PROGESTIN REGULATION OF FOLLICULAR DYNAMICS IN BEEF CATTLE

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

PROGESTIN REGULATION OF FOLLICULAR DYNAMICS IN BEEF CATTLE

Presented by Mallory Elise Risley

A candidate for the degree of Master of Science

And hereby certify that in their opinion it is worthy of acceptance

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LIST OF ABBREVIATIONS

3β-HSD	3 beta- hydroxydehydrogenase
ACL	Accessory corpus luteum, accessory corpora lutea
AI	Artificial insemination
C	Celcius
CA	Corpus albican
CH	Corpus hemmoraghicum
CIDR	Controlled Internal Drug Release (s)
CL	Corpus luteum, corpora lutea
CV	Coefficients of variation
d	Day (s)
ECP	Estradiol cypionate
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
FTAI	Fixed-time artificial insemination
g	Grams
GC	Granulosa cell
GnRH	Gonadotropin-releasing Hormone
hCG	Human chorionic gonadotropin

hr	Hour (s)
i.m.	Intramuscular
IGF-1	Insulin like growth factor-1
IGFBP	Insulin like growth factor binding proteins
LH	Luteinizing hormone
LLC	Large luteal cell
MAP	Medroxyprogesterone acetate
MFA	Missouri Farmers Association
MGA	Melengestrol acetate
ml	Milliliter
mm	Milimeter
mos.	Months
ng	Nanogram
P4	Progesterone
PF	Persistent Follicle
pg	Picagram
PG	Prostaglandin, prostaglandin $F_{2\alpha}$
PRID	Progesterone Releasing Internal Device
RIA	Radioimmunoassay
SLC	Small luteal cell
StAR	Steroidogenic acute regualtory protein
STD	Sexually-transmitted disease
TC	Theca cell

PROGESTIN REGULATION OF FOLLICULAR DYNAMICS IN CATTLE

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Dr. Michael F. Smith, Thesis Supervisor

ABSTRACT

Progestins, such as melengestrol acetate (MGA) and Controlled Internal Drug Release (CIDR), are efficient at inhibiting ovulation and synchronizing estrus in cattle. However, treatment with MGA for an extended period of time (approximately 14 days [d] or greater) results in the formation of persistent follicles (PF) when administered in the absence of a corpus luteum (CL). A PF is characterized by a longer lifespan, larger maximum follicle diameter, and increased secretion of estradiol compared to a dominant follicle formed during a normal estrous cycle. Insemination following ovulation of a PF results in decreased pregnancy rates in cattle. The overall objectives for experiments described in chapter III are as follows: 1) To determine if the presence of a new or used CIDR in cycling heifers and postpartum cows, without a CL, would induce the formation of a PF, 2) To compare serum concentrations of progesterone (P4) in heifers and cows treated with a new or used CIDR to luteal phase concentrations of P4 in non-treated heifers and cows, and 3) To determine the effect of length of storage of a used CIDR on circulating concentrations of P4 in cycling beef heifers and postpartum beef cows. Heifers (experiment 1) and cows (experiment 2) were presynchronized with a CIDR for 7 d, prostaglandin $F_{2\alpha}$ (PG) on d 6 and GnRH 48 hour (hr) after CIDR removal and

allocated into four treatment groups: Control, MGA, New CIDR and Used CIDR (new CIDR previously inserted for 7 d, disinfected, and stored). Progestin treatment began on d 4 post-estrus (d 0 = estrus) and PG was injected on d 6 to induce luteolysis. Progestin treatment, MGA or CIDR (New or Used), continued for 14 d and length of a follicular wave was defined as the interval from follicular recruitment to ovulation or initiation of a new wave. Serum concentrations of progesterone (P4) for heifers in the New and Used CIDR groups were similar ($P > 0.1$) throughout the 14 d treatment period but lower than in the Control group. Length of follicular waves were longer ($P < 0.05$) in the progestin-treated groups compared to the Control group. For heifers, dominant follicle diameter was greater ($P < 0.05$) in the Used CIDR group compared to Control heifers; whereas, there were no differences ($P > 0.63$) in cows treated with a new or used CIDR. In experiment 3, estrous cycling crossbred cows and heifers were treated for 14 d with a new CIDR, a used CIDR with no storage duration, or a used CIDR stored for 6 months (mos.). There was no significant difference ($P > 0.88$) among treatments in serum P4 concentration from d 4 to d 17.

The CO-Synch protocol (gonadotropin releasing hormone [GnRH-1], followed by PG 7 d later, and a second injection of GnRH [GnRH-2] plus artificial insemination (AI) 48 hr after PG) is commonly used for synchronization of estrus and ovulation in beef cattle. We hypothesized that a GnRH-induced accessory corpus luteum (ACL) is equally effective at inhibiting estrus and ovulation in heifers compared to cows. The objective of the experiment described in Chapter IV was to compare the ability of a GnRH-induced ACL to inhibit the expression of estrus and ovulation in cycling beef heifers and cows. Cycling heifers (14 to 20 mos. of age) and multiparous suckled cows were randomly

assigned to receive an injection of saline or GnRH on d 16 of the estrous cycle (d 0 = estrus) resulting in the following treatment groups: heifer-saline, heifer GnRH, cow saline, or cow GnRH (n= 7, 15, 9, and 17, respectively). In both heifers and cows, diameter of the largest follicle at GnRH-1 injection was similar ($P > 0.09$) among treatment groups. In the GnRH treatment groups, there were 10/15 (66.7%) heifers and 15/17 (88.2%) cows that responded to GnRH-1 (GnRH-Yes Response); whereas, 5 heifers and 2 cows did not respond (GnRH-No Response). There was no difference in the proportion of heifers or cows in estrus or ovulating before PG (d 23) or in interestrus interval for the Saline or GnRH-No Response groups; therefore, data from the saline and GnRH-No Response groups were combined and designated as the heifer saline or cow saline groups. The proportion of animals in estrus or ovulating before PG administration for the heifer-saline, heifer-GnRH-Yes Response, cow-saline, and cow-GnRH-Yes Response groups was $12/12^{a}$, $2/10^{b}$, $9/11^{a}$, and $1/15^{b}$, respectively ($P < 0.0001$) and mean interestrus interval was 20.25 ± 0.41^{a}, 24.2 ± 0.33^{b}, 21.0 ± 0.6^{a}, and 24.7 ± 0.21^{b} d, respectively ($P < 0.0001$). GnRH-induced formation of an ACL decreased the proportion of heifers and cows that displayed estrus or ovulated before PG administration and increased estrous cycle length. There was no difference ($P > 0.28$) in the effectiveness of the GnRH-induced CL to inhibit estrus and ovulation in heifers compared to cows.

CHAPTER I

INTRODUCTION

Artificial insemination (AI) facilitates widespread use of genetically superior sires and is a powerful tool for genetic improvement of a beef herd. However, the percentage of beef herds that utilize AI is relatively low (< 8%; NAHMS, 2008). Time and labor associated with detection of estrus under extensive conditions is a primary deterrent to the adoption of AI in beef cattle (NAHMS, 2008). Furthermore, problems with both estrous detection efficiency and accuracy will reduce estrous detection rate (Odde, 1990). Since pregnancy rate is the product of estrous detection rate and conception rate, a reduction in estrous detection rate will reduce pregnancy rate. To reduce or alleviate the problem of estrous detection, significant effort has been directed toward development of protocols that precisely control the time of ovulation and permit insemination of heifers or cows at a fixed-time (FTAI; Lamb et al., 2009). When using FTAI protocols, all animals are inseminated at a predetermined time and require no estrous detection. An additional benefit of FTAI protocols is the insemination and subsequent conception of heifers or cows that would not have been detected in estrus.

The development of FTAI protocols requires the management of both the luteal phase and follicular waves. The luteal phase can be manipulated by inducing premature luteolysis with prostaglandin $F_{2\alpha}$ (PG) and (or) administration of exogenous

(melengestrol acetate [MGA] or controlled internal drug release [CIDR]) or endogenous (accessory corpora lutea [ACL]) progestins (Martinez et al., 2000; Sirois and Fortune, 1990). Insemination of beef heifers at the synchronized estrus immediately following long-term administration of MGA (approximately 14 days [d]), results in reduced conception rates (Beal et al., 1988; Chenault et al., 1990; O'Brien and Zimbelman, 1970; Roche and Crowley, 1973). The formation of a persistent follicle (PF) containing an aged oocyte has been proposed to be a mechanism accounting for the decreased conception rates (Mihm et al., 1999; Savio et al., 1993). However, there is little research to show whether or not long-term administration of a CIDR produces the same effect on follicular growth in estrous cycling beef heifers and cows. The third chapter reports the effect of long term administration of MGA or a new or used CIDR on follicular dynamics in beef heifers and cows. The specific objectives were as follows: 1) To determine if the presence of a new or used CIDR in estrous cycling heifers and postpartum cows, without a corpus luteum (CL), will induce the formation of a PF, 2) To compare the pattern of circulating concentrations of estradiol and P4 in estrous cycling heifers and cows treated with a new or used CIDR to mid-luteal phase concentrations of the preceding hormones in non-treated heifers and cows, and 3) To determine the effect of length of storage of used CIDR on circulating concentrations of P4 in estrous cycling beef heifers and postpartum cows.

The CO-Synch protocol, or a variation thereof, is commonly used for FTAI in beef cattle and consists of an injection of gonadotropin releasing hormone (GnRH) on d -9 (GnRH-1), PG on d -2, and a second injection of GnRH (GnRH-2) plus AI 48 hours (hr) after PG (d 0). Preliminary data from our lab suggested that injection of GnRH-1

during the late luteal phase was more effective at delaying estrus and ovulation until after PG injection in postpartum cows compared to heifers (Atkins et al., unpublished data). Injection of GnRH or a GnRH agonist, during the luteal phase of heifers or cows, can induce ovulation of a dominant follicle, cause formation of luteal tissue, and synchronize a follicular wave (Twagiramungu et al., 1994). If the CL formed at the previous spontaneous ovulation is present (primary CL), then the GnRH-induced luteal tissue is referred to as an accessory CL (ACL). Protocols implementing FTAI commonly induce formation of ACL in cycling cows; however, cows and heifers frequently display estrus prior to an injection of PG. The experiments described in the fourth chapter focused on the ability of an ACL to delay estrus and ovulation in beef heifers and cows. The specific objectives were as follows: 1) To determine if there is a difference between heifers and cows in the ability of GnRH to induce ovulation on d 16 of the estrous cycle (d 0=estrus), 2) To determine if there is a difference in size of the preovulatory follicle for heifers and cows that ovulate versus those that do not ovulate in response to GnRH on d 16 post estrus, 3) To determine if there is a difference between heifers and cows in the ability of a GnRH-induced ACL on d 16 of the estrous cycle to delay luteolysis until after d 23 (PG injection) and to extend estrous cycle length, and 4) To determine if there is a difference in serum concentrations of P4 produced by the ACL of heifers and cows.

CHAPTER II

LITERATURE REVIEW

The emphasis in the beef industry has changed from producing a commodity (e.g. weaned calves) to producing a food item (beef). As a result, more profit is being realized by producers who place emphasis on the genetic improvement of their herds for economically relevant traits (e.g. calving ease, growth traits, and carcass traits). Currently, the most effective tool for genetic improvement is artificial insemination (AI). Even with the availability of AI, the number of beef producers that take advantage of this breeding method is relatively small (< 8%, NAHMS, 2008). The primary deterrent to adoption of AI by beef producers is the time and labor associated with implementing the technology, especially detection of estrus (NAHMS, 2008). Therefore, reducing or eliminating the need for estrus detection through effective fixed-timed AI (FTAI) protocols is essential for increasing the utilization of AI. In addition to genetic improvement, beneficial effects such as induction of cyclicity in anestrous animals (Lucy et al., 2001; Odde, 1990; Patterson et al., 1989a) and increasing the proportion of animals that conceive earlier in the breeding season (Lucy et al., 2001) are benefits of estrous synchronization. To effectively synchronize the estrous cycle, both the follicular and luteal phases must be managed. The latter phase is most commonly manipulated through the use of progestins/progesterone (P4) and prostaglandin $F_{2\alpha}$ (PG). This review of the

literature will focus on the physiology of the bovine estrous cycle, the use of progestins/P4 in managing the luteal phase of the estrous cycle, the development and problems associated with persistent follicles, and the formation and function of accessory corpora (ACL) lutea following gonadotropin releasing hormone (GnRH)-induced ovulation.

Hormonal Control of the Estrous Cycle

There are two types of sexual cycles in mammals; the menstrual cycle and the estrous cycle. The menstrual cycle pertains to humans and primates and is characterized by three well defined phases (follicular, luteal, and menses) with no set period of sexual receptivity (Rowell, 1963). The follicular phase (approximately 15 days [d] in length; Zeleznik and Benyo, 1994), is characterized by a period of follicular growth and maturation. This phase is also known as the proliferative phase due to the preparation of the endometrium for implantation by the fertilized embryo. Estradiol, a hormone important for uterine growth and development, is secreted during the proliferative phase (Anderson et al., 1975). Ovulation marks the end of the follicular phase and the beginning of the luteal phase (Zeleznik and Benyo, 1994). Following ovulation, a corpus luteum (CL) forms and secretes P4, a hormone necessary for the maintenance of pregnancy (diZerega and Hodgen, 1980). The luteal phase is limited to 14 to 16 d in length due to the finite lifespan of the CL (Zeleznik and Benyo, 1994) and is also referred to as the secretory phase due to the effects of P4 on the endometrium. Unlike ruminants, the functional CL in primates secretes both P4 and estrogen, specifically estrone (Butler

et al., 1975). The last phase, menses, is characterized by luteolysis and a decrease in P4 which precedes the sloughing of the endometrium. In the event that conception occurs, the embryo will produce human chorionic gonadotropin (hCG; signal for maternal recognition of pregnancy) and the CL will be rescued.

The second type of sexual cycle, estrous cycle, is also comprised of three phases: 1) the follicular phase, 2) estrus, and 3) the luteal phase. Unlike the menstrual cycle, there is a defined period of sexual receptivity (estrus) and no endometrial shedding (menses). The follicular phase begins with luteolysis and ends with the onset of estrus. The estrus phase is from the onset of estrus until ovulation and the luteal phase begins with ovulation and ends with the initiation of luteolysis. The preceding phases are described in more detail below.

Follicular phase:

The follicular phase in cattle is characterized by growth of a dominant follicle in preparation for ovulation. Growth of a preovulatory follicle is dependent upon the pulsatile secretions of luteinizing hormone (LH; Evans et al., 1997; Ginther et al., 2001), which is stimulated by GnRH (Mauer and Ripple, 1972). Gonadotropin releasing hormone, also known as luteinizing-hormone releasing hormone, is a decapeptide hormone that is produced by neurons in the hypothalamus and released into the hypothalmo-hypophyseal portal vessels, which drain to the anterior pituitary gland (Convey, 1973). It binds to its receptor, a seven trans-membrane domain receptor (Flanagan et al., 1997), located in the membrane of a gonadotrope and is responsible for

the secretion of follicle stimulating (FSH) and LH (see review by Gharib et al., 1990). Luteinizing hormone is secreted from the anterior pituitary in pulses (Schallenberger et al., 1984) which coincide with the pulsatile release of GnRH (Moenter et al., 1991). A transient rise in FSH is associated with the initiation of follicular waves (Ginther et al., 1996) and FSH is also necessary for follicular steroidogenesis (Wang and Greenwald, 1993). Follicle stimulating hormone release is unaffected by circulating concentrations of P4 (Schallenberger et al., 1984). However, FSH pulse secretion is inversely related to androgens (Evans et al., 1997) and androgens, as well as estradiol, reportedly inhibit FSH release.

Luteinizing hormone is also a key hormone involved in follicular steroidogenesis in cattle (Kinder et al., 1996) and is released in a pulsatile manner (Rahe et al., 1980). Pulses of LH increase in both frequency and amplitude following regression of the CL (Schallenberger et al., 1984) due to a reduction in the negative feedback of P4 (Goodman et al., 1980). Kesner et al., (1982) demonstrated an inhibition of LH secretion through negative feedback of P4 on GnRH. When P4 is high, during the mid-luteal phase of the estrous cycle, LH pulse frequency is reduced; however, pulse amplitude is elevated (Rahe et al., 1980; Walters et al., 1984). Conversely, when P4 is low during the early luteal phase, LH pulses increase in frequency and decrease in amplitude (Rahe et al., 1980; Walters and Schallenberger, 1984). Additionally, LH pulsatility is modified by both P4 and estradiol in the ewe (Goodman et al., 1980). Progesterone inhibits estradiol secretion indirectly through negative feedback on GnRH and LH secretion (Schallenberger et al., 1984). Both FSH and LH stimulate the synthesis and release of estradiol (Fortune and

Armstrong, 1978; Schallenberger et al., 1984) via the two cell – two gonadotropin concept as described below.

The Graafian (dominant) follicle consists of the theca and granulosa cells. These cell layers are responsible for producing both androgens (thecal layer) and estradiol (granulosa layer) in addition to bidirectional communication with the oocyte (Anderson and Albertini, 1976; Gilula et al., 1978). The theca layer is divided into the theca interna and the theca externa. The theca externa consists of connective tissue cells; whereas, theca interna cells can synthesize steroids and are surrounded by an extensive capillary network (Marion et al., 1968). The granulosa cells (GC) are separated from the theca cells (TC) by a basement membrane and are avascular until after the preovulatory gonadotropin surge (van Wezel and Rodgers, 1996). The TC and GC produce androgens and estrogens in response to LH and FSH, respectively (Fortune and Armstrong, 1978).

The two cell – two gonadotropin concept of estradiol synthesis begins with LH simulation of testosterone synthesis by the theca interna. Testosterone synthesis is initiated following conversion of cholesterol to pregnenolone within the mitochondria of TC by P450 side chain cleavage (Farkash et al., 1986). Steroidogenic acute regulatory protein (StAR), first discovered in murine Leydig cells, is required to transfer cholesterol from the outer to inner mitochondrial membrane (Stocco, 1997). The preceding process has been identified as a rate limiting step in steroidogenesis. The protein StAR, is synthesized in the cytosol of steroid producing tissues (Stocco, 2001). Regulation of StAR expression is species and tissue specific. Once inside the mitochondria, cholesterol is converted by P450 side chain cleavage to pregnenolone (Rouiller et al., 1990).

Pregnenolone is further converted to P4 by 3 beta- hydroxysteroid dehydrogenase (3β-HSD; Fortune, 1986) located on the smooth endoplasmic reticulum.

Within TC, P4 is converted by cytochrome P450 17 hydroxylase to androstenedione and subsequently, testosterone. This first step in the synthesis of estradiol is binding of LH to its receptor on the TC. Without LH, testosterone production is significantly decreased in the TC (Fortune and Armstrong, 1978). The addition of pregnenolone to TC results in more androstenedione production compared to addition of P4, providing evidence that the delta ^{5} pathway is preferred by TC for production of testosterone in cattle (Fortune, 1986). Granulosa cells produce limited amounts of androstenedione but aid in the production of this hormone by providing pregnenolone to the TC (Fortune, 1986). The TC produce only limited amounts of estradiol, even when supplied with the necessary androgens (testosterone) required by the GC (Fortune and Armstrong, 1978).

The next step in the two cell – two gonadotropin concept requires the movement of testosterone into the GC compartment. The process that allows significant quantities of testosterone to move into the granulosa compartment rather than entering the thecal vasculature is not clear. The binding of FSH to its receptors in the membrane of GC initiates the expression of aromatase, an enzyme that converts testosterone to estradiol. Production of estradiol dramatically increases with FSH stimulation and provision of androgens as a substrate (Fortune and Armstrong, 1978). Granulosa cells require androgens to produce estradiol; therefore, the addition of testosterone to GC can increase production (Fortune and Armstrong, 1978).

Follicular Waves

In domestic mammals (cattle, sheep, goats, and horses), follicles grow in consecutive waves (Evans, 2003; Ginther and Kot, 1994; Ginther et al., 1995; Ireland et al., 1979; Sirois and Fortune, 1988). Follicular waves begin prior to puberty in heifers (Adams et al., 1994; Evans et al., 1994) and continue to occur during the estrous cycle and throughout pregnancy until shortly before parturition (Ginther et al., 1996; Guilbault et al., 1986; Savio et al., 1990). Typically, there are two or three follicular waves per estrous cycle but rare occurrences of either one or four waves are possible (Sirois and Fortune, 1988). Cattle with three follicular waves tend to have a longer luteal phase than cattle with two waves (Ginther et al., 1989). Typically, initiation of waves occurs around d 2, 10, and 17 of the estrous cycle (d 0 = estrus) in cattle with three waves, rather than d 2 and 11 in cows with two follicular waves (Sirois and Fortune, 1988). In cattle with three waves, the dominant follicle in the second wave tends to be smaller (mean diameter = 10 mm) than during either the first or third (mean diameter = 13 mm) wave (Sirois and Fortune, 1988). Evans et al., (1997) demonstrated that the smaller second wave dominant follicle secreted less estradiol due to the negative feedback of P4 on LH. Savio et al., (1988) demonstrated that a second wave dominant follicle during a three wave cycle grows at a slower rate than the dominant follicle during either the first or third wave. The authors suggested that the difference in growth rate and diameter is likely due to the higher pulse frequency of LH during the early and late luteal phases compared to the mid-luteal phase.

A follicular wave is defined by three different stages: 1) recruitment, 2) selection, and 3) dominance (Sirois and Fortune, 1988). Follicles are initially recruited as a cohort and a single follicle is subsequently selected to become dominant (Goodman and Hodgen, 1983). Matton et al., (1981) demonstrated that larger follicles are able to inhibit the growth of medium to smaller sized follicles during the estrous cycle in cattle. This characteristic of having the ability to suppress growth of other follicles is defined as dominance (Goodman and Hodgen, 1983). Follicles are initially recruited in response to a transient increase in FSH (Ginther et al., 1996).

Currently, there are two general models of follicular selection in cattle (Lucy, 2007); 1) acquisition of LH receptors in the GC and 2) increased bioavailability of insulin like growth factor-1 (IGF-1) which increases the responsiveness of follicular cells to gonadotropin stimulation. In the first model, the selected follicle acquires LH receptors in the GC and becomes LH dependent; whereas, other follicles in the cohort remain FSH dependent and undergo atresia in the face of declining circulating concentrations of FSH. Large dominant follicles have the ability to suppress the growth of small and medium size follicles (Ireland et al., 1979) through secretion of estradiol and inhibin which have a negative feedback on FSH secretion (Goodman and Hodgen, 1983). Therefore, follicles requiring FSH stimulation become atretic and the dominant follicle inhibits new follicular wave formation (Goodman and Hodgen, 1983).

In the second model, free IGF-1 concentrations are elevated in the dominant follicle prior to selection compared to the subordinate follicles (Ginther et al., 2001). Factors, such as IGF-1, are associated with cell growth and proliferation (mitosis) particularly in TC and GC as well as estradiol production (Glister et al., 2001; Spicer and

Stewart, 1996). As reviewed by Beg and Ginther (2006), follicular IGF binding proteins (IGFBP) limit the availability of IGF to its receptor. However, IGFBP proteases such as pregnancy associated plasma proteins, located within the follicular fluid, can degrade these proteins liberating IGF-1. Therefore, IGF-1 can bind to its receptors and act upon the follicular cells. IGFBP protease activity is greater in selected follicles and increases the responsiveness of the follicular cells to gonadotropins. Ginther (2000) suggested that follicle selection may be dependent upon a combination of both models.

Increasing estradiol concentrations produced by the dominant follicle, are responsible for many reproductive characteristics: estrus behavior, induction of the preovulatory gonadotropin surge, sperm transport, change in uterine pH, and an increase in endometrial P4 receptors (Allrich, 1994; Hawk and Cooper, 1976; Kesner et al., 1982; Perry and Perry, 2008; Zelinski et al., 1982). Exogenous administration of estradiol increased sperm movement to the oviducts of sheep (Hawk and Cooper, 1975) and in the uteri of rabbits (Hawk and Cooper, 1976). The authors concluded that this is mediated by altering the direction of uterine contractions so that sperm movement is towards the oviducts in rabbits. Hawk (1983) reported that as estradiol increased, the intensity of uterine contractions increased; conversely, uterine contractions decreased during the luteal phase.

An inverse relationship between uterine pH at insemination and the probability of pregnancy was reported in cattle (Lares et al., 2007). Estradiol decreased uterine pH at estrus compared to the mid-luteal phase (Elrod and Butler, 1993). Perry and Perry (2008) injected estradiol cypionate (ECP) into cows and measured circulating concentrations of estradiol and uterine pH at estrus and subsequently compared uterine pH and circulating

concentrations of estradiol between treated and non-treated cows. They demonstrated that cows displaying standing estrus had lower uterine pH compared to cows that were not in standing estrus; these values were independent of treatment with ECP. Furthermore, results from this study demonstrated that increased concentrations of estradiol were consistent with a decrease in uterine pH. Sperm motility decreased when pH was lowered by 0.5 pH units (Jones and Bavister, 2000) which might increase sperm longevity in utero (Perry and Perry, 2008). Perry and Perry (2008) suggested that a decrease in uterine pH would temporarily reduce sperm motility and increase sperm lifespan. Acott and Carr (1984) also reported that sperm motility was decreased at a lower pH. Increasing sperm survival in utero may potentially improve pregnancy rates of cattle in estrus within 24 hr of FTAI.

Zelinski et al., 1980 reported that estradiol administration increased the number of P4 receptors within the endometrium of sheep during the mid-luteal phase of the estrous cycle. Aronica and Katzenellenbogen (1991) observed similar results in rats during estrus when estradiol concentrations were elevated. Additionally, estradiol has the ability to increase expression of estradiol receptors within the uterus (Xiao and Goff, 1999). Bovine estradiol receptor expression is greater during the late luteal phase compared to mid-luteal phase (Zelinski et al., 1982). These increases in receptor expression are consistent with rising estradiol concentrations responsible for controlling estrus in cattle (Allrich, 1994).

Estrus:

Estrus (d 0 of estrous cycle), the period of sexual receptivity, is characterized by a number of visual secondary signs (e.g. increased vocalization, increased activity and increased cervical mucus production [Alliston et al., 1958; Kiddy, 1977; Schon et al., 2007]). However, the key characteristic of estrus (standing estrus) is standing to be mounted (behavioral estrus; Howes et al., 1960). Cattle in the estrus phase increase mounting activity compared to the mid-luteal phase (Alexander et al., 1984). On average, standing estrus duration is 11 to 20 hr but can range from 0 to greater than 25 hr (White et al., 2002) . Heifers tend to exhibit standing estrus for a shorter duration (approximately 7 hr) than cows (approximately 14 hr; Walker et al., 1996). These data were consistent with results reported by Roelofs et al. (2005) that primiparous cows demonstrated estrus more intensely than multiparous cows. Additionally, there exists both seasonal and time of day effects on the duration of standing estrus expressed by cattle (Gwazdauskas et al., 1983; White et al., 2002). White et al., (2002) monitored estrus in beef cattle using the Heat Watch system during the winter, spring, and summer months. Their data demonstrated that cows were in estrus longer during the summer months compared to winter or spring and the interval between mounts was greater during the summer compared to the other time periods (White et al., 2002). Alternatively, At-Taras and Spahr (2001) reported that warmer temperatures decreased the duration of estrus activity. White et al., (2002) suggested the decrease in estrus activity of cattle in warmer climates is due to the lack of willingness, rather than a physiological reason. Floyd et al., (2009) reported that the number of mounts per female increased as the number of cows in estrus

increased; therefore, grouping cows together that are in estrus at one time can aid in estrus detection.

Estrus is controlled by circulating concentrations of estradiol (Allrich, 1994) produced by the large dominant follicle. Estradiol concentrations peak approximately 7 hr after the onset of estrus (Saumande and Humblot, 2005). This increase in estradiol is necessary for the preovulatory LH surge (Kesner et al., 1981). Peak concentration of LH occurs approximately 10 hr after the onset of estrus (Saumande and Humblot, 2005). *In vivo,* estradiol stimulates GnRH secretion by the hypothalamus through a positive feedback mechanism (Moenter et al., 1991). An increase of GnRH is necessary for the LH surge (Bowen et al., 1998) which is required for ovulation (Henricks et al., 1970). In preparation for the LH surge, GnRH concentrations increased approximately 40 fold compared to basal concentrations (Moenter et al., 1991) in association with an increase in estradiol. During this time, GnRH pulse frequency increased and amplitude decreased, resulting in an accumulation of GnRH within hypothalamus (Moenter et al., 1991). Pulsatile secretion of LH was preceded by pulsatile secretion of GnRH (Moenter et al., 1991). During the surge, LH is released in a pulsatile manner consisting of high frequency, low amplitude pulses (Rahe et al., 1980). Following the LH surge, gonadotrophes continue to release LH into the blood stream as a result of GnRH stimulation, even after the surge is observed (Moenter et al., 1991). In cattle, Walters and Schallengberger (1984) observed an increase in estradiol until the preovulatory surge at which point, estradiol decreased. This decrease in estradiol was due to the inhibition of estradiol production following the onset of the gonadotropin surge (Komar et al., 2001).

The concentration of LH following the surge returns to baseline approximately eight to ten hr after initial release (Rahe et al., 1980).

Decreases in LH are observed following a transient increase in P4 (Kesner et al., 1982). P4 increases following ovulation (Milvae and Hansel, 1983) and inhibits pulse frequency of LH (Rahe et al., 1980). Therefore, decreases in LH concentration and pulse frequency are observed with the increase in P4 after ovulation (Kesner et al., 1982). On average, cattle ovulate approximately 30 hr after the beginning of estrus (Roelofs et al., 2005; White et al., 2002); however, there are different reports of exact ovulation time in cattle. Saumande and Humblot (2005) reported that ovulation occurs at approximately 40 and 30 hr after onset of estrus and after LH peak, respectively. Alternatively, Walker et al., (1996) reported that ovulation occurred at a mean time of 27.6 hr after the onset of estrus. The authors also stated that longer intervals from onset of estrus to ovulation were associated with increased duration of estrus expression. This phenomenon was suggested to be a function of amount of estradiol and duration of exposure to estradiol.

Luteal Phase:

The luteal phase begins following ovulation and ends with the onset of luteolysis. There are three stages of luteal development during the luteal phase of the estrous cycle; 1) corpus hemorrhagicum (CH), 2) corpus luteum (CL), and 3) corpus albican (CA). The CH is a red or "bloody" structure that develops during the first d after estrus and is non-responsive to PG (Louis et al., 1973) until d 5 or later in cattle. Prostaglandin is the luteolytic signal from the uterus (McCracken et al., 1981). Blood vessels begin to form

and infiltrate the new structure to provide blood flow to the developing CH through angiogenesis. Typically, the lifespan of the CH is around 3 d at which point, it is referred to as a CL, the longest stage of the luteal phase. Average luteal lifespan is approximately 17 d. Then the CL is lysed and transitions into a CA, the last stage of the luteal phase.

The CL is a major source of P4 during the luteal phase. The target organs of P4 include the hypothalamus, pituitary, ovary, uterus, and mammary gland. If conception occurs, the CL continues to produce P4 and maintain pregnancy. However, if conception does not occur, PG will be produced by the uterus and regress the CL (Schallenberger et al., 1984). During the luteal phase, P4 exerts negative feedback on the hypothalamus reducing GnRH secretion which causes a transient decrease in LH (Schallenberger et al., 1984). Progesterone inhibits the expression of estrus and ovulation by reducing secretion of LH (Kinder et al., 1996) and estradiol (Short et al., 1973). Increased concentrations of P4 are associated with high amplitude, low frequency pulses of LH (Rahe et al., 1980). Conversely, when P4 concentration is decreased, LH increases in frequency and decreases in amplitude (Schallenberger et al., 1984). The release of LH during low circulating concentrations of P4 is necessary for estradiol production. Synthesis of estradiol is inhibited through negative feedback of P4 on the hypothalamus and anterior pituitary, thus decreasing LH stimulation of the thecal cells (two-cell, two gonadotropin concept). However, once the CL is lysed, this negative feedback mechanism is removed and both estradiol and LH concentrations increase.

There are two major types of luteal cells that originate from the recently ovulated follicle and are responsible for producing and secreting P4 (Ursely and Leymarie, 1979). The TC and GC of the follicle differentiate into small luteal cells (SLC) and large luteal

cells (LLC), respectively (Alila and Hansel, 1984). The majority of circulating P4 (> 80%) is produced by the LLC (Niswender et al., 1985). There are many functional differences between SLC and LLC (Fitz et al., 1982). The authors studied the differences between the two cell types of the sheep CL and reported the following results: LLC have higher basal secretion of P4 compared to SLC, LLC had more PG receptors than SLC, and LH stimulation is needed to drive SLC production of P4 but it is not needed for LLC P4 production. These data suggested PG-induced luteolysis of SLC is dependent upon communication between LLC and SLC.

The biosynthesis of P4 by the CL is similar to P4 synthesis during the first step of the two-cell, two gonadotropin concept. It begins with the transport of cholesterol into the mitochondria by StAR, the rate-limiting step in P4 biosynthesis (Niswender, 2002). Once inside the mitochondria, cholesterol is converted to pregnenolone by P450 side chain cleavage. Pregnenolone is transported out of the mitochondria and to the endoplasmic reticulum where it is further converted to P4 by 3β-HSD.

Use of progestins/progesterone for synchronization of estrus

Previous research has shown that P4 administration can inhibit estrus and ovulation in cattle (Sirois and Fortune, 1990; Zimbelman and Smith, 1966a). As a result, exogenous progestins are used as tools in synchronization protocols (Odde, 1990). In prepubertal heifers and anestrous cows, progestin treatment can simulate the short luteal phase which occurs before the first ovulatory estrus (Patterson et al., 1989a; Smith et al., 1987). Currently, there are two commercially available progestins that are used for

estrous synchronization: 1) Melengestrol Acetate (MGA) and 2) Controlled Internal Drug Release (CIDR).

Melengestrol Acetate:

Melengestrol acetate is a synthetic, orally-active progestin that inhibits both estrus and ovulation in cattle (Zimbelman and Smith, 1966b). It also is capable of inducing cyclicity in non-cycling heifers (Imwalle et al., 1998; Patterson and Corah, 1992). Melengestrol acetate is approved by the Food and Drug Administration (FDA) for use in heifers (Federal Register, 1997) and acts primarily as a progestin with an affinity for the P4 receptor that is 11-times greater than P4; therefore, circulating concentrations of MGA at the dose used in estrous synchronization protocols (0.5 mg/hd/d) are able to be lower than mid-luteal phase concentrations of P4 (Perry et al., 2005).

Zimbelman and Smith (1966a) discussed the chemical alterations to medroxyprogesterone acetate (MAP), another progestin previously used for the inhibition of estrus and ovulation in ewes (Fitzgerald et al., 1985), to produce MGA. These alterations increase the potency of MGA compared to MAP (Zimbelman and Smith, 1966a). The authors described the following chemical modifications that altered the chemical structure of MAP to produce MGA; 1) an addition of 6-dehydro to MAP so that it becomes 6-dehydro-MAP and 2) addition of a methylene on the 16 position to change 6-dehydro-MAP to 6α-methyl-6-dehyro-16-methylene-17-acetoxyprogesterone. The authors of this paper also discussed unpublished data that showed a 16-methyl alteration was not as effective as a 16-methylene addition. The modifications are required for an

increased potency of MGA with the later modification producing the greatest increase in effectiveness. Melengestrol acetate is several hundred times more potent than MAP when fed orally (Zimbelman and Smith, 1966a). However, MAP is more effective than MGA when injected intravenously. By way of explanation, Zimbelman and Smith (1966b) hypothesized that ruminal microbes convert MGA to a different compound that is increased in potency. In a review, Patterson et al. (1989) stated that further studies unsuccessfully supported or rebutted this claim.

The chemical structure of MGA reduces the rate of metabolism in the liver compared to progesterone. It is essential that individual animals consume the required amount of MGA (0.5 mg/hd/d) for treatment to be effective. Therefore, adequate bunk space and 0.91 to 1.8 kg of carrier is necessary for proper application of MGA to a breeding herd. Unequal intake among groups treated with MGA results in decreased efficacy overall. If the proper dose (0.5 mg/hd/d) of MGA is consumed, the peripheral concentration of circulating MGA is 0.03 ng/mL (Hageleit et al., 2000). However, due to individual differences of MGA metabolism, there exists variation in circulation concentrations (Perry et al., 2005). Research has shown that a dose of 0.25 mg of MGA fed daily is sufficient at inhibiting estrus but 0.5mg/hd/d in a single feeding is required to inhibit both estrus and ovulation 100% of the time (Zimbelman and Smith, 1966a). Pregnancy can be maintained with MGA fed at a dose equal to or greater than 1mg/d in the absence of both ovaries (Zimbelman and Smith, 1966c). However, if one ovary is removed, 4mg/d must be fed to avoid termination of the pregnancy (Zimbelman and Smith, 1966c). The authors suggested the increased requirement of MGA when one ovary is removed is due to the increased estrogenic activity of the remaining ovary. Treatment

with MGA causes increased follicular growth and estradiol production (Zimbelman and Smith, 1966c), thus maintenance of pregnancy with one intact ovary requires an increased level of progestin compared to removal of both ovaries to inhibit estrogenic affects of the large follicle on the remaining ovary.

In addition to being a tool in estrous synchronization, MGA has been incorporated into feedlot diets to increase rate of gain (Bloss et al., 1966). Cattle on high energy diets containing MGA tend to have increased weight gains and improved feed efficiencies compared to diets without MGA (Bloss et al., 1966). There are two ways that MGA can increase rate of gain: 1) prevent estrus and 2) anabolic effect of estradiol. A problem with heifers in feedlots is the occurrence of estrus expression during which time, feed consumption is lowered. However, with the addition of MGA, estrus is suppressed and a decrease in feed intake is not observed (O'Brien et al., 1968). Additionally, increased follicular estradiol production in heifers fed MGA has an effect similar to growth promoting implants, resulting in increased weight gains compared to non-treated heifers. In an experiment conducted by Bloss et al. (1966), heifers treated with MGA for 98 d weighed 7.2% more than non-treated heifers. In addition to this first study, Bloss and his colleagues noted non-sexually mature animals had weight gains that were 6.2% higher than sexually matured animals after a 111 d treatment period. The preceding results are likely due to the increased circulating concentrations of estradiol produced by the dominant follicle that persists on the ovary of heifers treated with MGA without a corpus luteum (Sirois and Fortune, 1990; Zimbelman and Smith, 1966b). Any direct estrogenic effect caused by MGA would require an oral dose 1,000 times higher than the currently fed dose (0.5mg/hd/d; Perry et al., 2005). In the studies completed by Bloss et al. (1966),

individual animals were randomly selected and ovariectomized prior to the experiment. These animals had lower weight gains and poorer performance than those with intact ovaries supporting the claim that estradiol from an ovarian another source (the dominant follicle), not MGA, promotes weight gain.

Although effective at inhibiting estrus and ovulation, long-term administration of MGA (14 d or greater), in the absence of a CL, results in a lower pregnancy rate when insemination occurs at the synchronized estrus (Patterson et al., 1989a). However, fertility at the subsequent estrus is not negatively affected (Patterson et al., 1989a). Conversely, short-term treatment (9 to 12d) of P4 does not negatively affect pregnancy rates (Roche, 1974). This is only true if treatment is administered early in the estrous cycle (Patterson et al., 1989b). If administered during the latter portion of the estrous cycle, MGA treatment can negatively influence pregnancy rates in cattle during the synchronized estrus (Patterson et al., 1989b). Patterson et al. (1989b) conducted a study in which MGA was fed for 7 d starting on d 0 to 5, d 6 to 11, d 12 to 16, and d 17 to 21 of the estrous cycle with PG administration at the end of MGA treatment and conception rates for each group were 82%, 69%, 42% and 21% respectively. Conception rates declined when MGA treatment was initiated later in the estrous cycle. The decrease in pregnancy rates following P4 treatment was due to the formation of persistent follicles (Patterson et al., 1989a).

Persistent Follicle:

Long-term treatment (approximately 14 d or greater) with progestins, result in good synchrony of estrus (Hill et al., 1971); however, fertility at the synchronized estrus is reduced during extended exposures to progestins in the absence of a CL (Sirois and Fortune, 1990). Short-term progestin treatment (approximately 7 d) does not negatively affect fertility (Roche, 1974), although the synchrony is poor, unless PG is administered at the end of progestin treatment. Previous research has demonstrated that persistent follicles (PF) form during long-term progestin treatment in the absence of a CL (Cooperative Regional Research Project, 1996; Yelich et al., 1997; Zimbelman and Smith, 1966b). Persistent follicles are characterized by four main attributes; 1) increase in estradiol production, 2) larger follicle diameter, 3) increase in duration of growth, and 4) contain aged-oocytes. This type of follicle can disrupt normal follicular dynamics by suppressing FSH and delaying new follicular wave initiation (Lamond et al., 1971; Yelich et al., 1997).

Long-term treatment with MGA results in the formation of PF (Zimbelman and Smith, 1966b). Due to the increased affinity of MGA for the P4 receptor (Perry et al., 2005), MGA can inhibit estrus and ovulation at lower circulating concentrations (0.3 ng/ml; Hageleit et al., 2000) than P4. Rahe et al., (1980) demonstrated that low concentrations of P4 (e.g. early or late luteal phase) increase LH pulse frequency, but decrease LH pulse amplitude. The release of LH during MGA treatment increases in frequency (Custer et al., 1994). In another study conducted by Kojima et al. (2003), cows were treated with various levels of progestins with the lowest level resulting in high

frequency, low amplitude pulses of LH. This type of LH pulsatility drives follicular growth, estradiol secretion, and the formation of PF (Kinder et al., 1996). Increased growth of a dominant follicle is positively correlated with increased estradiol production (Kojima et al., 1995) when circulating P4 concentrations are low. Persistent follicles are larger in size (diameter) compared to non-persistent follicles during MGA treatment (Zimbelman and Smith, 1966b) due to an increase in lifespan. However, PF lifespan seems to be limited in that dominance is eventually lost (Anderson and Day, 1994; Kojima et al., 2003). These follicles secrete increased quantities of estradiol which increase LH secretion (Sirois and Fortune, 1990). Increased estradiol production by PF is associated with lower conception rates (Kojima et al., 1995; Patterson et al., 1989a; Stock and Fortune, 1993). Inhibin production also increases in addition to estradiol and has the ability to prevent the initiation of a new follicular wave (Knight and Glister, 2001).

Treatment with MGA, in the absence of a CL, causes alterations in LH pulsatility that can affect oocyte development (Mihm et al., 1994). Reductions in fertility are the result of the pre-mature resumption of meiosis by the oocyte within the follicle (Mihm et al., 1994). The pre-mature resumption of meiosis is likely due to the exposure of the persistent follicle and oocyte to high frequency pulses of LH over sustained period of time. Aged-oocytes that ovulate from PF negatively affect overall pregnancy rates (Kinder et al., 1996). Fertility is reduced due to the condensation of the chromosomes and the transition from metaphase I into metaphase II (Mihm et al., 1994). Revah and Bulter (1996) reported similar results in bovine oocytes under long-term exposure to progestins. After nine days of progestin exposure (and increased estradiol), oocytes had expanded cumulus cells and germinal vesicle break down; both are key events in oocyte nuclear

maturation. These processes normally occur following the LH surge; however, the follicle ovulates as a result. Persistent follicles persist until they turnover or until the progestin is removed and ovulation occurs (O'Brien and Zimbelman, 1970; Sirois and Fortune, 1990). Even though the follicle ovulates, PF result in aged-oocytes that are unable to continue beyond the 16-cell stage and embryo recovery rates were lower in cattle with PF (Ahmad et al., 1995).

There also are reports that increased estradiol production caused negative modifications to the uterine environment. A study completed by Safro et al., (1990), using the mouse model, demonstrated that the ratio of estradiol to P4 concentration is important for proper embryonic implantation. When estradiol is high compared to P4, implantation was inhibited; whereas, equal amounts of P4 and estradiol had no effect (Safro et al., 1990). Delayed ovulation in rats decreased normal implantation rate of embryos suggesting uterine environment plays a role in healthy implantation (Butcher et al., 1969). Similarly, Butcher and Pope (1979) showed that delayed ovulation in rats caused increased estradiol production which was associated with improper implantation, increased embryo mortality, and developmental anomalies.

Controlled Internal Drug release:

A CIDR is a T-shaped, intra-vaginal device inserted into a cow or heifer to simulate the presence of a CL. The design is a synthetic nylon backbone coated in silicone that is embedded with 1.38 g (North America; Pharmacia Animal Health, Kalamazoo, MI) or 1.9 g (Canada; Bioniche Animal Health, Belleville, ON, Canada) of

P4. Overtime, P4 is slowly released and absorbed through the vaginal tissue into the blood stream. Unlike MGA, CIDR are legal for use in both lactating and non-lactating cows and heifers. However, CIDR are approved by the Food and Drug Administration for one-time use and are capable of initiating cyclicity in non-cycling animals, similar to MGA. Both anestrous cows (Cerri et al., 2009; Fike et al., 1997; Sá Filho, et al., 2009) and pre-pubertal heifers (Lucy et al., 2001) can be induced to begin cycling with CIDR treatment.

In combination with PG, CIDR, are very effective at synchronizing estrus (Mapletoft et al., 2003; Roche, 1974). The addition of a CIDR in estrous synchronization protocols increases pregnancy rates compared to non-treated cows (Martinez et al., 2002). Additionally, there are no differences in pregnancy rates in beef cattle treated with a new, once-used, or twice-used CIDR (Colazo et al., 2004). Previous research has shown that there is a sufficient amount of P4 within a CIDR following a 9 d insertion (Meneghetti et al., 2009). However, re-using CIDR creates a concern regarding spread of disease since CIDR are difficult to sanitize completely and there is risk for sexually-transmitted disease transmission. Zuluaga and Williams (2008) sterilized CIDR using an autoclave. CIDR were re-inserted into ovariectomized cows and blood samples were collected to measure serum concentrations of P4 in three treatment groups; 1) New CIDR, 2) Disinfected (chlorhexidine gluconate) and 3)Autoclaved CIDR. Results showed that treatment with Autoclaved CIDR resulted in higher concentrations of P4 compared to the other treatment groups. The authors suggest the increase in P4 was due to a modification of the CIDR insert or the arrangement of P4 within the insert. Nonetheless, disease transmission is

possible with the re-use of CIDR and it is not recommended to re-use CIDR due to the possibility of spreading disease.

In CIDR based estrous synchronization protocols, PG is often used to regress the CL enabling estrus and ovulation following the removal of the progestin. Decreased P4 following PG injection results in increased LH pulse frequency (Rahe et al., 1980) thus driving follicular growth and steroidogenesis (Kinder et al., 1996; Schallenberger et al., 1984). In the presence of an external progestin, PF form in the absence of a CL.

Accessory CL:

In addition to controlling the luteal phase with progestins and PG, it's equally important to manipulate a follicular wave to ensure that a competent oocyte will be ovulated during estrus. Follicular waves can be manipulated by inducing ovulation with GnRH or human chorionic gonadotropin (hCG; Bo et al., 1995) or by negative feedback on FSH or LH following injection of estradiol or P4 (Bo et al., 1995; Anderson and Day, 1994), respectively. When administered during an estrus synchronization protocol, GnRH is commonly combined with progestin (MGA or CIDR) treatment resulting in the synchronization of both follicular waves and the luteal phase. In addition to GnRH, hCG (a gonadotropin) is administered to cattle to induce ovulation (Kerbler et al., 1997). If GnRH is administered at the start of short-term (7 d) progestin treatment, fertility is not adversely affected following progestin removal (Martinez et al., 2002). Ovulation will occur in dominant follicles greater than 10 mm in diameter following injection of GnRH or hCG (Twagiramungu et al., 1994). As a result, a CL will develop and produce P4. If

GnRH or hCG is administered during the luteal phase, an accessory CL will form and secrete P4 (Kerbler et al., 1997). Heifers injected with hCG on d 5 of the luteal phase have higher concentrations of P4 compared to heifers injected with saline as a result of ACL formation (Diaz et al., 1998). However, lifespan of the hCG-induced ACL is shorter (regression occurs in approximately one week) compared to spontaneous CL (Stevenson et al., 2008). Sianangama and Rajamahendran (1996) demonstrated that hCG-induced ACL were functional, however, they tended to secrete lower circulating concentrations of P4 compared to spontaneous CL.

Prostaglandin is incorporated into synchronization protocols to regress the CL and eliminate endogenous P4 production. Without PG prior to progestin removal, inhibition of ovulation and estrus will occur due to the elevated concentrations of endogenous P4. Accessory CL are responsive to PG by 2 d following hCG-induced formation (Howard and Britt, 1990); whereas, spontaneous CL are not responsive until d 5 after induction (Lauderdale, 1972). Howard and Britt (1990) suggested one reason for the increased sensitivity of the ACL to PG is because the ACL develops in the presence of elevated concentration of P4 compared to the endocrine environment during development of the spontaneous CL. However, this claim was not supported by findings of Battista et al., (1984) who demonstrated that exogenous P4 injected into heifers elevated serum concentrations of P4 but did not increase the sensitivity of the spontaneous CL to PG on d 3 of the estrous cycle compared to non-treated heifers. Wiltbank and Tsai (1998) demonstrated that the inability of the early bovine CL to respond to PG is possibly due to the altered gene expression of intra-PG synthesis; a process that seems to be needed for

luteolysis and requires further research. Currently, there is little research on GnRH-induced ACL development.

CHAPTER III

EFFECT OF PROGESTIN TREATMENT ON FORMATION OF PERSISTENT FOLLICLES IN CYCLING HEIFERS AND POSTPARTUM BEEF COWS

ABSTRACT

Progestins (melengestrol acetate [MGA] and Controlled Internal Drug Release [CIDR] inserts) are frequently used to effectively control estrus and ovulation in beef cattle. Treatment of an estrous cycling cow with MGA for 14 days (d), in the absence of a corpus luteum (CL), induces formation of persistent follicles (PF) and a corresponding reduction in fertility. The overall objectives were to determine if the presence of a new or used CIDR in estrous cycling heifers and postpartum cows, without a CL, would induce the formation of PF, to compare serum concentrations of progesterone (P4) and estradiol in heifers and cows treated with a new or used CIDR to luteal phase concentrations of P4 and estradiol in non-treated heifers and cows, and to determine the effect of length of storage of used CIDR on circulating concentrations of P4 in cycling beef heifers and postpartum beef cows. Estrous cycling heifers were allocated by age, weight, and breed into four treatment groups: Control (n = 8), MGA (n = 3; 0.91 $kg^{-1}hd^{-1}d$), New CIDR (n = 7; 1.38 g P4), and Used CIDR (n = 7; new CIDR previously inserted into cows for 7 d and subsequently stored for 6 months [mos.]). Postpartum estrous cycling cows were allocated by d postpartum, age and body condition score to the following treatments: Control (n=10), MGA (n = 10; 0.91 $kg^{-1}hd^{-1}d$), New CIDR (n = 8; 1.38 g P4), and Used

CIDR (n = 6; new CIDR previously inserted into cows for 7 d and subsequently stored for approximately 6 mos.) Both heifer and cow groups were presynchronized with a CIDR for 7 d, prostaglandin $F_{2\alpha}$ (PG) was administered on d 6, and GnRH was administered 48 hours (hr) after CIDR removal. Progestin treatment began on d 4 post-estrus and PG was injected on d 6 to induce luteolysis (d 0 = estrus). Progestin treatment, MGA or CIDR, continued for 14 d and length of a follicular wave was defined as the interval from follicular recruitment to ovulation or initiation of a new wave. In heifers, length of the first follicular wave (d) was 9.6^{a}, 19.3^{b}, 16.6^{bc}, and 16.1^{c} ($^{abc}P < 0.05$) and maximum diameter (mm) of the dominant follicle was 14.3^{a}, 20.2^{b}, 16.0^{a} and 18.3^{b} for the Control, MGA, New CIDR, and Used CIDR groups, respectively; ($^{ab}P < 0.002$). Dominant follicle diameter was greater ($P < 0.05$) in the Used CIDR group compared to the New CIDR group after d 10 of treatment but similar to the MGA group. Serum concentrations of P4 in the New and Used CIDR groups were similar ($P > 0.05$) throughout the 14 d treatment period but lower than in the Control group. In postpartum cows, length of first follicular wave (d) was 9.6^{a}, 14.2^{b}, 14.5^{b}, and 13.3^{b} ($^{ab}P < 0.05$) and maximum diameter (mm) of the dominant follicle was 14.0^{a}, 17.3^{b}, 17.1^{b}, and 17.0^{b} ($^{ab}P < 0.02$) in the Control, MGA, New CIDR, and Used CIDR groups, respectively. Maximum diameter of the dominant follicle was similar ($P > 0.1$) in the MGA, New CIDR, and Used CIDR groups. Serum concentrations of P4 in the New and Used CIDR groups were similar ($P > 0.1$) throughout the 14 d treatment period but lower than in the Control group. Serum estradiol concentrations were significantly ($P < 0.05$) lower for heifers in the Control group compared to heifers treated with MGA, New CIDR, and Used CIDR from d 9 until d 15. Serum estradiol concentrations for cows in the Control group were

significantly lower ($P < 0.05$) compared to concentrations of cows treated with MGA, New CIDR or Used CIDR. There were no differences ($P > 0.05$) among treated cows with MGA, New, or Used CIDR. There were no significant differences ($P > 0.88$) in serum concentrations of P4 of animals treated with a new CIDR, used CIDR with no storage (CIDR-0), or used CIDR stored for 6 mos. (CIDR-6). In summary, treatment with a new or used CIDR induced formation of PF in estrous cycling heifers and postpartum beef cows and serum concentrations of P4 were not between the New and Used CIDR groups.

INTRODUCTION

Expanded use of artificial insemination (AI) and adoption of emerging reproductive technologies for beef heifers and cows require precise methods of ovulation control that do not compromise fertility. Effective control of ovulation requires the synchronization of follicular waves and timing of luteolysis. Efforts to develop more effective AI protocols have utilized progestins (melengestrol acetate [MGA] and Controlled Internal Drug Release [CIDR]) to synchronize ovulation in cycling and anestrous cattle. The preceding products are commercially available and have the same biological action as progesterone (P4). Melengestrol acetate is orally active and administered in the feed; whereas, CIDR are inserted into the vagina for 7 to 14 days (d). Previous studies demonstrate that long-term treatment with MGA, in the absence of a corpus luteum (CL), causes formation of a persistent follicle (PF) that results in a low conception rate after ovulation. The effect of a new or used CIDR on follicular dynamics and formation of a PF has not been thoroughly examined in beef cattle. For purposes of

this study, the term "used CIDR" will refer to a CIDR that had been previously inserted into a cow for 7 d and subsequently stored. The specific aims of this study were to 1) To determine if the presence of a new or used CIDR, in estrous cycling heifers and postpartum cows, without a CL, will induce the formation of a PF, 2) To compare the pattern of circulating concentrations of P4 and estradiol in heifers and cows treated with a new or used CIDR to mid-luteal phase concentrations of the preceding hormones in non-treated heifers and cows, and 3) To determine the effect of length of storage of used CIDR on circulating concentrations of P4 in estrous cycling beef heifers and cows.

MATERIALS AND METHODS

Experiment 1

Estrous cycling Angus and Simmental crossbred heifers (n = 32) were allocated by age, weight, and breed into the following treatment groups: Control (n = 8), MGA (n = 3; 0.91 $kg^{-1}hd^{-1}d$), New CIDR (n = 7; 1.38 g P4) and Used CIDR (n = 7; new CIDR previously inserted into cows for 7 d and subsequently stored for 6 mos.). There were 7 heifers that were removed from the project and not included in the analysis for the following reasons: 1) ovulated during MGA treatment (n = 4), 2) did not respond to GnRH during pre-synchronization (n = 1), and 3) had 2 dominant follicles during treatment (n = 2). Each group was presynchronized with the CIDR-prostaglandin $F_{2\alpha}$ protocol (CIDR insertion for 7 d; injection of 25 mg of prostaglandin $F_{2\alpha}$ (PG) [Lutalyse; Upjohn, Kalamazoo, Michigan] i.m. on d 6 of CIDR treatment; gonadotropin releasing hormone (GnRH) treatment 48 hr after CIDR removal) to induce ovulation of a

preovulatory follicle and synchronize the luteal phase before the initiation of treatment. Estrus was detected by identifying activated KMAR heat mount patches. In the MGA, New CIDR, and Used CIDR groups, treatment (MGA or CIDR) began on d 4 post-GnRH (d 0) and PG was injected on d 6 (Figure 3.1). Treatment with MGA or CIDR continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Heifers in the MGA group were fed 0.91 $kgs^{-1}hd^{-1}d$ of carrier containing 0.55 mg MGA kg^{-1} (Cattle Charge – Estrus Control; MFA, Columbia, Missouri). All other heifers were fed carrier (0.91 $kgs^{-1}hd^{-1}d$; Cattle Charge; MFA, Columbia, Missouri) alone during the period of progestin treatment.

Blood sampling: Daily blood samples were collected from d 4 until each heifer or cow completed the experiment (see ultrasonography below). Blood samples were collected by jugular venipuncture into 10 ml vacutainer tubes (Fisher Scientific, Pittsburgh, Pennsylvania). Blood was allowed to clot, stored at 4^0C for 24 hr, and serum was harvested for determination concentrations of P4 and estradiol by radioimmunoassay (RIA). Serum P4 concentrations were measured from d 4 until completion of the project (see ultrasonography below) using a Coat-a-count kit (Diagnostic Products Corporation, Los Angeles, California; Kirby et al., 1997). Intra- and interassay coefficients of variance (CV) and assay sensitivity were 4.8%, 4.2% and 0.1 ng/ml, respectively. Serum estradiol concentrations were measured by RIA (Kirby et al., 1997) from d 4 until animals completed the project (see ultrasonography below). Intra- and interassay CV and assay sensitivity were 13.3%, 5.6%, and 0.25 pg/ml, respectively.

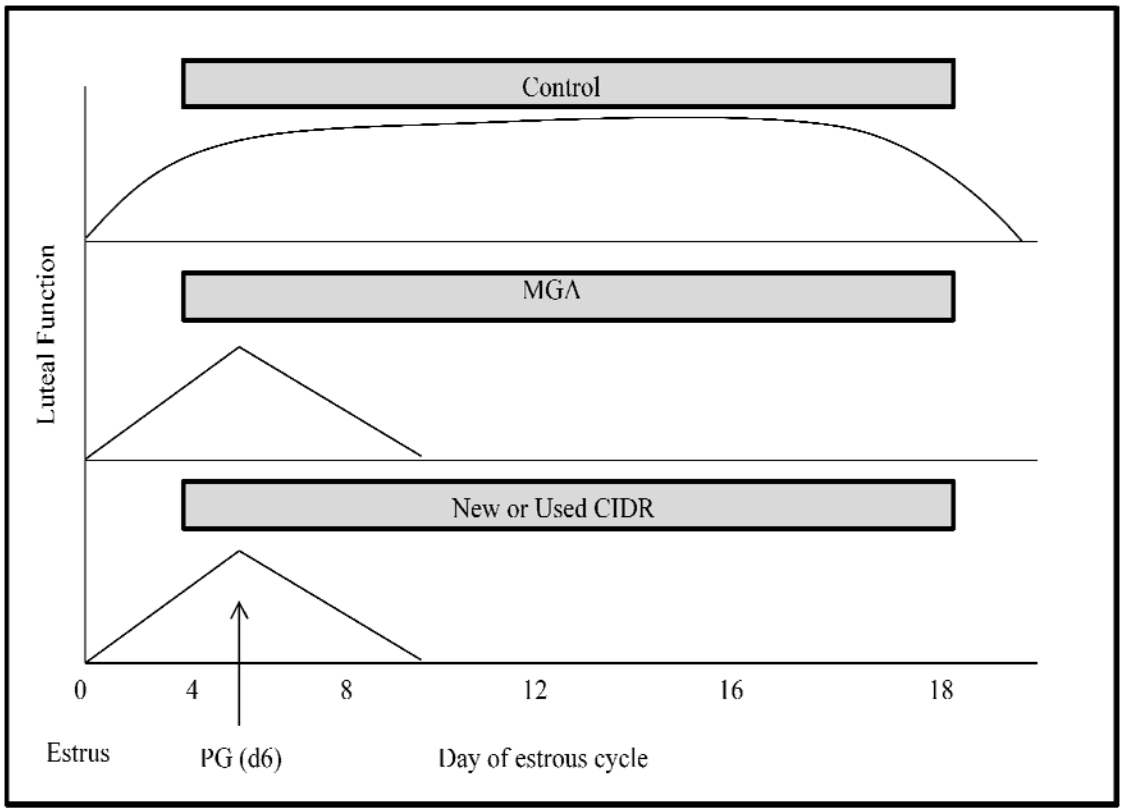

Figure 3.1. Experimental design for experiments 1 and 2. Treatment began on d 4 post-estrus (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). PG was administered to the MGA and CIDR groups on d 6.

Ultrasonography: The ovaries of all heifers and cows were examined on d 0 to determine if a dominant (preovulatory) follicle was present and on d 2 to verify that the follicle ovulated and that a new follicular wave was recruited. Ovaries of each cow were examined daily beginning on d 4 by transrectal ultrasonography with an Aloka 500V ultrasound with a 7.5-MHz transrectal linear probe (Aloka, Wallingford, Connecticut). Follicle diameter was determined by measuring diameter of the follicle at the widest point and then at a right angle to the first measurement. Cows were examined daily from d 4 post-estrus (beginning of MGA or CIDR treatment) until turnover of the dominant follicle or ovulation. Dominant follicles were defined as having a diameter ≥ 9 mm with other follicles regressed or regressing. Dominance was considered to have ended when a new follicular wave emerged (indicative of dominant follicle turnover), display of estrus, or ovulation occurred. Length of a follicular wave was defined as the interval from initiation of a follicular wave (d 2 = recruitment) until turnover of the dominant follicle or ovulation.

Statistical analysis: Effect of treatment on length of follicular wave and maximum diameter of the dominant follicle was analyzed by one-way analysis of variance (PROC GLM; Littell et al., 1998) with SAS (SAS Inst. Inc., Cary, North Carolina). Rate of follicular growth was analyzed using polynomial regression in SAS and higher order effects were removed as they were not significant ($P > 0.05$). An effect of treatment on serum concentrations of P4 and estradiol were analyzed starting from treatment initiation (d 4 of the estrous cycle) through completion of the project (see ultrasonography) by analysis of variance for repeated measures (PROC MIXED; Littell et al., 1998). The

model included P4 or estradiol as the dependent variable, treatment group and day as the independent variables, and all respective interactions.

Experiment 2

Estrous cycling postpartum crossbred cows (n = 41) were allocated by days postpartum, age and body condition score into four treatment groups: Control (n = 10), MGA (n = 10; 0.91 $kgs^{-1}hd^{-1}d$), New CIDR (n = 8; 1.38 g P4), and Used CIDR (n = 6; new CIDR previously inserted into cows for 7 d and subsequently stored for 6 mos.). There were 7 cows that were removed from the project and not included in the analysis for one of two reasons: 1) appeared to have not responded to PG (n = 6) or 2) not observed in estrus following progestin treatment removal (n = 1). Each group was presynchronized with the CIDR-PG protocol (CIDR insertion for 7 d and injection of 25 mg of PG [Lutalyse; Upjohn, Kalamazoo, Michigan] i.m. on d 6 of CIDR treatment and GnRH treatment 48 hr after CIDR removal) to induce ovulation of a preovulatory follicle. Estrus was detected by identifying activated KMAR heat mount patches. In the MGA, New CIDR, and Used CIDR groups, treatment (MGA or CIDR) began on d 4 post-GnRH (d 0 = estrus) and PG was injected on d 6. Treatment continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Cows in the MGA group were fed 0.91 $kg^{-1}hd^{-1}d$ of carrier containing 0.55 mg MGA kg^{-1} (Cattle Charge – Estrus Control; MFA, Columbia, Missouri). All other cows were fed carrier (0.91 $kg^{-1}hd^{-1}d$; Cattle Charge; MFA, Columbia, Missouri) alone during the period of MGA or CIDR treatment. Daily blood samples were collected, ultrasonography was performed, and the data analyzed as

described for experiment 1. Progesterone intra- and interassay coefficients of variance (CV) and assay sensitivity were 3.3%, 10.4% and 0.1 ng/ml, respectively. Estradiol intra- and interassay CV and assay sensitivity were 11.7%, 5.96%, and 0.25 pg/ml, respectively.

Experiment 3

Estrous cycling crossbred cows and heifers (n = 23) were allocated by weight, breed, and parity into four groups: 1) New CIDR (n = 8; 1.38 g P4), 2) Used CIDR-0 (n = 5; new CIDR previously inserted into cows for 7 d and immediately transferred into cows on experiment), and 3) Used CIDR–6 (n = 9; new CIDR previously inserted into cows for 7 d and subsequently stored for approximately 6 mos. before insertion into cows on experiment). One animal in the CIDR-0 group was removed from the project following loss of a CIDR after 10 d of treatment. Animals in each group were presynchronized with an injection of GnRH and insertion of a CIDR on d -9, injection of PG on d -3, CIDR removal on d -2 and GnRH 54 hr after CIDR removal (d 0). Treatment with a CIDR (new or used) began on d 4 and PG was administered on d 6. All CIDR were removed 15 d after insertion.

Blood sampling: Daily blood samples were collected from d 4 until one day post-treatment removal (d 20). Blood samples were collected by jugular venipuncture into 10 ml vacutainer tubes (Fisher Scientific, Pittsburgh, Pennsylvania). Blood was allowed to clot, stored at 4^0C for 24 hr, and serum harvested for determination of serum

concentrations of P4 by RIA. Serum P4 concentrations were measured from d 4 until CIDR removal on d 19 using a Coat-a-count kit (Diagnostic Products Corporation, Los Angeles, California; Kirby et al., 1997). Intra- and interassay CV and assay sensitivity were 2.23%, 6.3%, and 2.24%, respectively.

Ultrasonography: The ovaries of all cows and heifers were examined by transrectal ultrasonography on d -9 during the pre-synchronization period to determine estrous cyclicity status and again on d 0 to determine the presence of a preovulatory follicle. On d 2, all ovaries were examined to confirm ovulation and then again on d 6 to verify the development of a CL. On d 9 (3 d post-PG) ovaries were examined to determine if the CL had regressed.

Statistical analysis: Serum concentrations of P4 from d 4 until d 19 were analyzed by analysis of variance for repeated measures (PROC MIXED; Littell et al., 1998) by using SAS (SAS Inst. Inc., Cary, North Carolina). The model included P4 as the dependent variable, treatment group and d as the independent variables, and all respective interactions. Rate of follicular growth was analyzed by polynomial regression in SAS.

Results

Experiment 1

Dominant Follicle Diameter and Length of Wave in Heifers

Mean length of the follicular wave was prolonged ($P < 0.05$) for the MGA, New CIDR, and Used CIDR groups compared to the Control group. Length of follicular wave was similar ($P > 0.07$) for heifers treated with MGA compared to a new CIDR and for a new CIDR compared to a used CIDR ($P > 0.72$). However, heifers treated with MGA had a longer wave compared to heifers treated with a used CIDR ($P < 0.05$; Table 3.1). There was an effect of day ($P < 0.0001$) on rate of follicular growth among the treatment groups from d 4 to d 10; however, there were no effect of treatment ($P > 0.38$) or treatment by day interaction ($P > 0.47$) for follicular growth rate among the experimental groups. However, dominant follicle growth continued after d 10 in the majority of the heifers in the progestin-treated groups compared to the Control group (Figure 3.2).

Mean maximum follicle diameter for heifers in the Control, MGA, New CIDR, and Used CIDR groups was $14.3 \pm 0.33\text{mm}^{a}$, $20.2 \pm 0.9\text{mm}^{b}$, $16.0 \pm 0.91\text{mm}^{a}$, and $18.3 \pm 1.1\text{mm}^{b}$, respectively (^{ab} $P < 0.05$; Table 3.1). Follicle diameter was similar ($P > 0.2$) in MGA and Used CIDR groups but larger than in the Control and New CIDR groups. Additionally, treatment with MGA extended the length of the follicular wave ($P < 0.0001$) and increased dominant follicle diameter ($P = 0.0005$) compared to the Control group (Figure 3.2); whereas, treatment with a new CIDR, extended the length of the

Table 3.1 Effect of treatment on length of a follicular wave and maximum follicle diameter in heifers.

Treatment [1]	No.	Age, mos.	Wt, kg	Length of Follicular Wave, d [2]	Maximum Follicle Diameter, mm [3]
Control	8	19.6 ± 0.19	509.6 ± 14.20	9.6 ± 0.32 [a]	14.3 ± 0.33 [a]
MGA	3	19.7 ± 0.3	461.1 ± 5.700	19.3 ± 0.33 [b]	20.2 ± 0.90 [b]
New CIDR	7	19.6 ± .16	502.5 ± 17.20	16.6 ± 1.13 [bc]	16.0 ± 0.91 [a]
Used CIDR	7	19.6 ± .14	508.4 ± 9.900	16.1 ± 0.92 [c]	18.3 ± 1.10 [b]

[abc] Within a column, means without a common superscript, differ ($P < 0.05$).
[1] Treatment was administered on d 4 (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Heifers in the MGA, New CIDR, and Used CIDR groups received PG on d 6.
[2] Length of a follicular wave was defined as the interval from initiation of a follicular wave (d 2 = recruitment) until turnover of the dominant follicle, estrus, or ovulation.
[3] Diameter of the dominant follicle was measured daily beginning on d 4 post-estrus by transrectal ultrasonography. Follicle size was determined by measuring diameter of the follicle at the widest point and at a right angle to the first measurement.

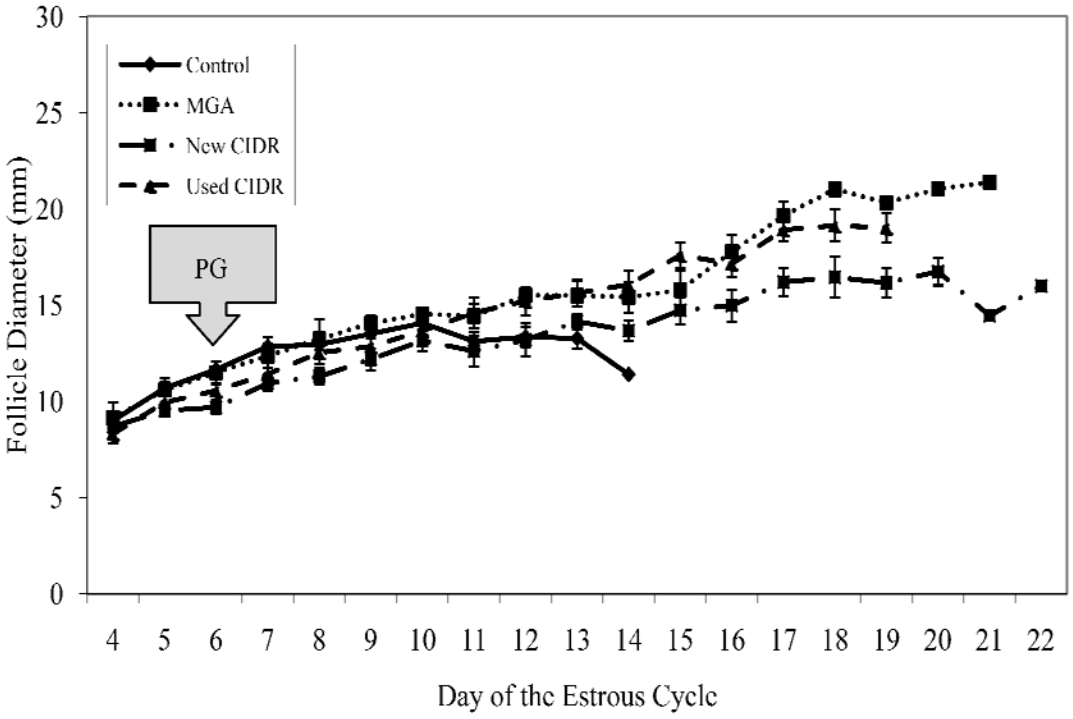

Figure 3.2. Mean (± SEM) dominant follicle diameter for heifers in the following treatment groups; 1) Control (n = 8), 2) MGA (n = 3; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 7; 1.38 g progesterone), and 4) Used CIDR (n = 7; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Treatment was administered starting on d 4 of the estrous cycle (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Follicle diameter was monitored until turnover, standing estrus, or ovulation. There was no effect of treatment ($P > 0.38$) or treatment by day interaction ($P > 0.47$) on follicle growth from d 4 to d 10; however, there was an effect of day ($P < 0.0001$).

follicular wave ($P < 0.0001$) but not follicle diameter ($P > 0.14$) compared to the Control group.

Steroid Hormone Changes

In the Control and MGA groups, changes in mean serum concentrations of P4 during the treatment period were as expected with heifers in the Control group exhibiting normal luteal phase concentrations of P4. Heifers in the MGA group also exhibited normal luteal phase concentrations of P4 until injection of PG (d 6), after which P4 concentrations remained at baseline (Figure 3.3). Following CIDR insertion (d 4), both the New and Used CIDR groups had an initial increase ($P < 0.0001$) in serum concentrations of P4 on d 5 and d 6 compared to the Control and MGA groups. Concentrations of P4 steadily declined from d 6 (PG administration) until CIDR removal on d 18. Serum concentrations of P4 in both CIDR groups were lower ($P < 0.05$) than the Control group and greater ($P \leq 0.05$) than the MGA group from d 7 to 17 (Figure 3.3). There was no difference ($P > 0.22$) in serum concentrations of P4 between the two CIDR treatments from d 4 until d 17.

Serum concentrations of estradiol for the heifers in the Control group were significantly ($P < 0.05$) lower compared to the MGA, New CIDR, and Used CIDR groups on most days from d 9 to 15 (Figure 3.4). Serum estradiol concentrations for heifers treated with MGA were higher ($P < 0.05$) than serum estradiol concentrations of heifers in the Control or CIDR groups on most days between d 9 and d 15. Serum estradiol

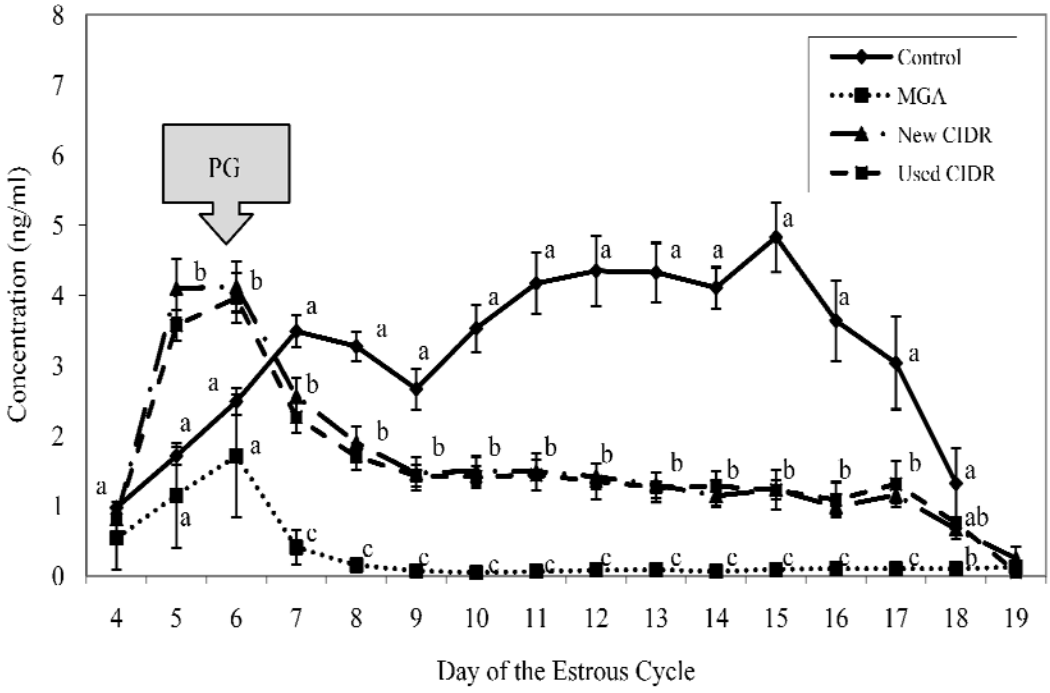

Figure 3.3. Mean (± SEM) serum concentrations of progesterone for heifers in the following treatments: 1) Control (n = 8), 2) MGA (n = 3; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 7; 1.38g P4), and 4) Used CIDR (n = 7; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Treatment was administered on d 4 of the estrous cycle (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Serum concentrations of progesterone within a day having different superscripts differ (^{abc} $P < 0.05$).

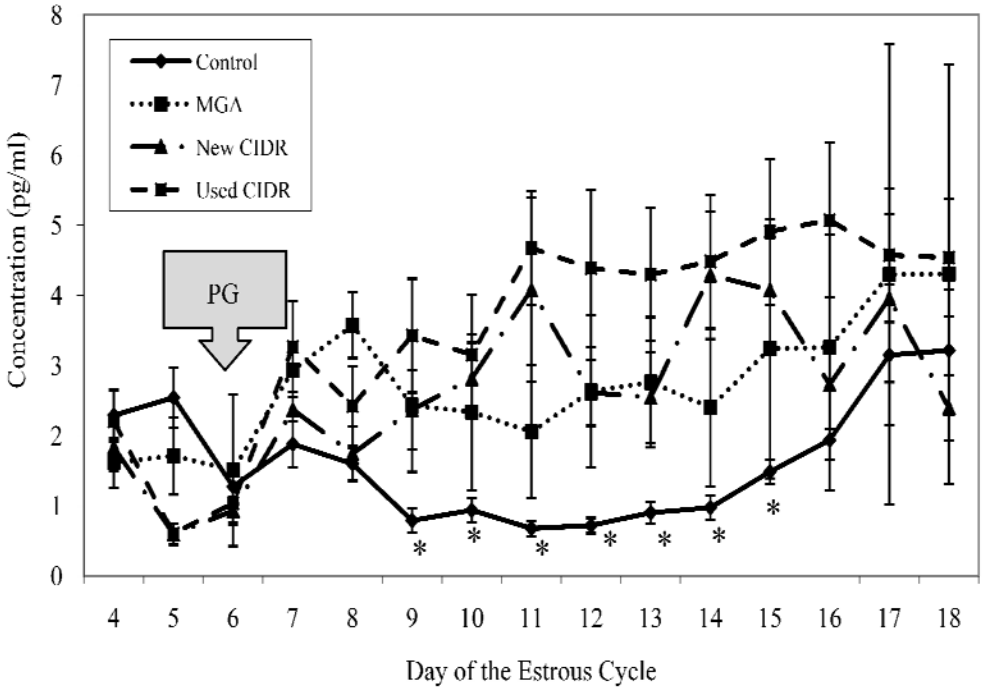

Figure 3.4. Mean (± SEM) serum concentrations of estradiol for heifers in the following treatments: 1) Control (n = 8), 2) MGA (n = 3; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 7; 1.38g progesterone), and 4) Used CIDR (n = 7; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Treatment was administered on d 4 of the estrous cycle (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). On d 9 to 15, serum concentrations of estradiol were lower (* $P < 0.05$) on most days in the Control group compared to the MGA, New CIDR, and Used CIDR groups. Serum concentrations of estradiol in the MGA group were greater ($P < 0.05$) than the New CIDR and Used CIDR groups on most days from d 9 to 15. There was no difference ($P > 0.05$) in serum concentrations of estradiol between the New CIDR, and Used CIDR groups between d 9 to 15.

concentrations were similar (P > 0.2) for heifers treated with New or Used CIDR from d 4 (start of treatment) to d18 (CIDR removal).

Experiment 2

Dominant Follicle Diameter and Length of Wave

Mean length of the follicular wave was prolonged (P < 0.05) for the MGA, New CIDR, and Used CIDR groups (14.2 ± 1.3d[a], 14.5 ± 1.3d[a], and 13.3 ± 1.4d[a], respectively) compared to the Control group (9.6 ± 0.31d[b]; [ab] P < 0.0001; Table 3.2). There was no difference (P > 0.63) among the progestin-treated groups. There was an effect of day (P < 0.0001) on rate of follicular growth among the treatment groups from d 4 to10; however, there were no effect of treatment (P > 0.51) or treatment by day interaction (P > 0.27) for follicular growth rate among the experimental groups (Figure 3.5).

Cows in the Control group had a mean maximum dominant follicle diameter of 14.0 ± 0.4 mm; whereas, mean maximum follicle diameter for cows treated with MGA (17.3 ± 0.68 mm) was larger (P < 0.05) during the treatment period (Figure 3.5). Treatment with a new CIDR (17.1 ± 0.77 mm) and used CIDR (17.0 ± 1.4 mm) resulted in mean maximum follicle diameters similar (P > 0.76) to treatment with MGA. There were no differences (P > 0.76) in mean follicle diameter among progestin-treated cows.

Table 3.2 Effect of treatment on length of a follicular wave and maximum follicle diameter in postpartum cows.

Treatment [1]	No.	Age (yrs)	BCS	Days Postpartum	Length of Follicular Wave, d[2]	Maximum Follicle Diameter, mm [3]
Control	10	5.6 ± 0.7	5.2 ± 0.18	55.7 ± 0.98	9.6 ± 0.31 [a]	14.1 ± 0.4 [a]
MGA	10	6.1 ± 0.8	5.3 ± 0.2	57.5 ± 0.98	14.2 ± 1.3 [b]	17.3 ± 0.68 [b]
New CIDR	8	6.1 ± 1.0	5.3 ± 0.19	54.4 ± 0.73	14.5 ± 1.3 [b]	17.1 ± 0.77 [b]
Used CIDR	6	4.1 ± 1.3	5.4 ± 0.2	55.7 ± 0.88	13.3 ± 1.4 [b]	17.0 ± 1.4 [b]

[ab] Within a column, means without a common superscript differ ($P < 0.05$).
[1] Treatment was administered on d 4 (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Heifers in the MGA, New CIDR, and Used CIDR groups received PG on d 6.
[2] Length of a follicular wave was defined as the interval from initiation of a follicular wave (d 2 = recruitment) until turnover of the dominant follicle, estrus, or ovulation.
[3] Diameter of the dominant follicle was measured daily beginning on d 4 post-estrus by transrectal ultrasonography. Follicle size was determined by measuring diameter of the follicle at the widest point and at a right angle to the first measurement.

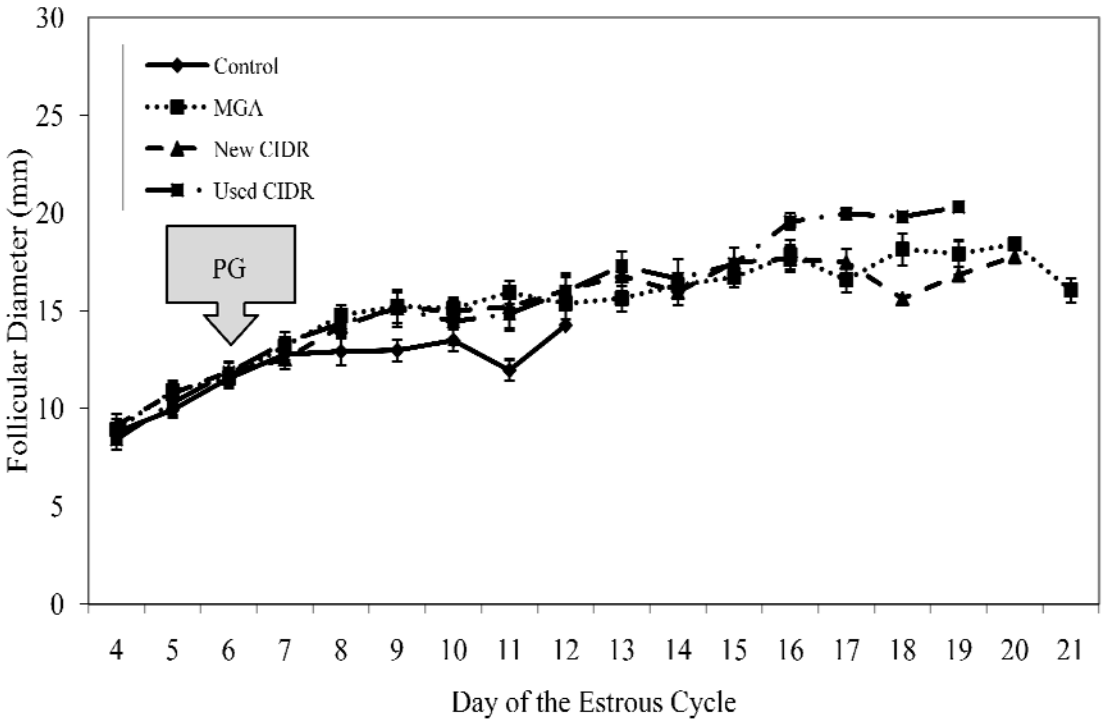

Figure 3.5. Mean (± SEM) dominant follicle diameter for postpartum cows in the following treatments; 1) Control (n = 10), 2) MGA (n = 10; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 8; 1.38 g progesterone) and 4) Used CIDR (n = 6; new CIDR previously inserted into cows for 7 d, disinfected, and stored). Treatment was administered starting on d 4 of the estrous cycle (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Follicle diameter was monitored until turn over, standing estrus, or ovulation. There was no effect of treatment ($P > 0.51$), or treatment by day interaction ($P > 0.27$) on follicle growth from d 4 to 10; however, there was an effect of day ($P < 0.0001$).

Steroid Hormone Concentration

Mean serum concentrations of P4 in the Control group followed a normal pattern for a non-pregnant cow during the luteal phase. Mean serum concentrations of P4 for cows treated with a new CIDR increased to a higher ($P < 0.05$) concentration compared to cows treated with a used CIDR on d 5 (Figure 3.7). There were no significant differences ($P > 0.05$) in the progestin treated cows from d 17 to 19 (Figure 3.8).

Mean serum concentrations of estradiol for cows in the Control group were significantly lower ($P < 0.05$) than cows treated with MGA, a new CIDR, or a used CIDR on most days from d 6 to 11(Figure 3.9). Likewise, there were no differences ($P > 0.05$) among cows treated with MGA compared to a new or used CIDR. The exception was on d 13 when treatment with a new CIDR resulted in higher ($P = 0.03$) concentrations of estradiol than treatment with MGA or a used CIDR.

Experiment 3

There was no effect of treatment ($P > 0.66$) on serum concentrations of P4 for cows with a new CIDR, used CIDR stored for 6 months (CIDR-6), or used CIDR with no storage (CIDR-0; Figure 3.10). Although, there was a significant effect of day ($P < 0.0001$), there was no treatment by day interaction ($P > 0.88$).

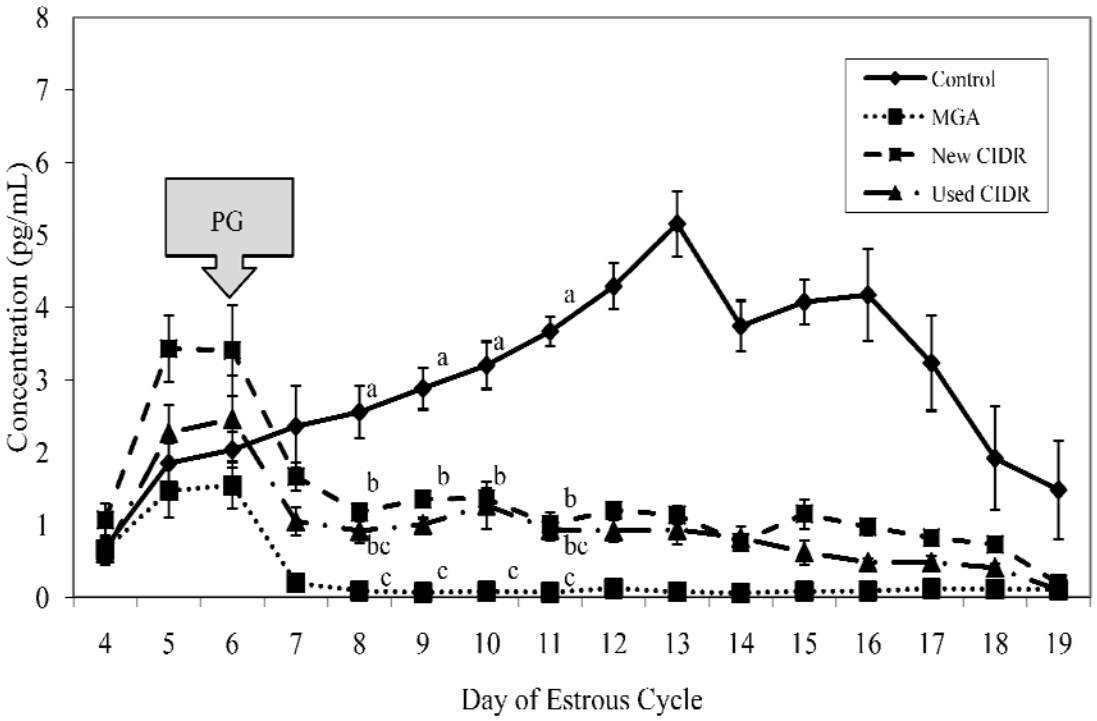

Figure 3.6. Mean (±SEM) serum concentrations of progesterone for postpartum cows in the following treatments; 1) Control (n = 10), 2) MGA (n = 10; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 8; 1.38 g progesterone) and 4) Used CIDR (n = 6; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Treatment was administered starting on d 4 of the estrous cycle (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Serum concentrations of progesterone within a day having different superscripts differ (^{abc} $P < 0.05$).

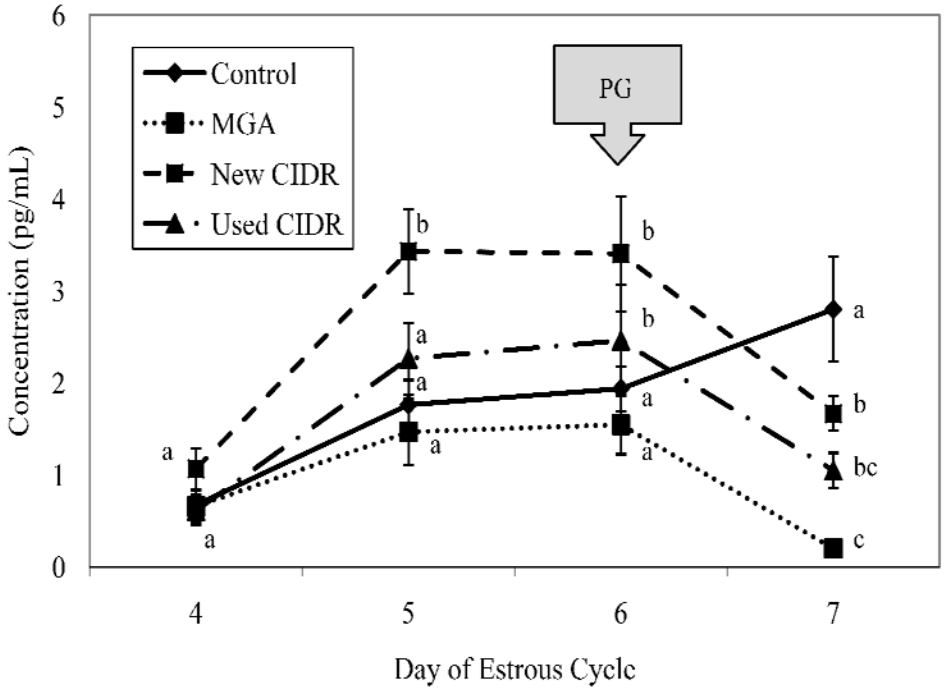

Figure 3.7. Mean (± SEM) serum concentrations of progesterone from d 4 to 7 (d 0 = estrus) for postpartum cows in the following treatments; 1) Control (n = 10), 2) MGA (n = 10; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 8; 1.38g progesterone) and 4) Used CIDR (n = 6; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Serum concentrations of progesterone within a day having different superscripts differ (^{abc} $P < 0.05$).

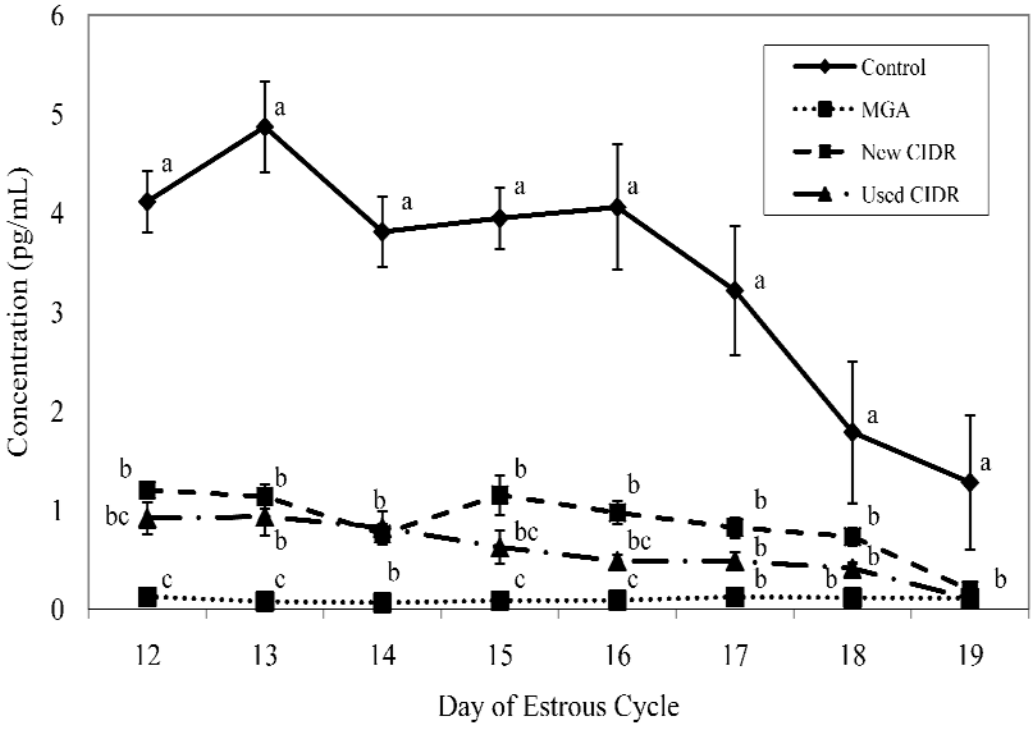

Figure 3.8. Mean (± SEM) serum concentrations of progesterone from d 12 to 19 for postpartum cows in the following treatments; 1) Control (n = 10), 2) MGA (n = 10; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 8; 1.38g progesterone) and 4) Used CIDR (n = 6; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Serum concentrations of progesterone within a day having different superscripts differ (^{abc} $P < 0.05$).

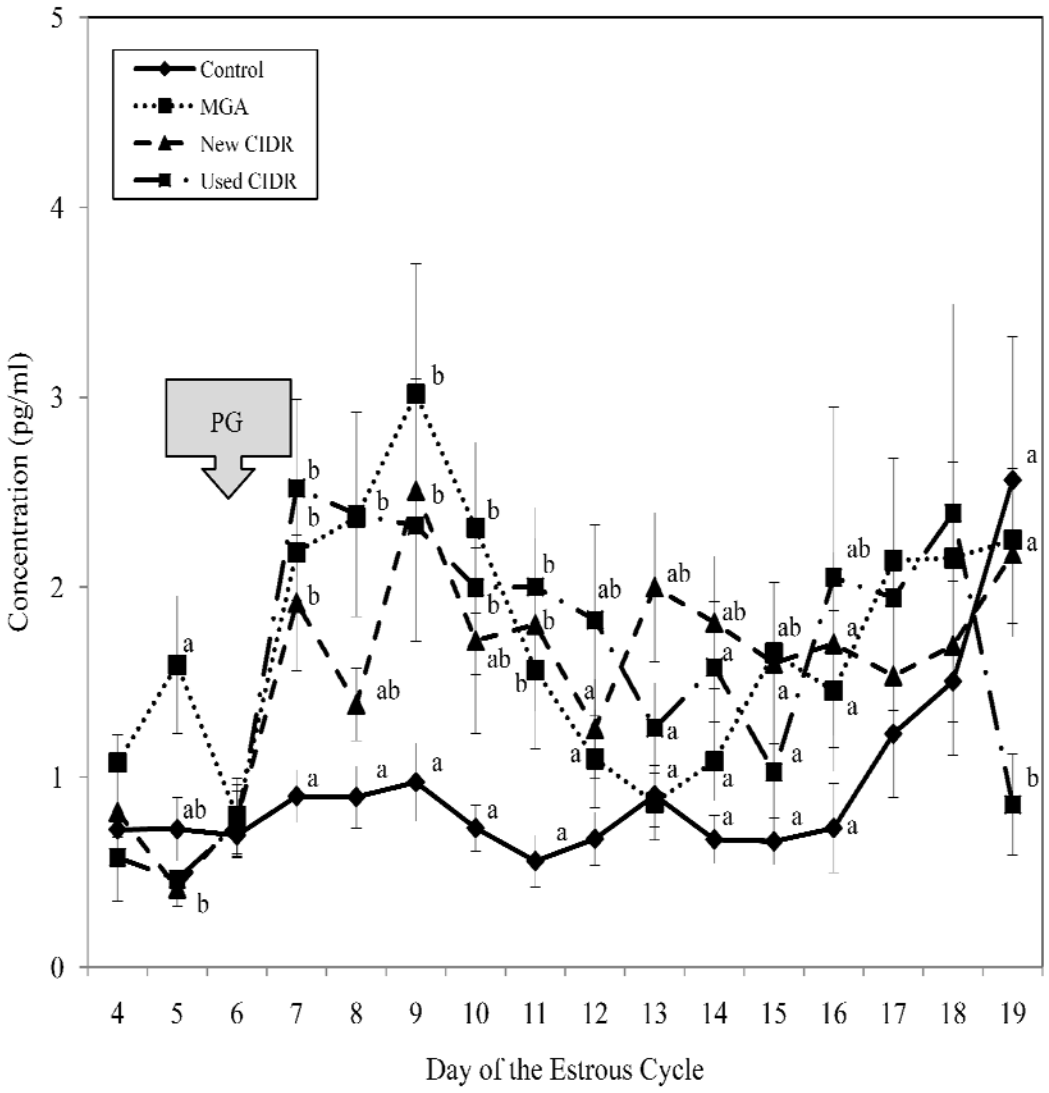

Figure 3.9. Mean (± SEM) serum concentrations of estradiol for postpartum cows in the following treatments; 1) Control (n = 10), 2) MGA (n = 10; 0.91 $kgs^{-1}hd^{-1}d$), 3) New CIDR (n = 8; 1.38 g progesterone) and 4) Used CIDR (n = 6; new CIDR previously inserted in cows for 7 d, disinfected, and stored). Treatment was administered starting on d 4 of the estrous cycle (d 0 = estrus) and continued until d 17 (last MGA feeding) or d 18 (CIDR removal). Serum concentrations of estradiol within a day having different superscripts differ (^{ab} $P< 0.05$).

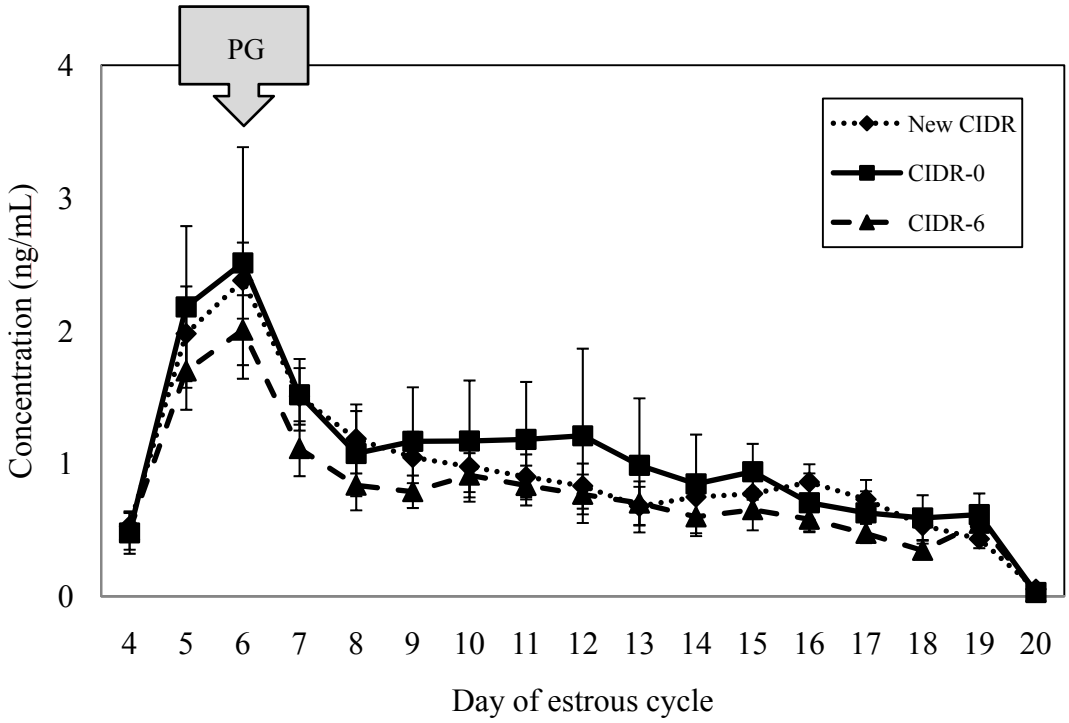

Figure 3.10. Effect of treatment on mean (± SEM) serum concentrations of progesterone for heifers and cows in the following treatments; 1) New CIDR (n = 8), 2) CIDR-0 (n = 4; new CIDR previously inserted into cows for 7 d and then immediately inserted into another cow following removal with no storage) and 3) CIDR-6 (n = 9; new CIDR previously inserted into cows for 7 d and stored for a period of 6 months). CIDR were inserted on d 4 of the estrous cycle and were removed on d 19. There was no effect of treatment ($P > 0.66$), day ($P < 0.001$) or treatment by day interaction ($P > 0.88$).

DISCUSSION

The ability to synchronize follicular waves in combination with controlling luteal lifespan has facilitated the development of protocols for fixed-time artificial insemination (FTAI). Short- (5 or 7 d) or long-term (14 d) progestin treatments have been used to synchronize estrus and ovulation in beef cattle (Patterson et al., 1989a; Sirois and Fortune, 1990; Zimbelman and Smith 1966a). FTAI protocols that include long-term (14 d) CIDR treatment have been developed for both heifers and postpartum cows; however, the effects of CIDR treatment, in the absence of a CL, on follicular dynamics in beef heifers and cows have not been determined. Progestin treatment (MGA), in the absence of a CL, can cause formation of persistent follicles (PF) and a subsequent reduction in fertility (Patterson et al., 1989b; Yelich et al., 1997; Zimbelman and Smith, 1966b). Decreased pregnancy rates following ovulation of a PF are reportedly due to premature resumption of meiosis resulting in fertilization of an aged oocyte (Mihm et al., 1999).

PF have been characterized as having a prolonged lifespan, larger diameter, and increased estradiol secretion compared to dominant follicles that develop during the luteal phase (Fralix, et al., 1996; Kojima et al., 1995; Sirois and Fortune, 1990). In beef cattle, inclusion of a 7 d CIDR, in combination with an injection of PG, did not reduce pregnancy rates compared to PG treatment alone (Lucy et. al., 2001); therefore, it is possible that CIDR treatment does not result in formation of PF. A major objective of the current study was to examine the effect of a new versus a used CIDR, in the absence of a CL, on follicular dynamics and estradiol secretion in beef heifers and postpartum cows to determine if a PF forms in the presence of one or both progestin treatments.

In the present study, treatment of heifers or cows with a new or used CIDR for 14 d resulted in the development of PF based on extension of the first follicular wave, increased dominant follicle diameter, and increased circulating concentrations of estradiol compared to the dominant follicle of the first follicular wave in the Control group. These findings are similar to Sirois and Fortune (1990) who observed prolonged follicular waves during treatment with different levels of P4 supplementation: 1) no CIDR, 2) a single CIDR, or 3) two CIDR. These authors observed the formation of PF in heifers treated with a single CIDR compared to heifers treated with a blank CIDR or two CIDR. Additionally, heifers treated with a single CIDR had increased circulating concentrations of estradiol compared to heifers with a blank CIDR or two CIDR. Differences in length of follicular waves and estradiol production among the treatment groups were presumed to be the result of varying levels of P4, since heifers treated with one CIDR had lower ($P < 0.003$) concentrations of circulating P4 compared to heifers receiving two CIDR.

Low circulating concentrations of progesterone (1 to 3 ng/ml) were successful at inhibiting estrus and ovulation in cattle (Adams et al., 1992; Savio et al., 1993; Sirois and Fortune, 1990). It was hypothesized that a used CIDR, previously inserted into a heifer or cow for 7 d, would contain less P4 at the end of treatment than a new CIDR but still be effective at inhibiting estrus and ovulation. Therefore, we expected serum concentrations of P4 to be lower in heifers and cows treated with a used versus a new CIDR. However, there was no difference ($P > 0.2$) in the serum concentrations or pattern of secretion of P4 for animals receiving a new or used CIDR. These data do not agree with a study by Herrmann and Wallace (2007) who demonstrated that there were differences in P4 concentrations of dairy cows treated for 7 d with new or used CIDR (previously inserted

for 7 d). Cows treated with used CIDR in the trial by Herrmann and Wallace (2007) had circulating P4 concentrations that were similar ($P = 0.95$) to cows prior to CIDR insertion (basal P4; no treatment). Alternatively, Meneghetti et al., (2009) demonstrated that a CIDR (1.9 g P4) inserted for 9 d per treatment could be used successfully four times, without negatively affecting pregnancy rates. These data suggest that a CIDR, containing 1.9 g of P4, can prevent estrus and ovulation for greater than 4 weeks. Our findings indicate there is a sufficient amount of P4 in a CIDR (containing 1.38 g P4; following insertion for 7 d) to inhibit estrus and ovulation in a majority of heifers and cows for 14 d.

Progesterone content of a CIDR (1.9 g) can be reduced to 1.38 g and still be effective (Rathbone et al., 2002). Therefore, it seems there is enough P4 remaining in a CIDR (0.72 g; Rathbone et al., 2002) after insertion for 7 d for an additional 7 d treatment period. Data from the current study indicate there was no difference in circulating concentrations of progesterone over 14 d between the new CIDR and used CIDR groups. Furthermore, at CIDR insertion for heifers in the current study, both new and used CIDR groups had a similar initial increase in P4 compared to the controls ($P > 0.22$). Zuluaga and Williams (2008) observed similar results when comparing used CIDR that were disinfected to new or autoclaved CIDR. Treatment with a disinfected or autoclaved used CIDR resulted in initial increases in P4 concentrations following insertion. Interestingly, treatment with an autoclaved CIDR resulted in higher serum P4 concentrations compared to a new or disinfected CIDR during the first 8 hr of treatment. Treatment with the disinfected CIDR resulted in the lowest ($P < 0.05$) circulating P4 concentrations of the

three treatment groups during the initial 8 hr. Since blood samples were collected daily, instead of hourly in the current study, this comparison could not be made.

The initial increase in P4 following insertion of a new CIDR is generally thought to be due to the movement of P4 located on or near the surface of the CIDR into the blood stream. Therefore, it was surprising that this increase also occurred following insertion of a used CIDR. Perhaps storage of a used CIDR has an affect on release of P4 following insertion. As a result, we decided to compare the effect of inserting a CIDR directly from one animal into another with no storage to a used CIDR that had been stored for 6 mos. on serum concentrations of P4. The results from this study indicate that there was an increase in serum concentrations of P4, even in the absence of storage.

In addition to similar circulating concentrations of serum P4 between the new and used CIDR groups, there was essentially no difference in follicular dynamics of heifers or cows treated with a new or used CIDR. Heifers treated with a New CIDR had a smaller maximum follicle size compared to MGA or used CIDR treated heifers; a surprising finding as there was no difference in serum concentrations of P4 between the CIDR treated groups. Why the heifers treated with a new CIDR had a smaller maximum follicle size is unknown. Nonetheless, treatment with progestins in the current experiment resulted in the formation of PF compared to non-treated animals (Control group).

Formation of PF is generally believed to be the result of increased pulse frequency of luteinizing hormone (LH) due to the presence of low circulating concentrations of P4. During the development and demise of the CL, when P4 concentrations were lower than mid-luteal concentrations, Rahe et al., (1980) reported that LH pulsatility increased in frequency and decreased in amplitude. Pulses of LH that

are high in frequency and low in amplitude have been shown to stimulate follicular growth in cattle (Kinder et al., 1996). Serum concentrations of LH were not measured in the current study; however, it's likely that increased LH pulsatility in the MGA, New CIDR, and Used CIDR groups had a role in the prolonged length of the follicular wave, increased follicular diameter, and increased circulating concentrations of estradiol.

Treatment with MGA, a New CIDR, or a Used CIDR, increased serum estradiol concentrations compared to Control animals. Our findings support previous data that long-term treatment with MGA, in the absence of a CL, increased circulating concentrations of estradiol (Zimbelman and Smith, 1966c) which was likely due to increased pulse frequency of LH (Kinder et al., 1996). Luteinizing hormone is known to stimulate estradiol production via the two-cell two-gonadotropin concept (Fortune and Armstrong, 1978). Custer et al., (1994) demonstrated that MGA increased LH pulse frequency and Ireland and Roche (1982) reported that treatment with P4 releasing intravaginal devices (PRID) increased LH pulse frequency in the presence of lowered (1 to 2 ng/ml) concentrations of P4. We observed that animals treated with a CIDR (new or used) resulted in circulating concentrations of progesterone that were significantly lower than mid-luteal phase concentrations (Control group) and that were similar to the concentrations reported by Ireland and Roche (1982).

In summary, long-term use of a new or used CIDR, in the absence of a CL, caused the formation of PF in estrous cycling heifers and postpartum beef cows. Serum concentrations of P4 were not different between the New and Used CIDR groups, regardless of CIDR storage length. Serum estradiol concentrations are increased during treatment with MGA , New CIDR, or Used CIDR in both heifers and cows.

CHAPTER IV

EFFECT OF GnRH- INDUCED ACCESSORY CL ON DELAY OF ESTRUS AND OVULATION IN BEEF HEIFERS AND COWS

Abstract

The CO-Synch protocol (gonadotropin releasing hormone [GnRH-1], followed by prostaglandin F2α [PG] 7 days [d] later, and a second injection of GnRH [GnRH-2] plus AI 48 hours [hr] after PG) is commonly used for synchronization of estrus and ovulation in beef cattle. Preliminary data from our lab suggested heifers injected with GnRH-1 during the late luteal phase were more likely to ovulate or display estrus early (before PG) compared to cows. We hypothesized that a GnRH-induced accessory corpus luteum (ACL) is equally effective at inhibiting estrus and ovulation in heifers compared to cows. Therefore, the objective of this study was to compare the ability of a GnRH-induced ACL to inhibit the expression of estrus and ovulation in estrous cycling beef heifers and cows. Estrous cycling heifers (14 to 20 months [mos.] of age) and multiparous suckled cows were separated by parity and then randomly assigned to receive an injection of saline or GnRH on d 16 of the estrous cycle resulting in the following treatment groups: heifer-saline, heifer GnRH, cow saline, or cow GnRH (n = 7, 15, 9, and 17, respectively). Transrectal ultrasonography was used to verify ovulation following GnRH-1 and to monitor ACL development. Heifers and cows received PG 7 d after GnRH-1 or saline treatment.

Heifers and cows that were not in estrus by 48 hr after PG were given a second GnRH injection (GnRH-2) and considered to have an estrous cycle length of 25 d. Estrus was detected three times daily throughout the treatment period. In the GnRH treatment groups, there were 10/15 (66.67%) heifers and 15/17 (88.2%) cows that responded to GnRH-1 (GnRH-Yes Response); whereas, 5 heifers and 2 cows did not respond (GnRH-No Response). The was no difference ($P > 0.12$) in the proportion of animals in estrus or ovulating before PG administration for the heifer-saline, heifer GnRH-No Response, cow saline, and cow GnRH-No Response, groups (7/7, 5/5, 8/9, or 1/2, respectively. Furthermore, interestrus interval was similar ($P > 0.2$) for the heifer-saline, heifer GnRH-No Response, cow saline, and cow GnRH-No Response, groups (19.9 ± 0.46, 20.8 ± 0.73, 20.7 ± 0.53 , or 22.5 ± 2.5, respectively). Therefore, data from the saline and GnRH-No Response groups were combined into the heifer saline or cow saline groups. The proportion of animals in estrus or ovulating before PG administration for the heifer-saline, heifer GnRH-Yes Response, cow-saline, and cow GnRH-Yes Response groups was $12/12^{a}$, $2/10^{b}$, $9/11^{a}$, and $1/15^{b}$, respectively ($P < 0.001$) and mean interestrus interval was (20.25 ± 0.41^{a}, 24.2 ± 0.33^{b}, 21.0 ± 0.58^{a} , or 24.7 ± 0.21^{b}, respectively ($P < 0.0001$). In summary, GnRH-induced formation of an ACL decreased the proportion of heifers and cows that displayed estrus or ovulated before PG administration and increased estrous cycle length. There was no difference ($P > 0.28$) in the effectiveness of the GnRH-induced ACL to inhibit estrus and ovulation in heifers compared to cows.

INTRODUCTION

Estrous synchronization can facilitate the implementation of an artificial insemination (AI) program in beef cattle. Synchronization of estrus and ovulation reduces or eliminates the necessity for detection of estrus, which is a common deterrent to the implementation of an AI program. Recent advances in the synchronization of follicular waves, in combination with the ability to control luteal lifespan, has resulted in protocols that permit insemination of heifers and cows at a predetermined time. Most fixed-time AI (FTAI) protocols are based on the following sequence of injections: GnRH - PG - GnRH. The initial injection of GnRH (GnRH-1) during the luteal phase can induce ovulation of a dominant follicle, induce formation of an ACL and initiate a new follicular wave. Seven d later, animals receive an injection of PG to induce regression of the original (primary corpus luteum [CL]) and ACL. A second injection of GnRH (GnRH-2) is administered in conjunction with insemination 48 hr after PG injection. GnRH induction of an ACL, in the late luteal phase of the estrous cycle, can delay expression of estrus until PG injection on d 7 of the protocol (d 0 = GnRH-1 injection) due to the progesterone (P4) produced by the ACL. However, previous research in our lab suggests that GnRH-induced formation of an ACL on d 18 post-estrus results in an ACL that produces less P4 and is less effective at delaying estrus in heifers compared to cows (Figure 4.1). The experiments described in this chapter focused on the ability of an ACL to delay estrus and ovulation in beef heifers and cows. The specific objectives were as follows: 1) To determine if there is a difference between heifers and cows in the ability of GnRH-1 to induce ovulation on d 16 of the estrous cycle (d 0 = estrus), 2) To determine if there is a difference in dominant follicle size for heifers and cows that ovulate versus those that do not ovulate in response

Figure 4.1. Serum concentrations of progesterone following GnRH-induced ACL formation on d 18 of the estrous cycle for heifers and cows (Atkins et al., unpublished data).

to GnRH-1 on d 16 post estrus, and 3) To determine if there is a difference between heifers and cows in the ability of a GnRH-induced ACL on d 16 of the estrous cycle to delay luteolysis until after d 23 (PG injection) and to extend estrous cycle length.

MATERIALS AND METHODS

Experiment 1

Estrous cycling multiparous crossbred cows and heifers (n = 49) were divided into parity groups. Animals were kept on the same farm premises but were housed in two locations within the farm's boundaries. Heifers and cows were allocated by parity, date of birth, weight (heifers), and body condition score (cows) into one of two groups 1) Saline or 2) GnRH to receive an injection on d 16 of the estrous cycle. There were 10 animals that were not included in the analysis for the following reasons: 1) two primary CL present (n = 3), 2) difficulty in determining presence of ACL (n = 5), 3) two dominant follicles present (n = 2), and 3) displayed estrus on day of GnRH-1 injection (n=1).

All animals were presynchronized with the CIDR- PG protocol (CIDR insertion for 7 d and injection of 25 mg of PG [Lutalyse; Upjohn, Kalamazoo, Michigan] i.m. on d 6 of CIDR treatment). KMAR heat detection patches were used as an aid in estrus detection. All animals were monitored for estrus three times daily and standing estrus was designated as d 0. Starting on d 10 of the cycle, daily blood samples were collected (see blood sampling below) as well as ovarian follicular observation (see ultrasonography below). Saline or GnRH-1 was administered to heifers and cows on d 16 of the estrous cycle to induce an ACL or as a control injection, respectively. KMAR patches were

reapplied to aid in estrus detection at time of GnRH-1 or saline injection. Cows and heifers were monitored three times daily until expression of estrus or GnRH-2. On d 23, PG was given to any cow or heifer that had not been observed in estrus. Cows and heifers that did not respond to GnRH-1 were not given PG on d 23 but instead were monitored until estrus was observed or ovulation occurred.

Blood sampling: Daily blood samples were collected from all cows and heifers beginning on d 10 of the estrous cycle until completion of the project (see ultrasonography below). Blood samples were collected by jugular venipuncture into 10 ml vacutainer tubes (Fisher Scientific, Pittsburgh, Pennsylvania). Blood was allowed to clot, stored at 40^0C for 24 hr, and serum harvested for determination of serum concentrations of P4 by radioimmunoassay (RIA). Serum P4 concentrations were measured from d 10 until completion of project (see ultrasonography below) using a Coat-a-Count (Diagnostic Products Corporation, Los Angeles, California; Kirby et al., 1997). Intra- and interassay coefficients of variation (CV) and assay sensitivity were 2.84%, 0.73%, and 0.1 ng/ml, respectively.

Ultrasonography: All cows and heifers were examined by transrectal ultrasonography by using an Aloka 500V ultrasound with a 7.5-MHz transrectal linear probe (Aloka, Wallingford, Connecticut) every other day to monitor CL development starting on d 10 of the estrous cycle. CL diameter was determined by measuring the CL at the widest point and then at a right angle to the first measurement. Any follicle (> 6 mm) was also measured in the same fashion. On the day of estrus following either GnRH-1 or saline

treatment, dominant follicle diameter was measured by the same procedure as CL diameter. Ultrasound was also preformed on the day of PG injection (d 23). Daily ultrasound was performed on those cows and heifers that had not ovulated or been observed in estrus by the time of PG, and ultrasonography continued until estrus detection or ovulation of the dominant follicle. Cattle were finished with the project once the cow or heifer came into standing estrus, was observed to have ovulated, or inseminated at GnRH-2.

Statistical analysis: For statistical analysis purposes, heifers (n = 5) and cows (n = 2) in the GnRH groups that did not respond to GnRH-1 on d 16 will be referred to as GnRH-No Response, while those that did respond will be referred to as the GnRH-Yes Response and were analyzed separately. Interestrus interval was defined as the interval from estrus to the subsequent estrus or ovulation (spontaneous or induced by GnRH-2). The day that luteolysis was complete was designated as the day that progesterone decreased below 1 ng/ml. Maximum follicle diameter at Saline or GnRH-1 (d 16), interestrus interval, and largest follicle diameter at estrus or GnRH-2 (d 25) were analyzed by one-way analysis of variance (PROC GLM) in SAS. The number of heifers and cows that underwent luteolysis prior to PG (d 23) was analyzed using Chi-square (PROC FREQ) in SAS. Serum P4 concentrations from d 10 to luteolysis, concentrations of P4 during luteolysis, and concentrations of P4 produced by the ACL were all analyzed by analysis of variance for repeated measures (PROC MIXED) in SAS (SAS Inst. Inc., Cary, North Carolina).

RESULTS

The proportion of heifers and cows that ovulated in response to GnRH-1 (GnRH-Yes Response) on d 16 of the estrous cycle was similar (10/15 [66.67%] and 15/17 [88.2%], respectively [P > 0.05]; Table 4.1). Mean follicle diameter at GnRH-1 injection for heifers and cows was similar (P> 0.09) within parity for the Saline, GnRH-No Response, and GnRH-Yes Response groups (Table 4.1). The was no difference (P > 0.12) in the proportion of animals in estrus or ovulating before PG administration for the heifer-saline, heifer GnRH-No Response, cow saline, and cow GnRH-No Response, groups (7/7, 5/5, 8/9, or 1/2, respectively). Furthermore, interestrus interval was similar (P > 0.2) for the heifer-saline, heifer GnRH-No Response, cow saline, and cow GnRH-No Response, groups (19.9 ± 0.46, 20.8 ± 0.73, 20.7 ± 0.53 , or 22.5 ± 2.5, respectively). Therefore, data from the saline and GnRH-No Response groups were combined into the heifer saline or cow saline groups. However, the proportion of heifers and cows in the GnRH-Yes Response that underwent luteolysis before PG injection was lower (P < 0.0001) than the Saline or GnRH- No Response groups. There was no difference (P > 0.35) between heifers and cows in the proportion that underwent luteolysis before PG in the GnRH-Yes Response groups.

Interestrus interval for heifers in the Saline, GnRH-No Response and GnRH-Yes Response was 19.9 ± 0.46^{a}, 20.8 ± 0.73^{a}, and 24.2 ± 0.31^{b} d, respectively (P < 0.05; Table 4.1). Likewise, interestrus interval for cows in the Saline, GnRH-No, and GnRH-Yes groups was 20.7 ± 0.53^{a}, 22.5 ± 2.5^{a}, and 24.7 ± 0.21^{b} d, respectively (P < 0.05; Table 4.1). Both the heifers and cows that responded to GnRH-1 (GnRH-Yes) had longer (P <

Table 4.1. Mean follicle diameter at GnRH-1, incidence of estrus prior to PG, interestrus interval, and follicle diameter at GnRH-2 or estrus for heifers and cows treated with Saline or GnRH-1 on d 16 of the estrous cycle, PG on d 23, and GnRH-2 on d 25.

	Treatment [1]	no.	Mean diameter of largest follicle (d16) [2]	Estrus prior to PG [3]	(%)	Interestrus Interval[4]	Largest follicle diameter at GnRH-2 or heat[5]
Heifer							
	Saline	7	10.3	7/7[a]	(100.0)	19.9 ± 0.46 [a]	13.2 ± 0.52 [a]
	GnRH-No Response	5	11.5	5/5[a]	(100.0)	20.8 ± 0.73 [a]	13.02 ± 0.21 [a]
	GnRH-Yes Response	10	11.7	2/10 [b]	(20.0)	24.2 ± 0.30 [b]	15.14 ± 0.52 [b]
Cow							
	Saline	9	12.2	8/9[a]	(88.9)	20.7 ± 0.53 [a]	15.3 ± 0.52 [ab]
	GnRH-No Response	2	11.9	1/2 [a]	(50.0)	22.5 ± 2.5 [a]	13.38 ± 1.4 [a]
	GnRH-Yes Response	15	12.4	1/15[b]	(7.0)	24.7 ± 0.21 [b]	16.6 ± 0.65 [b]

[ab] Within a column, means without a common superscript letter, differ ($P < 0.05$).
[1] Treatment with saline or GnRH-1 was administered on d 16 of the estrous cycle. Heifers and cows not responding to GnRH (GnRH-No Response) were analyzed separately.
[2] Mean follicle diameter at treatment with saline or GnRH-1 (d 16) was determined by measuring diameter of the largest follicle at the widest point and then at a right angle to the first measurement. There were no differences within parity.
[3] Estrus prior to PG (d 23) was defined as display of standing estrus prior to injection with PG on d 23 of the estrous cycle. Heifers and cows observed in estrus prior to PG were not injected on d 23.
[4] Interval from one estrus to the next.
[5] Injection of GnRH-2 on d 25.

0.02) interestrus intervals compared to the saline and GnRH-No Response groups. The largest follicle diameter at estrus or GnRH-2 for heifers and cows in the Saline, GnRH-No Response, and GnRH-Yes Response groups was 13.2 ± 0.52 [a] mm, 13.02 ± 0.21 [a] mm, and 15.14 ± 0.52 [b] mm or 15.33 ± 0.52 [ab] mm, 13.38 ± 1.4 [a] mm, and 16.6 ± 0.65 [b] mm, respectively ([ab] $P < 0.04$; Table 4.1). Serum concentrations of P4 from d 10 to 25 for the Saline, GnRH-No Response, and GnRH-Yes Response groups are depicted in Figures 4.2 and 4.5, respectively. In heifers and cows, serum concentrations of P4 from d 10 to 19 and change in P4 during luteolysis were similar ($P > 0.15$) for the saline and GnRH-No Response groups (Figure 4.3, 4.4 and 4.6, 4.7; respectively). Furthermore, serum P4 concentration, produced by the ACL, did not differ ($P > 0.28$) between heifers and cows (Figure 4.8). In the GnRH-Yes Response groups, there also was no effect of day ($P > 0.33$) on serum concentrations of P4, nor a parity by day interaction ($P > 0.28$).

DISCUSSION

The CO-Synch protocol consists of a GnRH – PG – GnRH injection sequence that is designed to synchronize estrus and ovulation in beef cattle to facilitate FTAI. The purpose of the first GnRH injection (GnRH-1; d -9) is to induce ovulation of a dominant follicle and thereby synchronize a follicular wave. Prostaglandin is administered 7 d later (d -2) to regress the primary CL (from previous estrus) and an ACL, assuming the animal ovulated to GnRH-1 during the luteal phase. A second GnRH injection (GnRH-2) and insemination generally occurs 48 to 60 hr after PG (d 0). A drawback to this protocol is that 5 to 15% of animals show estrus on or before the PG injection (Kojima et. al., 2000); however, this problem can be alleviated by presence of a CIDR between GnRH-1 and PG

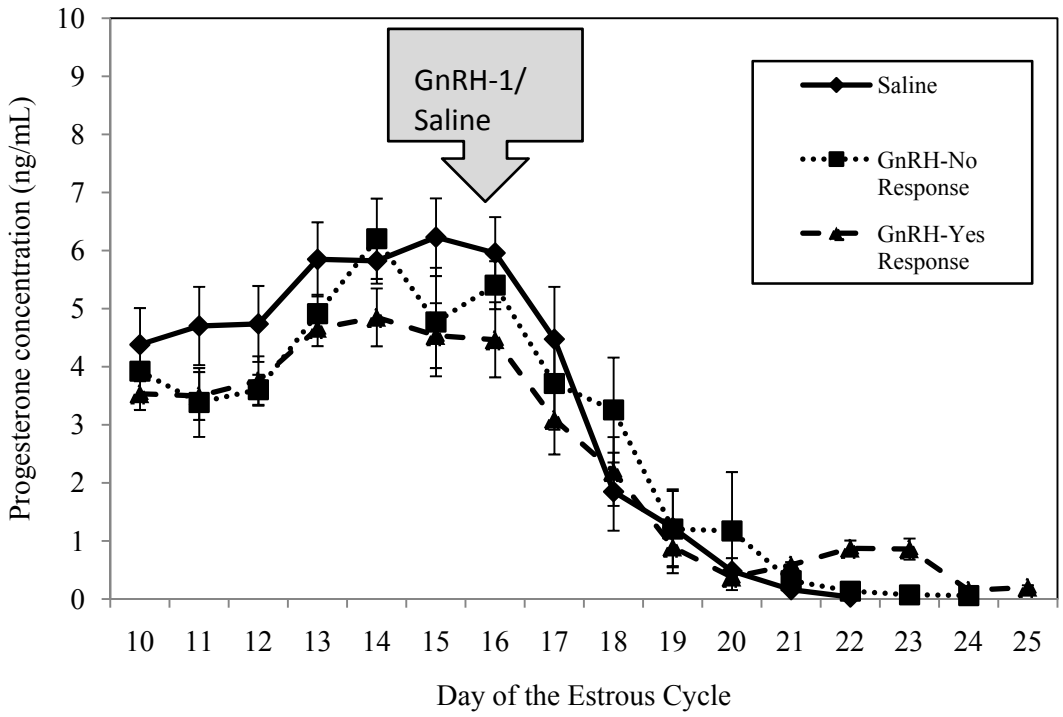

Figure 4.2. Mean (± SEM) serum concentrations of progesterone from d 10 to 25 for heifers receiving saline (n = 7) or the CO-Synch protocol (n = 16; GnRH-1 on d 16, PG on d 23, and GnRH-2 plus AI on d 25).

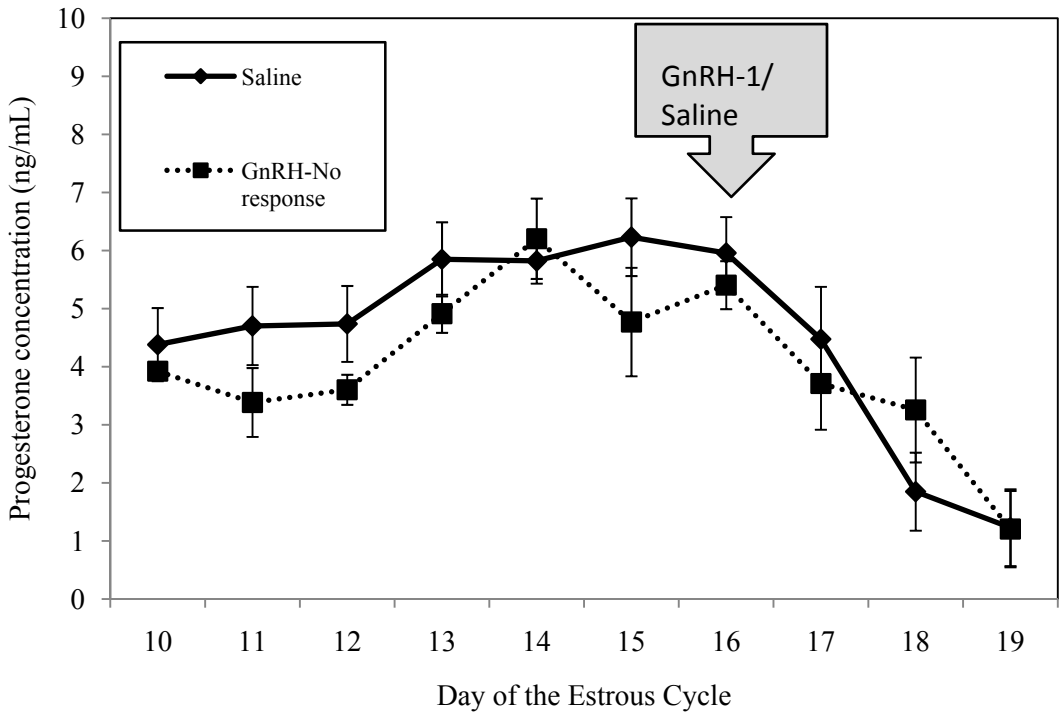

Figure 4.3. Mean (± SEM) serum concentrations of progesterone from d 10 until luteolysis (d 19) for heifers injected with Saline (n = 7) or GnRH-1 (n = 5) on d 16 of the estrous cycle. Heifers injected with GnRH-1 but that did not develop an ACL were designated as GnRH-No Response. There was no effect of treatment ($P > 0.15$) or treatment by day interaction ($P > 0.38$); however, there was an effect of day ($P < 0.0001$).

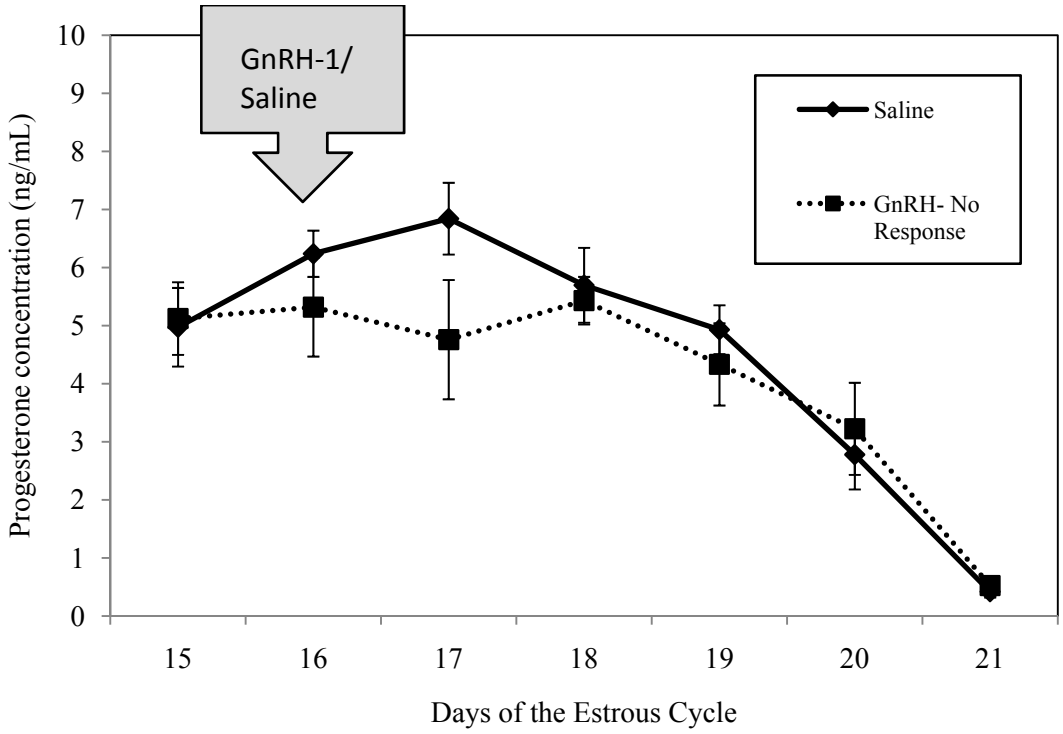

Figure 4.4. Mean (± SEM) serum concentrations of progesterone during luteolysis of heifers injected with Saline (n = 7) or GnRH-1 (n = 5) on d 16 of the estrous cycle. Heifers injected with GnRH-1 but that did not develop an ACL were designated as GnRH- No Response. Serum concentrations of progesterone were standardized to the first day that progesterone decreased below 1 ng/ml (d 21). There was no effect of treatment ($P > 0.36$) or treatment by day interaction ($P > 0.08$); however, there was an effect of day ($P < 0.0001$).

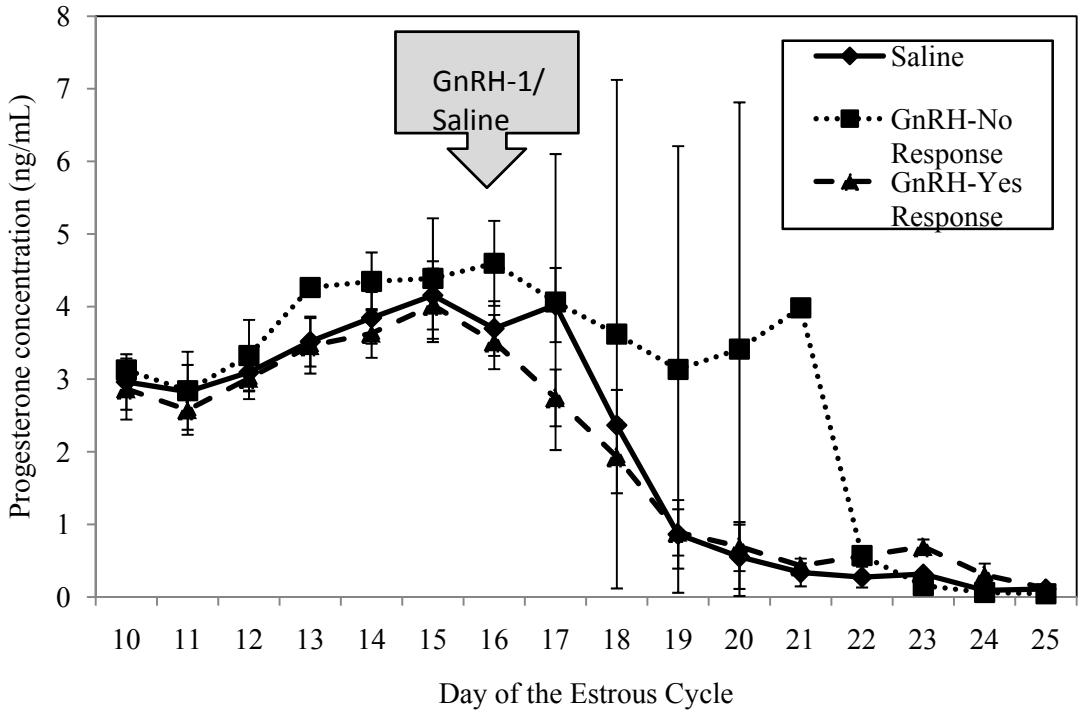

Figure 4.5. Mean (± SEM) serum concentrations of progesterone from d 10 to 25 for cows receiving saline (n = 9) or the CO-Synch protocol (n = 17; GnRH-1 on d 16; PG on d 23, and GnRH-2 plus AI on d 25).

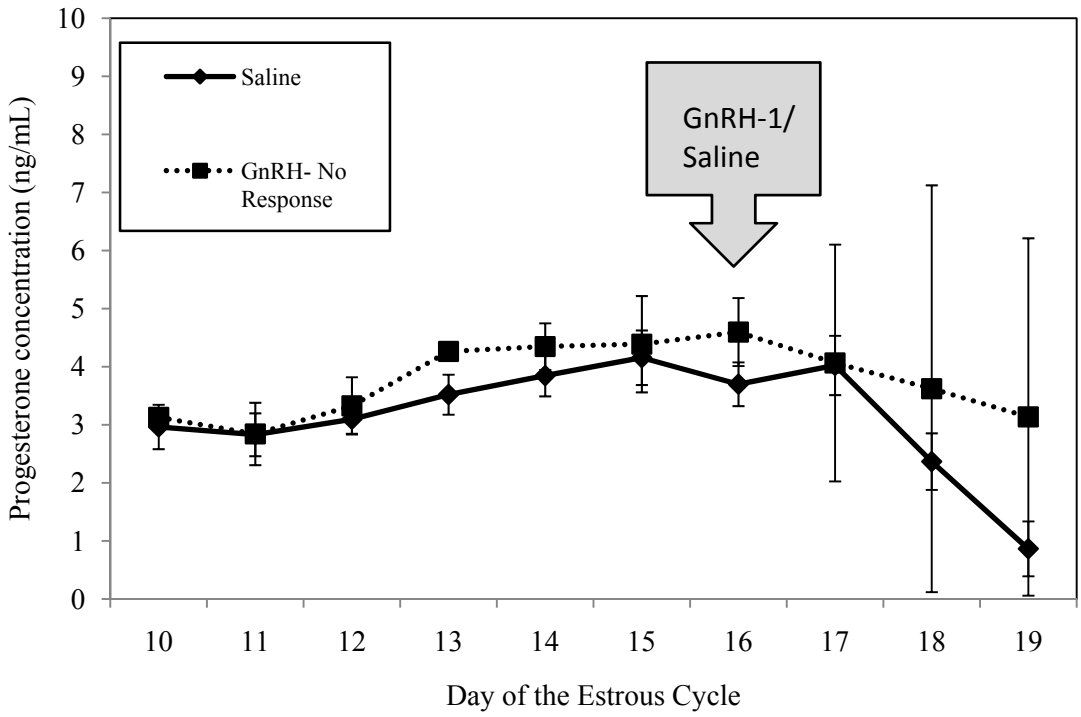

Figure 4.6. Mean (± SEM) serum concentrations of progesterone from d 10 until luteolysis (d 19) for cows injected with Saline (n = 9) or GnRH-1 (n = 2) on d 16 of the estrous cycle. Cows injected with GnRH but did not develop an ACL were designated as GnRH- No Response. There was no effect of treatment ($P > 0.46$) or treatment by day interaction ($P > 0.74$); however, there was an effect of day ($P < 0.0001$).

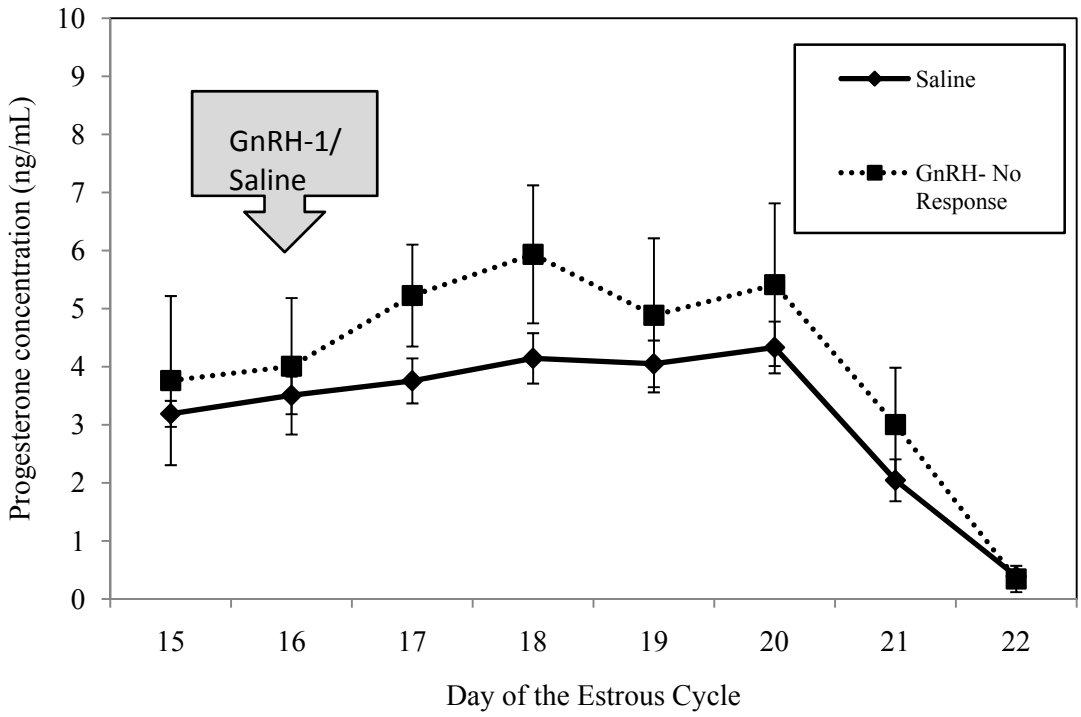

Figure 4.7. Mean (± SEM) serum concentrations of progesterone during luteolysis for cows injected with Saline (n = 9) or GnRH-1 (n = 2) on d 16 of the estrous cycle. Cows injected with GnRH but that did not develop an ACL were designated as GnRH- No Response. Serum concentrations of progesterone were standardized to the first day that progesterone decreased below 1 ng/ml (d 22). There was no effect of treatment ($P > 0.22$) or treatment by day interaction ($P > 0.16$); however, there was an effect of day ($P < 0.0001$).

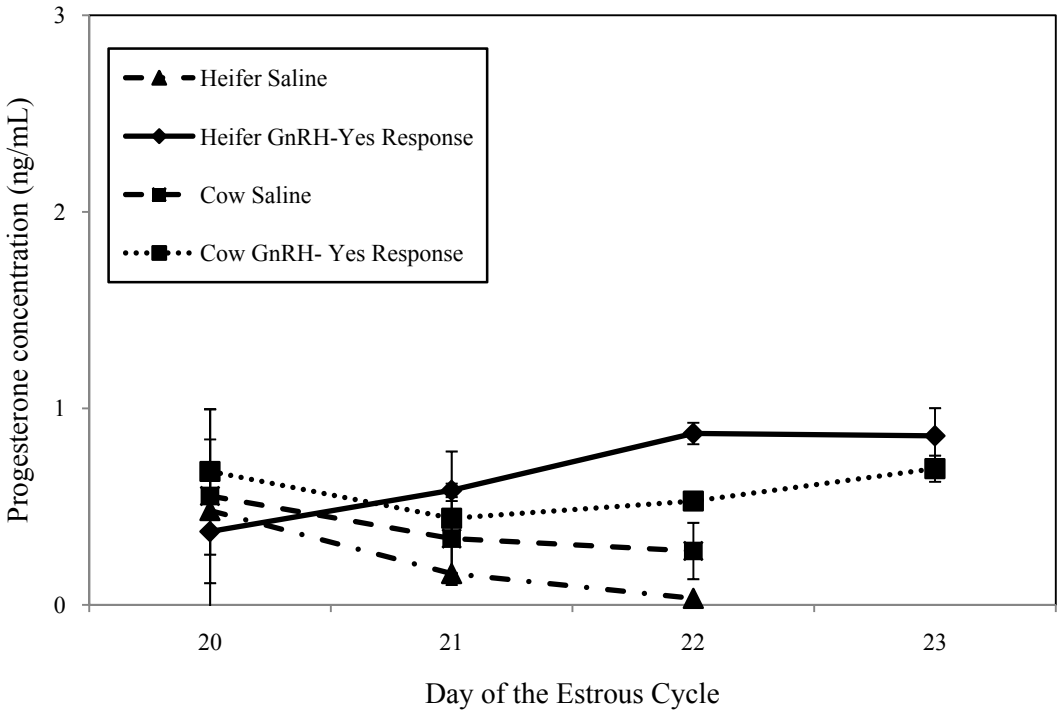

Figure 4.8. Mean (± SEM) serum concentrations of progesterone for heifers (n = 10) and cows (n = 15) injected with GnRH-1 on d 16 of the estrous cycle and that formed an ACL. There was no affect of parity (P > 0.28), day (P > 0.33), or parity by day interaction (P > 0.29) between heifers and cows treated with saline or GnRH-1 on d 16 of the estrous cycle.

(CO-Synch + CIDR; Lamb et al., 2001). Pregnancy rates following the CO-Synch + CIDR protocol are typically higher than the CO-Synch protocol (Lamb et al., 2001). Improved pregnancy rate with CO-Synch + CIDR is likely due to prevention of estrus and ovulation prior to PG and a greater anestrous response to the CIDR.

Ovulatory response to GnRH-1 is dependent upon the presence of a dominant follicle (> 10 mm) at the time of injection (Sartori et al., 2001). The proportion of beef cows, dairy cows, beef heifers, and dairy heifers that ovulated to GnRH-1 was 66%, (Geary et al., 1998), 66% (Vasconcelos et al.,1999), 42% (Atkins et al., 2008) and 50% (Pursely et al., 1997), respectively. In the current trial, the proportion of heifers and cows that ovulated to GnRH-1 was 67% and 86.7%, respectively. The higher response to GnRH-1 for both heifers and cows in the present study compared to previous reports might be due to injection of GnRH-1 on d 16, a time when a second wave dominant follicle would likely be present. In the current study, follicular diameter at GnRH-1 did not differ ($P > 0.09$) within parity; the GnRH-Yes Response heifers and cows had similar dominant follicle size at GnRH-1 compared to the saline and GnRH-No Response groups. The lack of ovulatory response in the GnRH-No Response group (heifers [n = 5] or cows [n = 2]) on d 16 was probably because the largest follicle on d 16 had lost dominance and a new dominant follicle (> 10 mm) had not yet emerged. Sirois and Fortune (1998) reported that initiation of the third bovine follicular wave occurred around d 17. Consequently, the heifers and cows that did not ovulate in the GnRH-No Response groups may have been having three follicular waves.

Assuming spontaneous luteolysis normally occurs around d 16 to 18 in cattle, Animals injected with GnRH-1 on or before d 9 to 11 will likely have a viable primary

CL at PG, which explains why few animals receiving GnRH-1 before mid-cycle display estrus before PG. However, heifers or cows injected after mid-cycle frequently require the presence of an ACL to inhibit estrus and ovulation until after the PG injection. Since a CL is not normally responsive to the luteolytic action of PG until d 4 d to 5 after ovulation, an ACL induced on d 16 should not undergo luteolysis at the same time as the primary CL. However, when a dominant follicle is induced to ovulate (GnRH-1) during the late luteal phase, heifers or cows may show estrus or ovulate by the day of PG administration for the following reasons: 1) there may not be sufficient time for an ACL to form, 2) the ACL may not produce adequate circulating concentrations of progesterone to inhibit estrus/ovulation, or 3) the ACL may regress at the same time as the primary CL.

Previous studies in our lab indicated that the proportion of heifers and estrous cycling postpartum cows detected in estrus by PG injection (CO-Synch protocol) following injection of GnRH-1 after d 12 was 54 % and 25%, respectively (Atkins et al., 2008, Atkins et al., 2009 submitted). In addition, serum concentrations of progesterone from an ACL (formed late in the luteal phase) appeared to be lower in heifers compared to cows (Atkins et al., unpublished data; Figure 4.1). Atkins et al., (2008) reported that the proportion of heifers in estrus between GnRH-1 and PG was decreased for animals that received GnRH-1 earlier (d 2, 5, or 10) compared to later (d 15 and 18) in the estrous cycle (d 0 = estrus). In the preceding study, GnRH-1 injections on d 15 or 18, resulted in 63 and 38% of heifers displaying estrus before PG, respectively; whereas, none of the heifers that received GnRH-1 on d 2, 5, or 10 were detected in estrus before PG. An explanation for the relatively large proportion of heifers that showed estrus on or before PG could be failure to form an ACL or failure of an ACL formed following GnRH-1 to

produce an adequate amount of P4 to inhibit estrus and ovulation. Although, injection in cycling postpartum cows on d 2, 5, or 10 may not have induced an ovulation; there most likely would have been a primary CL present that would have prevented estrus.

Heifers and cows in the saline and GnRH-No Response groups did not form ACL and displayed estrus sooner than the GnRH-Yes Response group. Without elevated concentrations of P4, estrus and ovulation were not inhibited. On the other hand, those that did form ACL and secreted P4 displayed estrus later than the Saline/GnRH-No Response. There were two heifers and one cow that formed ACL but that also displayed estrus by PG injection. It is not clear why these animals showed estrus in the presence of an ACL. The CL is a continuation of follicular maturation; therefore, the preovulatory endocrine environment may affect preparation of follicular cells for luteinization (as reviewed by McNatty, 1979). In humans, granulosa cells from follicles containing elevated concentrations of FSH and estradiol were reported to produce increased progesterone, *in vitro*, compared to granulosa cells from follicles having decreased concentrations of FSH and estradiol. Therefore, induction of ovulation in the presence of high P4 might compromise luteinization. Alternatively, Howard and Britt (1990) demonstrated that hCG-induced ACL were more responsive to the luteolytic action of PG and regressed during the first 5 d following formation; whereas, spontaneously-induced CL do not respond until d 5 or later (d 0 = estrus; Lauderdale, 1972). Howard and Britt (1990) proposed that formation of ACL during elevated concentrations of P4 may advance their responsiveness to PG compared CL that form spontaneously. In the present study, ACL formation was induced with GnRH and not hCG. Luteinizing hormone (LH) is known to have a shorter half life and to interact differently with the LH receptor

compared to hCG (Niswender et al., 1985). Therefore, it is not known whether GnRH-induced ACL would act like hCG-induced ACL.

Nonetheless, data from this study indicate that an ACL formed following GnRH-induced ovulation was capable of inhibiting estrus and ovulation similarly in both heifers and postpartum cows. In addition, serum concentrations of progesterone were similar following ACL formation for both heifers and cows and there was a similar extension of interestrus interval in both cases. As expected, circulating concentrations of progesterone were similar in the saline and GnRH-No Response groups indicating there was no effect of GnRH-1 on progesterone that was independent of ACL formation.

In summary, GnRH-1 (CO-Synch) induced ovulation and formation of ACL on d 16 in heifers and cows. Furthermore, GnRH-induced ACL did not differ between heifers and cows in the ability to inhibit estrus and ovulation prior to d 23 (PG injection).

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Appendix

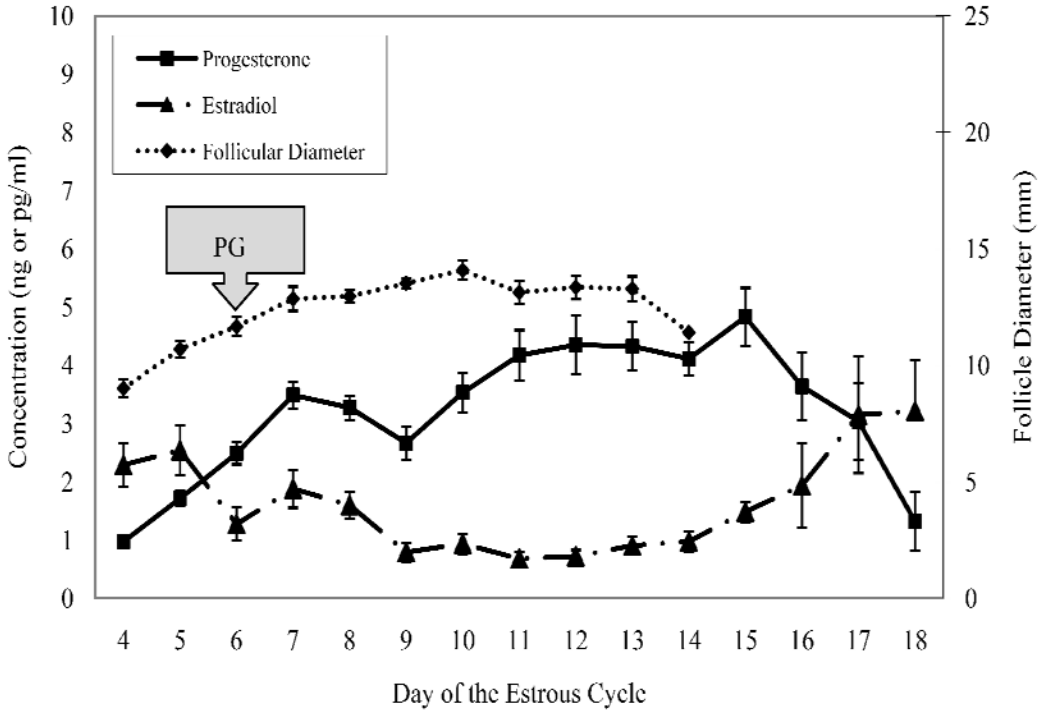

Figure A.1. Mean (± SEM) serum concentrations of progesterone (ng/ml) and estradiol (pg/ml) and dominant follicle diameter (mm) of heifers in the Control group (n = 8) during treatment period (d 4 to 18 of the estrous cycle; d 0 = estrus).

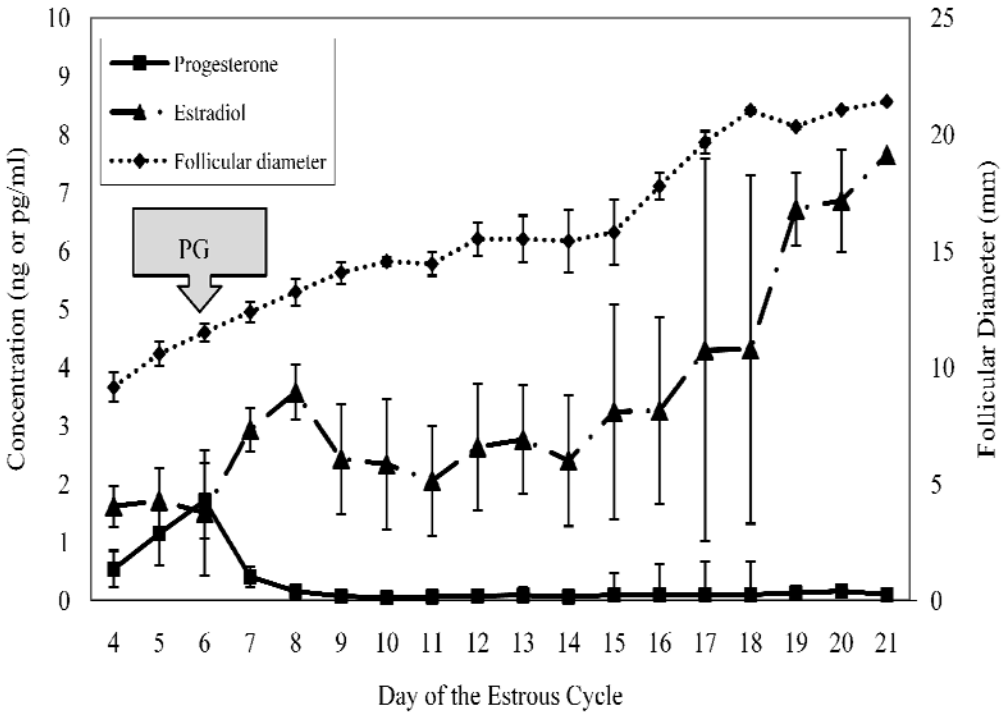

Figure A. 2. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of MGA treated heifers (n = 3) during treatment period (d 4 to 17 [last d of MGA feeding] of the estrous cycle; d 0 = estrus).

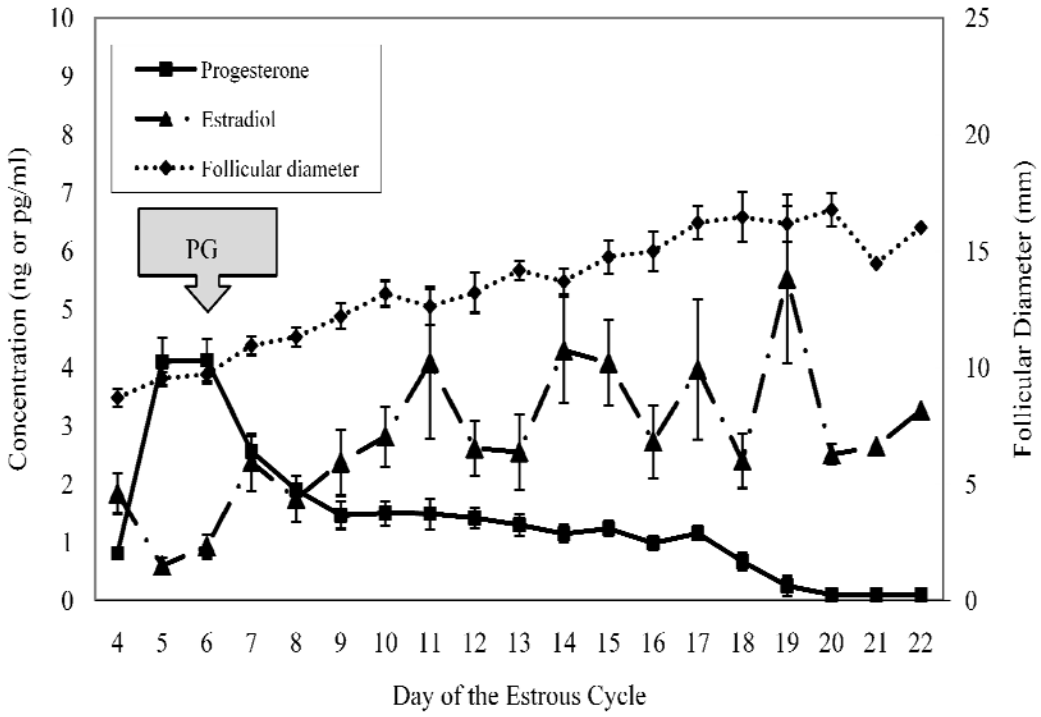

Figure A.3. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of heifers (n = 7) treated with a new CIDR (1.38 g). CIDR were inserted on d 4 of the estrous cycle (d 0 = estrus) and were removed on d 18.

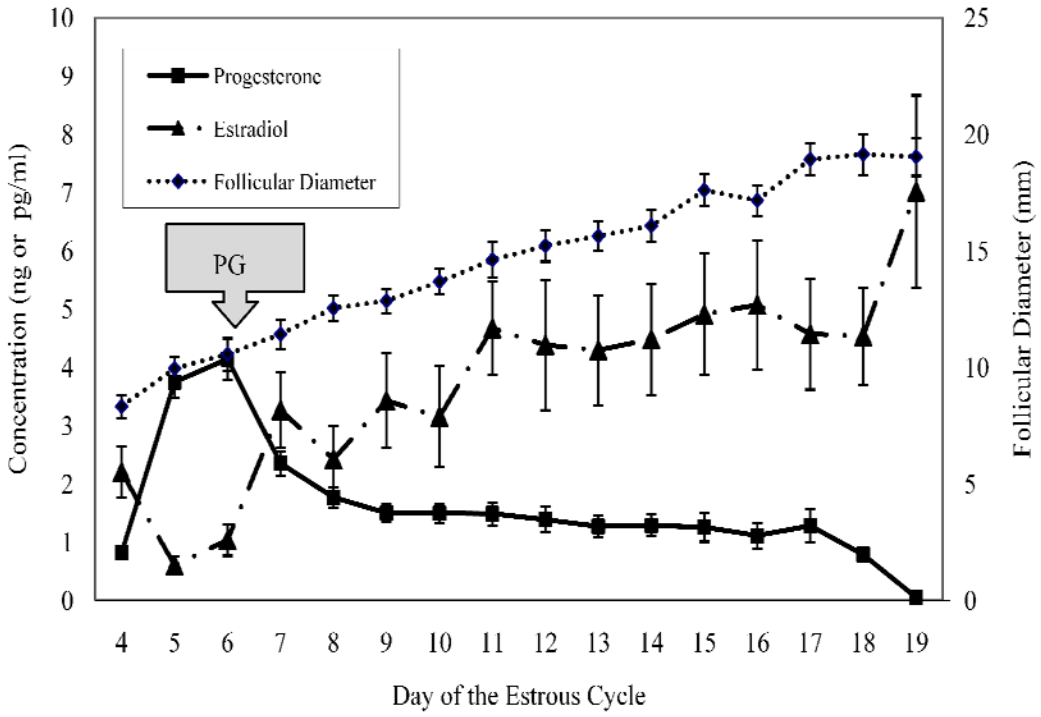

Figure A.4. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of heifers (n = 7) treated with a used CIDR (New CIDR previously inserted for 7 d, removed, disinfected, and stored). Used CIDR were inserted on d 4 of the estrous cycle (d 0 = estrus) and were removed on d 18.

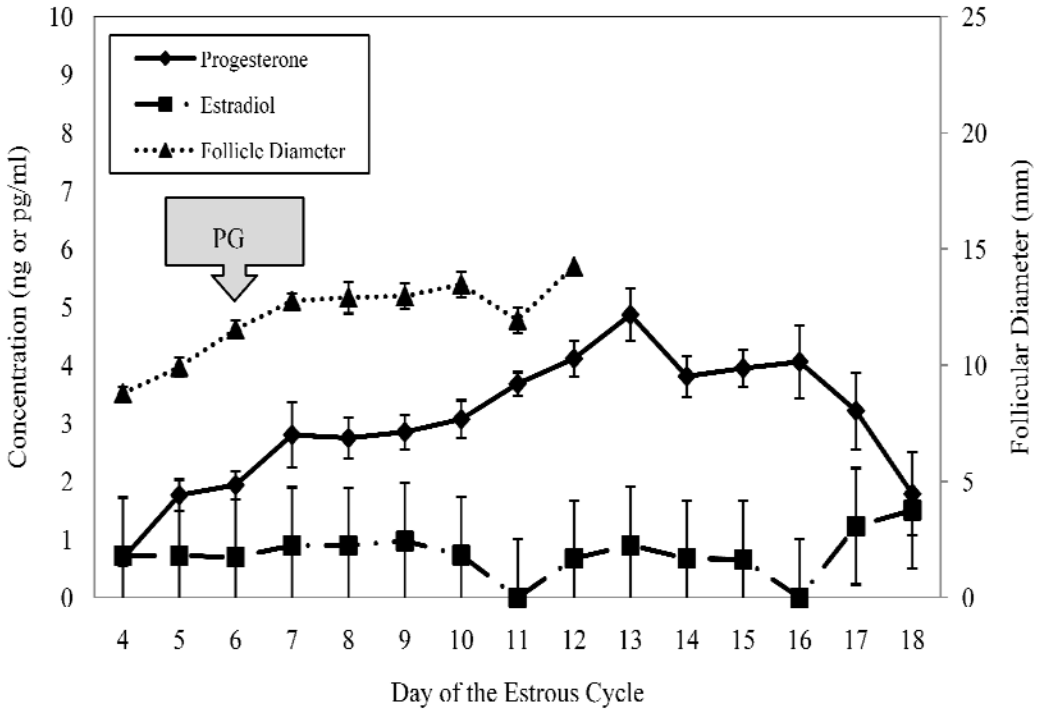

Figure A.5. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of postpartum cows (n = 10) in the Control group during treatment period (d 4 to 18 of the estrous cycle; d 0 = estrus).

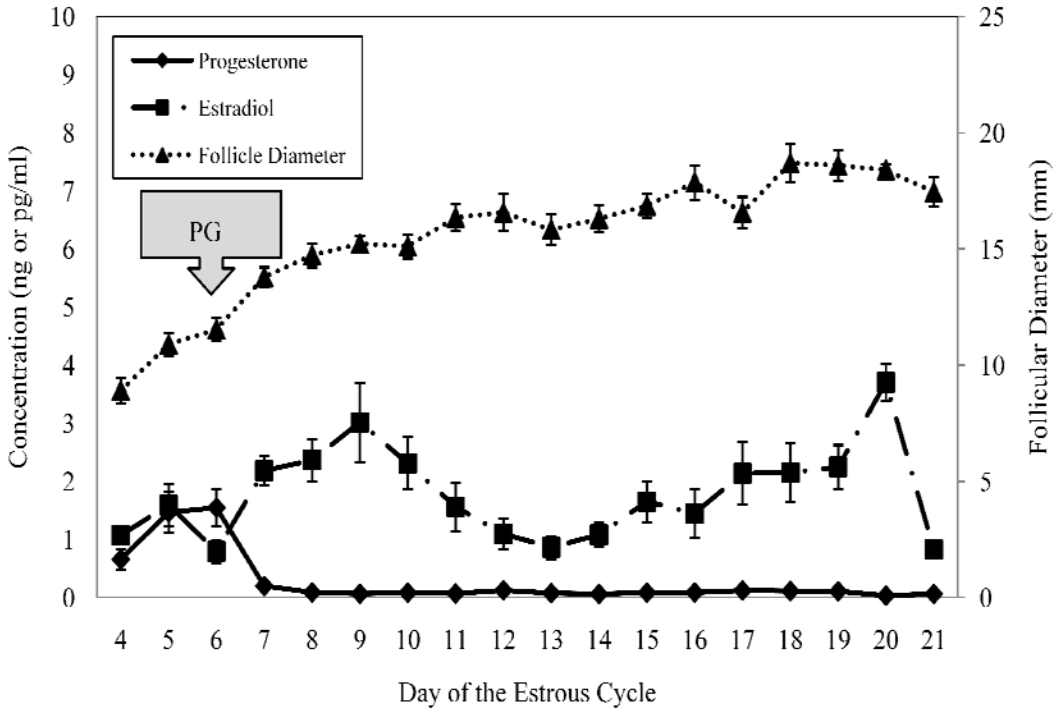

Figure A.6. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of postpartum cows (n = 10) in the MGA group during treatment period (d 4 to 17 [last d of MGA feeding] of the estrous cycle; d 0 = estrus).

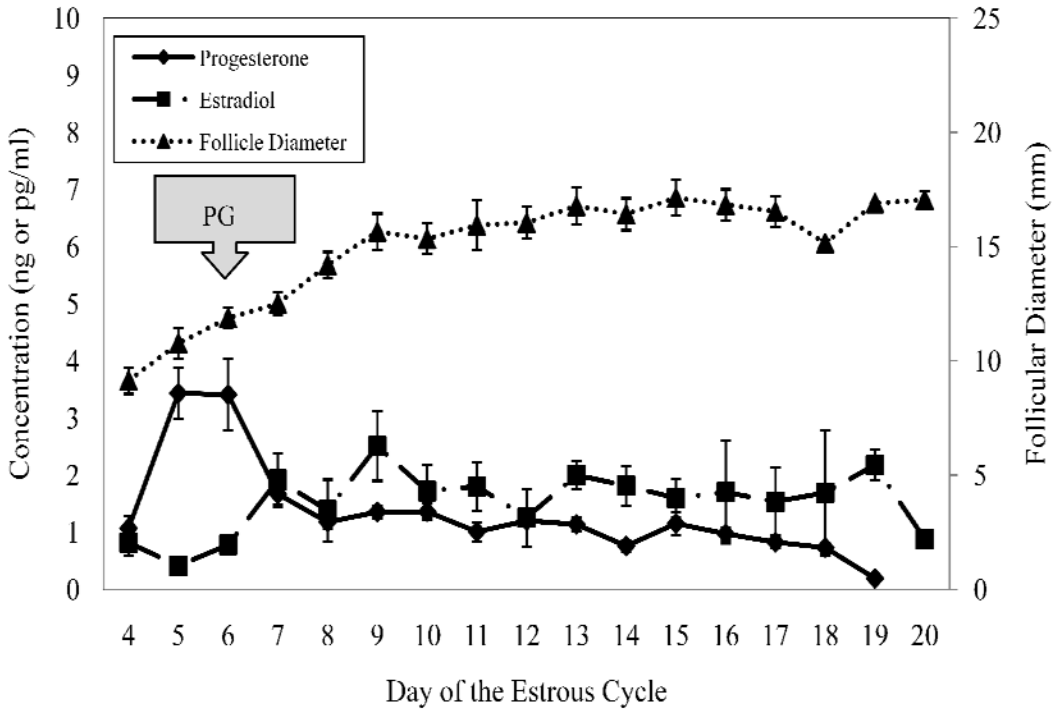

Figure A.7. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of postpartum cows (n = 8) in the New CIDR (1.38 g) group. CIDR were inserted on d 4 of the estrous cycle (d 0 = estrus) and were removed on d 18.

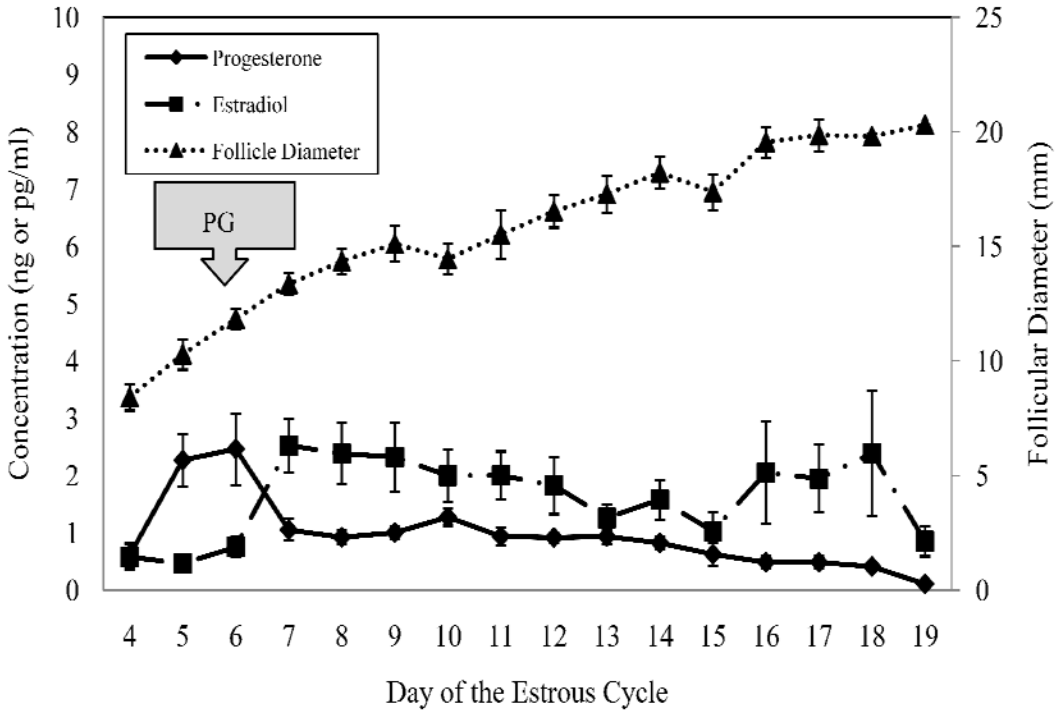

Figure A.8. Mean (± SEM) serum concentrations of progesterone (ng/ml), estradiol (pg/ml) and dominant follicle diameter (mm) of postpartum cows (n = 6) in the Used CIDR (New CIDR previously inserted for 7 d, removed and cleaned, then stored) group. Used CIDR were inserted on d 4 of the estrous cycle (d 0 = estrus) and were removed on d 18.

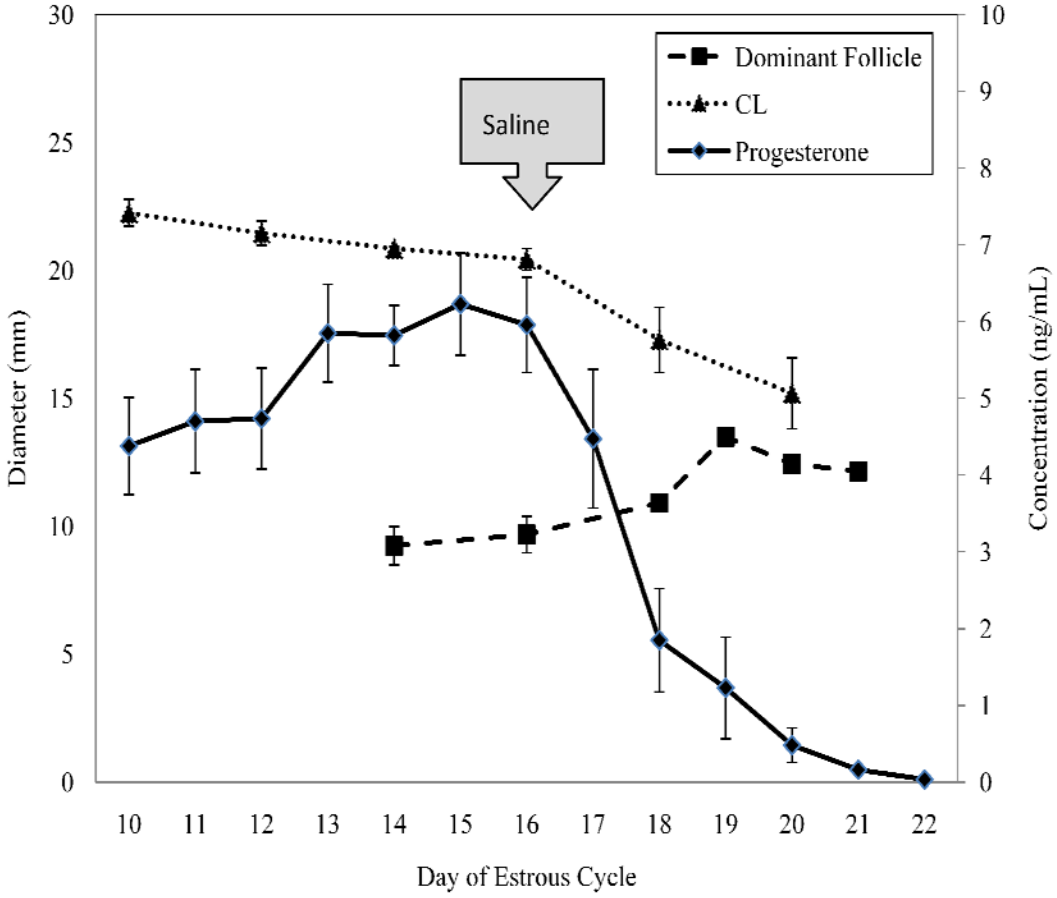

Figure A.9. Mean (± SEM) serum concentrations of progesterone, dominant follicle diameter, and luteal (CL) diameter of heifers (n = 7) injected with saline on d 16 of the estrous cycle (d 0 = estrus). All heifers exhibited estrus by d 23 and did not receive PG.

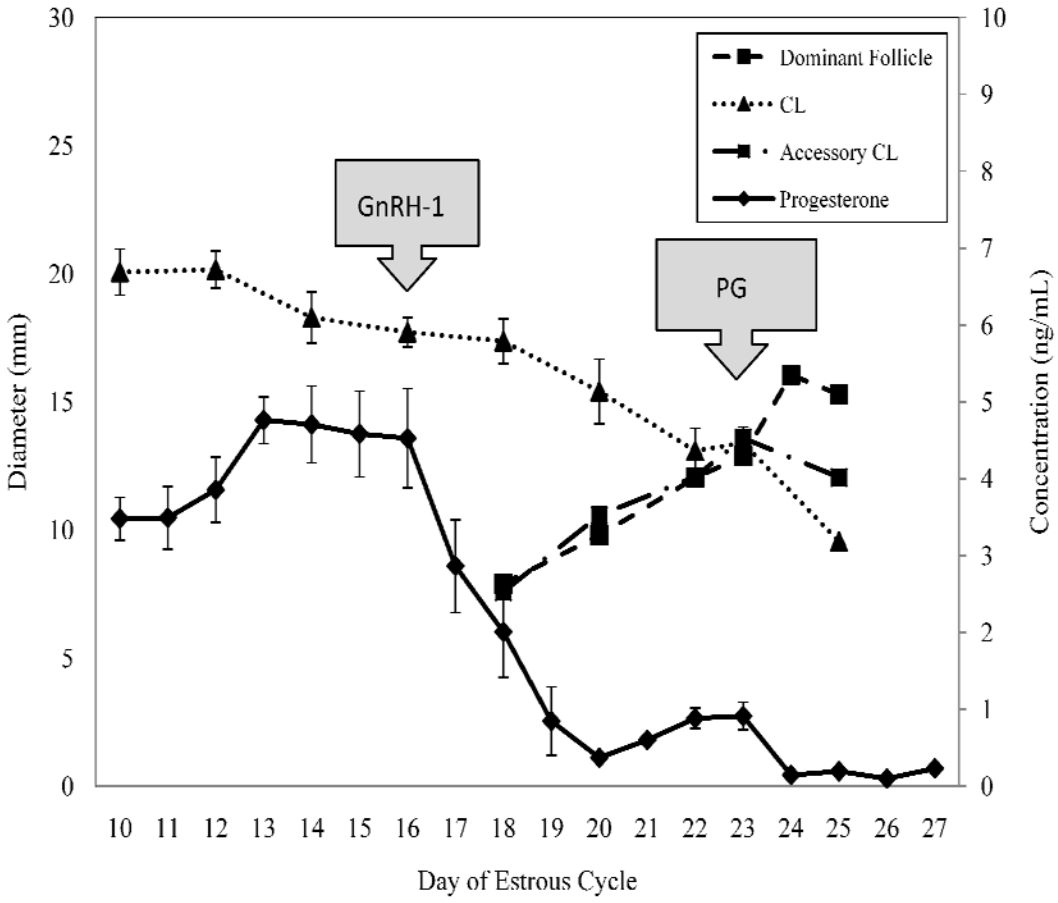

Figure A.10. Mean (± SEM) serum concentrations of progesterone, dominant follicle diameter, luteal (CL) diameter, and accessory CL diameter of heifers (n = 10) injected with GnRH on d 16 of the estrous cycle (d 0 = estrus). If heifers were not detected in estrus by d 23, they were injected with PG.

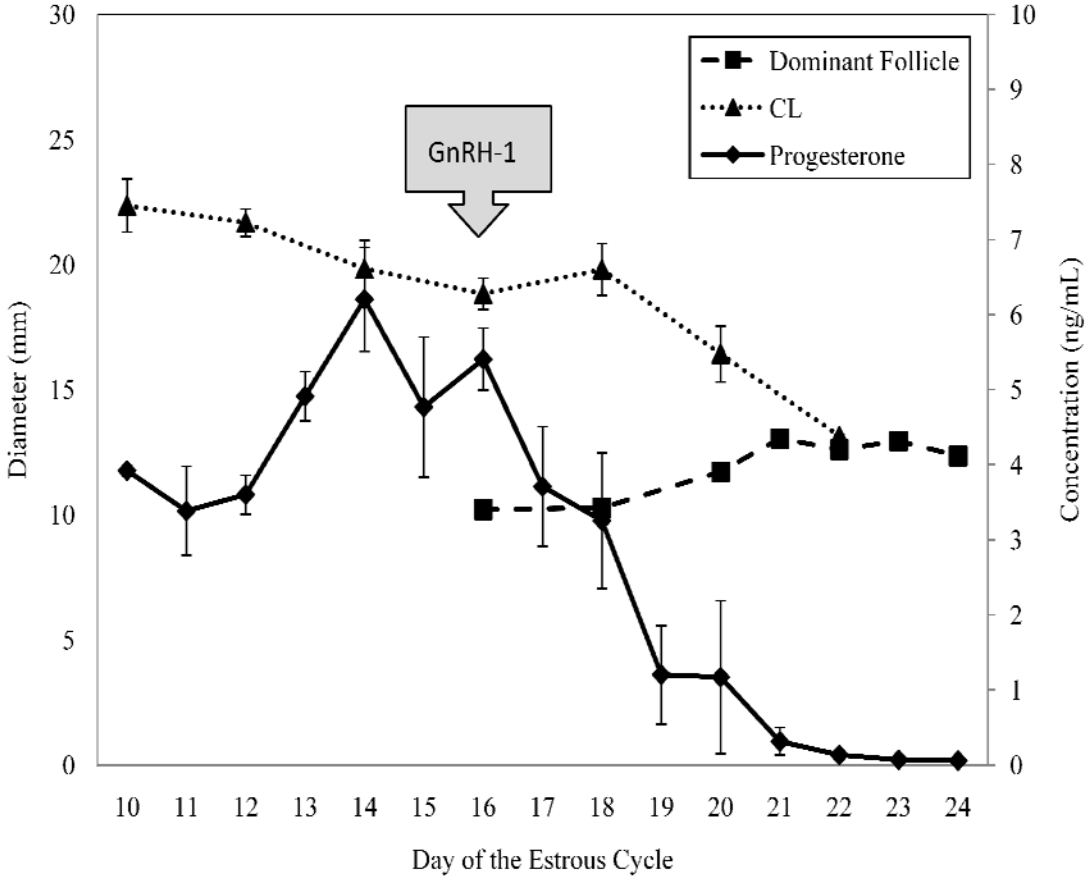

Figure A.11. Mean (± SEM) serum concentrations of progesterone, dominant follicle diameter, and luteal (CL) diameter of heifers (n = 5) injected with GnRH on d 16 of the estrous cycle but did not develop an accessory CL. If heifers were not detected in estrus by d 23, they were injected with PG.

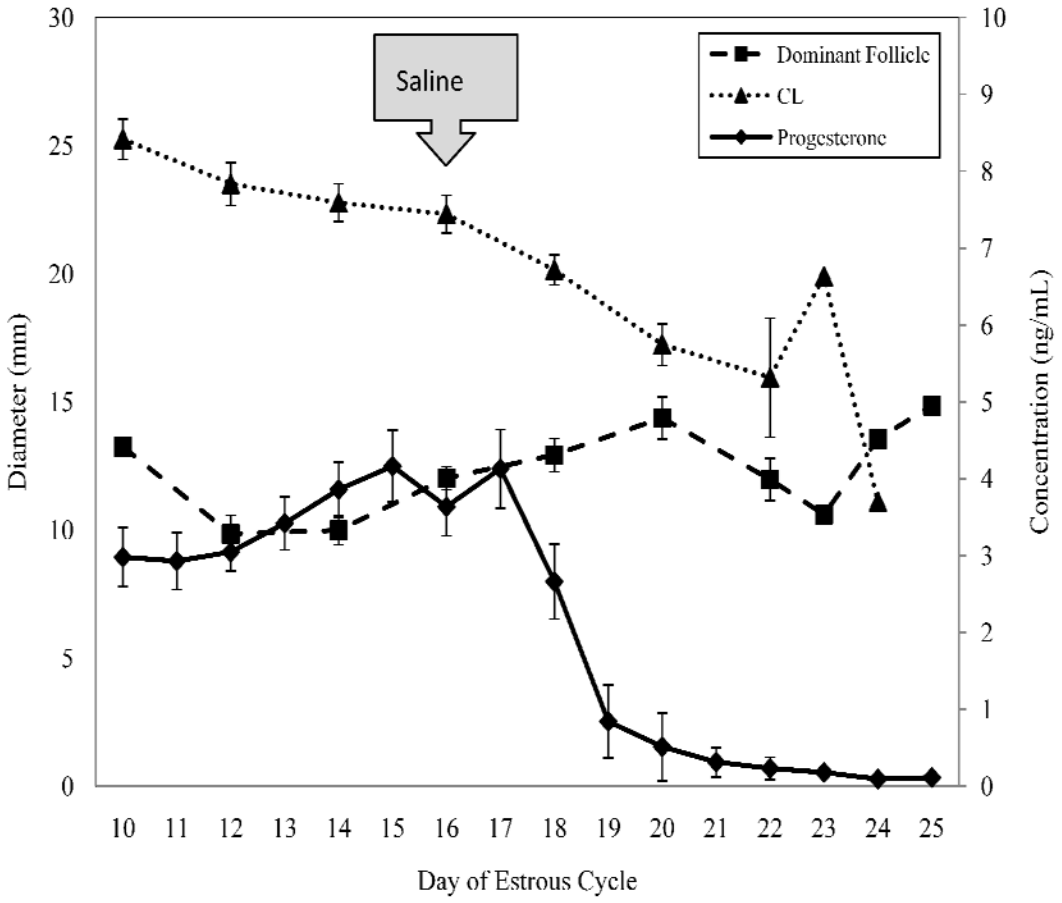

Figure A.12. Mean (± SEM) serum concentrations of progesterone, dominant follicle diameter, and luteal (CL) diameter of cows (n = 9) injected with saline on d 16 of the estrous cycle. If cows were not detected in estrus by d 23, they were injected with PG.

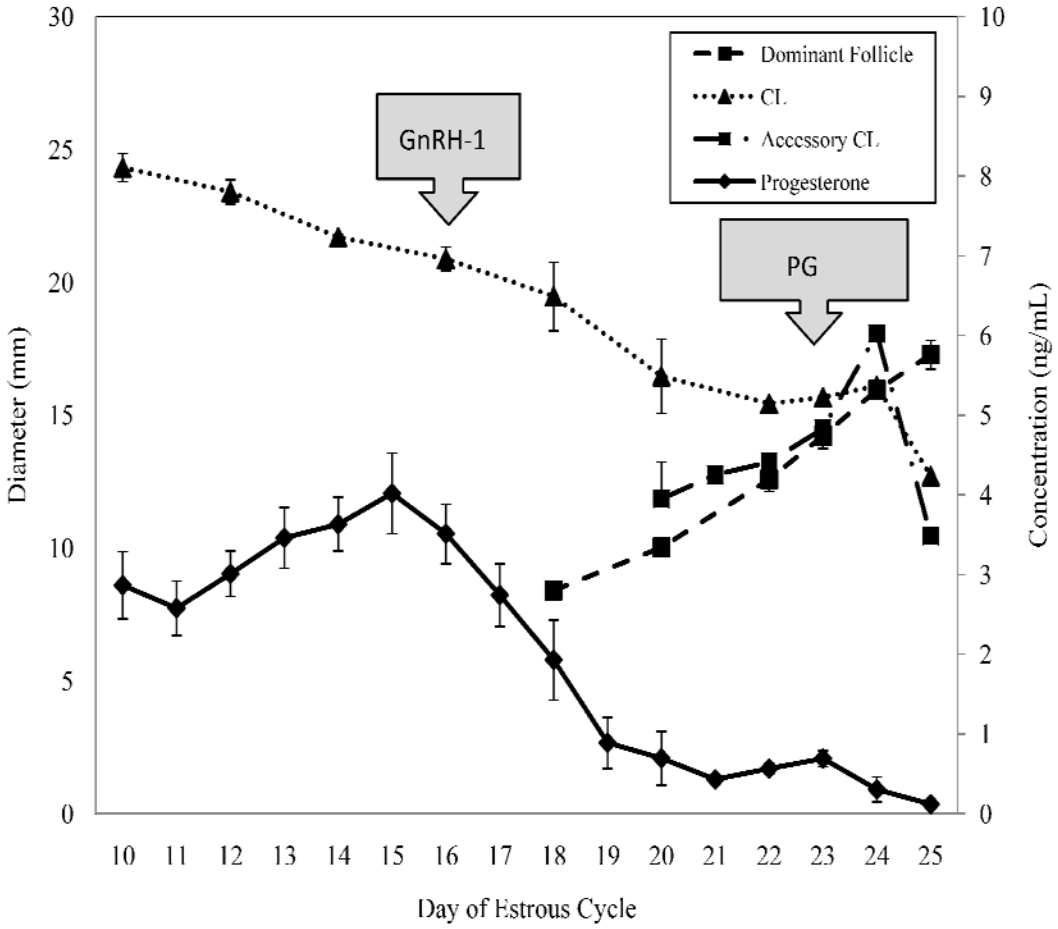

Figure A.13. Mean (± SEM) serum concentrations of progesterone, dominant follicle diameter, luteal (CL) diameter, and accessory CL diameter of cows (n = 15) injected with GnRH on d 16 of the estrous cycle. If cows were not detected in estrus by d 23, they were injected with PG.

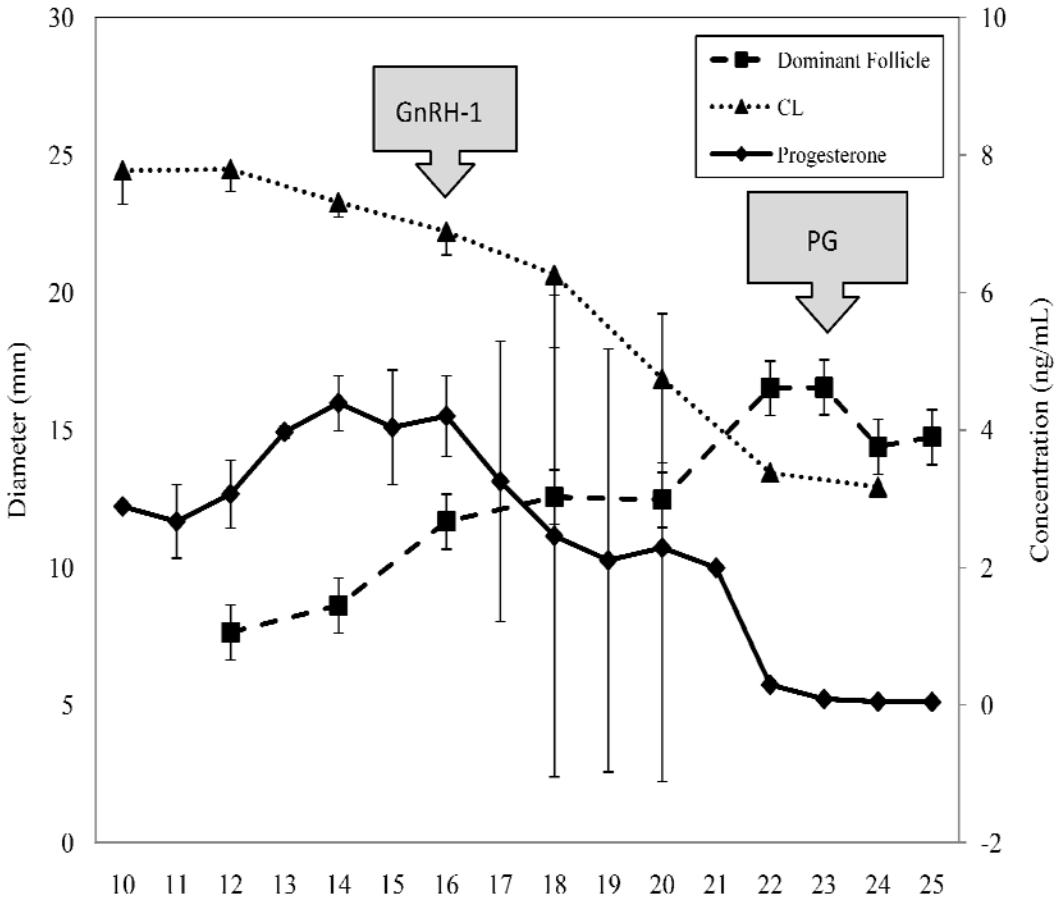

Figure A.14. Mean (± SEM) serum concentrations of progesterone, dominant follicle diameter, and luteal (CL) diameter of cows (n = 2) injected with GnRH on d 16 of the estrous cycle but did not develop an accessory CL. If cows were not detected in estrus by d 23, they were injected with PG.

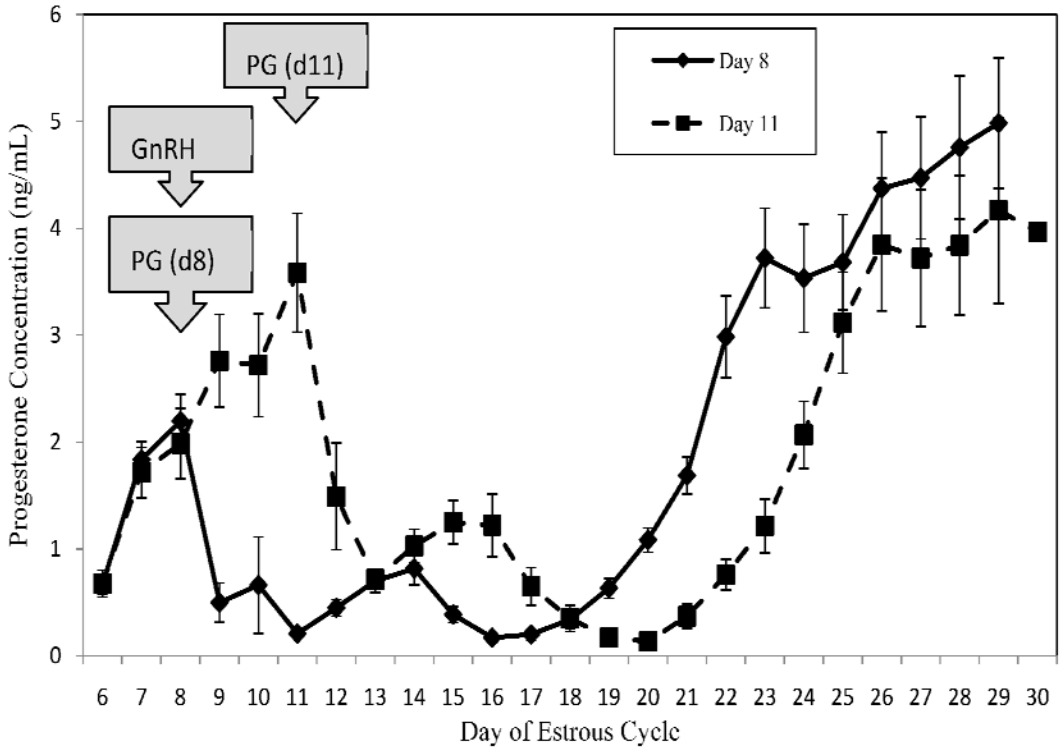

Figure A.15. Mean (± SEM) serum progesterone concentration of cows (n = 24) injected with GnRH on d 8 of the estrous cycle (d 0 = estrus) and injected with PG on d 8 or 11.

VITA

Mallory Risley, formerly Mallory Heaton, was born in Overland Park, Kansas but was raised in a rural town (Pittsfield) in West Central Illinois. She and her brother, Spencer, are the two kids of Mr. and Mrs. Peter and Paula Heaton, formerly of Jacksonville, Illinois, and Niagara Falls, New York, respectively. Mallory graduated in 2004 from Pittsfield High School and then attended the University of Missouri-Columbia in the fall of 2004. She graduated with a Bachelor of Science in December 2007 in Animal Science. She married her husband, Gavin Risley, formerly of Perry, Illinois in December 2007. As he is a student at Missouri Science and Technology in Rolla, Mo, they resided in Freeburg, Mo throughout their graduate careers. She graduated in December 2009 with a Master of Science in Reproductive Physiology from the University of Missouri-Columbia.