THE EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON FERTILITY OF BEEF COWS

By

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To my entire family and my wife Maria Fernanda
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THE EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON FERTILITY OF BEEF COWS

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Chair: Cliff G. Lamb
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Two experiments were conducted to evaluate whether Human Chorionic Gonadotropin (hCG) administered 7 days before initiating the CO-Synch + Controlled Internal Drug Releasing (CIDR) estrous synchronization protocol (Exp. 1 and 2), or replacing GnRH with hCG at the time of insemination (Exp. 1), would improve pregnancy rate to a fixed time artificial insemination (TAI) in suckled beef cows. In addition, we evaluated the effect of hCG on follicle dynamics, corpus luteum (CL) development, and concentration of progesterone (P4). In Exp. 1, cows were stratified by days postpartum, age, and parity and randomly assigned to one of four treatments in a 2x2 factorial arrangements: 1) Cows received GnRH at CIDR insertion (d -7) and 25 mg of prostaglandin F$_{2\alpha}$ (PGF) at CIDR removal (d 0), followed in 64 to 68 hr by a TAI with a second injection of GnRH at TAI (CG; n = 29); 2) same as CG but the second injection of GnRH at the time of insemination was replaced by hCG (CH; n = 28); 3) same as CG, but cows received hCG 7 d (d -14) prior to CIDR insertion (HG; n = 28); and 4) same as HG, but cows received hCG 7 d (d -14) prior to CIDR insertion (HH; n = 29). Pregnancy rates were 52%, 41%, 59%, and 38% for GG, GH, HG, and HH, respectively. Cows
receiving hCG (39%) in place of GnRH at TAI tended (P = 0.06) to have poorer pregnancy rates than those receiving GnRH (56%). Pre-treatment with hCG increased (P < 0.05) the percentage of cows cycling at d -7, increased (P < 0.02) P4 concentration on d -7 and decreased (P < 0.001) the size of the dominant follicle on d 0 and 3, compared to control. In Exp. 2, cows were stratified based on days postpartum, body condition score (BCS), breed type, and calf sex and then assigned to the CG (n = 102) or HG (n = 103) treatments. Overall pregnancy rates were 51%, but no differences in pregnancy rates were detected between treatments, breed, days postpartum, or calf sex. Pre-treatment with hCG increased (P < 0.05) the percentage of cows cycling on d -7 and increased (P < 0.05) concentrations of P4 on d -7. We concluded that pre-synchronization with hCG 7 d prior to initiation of the CO-Synch + CIDR protocol failed to enhance pregnancy rates, but replacing GnRH with hCG at the time of AI may reduce pregnancy rates.
CHAPTER 1
INTRODUCTION

The goal of cow-calf operations is to produce one calf per cow per year. This requires significant management oversight to ensure that these goals are achievable. The gestational length of beef cattle averages approximately 285 d (Lush, 1937; Rife et al., 1943; Foote et al., 1960), which results in less than 85 d in which a cow must conceive to achieve an annual calving rate of one calf per cow per year. After parturition, the uterus needs to involute and recover from gestation and parturition in order for establishment and maintenance of pregnancy (Buch et al., 1955). Complete uterine involution occurs within 20 to 50 days in beef cows (Perkins and Kidder, 1963). The opportunity for conception during the period of uterine involution is poor. Therefore, in addition to the 285 d of gestation and 30 d of uterine involution the result is that within less than 60 d cows must initiate postpartum estrous cycles and conceive to maintain a yearly calving interval. Therefore, producers need to incorporate reproductive management procedures to improve cow reproductive efficiency and reduce the length of postpartum anovulation. However, incorporation of reproductive management procedures does not overcome sound nutritional management to ensure that cows are capable of initiating estrous cycles early postpartum.

One method of enhancing reproductive efficiency is to utilize estrous synchronization (ES) and artificial insemination (AI). Effective fixed-time AI (TAI) systems have been developed (Larson et al., 2006) to reduce the labor associated with insemination of cows. To ensure the greatest response to ES and AI, increasing the percentage of cows cycling at the initiation of the breeding season is paramount (Lamb et al., 2009). The technology now exists to successfully achieve acceptable fertility in
replacement beef heifers and postpartum beef cows after applying TAI protocols, especially considering that, in many cases, a high percentage of females may not be cycling at the onset of treatment (Stevenson et al., 2000). Initiation of fertile ovulation in noncycling cows is likely the primary manner in which beef producers may improve fertility in response to estrous synchronization and TAI protocols (Lamb et al., 2009). Use of progestin-based presynchronization before implementation of a TAI protocol for cows and heifers enhances fertility (Patterson et al., 1995; Busch et al., 2007). In contrast, supplementation of a progestin after TAI failed to improve fertility in beef cows (Larson et al., 2009), but administration of hCG to induce an accessory CL and increase concentrations of progesterone may effectively enhance pregnancy rates in beef cattle (Dahlen et al., 2009). Development of TAI protocols that reduce the hassle factors associated with ovulation synchronization and AI provide cattle producers efficient and effective tools for improving cattle genetics in their operations. Location variables, however, that may include differences in pasture and diet, breed composition, body condition, postpartum interval, climate, and geographic location, may affect the success of TAI protocols.
CHAPTER 2
LITERATURE REVIEW

The Bovine Estrous Cycle

The bovine estrous cycle involves several hormones, proteins and other factors produced by different organs of the body that play a significant role on certain tissues resulting in successful fertilization, gestation, and eventual birth of a new calf. It is well known that hypothalamic hormones (gonadotropin releasing hormone, GnRH), pituitary hormones (follicle stimulating hormone, FSH; luteinizing hormone, LH), ovarian hormones (progesterone, P4; inhibin; estradiol, E2), as well as uterine hormones (prostaglandin F2α, PGF), are the primary hormones responsible for initiating, maintaining, and ceasing follicular and luteal development.

The bovine estrous cycle normally ranges from 17 to 24 days (Wishart, 1972; Salisbury et al., 1978) and is commonly categorized into 4 stages: 1) proestrus: occurring on d 2 to 5 before estrus and is a period of transition from P4 dominance to E2 dominance and is initiated by corpus luteum (CL) regression by PGF secreted by the uterus; 2) estrus: The time that the dominant follicle is producing significant concentrations of E2, stimulating LH surge and subsequent ovulation 25 to 30 h after the onset of estrus (initiation of acceptance of mount; Christenson et al., 1975; Bernard et al., 1983); 3) metestrus: occurs between 1 and 5 d after estrus and is the transition of E2 dominance to P4 dominance with the initial development of the CL (the CL is still refractory to PGF); 4) diestrus: typically occurs between d 5 to 15 after estrus and is characterized by the fully developed CL with high concentrations of P4 and responsiveness to PGF.
**Folliculogenesis and Follicular Waves**

**Primordial and primary follicles.** Follicular development is initiated by primordial follicles with a single layer of flattened granulosa cells (GC) without a zona pellucida (Roche et al., 1998). Primordial follicles are often referred to as quiescent, non-growing, or resting follicles (Ruth, 2002). However researchers have demonstrated DNA activity on GC of the primordial follicles (Oktay et al., 1995; Lundy et al., 1999). The initiation of follicle growth is characterized by proliferation of GC, changing from a flattened to cuboidal shape, enlargement of the oocyte, and formation of the zona pellucida (Ruth, 2002). Primordial follicles are differentiated from primary follicles following the change in shape of the GC from flattened to cuboidal (Roche et al., 1998). Granulosa cells appear to be essential for follicle development and oocyte growth (Ruth, 2002). The differentiation of GC is under influence of certain activation factors such as KL/cKit interaction, leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), bone morphogenic protein 4 (Bmp4) and others (Wandji et al., 1996). The proliferation of GC, hypertrophy of the oocyte, and appearance of the zona pellucida on the oocyte are characteristics of the secondary follicle (Roche et al., 1998). Growth and differentiation factor-9 (GDF-9) and bone morphogenic protein-15 (BMP-15) interact with KL (produced in the granulosa cells) controlling GC proliferation (Ruth, 2002). The secondary follicle also has an outer layer of cells noted as theca cells. Theca cells contain luteinizing hormone (LH) receptors and also are responsible for converting cholesterol into testosterone that later are converted into estradiol (E2) by the GC (Fortune, 1986; Ginther et al., 1996).

**Pre-antral and antral follicles.** The development of the secondary follicle to pre-antral and antral follicles is dependent upon the gonadotropin hormones, FSH and LH
(Fortune, 1994). Lack or suppression of FSH blocks new wave emergence (Turzillo and Fortune, 1993). Suppressing and abolishment of LH pulses (Prendiville et al., 1996) and blocking of follicular growth at 4 to 5 mm of diameter (Prendiville et al., 1995) was observed when heifers were immunized against gonadotropin releasing hormone (GnRH). Factors and hormones such as activin, IGF-1, IGFBP (Roberts and Echternkamp, 2003), insulin, and E2 up-regulate follicle function stimulating GC proliferation and FSH/LH receptors (Fortune, et al., 2004). Inhibin, follistatin, and anti-mullerian hormone (AMH) down regulate FSH follicle responsiveness (Kaneko et al., 1993,1997; Durlinger et al., 2001). The development of a cavity filled with fluid (antrum) is a characteristic of a tertiary or antral follicle. The fluid contains numerous hormones (i.e., E2 and P4), glycoconjugates, lipoproteins, glucose metabolites, amino acids and others that are necessary to assist with further follicle and oocyte development (Gerard et al., 2002).

**Follicular waves.** Follicular waves of the bovine estrous cycle were first described by Rajakosi (1960). Each follicular wave is initiated with the emergence of a cohort of follicles 4 to 5 mm in diameter (recruitment; Knopf et al., 1989). The cohort of follicles grows during the subsequent 2 to 3 d. A single follicle (8 to 9 mm in diameter) continues to grow (selection) and growth of the remaining follicles is terminated and they undergo atresia (Adams et al., 1992; Hamilton et al., 1995, Ginther et al., 1989). The selected follicle is the dominant follicle and continues to grow until it reaches the maximum diameter for 3 to 6 d before regressing in the presence of high P4 concentration (Adams et al., 1992; Hamilton et al., 1995). When luteolysis occurs while the dominant follicle is growing (low P4), the follicle is destined to ovulate rather than
undergo atresia (Kastelic et al., 1990). Beef females may have two (Ginther et al., 1989; Knopf et al., 1989; Rajamahendran and Taylor, 1991), three (Savio et al., 1988; Sirois and Fortune, 1988), or four (Rhodes et al., 1995) follicular waves during an estrous cycle.

**Hormonal Regulation of the Estrous Cycle**

**Follicular phase.** The follicular phase is described as a period when follicle growth, ranging from 3 to 5 d in length without CL activity. Therefore, lower concentration of P4 in the blood drives the dominant follicle to release high concentrations of E2, stimulating the cascade of GnRH and LH release from the hypothalamus and pituitary gland, respectively (Chenault et al., 1975; Imakawa et al., 1986).

Gonadotropin releasing hormone is produced by the hypothalamus and reaches the anterior pituitary via the portal blood system. The anterior pituitary is stimulated, producing and releasing gonadotropic hormones FSH and LH (Schally et al., 1971). Follicle stimulating hormone is important during the recruitment of a cohort of follicles (Ireland, 1987). Blood concentration of FSH rise immediately prior to initiation of each follicular wave, decreasing following deviation of the dominant follicle (Adams et al., 1992). During the first 3 d of the follicular wave, a cohort of growing follicles undergoes continuous selection. Only a few follicles reach a diameter greater than 3 mm and usually only a single follicle (dominant follicle) grows to more than 10 mm in diameter for each follicular wave (Matton et al., 1981). Deviation occurs when the divergence of growth rate between the two largest follicles within a wave occurs between d 2 and 4 after the emergence of a new follicular wave (Savio et al., 1988, 1993; Sirois and Fortune, 1988; Ginther et al., 1996). The dominant follicle has greater concentrations of
LH receptors (Xu et al., 1995a; Bao and Garverick, 1998) and estradiol-17β (Badinga et al., 1992; Fortune, 1994) in follicular fluid compared with the subordinated follicles. Increases in E2 are dependent upon gonadotropic stimulation (Schallenberger et al., 1984; Walters et al., 1984). The dominant follicle produces inhibin that reduces steroidogenesis and decreases E2 in subordinate follicles and also inhibits other follicles to develop (Kaneko et al., 1997). Estradiol (Butler et al., 1983; Quirk and Fortune, 1986; Price and Webb, 1988) and inhibin (Ireland et al., 1983; Kaneko et al., 1997) causes a negative feedback signal on the anterior pituitary resulting in decreased FSH release. With low concentrations of FSH the subordinate follicles cease to grow.

After deviation, LH stimulates growth of the dominant follicle either until the dominant follicle ovulates or initiates apoptosis and undergoes atresia (Schallenberger et al., 1984). Atresia of the dominant follicle occurs at the first follicular wave when a cow experiences two follicular waves and at the first and second follicular waves when a cow experiences three follicular waves. Ovulation occurs after development of the last follicular wave of the estrous cycle (Diaz et al., 1998). Greater concentrations of E2 enhances the amplitude of LH pulses stimulating the LH surge (Stumpf et al., 1991; Walters et al., 1984) and, consequently, ovulation occurs 25 to 35 h following the onset of the pre-ovulatory surge of LH (Chenault et al., 1975; Christenson et al., 1974; Bernard et al., 1983).

**Luteal phase.** The CL initiates secretion of P4 shortly after ovulation. Low concentrations of P4 are detected between d 2 and 3 and then begin to increase by d 4 of the estrous cycle. Between d 7 and 12 P4 reaches its highest concentration in blood (Donaldson and Hansel, 1965). Concentrations of P4 are maintained elevated until d 18
to 19 of the estrous cycle and decline thereafter if regression of the CL occurs (Wettemann et al., 1972; Echternkamp and Hansel, 1973; Walters et al., 1984).

Progesterone causes a negative feedback on the hypothalamus changing the pattern of GnRH release and consequently LH secretion (Beck et al., 1976; Stumpf et al., 1993). During the luteal phase, low concentrations of E2 and high concentrations of P4 in blood drives low frequency and high amplitude pulses of LH. During the follicular phase, high concentrations of E2 and low concentrations of P4 causes low amplitude and high frequency pulses of LH secretion, stimulating the LH surge (Rahe et al., 1980; Ireland and Roche, 1982; Lucy et al., 1992). High concentration of P4 blocks the LH surge and consequently ovulation (Short et al., 1979). The consequence of blocking the LH surge causes atresia of the dominant follicle and initiation of a new follicular wave. Ovulation occurs when PGF is secreted by the uterus and causes lyses of the CL (Niswender, 2000). The CL ceases to produce and secrete P4 resulting in a decline of systemic concentrations of P4. Low concentrations of P4 stimulate follicle growth and high frequency of LH pulses (Rahe et al., 1980). High LH pulsatility stimulates growth of the dominant follicle that increases the release of E2.

**Human Chorionic Gonadotropin (hCG)**

**Biological Function and Action of hCG**

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the human embryo soon after conception and by the syncytiotrophoblasts (trophoblasts cells that invade the uterine wall), which stimulates P4 production and sustain pregnancy early in gestation (Pierce and Parsons, 1981). Human chorionic gonadotropin has a molecular weight of 36.7 kDa and is composed of 244 amino acids, with an α and β subunit. The α subunit is 92 amino acids long and is identical to that of
LH, FSH, and thyroid-stimulating hormone (TSH). The β subunit is 145 amino acids in length and is unique to the hCG molecule (Tegoni et al., 1999). For use as an exogenous treatment, hCG can be extracted from urine of pregnant women or by genetic modification.

Human chorionic gonadotropin interacts with the LH/gonadotropin receptor on the ovary preventing CL demise and stimulates production of P4 (Pratt et al., 1982; Fricke et al., 1993; Schmitt et al., 1996). Kayisli et al. (2003) demonstrated that hCG may provide an immunological effect to enhance embryonic survival. They demonstrated that hCG-treated endometrial cells induced apoptosis in both endometrial and T-cells, suggesting that hCG may play a role in peritrophoblastic immune tolerance. This immune function improves the invasion of the trophoblast cells, which is responsible for fetal development in the endometrium (Kayisli et al., 2003).

The hCG molecule binds to the LH/hCG receptors of follicular cells and activates the G-protein attached to the receptor internally in the cell (Marsh, 1970). The G-protein, detaches the receptor and activates the cAMP second messenger system resulting in activation of protein kinase A (PKA; Hunzicker-Dunn M, 1981). After binding to cAMP protein kinase releases its catalytic unit and initiates phosphorilation of the proteins. Phosphorilated proteins bind to DNA and activate gene expression. The physiologic consequences of gene expression in follicular cells are follicle maturation, follicle ovulation, and CL formation in females (Flynn et al., 2008).

Human chorionic gonadotropin is widely used to determine pregnancy status in women and is the first practical indicator of implantation of the fertilized embryo (Hussa, 1981). Because of the similarity of the α LH subunit, hCG can be used to initiate
ovulation of follicles in other species, including cattle. Treatment with hCG has been used to improve fertility in woman (Filicori et al., 2005) and also has been used in cattle for the same purpose (Sheffel et al., 1982; Pratt et al., 1982; Price and Webb, 1989; Lamb et al., 2009). When administered to cattle, hCG increased blood concentrations of P4 by forming an accessory CL resulting in enhanced pregnancy rates in cattle (Fricke et al., 1993; Schmitt et al., 1996; Santos et al., 2001). In males, hCG stimulated Leydig cells (testicular cells) to produce testosterone (Langope, 1973), which is necessary for spermatogenesis (sperm production).

**Applications of hCG in cattle**

The goal of researchers developing reproductive management protocols systems are to enhance fertility in cattle by controlling the estrous cycle with applications of exogenous hormones. The protocols need to provide successful results with low variability in results and must be cost effective. Therefore, searching for less expensive and more efficient drug use for estrous synchronization systems that decrease labor and are easy for cattle producers has led to the incorporation of hCG into ovulation control systems.

**Exogenous Hormonal Control of Ovulation**

Exogenous gonadotropin agonists are able to cause ovulation of the dominant follicle. The primary hormone responsible for the development of a follicle after deviation to ovulation in cattle is LH (Ireland and Roche, 1983; Xu et al., 1995). When GnRH is released from the hypothalamus it stimulates the production and secretion of LH from the anterior pituitary. Therefore, exogenous GnRH has been used to initiate follicular waves in cattle by ovulating the dominant follicle to initiate the emergence of a new follicular wave (Garverick et al., 1980; Bao and Garverick, 1998; Sartori et al., 2001).
Additional gonadotropin agonists as well as equine chorionic gonadotropin (eCG; Laster et al., 1971; Langford, 1982; Cline et al., 2001), and hCG (Pratt et al., 1982; Sheffel et al., 1982) have been utilized for the same purpose.

The mechanism by which gonadotropin agonists promote ovulation varies by each pathway after administration. For example, exogenous administration of GnRH acts on the pituitary gland stimulating the secretion of FSH and LH. Therefore the mechanism of ovulation occurs via the pituitary-ovarian pathway (Hansel and Convey, 1983). Conversely, hCG acts directly on the ovary binding to the LH/hCG receptors, stimulating ovulation (Filicori et al., 2005).

**Ovulation Capacity of Gonadotropins**

The use of hCG for ovulation in cattle has been the target of research for decades. The primary focus of these researchers was to investigate the capability of hCG to induce ovulation/luteinization of follicles (Pratt et al., 1982; Sheffel et al., 1982; Price and Webb, 1989; Diaz et al., 1998; Fricke et al., 1993; Burns et al., 2008; Dahlen et al., 2009) as well as alter the secretory dynamics of the original and the induced accessory CL (Fricke et al., 1993, Schmitt et al., 1993, 1996; Santos et al., 2001). Administration of hCG has been used to replace GnRH because of its greater capacity to ovulate follicles.

In postpartum cows, no difference was detected in the number of cows that ovulated a follicle after receiving 200 µg of GnRH or 2,500 IU of hCG (Pratt et al., 1982). In addition, cows in both treatments had a greater number of CL 12 d after treatment compared to untreated. The capability of GnRH or hCG to ovulate follicles appears to vary depending the day of the estrous cycle (Price and Webb, 1989;
Vasconcelos et al., 1999). Fricke et al. (1993) observed an ovulation in all 5 cows when 1,500 IU of hCG was injected on d 6 of the estrous cycle. Similarly, administering 3,300 IU of hCG 5 d after AI in high producing lactating dairy cows resulted in 86.2% of cows that formed a new CL (Santos et al., 2001). In addition, administration of 3,300 IU of hCG in lactating Holstein cows induced follicles to ovulate in 78% of cows compared to 60% of cows receiving 100 µg of GnRH (Stevenson et al., 2006). A GnRH agonist (8 µg of Buserelin) was equally effective as 3,000 IU of hCG (1,000 IU administered i.v. plus 2,000 IU administered i.m.) at inducing accessory CL when injected on d 5 or 6 after estrus in dairy heifers (Schmitt et al., 1996). Dahlen et al., 2009 comparing two different doses of hCG (500 or 1000 IU) and GnRH (100 µg) given a random day of the estrous cycle observed a greater percentage of ovulations occurring in anestrous heifers receiving hCG (87.5 and 73.7% for 1,000 and 500 IU of hCG, respectively) compared to GnRH (43.8%).

Factors that Influence Initiation of Ovulation

Stage of the follicular wave: The ability of the follicle to ovulate appears to be dependent of the capacity of the LH to bind to LH receptors in mature follicles independently of the follicle size and day of the estrous cycle. Large follicles that lose their dominance and initiate atresia frequently do not respond to treatment and fail to ovulate. The responsiveness to gonadotropin treatment is influenced by growing, static, or regressing phases of the follicular wave (Roche et al. 1998). When cows with follicles larger than 10 mm in diameter received GnRH, ovulation rates varied depending on the phase of the follicular wave (Pursley et al. 1995). It appears to be essential that the dominant follicle achieves ovulatory capacity and responsiveness to LH when LH
receptors are up-regulated. Ovulation rates of follicles were lowest between day 10 and 16 of the estrous cycle and were associated with follicles that were no longer dominant and failed to respond to LH (Ireland and Roche, 1993; Roche et al., 1998; Vasconcelos et al., 1999).

**Presence of estrogen:** Estrogen active follicles (those with high ovulation capacity) have more receptors for hCG and LH on granulosa and theca cells than E2 inactive follicles (those with lower ovulation capacity; Ireland and Roche, 1983). Filicori et al. (1999) observed that hCG, at low doses (50 IU), can be used through mid to late follicular phase in woman to mimic the effect of LH on follicular development. The hCG binds to LH receptors on granulosa and theca cells increasing E2 production. This mechanism could be responsible for improving follicle development, increasing follicle size, increasing volume of the follicular fluid, and enhancing estrogen synthesis (Filicori et al., 1999).

**Half-Life of exogenous gonadotropins:** The half-life of exogenous GnRH and hCG differs in cattle. Also the amount and time-to-peak of LH release from the anterior pituitary varies with different types and doses of GnRH (Chenault et al., 1990). Chenault et al. (1990) observed a dose dependent increase of LH concentration in blood after GnRH administration. Testing different sources and doses of GnRH (fertirelin 25, 50, 100, and 200 µg; gonadorelin 100, 250, and 500 µg; buserelin 10 and 20 µg) they reported that the time to peak of LH was 87 min for fertirelin, 148 min for buserelin, and 65 min for gonadorelin. Interestingly, the authors noted that fertirelin acetate was 4 to 10 times more potent than gonadorelin, buserelin was 10 to 20 times more potent than fertirelin and the highest recommended doses for gonadorelin (500 µg) for use in cattle
induced a significantly lower LH peak than the lowest dose of buserelin (10 µg). In contrast, exogenous hCG was detected more than 30 h after administration to cattle (Schmitt et al., 1996).

It is hypothesized (Bousfield et al., 1996) that the longer half-life of hCG compared with LH is due to the longer hCG β subunit compared with LH β subunit (145 vs. 121 amino acids, respectively). The extended half-life of hCG could potentially influence follicle maturation, improving the ovulatory capacity of small follicles compared with 4 h of LH stimulus after GnRH injection. Karsch et al., (1997) tested the efficacy of E2 to stimulate GnRH release and reported that pulses of GnRH rise just before the LH surge and remain high (10 to 25 pg/min) for at least 10 more hours after LH concentration drops in ewe. They observed that at least some concentrations (not well determined) of GnRH was required to promote the LH surge in ewes.

**Luteinization of the Dominant Follicle**

Luteinizing hormone plays an important role in follicle deviation, maturation and ovulation. In addition, LH is responsible for luteinization of the granulosa and theca cells after ovulation of a follicle (Farin et al., 1988). Luteinization is characterized by transformation of granulosa and theca cells into luteal cells (Donaldson and Hansel, 1965). The LH surge drives the transformation of the luteal cells and the acquisition of capacity to synthesize steroid hormones as P4 and E2 (Farin et al., 1988). Niswender et al. (2000) described that the luteinization of the granulosa cells involves accumulation of lutein (yellow pigment), vacuolization, hypertrophy of the endoplasmatic reticulum, and enlargement of the cell. The granulosa cells become “large luteal cells” and are the most responsible for P4 and E2 synthesis. The theca cells become “small luteal cells”
and are less steroidogenic active and have no secretory granules (Niswender et al., 2000). Luteinization of granulosa and theca cells forms the CL. The development and maintenance of the CL is also dependent upon LH. Hypophysectomy (Denamur et al., 1966) or injection of anti-bovine luteinizing hormone (Fuller and Hansel, 1970), both decreasing LH, causes reduction in P4 production in sheep.

Since 1931, hCG has been demonstrated to have an effect on the life-span of the CL (Katzman et al., 1931). The life of the bovine CL was prolonged by daily injections of hCG from d 15 to 26 after estrus in cycling heifers (Wiltbank et al., 1961).

**Luteinization of the CL following gonadotropin treatment.** Administration of GnRH or hCG on d 5 of the estrous cycle caused ovulation of the dominant follicle and appearance of a new CL (Schmitt et al., 1996a,b). Pratt et al. (1982) reported that the life-span of the CL induced by GnRH was shorter than that induced by hCG even though plasma concentrations of P4 increased in both GnRH and hCG treated cattle when an accessory CL was formed. Interestingly, the difference in concentrations of plasma concentrations of P4 between the GnRH and control group was observed 6 d after treatment on d 11 of the estrous cycle, whereas the difference between hCG and control group was first observed as early as 3 d after treatment on d 8 of the estrous cycle. In addition, the authors noted that hCG treated cows had consistently greater concentrations of P4 than cows treated with GnRH. Therefore, they hypothesized that the difference in concentrations of P4 may be related to the differing structures of LH and hCG.

The high concentrations of P4 in blood during the luteal phase decreases gene expression for encoding the β-subunit of LH (Brann et al., 1993); therefore, when GnRH
is administrated the anterior pituitary gland requires sufficient stores of LH to be released. However, hCG mimics LH and acts directly on LH receptors, bypassing the pituitary pathway (Filicori et al., 2005). When a new CL is formed concomitant with an old active CL, the LH release could be impaired by high concentrations of P4 (Rahe et al., 1980; Savio et al., 1993).

**Characteristics of the CL after gonadotropin treatment:** Schmitt et al. (1996a) observed that hCG-induced CL contain more luteal cells than GnRH-induced CL. The authors observed that CL formed after hCG injection were heavier than GnRH-induced CL but no difference in the number of luteal cells per surface area were observed. These findings may be responsible for greater concentrations of P4 after injection of hCG than GnRH between d 6 and 13 of the estrous cycle reported in their results. Schmitt et al. (1996a) also reported that induced CL cells in culture medium produced more P4 for the hCG-induced CL than GnRH-induced CL when exposed to various doses of LH. However, plasma concentrations of P4 between d 14 and 17 of the estrous cycle did not differ between hCG, GnRH, or controls (no treatment) applied on d 5 of the estrous cycle when the newly formed accessory CL was removed on day 13. In addition, a greater percentage of stage 1 and 2 luteal cells (characteristic of a developing CL) on original CL was observed, indicating that LH and hCG had a stimulatory effect on granulosa and theca cells of the original CL. The hypothesis proposed by Fricke et al. (1993) that concentrations of P4 differs from GnRH and hCG because of modification of the original CL was not observed by Schmitt et al. (1996a). When a single dose of hCG (1,000, 2,000, or 4,000 IU) was administered to heifers 3 d after ovulation, an increase in size of the original CL removed on d 9, 10, or 11 of the
The estrous cycle was observed (Veenhuizen et al., 1972). The size of the luteal tissue was related to enlargement of granulosa cells (large luteal cells, LLC) and multiplication of theca cells (small luteal cells, SLC). Luteinizing hormone and hCG have been reported to stimulate other factors such as fibroblast growth factor, vascular endothelial growth factor (VEGF), and growth hormone (GH) all of which may be responsible for modification of granulosa and theca cells (Grazul-Bilska et al., 1995). The differences in dynamics of LH-like exposure plus the extended half-life of hCG may contribute to greater stimulation of the LH receptors. Therefore, perhaps hCG induces a greater luteinization of the pre-ovulatory follicle and subsequent differentiation into an active CL than does GnRH. Although the effect of LH or hCG on CL development is essential, increased LH exposure during the early luteal phase did not reduce the incidence of short estrous cycles in postpartum beef cows (Carruthers et al., 1986).

**Luteolysis**

Gonadotropin hormones, such as LH or hCG, have been reported to be essential for formation and maintenance of the CL (Wiltbank et al., 1961; Fricke et al., 1993; Burke et al., 1994; Peters et al., 1994; Grazul-Bilska et al., 1995; Diaz et al., 1998). The luteolytic effect on the CL was initially associated with lack of gonadotropins (Wiltbank et al., 1961). Prior to these reports, estrogen was associated with concentrations of LH when observations indicated that low concentrations of estrogen during mid-cycle would decrease concentrations of LH and consequently cause regression of the CL (Moss et al., 1954). Subsequently, researchers demonstrated the luteolytic potential of PGF in rats (Pharriss and Wyngarden, 1969). The interaction between PGF synthesis and E2 was demonstrated when injection of tamoxifen (an E2 antagonist), followed by treatment of E2 during the luteal phase of heifers caused an inhibition of E2-induced LH
secretion and inhibited behavioral estrus and ovulation (Jacobs et al., 1988). When heifers were treated with tamoxifen they had a decrease in the PGF metabolite (PGFM) indicating an estrogen-dependent release of PGF but did not block luteolysis. The reduction in concentrations of P4 provoked by low E2 in rabbits indicates that E2 is an important luteotropic hormone in some species (Keyes et al., 1983; Wiltbank et al., 1989). Low concentrations of E2 and consequently low pulses of LH provide different actions on P4 release by the CL and seem to be dependent on the stage of development of the CL. Treatment with LH or GnRH antagonist before d 12 of the estrous cycle reduced concentrations of P4, but after d 12 no effect was detected (Peters et al., 1993).

Intra-vaginal doses of 25 µg of cloprostenol (PGF analogue) in rats caused complete disintegration (a sign of death) of some luteal cells by 26 h after the administration (Hernando et al., 1976). In addition, a decrease of 70% in CL volume over 24 h and a 50% decline in serum P4 within 12 h was detected after intrauterine PGF administration (Louis et al., 1974). Luteal cells as large luteal cells (LLC) and small luteal cells (SLC) have distinct responsiveness to PGF. The SLC contain more LH receptors (Fitz et al., 1993) and also are six-fold more LH sensitive than LLC (Hansel and Dowd, 1986). The stage of development of luteal cells, the modification of SLC to LLC, and the changes in ratio of luteal cells from SLC to LLC caused by administration of GnRH or hCG early in the estrous cycle, may impact luteolysis. This impact may delay the process of CL regression, and extend the length of the estrous cycle (Donaldson and Hansel, 1965, Schmitt et al., 1996b). Even though high doses of LH does not increase P4 synthesis in these cells at late stages of the estrous cycle (Hansel
et al., 1991), increasing the size of the luteal cells (indicative of healthiness) by administration of hCG or GnRH at d 5 of the estrous cycle could prevent aging of the cells and delay CL regression (Schmitt et al., 1996b).

Administration of PGF has a negative effect on blood flow of the CL. Initially there is an increase in blood flow followed by a immediately decrease, causing luteolysis by decreasing nutrients, substrates for steroidogenesis, and luteotropic support for the CL (Phariss et al., 1969). The deprivation of CL blood flow occurs prior to morphological changes of the luteal cells (apoptosis) and also before a decline in LH receptors (Spicer et al., 1981). There is an indication that decreasing blood supply seems to be the primary cause of decreasing concentrations of P4 in blood (Niswender et al., 2000). Luteal cells undergo apoptosis when PGF is synthesized (Fitz et al., 1993). Losses of capacity to synthesize P4 followed by death of luteal cells are the primary characteristics of luteolysis.

**Role of Progesterone in Cattle**

**Progesterone During the Estrous Cycle**

The primary function of P4 is to prepare the uterus for embryo implantation and maintenance of pregnancy during gestation (Niswender et al., 2000; Stocco et al., 2007). The first ovulation in pre-pubertal heifers and postpartum cows frequently is accompanied by a short estrous cycle with the CL regressing 7 to 10 d after ovulation (Ramirez-Godinez et al., 1981; Perry et al., 1991). In addition, a short-term increase in blood concentrations of P4 was noted prior to the first observed estrus (Wettemann, 1980). Ramirez-Godinez et al. (1981) hypothesized that P4 was necessary prior ovulation in order to maintain the normal life span of the CL after ovulation. They observed that greater than 80% of cows treated with an ear implant containing 6 mg of
norgestomet (a progestin) prior to first ovulation, had a subsequent estrous cycle of normal length. An increased in P4 blood concentration was associated with silenced estrous prior to first ovulation. Second ovulation is usually preceded by estrous display. (Stevenson and Britt, 1979; Perry et al., 1991).

**Progesterone effect on Hypothalamus and Anterior Pituitary**

Progesterone appears to have distinct effects on the hypothalamus and anterior pituitary. Progesterone has been demonstrated to be an essential hormone to influence the length of the subsequent estrous cycle after ovulation (Sá Filho et al., 2009). During the luteal phase, P4 is elevated and inhibits GnRH secretion (Karsh et al., 1977). Progesterone diminishes the GnRH responsiveness of the anterior pituitary by decreasing the number of GnRH receptors; consequently decreasing the quantity of LH released (Janovick et al., 1996). Conversely, P4 during the luteal phase primes the hypothalamus resulting in an up regulation of E2 receptors, improving the positive feedback effect of E2 on GnRH secretion by enhancing the magnitude of the GnRH surge during the follicular phase in the ewe (Janovick et al., 1996). The magnitude of GnRH secretion but not LH was always larger when animals had been pretreated with P4 and stimulated with E2 (Caraty and Skinner, 1999). When E2 was administered in the presence of elevated P4, no GnRH surge was induced (Kasa-Vubu et al., 1992; Janovick et al., 1996; Caraty and Skinner, 1999). In addition, the hypothalamic signal for the LH surge in the ewe was initiated by a sharp increase in GnRH secretion (Karsch et al., 1997). Bowen et al. (1998), demonstrated that only a small portion of the GnRH surge was necessary to cause a full LH surge in the ewe and GnRH release during a normal estrous cycle is well above the quantity necessary to induce an LH surge.
Progesterone and Pregnancy Maintenance

The size of the ovulatory follicle prior to ovulation is related to the size of the CL formed and the concentration of P4 in serum after luteinization (Vasconcelos et al., 2001). Stevenson et al. (2006) reported that pregnancy loss was greater in cows that had smaller follicles at AI, which could be explained by the fact that the subsequent CL formed from smaller follicles may have fewer luteal cells that secrete less P4. Further research demonstrated that lower concentrations of E2 were produced when follicles of smaller diameter were induced to ovulate (Vasconcelos et al., 2001). Low E2 from small ovulating follicles likely results in low P4 secretion from the subsequently formed CL (Vasconcelos et al., 2001) but the diameter of the follicle was not critical to pregnancy (Mussard et al., 2007). In addition, the cross-sectional area of the CL and concentrations of P4 was reduced in animals that were induced to ovulate prematurely (Burke et al., 1994; Vasconcelos et al., 2001). Ovulation of smaller follicles resulted in poorer fertility than those ovulating larger follicles when proestrus was limited (Peters and Pursley, 2003). It appears that the fertility of the follicle depends upon endocrinological, physiological, and environmental factors such as hormonal concentrations, temperature, breed, animal size and other factors that could influence the size and timing of ovulation of the ovulatory follicle for each female.

Exogenous Sources of Progesterone

The use of exogenous sources of P4 or progestins for cattle was initiated by the understanding that P4 primes the hypothalamus and promotes the development of normal length estrous cycles (Ramirez- Godinez et al., 1981, Perry et al., 1991; Sa Filho et al., 2009), prevents ovulation, improves synchrony of ovulation, and stimulates cyclicity in pre-pubertal heifers and anestrous cows (Mackey et al., 2000; Lamb et. al.,
numerous companies initiated the development of exogenous forms of progestins for use in cattle to mimic the release of progesterone by the CL. Intra-vaginal devices such as the CIDR® (EAZI-BREED™ CIDR®; 1.38 g of progesterone; Pfizer Animal Health, New York, NY) or PRID (Progesterone Release Intravaginal Device) and ear implant such as Norgestomet® (Crestar, Intervet, Boxmeer, The Netherlands) release progesterone or progestins that increase P4 blood concentration over a sustained period of time. Melengestrol acetate (MGA, Pfizer Animal Health, New York, NY) is an orally active progestin that can be mixed in feed at 0.5 mg per head per day and also acts as progestin source. These exogenous P4 products have led to the development of estrous synchronization protocols that are effective in both cycling and noncycling cows.

Exogenous Hormonal Control of Estrous Cycles

Presynchronization

The utilization of synchronization protocols to synchronize ovulation has been largely used by researchers (Pursley et al., 1995; Geary et al., 2001; Bridges et al., 2008). The protocols reset follicular growth on the ovary, initiating a new follicular wave, regress the original and/or induced CL with PGF 5 to 7 days later. Ovulation is synchronized with injection of GnRH before or at TAI. Ovulation to the first GnRH is important to improve synchronization of follicular wave (Vasconcelos et al., 1999). Inducing ovulation with PGF and GnRH prior to the Ovsynch protocol, Belo et al. (2006) reported an increase in ovulation to the first GnRH of Ovsynch (84.6 vs. 53.8% for presynchronized and controls, respectively). They also reported greater synchronization rate (96 vs. 69%) and greater response to PGF (92.7 vs. 77.1%) for presynchronized compared to control. Although, ovulation rates were not statistically different (P = 0.53)
to the first GnRH (71.8 vs. 66.7% for presynchronized and double-Ovsynch, respectively), pregnancy rates were higher in double-Ovsynch compared to presynch (65.2 vs. 45.2%) primiparous cows. This likely occurred because of induction of ovulation in non-cycling cows (Souza et al., 2008). Follicles that do not respond to the first ovulation persist on the ovary (persistent follicle) when P4 such as a CIDR is applied and CL is not present or not functional on the ovary (Kinder et al., 1996). Follicles may potentially ovulate before planned or fail to ovulate at TAI due to reduced dominance (Vasconcelos et al., 1999), lowering pregnancy rates to TAI (Kinder et al., 1996).

**Synchronization of Estrous and Ovulation**

Initial estrous synchronization protocols failed to address the primary limitation to synchronizing estrus; the lack of normal estrous cycles in pre- or peri-pubertal heifers or postpartum anestrus in suckled cows. In addition, these programs failed to manage follicular waves, thus resulting in less synchronous control of both follicular maturation and CL regression. As a result, the onset of estrus occurred over more days during the synchronized period in which detection of estrus was necessary. Lack of synchrony of estrus and ovulation ultimately precluded TAI reaching acceptable pregnancy rates.

More recent developments focused on both CL and follicle control in convenient and economical protocols to synchronize ovulation. Current research has focused on the development of methods that effectively synchronize estrus in postpartum beef cows and replacement beef heifers by decreasing the period of time during which detection of estrus is required, thus facilitating the use of TAI (Geary et al., 2001a,b; Lamb et al., 2001; 2006, Larson et al., 2006; Busch et al., 2007). This new generation of ovulation-synchronization protocols uses 2 strategies that are key factors for
implementation by producers because they: 1) minimize the number and frequency of handling cattle and 2) eliminate detection of estrus by employing TAI.

The most common systems to synchronize estrus or ovulation in beef cattle in the United States are defined below.

**Protocols Requiring Detection of Estrus**

*Two-injection PG:* Two injections of PGF administered 11 to 14 d apart followed by detection of estrus (Lauderdale et al., 1974).

*CIDR-PG:* A CIDR is inserted for 7 d with PGF administered at CIDR removal, followed by detection of estrus (Lucy et al., 2001).

*Select Synch:* A GnRH injection followed by an injection of PGF 7 d later followed by detection of estrus (Twagiramungu et al., 1995).

*Select Synch + CIDR:* Same as Select Synch, plus a CIDR inserted for 7 d between GnRH and PGF (Larson et al., 2006).

*7-11 Synch:* Females consume MGA for 7 d with PGF administered on the last day of feeding, followed by the initiation of Select Synch 4 d later (Wood-Follis et al., 2004).

*MGA-PG:* Females consume MGA for 14 d with PGF administered 19 d after withdrawal of MGA from the feed followed by detection of estrus (Lamb et al., 2000).

**Protocols Using TAI:**

*Ovsynch:* A GnRH injection followed by PGF 7 d later, plus a second GnRH injection administered at 48 h after PGF and TAI 16 h after the second GnRH (Pursley et al., 1995).

*CO-Synch:* GnRH is administered followed in 7 d by administration of PGF, plus TAI and another GnRH 48 h later (Geary et al., 2001).
5-d CO-Synch + CIDR: GnRH is administered at CIDR insertion followed by PGF 5 d later and CIDR removal. A second injection of PGF is administered 12 h after CIDR removal. Fixed-TAI is performed 72 h after the first PGF concomitantly with another GnRH injection (Bridges et al., 2008).

7-d CO-Synch + CIDR: GnRH is administered at CIDR insertion followed by PGF 7 d later and CIDR removal. A TAI is performed 60 to 66 h after PGF concomitantly with another GnRH injection (Lamb et al., 2001; Larson et al., 2006).
CHAPTER 3
PRESYNCHRONIZATION WITH HCG 7 D PRIOR TO ESTROUS SYNCHRONIZATION AND REPLACEMENT OF GNRH WITH HCG AT FIXED-TIME AI (TAI) IN SUCKLED BEEF COWS

Introduction

The primary sources of revenue for cow-calf operations arise primarily from marketing of calves, but also from the sale of cull cows. To obtain greater revenue, producers need to improve production efficiency (nutrition and reproduction) to cull fewer cows, improve the quality of calves produced, and increase the rate of calves per cow born in a herd per year (Russell, 2001). Artificial insemination (AI) and estrous synchronization (ES) are management procedures that not only improve the genetics of the herd but also shorten the breeding season and provide more opportunities for cows to become pregnant during the breeding season (Odde, 1990). The use of AI and ES in conjunction with detection of estrus has limited application in beef production programs because time and labor associated. The use of more recently developed fixed-time AI (TAI) protocols that eliminate detection of estrus overcome many of the drawbacks of detection of estrus protocols (Patterson et al., 1995; Lamb et al., 2001; Geary et al., 2001; Day et al., 2005; Larson et al., 2006; Bridges et al., 2008). These protocols increase the proportion of cows that become pregnant on the first day of the breeding season and also increase the number of calves born from AI (Lamb, 2006; Larson et al., 2006). However, opportunities exist to further enhance fertility to current systems by use of pre-synchronization with exogenous hormones that synchronize follicular waves and initiate cyclicity in noncycling cows.

Inclusion of human chorionic gonadotropin (hCG) into ES protocols for beef cows may enhance overall pregnancy rates to TAI. Human chorionic gonadotropin is
secreted by the trophoblasts cells of human embryos, beginning 6 to 8 d after fertilization. When administered to cattle, hCG enters the circulation and acts directly on the ovary by binding to the LH receptors present on mature follicles. Ovulation, follicular wave emergence, and CL formation result from administration of hCG (Diaz et al., 1998; Niasari-Naslaji et al., 1996). When hCG was administered to beef cows, CL development resulted from follicles < 10mm in diameter (Sheffel et al., 1982) and 6 of 18 lactating dairy cows treated with hCG having follicles < 10 mm in diameter ovulated compared to 0 of 13 treated with GnRH (Stevenson et al., 2008). Thus, hCG may have greater capacity to luteinize preovulatory follicles compared to GnRH (Schmitt et al., 1996).

Therefore, two experiments were designed to evaluate the effectiveness of hCG when applied prior to initiation of an ES protocol and at TAI to improve pregnancy rates in suckled beef cows. In addition, the experiments evaluated the effect of hCG pretreatment on stimulation of cyclicity, follicular development, concentrations of progesterone (P4), and corpus luteum (CL) development.

**Materials and Methods**

Two experiments were conducted to evaluate the influence of hCG administered 7 d before the initiation of the CO-Synch + CIDR protocol (Exp. 1 and 2) and replacement of GnRH with hCG at the time of TAI (Exp. 1). Experiment 1 was conducted at the North Central Research and Outreach Central in Grand Rapids, MN during the breeding season between May and July of 2007. Exp. 2 was conducted at the North Florida Research and Education Center in Marianna, FL, between February and April of 2008. Both experiments were conducted with Institutional Animal Care and Use Committee (IACUC) approval.
Animals and Treatments

Experiment 1. One hundred fourteen suckled purebred Angus cows were maintained on legume and grass pasture during the entire breeding season. Mean body condition score (BCS; scale of 1 to 9; 1 = emaciated, 9 = obese; Whitman, 1975) was 6.0 with a range of 4.0 to 7.0. Mean days postpartum (DPP) were 81.3 with a range of 33 to 123. Cows were stratified by DPP and parity before assigned to one of four treatments (Figure 1): 1) Cows received the CO-Synch + CIDR protocol (Larson et al., 2006), which included 100 µg of GnRH i.m. (2 mL of Cystorelin®, Merial, Duluth, GA) and a CIDR (EAZI-Breed CIDR containing 1.38 g of P4; Pfizer Animal Health, New York, NY ) insert followed in 7 d by 25 mg prostaglandin F$_2\alpha$ (PGF; 5 mL of Lutalyse®; Pfizer Animal Health) and CIDR removal, followed in 64 to 68 h by TAI and a second injection of GnRH (CG; n = 29); 2) CG but the second injection of GnRH at the time of TAI was replaced by 1,000 IU of hCG i.m. (1 mL of Chorulon®; Intervet, Millsboro, DE; CH; n = 28); 3) CG plus 1,000 IU of hCG administered 7 d (d -14) prior to CIDR insertion (HG; n = 28); and, 4) HG plus the second GnRH was replaced by 1,000 IU of hCG at the time of TAI (HH; n = 29).

Experiment 2. Two hundred and fifty-nine Angus and Brangus cows, from three separate herds were used in this study. Herd size was 124, 80, and 57 cows for herds 1, 2, and 3, respectively. Mean BCS was 5.7 with a range of 4.0 to 8.0, mean DPP was 70.2 with a range of 32 to 113, and mean parity was 3.7 with a range of 1 to 10. Cows were stratified based on DPP, parity, and calf sex prior to assignment to the CG (n = 130) and HG (n = 129) treatments described for Exp. 1.
Blood Samples and Analysis

Blood samples were collected via coccygeal venipuncture using Vacutainer tubes (Becton & Dickinson Vacutainer Systems, Rutherford, NJ) on d -24, -14, -7, 0, 3, 10, 16, and 29 in Exp. 1 and on d -24, -14, -7, 0, and 3 in Exp. 2. Blood was refrigerated for 24 to 30 hr after collection, centrifuged, and serum was placed in sample vials and frozen for later analysis. Concentrations of progesterone in samples were analyzed in duplicate by radioimmunoassay using progesterone kits (Coat-A-Count; Diagnostic Products Corp. Los Angeles, CA). For Exp. 1, the assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 µl. Assay tubes for the standard curve contained 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity for a 100-µl sample was 0.1 ng/mL. Pooled samples revealed that the intra- and inter-assay coefficient of variation were 4.7 and 3.9% for two assays, respectively. For Exp. 2, the assay tubes for the standard curve contained 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 ng/tube. Assay sensitivity for a 100-µl sample was 0.01 ng/mL. Pooled samples revealed that the intra- and inter-assay coefficient of variation were 6.2 and 7.4% for six assays, respectively. The cow was considered to be cycling at the initiation of treatments if at least 1 of 2 blood samples had concentrations of progesterone ≥ 1 ng/mL (Perry et al., 1991) for samples collected on d -24 and d -14. All remaining serum samples were used to assess response to treatments.

Ultrasound of Ovarian Structures and Pregnancy Diagnosis

Ovaries of all cows in Exp. 1 were examined on d -14, -7, 0, 3, and 10 by transrectal ultrasonography (7.5-MHz linear array transducer, Aloka 900V, Corimetrics Medical Systems, Inc., Wallingford, CT) and mapped to monitor changes in CL and
follicles in response to treatment. The vertical and horizontal diameter of the largest follicle on each ovary and all CL were measured and recorded. Volume of CL tissue was calculated using the formula \( V = \frac{4\pi r^3}{3} \) where \( r = \frac{1}{2} \) of the average value for vertical and horizontal CL measurements. In cases where CL had fluid filled cavities, the volume of the cavity was subtracted from the total volume of the CL, resulting in a value that reflected the actual volume of CL tissue present.

In Exp. 1 and 2, transrectal ultrasonography (5.0-MHz linear array transducer, Aloka 500V) was used on d 29 to determine presence of a viable conceptus by heartbeat.

**Statistical Analysis**

Serum concentration of P4, follicle size and CL volume were analyzed by analysis of variance for repeated measures using procedure PROC MIXED (SAS inst. Inc., Cary, NC). The statistical model was:

**Exp 1.**

\[
Y = \mu + \text{PRETRT}_i + \text{TAITRT}_j + \text{COMTRT}_k + \text{DAY}_l + \text{CYCL}_m + \text{PRETRT}^{*}\text{DAY}_{il} + \\
\text{TAITRT}^{*}\text{DAY}_{jl} + \text{CYCL}^{*}\text{DAY}_{ml} + \text{PRETRT}^{*}\text{CYCL}^{*}\text{DAY}_{ilm} + \text{TAITRT}^{*}\text{CYCL}^{*}\text{DAY}_{jml} + E_{ijklm}
\]

**Exp 2.**

\[
Y = \mu + \text{PRETRT}_i + \text{DAY}_j + \text{CYCL}_k + \text{PRETRT}^{*}\text{DAY}_{ij} + \text{CYCL}^{*}\text{DAY}_{kj} + \\
\text{PRETRT}^{*}\text{CYCL}^{*}\text{DAY}_{ikj} + E_{ijk}
\]

where:

- \( Y \) = response variable (progesterone concentration, follicle diameter, CL volume)
- \( \mu \) = overall mean
- PRETRT = fixed effect of hCG pre-treatment at d -14
TAITRT = fixed effect of hCG treatment at TAI
DAY = fixed effect of day
CYCL = fixed effect of cycling status
PRETRT*DAY = effect due interaction of pre-treatment by day
TAITRT*DAY = effect due interaction of treatment at TAI by day
CYCL*DAY = effect due interaction of cycling status by day
PRETRT*CYCL*DAY = effect due interaction of pre-treatment by cycling status by day
TAITRT*CYCL*DAY = effect due interaction of treatment at TAI by cycling status by day
E = residual error

Repeated measures for follicle size were only analyzed for d 0 and 3 when the same follicle was present in both days. The interaction between pre-treatment x treatment x day was not significant and was removed from the model. Procedures GENMOD and GLM of SAS (SAS Inst. Inc., Cary, NC) were used to analyze all categorical data. Pearson correlation and logistic regression for linear effect was used to evaluate correlation between follicle diameter, P4 concentration and CL volume. Relationship between follicle, body condition scores and DPP were included in the model as regression covariates. Results are reported as least square means. Means were separated using LSD (Snedecor and Cochran, 1989). Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $\leq 0.10$. 

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Results

Experiment 1

No interaction between pre-treatment and treatment at TAI was observed for concentrations of P4, follicle size, and CL volume. Therefore, this interaction was removed from the model and the model evaluated the effect of pre-treatment hCG (HG and HH) against pre-treatment control (CG and CH) on d -7, 0 and 3. A day by pre-treatment and cycling status by pre-treatment interaction (P < 0.05) was observed for concentrations of P4, a cycling status by day and pre-treatment by day interaction (P < 0.05) was observed for follicle diameter, and a pre-treatment by cycling status by day interaction (P < 0.05) was observed for CL volume. No pre-treatment by treatment or pre-treatment by cycling status by day interaction existed for concentrations of P4 or follicle diameter. Therefore, the data were pooled for cows that received either pre-treatment hCG (HG and HH) or pre-treatment control (CG and CH) groups on d -14, -7, 0 and 3.

Follicular dynamics. Follicle diameter was greater (P < 0.01) for pre-treatment control than pre-treatment hCG cows on d 0 (14.4 ± 0.5 vs. 11.9 ± 0.5 mm for pre-treatment control and pre-treatment hCG, respectively) and d 3 (17.0 ± 0.5 vs. 14.5 ± 0.5 mm for pre-treatment control and pre-treatment hCG, respectively; Figure 2). There was a statistically difference (P = 0.05) of follicle diameter to be greater for noncycling than cycling cows on d 3 (16.6 ± 0.7 vs. 14.9 ± 0.4 mm; for noncycling and cycling cows, respectively; Figure 3).

On d 0, pre-treatment control noncycling cows (15.2 ± 0.9 mm) had follicles with greater (P < 0.05) diameter than pre-treatment hCG noncycling cows (12.0 ± 0.9 mm)
and pre-treatment hCG cycling cows (11.7 ± 0.6 mm). In addition, pre-treatment control cycling cows (13.6 ± 0.6 mm) had greater (P < 0.02) follicles than pre-treatment hCG cycling cows (11.7 ± 0.6 mm; Figure 4). On d 3, follicle diameter was greater (P < 0.01) for pre-treatment control noncycling cows (18.3 ± 0.9 mm) than pre-treatment control cycling (15.7 ± 0.6 mm), pre-treatment hCG noncycling (14.9 ± 0.9 mm), and pre-treatment hCG cycling cows (14.1 ± 0.6 mm; Figure 5).

No differences existed in follicle diameter between treatments, among days or the interaction after cows received either GnRH or hCG at TAI.

Concentrations of Progesterone. Overall, 55% of cows were cycling by d -14 and it did not differ between treatments; however, the percentage of cycling cows tended (P = 0.10) to increase by d -7 after receiving pre-treatment hCG (78.9%) compared to pre-treatment control (64.9%; Table 1). Concentrations of P4 was assessed on d -14 -7, 0, 3, 10, 16 and 29 to evaluate differences between pre-treatment, cycling status, treatment at TAI, and their respective interactions. A pre-treatment × day and cycling status × day interaction was observed (P < 0.05). Pre-treatment hCG cows (2.4 ± 0.3 ng/ml) had elevated (P = 0.02) concentrations of P4 compared to pre-treatment controls (1.6 ± 0.3 ng/ml) on d -7 (Table 1).

Cycling cows (2.4 ± 0.2 and 3.2 ± 0.2 ng/ml) had greater (P < 0.001) concentrations of P4 than noncycling cows (0.2 ± 0.4 and 0.8 ± 0.4 ng/ml) on d -14 and d -7, respectively (Figure 6). Pre-treatment hCG cycling cows (3.7 ± 0.3 ng/ml) had greater (P <0.01) concentrations of P4 than pre-treatment control cycling cows (2.7 ± 0.3 ng/ml) and both were greater than pre-treatment control noncycling (0.4 ± 0.5 ng/ml) and pre-treatment hCG noncycling cows (1.2 ± 0.5 ng/ml; Figure 7) on d -7. Pre-
treatment cycling (3.2 ± 0.3 ng/ml) cows tended (P = 0.06) to have greater concentrations of P4 than pre-treatment control noncycling cows (2.3 ± 0.5 ng/ml) on d 0 (Figure 8).

Ovulation rate to the first GnRH on d -7 was 42% (24/57) for control and for the pre-treatment hCG cows 49% (28/57). Interestingly, of the 63% (36/57) of pre-treatment hCG cows that ovulated on d -14, only 39% (14/36) subsequently ovulated to GnRH on d -7, whereas of the pre-treatment hCG cows that did not ovulate on d -14, 67% (14/21) subsequently ovulated to GnRH on d -7. In the pre-treatment control group, 21% (12/57) of cows ovulated between d -14 and d -7, and 50% (6/12) subsequently ovulated on d -7. Ovulation on d 3 was not different between hCG and GnRH at time AI (89.5%).

No differences were observed in concentrations of P4 between treatments, among days or the interaction after cows received either GnRH or hCG at TAI.

CL characteristics. There was a pre-treatment × cycling status × day interaction (P < 0.05) for CL volume. On d -7 pre-treatment control noncycling (6.7 ± 1.5 cm³) and pre-treatment hCG cycling (6.6 ± 0.5 cm³) cows had greater CL volume than pre-treatment hCG noncycling cows (3.5 ± 0.9 cm³), whereas pre-treatment control cycling cows were intermediate (5.8 ± 0.5 cm³; Figure 10). On d 0 pre-treatment hCG cycling cows (5.4 ± 0.5 cm³) had greater CL volume than pre-treatment hCG noncycling cows (2.9 ± 0.8 cm³) and pre-treatment control noncycling cows (3.2 ± 1.0 cm³) whereas pre-treatment control cycling cows was intermediate (4.5 ± 0.6 cm³; Figure 11).

When Pearson correlation coefficients were assessed between follicle diameter on d 3 and CL volume on d 10, there was a positive relationship (0.44, P < 0.001).
There also was a positive relationship \( (P = 0.01) \) for follicle diameter on d 3 and concentrations of P4 on d 10, with a correlation coefficient of 0.24.

No differences were observed in CL characteristics between treatments, among days or the interaction after cows received either GnRH or hCG at TAI.

*Pregnancy rate.* Overall, pregnancy rate was 47.4% and it was not altered by presynchronization with hCG. However, pregnancy rate tended \( (P = 0.06) \) to be reduced in cows receiving hCG at TAI (38.6%) compared to those receiving GnRH at TAI (56.1%; Table 2).

**Experiment 2**

Human chorionic gonadotropin increased the proportion of noncycling cows with elevated P4 \( (> 1 \text{ ng/ml}) \) compared with control. In addition, a pre-treatment by day interaction was observed \( (P < 0.05) \). Concentrations of P4 were greater \( (P < 0.01) \) on d -7 for HG \( (2.0 \pm 0.2 \text{ ng/ml}) \) than CG \( (1.3 \pm 0.2) \), but were similar between treatments on d 0 and 3 (Table 3).

Pregnancy rates were similar between CG (53.1%) and HG (54.3%; Table 3). Pregnancy rates tended \( (P = 0.11) \) to be greater for cycling (62.5%) than noncycling cows (47.6%); however, pre-treatment with hCG administered 7 d prior to CIDR insertion failed to increase pregnancy rates in noncycling cows.

**Discussion**

Injection of hCG resulted in ovulation of the dominant follicle and subsequent formation of an accessory CL when an existing CL was present on the ovary (Schmitt et al., 1996ab; Diaz et al., 1998; Santos et al., 2001; Dahlen et al., 2009). Greater concentrations of P4 were observed in cows with an induced CL than cows without an accessory CL. Schmitt et al. (1996a,b) demonstrated that hCG (3,000 IU) or GnRH
(buserelin, 8µg) injected on d 5 of the estrous cycle induced an accessory CL and elevated the concentrations of P4 compared to controls. When the accessory CL was removed on d 13 there were no detectable differences in concentrations of P4 of the original CL in both groups, demonstrating that the P4 produced by the accessory CL was responsible for the increase in P4. In both Exp. 1 and 2, half of the cows were pre-synchronized with 1,000 IU of hCG at random stages of the estrous cycle. Pre-treatment with hCG induced noncycling and cycling cows to ovulate follicles (Exp. 1) and subsequently increased the percentage of cows with elevated P4 7 d later (Exp. 1 and 2). The incidence of gonadotropin induced ovulation of the dominant follicles may vary depending on the stage of the estrous cycle (Price and Webb, 1989; Vasconcelos et al., 1999). However, our results indicated that injection of hCG in cows at random stages of the estrous cycle and of unknown cycling status increased the percentage of cows with elevated P4 7 d later and increased the percentage of cycling cows after administration of hCG. In addition, cows receiving hCG that ovulated a follicle had greater concentrations of P4 than cows that did not ovulate a follicle, indicating that the formation of the accessory CL likely was responsible for elevating the concentration of P4.

Concentrations of P4 were greater on d -7 for cows receiving hCG than controls but no differences were observed on subsequent days. The similar concentrations of P4 on d 0 for both treatments could be due to ovulation of follicles on d -7 after administration of GnRH in both groups (45.6 and 49.1% of ovulation on d -7 for control and hCG-pretreatment, respectively). Schmitt et al. (1996) observed that concentration of P4 was elevated after induction of an accessory CL on d 5. This data could explain
the greater concentrations of P4 of the pre-treatment hCG cycling cows on d -7. Although, P4 concentrations were greater for the pre-treatment hCG group compared to the pre-treatment control group on d -7, the total CL volume was similar. A possible explanation for this may be that the new CL formed after hCG may not be fully developed 7 d later, even though the new CL has the capability to produce P4, the total luteal cell mass between treatments was likely insufficient to differentiate, statistically.

On d 0, CL volume did not differ between pre-treatment and control. Diaz et al. (1998) reported that an hCG induced CL at d 5 of the estrous cycle was smaller than the original CL from d 3 to 8 after ovulation. Forty eight percent of cycling cows were in the later stages of the estrous cycle on d -7 and could have had their CL regressing between d -7 and 0. Noncycling cows that ovulated after pre-treatment with hCG on d -14 may have experienced a short estrous cycle and the CL could have regressed between d -7 and 0. The CL induced by GnRH on d -7 on cows that had previous ovulation between d -14 and -7 could have been smaller than cows that did not ovulated previously of GnRH due to smaller follicle on d -7 (Vasconcelos et al., 2001).

It has been shown that intravaginal releasing device inserted after ovulation did (Burke et al., 1994, Menchaca et al., 2001) or did not (Beltman et al., 2008) alter the development of the CL when induced concomitant of CIDR insertion. The P4 device may have caused an inhibitory effect on the development of the new CL induced by GnRH. These factors could explain the lack of difference in CL volume between control and hCG pre-treatment on d 0.

Follicle diameter was smaller for cows pre-treated with hCG than controls on d 0 and 3. High concentration of P4 from the CL induced by hCG pre-treatment on d -14 in
addition to the P4 from the new CL induced by GnRH and the P4 released by the CIDR inserted on d -7, could alter LH pulsatility pattern which affect negatively follicle growth rate. These data concur with other studies that demonstrated that high concentrations of P4 resulted in reduced growth of the dominant follicle and induced follicle turnover (Savio et al., 1993; Burke et al., 1994) possibly because of reducing LH secretion (Kinder et al., 1996). In our study, control noncycling cows had the lowest P4 on d -7 and had the greatest follicle diameter on d 0 and 3, whereas pre-treatment hCG cycling cows had the highest P4 on d -7 and the lowest follicle diameter on d 0 and 3.

Pregnancy rate was not affected by presynchronization with hCG; however hCG at TAI tended (P = 0.06) to decrease pregnancy rate. The capacity of hCG to induce ovulation of small follicles has been reported (Stevenson et al., 2008). Ovulation of small follicles may result in poorer fertility possibly because of smaller CL with lower capacity to produce P4 (Vasconcelos et al., 2001). In addition, hCG acting directly on the ovary bypasses the hypothalamic-pituitary axis, does not induce GnRH pulses (Filicori et al., 2005), therefore does not induce FSH or LH secretion from the pituitary. The LH produced by the pituitary is essential for oocyte meiotic maturation and ovulation (Schimada et al., 2003). Treatment with hCG does not result in terminal maturation of the dominant follicle due to the lack of appropriate LH pulses, potentially resulting in decreased pregnancy rates (Yavas et al., 2000).

Concentration and duration of E2 production from mature follicles might be important to stimulate E2 and P4 receptors on endometrial cells (Zeliski et al., 1980). Receptors for LH are also present in the uterus and exert direct and indirect effects (via steroid production of the ovary) essential for LH signaling to initiate pregnancy (Rao et
In cycling cows, injection of exogenous GnRH induce LH release, follicle growth and ovulation of a dominant follicle ≥10 mm in diameter and also increased E2 release (Yavas et al., 2000). Ovulation of premature follicles by hCG injection, perhaps did not have sufficient associated E2 production, consequently less stimulation of E2 and P4 receptors of the uterus, affecting pregnancy.

An additional factor that could result in the decreased pregnancy rate is the possible action of the hCG directly on the oocyte. Oocytes contain LH receptors and may be activated by LH as well as hCG (Filicori et al., 2005). The addition of FSH to maturation media induced a significant increase in the binding of hCG to complex granulosa/oocyte cells (Shimada et al., 2003). Further, the development of the antral follicles drastically changes soon after injection of hCG at 72 h after an injection of eCG in sows (Funahashi et al., 1996). Therefore, hCG may not provide the necessary E2 needed to stimulate FSH secretion, increasing LH receptors in the cumulus oocyte complex, increasing responsiveness of the follicle to LH and resulting in oocyte maturation and ovulation of a healthy follicle.

**Conclusion**

In conclusion, pre-treatment with hCG 7 d prior to initiation of estrous synchronization induced an accessory CL, increase concentrations of P4, and decrease the diameter of the dominant follicle at TAI, but did not alter pregnancy rates. However, although no differences occurred in follicle diameter, concentrations of P4, or subsequent CL development, replacing GnRH with hCG at TAI, tended to decrease pregnancy rates.
Table 3-1. Concentrations of progesterone (P4), percentage of cycling, and pregnancy rates in cows assigned to untreated control or human chorionic gonadotropin (hCG) pre-treatment on d -14 in Exp. 1.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (CG and CH)</th>
<th>hCG (HG and HH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4 on d -14</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>P4 on d -7</td>
<td>1.6 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>P4 on d 0</td>
<td>2.5 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>P4 on d 3</td>
<td>0.2 ± 0.3</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Cycling cows on d -14 b</td>
<td>31/57 (54.4)</td>
<td>32/57 (56.1)</td>
</tr>
<tr>
<td>Cycling cows on d -7 c</td>
<td>37/57 (64.9) w</td>
<td>45/57 (78.9) x</td>
</tr>
<tr>
<td>Cows with &gt;1 ng/ml P4 on d -14 d</td>
<td>26/57 (45.6)</td>
<td>27/57 (47.4)</td>
</tr>
<tr>
<td>Cows with &gt;1 ng/ml P4 on d -7 d</td>
<td>23/57 (40.4) y</td>
<td>36/57 (63.2) z</td>
</tr>
<tr>
<td>Pregnancy rates</td>
<td>26/57 (45.6)</td>
<td>28/57 (49.1)</td>
</tr>
</tbody>
</table>

aCows were assigned to receive no treatment or hCG on d -14.
bNumber and percentage of cows cycling on d -14 based on two blood samples taken on d d -25 and -14.
cNumber and percentage of cows cycling on d -7 based on three blood samples taken on d d -25, -14, and -7.
dCows with concentrations of P4 >1 ng/ml on d-14 or -7.
w x Tendency for percentages to differ (P = 0.10).
y z Percentages within row differ (P < 0.05).
Table 3-2. Concentrations of progesterone (P4) and pregnancy rates in cows assigned to receive either GnRH or hCG at TAI on d 3 in Exp. 1.

<table>
<thead>
<tr>
<th>Item</th>
<th>GnRH (CG and HG)</th>
<th>hCG (CH and HH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4 on d 3</td>
<td>0.1 ± 0.2</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>P4 on d 10</td>
<td>2.4 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>P4 on d 16</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>P4 on d 29</td>
<td>2.8 ± 0.4</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Pregnancy rates</td>
<td>32/57 (56.1)</td>
<td>22/57 (38.6)</td>
</tr>
</tbody>
</table>

*Cows were assigned to receive GnRH or hCG on d 3 (at the time of TAI).

*xy*Tendency for percentages to differ (P = 0.06).
Table 3-3. Pregnancy rates and percentage of cycling cows in cows assigned to untreated control or human chorionic gonadotropin (hCG) pre-treatment on d -14 in Exp. 2.

<table>
<thead>
<tr>
<th>Treatments&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CG</th>
<th>HG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
<td><strong>ng/ml</strong></td>
<td><strong>no./no. (%)</strong></td>
</tr>
<tr>
<td>P4 on d -14</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>P4 on d -7</td>
<td>1.3 ± 0.2&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2.0 ± 0.1&lt;sup&gt;z&lt;/sup&gt;</td>
</tr>
<tr>
<td>P4 on d 0</td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>P4 on d 3</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Cycling cows on d -14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31/130 (23.8)</td>
<td>45/129 (34.1)</td>
</tr>
<tr>
<td>Cycling cows on d -7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49/130 (37.7)&lt;sup&gt;w&lt;/sup&gt;</td>
<td>79/129 (61.2)&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cows with &gt;1 ng/ml P4 on d -14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29/130 (22.3)</td>
<td>29/129 (22.5)</td>
</tr>
<tr>
<td>Cows with &gt;1 ng/ml P4 on d -7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35/130 (26.9)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>58/129 (45.0)&lt;sup&gt;z&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnancy rates</td>
<td>69/130 (53.1)</td>
<td>70/129 (54.3)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cows were assigned to receive no treatment or hCG on d -14.
<sup>b</sup>Number and percentage of cows cycling on d -14 based on two blood samples taken on d -25 and -14.
<sup>c</sup>Number and percentage of cows cycling on d -7 based on three blood samples taken on d -25, -14, and -7.
<sup>d</sup>Cows with concentrations of P4 >1 ng/ml on d-14 or -7.
<sup>w</sup>Tendency for percentages to differ (P = 0.10).
<sup>x</sup>Percentages within row differ (P < 0.05).
Figure 3-1. Experimental protocol for treatments to synchronize ovulation in Exp. 1 (CG, CH, HG, and HH) and Exp. 2 (CG and HG). Blood (B) samples were collected on d -25, -14, -7, 0, 3, and 10, in Exp. 1 and on d -25, -14, -7, 0, and 3 in Exp. 2. Ultrasonography (US) of ovarian follicles was performed on d -14, -7, 0, and 3 in Exp. 1. BCS = body condition score; GnRH = gonadotropin-releasing hormone; CIDR = controlled internal device release; PGF = Prostaglandin F2α; TAI = fixed-timed AI.
Figure 3-2. Experiment 1: Diameter of the largest follicle present on the ovary associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14. * Pre-treatment with hCG differs from controls (P < 0.001).
Figure 3-3. Experiment 1: Diameter of the largest follicle present on the ovary associated with cows determined to be cycling or noncycling on d -14 relative to PGF. * Cycling cows differ from noncycling cows (P < 0.05).
Figure 3-4. Experiment 1: Follicle diameter on d 0 (day of PGF) associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14 and in cows determined to be cycling or noncycling on d -14. x,y,zMeans differ (P < 0.05).
Figure 3-5. Experiment 1: Follicle diameter on d 3 (day of the AI) associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14 and in cows determined to be cycling or noncycling on d -14. \( x, y, z \) Means differ (P < 0.01).
Figure 3-6. Experiment 1: Concentrations of P4 for cows determined to be cycling and noncycling on d -14. *Concentrations differ (P < 0.001) between cycling and noncycling cows.
Figure 3-7. Experiment 1: Concentrations of P4 on d -7 associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14 and in cows determined to be cycling or noncycling on d -14. x,y,zMeans differ (P < 0.01)
Figure 3-8. Experiment 1: Concentrations of P4 on d 0 associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14 and in cows determined to be cycling or noncycling on d -14. **z** Means tend to differ (P =0.06)
Figure 3-9. Experiment 1: Volume of CL structures associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14.
Figure 3-10. Experiment 1: Volume of the CL on d -7 associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14 and in cows determined to be cycling or noncycling on d -14. x,y Means differ (P < 0.01).
Figure 3-11. Experiment 1: Volume of the CL on d 0 associated with cows receiving pretreatment with 1,000 IU of hCG or no treatment (control) on d -14 and in cows determined to be cycling or noncycling on d -14. x,y means differ (P < 0.01).
REFERENCES


BIOGRAPHICAL SKETCH

Guilherme H.L. Marquezini was born in Vilhena/ Rondônia, Brazil in Nov 03/1980. He is the middle son of Paulo Sérgio Marquezini and Ana Maria Marquezini and obtained his B.S. in Veterinary Medicine from the São Paulo State University (UNESP) in 2004. In 2009, Guilherme moved to Gainesville, FL to begin his Master of Science program at the University of Florida - Animal Sciences, advised by Dr. Cliff Lamb. His research focused on beef cattle reproduction. After graduation, Guilherme will pursue his PhD degree again at University of Florida, further investigating the findings obtained during his MS program.