, 1990; Mihm et al., 1994), and cannot undergo ovulation (Cook et al., 1990). Therefore, these results suggest that persistent dominant follicles that develop during a long-term MGA treatment have altered ovulatory capacities and the capacity to ovulate is probably influenced by the period of time that the follicles are under the influence of a low progesterone environment. Consequently, if the goal is to initiate ovulation in a majority of follicles to synchronize follicle development after an MGA treatment, it may prove advantageous to use short-term (7-9 d) MGA treatments to decrease the incidence of large persistent dominant follicles present during long term (14 d) MGA treatments. Additional research will need to be conducted to evaluate this.

Estrous response during the five days after MGA withdrawal for the G10 heifers was only 65.2%, which is similar to the seven day estrous response observed in Angus and Brangus heifers (See Chapter 3), but slightly less than *Bos taurus* heifers treated with a 14 d MGA treatment (Yelich et al., 1997). One explanation for the decreased estrous response could be the incidence of a "silent estrus", which is a frequent occurrence in cattle of *Bos indicus* breeding (Galina et al., 1982; Orihuela et al., 1983) and was observed in similar study conducted in our lab (See Chapter 3). This also appears to be the case in Experiment 1, since two of five G10 heifers that were not observed in estrus had a CL at GnRH. The ovulation rate to GnRH was only 47.8% in G10 heifers compared to 76.0% for the G3 heifers. Even though all SOF groups except the d 2 group had a follicle diameter > 10 mm for the G10 treatment, it did not equate into a large percentage of follicles ovulating to GnRH for the G10 treatment. The decreased ovulation to GnRH suggest that there was significant asynchrony of follicle development by d 10 after MGA withdrawal resulting in a limited number of follicles in the growing phase that were

capable of ovulating to GnRH (Moreira et al., 2000). Part of the reason for the follicle asynchrony was dictated by the low estrous response observed during the 5 d after MGA withdrawal. This is supported by the observation that more heifers that exhibited estrus after MGA withdrawal ovulated to GnRH compared to heifers not exhibiting estrus within 5 d of MGA withdrawal. Hence, the effectiveness of administering GnRH 10 d after MGA withdrawal is largely predicated on heifers exhibiting estrus within 5 d of MGA withdrawal. Certainly, d 10 is not the appropriate time to administer GnRH and it is unclear if waiting until d 12 would have provided a better response.

There was a similar percentage of G3 and G10 heifers with a functional CL at PG, but SOF development influenced the percentage of heifers with a functional CL. It should be noted that two G3 heifers in the d 2 group that did not ovulate to GnRH, had functional CL's at PG. The two heifers must have ovulated sometime after GnRH and been in the early luteal phase at PG. The d 14 heifers had significantly lower percentage of functional CL at PG than all other SOF groups for both the G3 and G10 treatments. Not only did GnRH not initiate ovulation in the d 14 SOF group in the G3 treatment, the presence of a persistent dominant follicle altered follicle development enough so that there was not a new follicle wave available to ovulate by d 10 after MGA in the G10 treatment. Clearly dealing with the persistent dominant follicle in cattle of *Bos indicus* breeding is difficult. It is unknown if similar responses are observed in *Bos taurus* cattle treated with a 14 d MGA treatment.

Heifers in the G10 treatment had increased progesterone concentrations at PG compared to G3 treated heifers, which was likely due to the increased incidence of heifers in the G10 treatment having two CL on their ovaries (Diaz et al., 1998) compared to G3 treated heifers. Therefore, some of the G10 heifers would have a CL from the estrus after MGA withdrawal and

a CL from the GnRH treatment. Two G10 heifers in the d 14 SOF group, one of which ovulated prior to GnRH and the other to GnRH, did not have a functional CL at PG. Lemaster et al., (1999) reported the presence of luteal tissue without any progesterone production in frequently worked cattle of *Bos indicus* breeding. However, since both heifers exhibited estrus shortly after PG, both heifers may have undergone a short estrous cycle accompanied by a short-lived luteal structure.

Treatment did not influence the diameter of the largest follicle at PG. However, diameter of the largest follicle of the *Bos indicus* × *Bos taurus* heifers in the present experiment are considerably less than the diameters observed by Wood et al. (2001) in MGA-G-PG treated *Bos taurus* heifers. Wood et al. (2001) reported a high percentage of heifers ovulating to GnRH administered 12 d after a 14 d MGA treatment resulting in a new follicle wave that was reaching maximal diameter when PG was administered 7 d after GnRH. In the current study, a high percentage of heifers that received GnRH 10 d after MGA did not ovulate to GnRH, which probably resulted in asynchronous follicle wave development when PG was administered 7 d after GnRH resulting follicles of various sizes. Diameters of the largest follicles at PG were greatest in d 14 SOF heifers for both the G3 and G10 treatments. Because daily ultrasound examinations were not conducted between MGA withdrawal and PG, it is difficult to determine if the largest follicle present at PG was either from a new follicle wave initiated by the GnRH treatment or the presence of persistent dominant follicles that were present at MGA withdrawal and still on the ovaries at PG. It is interesting to note that progesterone concentrations were < 1 ng/mL for G3 heifers in the d 14 SOF group, which may have resulted in increased pulsatile secretion of LH secretion resulting in increased follicle development (Sirois and Fortune, 1990; Kojima et al., 1992).

The G3 treatment proved to be more effective in synchronizing estrus as 76% of the heifers exhibited estrus during the three-days after PG, which was similar to the estrous response observed by Wood et al. (2001) in *Bos taurus* heifers receiving the MGA-G-PG treatment where GnRH was administered12 d after MGA withdrawal. It is interesting to note that all G3 heifers in the d 14 SOF group exhibited estrus by 3 d after PG. This suggests that the d 14 SOF group that did not respond to GnRH must have initiated a new follicular wave that was capable of initiating estrus and ovulating by approximately 12 d after MGA withdrawal. A similar type of follicle growth pattern was also observed for heifers that developed persistent dominant follicles during a 14 d MGA treatment and failed to ovulate the follicles within 5 d after MGA withdrawal (See Chapter 3). In comparison to other studies, the G3 treatment produced a substantially greater 3 d estrous response than the MGA-PG system in *Bos taurus* (Wood et al., 2001; Bridges et al., 2005) and *Bos indicus* × *Bos taurus* (Stevenson et al., 1996; Bridges et al., 2005) heifers. The percentage of heifers exhibiting estrus within the 7 d after PG is similar to other studies synchronizing *Bos taurus* heifers with either the MGA-PG (Brown et al., 1988, Lamb et al., 2000) or the MGA-PG system with GnRH (Wood et al., 2001). It should be noted that all of the heifers used in the present study were going through normal estrous cycles at the start of the experiment. Therefore, inducing an effective estrous response in *Bos indicus* × *Bos taurus* heifers with the MGA-PG estrous synchronization maybe predicated more by the estrous cycling status of heifers (Brown et al., 1988; Patterson et al., 1989) than by manipulation of the follicle wave dynamics of the heifers. Additional research will need to be conducted to completely characterize what effects cycling status (cycling vs., non-cycling) has on modifying follicle dynamics in the MGA-PG synchronization protocol.

Interval from PG to the onset of estrus in the current study was comparable to MGA-G-PG (GnRH administered 12 d after MGA withdrawal) treated *Bos taurus* heifers (Wood et al., 2001), but increased compared to MGA-PG treated Angus and Brangus heifers in chapter 3. Additionally, heifers that would have begun MGA at d 14 of their estrous cycle had the shortest interval from PG to estrus regardless of GnRH treatment. One reason for the shorter interval was the fact that the diameter of the dominant follicle at PG was greatest in d 14 SOF group regardless of GnRH treatment, which is supported by the observation made by Sirois and Fortune (1988) where size of the eventual ovulatory follicle at PG was negatively correlated with the interval to estrus.

The percentage of heifers with a functional CL 8 d after an observed estrus was similar between G3 and G10 treatments. It should be noted that three G10 treated heifers not detected in estrus had a CL present 10 d following PG. Furthermore, there were some abnormalities in ovulation and luteal development that occurred in the G3 heifers. Two G3 heifers that exhibited estrus failed to ovulate, which was confirmed by lack of a CL and progesterone concentrations < 1ng/mL 8 d after the onset of estrus. Certainly, some of the G10 heifers had a "silent estrus" (Galina et al., 1996) but it is not clear why the G3 heifers failed to ovulate and form a CL. The frequent working of the heifers could have resulted in abnormal luteal development where a CL is formed but it does not secrete any progesterone (Lemaster et al., 1999).

Results from Experiment 1 suggested that administering GnRH 3 d after a 14 d MGA treatment resulted in the most synchronous estrus. Therefore, the objective of Experiment 2 was to evaluate the effectiveness of the G3 (MGA-G-PG) treatment compared to a MGA-PG treatment in a field trial utilizing yearling *Bos indicus* × *Bos taurus* and *Bos taurus* heifers.

The three-day estrous response of the *Bos indicus* × *Bos taurus* heifers in Experiment 2 was similar between the MGA-PG and the MGA-G-PG treatments and comparable to a report by Bridges et al. (2005) but substantially less compared to a report by Stevenson et al. (1996) in *Bos indicus* × *Bos taurus* heifers synchronized with the MGA-PG system. One factor that influences estrous response is the number of heifers going through estrous cycles at the start of the synchronization treatment (See Chapter 3; Brown et al., Patterson and Corah, 1992). The importance of having heifers going through estrous cycles is also supported by the results of Experiment 1 where 72% of the heifers exhibited estrus in the first 3 d after PG. Although, estrous cycling status was not determined in Experiment 2, the RTS data from Location 2 suggest that cycling status had a major influence on estrous response. Heifers with a RTS of 4 and 5, indicative of heifers that are probably going through normal estrous cycles, had an estrous response of 70% in the MGA-G-PG heifers compared to 42.9% in MGA-G-PG heifers with a RTS of 3.

Conception rates were similar between the MGA-PG and the MGA-G-PG treatments. Although no studies utilizing a similar MGA-G-PG treatment are available for comparison, conception rates for the MGA-G-PG were similar to *Bos indicus* × *Bos taurus* heifers synchronized with the MGA-PG treatment (Bridges et al., 2005). However, there was a significant treatment by location interaction indicating that the treatment responses were different between locations. Exact reasons for the differential response to treatments between locations are unclear. It does not appear that AI technician or AI sire had an effect on conception rates, since a single AI technician inseminated all heifers at both locations and AI sires were equally stratified across treatments. However, when conception rates were evaluated within Location 2 for the *Bos indicus* × *Bos taurus* heifers, the MGA-PG heifers had a 28% greater

conception rate compared to the MGA-G-PG. The trend for decreased conception rates for the MGA-G-PG was consistent across the three RTS. Additionally, the MGA-G-PG Angus heifers had a lower conception rate compared to MGA-PG Angus heifers. The similar trend of decreased conception rates in both the Angus and *Bos indicus* × *Bos taurus* heifers is of concern. It is possible that some of the heifers that exhibited estrus after PG could be heifers that did not ovulate to GnRH but the heifers initiated a new follicle wave that initiated the expression of estrus and ovulation around the time of PG. The questions that need to be answered are if these follicles are fertile or not and if the lack of progesterone exposure before estrus and ovulation resulted in short estrous cycles after the PG (Berardinelli et al., 1979; Evans et al., 1994).

One of the reasons that producers like to use the MGA-PG system is that it consistently results in excellent conception rates during the synchronized estrus (Brown et al., 1988; Lamb et al., 2000) in heifers of *Bos taurus* breeding. In contrasts, conception rates of *Bos indicus* × *Bos taurus* heifers synchronized with MGA-PG protocols (See Chapter 3; Bridges et al., 2005) are highly variable and are still considerably less than studies in *Bos taurus* heifers synchronized with MGA-PG systems (Brown et al., 1988; Lamb et al., 2000). Age at puberty may have something to do with this since cattle of *Bos indicus* breeding (Plasse et al., 1968; Baker et al., 1989) attain puberty at older ages than *Bos taurus* breeds (Wiltbank et al., 1966; Baker et al., 1989). As a result, reaching puberty at older ages may have decreased the number of estrous cycles heifers had before synchronization and AI resulting in decreased conception rates (Byerley et al., 1987; Galina et al., 1996).

A similar percentage of MGA-PG and MGA-G-PG treated *Bos indicus* × *Bos taurus* heifers became pregnant to the timed-AI, but the timed-AI pregnancy rate was considerably less compared to an experiment conducted by Bridges and coworkers (2005) in yearling *Bos indicus*

× *Bos taurus* heifers synchronized with an MGA-PG protocol receiving consecutive split treatments of PG. Additionally, timed-AI pregnancy rates were substantially reduced in MGA-G-PG compared to MGA-PG treated Angus heifers. The reduced timed-AI pregnancy rate in the MGA-G-PG compared to MGA-PG treatment may be a result of when the timed-AI was conducted relative to the ability of GnRH to induce ovulation (Moreira et al., 2000). Furthermore, the timed-AI groups also contain heifers that are not cycling, did not respond to PG, or did not have any follicles large enough to ovulate to GnRH. Any of these three scenarios would result in decreased pregnancy rates to the timed-AI

Synchronized pregnancy rates were similar between the MGA-PG and MGA-G-PG treated *Bos indicus* × *Bos taurus* heifers in Experiment 2, which are similar to synchronized pregnancy rates of MGA-PG treated *Bos indicus* × *Bos taurus* heifers (Bridges et al., 2005). As observed with estrous response, cycling status probably has more influence on synchronized pregnancy rates than any other single variable (Brown et al., 1988; Patterson et al., 1989). This can be partially explained by the reproductive tract score data from Location 2. Heifers with a RTS of 4 or 5, indicative of heifers that, by definition, are going through normal estrous cycles, had a synchronized pregnancy rate of 44.4% across the MGA-PG and MGA-G-PG treatments compared to 34.0% in the heifers with a RTS of 3. For Angus heifers, synchronized pregnancy rates tended to be greater in MGA-PG compared to MGA-G-PG. The major reasons for the decreased synchronized pregnancy rate for the Angus heifers were due to the numerically lower conception rate and the significantly lower timed-AI pregnancy rate for the MGA-G-PG heifers compared to the MGA-PG heifers.

In summary, the G3 treatment improved the synchrony of estrus compared to G10 treatment in *Bos indicus* × *Bos taurus* heifers in Experiment 1. Stage of follicle development at

the end of a 14 d MGA treatment had significant effects on largest follicle size at MGA withdrawal and the subsequent estrous response after MGA withdrawal. Heifers that developed a long-term (> 9 d) persistent dominant follicle under the influence of a low progesterone environment had a detrimental effect on the subsequent estrous response after MGA withdrawal and ability to ovulate to GnRH, suggesting that long-term persistent follicles have a reduced capacity to ovulation capacity. Additional experiments will need to be evaluated to either regress or ovulate long-term persistent follicles developed under a 14 MGA environment, or utilization of short-term (< 9 d) progestin treatments may need to be used to prevent the occurrence of long-term persistent follicles. In Experiment 2, the MGA-G-PG (G3) protocol failed to increase estrous response over the MGA-PG system in *Bos indicus* × *Bos taurus* heifers. Synchronized pregnancy rates of the MGA-G-PG and MGA-PG systems were similar but the synchronized pregnancy rates from Experiment 2 are still considerably less compared to other reports in *Bos taurus* and *Bos indicus* × *Bos taurus* heifers synchronized with the MGA-PG system.

Implications

The addition of GnRH 3 d after withdrawal of a 14 d MGA treatment resulted in a greater synchrony of estrus compared to GnRH 10 d after MGA. However, heifers that would have started MGA late in the luteal phase of the estrous cycle and developed persistent dominant follicles did not respond well to the GnRH treatments. Therefore, future research needs to address dealing with persistent dominant follicles developed in low progesterone environments in relation to their prevention and (or) removal from the ovary in heifers of *Bos indicus* × *Bos taurus* breeding. The estrous response, conception rate, and synchronized pregnancy rate were similar for MGA-G-PG compared to the traditional MGA-PG system. Therefore, administering

GnRH 3 d after a long term MGA treatment does not appear to improve the effectiveness of the MGA-PG estrous synchronization system in heifers of *Bos indicus* × *Bos taurus* breeding.

Table 4-1. The effect of stage of follicle (SOF) development during a 14 d melengestrol acetate (MGA) treatment on progesterone concentration (LSM ± S.E.) at MGA withdrawal, diameter of the largest follicle at MGA withdrawal (LSM ± S.E.), and three day estrous response following MGA withdrawal. Data are combined for G3 and G10 heifers (Experiment 1). [a]

SOF	n	Progesterone concentration, ng/mL	Diameter of largest follicle, mm	Three day estrous response, % [b]
d 2	12	5.5 ± 0.4 [c]	12.6 ± 1.0 [c]	8.3 [c]
d 6	11	0.3 ± 0.5 [d]	13.8 ± 0.9 [c]	54.5 [d]
d 10	15	0.2 ± 0.4 [d]	16.4 ± 0.8 [d]	53.3 [d]
d 14	10	0.3 ± 0.5 [d]	20.4 ± 1.0 [e]	20.0 [c]
P-value		0.001	0.001	0.02

[a] See material and methods for SOF descriptions.
[b] Estrous response is the total number of heifers exhibiting estrus out of the total number treated within 3 d of MGA withdrawal.
[c,d,e] Means within a column without a common superscript differ ($P < 0.05$)

Table 4-2. Effect of treatment (T) and stage of follicle (S) development on largest follicle diameter at GnRH (LSM ± SE), diameter of follicle ovulating to GnRH (LSM ± SE), and ovulation rate for heifers receiving GnRH either 3 d (G3) or 10 d (G10) after withdrawal of a 14 d melengestrol acetate (MGA) treatment (Experiment 1).

	Stage of follicle development group [a]				P-values		
Variable	2	6	10	14	T	S	T × S
Largest follicle GnRH, mm					0.01	0.01	0.21
G3 (25)	12.5 ± 1.3 (6)^{d,x}	13.2 ± 1.3 (5)^{d,x}	13.3 ± 1.2 (8)^{d,x}	20.2 ± 1.4 (5)^{e,x}			
G10 (23)	9.7 ± 1.3 (6)^{d,x}	12.6 ± 1.4 (5)^{e,f,x}	12.0 ± 1.2 (7)^{e,f,x}	14.2 ± 1.4 (5)^{f,y}			
Follicle ovulating to GnRH, mm					0.32	0.20	0.11
G3 (15) [b]	12.8 ± 1.0 (4)	15.0 ± 1.0 (4)	15.6 ± 0.9 (5)	11.5 ± 1.4 (2)			
G10 (11)	11.3 ± 1.2 (3)	13.7 ± 1.2 (3	12.0 ± 1.2 (3)	14.5 ± 1.4 (2)			
Ovulation rate, % [c]					0.02	0.12	0.34
G3 (25)	66.7 (6)^{d,x}	100.0 (6)^{e,x}	87.5 (8)^{d,x}	40.0 (5)^{d,x}			
G10 (23)	50.0 (6)^{d,x}	60.0 (5)^{d,y}	42.9 (7)^{d,y}	40.0 (5)^{d,x}			

[a] See material and methods for description of stage of follicle development at MGA withdrawal.
[b] Four G3 heifers exhibited estrus before GnRH so their data were not include in the size of follicle ovulating to GnRH analysis but the heifers were included in the ovulation rate data.
[c] Ovulation rate for G3 defined as number of heifers ovulating after an observed estrus within 3 d after MGA withdrawal and (or) GnRH 3 d after MGA withdrawal divided by the total in the group. Ovulation rate for G10 treatment defined as the number of heifers ovulating to GnRH 10 d after MGA withdrawal divided by the total in the group
^{d,e,f}Means within a variable and row without a common superscript differ ($P < 0.05$).
^{x,y,z}Means within a variable and column without a common superscript differ ($P < 0.05$)

Table 4-3. Percentage of heifers with a functional corpus luteum (CL), progesterone concentration (LSM ± S.E.), and diameter of the largest dominant follicle at prostaglandin $F_{2\alpha}$ (PG: LSM ± S.E.) for G3 and G10 heifers across different stages of follicle (SOF) development (Experiment 1).[a]

Treatment (SOF)	n	Functional CL at PG, %[b]	Progesterone concentration at PG, ng/mL	Diameter of the largest follicle at PG, mm
G3 mean	25	84.0	4.3 ± 0.8	12.8 ± 0.6
d 2	6	100.0	5.6± 1.6	12.5 ± 1.2
d 6	6	100.0	5.0 ± 1.6	12.2 ± 1.2
d 10	8	87.5	5.7 ± 1.4	10.3 ± 1.1
d 14	5	40.0	1.0 ± 1.7	16.2 ± 1.4
G10 mean	23	78.3	7.7 ± 0.8	13.2 ± 0.6
d 2	6	100.0	8.0 ± 1.6	13.7 ± 1.2
d 6	5	80.0	9.1 ± 1.7	12.2 ± 1.4
d 10	7	85.7	9.8 ± 1.5	12.1 ± 1.1
d 14	5	40.0	4.0 ± 1.7	14.6 ± 1.4
P-values				
Treatment		0.59	0.01	0.67
SOF		0.01	0.01	0.01
Treatment × SOF		0.89	0.93	0.54

[a] Heifers administered MGA for 14 d followed by GnRH either 3 (G3) or 10 d (G10) after MGA withdrawal. Seven days after GnRH, heifers received PG.
[b]Functional CL at PG defined as a heifer having progesterone concentrations ≥ 1 ng/mL combined with the presence of a CL as determined by ultrasonography

Table 4-4. Three-day estrous response, total estrous response, and interval from prostaglandin $F_{2\alpha}$ (PG) to onset of estrus following PG treatment for G3 and G10 heifers across different stages of follicle (SOF) development (Experiment 1).[a]

Treatment (SOF)	n	Three-day estrous response, %[b]	Total estrous response (%)[c]	Interval from PG to estrus, h
G3 mean	25	76.0	92.0	71.7 ± 4.6
d 2	6	50.0	83.3	79.2 ± 9.9
d 6	6	83.3	100.0	72.0 ± 9.0
d 10	8	75.0	87.5	75.4 ± 8.3
d 14	5	100.0	100.0	60.0 ± 9.9
G10 mean	23	43.5	82.6	82.1 ± 5.1
d 2	6	33.3	83.3	103.2 ± 9.9
d 6	5	40.0	80.0	81.0 ± 11.0
d 10	7	42.9	85.7	84.0 ± 9.0
d 14	5	60.0	80.0	60.0 ± 11.0
P-values				
Treatment		0.01	0.12	0.14
SOF		0.17	0.67	0.04
Treatment × SOF		0.63	0.52	0.69

[a]Heifers administered melengestrol acetate (MGA) for 14 d followed by GnRH either 3 (G3) or 10 d (G10) after MGA withdrawal. Heifers received 12.5 mg of PG on both d 7 and 8 after GnRH.
[b]Three-day estrous response is the number of heifers observed in estrus within 3 d of the initial PG treatment divided by the total number of heifers treated.
[c]Total estrous response is the number of heifers observed in estrus within 7 d of PG treatment divided by the total number of heifers treated.

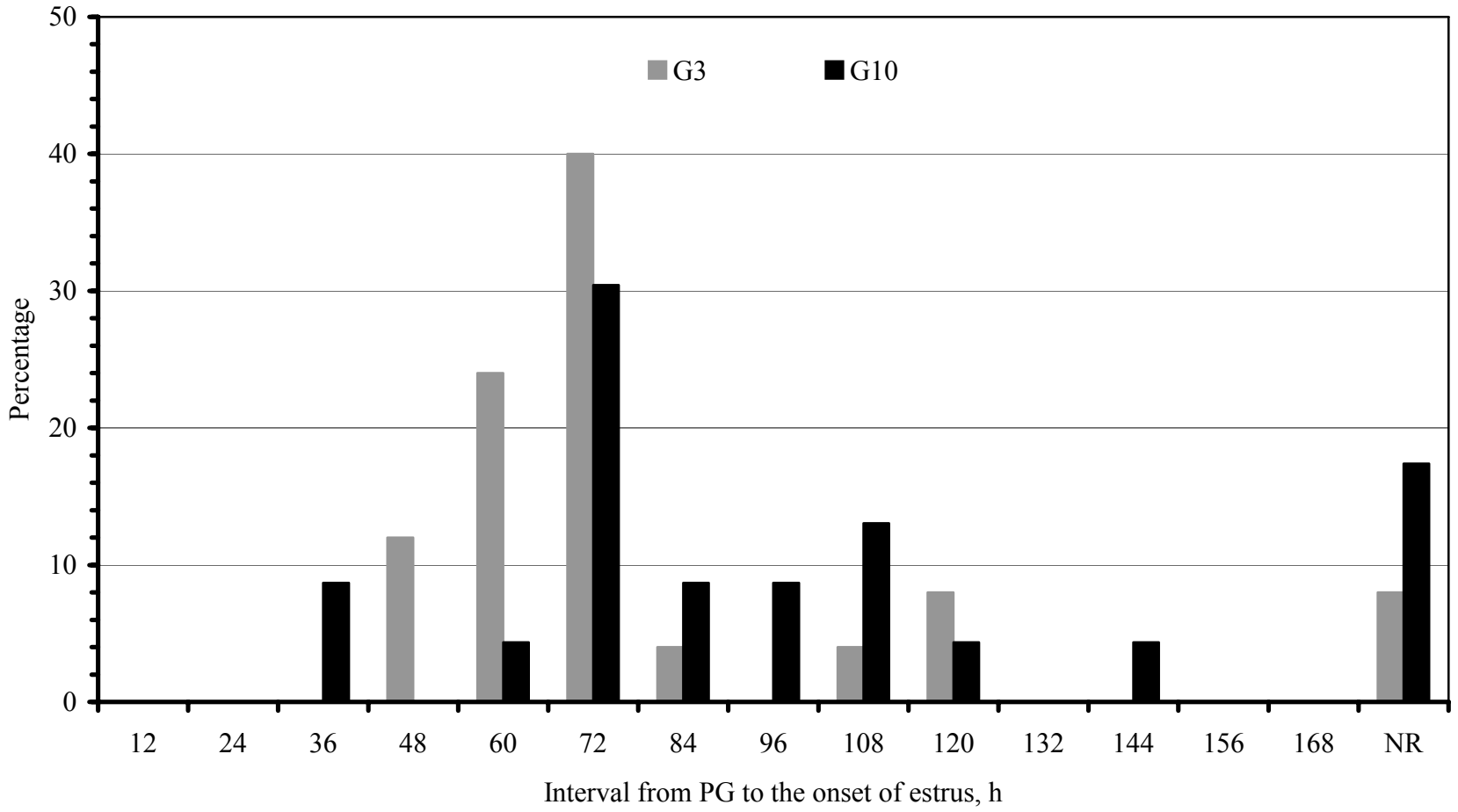


Figure 4-1. Estrous response, expressed as a percentage of the total number of heifers in a group, during the 7 d after the initial PG treatment for G3 (n = 25) and G10 (n = 23) treatments. NR = no estrous response (Experiment 1) .

Table 4-5. Estrous, conception and pregnancy rates of *Bos taurus* x *Bos indicus* heifers synchronized with combinations of melengestrol acetate (MGA), GnRH (G), and prostaglandin $F_{2\alpha}$ (PG) at two locations (LOC) (Experiment 2).

Treatment (TRT)[a]	n	LOC	Estrous Response (%)[b]	Conception rate (%)[c]	Timed-AI pregnancy rate (%)[d]	Synchronized Pregnancy rate (%)[e]	30 d pregnancy rate (%)[f]
MGA-PG	89	1	38/89 (42.7)	16/38 (42.1)	13/51 (25.5)	29/89 (32.6)	62/89 (69.7)
MGA-PG	58	2	33/58 (57.0)	23/33 (69.7)	4/25 (16.0)	27/58 (46.6)	41/58 (70.7)
Overall means	147		71/147 (48.3)	39/71 (54.9)	17/76 (22.4)	56/147 (38.1)	103/147 (70.1)
MGA-G-PG	89	1	47/89 (52.8)	28/47 (59.6)	7/42 (16.7)	35/89 (39.3)	62/89 (69.7)
MGA-G-PG	59	2	37/59 (62.7)	16/37 (43.2)	5/22 (22.7)	21/59 (35.6)	43/59 (72.9)
Overall means	148		84/148 (56.7)	44/84 (52.4)	12/64 (18.8)	56/148 (37.8)	105/148 (71.0)
P-values							
TRT			0.18	0.55	0.91	0.74	0.84
LOC			0.04	0.46	0.83	0.38	0.69
TRT × LOC			0.73	0.01	0.28	0.13	0.84

[a] Both treatments received MGA for 14 d. MGA-PG heifers received PG 19 (12.5 mg) and 20(12.5 mg) d after MGA withdrawal. The MGA-G-PG heifers received GnRH (100 μg) 3 d after MGA withdrawal with PG 7 (12.5 mg) and 8 (12.5 mg) d after GnRH. Estrus was detected for 3 d, and heifers exhibiting estrus were AI 8-12 h later. Heifers not displaying estrus were timed-AI 72-80 h and received GnRH.
[b] Percentage of heifers displaying estrus 3 d after PG of the total treated.
[c] Percentage of heifers that were pregnant to AI of the total that exhibited estrus and were AI.
[d] Percentage of heifers pregnant to timed-AI 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 synchronized breeding of the total treated.

Table 4-6. Estrous, conception and pregnancy rates of Angus heifers in Location 1 synchronized with combinations of melengestrol acetate (MGA), GnRH (G), and prostaglandin $F_{2\alpha}$ (PG) (Experiment 2).

Treatment [a]	n	Estrous response (%)[b]	Conception rate (%)[c]	Timed-AI pregnancy rate (%)[d]	Synchronized pregnancy rate (%)[e]	30 d pregnancy rate (%)[f]
MGA-PG	27	15/27 (55.6)	6/15 (40.0)	5/12 (41.7)	11/27 (40.7)	19/27 (70.4)
MGA-G-PG	30	22/30 (73.3)	6/22 (27.3)	0/8 (0.0)	6/30 (20.0)	20/30 (66.7)
P-values						
Treatment		0.16	0.42	0.01	0.09	0.76

[a] Both treatments received MGA for 14 d. MGA-PG heifers received PG 19 (12.5 mg) and 20 (12.5 mg) d after MGA withdrawal. The MGA-G-PG heifers received GnRH (100 μg) 3 d after MGA withdrawal with PG 7 (12.5 mg) and 8 (12.5 mg) d after GnRH. Estrus was detected for 3 d, and heifers exhibiting estrus were AI 8-12 h later. Heifers not displaying estrus were timed-AI 72-80 h and received GnRH.
[b] Percentage of heifers displaying estrus 3 d after PG of the total treated.
[c] Percentage of heifers that were pregnant to AI of the total that exhibited estrus and were AI.
[d] Percentage of heifers pregnant to timed-AI 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 synchronized breeding of the total treated.

Table 4-7. Estrous, conception, timed-AI, pregnancy rates by treatment (TRT) and reproductive tract score (RTS) for *Bos taurus* × *Bos indicus* heifers synchronized with combinations of melengestrol acetate (MGA), GnRH (G), and prostaglandin $F_{2\alpha}$ (PG) at Location 2 (Experiment 2).[a]

RTS	TRT	Estrous response (%)[b]	Conception rate (%)[c]	Timed-AI pregnancy rate (%)[d]	Synchronized pregnancy rate (%)[e]	30 d pregnancy rate (%)[f]
3	MGA-PG	15/26 = 57.7	10/15 = 66.7	0/11 = 0.0	10/26 = 38.5	17/26 = 65.4
	MGA-G-PG	9/21 = 42.9	3/9 = 33.3	3/12 = 25.0	6/21 = 28.6	13/21 = 61.9
4	MGA-PG	12/19 = 63.2	8/12 = 66.7	1/7 = 14.3	9/19 = 47.4	14/19 = 73.7
	MGA-G-PG	14/21 = 66.7	6/14 = 42.9	1/7 = 14.3	7/21 = 33.3	19/21 = 90.5
5	MGA-PG	5/11 = 45.5	4/5 = 80.0	3/6 = 50.0	7/11 = 63.6	9/11 = 81.8
	MGA-G-PG	9/12 = 75.0	4/9 = 44.4	1/3 = 33.3	5/12 = 41.7	7/12 = 58.3
Total	MGA-PG	32/56 = 57.1	22/32 = 68.8	4/24 = 16.7	26/56 = 46.4	40/56 = 71.4
	MGA-G-PG	32/54 = 59.3	13/32 = 40.6	5/22 = 22.7	18/54 = 33.3	39/54 = 72.2
P-values	TRT	0.51	0.02	0.27	0.12	0.95
	RTS	0.37	0.75	0.11	0.31	0.11
	TRT × RTS	0.20	0.90	0.15	0.91	0.17

[a] Both treatments received MGA for 14 d. MGA-PG heifers received PG 19 (12.5 mg) and 20(12.5 mg) d after MGA withdrawal. The MGA-G-PG heifers received GnRH (100 μg) 3 d after MGA withdrawal with PG 7 (12.5 mg) and 8 (12.5 mg) d after GnRH. Estrus was detected for 3 d, and heifers exhibiting estrus were AI 8-12 h later. Heifers not displaying estrus were timed-AI 72-80 h and received GnRH.

[b] Percentage of heifers displaying estrus 3 d after PG of the total treated.

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

[d] Percentage of heifers pregnant to timed-AI 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 synchronized breeding of the total treated.

CHAPTER 5
SUMMARY

The primary objective of Experiment 1 (Chapter 3) was to evaluate follicular development in yearling Angus and Brangus heifers during the 19 d period between MGA withdrawal and PG, and to evaluate the follicle development and estrous response following PG. A primary objectives of Experiments 2 and 3 (Chapter 4) were to determine the optimal timing to implement GnRH during the period from MGA withdrawal to PG and to evaluate the subsequent estrous response and fertility in *Bos indicus* × *Bos taurus* heifers.

In Experiment 1, yearling Angus and Brangus heifers were synchronized with the MGA-PG system with PG was administered 19 after MGA withdrawal. During the period between MGA withdrawal and PG, follicle development patterns were characterized by daily ultrasonography and the follicle development patterns were different between Angus and Brangus heifers. Factors contributing to the differences in follicle development were the decreased number of Brangus heifers that exhibited estrus during the 7 d after MGA withdrawal compared to Angus heifers and the increased incidence of three and four follicle wave patterns in Brangus compared to Angus heifers. From days 9 to 13 after MGA withdrawal, the percentage of follicle ≥ 10 mm steadily increased to 100% by d 13 in Angus heifers but increased in Brangus heifers up to d 10 but started to decrease to a low of 50% by d 13 in Brangus heifers. Although follicle development between MGA withdrawal and PG was different between Angus and Brangus heifers, diameter of the largest follicle at PG, estrous response, and interval from PG to estrus were similar between Angus and Brangus heifers. Conversely, when the number of follicle waves was evaluated, there was an interaction between breed and number of follicle waves on the interval from PG to estrus. Angus heifers displaying three follicle waves had a longer interval from PG to estrus compared to two wave Angus and Brangus and three wave

Brangus heifers. Therefore, the variation in follicle wave development between MGA withdrawal and the PG administered 19 d later needs to be altered in order to improve the synchrony of estrus following PG.

In a recent report by Wood and coworkers (2001), GnRH was included in the MGA-PG estrous synchronization system 12 d after MGA withdrawal in *Bos taurus* heifers to synchronize follicle development at the PG treatment. However, it does not appear that administering GnRH 12 d after MGA withdrawal would be as effective in Brangus heifers due to a decreased percentage of Brangus heifers with large growing follicles capable of ovulating to GnRH 12 d after MGA withdrawal. It appears that introducing a GnRH treatment would need to occur approximately 10 d after MGA withdrawal, when a high percentage of Brangus have large growing follicles capable of ovulating to GnRH. Furthermore, it may actually be better to administer GnRH soon after MGA (3 to 4 d) withdrawal where most heifers have large follicles and variation in follicle development is minimal.

In Experiment 2, cycling *Bos indicus* × *Bos taurus* heifers that were on day two of the estrous cycle were administered GnRH either 3 (G3) or 10 (G10) d after the last day of a 14 d MGA treatment followed by PG 7 d after GnRH. In addition, three groups of heifers received two consecutive PG treatments at predetermined days during MGA to imitate heifers starting MGA at different stages of the estrous cycle (SOC). The four stages included d 2, 6, 10, and 14. Administering GnRH three days after the last day of MGA withdrawal was more effective in initiating ovulation compared to d 10 after MGA withdrawal. The decreased ovulation rate to GnRH in G10 likely resulted in asynchronous follicle development at PG, which was partially due to the poor estrous during the five days after MGA withdrawal. Furthermore, the GnRH treatment was not very effective at inducing ovulation in long-term persistent follicles (d 14

SOC) regardless of treatment, which demonstrated the negative effects persistent follicles have on an estrous synchronization system. The synchrony of estrus following PG was substantially improved for G3 compared to G10 as evidence by the greater 72 h estrus response of the G3 treated heifers.

Because administering GnRH 3 d following MGA withdrawal provided the greatest synchrony of estrus when PG was administered 7 d after GnRH, it was decided to evaluate the fertility of the G3 treatment in a field trial in Experiment 3. Experiment 3 was conducted to evaluate the MGA-G-PG (G3) system compared to the traditional MGA-PG system in Angus and *Bos indicus* × *Bos taurus* heifers. The only exception was heifers received estrus detection and AI for 72 h, at which time all non-responders were timed-AI and received GnRH. Unlike Experiment 2, the MGA-G-PG treatment failed to increase the percentage of *Bos indicus* × *Bos taurus* heifers in estrus within 72 h after PG. Conception, timed-AI pregnancy, and synchronized pregnancy rates were similar for MGA-G-PG and MGA-PG treatments.

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BIOGRAPHICAL SKETCH

Steaven A. Woodall, Jr. was born in Tampa, Florida on December 02, 1978 to Steve and Paula Woodall of Plant City, Florida. Steaven has one sibling, Priscilla, and he is the oldest of the two children. Steaven attended several schools during his childhood and graduated from Durant Senior High School where he was an active member of the Durant FFA. Throughout his high school years, Steaven was a member of the Florida Junior Limousin Association and was active in showing cattle throughout the Southeast. After high school, Steaven was employed by Sun State International Trucks while attending Hillsborough Community College on a Florida Bright Futures Scholarship, where he received his A.A. degree. In August 2002, Steaven enrolled at the University of Florida to pursue his B.S. At the University of Florida, Steaven joined the Alpha Gamma Rho fraternity, where he held the office of Vice Noble Ruler of Management and Operations, and served as kitchen manager. Steaven graduated in August 2004 and accepted a graduate assistant position, starting the next semester, in the Department of Animal Sciences at the University of Florida under the direction of Dr. Joel Yelich. In addition to his own research duties, Steaven had the opportunity to conduct a laboratory section of the Reproductive Physiology course as well as assist research in many aspects of reproductive physiology. Steaven's future plans are to pursue a career in the beef cattle industry focusing on bovine reproduction.