USE OF EQUINE CHORIONIC GONADOTROPIN, TEMPORARY CALF REMOVAL, AND PROGESTERONE FOR ESTROUS CYCLE CONTROL OF SUCKLED BEEF COWS AND REPLACEMENT HEIFERS

By

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ACTH	adrenocorticotropic hormone
ADG	average daily gain
AGRP	agouti-related protein
AI	artificial insemination
ANOVA	analysis of variance
APP	acute phase protein
ARC	arcuate nucleus
AVPV	anteroventral paraventricular nucleus
BCS	body condition score
BW	body weight
cAMP	cyclic adenosine monophosphate
CIDR	controlled internal drug release
CL	corpus luteum
CNS	central nervous system
CR	calf removal
CRH	corticotropic releasing hormone
DF	dominant follicle
DPP	days postpartum
DYN	dynorphins
E	estradiol 17β
eCG	equine chorionic gonadotropin
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbant assay
EOP	endogenous opioids

ER	estrogen receptor
ERK	extracellular-signal regulated kinase
ET	embryo transfer
ET-2	endotelin-2
FP	prostaglandin receptor
FSH	follicle stimulating hormone
GH	growth hormone
GnRH	gonadotropin releasing hormone
HPO	hypothalamus-pituitary-ovary axis
HSP	heat shock protein
IAP	intestinal alkaline phosphatase
IVF	in vitro fertilization
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL	interleukin
INF-τ	interferon-tau
LH	luteinizing hormone
LH-RH	luteinizing hormone releasing hormone
LPS	lipo-polysaccharide
MAPK	mitogen-activated protein kinase
MCH	melanin-concentrating hormone
ME	median eminence
MGA	melengestrol acetate
MGC	mural granulosa cell
mRNA	messenger ribonucleic acid

MSH	melanocyte-stimulating hormone
NASS	national agriculture statistical service
NHAMS	national animal health monitoring system
NKB	neurokinin-B
NOS	nitric oxide synthase
NPY	neuropeptides Y
OX-A	orexin-A
P	progesterone
PBW	percent of body weight
PG	prostaglandin $F_{2\alpha}$
PMSG	pregnant mare serum gonadotropin
POA	pre-optic area
PR	progesterone receptor
PRL	prolactin
RIA	radioimmunoassay
SRBC	sheep red blood cell
TNF-α	tumor necrose factor
TSH	thyroid-stimulating hormone

Abstract of Dissertation Presented to the Graduate School
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USE OF EQUINE CHORIONIC GONADOTROPIN, TEMPORARY CALF REMOVAL, AND PROGESTERONE FOR ESTROUS CYCLE CONTROL OF SUCKLED BEEF COWS AND REPLACEMENT HEIFERS

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This project was conducted to evaluate alternative breeding management systems attempting to improve fertility in suckled beef cows and to evaluate the consequences on subsequent performance of the calf. Two experiments were conducted to evaluate changes in follicular development in peripubertal beef heifers treated with a controlled internal drug release. These studies improved our knowledge about the reproductive endocrinology and physiology in beef cows and heifers, pointing out some positive and negative outcomes in hormone pattern release, follicle dynamics, and fertility when these techniques are applied.

Study one consisted of two experiments using 679 suckled beef cows from six different locations. Cows were enrolled in a 7-d CO-Synch + CIDR estrous synchronization protocol (i.e., 100 μg injection of gonadotropin releasing hormone [GnRH] at controlled internal drug release [CIDR] insertion [d -7] with 25 mg injection of prostaglandin [PG] at CIDR removal [d 0], followed by injection of GnRH and timed artificial insemination [TAI] on d 3). In the first experiment cows received the following treatments: 1) control (Control; n = 156); 2) calves were separated from their dams

between d 0 and 3 (CR72; n = 168). In experiment 2 cows received one of four treatments: Control (n = 103); 2) CR72 (n = 104); 3) similar to Control with addition of CR between d 0 and 2 (CR48A; n = 95); and 4) similar to Control with addition of CR between d 1 and 3 (CR48B; n = 53). The results indicate that cows receiving temporary CR (0.42 ± 0.15 mm/d) tended (*P* = 0.06) to have greater follicle growth rate than cows without calf removal (0.02 ± 0.15 mm/d). In addition, CR tended to improve pregnancy rates with TAI at one location (53% vs. 38% for Control and CR, respectively; *P* = 0.06); however, CR had no significant effect on pregnancy rates at the other locations. Moreover, 48 and 72 h of CR resulted in similar pregnancy rates when compared to each other, but not to subsequent calf performance assessed by live body weight (BW) 33 and 63 d after CR. Young (25 to 60 d of age) and old (> 80 d of age), but not medium (61 to 80 d of age) calves exposed to 72 h of CR were 8 kg lighter 63 d after CR than contemporary age Control calves. Calves exposed to 48 h of CR had similar weights compared to Control calves, 63 d following CR. We conclude that CR may be a good alternative to improve fertility of beef cows and 48 or 72 h of CR may have similar effects on pregnancy rates, and reduce the negative impact on calf performance.

The objective of the second study was to evaluate whether equine chorionic gonadotropin (eCG) or temporary CR altered follicle development and serum hormone concentrations (estradiol 17β [E] and luteinizing hormone [LH]) prior to TAI. We evaluated the effect of treatments on pregnancy rate and ovulation rate. Two experiments were conducted using five hundred forty-eight suckled beef cows. The cows were enrolled in a 7-d CO-Synch + CIDR protocol and assigned to treatments on d 0. In Experiment 1, Thirty-five cows received blood sample collection every 4 h from d

0 to 4 and ovary ultrasonography scanning once daily from d 0 to 4. Cows were assigned in a 2 × 2 factorial arrangment of experimental design to the following treatments: 1) regular CoSynch + CIDR protocol (Control; n = 9); 2) same as Control but calves were removed from their dams for 72 h between d 0 and d 3 (COCR; n = 9); 3) same as Control but cows received 400 IU of eCG on d 0 (COeCG; n = 9); 4) same as COCR but cows received an additional 400 IU of eCG on d 0 (eCGCR; n = 8). The results indicate that cows treated with eCG had greater follicle diameter on d 3 (14.9 ± 0.5 mm) compared to cows receiving no eCG (13.1 ± 0.5 mm). Pregnancy rates (41% vs. 12%), ovulation rate by d 4 (64% vs. 27%) and peak of LH within 72 h after PG (52% vs. 16%) were greater for cows exposed to CR than cows not exposed to CR. In Experiment 2, cows received the Control (n = 261) and COeCG (n = 252) treatments detailed in experiment 1, however the interval from PG to TAI was 66 h and 200 IU of eCG were administered to the COeCG group. Treating cows with 200 IU of eCG (43%) did not improve pregnancy rates to TAI compared to the CO-Synch + CIDR protocol (50%).

In the third study, two experiments were conducted using one hundred beef heifers. The objective was to evaluate the incidence of a persistent follicle when a once-used or new CIDR was inserted, as well as the incidence of follicles turnover following insertion of a second new CIDR 8 or 10 d later. In Experiment 1, all heifers received PG on d -9 (relative to treatments on d 0) and on d -8 heifers were randomly assigned in a 2 × 3 factorial arrangement to receive one of six treatments; 1) heifers received either a new (NewSal; n = 9) or once-used (for 7 d; UsedSal; n = 10) CIDR inserted on d -8 and 2 mL i.m. of saline on d 0; 2) heifers received either a new (NewAsp; n = 10) or once-

used (UsedAsp; n = 10) CIDR on d -8 and aspiration of all follicles ≥ 5mm on d 0; and, 3) heifers received either a new (NewCIDR; n = 10) or once-used (UsedCIDR; n = 10) CIDR on d -8 and a second additional new CIDR on d 0. All CIDR were removed on d 3. Therefore the main effect of type of CIDR (inserted on d -8) and treatments (d 0) were analyzed. A follicle was deemed persistent when the same follicle was observed on d -8 and d 0. Follicle turnover was defined as the largest follicle on d 0 that regressed by d 5 and new follicular wave was initiated by d 5. The results indicate that new (10 of 25; 40%) and once-used (11 of 27; 40%) CIDR were equally effective at inducing persistent follicles. Follicular aspiration (18 of 18; 100%) had a greater capacity to induce follicle turnover compared to those heifers receiving an additional new CIDR (12 of 19; 63%) or saline (8 of 18; 44%). In Experiment 2, heifers received an injection of PG on d -12 and on d -10 all follicles ≥ 5 mm were aspirated and a once-used CIDR was inserted. On d 0 heifers were assigned to 2 treatments: 1) heifers received a 2 mL i.m. injection of saline (UsedSal; n = 21) or 2) heifers received an additional new CIDR (UsedCIDR; n = 20). Follicle turnover was defined as the largest follicle on d 0 that regressed by d 4 and new follicular wave was initiated by d 4. The incidence of follicle turnover was similar between UsedCIDR (10 of 18; 55%) and UsedSal (15 of 19; 78%). We conclude that new or once-used CIDR were equally effective at inducing persistent follicles and the additional CIDR or saline were equally effective at inducing follicle turnover of a growing follicle.

CHAPTER 1
INTRODUCTION

Artificial insemination (AI) has been a long-standing practice to improve cattle fertility dating back to reports from 1889. The first AI cooperative in the US was established at the New Jersey State College of Agriculture in 1938. Since then, the AI technique has been used to spread genetic cattle lineage around the world improving fertility and production efficiency of animals. The AI technique enables a tremendous increase in the capability of a single sire to spread its genetics compared to natural service (Foote, 1998). The technique of AI was introduced to US cattle producers in the 1930s and by 1970, about 46% of the dairy female cattle in the US were being inseminated (Foote 2002). Other techniques such as embryo transfer (ET) and in vitro fertilization (IVF) have the capacity to disseminate genetics, but the high cost and low efficiency limits their commercial use to the same magnitude as AI.

There are 30.9 million beef cows in the United States, with ≈ 8% exposed to AI each year (NAHMS, 2011). The reason for the low acceptance by cattle producers likely is due to the intensive labor/time and high cost required to perform AI successfully. However, advancements in research, availability of commercial hormones and a better understanding of endocrinology and physiology of cattle, AI has become easier and more applied to the field. The possibility of administration of exogenous hormones to manipulate the estrous cycle has allowed for more control of the time of insemination, removing the need for detection of estrus.

Improvements in methods to study the estrous cycle, such as analysis of real time ultrasonography and quantification of concentrations of hormones in blood serum or plasma (RIA or ELISA) has enabled researchers to better understand the pattern of

hormone release and development of ovarian structures during the estrous cycle. The bovine estrous cycle relies mainly on the function and communication among four structures: 1) hypothalamus, 2) pituitary, 3) ovary, and 4) uterus. The first three structures are often referred to as the hypothalamus-pituitary-ovary (HPO) axis. These four structures are responsible for synthesizing and releasing essential hormones to initiate, maintain, and terminate the estrous cycle. The interaction between these hormones occurs by an endocrine system promoting a positive or negative feedback among specific hormones. The synergism between the hormonal feedbacks is essential for proper development of ovarian structures, such as follicles or corpora lutea (CL). Intrinsic factors, such as hormones, plus external factors, such as the environment and the maternal bond between calf and dam positively or negatively affect fertility. The maternal bond during suckling is an important interaction that is responsible for the delay in postpartum return to cyclicity, and may adversely affect overall fertility of beef cows.

This dissertation consists of five chapters. Chapter 2 is the literature review, which summarizes the main reproductive functions of the HPO axis and uterus, as well as the interaction between the hormones controlling the estrous cycle of cows. In addition, Chapter 2 reviews the maternal bond between cow and calf and its effect on fertility and calf growth performance, plus focusing on additional literature regarding the physiologic mechanisms associated with the suckling interaction and reproductive performance of cows. Chapter 3 is the primary focus of my research, aimed at the effect of temporary separation of the maternal bond between cow and calf, prior to AI, and its effect on fertility of the cows and growth performance of the calf. In addition, the project objective

is to clarify the optimal interval of temporary calf removal that may be beneficial to cow fertility with minimum effects on calf performance. Chapter 4 describes the effect of equine chorionic gonadotropin and/or calf removal on luteinizing hormone and concentrations of estradiol in blood and the impact on ovulation in suckled beef cows exposed to a fixed-time artificial insemination protocols. Chapter 5 describes the capability of low or high concentrations of progesterone from an external progesterone releasing device to induce persistency and turnover of follicles in pre-pubertal beef heifers.

CHAPTER 2
LITERATURE REVIEW

Reproductive Organs and their Respective Hormones

The oocyte is the female germ cell produced in the ovary during gametogenesis. A single oocyte is embedded in a follicle. Follicle development comprises four categories: 1) primordial; 2) primary; 3) secondary; and 4) tertiary or Graafian follicles. The hypothalamus-pituitary-ovary (HPO) axis and uterus are responsible for stimulating the development of follicles via hormonal secretion. Follicles grow in a wave-like pattern (Quirk et al., 1986), which terminate with ovulation and expulsion of the oocyte into the uteruine oviduct, or natural regression with infiltration of inflammatory cells into the follicle (Wu et al., 2004). For a follicle to be selected, matured, and ovulated the HPO axis and uterus, must work synergistically. Hormones are stimulated and inhibited throughout an estrous cycle; therefore, each organ is essential at all stages of the estrous cycle.

The following narrative will summarize the individual functions and synthesis of reproductive hormones of the HPO axis and uterus, followed by a summary of the interaction of the endocrine and physiological events controlling the entire estrous cycle.

Hypothalamus

The hypothalamus is located ventral to the thalamus and forms the ventral section of the diencephalon. The hypothalamus is responsible for secreting hormones called hypothalamic-releasing hormones. Food and water intake, temperature, sleep, release and inhibition of reproductive hormones are some of the functions of the hypothalamus. In the past century, numerous research reports have been performed to better understand the hormonal hypothalamic control over the pituitary secretion of

gonadotropic hormones. It is well established that hypothalamic gonadotropin releasing hormone (GnRH) stimulates the pituitary to release gonadotropic hormone, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) and in contrast, the inhibition of GnRH dramatically decreases the release of these hormones (Schally et al., 1971; Ginther et al., 2011). Gonadotropic hormones travel via blood reaching the ovary and stimulating the development of structures such as follicles and the corpus luteum (CL). These structures are responsible for synthesizing the steroid hormones estradiol (E) and progesterone (P). These steroid hormones have the ability to regulate the secretion of GnRH by a positive or negative feedback on the hypothalamus (Senger, 2011). However, the mechanism of action between E and P with GnRH neurons is intriguing because GnRH neurons do not express either estradiol receptors (ER; Shivers et al., 1983; Herbison and Theodosis, 1992; Constantin, 2011) or progesterone receptors (PR; Skinner et al., 2001; Constantin, 2011) on their membrane. The lack of those receptors indicates that the regulation of GnRH secretion by sex steroids likely occurs prior to stimulation of GnRH neurons. Therefore, perhaps other neurons could be the link that could be mediating this feedback regulation. More recent research appears to clarify, at least in part, this feedback mechanism by the discovery of an intermediate neuron that expresses E and P receptors termed the KiSS-1 neuron (Lee et al., 1999; Roa and Tena-Sempere, 2007; Castellano et al., 2009).

The KiSS-1 neuron synthesizes the neuropeptide kisspeptin that is encoded by the KiSS-1 gene. When KiSS-1 is stimulated, kisspeptin is released and stimulates GnRH neurons located at the pre-optic area (POA) of the brain. Kisspeptin connects with its G-protein coupled receptor ligand, GPR54, expressed by GnRH neurons, which

stimulates the synthesis and release of GnRH peptide into the median eminence region between the hypothalamus and pituitary (Lee et al., 1999; Irwig et al., 2005). Once in the hypophyseal portal vessels, GnRH is transported to the anterior pituitary, stimulating FSH and LH secretion. The mutation of the GPR54 receptor is associated with dramatic decreases in LH and FSH secretion because of impairment of stimulation on GnRH neurons and the reduction in secretion of GnRH (Irwig et al., 2005; Messager et al., 2005; Shahab et al., 2005). The administration of exogenous kisspeptin rapidly elevates plasma concentrations of LH and FSH (Gottsch et al., 2004; Irwig et al., 2005; Messager et al., 2005; Matsui et al., 2004; Jayasena et al., 2009a, b). Pituitary gonadotropes also express GPR54 (Muir et al., 2001; Navarro et al., 2005); however, the increase in LH and FSH secretion by kisspeptin administration can be blocked by a GnRH antagonist, demonstrating that kisspeptin acts through GnRH neurons and not directly on pituitary receptors (Gottsch et al., 2004; Matsui et al., 2004: Irwig et al., 2005; Shahab et al., 2005; Dungan et al., 2007). The KiSS-1 neuron is expressed in several organs of the body, but for the purpose of this dissertation, the hypothalamic region will be the primary focus. In the hypothalamus, KiSS-1 is mainly expressed in the arcuate nucleus (ARC) and in the anteroventral paraventricular nucleus (AVPV; Smith, 2008). However, the expression intensity and the function of kiSS-1 on those regions vary by species and gender.

It is still not clear how the feedback mechanism between E and KiSS-1 neurons communicate. In some species, such as rats, KiSS-1 is expressed in both the ARC and AVPV nucleus (Gottsch et al., 2004; Smith et al., 2005ab) whereas in ewes, KiSS-1 is expressed in the ARC and not in the AVPV (Estrada et al., 2006; Smith et al. 2007,

2009). In rats, the KiSS-1 gene expression varies in different regions of the hypothalamus depending on the phase of the estrous cycle. For instance, during estrus (high E and low P), E up-regulates the KiSS-1 gene at the AVPV and down-regulates KiSS-1 gene at the ARC (Smith et al., 2006a). The up-regulation of KiSS-1 gene in AVPV during estrus indicates that this region is critical for the pre-ovulatory surge of LH in rats (Estrada et al., 2006; Wintermantel et al., 2006). It appears that in rats, the negative feedback of E inhibits kisspeptin release at the ARC nucleus, while the positive feedback of E stimulates AVPV nucleus KiSS-1 neurons (Smith et al., 2005a, 2006a, 2007). However, in ewes, the inhibition and stimulation of KiSS-1 neurons appears to occur mainly at the ARC (Estrada et al., 2003; Smith et al., 2007, 2009). It is important to note that the AVPV area is one of the few dimorphic regions of the body between male and female primates and rats (Simerly, 1998), being much larger in females than males. This dimorphism is necessary for positive feedback of E on the KiSS-1 neuron in the AVPV nucleus in females. Estradiol stimulation causes a greater release of kisspeptin from the AVPV nucleus driving the LH surge during estrus in females (Smith, 2008). However, the absence of sex steroids such as E or testosterone, up-regulates the KiSS-1 gene on the ARC and down-regulates at the AVPV. Moreover, the replacement of sex steroids causes the reverse effect (Smith, 2008). In primates and rats during diestrus (with low concentrations of E), the ARC nucleus is stimulated and releases kisspeptin, resulting in only basal concentrations of LH in blood. Whereas, during estrus (with high concentrations of E), the AVPV nucleus is stimulated, causing the pre-ovulatory surge of LH. This observation indicates that the ARC nucleus is

related to basal release of LH while AVPV nucleus is related to the LH surge (Lehman et al., 2010; Smith et al, 2009).

The secretion of GnRH from GnRH neurons is not only regulated by the KiSS-1 neuron but also by endogenous opioid peptides (EOP). The opioids bind to opioid receptors expressed in specific neurons of the central and peripheral nervous system as well as the gastrointestinal tract, causing several outcomes such as sedation, respiration depression, constipation, euphoria, and analgesia (Dhawan et al., 1996; Bodnar and Klein, 2004). Examples of EOP are dynorphins, enkephalins, endorphins, endomorphins, and nociception (Bodnar and Klein, 2004). The EOP are released in several regions of the hypothalamus such as ARC, AVPV, POA and ME area (Bodnar and Klein, 2004). Release of specific opioids, such as β-endorphins, in the POA and ME inhibits GnRH secretion and consequently decreases LH and FSH in blood (Barb et al., 1994). In addition, suckling stimulation is positively related to release of EOP in the brain (Gordon et al., 1987), which subsequently has a negative impact on fertility.

In summary, KiSS-1 neurons are essential for stimulation of GnRH secretion and consequently FSH and LH release. Eliminating the KiSS-1 gene results in infertility and administration of kisspeptin reverses the effect. Estradiol and P modulates the secretion of GnRH by inhibiting or stimulating kisspeptin release from KiSS-1 neurons at different regions of the hypothalamus.

Pituitary gland

The pituitary is located under the hypothalamus in a small bone cavity (sella turcica) at the base of the brain (Senger, 2011). It is divided into an anterior and posterior portion. The posterior pituitary releases oxytocin and antidiuretic hormone (ADH). The anterior pituitary synthesizes and releases growth hormone, known as

somatotropin (GH), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), prolactin (PRL), melanocyte-stimulating hormone (MSHs), and FSH and LH (Senger, 2011). The gonadotropin hormones are paramount in regulating gonad development and consequently fertility (Desjardins 1981; Clarke et al., 1983; Gharib et al., 1990). Secretion of GnRH from the hypothalamus enters the hypophyseal portal vessels and reaches the anterior pituitary gonadotropes to stimulate the release of LH and FSH (Schally et al., 1971). The development of gonadal structures such as follicles and CLs stimulates the synthesis of gonadal steroid hormones (E and P) that modulates GnRH secretion (Chenault et al., 1975; Imakawa et al., 1986). Gonadotropin releasing hormone pulses are in low frequency and high amplitude during diestrus due to high P and low E concentrations in blood, favoring FSH release. However, during the follicular phase (proestrus and estrus) characterized by low P and high E, these hormones drive high frequency and low amplitude pulses of GnRH, favoring the LH surge (Rahe et al., 1980; Lucy et al., 1992). The secretion of FSH stimulates the growth of small young follicles (< 3 mm of diameter; Ireland, 1987). Growing follicles acquire the capacity to synthesize E and inhibin. Inhibin (Ireland et al., 1983; Kaneko et al., 1997) and E (Butler et al., 1983; Quirk and Fortune, 1986; Price and Webb, 1988) causes a negative feedback in the hypothalamic neurons decreasing GnRH secretion, impairing additional young follicles to develop. Few follicles of the cohort of follicles achieve a diameter > 5 mm and, in cattle, generally a single follicle acquires the ability to become dominant (Matton et al., 1981). Eventually, the dominant follicle (DF) ovulates, stimulated by an LH surge, and the remaining follicular cells are luteinized, resulting in the development

of the CL. Therefore, the pituitary is critical for the secretion of gonadotropins to stimulate growth of follicles and CL development (Ginther et al., 1989, 1996).

Ovary

The ovary is located at the ovarian fossa near the lateral wall of the pelvis. Two distinct layers, the inner and the outer layer characterize the ovary. Follicles, CL, and stroma comprise the outermost layer. The inner layer is the ovarian medulla and is devoid of follicles and CL (Adams et al., 2008; Senger, 2011). Follicles are structures that contain the female germ cell (oocyte). Within the follicle there are granulosa and theca cells responsible for nourishment of nutrients and hormones responsible for follicle maintenance and growth (Orisaka et al., 2009).

Gonadotropin hormones from the pituitary bind to receptors on follicular cells. Follicle stimulating hormone increases the number of FSH receptors on granulosa cells and LH receptors on theca cells as well as increases in aromatase activity (converts androgen to estrogen; Callard et al., 1978). Luteinizing hormone binds to theca cells stimulating specific enzymes activity such as P50, 3β-HSB, and 17-keto-reductase, responsible for conversion of cholesterol to testosterone. Testosterone is converted to E in granulosa cells by aromatization (Two-cell, two-gonadotropin theory; Armstrong et al., 1979; Hillier et al., 1994). Estrogen is released into blood causing a cascade of events in the hypothalamus as discussed previously. Greater concentrations of E in an environment of low P cause a positive feedback on LH secretion stimulating the growth of larger (> 5 mm of diameter) follicles in the ovary (Ireland and Roche, 1982; Lucy et al., 1992). As a result of high E, LH is released at high pulse frequency causing an LH surge resulting in ovulation of the DF and luteinization of the remaining theca and

granulosa cells of the follicle, resulting in the formation of the CL (Rahe et al., 1980; Ireland and Roche, 1982; Lucy et al., 1992).

Theca cells transform into small luteal cells (SLC), which comprise 70% of the steroidogenic cells and are responsible for 30% of the P synthesized by the CL in response to LH (Niswender et al., 2000, 2002). Granulosa cells differentiate into large luteal cells (LLC), which comprise 30% of the steroidogenic cells, but synthesize 70% of the P independent of LH (Farin et al., 1988; Niswender et al. 2000). The CL is heterogeneous tissue constituting cells such as pericytes, fibricytes, nerves, smooth muscle, and P synthesizing cells (SLC and LLC; Farin et al., 1988). In addition, 22% of the bovine CL tissue is composed of capillary lumina, demonstrating the high vascularization of the CL (Niswender et al., 2000). Luteolysis (regression of the CL) occurs by the action of prostaglandin (PG), which binds to PG receptors (FP) at the membrane of the large luteal cells, elevating the free intracellular Ca^{2+} and activating protein kinase C (PKC), causing cell death (Niswender, 2002). In addition, luteolysis is accompanied by the disruption of the vasculature and decrease in P synthesis (Niswender et al., 2000). According to Ginther (1974) and O'Shea and McCoy (1988) the decrease in P secretion is observed before morphological changes in the CL tissue.

Uterus

The uterus is one of the major reproductive organs of the female mammal, responsible for housing and nourishing the conceptus from fertilization to parturition (Senger, 2011). Progesterone is responsible for physiological modifications in the uterine wall such as mitosis of the endometrium cells, and proliferation of the glandular and vascular tissues, preparing the uterus for embryonic attachment (Jeng et al., 2007). Concentrations of P in blood increases from d 1 to approximately d 12 of the estrous

cycle in cows (Donaldson and Hansel, 1965), reaching a plateau and remaining elevated until d 16 to 18 (Echternkamp and Hansel, 1973; Walters et al., 1984). It has been reported that normal concentrations of P are required to optimize conditions in the uterus for embryo elongation and development (Garret et al., 1988; Carter et al., 2008). Furthermore, the supplementation of P during early (d 3 to 9) but not later (d 12 to 16) stages of the estrous cycle promotes better synchrony of the down regulation of PR and up-regulations of genes related to embryonic elongation in cattle, which leads to less embryo mortality (Mann et al., 2006; Forde et al., 2011).

The uterus also plays an important role in regulation of the estrous cycle. The presence or absence of the conceptus dictates the persistence or disappearance of the CL, respectively. The elongating conceptus in the uterine horn releases interferon-τ (INF-τ) around d 16 of the estrous cycle, which is a paracrine signal blocking E and oxytocin receptor accumulation in the luminal epithelium of the uterine wall (Thatcher et al., 1995; Mann et al., 1999, 2006). The blockage of these receptors inhibits the secretion of PG from endometrial cells, preventing luteolysis (Spencer et al., 2007). In contrast, when pregnancy is not recognized, the expression of E and oxytocin receptors in the endometrium increases, activating and stimulating PG release causing luteolysis (Wathes and Lamming, 1995**)**. The lysis of the CL and lower concentration of P, diminish the negative feedback on GnRH and LH secretion as described previously, inducing ovulation and initiation of a new estrous cycle.

The Bovine Estrous Cycle

Follicular waves

The bovine estrous cycle is characterized by a sequence of several (2 to 4) follicular waves, which are regulated by changes in frequency and amplitude of pulses

of several hormones (Rahe et al., 1980; Savio et al., 1990). Follicular waves can be defined as a sequence of events related to follicle development and is categorized into 4 stages: 1) recruitment; 2) selection and deviation; 3) dominance; and, 4) atresia. The inhibitory or stimulatory hormonal stimulus dictates the fate of the follicles.

The ovary is colonized by several primordial follicles, defined as an oocyte surrounded by a single layer of squamous cells arrested at the diplotene stage of prophase-I of meiosis, but originating from an oogonia during embryonic development (Adams et al., 2008). A few primordial follicles are activated, independently of gonadotropins, by follicular factors such as insulin, kit ligand, testosterone, fibroblast growth factor-7, and vascular endothelium growth factor (Fortune et al., 2000). The activation leads to an increase in mitotic division of the granulosa cells and differentiation into primary and secondary follicles (several layers of cuboidal pre-granulosa cells). The secondary follicles differentiate into tertiary or Graafian follicles that are characterized by granulosa cell proliferation and antrum formation (Fortune et al., 2000). The development of the antral follicle is further stimulated by gonadotropins.

The follicular wave is initiated by a surge of FSH, which stimulates the growth of multiple small follicles (< 3 mm of diameter; Ireland, 1987). The growing follicles progress through a selection process until deviation, where the growth rate of the two largest follicles becomes evident (Ireland et al., 1987). The selected follicle becomes dominant and continues to grow under LH stimulation (Prendiville et al., 1996). A follicle becomes dominant because its greater concentration of LH receptors (Xu et al., 1995; Bao and Garverick, 1998), greater steroidogenic capacity and insulin-like growth factor (IGF-I) concentration, and decreased concentrations of specific IGF-I binding proteins

(IGFBP) compared to subordinate follicles (Gerard et al., 2002; Fortune, et al., 2004). The DF synthesizes and secretes E (Butler et al., 1983; Price and Webb, 1988) and inhibin (Ireland et al., 1983; Kaneko et al., 1997). Both hormones are released into blood circulation reaching the hypothalamus and pituitary causing a negative feedback on GnRH. The negative feedback modifies GnRH secretion patterns decreasing FSH supply, blocking the selection of other follicles (Bao and Garverick, 1998). The estradiol secreted by the DF acts as a self-stimulator promoting self-growth by increasing LH pulsatility. The fate of the DF depends on the concentration of P in the blood. Two outcomes may occur with the DF: 1) when there are high concentrations of P and E the DF becomes atretic and dies; and 2) when P is low and E is high the DF ovulates and its cells become luteinized (Stumpf et al., 1991). In either case, a new follicular wave is initiated.

Phases of the estrous cycle

The bovine estrous cycle may be defined as the interval between two consecutive follicular ovulations and may be divided into four distinct phases: 1) metestrus; 2) diestrus; 3) proestrus; and 4) estrus (Senger, 2011). Each phase is represented by different stages of follicular waves and differences in concentrations of hormones. Follicular waves are influenced by the estrus phase and the fate of the DF is dependent on the phase of the estrous cycle at the moment of dominance (Senger, 2011).

Metestrus is the phase of the estrous cycle that occurs immediately after ovulation. The absence of large follicles following ovulation and the initiation of luteinization process of the follicular cells characterize this phase as having low E and low P. Metestrus extends for a period of 3 to 5 d days after ovulation. Therefore, metestrus coincides with the follicular phase during the period from selection to deviation of the

first follicular wave and the early development of the CL following ovulation (Senger, 2011).

Diestrus is initiated after metestrus and extends as long as the CL is active on the ovary synthesizing P. Diestrus is defined as the period when P reaches concentrations exceeding 1 ng/mL and it ends when P begins to decline. Diestrus usually begins on d 4 to 5 after ovulation and ends between d 15 and 18 of the estrous cycle. As many as 2 to 3 follicular waves may occur during diestrus (Senger, 2011). The high steroidogenic capacity of the DF and the synthesis of P by the CL characterize diestrus as having high P and sporadically elevated E (depending on the presence or absence of a DF).

Proestrus is initiated after diestrus between d 16 and 18 of the estrous cycle. Pregnancy recognition usually occurs in the presence of a viable conceptus between d 15 and 18 of the estrous cycle. The absence of the conceptus or the lack of pregnancy recognition enables the uterine endometrium to secrete PG causing lysis of the CL and initiation of proestrus (Senger, 2011). At this point, either a large follicle is present or a new follicle has been selected and deviated to become dominant; therefore, proestrus is characterized by decreasing concentrations of P and increasing concentrations of E. The proestrus phase extends for 2 to 3 days and ends when the estrus is initiated.

Estrus is the period of the estrous cycle when the female initiates sexual receptiveness (stands to be mounted) and is characterized by low P and high E. Increasing E synthesis of the growing DF stimulates frequency of pulses of LH, inducing an LH surge and ovulation. The high E concentrations in blood stimulate estrus behavior. The estrus phase usually lasts 8 to 24 hours in cattle (Senger, 2011).

Hormonal manipulation of the estrous cycle

Numerous laboratories have isolated natural hormones from live animals and/or synthesized artificial hormones for manipulation of the estrous cycle, such as GnRH, FSH, LH, PG, and progestins. With an enhanced understanding of the natural dynamics of the estrous cycle and the availability of these hormones, it is now possible to manipulate the estrous cycle and schedule the exact time and day of artificial insemination (TAI). An estrous synchronization program facilitates improved control of mating sires with dam, improvement in genetics of the herd, and enhances herd homogeneity (Lamb et al., 2010). However, estrous synchronization may be costly, labor intensive, time consuming, and in some cases inefficient (Johnson and Jones 2008).

The most common reproductive management tool for breeding cows on a cow/calf operation is natural mating using live bulls. Using a natural mating system, the control of the day of conception is difficult to determine. Accuracy of determining day of conception may be improved by using a program involving frequent pregnancy diagnosis using ultrasound or rectal palpation (Beal et al., 1992). Variables such as cow-to-bull ratio, number of cows in the operation, and cost of the supplies and hormones need to be considered when electing to utilize natural mating (Johnson and Jones, 2008). With the advance in development of frozen-thawed semen technology, it has become possible to AI cows and decrease the number of bulls utilized during the breeding season (Johnson and Jones, 2008).

Initial AI programs relied on detection of estrus two or more times per day with AI performed 12 to 16 h after initiation of estrus (AM/PM rule; Trimberger and Davis, 1943). However, this type of management without estrous synchronization is labor

intensive and relies on a trained AI technician, facilities in good working condition, and conducive environmental conditions. Therefore, AI alone may be inefficient in many beef production systems (Johnson and Jones, 2008).

In an attempt to reduce or eliminate the detection of estrus and to minimize labor and time associated with AI, researchers have developed estrus or ovulation control programs.

One of the first attempts to synchronize estrus and evaluate fertility occurred when a single injection of 30 mg of PG was administered to heifers (Lauderdale et al., 1974). Heifers were detected for estrus and AI was performed 12 h after observed estrus. Conception rates (percentage of females pregnant compared to females inseminated) did not differ between control females (21 d of detection of estrus and AI) and treated females receiving a single injection of PG (53.3 vs. 52.2%). However, the luteolytic effect of PG is only effective during diestrus when the CL is present and responsive to PG, therefore a certain percentage of females (those in anestrus or prepubertal) do not respond to PG treatment (Wiltbank et al., 1995; Weems et al., 2006). During proestrus and estrus, a natural luteolysis occurs; therefore, treatment with exogenous PG has synergistic action with the physiological PG that is synthesized by the uterus. According to Lucy et al. (1992) in a group of cyclic cows, 67% were between d 7 and 20 of the estrous cycle and responsive to PG. Moreover, no estrus response would be observed if a treatment of PG were administered to a group of cows in anestrous, because of the absence of a CL (Macmillan and Henderson, 1983).

Earlier studies observed that treatment with PG did not induce luteolysis in cows during metestrus (Lauderdale et al., 1974; Cooper and Rowson, 1975; Lauderdale,

1975). Recent studies evaluated the refractoriness of the young CL by the luteolytic action of PG and observed that administering PG during metestrus decreases the expression of vasoactive mediator of luteolysis, whereas it stimulates angiogenic factors and the IGF-I system (Wiltbank et al., 1995). It was observed that administration of PG on d 4 of the estrous cycle had no effect in P concentration in the blood (Braun et al., 1988). In addition, luteolysis and pregnancy rates are enhanced when administration of PG on d 5 of the estrous cycle is performed using 2 doses with at least a 6 h interval between PG injections (Kasimanickam et al., 2009; Peterson et al., 2011). Nevertheless, synchronization of estrus in cows with a single injection of PG requires extensive visual detection of estrus prior to AI and the luteolytic action of the PG is restricted to cows during diestrus.

Studies have demonstrated that blood concentrations of LH increase following administration of GnRH (Carter et al., 1980; Zaied et al., 1980; Williams et al., 1982). Administration of GnRH induces an LH surge causing ovulation of the DF within 24 to 32 h (Pursley et al., 1995). Therefore, a combination of GnRH plus PG was hypothesized to be an effective strategy to synchronize the follicular wave. An injection of GnRH 7 d before PG is capable of ovulating follicles greater than 10 mm in diameter that are responsive to LH (Pursley et al., 1995), inducing the subsequent development of a new CL and initiation of a new follicular wave within 2 d after GnRH injection. The new CL is regressed 5 to 7 d following ovulation if the cows are treated with PG. The absence of the CL after luteolysis and low P concentration enable the growing follicle to ovulate (Pursley et al., 1995). However, a drawback of such a protocol is the necessity for detection of estrus.

In an attempt to eliminate detection of estrus, a second injection of GnRH 48 h after PG was applied to induce the ovulation of the DF (Pursley et al., 1995). With the second injection of GnRH and induction of ovulation, TAI may be performed (Ovsynch; Pursley et al., 1995). Therefore, complete estrous synchronization relies on a follicle to ovulate to the first GnRH, luteolysis of the CL, and a second ovulation to the second GnRH. However, the response to the first injection of GnRH was 54% in dairy cows (Bello et al., 2006) and 31% in anestrous beef cows (Geary et al., 2001b) and varies according to the day of the estrous cycle (Price and Webb, 1989; Roche et al. 1992; Vasconcelos et al., 1999; Atkins et al., 2008), diameter of the DF (Roche et al., 1992), and stage of follicular development (Pursley et al. 1995; Roche et al., 1992). Cows that failed to respond to the first GnRH have poorer synchrony of an emerging follicular wave resulting in poorer fertility than cows that responded to GnRH (Vasconcelos et al., 1999). In the Ovsynch protocol (Pursley et al., 1995), the first GnRH injection is administered to induce ovulation of the DF, resetting the follicular wave; however, when ovulation fails, the follicle may ovulate at unexpected intervals prior to AI (Lamb et al., 2001; Kojima et al., 2003). When cows ovulate prior to AI the opportunity for successful fertilization is impaired.

To prevent ovulations from occurring prior to administration of PG inclusion of an intra-vaginal slow release P device has been used between the first injection of GnRH and PG (Lamb et. al., 2001, 2006; Larson et al., 2006; Bridges et al., 2008; Busch et al., 2008). These researchers evaluated the inclusion of the P releasing device into the existing estrous synchronization protocols or used the device as a new tool to develop new estrous synchronization programs. The inhibition of follicular ovulation is caused by

the increase in P with the insertion of the device, ultimately preventing a spontaneous LH surge. Conception rates comparing protocols with or without exogenous sources of P resulted in fertility that was improved when the device was applied (Lamb et al., 2001, 2006; Larson et al., 2006). In addition, conception rates of anestrus cows synchronized with the P device was similar to cyclic cows (Stevenson et al., 2000; Lamb et al., 2001; Busch et al., 2008).

The first ovulation in prepubertal heifers and postpartum cows frequently occurs in the absence of estrus expression (Perry et al., 1991). In addition, the duration of the CL life span from first ovulation follicles tends to be shorter compared to CL from subsequent ovulations (Werth et al., 1996; Sá Filho et al., 2009b). Consequently, early embryo mortality occurs due to premature regression of the CL before maternal recognition (Werth et al., 1996). It has been observed that pre-exposure of the hypothalamus to P prior to first ovulation reduces the occurrence of short cycles, resulting in the formation of a CL with normal life span (Werth et al., 1996). The overall improvement in conception rates using a P device between first GnRH and PG may be associated with the prevention of early ovulations prior to TAI and reducing the incidence of short cycles (Ramirez-Godinez et al., 1981; Fike et al., 1997).

Hypothalamic exposure to P appears to be necessary for the E-induced LH surge. In an attempt to induce follicular cysts in dairy cows, Gumen and Wiltbank (2002) demonstrated that the LH surge was induced by administration of E only when the hypothalamus had previously been exposed to P. However, the LH surge was induced in all cows by a GnRH injection, regardless of previous exposure to P. These data indicate that P was necessary to prime the hypothalamic neurons for the LH surge to be

induced by E, however the pituitary response to gonadotropins was not affected in the absence of P. The mechanism that explains the action of the P on the hypothalamus remains uncertain; however, there is an indication that P increases E receptor (ER-α) expression in hypothalamic neurons in rats (Simerly et al., 1996) and ewes (Blache et al., 1994; Caraty and Skinner, 1999). More recent studies demonstrated that kisspeptin neurons in the AVPV and ARC nucleus of rats express both the ER-α and PR on their membrane and the percentage of colocalization of the receptors and the kisspeptin neurons are greater in the AVPV (Smith et al., 2005, 2006; Clarkson et al., 2008, Clarkson and Herbison, 2009). Previously, we discussed that KiSS-1 neurons in the AVPV nucleus in rats and in the ARC nucleus in ewes was related to the positive feedback of E, stimulating GnRH secretion and inducing an LH surge (Estrada et al., 2006; Wintermantel et al., 2006). In addition, PR was up-regulated by E and was required for stimulation of the LH surge and ovulation in mice (Moffatt et al., 1998). Moreover, treatment with both E and P enhance kisspeptin-induced LH release in female ovariectomized rats (Roa et al., 2006).

The action of P is also important in the ovary and fertility may be compromised in the absence of intrafollicular P or PR (Loutradis et al., 1991). At ovulation, P acts specifically during follicular rupture, but follicle maturation and luteinization appear not to be affected (Robker et al., 2000). The signaling of P is essential for ovulation (Conneely, 2010), and the administration of P antagonists after the LH surge inhibited ovulation in mice (Loutradis et al., 1991).

The LH binds to mural granulosa cells (MGC) following the preovulatory surge of LH inducing gene expression required for cumulus cell (cells that surround the oocyte)

expansion, release of the oocyte and luteinization (Conneely, 2010). Cumulus cells and the oocyte do not express LH receptors; therefore the signal from LH to stimulate gene expression depends on the paracrine (cell to cell) signal (Kim et al., 2009; Conneely, 2010). The apparent lack of PR in PR-deficient females impairs pore formation in the apical follicle wall and the oocyte is not expelled, remaining trapped in the follicle (Robker et al., 2000). The LH surge induces a transient rise in PR mRNA in MGC and the paracrine signal via PR may be essential for follicle rupture and ovulation (Natraj and Richards, 1993; Robker et al., 2000; Russell and Robker, 2007). The expression of endotelin-2 (ET-2), a potent vasoactive molecule, and the epidermal growth factor (EGF) family, that regulates cumulus cell expansion, are both stimulated by LH and are required for ovulation. In addition, ET-2 and EGF are down-regulated in the follicle of PR-deficient females, indicating that their expression downstream of LH likely is co-regulated by PR signaling (Park et al., 2004; Palanisamy et al., 2006).

High concentrations of P inhibits GnRH and LH pulses; however, low concentrations of P has the opposite effect, stimulating follicle growth by increasing LH pulsatility (Adams et al., 1992; Bergfeld et al., 1996), and concentrations of E in blood (Ireland and Roche, 1982). Increasing E secretion and stimulation of LH pulses may be beneficial to follicle development, unless it is present for extended periods of time (Savio et al., 1993; Anderson and Day, 1994). The continued growth of follicles in the absence of follicle turnover or ovulation results in a persistent follicle. Fertility of a persistent follicle is decreased as a result of poor oocyte quality (Savio et al., 1993; Mihm et al., 1994; Stock and Fortune, 1993; Wehrman et al., 1993). Follicles that fail to ovulate to the first GnRH in the 7-d CO-Synch + CIDR protocol (Lamb et al., 2006;

Larson et al., 2006) may become persistent during the 7 d period in which the CIDR is present, thereby lowering the fertility to TAI.

The proestrus phase in the 7-d CoSynch + CIDR protocol may be defined as the interval from the administration of PG (initiation of decreasing P) to the second injection of GnRH (initiating the LH surge), which may have a duration of 60 to 72 h (Lamb et al., 2001; Larson et al., 2006; Bridges et al., 2008). Follicular steroidogenic activity is positively and highly correlated with growth of the DF, likelihood of subsequent ovulation, and oocyte quality (Tarlatzis et al., 1993; Revelli et al., 2009). Estrogen-active follicles have greater concentrations of E and a greater E:P ratio in follicular fluid compared to estrogen-inactive follicle (Ireland and Roche, 1982, 1983). It has been demonstrated that the induction of ovulation of smaller follicles (11.5 ± 0.2 vs. 14.5 ± 0.4 mm) with GnRH resulted in formation of smaller CL and lower concentrations of P on d 7 and 14 after ovulation (Vasconcelos et al., 1999). Moreover, P from d 3 to 15 after ovulation was decreased in cows induced to ovulate small follicles (11 mm) compared to cows that had spontaneously ovulating follicles (Busch et al., 2008). These data indicate that steroidogenic activity and the natural LH surge was more critical than the diameter of the dominant follicle for subsequent development of a normal CL.

For many years researchers have evaluated strategies to improve follicle development during proestrus with gonadotropin hormones, such as equine chorionic gonadotropin (eCG; Duffy et al., 2004; Peres et al., 2009; Pinheiro et al., 2009; Souza et al., 2009; Small et al., 2009; Sá Filho et al., 2010; Pegorer et al., 2011; Sales et al., 2011) or steroid hormones, such as E (Jacobs et al., 1988; Stumpf et al., 1991; Sá Filho et al., 2009a,b). More recently, research has focused on increasing the length of the

proestrus in estrous synchronization protocols from 66 h (7-d CoSynch + CIDR) to 72 h (5 d CoSynch + CIDR) attempting to improve fertility (Bridges et al., 2008; Busch et al., 2008; Wilson et al., 2010).

Equine chorionic gonadotropin (eCG) and its effect on follicle development

The embryonic allantochorionic girdle cells in the endometrial cups of a pregnant mare synthesize pregnant mare serum gonadotropin (PMSG) or equine chorionic gonadotropin (eCG), a glycoprotein hormone (Allen and Moor, 1972). The eCG molecule, like other gonadotropins (FSH and LH), is composed of two subunits; α and β subunit (Pierce and Parson, 1981). Equine chorionic gonadotropin can bind to both FSH and LH receptors in follicular cells of species other than the equine, where eCG only has LH-like activity (Stewart et al., 1976; Combarnous et al., 1978). The first increase in concentrations of P in mares is due to luteinization of the ovulary follicle following ovulation. A second increase in P is observed at approximately d 37 of gestation, 2 d after first detection of eCG in the blood (Allen and Moor, 1972). Concentrations of eCG are first detected at approximately d 35 of gestation and decrease at approximately d 120 (Allen and Moor, 1972). Equine chorionic gonadotropin induces the formation of multiple accessory CL at ≈ d 40 of gestation. Therefore, eCG has lutetrophic action because the second elevation in P has been associated with an increase in diameter of the original CL stimulated by eCG on d 37 (Wilsher and Allen, 2009).

Forty-one percent of the eCG molecule is comprised of carbohydrates, with the glycans terminated by sialic acids, thus eCG has a prolonged in vivo half-life exceeding 60 h in ewes (McIntosh et al., 1975; Wehbi et al., 2010). Because of the ability of eCG to bind to FSH and LH receptors in follicular cells (Combarnous et a., 19780) and its prolonged half-life, the hormonal treatment of eCG has been used to increase the

number of ovulatory follicles (Nasser et al., 2004; Baruselli et al., 2011; Sudano et al., 2011) as well as to improve growth of DF prior to ovulation in cattle (Duffy et al., 2004; Peres et al., 2009; Pinheiro et al., 2009; Souza et al., 2009; Small et al., 2009; Sá Filho et al., 2010; Pegorer et al., 2011; Sales et al., 2011) and ewes (Roy et al., 1999).

An immunogenic response may be induced in cows receiving eCG, which may decrease the ability for eCG to bind to the receptor, resulting in a compromise in efficacy when administered repeatedly (Roy et al., 1999). In contrast, recent research demonstrated that some specific complex eCG/anti-eCG antibodies form following eCG treatment, enhancing cAMP production in *in vitro* cultured granulosa cells, which indicates a potential positive effect on follicle development (Wehbi et al., 2010). Moreover, steroidogenic capacity of granulosa cells assessed by ERK1/2 MAPK phosphorylation (steroidogenesis is regulated by ERK MAPK activation) was increased when treated with eCG and specific eCG/anti-eCG antibody complex, but not with the antibody itself (Wehbi et al., 2010). Nevertheless, several *in vivo* studies demonstrated that administration of eCG causes multiple ovulations when injected in the absence of a DF (Duffy et al., 2004; Sales et al., 2011) or stimulates DF growth prior to ovulation (Baruselli et al., 2004; Small et al., 2009; Meneghetti et al., 2009; Sá Filho et al., 2010). In addition, the response to eCG treatment appeared to be exacerbated in low body condition score (Bó et al., 2007; Souza et al., 2009) and anestrous (Baruselli et al., 2004) cows. Taken together, these data indicate that eCG, although inducing an immune response, may be beneficial in estrous synchronization protocols to improve fertility in cattle.

Interaction of cow/calf bond and fertility

Resumption of postpartum estrous cycles relies on increasing LH pulse frequency and overall concentration of LH in blood and the increasing LH secretion stimulates follicle growth and ovulation (Yavas and Walton, 2000). The development of the CL characterizes cows that have resumed postpartum estrous cycles. Several factors such as body condition score (Rutter and Randel, 1984), energy restriction (Dunn and Kaltenbach, 1980), dystocia (Nakao et al., 1997), and diseases (Curtis et al., 1983; Rutigliano et al., 2008) can contribute to decrease or increase in the interval from calving to the first postpartum ovulation. In some species, such as swine, suckling stimulation completely blocks resumption of estrous cycles (Armtrong et al., 1979; De Rensis et al., 1993; Gerritsen et al., 2008), whereas in other species, such as the bovine, suckling stimulation merely delays resumption of estrous cycles (Williams and Griffith, 1995). In cattle, not only the physical suckling stimulation but also the maternal bond between the cow and the newborn, influences fertility. In suckled beef cows, the mean interval from parturition to the resumption of estrous cycles ranges from 29 to 67 d, however it might be extended in certain situations such as when cattle do not receive adequate feed resources (Yavas and Walton, 2000)

Postpartum anestrous is the major contributor to infertility in cattle (Short et al., 1990). The resumption of cyclicity earlier in the postpartum period increases the number of estrous cycles and the chances of a cow to become pregnant during the breeding season. Reducing the postpartum interval may be accomplished by managing nutrition, BCS, disease, and the suckling interaction between cow and calf (Crowe, 2008).

Cows nursing their suckling calves experienced longer intervals to first postpartum estrus than cows whose calves were weaned (Graves et al., 1968; Wagner and

Oxenreider, 1971). Tactile, olfactory, auditory, and vision of a cow with her calf are negatively correlated with number and frequency of LH pulses (Williams and Griffith, 1995; Lamb et al., 1997). Interestingly, not only cows nursing their own calves but also cows nursing an alien calf (unrelated calf) had a delayed postpartum interval 14 to 38 d longer than cows whose calves were weaned (Lamb et al., 1997). However, restricted physical contact by only head and neck between a cow and her own calf was not sufficient to delay the postpartum interval to first ovulation (Lamb et al., 1997). It was observed that frequency of pulses and overall blood concentrations of LH were increased in cows whose calves were weaned or suckled by an alien calf; however, no alterations were observed in cows suckled by their own calf (Silveira et al., 1993). In addition, the authors observed that at 10 d after initiation of treatments (treatments initiated on d 16 postpartum) a greater proportion of cows in the alien (72%) and weaned (75%) treatments had CL activity compared to cows that had ad libitum suckling by their own calves (13%).

The suckling effect by a cow's own calf on days to first postpartum estrus was assessed in cows that received ad libitum suckling, cows that were restricted to once-daily suckling, and unlimited restricted contact (head and neck only), or cows restricted to once-daily suckling and no additional contact, starting at 30 d postpartum (Stagg et al., 1998). The results indicated that days from calving to first estrus were reduced in cows that were restricted to once-daily suckling and no additional contact (51 d) compared to cows restricted to once-daily suckling with unlimited restricted contact (62 d), and cows with ad libitum suckling (79 d). The postpartum interval in Brahman × Hereford first parity cows was greater for those cows exposed to ad libitum suckling

(168 d) than those restricted to once-daily suckling (69 d; Randel, 1981). Once-daily (81%) and twice-daily (84%) suckling during 24 d, initiated 11 d before the breeding season (BS), increased the percent of cows exhibiting estrus during the first 3 wk after the initiation of treatment compared to those receiving ad libitum suckling (64%; Odde et al., 1986).

There is strong evidence that the presence of a calf and suckling stimulation affect fertility by delaying the interval from calving to initiation of estrous cycles. However, management of suckling may partially overcome the negative effects of ad libitum suckling and reduce the interval to initiation of postpartum estrous cycles. The overall effect of suckling stimulation may be attributed to three distinct factors: 1) the physical presence of the calf; 2) physical stimulation of the mammary gland; and, 3) removal of milk, which alters the total daily energy requirement of the cow; however, milk removal itself with adequate nutrition does not delay the postpartum interval to first ovulation (Lamb et al., 1999).

Relationship between milk production and LH secretion

The shape of the lactation curve differs between dairy and beef cows. In dairy cows, milk production increases from d 1 to 60 after parturition, followed by a gradual decrease (Bauman et al., 1985). Alternatively, in beef cows, there is a linear decrease from parturition to the end of lactation (Gaskins and Anderson, 1980). Removal of milk from the mammary gland stimulates additional milk synthesis (Wilde et al., 1999) that increases the overall requirement of daily energy necessary for maintenance and lactation (Bauman and Currie, 1980). The increase in energy required for lactation results in an increase in dry matter intake or mobilization of body energy reserves (such as body fat) in order to supply energy to the tissues of the body (Bauman and Currie,

1980; Taylor et al., 2004). In high producing dairy cows, feed consumed is unable to supply the total demand of energy utilized for milk synthesis and maintenance of tissues, consequently body energy reserves, mostly from fat tissue mobilization, are utilized resulting in decreasing BCS (Bauman and Currie 1980; Tamminga et al., 1997). Mobilization of fat reserves in beef cows also occurs, but with less severity than dairy cows due to lower milk production.

Body condition score during the peripartum period also affects fertility. Cows calving in poor BCS experience longer postpartum intervals to first estrus than those cows calving in moderate to good BCS (Rutter and Randel, 1984). The quantity of fat tissue is an indicator of BCS and energy reserves, but is not only an energy source tissue but also an important endocrine organ that synthesizes several hormones (Kershaw and Flier, 2004). Leptin is synthesized mainly by white adipose tissue, commonly referred to as fat tissue (Friedman and Halaas, 1998; Ahima and Flier, 2000). Leptin regulates body energy metabolism and food intake (Coleman et al., 1978). Concentration of leptin in the blood varies depending on the needs of energy on tissues of the body. High concentrations of leptin stimulate the central nervous system stimulating the sense of satiety (Brogan et al., 1999**)**. Leptin inhibits the hunger stimulator neuropeptide Y (NPY) located in the hypothalamus; therefore, high leptin causes a decrease in feed intake and increase energy expenditures.

In cows during periods of positive energy balance (later stages of lactation), concentrations of leptin in blood is greater than in cows during early stages of lactation, when cows are experiencing a negative energy balance (Kadokawa et al., 2000; Sadri et al., 2011). The low concentrations of leptin stimulate appetite and feed intake in order

to acquire nutrients necessary for the metabolism (Barb et al., 2001a,b). In addition, concentrations of leptin in the blood is also correlated with gonadotropin secretion, therefore hormones controlling reproduction may be linked by nutritional status via leptin (Barb et al., 2001a,b).

Concentrations of leptin are positively correlated with amplitude (r = 0.73) and frequency (r = 0.53) of LH pulses (Kadokawa et al., 2006). Abnormalities in the leptin receptor have been related to obesity and infertility in rats and humans (Barash et al., 1996; Sone and Osamura, 2001). The interval from parturition to first ovulation was positively correlated (r = 0.83) with the leptin nadir, indicating that a delay in the rise of leptin in blood is associated with an increase in the interval to first ovulation (Kadokawa et al., 2000). Early lactation females have low concentrations of leptin compared to non-lactating females and may be one link between negative energy balance and low concentrations of LH (Kadokawa et al., 2000).

Using rats as a model, Brogan et al. (1999) evaluated the effect of suckling stimulation and milk removal from the mammary gland on concentrations of leptin and patterns of LH pulses in the blood. Rats suckled by their own pups (resulting in milk production) had a decrease in concentrations of leptin in the blood. In contrast, rats suckled by alien pups (resulting in no milk production) experienced an increase in leptin in the blood. Interestingly, frequency, but not amplitude, of LH pulses decreased in rats suckled by their own or alien pups. These results indicate that milk removal (suckled by own pups) decreased concentrations of leptin, and that physical suckling stimulation, even with elevated concentration of leptin (suckled by alien pups) was able to decrease frequency of LH pulses (Brogan et al., 1999).

The effect of frequency, method of milk removal (machine or own calf), and calf presence on the postpartum interval from calving to first ovulation was evaluated in beef cows. Postpartum interval was extended when milk was removed by a calf suckling 2× daily or ad libitum, but not by machine milking 2× or 5× daily even with their own calf present with restricted contact (Lamb et al., 1999). In addition, presence of a muzzled calf (not suckling) or chronic hand milking (8× daily) was not able to suppress the elevation in concentrations of LH on d 19 postpartum, whereas concentrations of LH was suppressed in cows suckled by a calf (Williams et al., 1987).

In postpartum mastectomized cows (no milk production) whose calves were weaned at 12 h after parturition or mastectomized cows with ad libitum “suckling” (calves suckled the inguinal area where the mammary gland was located) by their calves, no cows “suckled” by calves had ovulated by d 49 postpartum, but all cows without calves ovulated between d 14 and 22 postpartum (average of 16 d; Viker et al., 1989). This experiment was repeated with the addition of two additional treatments that included intact suckled cows (producing milk) with ad libitum suckling and an additional treatment with mastectomized cows that had restricted contact (head and neck only) with their own calf (Viker et al., 1993). Mastectomized and intact cows with ad libitum suckling had delayed intervals to first ovulation compared to the mastectomized cows that had their calves weaned or mastectomized cows with restricted access to their calves (30. 29, 15 and 17 d of delay in first ovulation after initiation of treatment, for postpartum mastectomized ad libitum “suckling”, intact ad libitum suckling, mastectomized restricted contact, and mastectomized weaned treatments, respectively).

Collectively, these data indicate that presence of a suckling calf or nonsuckling calf with or without milk removal may prolong the interval from parturition to first ovulation in beef cows. The physiological mechanisms associated with physical suckling stimulation on LH secretion is still not well understood; however, reports have indicated that the release of endogenous opioids such as β-endorphin (Gordon et al., 1987) and leptin (Brogan et al., 1999) may alter neuropeptides while suckling and regulate LH secretion.

Suckling and its physiological effect on LH secretion

The resumption of cyclicity is dependent on LH accumulation in the pituitary after parturition and is influenced by intermittent secretion of GnRH from the hypothalamus (Short et al., 1972; Butler and Smith, 1989; Short et al., 1990). The storage of LH in the pituitary is depleted by parturition due to high concentrations of steroid hormones (mainly E) released during parturition (Chamley et al., 1974; Azzazi et al., 1983). In addition, the release of LH after administration of GnRH is lower on d 3 and 10 compared to d 20, 30, and 40 days postpartum (Fernandes et al., 1978), and an increase in LH release after GnRH injection was correlated with an increase in days postpartum in both suckled (r = 0.73) and nonsuckled (r = .61) cows (reviewed by Williams, 1990).

The restoration of LH to normal concentrations in the pituitary occurs within 3 to 4 wk postpartum in beef cows (Riley et al., 1981; Moss et al., 1985); however, the quantity of LH released by the pituitary remains suboptimal to promote ovulation (Yavas and Wallon, 2000). The secretion of LH is directly dependent on stimulation by GnRH from the hypothalamus and the content of GnRH in the hypothalamus appears to remain constant during the postpartum period in cows (Nett et al., 1988) and in sheep (Crowder et al., 1982). Therefore, low release of LH during the early postpartum period is not due

to the lack of hypothalamic GnRH content but appears to be associated with low stores of LH stored in the pituitary or the low supply of GnRH peptides reaching the pituitary.

Several observations demonstrate that suckling stimulation inhibits GnRH release and decreases LH secretion in rats (Brogan et al., 1999), ewes (Malven and Hudgens, 1987), pigs (Cox and Britt, 1982; De Rensis et al., 1993), and cows (Myers et al., 1989; Rund et al., 1989). The discovery of a relationship between EOP such as morphine and β-endorphin and LH secretion has generated the hypothesis that suckling stimulates EOP secretion, which negatively affects LH release. Endogenous opioids are peptides that work as neurotransmitters in the brain. The opioid β-endorphin is a cleavage product of proopiomelanocortin (POMC), which also is the precursor to adrenocorticotrophic hormone (ACTH) and a-melanocyte-stimulating hormone (a-MSH; Sarkar et al., 2008). β-endorphin neurons have been identified throughout the central nervous system (CNS) including the paraventricular nucleus (PVN) and arcuate nucleus (ARC; Akil et al., 1984; Sakar et al., 2008). In the PVN, β-endorphin neurons innervate corticotropic-releasing hormone (CRH) neurons important during stress (Kawano and Masuko, 2000). During stress CRH stimulates the release of ACTH, which stimulates cortisol secretion from the adrenal gland, elevating cortisol in the blood (Kawano and Maksuko et al., 2000). Simultaneously, β-endorphins also are synthesized and released to inhibit the secretion of CRH to minimize the stimulation of cortisol (Kawano and Maksuko et al., 2000).

The ARC nucleus is highly populated by β-endorphin neurons, from which the axons of these neurons extend to the POA region of the hypothalamus (Bakkali-Kassemi et al., 2011). The POA is the primary region innervated by GnRH neurons

(Estrada et al., 2006). An early study demonstrated that an injection of morphine inhibited LH secretion (Barraclough and Sawyer, 1955). Subsequently, several researchers demonstrated that the inhibition might be reversed by injection of opioid antagonists such as Nalaxone (Packman and Rothchild, 1976). An injection of β-endorphin to the third ventricular region blocked the LH surge and ovulation, but did not block LH release in response to an LH releasing hormone (LH-RH) injection (Kubo et al., 1983) indicating that β-endorphin acts on the suprahypophyseal area and not on the pituitary. In addition, injection of β-endorphin at the third ventricle did not inhibit ovulation when the median pre-optic area (MPO) and median eminence area (ME) were electrochemically stimulated (Kubo et al., 1983). Moreover, the release of β-endorphin in the hypophyseal portal blood vessels (vessels connecting the hypothalamus and pituitary) of ewes, increased in response to suckling bunts (Gordon et al., 1987).

Injection of opioid antagonists, Nalaxone or Nalmefene, increased secretion of LH during nonsuckling and suckling stimulation and blocked the negative effects of morphine on LH secretion (Whisnant et al., 1986ab; Malven and Hudgens, 1987; Myers et al., 1989). However, the response of LH secretion to Naloxone administration depends on the dose and the interval postpartum when Nalaxone is administered (Whisnant et al., 1986a). Injection of 200 mg of Nalaxone increased LH secretion at 42 d postpartum, but did not have an effect when a similar dose was injected on d 14 and 28 postpartum (Whisnant et al., 1986a). A greater dose (400 or 800 mg) of Nalaxone also was necessary to increase concentrations of LH at 14 d compared to 28 d postpartum (Whisnant et al., 1986a). The authors concluded that the negative effect of EOP on LH secretion differed depending on the postpartum interval.

Temporary CR increased LH secretion and LH pulse frequency remained high for up to 144 h after calf removal, but returned to baseline concentrations when cows were suckled (Edwards, 1985; Shively and Williams, 1989). When calves were removed from cows 48 h before Nalaxone injection, calf removal alone was able to increase LH secretion with no additional increase in LH pulses with the additional injection of Nalaxone (Whisnant et al., 1986a).

The effect of leptin and β-endorphin on GnRH neurons appears to be interrelated with other factors, such as other neurons and others peptides. Leptin decreases during early lactation increasing appetite and voluntary feeding intake (Bauman and Currie, 1980; Bell, 1995; Xu et al., 2009). In addition, concentrations of insulin declined during early lactation, decreasing glucose uptake by peripheral tissues and increasing the availability of glucose for the mammary gland where uptake is independent of insulin (Bauman and Currie, 1980; Bell and Bauman, 1997; Xu et al., 2009). Declining concentrations of leptin decreases the inhibition of orexigenic (appetite stimulator) neuropeptides such as NPY, agouti related protein (AGRP), melanin concentrating hormone (MCH) and orexin-A (OX), stimulating appetite and hyperphagia (Xu et al., 2009). Stimulation of NPY neurons by decreasing leptin results in inhibition of GnRH secretion (Smith et al., 2010). This evidence can be enforced by the observation that the cell body of NPY neurons is located at POA and ME where high concentrations of GnRH neurons are located (Smith et el., 2010).

The neuropeptide Y and MCH regulates KiSS-1 neurons, the upstream regulator of GnRH secretion. Cell bodies of GnRH neurons coexpress most of the orexigenic neuropeptide receptors (Campbell et al., 2003). Increased concentrations of NPY in the

ARC and neuropeptide MCH in the POA during lactation inhibit GnRH secretion, thereby indicating a potential link among the state of energy balance, food intake, and fertility (Xu et al., 2009). Interestingly, restoration of leptin and insulin to normal concentrations decreases NPY expression in the ARC but not in dorsomedial hypothalamus in rats (DMH; Xu et al., 2009). Reports are contradictory on the ability of postpartum restoration of leptin to block the inhibitory effects of negative energy balance on GnRH and LH secretion. Administration of leptin did not restore serum LH concentrations in blood during early lactation (negative energy balance state) in rats (Xu et al., 2009). In contrast, there is evidence that administration of leptin increases kisspeptin release, consequently GnRH and LH release, in feed restricted rats (Castellano et al., 2009) and also increases LH pulse frequency in fasted male sheep (Nagatani et al., 2000). In addition, concentrations of leptin were not altered during breast-feeding in women and the length of lactational amenorrhea (absence of menstrual period during pregnancy or lactation) was related to the intensity of suckling (McNeilly, 2001). The combined effects of suckling stimulation and low concentrations of leptin during lactation confound the ability to assess their individual contributions to altering LH secretion. A recent report (Donato et al., 2009), indicated that the hypothalamic ventral premammillary nuclei (PMV) expressed high concentrations of leptin receptors and was connected with important areas related to GnRH secretion such as the POA, ARC, and AVPV. Lesions in this area of the CNS blunt the increase in LH of fasting animals when injected with leptin.

The primary stimulator of the GnRH neuron is kisspeptin and during lactation injection of kisspeptin stimulates LH secretion (Yamada et al., 2007). However, the

regulation of kisspeptin release during lactation is complex and not well understood. Important peptides such as neurokinin B (NKB) and Dynorphin (DYN) are highly associated with KiSS-1 regulation. There are a high population of KiSS-1 neurons in the ARC that coexpress NKB and DYN, namely KND neurons (Navarro et al., 2009), and both neuropeptides play a role in the regulation of the GnRH neuron. The KND neurons may also coexpress receptors for NKB (NK3) and DYN (KOR) on their own membrane (Navarro et al., 2009). There may be an autoregulation by the KND neuron, demonstrating that NKB stimulates while DYN inhibits secretion of kisspeptin. During lactation, inhibition of NKB with normal concentrations of DYN inhibits kisspeptin release, thus decreasing secretion of GnRH (Navarro et al., 2009).

In summary, there are several factors during lactation that combine to deplete GnRH and subsequent secretion of LH, resulting in a delay to the onset of postpartum estrous cycles. During periods when requirements for energy are high (i.e., lactation) more energy needs to be directed towards the energy sink (i.e., towards production of milk). During periods of increased energy requirements, concentrations of leptin and insulin drop to signal the need for increased energy retention. Therefore, the hunger system is activated stimulating hyperphagia and the reproductive system is compromised. Ultimately, milk synthesis and removal with physical suckling stimulation have important roles in regulation of fertility. Therefore, the observation that calf removal (CR) increases concentrations of LH and the importance of LH in follicle development, researchers have focused on CR during estrous synchronization as a tool to improve fertility in beef cows (Kiser et al., 1980; Geary et al., 2001a; Meneghetti et al., 2009; Sá Filho et al., 2009b, c).

Estrous synchronization protocols using CR to improve fertility

Numerous studies have evaluated the benefits of using temporary calf removal (CR) during estrous synchronization. The improvement in fertility using estrous synchronization with CR has (Smith et al., 1979; Kiser et al., 1980; Yelich et al., 1995; Geary et al., 2001a; Sá Filho et al., 2009b, c, d; Vasconcelos et al., 2009) or has not (Geary et al., 2001b; Pinheiro et al., 2009) demonstrated an improvement in pregnancy rates compared to protocols without CR. Temporary CR of differing duration may also have differing effects on secretion of LH, and consequently on fertility, but in most estrous synchronization systems CR has been performed for 48 h. However, it has been demonstrated that the maximum LH rise occurs within 48 h of CR, but there is a linear increase in LH pulses when CR removal occurs for as long as 144 h (Shively and Williams, 1989). Therefore, it would be reasonable to propose that extending the duration of temporary CR would enhance fertility, even though concentrations of LH decline when cows and calves are reintroduced (Shively and Williams, 1989). This observation may imply that ovulation may be negatively affected when cows and calves are reintroduced prior to initiation of the LH surge or complete stimulation of LH on ovulatory follicles. However, increasing the duration of temporary CR may have subsequent negative effects on calf performance, the maternal bond, or the cow's future milk production.

Calf removal and calf performance

The evaluation of the effect of short-term CR on subsequent calf performance has been a focus of few experiments. The effect on cow fertility rather than calf performance is the focus of most experiments performing temporary CR. However, there are conflicting reports on calf performance of calves exposed to temporary CR during

estrous synchronization. In one report (McCartney et al., 1990), a 5% decrease in weaning weights for calves that were temporary removed for 48 h was noted compared with calves that were not exposed to CR. Evaluating four suckling treatments (once or twice daily suckling, 48 h CR, and no separation), Odde et al., (1986) reported lower weight gain for once-daily suckling and 48 h CR during the first month of the breeding season compared to no CR and twice-daily suckling calves. In contrast, body weight of calves weighed one and three weeks after 48 h of CR did not differ from controls, and no difference in 205-d adjusted weaning weights (Beck et al., 1979). Sá Filho et al., (2009d) observed that calves exposed to 48 h of CR between 39 and 68 d of age were lighter at weaning (240 d of age) compared to calves not exposed to CR. However, calves exposed to CR gained more body weight and had greater average daily gain (ADG) from weaning to 420 d of age than calves not exposed to CR, resulting in similar body weight at 420 d of age between treatments. However, these studies did not evaluate whether calves of different ages would respond to CR with differing performance. In addition, these studies primarily focused on 48 h CR and no known studies have demonstrated the effects of temporary CR on calf performance for longer durations. Calf removal may directly influence calf health or dam milk production indirectly affecting calf performance. Neither of these two factors has been researched.

Effects of CR on milk production

Milk yield assessed one to three wk after removing calves for 48 h did not differ between cows whose calves were or were not removed (Beck el al., 1979). In addition, milk production, and duration of suckling were taken two wk after treatments (once and twice daily suckling, 48 h of CR, and control) and no differences were observed (Odde et al., 1986). In contrast, in other species, such as mice and rats, the cessation of

milking might have severe modifications on mammary gland tissue. In rodents, removal of pups and sudden cessation of suckling caused rapid alteration in milk synthesis, patterns of hormone release, and changes in secretory cells, specific enzyme activity and gene expression (Wilde et al., 1999). Cessation of suckling caused accumulation of milk in the ducts and cisterns of the mammary gland. After 24 h of pup removal apoptosis of cells is initiated with a decrease in galactopoietic hormones, lipogenic enzymes (acetyl-CoA Carboxylase) and α, β, and γ- casein mRNA abundance, indicating involution of the mammary gland (Wilde et al., 1999).

In ruminants, the indicator of mammary gland involution after cessation of milking appears to be more delayed than in rodents (Goodman and Schanbacher, 1991). However, after 3 d of no milk removal a decrease in α-lactalbumin mRNA was noticed and after 7 d there was a reduction in expression of α-casein and α-lactalbumin mRNA (85 and 99%, respectively) and a 20-fold increase in lactoferrin mRNA abundance (protein secreted during the dry period and mammary gland involution; Wilde et al., 1997). In addition, one week after cessation of milking the proportion of secretory alveoli and stromal tissue changed markedly, increasing stromal infiltration in alveoli regions (Wilde et al., 1997). In rodents, the alveoli structure of the mammary gland was almost totally degenerated after 4 d of pup removal (Wilde et al., 1999). The precise timing of involution in mammary gland structures, or cessation of milk production by the secretory cells after calf removal continues to be studied. There are no studies addressing the impact of temporary CR performed early or late in lactation on milk production and mammary gland involution in beef cows.

Short-term feed deprivation and calf performance

Hormones, such as IGF-I and insulin, are present in the blood and are correlated with nutritional status of cattle (Wang et al., 2003). Insulin-like growth factor-I is a growth promoting peptide synthesized by the liver by stimulation of growth hormone (GH) on the anterior pituitary (Copeland et al., 1980). Since IGF-I has an anabolic effect its synthesis can be reduced when cattle experience undernutrition (Wang et al., 2003). Body size and growth of domestic animals have been correlated to IGF-I (Hossner et al., 1997), and IGF-I is positively correlated with growth (Ronge and Blum 1989; Roberts et al., 1990) and growth rate (Bishop et a., 1991) in ruminants. In addition, food deprivation was associated with a decrease in total blood concentrations of IGF-I and total IGF-I mRNA in the liver (Wang et al., 2003).

Food deprivation not only regulates hormones related to growth but also may affect the gastrointestinal tract. The intestinal mucosa villous-crypts have been reported to atrophy after 1.5 d of fasting in weaned pigs and a reduction of total activity of intestinal alkaline phosphatase (IAP) was observed (Lallès and David, 2011). Intestinal alkaline phosphatase is responsible for controlling intestinal homeostasis (Lallès, 2010), and detoxifies bacteria lipo-polysaccharides (LPS), reduces LPS-induced inflammation and limits trans-mucosal passage of bacteria (Goldberg et al., 2008). However, although refeeding for 2.5 d after fasting restores most normal characteristics of the small intestine, IAP activity was only restored by 50% and villus length remained short. Proteins related with stress of cell and tissue homeostasis, such as heat shock protein (HSP) and nitric oxide synthase (nNOS) are up-regulated after fasting (Lallès and David 2011). Intestinal cells are protected by HSP against endotoxins and reactive oxygen species (ROS), as well as control of protein traffic in the cell having anti-apoptotic

properties (David et al., 2002; Petrof et al., 2004; Otaka et al., 2006; Arya et al., 2007; Lanneau et al., 2007). An increase in HSP during fasting may be a mechanism of protection related to the absence of food and the threat of the gastric juice to stomach cells (Marruchella et al., 2004; Ebert et al., 2005).

In addition to the effect on growth performance, food deprivation may potentially impair healthy or deplete the total number and/or functional competence of immune cells (Fernandez-Riejos et al., 2010). Several studies have been conducted in chickens evaluating fasting conditions and immune responses (Spalatin and Hanson, 1974; Ben Nathan et al., 1977; Klasing, 1988). Chicks fasted for 24 h followed by an injection of sheep red blood cells (SRBC) followed by normal feed consumption increased anti-SRBC titers 7 d, but not 14 d later compared to those not exposed to feed deprivation (Klasing, 1988). In addition, chicks fasted for 24 or 48 h had an increase in feed consumption during the first (39 and 32% for 24 and 48 h deprivation, respectively) and second (12 and 22% for 24 and 48 h deprivation, respectively) days of the return to normal feed availability compared to chicks that did not experience feed deprivation (Klasing, 1988). Chicks deprived of water and feed for 24 h had greater response to Newcastle Disease vaccination than chicks with *ad libitum* access to feed (Spalatin and Hanson, 1974**)**. In contrast, chicks deprived of feed and water for 24 h prior to *Escherichia coli* or SRBC had lower anti-body responses than *ad libitum*-fed and water controls (Ben Nathan et al., 1977). Forty-eight hour water and food deprivation resulted in decreased body, liver, Bursa of Fabris, spleen, and thymus weight of chickens and fasting lowered the white blood cell count (Ben Nathan et al., 1977). The response to *E. coli* vaccine was also diminished (lower serum antibody titer) in chicks deprived of food

and water and starvation decreased leukocyte count indicating that some immune cells may be affected by feed restriction (Ben Nathan et al., 1977).

Several stress conditions such as feed deprivation, injury, restraint, castration, transport, disease, and commingling may alter specific blood hormone concentrations as well as blood immune cell counts, that might have an effect on animal performance (Grandin, 1997; Stafford et al., 2002; Arthington et al., 2005, 2008; Qiu et al., 2007; Carroll et al., 2009). Under stress, nerve cells of the hypothalamus release corticotrophin releasing factor (CRF) that stimulates the release of ACTH from the anterior pituitary. The release of ACTH into the blood acts on the adrenal gland, stimulating the release of cortisol (Kopin, 1980). Animals regarded as more temperamental had greater concentrations of cortisol, which has been negatively correlated with growth performance in feedlot cattle (Grandin, 1997; Voisinet et al., 1997).

Acute stress such as transportation or challenge with an injection of lipopolysaccharide (LPS) stimulates the release of pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF-α) in blood (Breazile, 1996; Arthington et al., 2003, 2005; Carroll et al., 2009). These cytokines stimulate the release of acute phase proteins (APP) from the liver, which is the first mechanism of an inflammatory response (Breazile, 1996). During an inflammatory response or stress, energy expenditure may be shifted towards synthesis of APP and immune cell enforcement and function, decreasing performance of cattle (Carroll et al., 2009). Interestingly, early-weaned (80 d of age) calves had a lower response to LPS challenge, having lower serum concentrations of TNF-α, IL-1 and IL-6 after LPS

injection than normal-weaned (250 d of age) calves (Carroll et al., 2009). In addition, early-weaned calves (89 d of age) had decreased elevations of the APP ceruloplasmin and haptoglobin after transportation and better feedlot performance than normal-weaned (300 d of age) calves, indicating an altered immune system competence between the two weaning ages (Arthington et al., 2005).

The concentrations of leptin in the blood may be a link between feed deprivation and immune cell competence. Previously it was discussed that negative energy balance and feed deprivation cause a decrease in concentrations of leptin to stimulate hunger and feeding intake. However, low concentrations of leptin may compromise immunity and the response to inflammation and hematopoiesis (synthesis of blood cells; Fantuzzi and Faggioni, 2000). Moreover, the declining leptin during starvation may result in suppression of immunity (Lord et al., 1998). The $CD4^+$ and $CD8^+$ T immune cells express leptin receptors (Lord et al., 1998) and in vitro studies indicate that leptin stimulated the proliferative response of $CD4^+$ T lymphocyte cells (Lord et al., 1998). In addition, leptin receptor deficient mice had a reduction in size of the thymus (the primary organ of the immune system) and had defective cell-mediated immunity (Lord et al., 1998; Howard et al., 1999). Moreover, malnourished infants, with low levels of plasma leptin, had impaired immune responses (Palacio et al., 2002).

Taken together, the observations resulted from temporary feed deprivation may alter gastrointestinal tract characteristics and function, decrease immune system competence, and negatively affect calf performance.

CHAPTER 3
EFFECTS OF TEMPORARY CALF REMOVAL PRIOR TO FIXED-TIME AI (TAI) ON PREGNANCY RATES AND SUBSEQUENT CALF PERFORMANCE IN SUCKLED BEEF COWS

Two experiments were conducted to determine the effect of calf removal (CR) on pregnancy rate (PR) and calf performance in suckled beef cows. Cows in both experiments were synchronized with the 7-day CO-Synch + CIDR protocol (i.e., 100 µg injection of GnRH at CIDR insertion [d -7] with 25 mg injection of PG at CIDR removal [d 0], followed by injection of GnRH and TAI on d 3). Cows were blocked by location (6 locations) and stratified by d postpartum (DPP), BCS, and parity and assigned to one of two treatments in Exp. 1; control (Control; n = 156); 2) calves were separated from their dams between d 0 and 3 (CR72; n = 168); and one of four treatments in Exp. 2; Control (n = 103); 2) CR72 (n = 104); 3) similar to Control with addition of CR between d 0 and 2 (CR48A; n = 95); and 4) similar to Control with addition of CR between d 1 and 3 (CR48B; n = 53). Transrectal ultrasonography was used to assess follicle dynamics and corpus luteum (CL) development on d 0, 1, 2, 3, 4 and 10 and to determine pregnancy status on d 30. Blood samples were collected on d -14, -7, 0, 3, and 10 to determine progesterone (P) and estradiol (E) concentrations. Calves were blocked by age as young (25 to 59 d), medium (60 to 79 d), and old (> 80 d) and were weighed on d 0, 3, 33, and 63. In Exp. 1, pregnancy rate (PR) at one location tended (P = 0.06) to be greater in cows exposed to the CR72 (53%) than Control (38%) treatment. Overall PR did not differ (P = 0.58) among treatments in the other 2 locations in Exp.1 and averaged 53%. Follicle growth rate from d 0 to 3 tended (P = 0.06) to be greater for CR72 (0.42 ± 0.15 mm/d) compared to Control (0.02 ± 0.15 mm/d), but there were no differences in follicle diameter on d 0 or 3 (P > 0.10). Young (-3.9 ± 0.3%) and old (-3.1

± 0.4%) calves lost greater ($P < 0.001$) percent of body weight (PBW) during CR than medium (-1.6 ± 0.3%) age calves exposed to CR72. In Exp. 2, PR were similar among all 3 locations (49%; $P = 0.15$). Concentrations of E were greater ($P < 0.001$) in the CR72 (14.6 ± 1.6 pg/mL) treatment at 24 h after PG than Control (7.4 ± 1.4 pg/mL), CR48A (8.4 ± 1.6 pg/mL), and CR48B (7.7 ± 1.6 pg/mL). Differences in follicle diameter were not significant. Young (-4.8 ± 0.6%) and medium (-3.0 ± 0.5%) calves lost a greater ($P < 0.01$) PBW during CR than old (-1.4 ± 0.6%) calves within the CR72 treatment. Calves exposed to CR48 (-2.2 ± 0.6%, -1.1 ± 0.6%, and -2.4 ± 0.6% PBW change for young, medium, and old, respectively) lost more weight than calves in the Control group (3.7 ± 0.4%, 1.7 ± 0.5%, and 2.1 ± 0.5% PBW change for young, medium, and old, respectively). We conclude that CR may be used as a tool to enhance fertility in beef cows, stimulating follicle growth and, although inconsistent results, increasing PR to TAI. However, CR had a negative impact on subsequent calf performance, which differed depending on the duration and age of the calf when exposed to CR.

Introductory Comments

Luteinizing hormone is depleted in the pituitary as a result of massive releases of steroid hormones during parturition (Riley et al., 1981; Moss et al., 1985). The presence of a calf physically suckling extends the number of days postpartum (DPP) to resumption of cyclicity in beef cows (Wagner and Oxenreider, 1971; Mukasa-Mugerwa et al., 1991; Viker et al., 1989, 1993; Lamb et al., 1997, 1999). Endogenous opioids, such as β-endorphins, are released during suckling and inhibit GnRH secretion and decrease frequency of LH pulses in ewes (Gordon et al., 1987). Lowering suckling frequency or temporarily withdrawing the suckling stimulus improved LH secretion

(Williams et al., 1987, 1995; Silveira et al., 1993), shortened the interval from calving to first ovulation (Graves et al., 1968; Oxenreider, 1968), and increased pregnancy rates (PR) earlier in the breeding season (Vasconcelos et al., 2009). However, when suckling was resumed, LH pulsatility returned to baseline concentrations (Shively and Williams, 1989; Edwards, 1985).

Numerous reports have indicated an improvement in PR to TAI in beef cows when exposed to temporary calf removal (CR; Geary et al., 2001; Baruselli et al., 2004; Duffy et al., 2004; Meneguetti et al., 2009; Sá Filho et al., 2009a; Small et al., 2009; Vasconcelos et al., 2009; Sá Filho et al., 2010). However, additional reports indicate that CR may (McCartney et al., 1990; Sá Filho et al., 2009b) or may not (Beck et al., 1979) affect subsequent calf performance.

An indirect effect of temporary CR on calf performance could be related to the decrease in milk production resulting from the cessation of milk withdrawal after weaning. In ruminants, one week after cessation of milking withdrawal, α-Lactalbumin and β-Casein mRNA decreased 85 and 99%, respectively, and lactoferrin mRNA increased 20-fold (Goodman and Schanbacher, 1991).

Two experiments were designed to evaluate the effect of CR on cow fertility and calf performance. Our objective was to evaluate duration (48 vs. 72 h) of CR on plasma concentrations of estradiol (E), growth of the pre-ovulatory follicle, and PR at TAI. In addition, we evaluated the effect of CR on subsequent calf performance by assessing calf weight and dam milk production. We hypothesized that CR would stimulate the growth of the pre-ovulatory follicle elevating E concentrations, increasing follicle growth

and resulting in greater PR. In addition, we hypothesized that CR would not have any negative effects on milk production or subsequent calf performance.

Materials and Methods

Six hundred seventy-nine suckled beef cow and calf pairs composed of Angus and Brangus breeds were enrolled in the study. Two experiments were conducted during 3 years at 6 separate locations. Exp. 1 was conducted during the 2008 and 2009 breeding seasons at three locations (Loc-1, FL; Loc-2, MN; and Loc-3, MN). Exp. 2 was conducted during the 2011 breeding season at three locations (Loc-4, FL; Loc-5, ND; and Loc-6, MS). Both experiments were conducted with Institutional Animal Care and Use Committee (IACUC) approval.

Animals and treatments

Experiment 1. Three hundred twenty-four cow and calf pairs were assigned to treatments. Angus and Brangus crossbred cows were used at Loc-1 (n = 99). Mean BCS (scale of 1 to 9; 1 = emaciated, 9 = obese; Whitman, 1975) was 5.3 ± 0.7 (mean ± SD) and mean DPP were 56 ± 22 (mean ± SD). Purebred Angus cows were used at Loc-2 (n = 108) with mean DPP of 69 ± 21 (mean ± SD) and Loc-3 (n = 117) with mean DPP of 68 ± 19 (mean ± SD).

Cows were stratified by parity, DPP, and calf gender and then assigned to 1 of 2 treatments: 1) cows received the 7-d CO-Synch + CIDR protocol (Larson et al., 2006), which included 100 µg of GnRH i.m. (2 mL of Cystorelin®, Merial, Duluth, GA, d -7) and a CIDR (EAZI-Breed CIDR containing 1.38 g of P; Pfizer Animal Health, New York, NY) insert followed in 7 d by 25 mg PG i.m. (5 mL of Lutalyse®; Pfizer Animal Health) and the CIDR was removed (d 0), followed in 72 h (d 3) by TAI and a second injection of GnRH (Control; n = 156); 2) similar as Control but calves were removed from their dams

for 72 h between d 0 and 3 (CR72; n = 168). Calves were weighed on d 0, 3, 33, and 63. During CR, calves were confined with *ad libitum* access to water and hay and were located no closer than 100 m from their dams (Figure 3-1).

Experiment 2. Three hundred fifty-five cow and calf pairs (n = 206, 75, 74 for Loc-4, Loc-5, and Loc-6, respectively) with mean (mean ± SD) DPP of 71 ± 38 (Loc-4 = 56 ± 18 d; Loc-5 = 74 ± 14 d; Loc-6 = 82 ± 15) were used. Cows were stratified by parity, DPP and calf gender before being assigned to one of four treatments: 1) same as Control described on Exp. 1 (Control; n = 103); 2) same as CR72 described on Exp. 1 (CR72; n = 104); 3) similar to CR72 but calves were removed on d 0 and returned to suckle on d 2 (CR48A; n = 95); and, 4) similar to CR72, but calves were removed on d 1 and returned to suckle on d 3 (CR48B; n = 53).

Calves were weighed on d 0, 3, 33, and 63. During CR, calves were confined with *ad libitum* access to water and hay and were located no closer than 100 m from their dams.

At Loc-6 (Exp. 2) 20 d before CR, calves received a creep feed, composed of a 50:50 ratio of soybean hull pellets and corn glutten pellets. Calves had *ad libitum* access to creep feed throughout the experiment (Figure 3-1).

Ultrasound scanning and blood sample collection

In Experiment 1, at Loc-1, transrectal ultrasonography (5.0-MHz linear array transducer, Aloka 500V) was performed on d 0, 3, and 10. In Exp. 2, at Loc-4, transrectal ultrasonography (7.5-MHz linear array transducer, Aloka 500V, Instrument of Science and Medicine, Vancouver, BC, Canada) was performed on d -14, -7, 0, 1, 2, 3, and 4. Ultrasonography was used to monitor growth of the dominant follicle (DF) and CL volume. The vertical and horizontal diameter of the largest follicle on each ovary and all

CL were measured and recorded. The diameter of the DF was calculated by the average value for the vertical and horizontal diameter measurements. Volume of CL tissue was calculated using the formula V = 4/3πr3 where r = ½ of the average value for vertical and horizontal diameter measurements. In cases where CL had fluid filled cavities, the volume of the cavity was subtracted from the total volume of the CL, resulting in a value that reflected the actual volume of CL tissue present. Ovulation was determined when the DF disappeared from the location determined by the previous scan, a CL developed at the same area, and concentration of progesterone (P) increased by d 10. In both Exp. 1 and 2, pregnancy diagnosis was performed on d 33 and 63 after TAI.

In Experiment 1 at Loc-1 and in Experiment 2 at all locations, blood samples were collected via coccygeal venipuncture using Vacutainer tubes (Becton & Dickinson Vacutainer Systems, Rutherford, NJ) from all cows on d -14, -7, 0, 3, and 10. In Exp. 2 a subset of 65 cows (Control, n = 20; CR72, n = 14; CR48A, n = 15; CR48B, n = 16) from Loc-4, were randomly selected and used to perform intensive ovary ultrasonography to evaluate follicle growth. Ultrasonography was performed once daily at 0800 h from d 0 to 4. In addition, blood samples were collected every 12 h from d 0 to 4 to determine plasma concentrations of E. Blood remained on ice for 2 h after collection and then centrifuged for 20 min at 1,500 × *g* at 4° C. After centrifugation a pipette was used to siphon plasma into polypropylene vials (12mm × 75mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), and then were stored at -20° C until analyses.

In Exp. 2, calves from Loc-4 were vaccinated on d 23 and 65 after CR with modified live virus (MLV) strains of infectious bovine rhinotracheitis (IBR), bovine virus

diarrhea (Type 1 and 2; BVD), parainfluenza3 virus (PI3), and bovine respiratory syncytial virus (BRSV; 2 mL i.m. of Bovi-Shield Gold 5, Pfizer Animal Health, New York, NY). Blood samples from 45 heifer calves (76 ± 21 d [mean ± SD] of age on d 23) were collected on d 23, 36, 65 and 87, to measure blood titers for IBR. Blood samples were left on ice for 2 h after collection and then centrifuged for 20 min at 1,500 × g at 4° C. After centrifugation a pipette was used to siphon plasma into polypropylene vials (12mm × 75mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), which were stored at -20° C until analyses. The samples were sent to a laboratory (Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL, 34741) for analyses of concentration of IBR antibody blood titer. Titers were evaluated using the serum neutralization method described by Rosenbaum et al., (1970). The lowest dilution tested was 1:4 and the greatest dilution was 1:256. Serum neutralization titers <4 were considered negative.

Hormone analyses

Concentrations of plasma P were analyzed in duplicate by RIA using progesterone kits (Coat-A-Count; Diagnostic Products Corp. Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 μL. Assay tubes for the standard curve contained 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20ng/tube. Assay sensitivity for a 100-μL sample was 0.1 ng/mL. The intra and inter-assay CV were 7,0% and 8.0%, respectively. Cows were considered to be cycling at the initiation of treatments if at least 1 of 2 blood samples had concentrations of P ≥ 1 ng/mL (Perry et al., 1991) for samples collected on d -14 and d -7 or if a CL was present on the ovary at one or both of these collection days when ultrasonography was performed.

Concentrations of plasma E were analyzed by RIA using a commercial kit (Estradiol Double Antibody, Siemens Healthcare Diagnostics, Los Angeles, CA), validated previously for use in bovine samples (Siddiqui et al., 2009). The sensitivity of the assay was 0.16 pg/mL calculated at 2 SD below the mean counts per minute at maximum binding. Samples were analyzed in duplicate and repeated when the coefficient of variation between duplicates was > 0.15. Plasma from a cow exhibiting standing activity and charcoal-stripped plasma from a male calf were used as positive and negative quality controls, respectively. High (10 pg/mL), moderate (5 pg/mL), and low (2.5 pg/mL) concentration samples were incorporated to each assay and used to evaluate the extraction. The intra-assay CV was 6, 15, and 6% for the first, second, and third assays, respectively; and the inter-assay CV was 9%.

Assessment of milk yield

In Experiment 2, a subset of 49 cows from Loc-4, (different cows used for ovary scanning and intensive blood collection), were used to evaluate milk production and components. Cows were segregated at first milking day (d -12) by calf age as young (n = 24; mean DPP of 19 ± 5 d [mean ± SD] with a range of 13 to 27 DPP), and old (n = 25; mean DPP of 68 ± 5 d [mean ± SD] with range of 57 to 80 DPP) to be milked on d -12, 36, 64 and 92. On the morning of milking, cows were separated from their calves. Each cow was restrained in a hydraulic chute and received 40 IU of oxytocin intravenously in the jugular vein. Cows were milked immediately as milk let-down occurred using a vacuum pump connected to a four claw milking machine. When all four quarters were dry, machine milking ceased and residual milk from all quarters was stripped by hand (procedure adapted from Marston et al., 1992). Cows were returned to their pens without their calves and were allowed *ad libitum* access to feed and water.

Following a minimum separation period of 6 h, cows were milked again as previously described. Milk was weighed and daily production (kg/d) was adjusted to a 24-h yield. Milk samples were collected and stored at 4°C for 24 h prior to being shipped to the laboratory (Southeast Milk, Inc, Belleview, FL USA) for analysis of fat, protein, lactose, solids not fat, and somatic cell count.

Statistical analyses

Experiment 1. was designed as a completely randomized block design. Cows were blocked by location and parity and assigned to treatments described previously. Binary responses (pregnancy rate at TAI) were analyzed using the GLIMMIX procedure of SAS (version 9.2; SAS/STAT, SAS Institute Inc., Cary, NC, USA). The model included the effect of treatment, parity, location, year, calf age, and the respective interactions. Body condition score (BCS) and cycling status were only assessed at Loc-1. Therefore, a separate analysis to evaluate PR was conducted for Loc-1 including the effect of treatment, parity, calf age, BCS, cycling status, and respective interactions. In addition, follicle diameter on d 0 and 3, concentrations of P on d 10 and CL volume on d 10 was assessed at Loc-1. These variables were analyzed by ANOVA using the GLIMMIX procedure of SAS assuming normal distribution. Follicle diameter on d 0 was used as a covariate in the analysis of follicle diameter on d 3. The model included the effects of treatment, cycling status, and respective interactions. In addition, Pearson correlation and logistic regression for linear effects were used to evaluate the correlations between follicle diameter on d 3, follicle growth rate, concentrations of P, and CL volume on d 10.

A separate analyze was conducted to evaluate the effect of treatments on calf performance. Calf weights were analyzed by ANOVA for repeated measures using

PROC MIXED procedure of SAS (SAS inst. Inc., Cary, NC) assuming normal distribution. The model included the effects of treatment, location, parity, calf gender, and calf age. Initial body weight (BW) measured on d 0, was used as a covariate parameter. Calf age was blocked and separated as young (range from 25 to 60 d), medium (range from 61 to 80 d), and old (> 80 d).

Experiment 2. was designed as a completely randomized block design. Cows were blocked by location and parity and randomly assigned to treatments described previously. Pregnancy rate were analyzed assuming binary distribution using the GLIMMIX procedure of SAS version 9.2 (SAS/STAT, SAS Institute Inc., Cary, NC, USA). The model included the effect of treatment, parity, location, cycling status and the respective interactions.

To evaluate the effect of duration of CR on calf performance, calves from treatments CR48A and CR48B were combined, because in both groups calves were removed for 48 h. Therefore, calf performance, IBR antibody blood titer, milk production, and milk property analyses included Control, CR48, and CR72 as treatment factors.

Analyses of concentrations of E, follicle diameter, calf antibody blood titers for IBR and calf body weight (BW) were conducted by ANOVA for repeated measures using PROC MIXED procedure of SAS (SAS inst. Inc., Cary, NC). The model to evaluate concentrations of hormones and follicle diameter included the effect of treatment, cycling status, and parity. The initial value on d 0 was used as a baseline and included in the model as a covariate. Pearson correlations and logistic regression for linear effects were used to evaluate correlations between follicle diameter on d 2 and concentrations of E at 36 h after PG. The model to evaluate calf performance included

the effect of treatment, gender, location, and age. Calf age was blocked and separated as young (range of 25 to 59 d), medium (range of 60 to 79 d), and old (≥ 80 d). The first measurement of calf BW and initial IBR blood titer were used as baseline measurements and included in the model as covariates. The results of serum neutralization analyses of IBR antibody blood titer were transformed using the logarithm function to approach normality, therefore for the titer level analysis, the reported values were log transformed and statistically evaluated.

Milk production and milk composition were analyzed by ANOVA using the GLIMMIX procedure of SAS assuming normal distribution. The model included the effect of treatment and age of calves (young and old) with the respective interaction.

Experiment 1 and 2. A combined final analysis was conducted to evaluate the main effect of treatments for Control and CR72 cows from Exp. 1 and 2. The GLIMMIX procedure of SAS, assuming binary distribution was used to analyze PR at TAI. The model included the effects of treatment, location, cycling status, and parity with the respective interactions. Body condition score and DPP were used as covariate parameters. In addition, an ANOVA for repeated measures using the PROC MIXED procedure of SAS was conducted to evaluate calf performance using calves exposed to Control and CR72 treatments from Exp. 1 and 2, from all locations except Loc-6. The model included the effect of treatment, gender, location, year, age, and the respective interactions.

Changes in PBW from d 0 to 3 were analyzed by ANOVA using GLIMMIX procedure of SAS, assuming normal distribution. The model for PBW analysis included

the effect of treatment, calf age, location, and the respective interactions. All locations were included, except Loc-6.

All the quantitative data were tested for normal distribution using the UNIVARIATE procedure of SAS and transformed when necessary. Pregnancy rates are reported as real means; however, all remaining measurements are expressed as least square means (LSM). Means were separated using LSD (Snedecor and Cochran, 1989) and significance was set at P ≤ 0.05 and tendencies were determined if $P > 0.05$ and ≤ 0.10.

Results

Experiment 1

Overall PR is summarized in Table 3-1. In Exp. 1, the treatment × location interaction tended ($P = 0.09$) to affect PR. Pregnancy rates at Loc-2 tended ($P = 0.06$) to be greater in cows exposed to CR72 (53%) compared to cows in the Control (38%) treatment. However, PR did not differ between treatments at Loc-1 (38% vs. 30% for CR72 and Control, respectively; $P = 0.28$) and at Loc-3 (63% vs. 74% for CR72 and Control, respectively; $P = 0.42$). Cows nursing old (56%) and medium (54%) age calves had greater ($P = 0.01$) PR than cows with young (29%) calves. There were no interactions ($P > 0.1$) between treatments, age, parity, year, or cycling status affecting PR.

Follicle diameter did not differ ($P = 0.21$) between treatments on d 0 (12.9 ± 0.4 mm vs. 12.1 ± 0.4 mm for Control and CR72, respectively) or on d 3 (12.7 ± 0.4 mm vs. 13.2 ± 0.3 mm; $P = 0.11$; Table 3-1); however, follicle growth rate between d 0 and 3, tended to be greater ($P = 0.06$) for cows exposed to CR72 (0.42 ± 0.15 mm/d) compared to those exposed to Control (0.02 ± 0.15 mm/d; Table 3-1). There was a negative and significant correlation (r = -0.63; $P < 0.0001$) between follicle diameter on

d 0 and follicle growth rate. Diameter of the DF on d 3 was not correlated (P = 0.21) with concentrations of P on d 10, but there was a positive correlation (r = 0.40, P < 0.001) between diameter of the DF on d 3 and CL volume on d 10. In addition, there was a positive correlation (0.30, P < 0.002) between concentrations of P and CL volume on d 10. There was a treatment × cycling interaction on concentration of P (P = 0.04) and CL volume (P = 0.05) on d 10. Progesterone concentrations were greater (P = 0.01) in noncycling cows exposed to CR72 (3.14 ± 0.29 ng/mL) than noncycling cows exposed to Control (2.02 ± 0.33 ng/mL) treatments. There were no differences (P = 0.26) on concentrations of P on d 10 between cycling cows exposed to CR72 (4.45 ± 0.82 ng/mL) and cycling cows exposed to Control (5.60 ± 0.63 ng/mL). Corpus luteum volume on d 10 tended to be greater (P = 0.06) on cycling cows exposed to Control (7.40 ± 0.91 cm^3) than cycling cows exposed to CR72 (4.61 ± 1.18 cm^3). No difference was observed on CL volume on d 10 between noncycling cows exposed to Control (5.42 ± 0.51 cm^3) or noncycling cows exposed to CR72 (5.74 ± 0.42 cm^3) treatments.

Calf performance was analyzed by change in PBW, BW, and average daily gain (ADG) during and after CR. Calf performance data are summarized on Table 3-2. During the period of CR, calves exposed to CR72 had a decrease of 2.8 ± 0.3 kg during the 72-h during CR period, whereas calves in the Control treatment had an increase of 2.9 ± 0.3 kg in the same period. There was a treatment × age interaction (P < 0.01) on PBW change during the CR period. Young (-3.9 ± 0.3%) and old (-3.1 ± 0.4%) calves lost a greater (P < 0.001) PBW during CR than medium (-1.6 ± 0.3%) age calves exposed to CR72. There was a three-way interaction of treatment × age × day (P < 0.01) for calf BW. Body weight of calves differed (P < 0.01) on d 33 for young calves

exposed to the CR72 (109 ± 2 kg) and Control (116 ± 2 kg) treatments and also differed ($P < 0.01$) for old calves exposed to the CR72 (159 ± 2 kg) and Control (164 ± 2 kg) treatments; however, the BW of medium calves exposed to Control (148 ± 2 kg) and CR72 (147 ± 2 kg) were similar ($P > 0.1$). In addition, BW of calves differed ($P < 0.01$) on d 63 for young calves exposed to the CR72 (133 ± 2 kg) and Control (142 ± 2 kg) treatments and also differed ($P < 0.01$) for old calves exposed to the CR72 (182 ± 2 kg) and Control (189 ± 2 kg) treatments; however, the BW of medium calves exposed to CR72 (177 ± 2 kg) and Control (176 ± 2 kg) were similar ($P > 0.1$; Table 3-2). Average daily gain was affected ($P = 0.03$) by calf age measured from d 3 to 63. Medium calves (0.95 ± 0.02 kg/d) had greater ($P = 0.009$) ADG than young calves (0.88 ± 0.01 kg/d), whereas old calves (0.91 ± 0.02 kg/d) had intermediate ADG. There was no statistical significance ($P > 0.10$) on the interaction treatment × age in ADG from d 3 to 63 (Table 3-2).

Experiment 2

Pregnancy rates differed by location (Loc-4, 39%; Loc-5, 56%; and Loc-6, 69%; $P < 0.001$), but were not affected by treatment (Table 3-1). Cows that were determined to have been cyclicing (52%) prior to initiation of treatments had similar ($P = 0.14$) PR than noncyclic cows (45%). No cyclicity status × treatment interaction existed ($P > 0.10$). In addition, cows with BCS ≥ 5 (52%) had greater ($P < 0.01$) PR than cows with BCS of < 5 (21%). Moreover, cows with old (59%) and medium (60%) age calves had greater ($P < 0.0001$) PR than cows with young calves (35%). There was no BCS × treatment interaction ($P > 0.10$).

There was a positive correlation (r = 0.27; *P* = 0.03) between follicle diameter on d 2 and concentrations of E at 36 h after PG. However, the diameter of the DF did not differ (*P* > 0.10) between treatments from d 0 to 3 (Figure 3-2). Concentrations of E also were greater (*P* = 0.02) at 24 h after PG for cows exposed to the CR72 (14.6 ± 1.6 pg/mL) compared to Control (7.4 ± 1.4 pg/mL), CR48A (8.4 ± 1.6 pg/mL), and CR48B (7.7 ± 1.6 pg/mL) treatments (Figure 3-3).

The effect of CR on calf performance was analyzed combining the treatments CR48A and CR48B (CR48). Therefore, the treatments included in the analysis were Control (no CR), CR48 (CR for 48 h) and CR72 (CR for 72 h). Calves from Loc-6 received creep feed prior and after CR. Therefore the calf performance analysis were conducted separately for Loc-6 and combined for Loc-4 and Loc-5. Calves, at Loc- 4 and 5, assigned to CR48 (-3.1 ± 0.3 kg) and CR72 (-3.3 ± 0.3 kg) lost weight between d 0 and 3, whereas calves exposed to the Control (1.7 ± 0.3 kg) gained weight; however at Loc-6 there was no weight loss between d 0 and 3 (2.4 ± 1.8 kg, 1.4 ± 1.4 kg, and 4.7 ± 1.7 kg weight gain for CR48, CR72 and Control, respectively). There was a treatment × day × age × location interaction (*P* < 0.01) for BW on d 33 and 63. Calves at Loc-4 and 5 performed similarly during and after CR. At these two locations, on d 33 and 63, young and old calves exposed to CR72 were lighter (*P* < 0.05) compared to younger and older calves exposed to Control, respectively (Table 3-3). Young calves exposed to CR48 (117 ± 1 and 143 ± 1 kg BW on d 33 and 63, respectively) were lighter than Control (120 ± 1 and ± 146 ± 1 kg on d 33 and 63, respectively) calves. Medium and old calves exposed to CR48 had similar (*P* > 0.10) weight on d 33 and 63 compared to Control (Table 3-3). Live weight for medium age calves did not differ (*P* > 0.1) among

treatments or days. Calf age tended (P = 0.07) to affect ADG from d 3 to 63. Medium (0.94 ± 0.03 kg/d) calves had greater (P < 0.001) ADG than young calves (0.86 ± 0.03 kg/d), while old calves (0.93 ± 0.03 kg/d) had intermediate ADG. There was no statistical significance (P > 0.10) on treatment × age on ADG from d 3 to 63.

At Loc-6, calves did not lose weight between d 0 and 3, regardless of treatment. No differences in BW within treatments or ages were found on d 33 and 63 (Table 3-3). However, there was a treatment × age interaction (P = 0.04) on ADG. Results of ADG are summarized on Table 3-3. Medium age calves exposed to CR48 (1.14 ± 0.04 kg/d) and to CR72 (1.10 ± 0.04 kg/d) had greater (P < 0.001) ADG than medium calves exposed to Control (0.95 ± 0.03 kg/d) between d 3 and 63. Old calves exposed to CR48 (1.07 ± 0.03 kg/d) had greater (P = 0.02) ADG than old calves exposed to Control (0.97 ± 0.03 kg/d) and to CR72 (0.96 ± 0.02 kg/d) between d 3 and 63.

Blood IBR antibody titer results are reported in Figure 3-4. All calves were serum positive at the day of first vaccination (d 23). Serum IBR antibody titers were greater (P = 0.04) in calves exposed to CR72 compared to other treatments on d 23. There was a tendency for a negative correlation (r = -0.27; P = 0.06) between PBW change during CR and serum IBR antibody titer on d 23. Greater (P = 0.01) percentage of calves exposed to CR48 (18 of 25; 72%) and Control (6 of 9; 66%) responded to the first vaccination (observed by the increase in blood titer in the subsequent blood sample) compared to CR72 (2 of 10; 20%). In all treatments serum IBR titer decreased from d 36 to 65, however it was more accentuated on calves in the Control, resulting in greater (P < 0.05) serum titers in calves exposed to CR72 and CR48 compared to Control on d 65.

Milk production and milk composition are summarized in Table 3-4. Calf removal had no effect ($P > 0.10$) on subsequent milk production of dams. Overall milk production decreased as lactation progressed ($P = 0.03$). Cows with young calves (7.9 ± 0.3 kg/d) had greater ($P < 0.05$) 24-h milk production compared to cows with old calves (6.5 ± 0.3 kg/d). Percentage of milk fat (4.3 ± 0.2%), milk protein (3.1 ± 0.1%), and milk lactose (4.9 ± 0.1%), Solids not fat (8.9 ± 0.2%), and somatic cell count (499 ± 130 cell/mL) did not differ ($P > 0.10$) between treatments.

Experiment 1 and 2

In a separate analysis, data of cows exposed to Control and CR72 treatments from Exp. 1 and 2 were combined. Pregnancy rates did not differ ($P = 0.92$) between treatments (138 of 272, 50% vs. 133 of 260, 51% for Control and CR72, respectively; Table 3-1).

Results of calf performance exposed to Control and CR72 including all locations except Loc-6 are summarized in Table 3-5. Analysis on calves within CR72 treatment indicates that young calves (-4.7 ± 0.2%) lost greater ($P < 0.01$) PBW than medium (-1.9 ± 0.2%) and old (-2.5 ± 0.3%) calves during the 3 d of CR. Young and old calves, but not medium calves, exposed to CR72 were lighter ($P < 0.01$) compared to calves exposed to Control on d 33 (118 ± 1 kg vs. 111 ± 1 kg for young, 157 ± 2 kg vs. 149 ± 2 kg for old calves exposed to Control and CR72, respectively) and 63 (144 ± 1 kg vs. 136 ± 1 kg for young, and 184 ± 2 kg vs. 175 ± 2 kg for old calves exposed to Control and CR72, respectively; Table 3-5). Medium age calves had similar weight compared to Control on d 33 (144 ± 2 kg vs. 145 ± 2 kg for medium calves exposed to Control and CR72, respectively) and d 63 (174 ± 2 kg vs. 176 ± 2 kg for medium calves exposed to Control and CR72, respectively; Table 3-5)

Medium age calves (0.97 ± 0.03 kg/d) had greater (P = 0.007) ADG than old calves (0.84 ± 0.04 kg/d), while young (0.90 ± 0.03 kg/d) had intermediate ADG between d 3 and 63. There was no statistical significance of treatment (P = 0.98) or treatment × age (P = 0.12) on ADG.

Discussion

Temporary weaning has been used concomitant with estrous synchronization protocols in beef cows as a tool to improve pre-ovulatory follicle growth prior to TAI to improve fertility (Smith et al., 1979; Kiser et al., 1980; Yelich et al., 1995; Geary et al., 2001; Sá Filho et al., 2009b,c,d; Vasconcelos et al., 2009). In the current study, CR for 72 h tended to increase PR at TAI in one location, but had no effect at the other five locations. Previous reports observed an increase in LH pulsatility when CR was applied to noncycling cows (Williams et al., 1987; Souza et al., 2004). In addition, CR increased PR in postpartum anestrous cows but not in cycling cows (Vasconcelos et al., 2009). The possibility exists that CR may improve fertility in acyclic cows. Both BCS and DPP are critical to increasing cycling status before initiation of the breeding season (Meneghetti and Vasconcelos, 2008; Vasconcelos et al., 2009). Cows at Loc-6 had a mean interval postpartum of 75 d at the beginning of the protocol, with 62% (46 of 74) of those cows that had resumed postpartum estrous cycles. At this location PR to TAI were the greatest (68%) compared to other locations, with no differences in PR between treatments. However, follicle growth rate was increased during the period of CR, and concentrations of E were greater at 24 h after PG for cows exposed to CR72 compared to other treatments. Therefore, CR may be an alternative tool to enhance PR and improve follicle development.

The effect of CR on calf performance was determined by the PBW change during CR and the subsequent BW gain. There is evidence that 48 h CR suckling once daily had a negative impact on calf performance (Odde et al., 1986; McCartney et al., 1990; Sá Filho et al., 2009b). In contrast, no differences in 205 d adjusted weaning weights were reported for calves that were either exposed to temporary CR or no CR (Beck et al., 1979). This study evaluated the impact of two durations of CR (48 and 72 h) on calves at different ages (young, medium, and old). We also assessed calf weights at 33 and 63 d after the return to suckling. Our results indicated that a significant location effect existed for calf performance. Location 6 managed calves differently compared to the remaining locations. At this location, calves received creep feed for 20 d before CR and continued receiving creep feed until weaning. Creep feeding may have inhibited the negative impacts of CR on weight loss and enhanced weight gain following CR. Calves at this location experienced no differences in ADG and BW during the entire experiment.

Additional analysis, excluding Loc-6, indicated that young calves exposed to CR72 had greater PBW loss during the period of removal compared to medium and old calves within the same treatment or exposed to CR48. Young and old calves exposed to CR72 were lighter on d 33 and 63 compared to contemporary calves exposed to the Control treatment. Calves from Loc-4 and Loc-5 exposed to CR48 had intermediate weights; however, young calves were also lighter at d 33 and 63 compared to Control calves. Feed deprived animals experience weight loss and alteration in metabolic hormones such as IGF-I and insulin (Sticker et al., 1995; Christensen et al., 1997). Insulin-like growth factors have anabolic action in the body, increasing body size and promoting

growth (Ronge and Blum 1989; Roberts et a., 1990; Bishop et al., 1991). Food deprivation decreases the concentrations of IGF-I in blood in humans (Clemmons et al., 1981), mice (McKnight and Goddard, 1989), rats (Maes et al., 1983), horses (Sticker et al., 1995), sheep (Gatford et al., 1997), and cattle (McGuire et al., 1995; Wang et al., 2003); however, IGF-I returns to normal concentrations following re-feeding (Christensen et al., 1997).

Fasting increases non-esterified fatty acids (NEFA) and blood urea nitrogen (BUN), which is an indicator of increased lipolysis and gluconeogenesis (Christensen et al., 1997). The concentrations of IGF binding protein 2 (IGFBP-2) increase during fasting in rats (Donavan et al., 1991) and cattle (McGuire et al., 1995). The increase in IGFBP-2 may provide protective action to preserve function and degradation of IGF-I (Donavan et al., 1991). Concentrations of insulin, glucose, and IGF-I decreased, and NEFA, growth hormone (GH), and IGFBP-2 increased during 2 d of feed deprivation in dairy cows (McGuire et al., 1995). During CR, even when hay and water is offered *ad libitum*, calves may experience feed deprivation.

Calf BW loss may be a result of the combination of a reduction in feed and water intake, but also potentially associated with loss in body tissue. The lack of intestinal content may damage the mucosa of the gastrointestinal (GI) tract. Weaned pigs exposed to 1.5 d of fasting had atrophy in the intestine mucosa villous-crypt (Lallès and David, 2011). In addition, young ruminants with a rudimentary reticulo-rumen relying mainly on milk as a source of nutrients may potentially have similar damage to the intestinal mucosa (Church, 1988). The damage to the intestinal mucosa might compromise digestion and absorption of nutrients, subsequently impairing performance.

In contrast, older calves with a GI tract more adapted to forage may consume and digest more feed than younger calves (Church, 1988; Davis and Drackley, 1998). In this study, on d 33 and 63, younger and older calves exposed to CR were lighter compared to young and old Control calves. However, ADG from d 3 to 63 did not differ between CR and Controls, indicating that calves performed similar after CR but no compensatory gain was evident in those calves exposed to CR.

In this study, young calves lost a greater PBW than older calves, while the medium aged calves were intermediate. Young animals have lower reserves of body fat and greater content of water relative to body weight (Kock and Preston, 1979). When wethers were deprived of feed and water for 3 d they lost 10% of their BW. Incidentally, 80% of the BW loss was body water and 20% was accounted for by GI tract solid content and body tissue loss, such as fat and muscle (Cole, 1995). In addition, feed restricted wethers had a decrease in weight of 13% of the GI tract tissue. Therefore, the greater loss of PBW in young calves during the 3 d of CR could be attributed to a greater loss of body water content compared to older calves.

The IBR serum titer was considered positive (>4) in all calves on the d of first vaccine. It has been demonstrated that calves acquire a passive immunity during the first day of life via colostrum consumption (Van Oirschot et al., 1999). Colostrum has immunoglobulin (IG) that protects the calves against infections during the period of active immunity (Larson et al., 1980; Stelwagen et al., 2009). The IG remains in the calf blood system for up to 6 to 8 mth after colostrum ingestion (Van Oirschot et al., 1999; Ridpath et al., 2003). In Exp. 2, cows received the IBR vaccination prior to calving. On d 23 of the experiment, when the calves received their initial vaccination to IBR they were

76 ± 21 d of age; therefore, there is a high probability that the antibody encountered in the serum was acquired by passive immunity.

It is well established that in chronic stress situations, concentrations of cortisol are elevated and may cause immunosuppression (Bayazit, 2009). Calves exposed to the CR72 treatment had greater serum IBR titer on d 23 compared to the Control and CR48 treatments, and lost a greater PBW during weaning. Perhaps, the stress during weaning and commingling with other calves, stimulating an active immune response to the IBR virus. In addition, 80% of the calves exposed to CR72 did not respond to the first vaccination, observed by no changes or a decrease in serum titer measured in the subsequent blood sample (d 36). Following vaccination, serum antibody titer should increase when the immune system is responsive (Howard, 1990; Ridpath et al., 2003), which was observed in most of the calves exposed to CR48 (72%) and Control (66%) following vaccination. Together commingling, high stress and weight loss during CR may have resulted in immunosuppression and exposure to the IBR virus, activating an immune response and elevating the serum titer observed on d 23 after CR in calves in the CR72 treatment.

Lactating female ruminants exposed to 3 d without milk withdrawal experience a reduction in α-Casein and α-Lactalbumin mRNA in the mammary gland (Wilde et al., 1997). In rats, the mammary gland alveoli structure is almost totally degenerated after 4 d of pup removal (Wilde et al., 1999). Therefore, to determine whether milk production of the cow was affected by CR, milk production in a subset of cows in Exp. 2 was assessed. The results indicated that cows early in lactation produce more milk than cows in late lactation; however, CR had no effect on milk production. Since suckling

behavior was not assessed in this study, change in suckling behavior cannot be discounted as a potential reason for the difference in performance between those calves exposed to CR and Controls. In addition, calves may be more effective at digesting forages and may decrease the number and duration of suckling events following CR. Therefore; the effects CR removal on subsequent suckling behavior needs further investigation.

Medium aged calves lost weight during CR; however, by d 33 the difference in BW between calves exposed to CR and Control had disappeared. Although milk production was not assessed in cows nursing medium aged calves, milk production of cows nursing older calves on d -12 was 9.2 ± 0.4 kg. Milk production on d -12 for cows nursing older calves represents the period when milk production is likely to be the greatest at all intervals when milk production was assessed. In addition, milk production at this interval also represents the period of time at which medium age calves would be suckling their dams during and shortly after exposure to CR. Therefore, there is a possibility that cows nursing medium age calves were producing the greatest quantity of milk when CR was performed. High milk production could be a factor associated with the negative effects of CR on subsequent calf performance of medium aged calves.

Conclusion

Calf removal elevates concentrations of E after 24 h, and increased growth rate of the DF from d 0 to 3, but did not increase the diameter of the pre-ovulatory follicle on d 3. Calf removal tended to enhance PR but was not consistent among locations. Calf performance was negatively affected by CR, and young calves had the greatest weight loss during CR. Young and old calves exposed to CR72 were lighter on d 63 compared to Control calves. Medium aged calves exposed to CR lost weight during CR, but did

not differ in BW on d 33. Milk production of cows exposed to 48 or 72 h CR was not affected. Therefore, CR had inconsistent results, enhancing PR in only one location in this study. In addition CR had a negative impact on subsequent calf performance, which differed depending on age of the calf when exposed to CR.

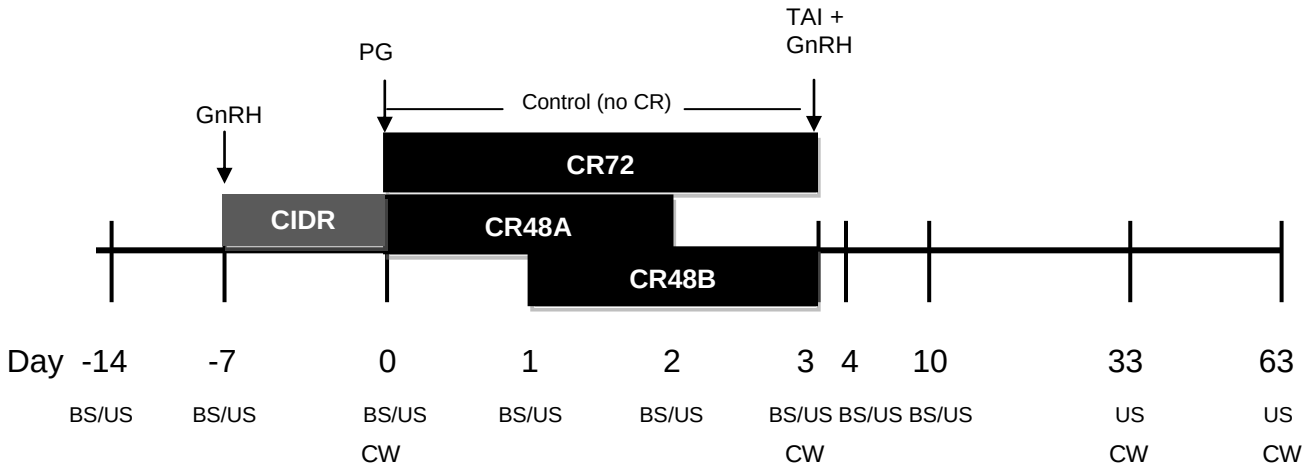

Figure 3-1. Experimental protocol. Cows received a 7-d CO-Synch + CIDR protocol (100 µg of GnRH i.m. [d -7] and a CIDR insert followed in 7 d by 25 mg PG [d 0] and CIDR removal, followed in 72 h [d 3] by TAI and an injection of GnRH). In Exp.1 (Control and CR72) and Exp. 2 (Control, CR72, CR48A, and CR48B). Calves in both experiments were weighed on d 0, 3, 33, and 63. Blood (BS) and ovary untrasonography (US) in Exp. 1 and 2 were performed on d -14, -7, 0, 3, and 10. In Exp. 2, BS in a subset of 65 cows from Loc-4 were performed every 12 h from d 0 to 4 and US were performed once daily from d 0 to 4. CIDR = controlled internal device release; PG = Prostaglandin $F_{2\alpha}$.; GnRH = gonadotropin releasing hormone; CR = calf removal; CW = calf live weight; TAI = fixed-time artificial insemination.

Table 3-1. Pregnancy rate, follicle diameter and follicle growth rate in Exp.1, and pregnancy rates to TAI in Exp. 2.

	Treatments[a]				
Item	Control[b]	CR72	CR48A	CR48B	Overall mean
Exp. 1					
Pregnancy rate, %	---------- no./no. (%) ----------				
Location 1	14/46 (30.4)	20/53 (37.7)	----	----	34/99 (34.3)[z]
Location 2	20/53 (37.7)[x]	29/55 (52.7)[y]	----	----	49/108 (45.4)[z]
Location 3	42/57 (73.7)	38/60 (63.3)	----	----	80/117 (68.4)[w]
Overall	76/156 (48.7)	87/168 (51.8)	----	----	163/324 (50.3)
Follicle diameter	---------- mm ----------				
d 0	12.9 ± 0.4	12.1 ± 0.4	----	----	----
d 3	12.7 ± 0.4	13.2 ± 0.3	----	----	----
	---------- mm/d ----------				
Follicle growth rate[c]	0.02 ± 0.15[x]	0.42 ± 0.15[y]	----	----	----
Exp. 2					
Pregnancy rate	---------- no./no. (%) ----------				
Location 4	22/52 (42.3)	19/53 (35.8)	21/48 (43.7)	19/53 (35.8)	81/206 (39.3)[y]
Location 5	17/26 (65.3)	14/27 (51.8)	11/22 (50.0)	----	42/75 (56.0)[w]
Location 6	17/25 (68.0)	18/24 (75.0)	16/25 (64.0)	----	51/74 (68.9)[w]
Overall	56/103 (55.7)	51/104 (49.0)	48/95 (50.5)	19/53 (35.8)	174/355 (49.0)
Exp. 1 and 2					
Pregnancy rate	132/259 (50.1)	138/272 (50.7)	----	----	270/531 (50.9)

[a]Calves were assigned to be separated from their dams for 72 h between d 0 and 3 (CR72), 48 h between d 0 and 2 (CR48A), 48 h between d 1 and 3 (CR48B), or no separation (Control).
[b]Calves were separated into ages from Young to Old as follows: young = 20 to 60, medium = 61 to 80, and old > 81 d of age)
[c]Follicle growth rate were calculated by the difference between follicle diameter on d 0 and 3 divided by 3.
[wz]Means within a column without a common superscript differ ($P < 0.05$).
[xy]Means within a row with different superscript tend to differ ($P = 0.06$).

Table 3-2. Percentage of body weight (PBW) change, calf body weight (BW) and average daily gain of calves associated with temporary calf removal in Exp. 1.

	Treatments[a]					
	Control[b]			CR72[b]		
Item	Young	Medium	Old	Young	Medium	Old
PBW[c], %						
d 0 to 3	4.6 ± 0.3^{w}	2.1 ± 0.3^{y}	2.0 ± 0.3^{y}	-3.9 ± 0.3^{x}	-1.6 ± 0.3^{z}	-3.1 ± 0.4^{x}
Calf BW[d], kg						
d 0	79 ± 2	116 ± 2	134 ± 2	81 ± 2	113 ± 2	135 ± 2
d 3	83 ± 2^{w}	118 ± 2^{w}	136 ± 2^{w}	78 ± 2^{y}	112 ± 2^{y}	131 ± 2^{y}
d 33	116 ± 2^{w}	148 ± 2^{w}	164 ± 2^{w}	109 ± 2^{y}	147 ± 2^{w}	159 ± 2^{y}
d 63	142 ± 2^{w}	176 ± 2^{w}	189 ± 2^{w}	133 ± 2^{y}	177 ± 2^{w}	182 ± 2^{y}
ADG[e], kg/d	0.88 ± 0.02	0.95 ± 0.03	0.91 ± 0.03	0.87 ± 0.03	0.95 ± 0.03	0.92 ± 0.03

[a]Calves were assigned to be separated from their dams for 72 h (CR72) or not (Control) between d 0 and 3.
[b]Calves were separated into ages from Young to Old as follows: young = 20 to 60, medium = 61 to 80, and old > 81 d of age).
[c]Percentage of body weight change, calculated from the weight gained or lost during CR divided by initial weight on d 0.
[d]Calf live weight.
[e]Average daily gain from d 3 to 63.
[wyxz] Means within a row and within age with different superscript letter differ ($P < 0.05$).

Table 3-3. Percent body weight (PBW) and calf body weight of calves exposed to temporary calf removal in Exp. 2.

	Treatments[a]								
	Control[b]			CR48[b]			CR72[b]		
Item	Young	Medium	Old	Young	Medium	Old	Young	Medium	Old
Calf BW[c], kg									
Loc-4 and 5[d]									
d 0	86 ± 1	114 ± 2	124 ± 2	86 ± 1	114 ± 1	124 ± 2	86 ± 1	114 ± 2	124 ± 2
d 3	87 ± 1[w]	115 ± 2[w]	127 ± 2[w]	82 ± 1[w]	112 ± 1[w]	121 ± 2[y]	81 ± 1[w]	110 ± 2[y]	123 ± 2[y]
d 33	120 ± 1[w]	141 ± 2	153 ± 2[w]	117 ± 1[y]	142 ± 1	151 ± 2[w]	113 ± 1[z]	139 ± 2	139 ± 2[y]
d 63	146 ± 1[w]	176 ± 2	186 ± 2[w]	143 ± 1[y]	171 ± 1	184 ± 2[w]	139 ± 1[z]	171 ± 2	175 ± 2[y]
PBW[e], %									
d 0 to 3	1.7 ± 0.4[w]	1.2 ± 0.6[y]	2.2 ± 0.6[w]	-4.8 ± 0.3[x]	-2.1 ± 0.5[z]	-2.6 ± 0.6[x]	-6.1 ± 0.4[v]	-3.1 ± 0.6[v]	-0.9 ± 0.6[z]
Calf BW[c], kg									
Loc-6[g]									
d 0	65 ± 5	95 ± 3	104 ± 3	65 ± 5	92 ± 3	103 ± 3	----	92 ± 4	105 ± 2
d 3	69 ± 5	101 ± 3	108 ± 3	71 ± 4	94 ± 2	103 ± 5	----	92 ± 3	107 ± 3
d 33	94 ± 5	132 ± 3	136 ± 3	95 ± 5	130 ± 3	134 ± 3		129 ± 4	136 ± 2
d 63	119 ± 5	161 ± 3	170 ± 3	123 ± 5	166 ± 3	170 ± 3		162 ± 4	167 ± 2
ADG[f], kg/d	0.79± 0.05[w]	0.95 ± 0.03[w]	0.97 ± 0.03[w]	0.83 ± 0.06[w]	1.14 ± 0.04[y]	1.07 ± 0.03[y]	----	1.10 ± 0.04[y]	0.96 ± 0.03[w]
PBW[g], kg									
d 0 to 3	5.8 ± 2.5[w]	7.3 ± 1.5[w]	3.8 ± 1.0[y]	8.0 ± 2.9[x]	2.3 ± 1.7[y]	0.3 ± 1.4[z]	----	0.1 ± 1.9[z]	2.9 ± 1.1[y]

[a]Calves were assigned to be separated from their dams for 72 h (CR72), 48 h (CR48A and CR48B) or not (Control) between d 0 and 3.
[b]Calves were separated into ages from young to old as follows: young = 20 to 60, medium = 61 to 80, and old > 81 d of age).
[c]Calf live weight.
[d]Data from locations 4 and 5 were combined.
[e]Percentage of body weight change, calculated from the weight gained or lost during CR divided by initial weight on d 0.
[f]Average daily gain from d 3 to 63.
[f]Average daily gain from d 3 to 63.
[g]Data from location 6.
[wyz] Means within a row and within age with different superscript letter differ ($P < 0.05$).

Table 3-4. Daily milk yields and milk composition of cows nursing young and old calves in Exp. 2.

	Treatments[a]		
Item	Control[b]	CR48	CR72
Milk yield, kg/d	7.6 ± 0.5	7.3 ± 0.4	7.2 ± 0.5
Milk Fat, %	4.3 ± 0.2	4.2 ± 0.1	4.4 ± 0.2
Milk protein, %	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
Milk Lactose, %	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.1
Solids non-fat, %	8.9 ± 0.1	8.9 ± 0.1	9.1 ± 0.1
Somatic cell count, cell/mL	724 ± 153	448 ± 107	366 ± 146
Milk Production, kg/d			
Young calves[c]			
d -12	7.8 ± 0.8	6.8 ± 0.6	6.8 ± 0.7
d 36	8.5 ± 0.8	7.3 ± 0.6	6.9 ± 0.7
d 64	8.3 ± 0.8	7.1 ± 0.6	6.6 ± 0.7
d 92	7.5 ± 0.8	6.6 ± 0.6	6.4 ± 0.7
Old calves[d]			
d -12	8.7 ± 0.8	9.7 ± 0.6	7.5 ± 0.7
d 36	7.1 ± 0.8	7.6 ± 0.6	7.5 ± 0.7
d 64	6.8 ± 0.8	6.6 ± 0.6	7.5 ± 0.7
d 92	6.6 ± 0.8	7.0 ± 0.6	9.1 ± 0.7

[a]Cows were assigned to receive 72 (CR72) or 48 h (CR48) temporary calf removal, or no (Control) weaning between d 0 and 3.
[b]Average milk yield assessed on d -12, 36, 64, and 92 relative to PG.
[c]Cows averaging 19 ± 5 d postpartum assessed on d -12.
[d]Cows averaging 68 ± 5 d postpartum assessed on d -12.

Table 3-5. Percentage body weight (PBW) change, calf body weight (BW) and average daily gain (ADG) in all locations except Loc-6 in Exp. 1 and 2.

	Treatments[a]					
	Control[b]			CR72[b]		
Item	Young	Medium	Old	Young	Medium	Old
PBW[c], %						
d 0 to 3	3.7 ± 0.2[w]	1.7 ± 0.3[y]	2.1 ±0.3[y]	-4.7±0.2[x]	-1.9 ± 0.2[z]	-2.5 ± 0.3[z]
Calf BW[d], kg						
d 0	83 ± 1	114 ± 2	127 ± 2	82 ± 1	114 ± 2	127 ± 2
d 3	86 ± 1[w]	116 ± 2[w]	130 ± 2[w]	79 ± 1[y]	112 ± 2[y]	124 ± 2[y]
d 33	118 ± 1[w]	144 ± 2	157 ± 2[w]	111 ± 1[y]	145 ± 2	149 ± 2[y]
d 63	144 ± 1[w]	174 ± 2	184 ± 2[w]	136 ± 1[y]	176 ± 2	175 ± 2[y]

[a]Calves were assigned to be separated from their dams for 72 h (CR72) or not (Control) between d 0 and 3.
[b]Calves were separated into ages from young to old as follows: young = 20 to 60, medium = 61 to 80, and old > 81 d of age).
[c]Percentage body weight change, calculated from the weight gained or lost during CR divided by initial weight on d 0.
[d]Calf live weight .
[wyxz] PMeans within a row and within age with different superscript letters differ ($P < 0.05$).

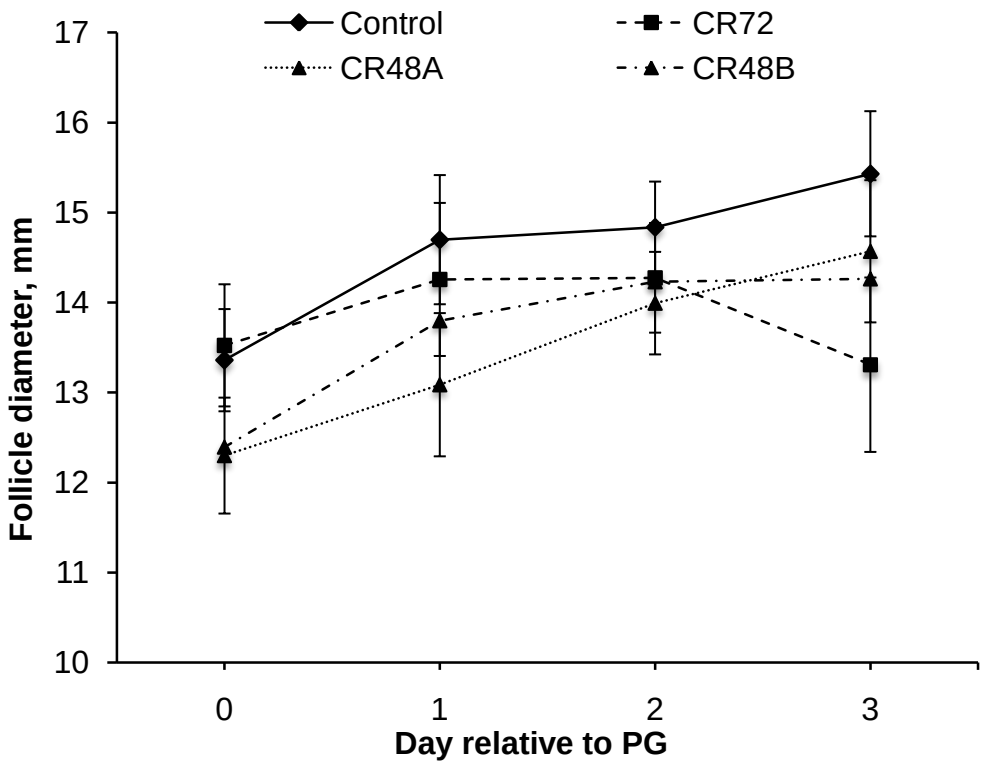

Figure 3-2. Exp. 2: Diameter of the largest follicle present on the ovary associated with cows receiving Control, CR72, CR48A and CR48B treatments on d 0.

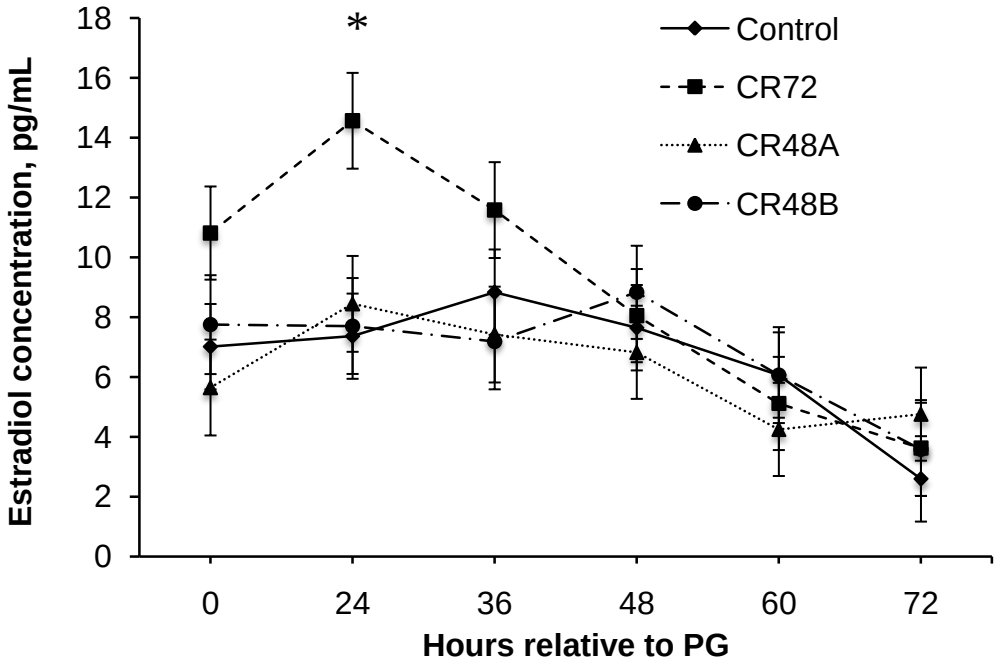

Figure 3-3. Exp. 2: Concentrations of E relative to PG (d0) for cows receiving Control, CR72, CR48A and CR48B treatments on d 0. *The CR72 treatment differed ($P < 0.01$) from Control, CR48A, and CR48B.

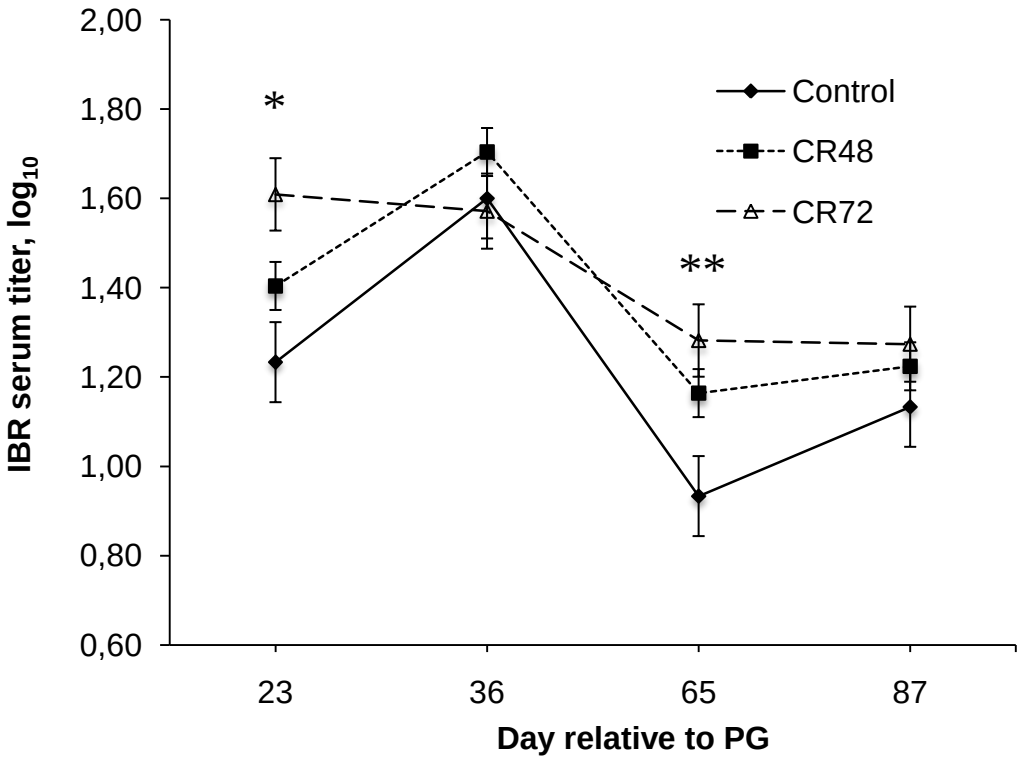

Figure 3-4. Exp. 2: Infectious Bovine Rhinotracheitis (IBR) blood titer on d 23 (first vaccination; Bovi-Shield Gold 5, Pfizer Animals Health, New York, NY; 2-mL i.m. dose), 36, 65 (second vaccination), and 87. *Serum IBR titers were greater (P < 0.01) for CR72 than Control, and CR48 on d 23. **Serum IBR titers were greater (P < 0.01) for CR72 and CR48 than Control.

CHAPTER 4
EQUINE CHORIONIC GONADOTROPIN INFLUENCES FOLLICLE DEVELOPMENT AND PREGNANCY RATES IN SUCKLED BEEF COWS WITH OR WITHOUT CALF REMOVAL

Two experiments were conducted to evaluate the effects of eCG, temporary 72-h calf removal (CR), or both, on dominant follicle (DF) diameter and pregnancy rates (PR) in suckled beef cows. For Exp. 1, we hypothesized that CR, eCG, or both at prostaglandin $F_{2\alpha}$ (PG) administration concomitant with synchronization of ovulation protocol would increase DF diameter and alter patterns of LH, estradiol (E), and progesterone (P) secretion. Thirty-five multiparous, suckled crossbred beef cows were assigned randomly to a 2 × 2 factorial arrangement of 4 treatments: 1) cows received 100 µg GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P (d -7), followed in 7 d by 25 mg PG and CIDR removal (d 0), followed in 72 h by GnRH and timed artificial insemination (timed AI; d 3; control; n = 9); 2) similar to Control, but calves were removed from their dams for 72 h between d 0 and 3 (COCR; n = 9); 3) similar to Control, but cows received 400 IU eCG on d 0 (COeCG; n = 9); 4) similar to COCR, but cows received 400 IU eCG on d 0 (eCGCR; n = 8). Blood sample collection and ovary scans were performed on d -14, -7, 0, 1, 2, 3, 4, and 10. Pregnancy rate, ovulation response by d 4, and peak concentrations of LH before 72 h after PG were greater ($P < 0.05$) for cows exposed to CR (COCR and eCGCR) than for cows not exposed to CR (Control and COeCG). Follicle diameter on d 3 was greater ($P = 0.02$) for cows receiving eCG (COeCG and COeCG; 14.9 ± 0.5 mm) than for cows receiving no eCG (Control and COCR; 13.1 ± 0.5 mm). Concentrations of E were greater ($P < 0.05$)

at 32 h for COCR (8.2 ± 1.0 pg/mL) and eCGCR (8.5 ± 0.9 pg/mL) than in control (4.9 ± 1.2 pg/mL) and COeCG (4.6 ± 1.1 pg/mL), and at 44 h after PG for eCGCR (11.7 ± 1.6 pg/mL) compared with control (6.9 ± 1.7 pg/mL), COCR (7.1 ± 1.5 pg/mL), and COeCG (7.5 ± 1.7 pg/mL). Concentrations of P on d 10 tended ($P < 0.10$) to increase when either CR (2.1 ± 0.3 vs. 2.8 ± 0.3 ng/mL, for no CR and CR, respectively) or eCG (2.1 ± 0.3 vs. 2.8 ± 0.3 ng/mL, for no eCG and eCG, respectively) was applied. In Exp. 2, we determined whether administration of 200 IU eCG improved PR in suckled beef cows. The control (n = 261) and COeCG (n = 252) treatments were similar to those previously described in Exp. 1; however, the interval from PG to fixed-time AI was 66 h and 200 IU of eCG were administered to the COeCG group. Pregnancy rates did not differ ($P > 0.10$) between COeCG (43%) and Control (50%). We conclude that eCG increased DF diameter and CR resulted in a greater percentage of cows experiencing LH peak before 72 h after PG and ovulation response; however, eCG failed to improve PR to timed AI.

Topic Overview

Equine chorionic gonadotropin is synthesized by endometrial cups, which originate from the trophoblastic epithelial cells during embryo development in horses (Allen and Moor, 1972). Synthesis of eCG begins approximately d 40 and is maintained through d 120 of gestation. Equine chorionic gonadotropin has LH-like actions in the equids, and both LH and FSH-like actions in others species, including the bovine (Soumano and Price, 1997). The eCG glycoprotein is able to bind to FSH and LH receptors located within the granulosa and theca cells of the ovary (Murphy and Martinuk, 1991). For that reason, eCG has been used in

combination with estrous synchronization and superovulation protocols in an attempt to improve follicle development and pregnancy rates (PR).

Treating cows with eCG in the absence of a dominant follicle (DF) promoted the growth of several follicles concomitantly and eventually resulted in multiple ovulations (Duffy et al., 2004). Greater DF growth rate was observed during the first 2 d after eCG treatment, resulting in an increased DF diameter at ovulation (Sá Filho et al., 2010). Cows in poor body condition and lacking normal estrous cycles (acyclic) seem to have an exaggerated response to an eCG treatment compared with cyclic cows in good body condition (Roche et al., 1992; Baruselli et al., 2004). This phenomenon may be a result of reduced concentrations of GnRH from the hypothalamus, reducing the release of LH from the pituitary, under those conditions (Yavas and Walton, 2000). Stimulation of follicle growth by eCG treatment increased the size of the DF at ovulation (Sá Filho et al., 2010), and increased concentrations of P after timed AI (Baruselli et al., 2004), thereby resulting in the subsequent formation of a larger corpus luteum (CL) post-ovulation. Larger CL synthesizes more P than smaller CL (Vasconcelos et al., 1999, 2001), thus CL quality may be a viable reason for improved PR at fixed-time AI. In contrast, not all studies in which cows were treated with eCG have resulted in increased PR (Pinheiro et al., 2009); therefore, it is not known whether effects of eCG on follicle and CL development may improved pregnancy outcomes.

Calf removal (CR) also may stimulate GnRH release. It has been shown that suckling results in reduced LH secretion from the anterior pituitary gland

(Radford et al., 1978; Dunlap et al., 1981). Suckling increased endogenous opioid concentrations in hypophyseal portal blood, inhibiting GnRH secretion and consequently pituitary FSH and LH release (Gordon et al., 1987). When used in combination with a timed AI protocol, temporary CR has been reported to be an inexpensive and effective manipulation to improve response to GnRH treatment, increase DF diameter before timed AI, and improve conception rates in suckled beef cows (Meneghetti et al., 2001; Baruselli et al., 2004; Duffy et al., 2004; Sá Filho et al., 2009; Pinheiro et al., 2009; Small et al., 2009; Sá Filho et al., 2010).

In 2 experiments we hypothesized that eCG and CR would increase DF diameter, estradiol (E) concentrations, and LH secretion; thereby increasing incidence of ovulation. The combination of both treatments (eCG plus CR) would further stimulate E and LH secretion, resulting in an additive effect for increased incidence of ovulation and improved synchrony of ovulation; and that eCG treatment would improve PR in suckled beef cows.

Materials and Methods

Experiment 1 (Exp. 1) was conducted at the North Florida Research and Education Center (NFREC) in Marianna, FL in April, 2009. Experiment 2 (Exp. 2) was conducted at three locations in Kansas, between April and June of 2009. Both experiments were performed in compliance with the University of Florida and Kansas State University Institutional Animal Care and Use Committee (IACUC) guidelines.

Animals and treatments

Experiment 1. In Exp. 1, 35 multiparous suckled crossbred beef cows with a mean BCS of 4.8 ± 0.7 (range 4 to 7), and 34 ± 7.3 d postpartum (range 19 to 40

d), were assigned randomly to a 2 x 2 factorial arrangement of 4 treatments: 1) cows received the 7-d CO-Synch + CIDR protocol (Larson et al., 2006), which included 100 µg of GnRH i.m. (d -7; 2 mL of Cystorelin, Merial, Duluth, GA) and a CIDR (EAZI-Breed CIDR containing 1.38 g of P; Pfizer Animal Health, New York, NY) insert followed in 7 d by 25 mg PG (d 0; 5 mL of Lutalyse; Pfizer Animal Health) and the CIDR was removed, followed in 72 h (d 3) by timed AI and a second 100 µg injection of GnRH (Control; n = 9); 2) similar to Control, but calves were removed from their dams for 72 h between d 0 and 3 (COCR; n = 9); 3) similar to Control, but cows received 400 IU of eCG (Pregnecol; Bioniche Animal Health, Atlanta, GA) on d 0 (COeCG; n = 9); and, 4) similar to COCR but cows received 400 IU of eCG on d 0 (eCGCR; n = 8). During CR, calves were housed in an open-faced barn with a*d libitum* access to perennial peanut hay and water, and were located no closer than 100 m from their dams (Figure 3-1).

In Experiment 2 (Exp. 2), 513 suckled beef cows (purebred and crossbred Angus, Simmental, and Hereford), at 3 different locations were enrolled in a 7-d CO-Synch + CIDR protocol. Cows were assigned randomly to a nontreated Control (n = 261) or to receive eCG (COeCG; n = 252) at the time of PG injection and CIDR removal (d 0), however, the interval from PG to timed AI was 66 h and 200 IU of eCG were administered to the COeCG group. Body condition scores averaged 5.7 ± 0.71 (range 3 to 7) and cows were 71.9 ± 16 d postpartum (range 17 to 99) at the time of AI (Figure 3-1).

Ultrasound scanning and blood sample collection

Ovaries of all cows in Exp. 1 were examined on d -7, 0, 1, 2, 3, 4 and 10 by transrectal ultrasonography (5.0-MHz linear array transducer, Aloka SSD-500,

Aloka Co. Ltd, Wallingford, CT) and all structures were mapped to monitor changes in CL volume and follicular diameter. The vertical and horizontal diameters of the largest follicle on each ovary and all CL present were measured and recorded. Volume of CL tissue was calculated using the formula $V = 4/3\pi r^3$ where r = ½ of the estimated diameter of the CL (average of vertical and horizontal diameter). In cases where CL had fluid-filled cavities, the volume of the cavity was subtracted from the total volume of the CL. Pregnancy diagnosis in Exp. 1 was performed 30 d after timed AI and in Exp. 2 was performed between 30 and 35 d and a second diagnosis between 60 and 65 d after timed AI.

Blood sample collection

Blood samples were collected via coccygeal vessel puncture using 10 mL Vacutainer tubes with no additive, silicone-coated/K_3EDTA interior (Becton & Dickinson Vacutainer Systems; Rutherford, NJ) on d -14, -7, every 4 h between d 0 and 3, every 2 h between d 3 and 4, and once on d 10 in Exp. 1. In Exp. 2, blood samples were collected on d -14, -7, 0, 3, and at both pregnancy diagnoses. Blood was refrigerated 24 to 30 h after collection, centrifuged for 20 min at 1,500 x *g* at 4° C. After centrifugation a pipette was used to siphon plasma into polypropylene vials (12 x 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), which were stored at -20° C until analyses.

Analysis of hormones

Concentrations of plasma P were analyzed by ELISA to determine cycling status. The ELISA procedure was adopted from that previously described by Rasmussen et al. (1996). Quality controls were established using 100 µL plasma

with a known P concentration of 2.5 ng/mL. Standards were determined with 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 ng/mL concentrations with a duplicate of each respective standard. Assay sensitivity for a 100 µL sample was 0.1 ng/mL. Pooled samples revealed that the intra- and inter-assay CV were 5.4 and 12.7% for 14 plates, respectively.

Plasma concentrations of LH were analyzed by RIA, using methodology described previously by Perry and Perry (2008). Inter- and intra-assay CV were 5.3 and 8.0%, respectively, and assay sensitivity was 0.125 ng/mL.

Commercial RIA kit (Estradiol Double Antibody, Siemens Healthcare Diagnostics, Los Angeles, CA) previously validated for use in bovine (Siddiqui et al., 2009) was used to analyze plasma concentrations of E. Sensitivity of the assay was 0.39 pg/mL, and the intra and inter-assay CV was 10 and 17%, respectively. Samples were analyzed in duplicate and reassayed when the coefficient of variation between duplicates was > 0.20. Plasma from a cow exhibiting estrus was used as positive and charcoal-stripped plasma from a male calf was used as negative quality controls, respectively.

In Exp. 2, blood samples were assayed for P using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA USA; Stevenson et al., 2011). The sensitivity of the assay was 0.004 ng/mL, and the intra- and inter-assay CV for 13 assays were 8.2 and 8.6%, respectively. Blood collected on d -14 and - 7 was used to verify the presence of a functional presence of a CL (when concentrations of P exceeded 1 ng/mL) at the onset of treatments. If any one of the first two samples contained serum P > 1

ng/mL (typical of cows in the luteal phase of the estrous cycle), cows were assumed to be cycling before the onset of treatments (d -7). If concentrations in the first two samples were <1 ng/mL, cows were considered to be noncycling.

Statistical analyses

Experiment 1 was designed as a 2 × 2 factorial arrangements of 4 treatments With CR and eCG as main effects. Cow was considered to be the experimental unit. Continuous data (follicle diameter, and concentration of P, E, and LH) were analyzed by ANOVA for repeated measures using the PROC MIXED procedure of SAS (SAS Inst. Inc.; Cary, NC), where day was the repeated factor and cow was the subject. Categorical data (PR, proportion of ovulation, and proportion of cows with a detectable LH peak before 72 h after PG) were analyzed using the PROC GLIMMIX procedure of SAS, specified as binary distribution. The model included main effects for CR and eCG and their respective interactions. Follicle diameter and concentrations of hormones on d 0 were used as a covariate in their respective analyses of subsequent measurements in time. Within cow, the time of peak of LH was calculated to be the interval between basal concentrations of LH and when the concentration of LH exceeded the basal concentrations by 5-fold, followed by a significant decrease in concentration before 72 h after PG.

Experiment 2 was designed as a completely randomized block design in which cow was the experimental unit. The LOGISTIC procedure of SAS (SAS Inst. Inc.) was used to analyze categorical data (PR, pretreatment cycling status, rate of luteolysis, and rate of pregnancy loss). A backward stepwise regression

model was used, and variables were sequentially removed from the model by the Wald statistic criterion when $P > 0.20$. The model for PR included treatment, technician, cycling status, location, parity, sire, and the respective interactions as explanatory variables. The GLM procedure of SAS (SAS Inst. Inc.) was used to analyze concentrations of P at the first and second pregnancy diagnosis. The model included treatment, cycling status, location and parity as independent variables.

Pregnancy rate, incidence of ovulation, and proportion of cows having a detectable LH peak before 72 h after PG are reported as actual proportions. In contrast, all remaining values are reported as LSM ± SE. Significance was established at $P < 0.05$ and a tendency was considered when $0.05 < P \leq 0.10$.

Results

Experiment 1

There was no statistical significance on the effect of treatments on ovulation rate between d 3 and 4 ($P = 0.62$), percentage of cows experiencing LH surge within 72 h after PG ($P = 0.57$), percentage of ovulation between d 3 and 10 ($P = 0.60$), and overall PR ($P = 0.25$; Table 4-1). The analysis of the main effects of CR and eCG indicated no statistical difference ($P = 0.85$) observed on the percentage of cows expressing an LH surge prior to the second GnRH between cows receiving (6 of 17; 36%) or not receiving (6 of 18; 33%) the eCG treatment. In contrast, a greater ($P = 0.02$) percentage of cows exposed to CR (9 of 17; 52%) had experienced an LH peak prior to the second GnRH than cows not exposed to CR (3 of 18; 16%; Table 4-1).

Cows exposed to CR (11 of 17; 64%) had greater (P = 0.02) proportion of ovulation between d 3 and 4 compared with cows not exposed to CR (5 of 18; 27%); however, cows treated with eCG (8 of 17; 47%) had similar (P = 0.82) proportion of ovulation between d 3 and 4 compared with cows not treated with eCG (8 of 18; 44%; Table 4-1).

A greater (P = 0.01) percentage of cows exposed to CR (17 of 17; 100%) had ovulated by d 10 compared with cows not exposed to CR (13 of 18; 72%). In contrast, cows receiving eCG (15 of 17; 88%) or not receiving eCG (15 of 18; 83%) had similar (P = 0.60) proportion of ovulation by d 10 (Table 4-1).

Cows that had an LH peak before the second GnRH (8 of 12; 66%) had greater (P < 0.0001) PR than cows with an LH peak induced by GnRH (1 of 22; 4%). In addition, PR were greater (P = 0.04) for cows exposed to CR (7 of 17; 41%) compared with cows not exposed to CR (2 of 17; 12%). Cows treated with eCG (5 of 17; 29%) had similar (P = 0.51) PR than cows not treated with eCG (4 of 18; 22%; Table 4-1).

There was a negative correlation (r = -0.42; P = 0.01) between ovulation to the first GnRH and ovulation to the second GnRH. As expected, there was also a positive correlation (r = 0.78; P < 0.001) between LH peak before the second GnRH and ovulation rate between d 3 and 4 (12 of 16; 75% vs. 0 of 19; 0% for ovulation between d 3 and 4 for cows experiencing LH peak prior to or after second GnRH injection, respectively).

There was no statistical significance (P = 0.99) on the effect of treatments on follicle diameter. In addition, follicle diameter on d 3 was not affected (P =

0.26) by the main effect of CR and was similar for cows exposed (14.2 ± 0.5 mm) or not exposed (13.5 ± 0.5 mm) to CR (Figure 4-2). In contrast, follicle diameter on d 3 was greater (P = 0.04) for cows exposed to eCG (14.7 ± 0.6 mm) compared with cows not exposed to eCG (13.0 ± 0.5 mm; Figure 4-3). Follicle growth rate on cows exposed to CR (0.77 ± 0.27 mm/d) were similar (P = 0.14) than cows not exposed to CR (0.23 ± 0.26 mm/d). In contrast, follicle growth rate was greater (P = 0.02) on cows exposed to eCG (0.93 ± 0.24 mm/d) compared with cows not exposed to eCG (0.07 ± 0.24 mm/d).

The releasing patterns and concentrations of hormones were affected by treatments. There was a significant interaction (P = 0.04) between CR and eCG on concentrations of E. The COCR (8.2 ± 1.0 pg/mL) and eCGCR (8.5 ± 0.9 pg/mL) treatments had greater (P = 0.03) concentrations of E after PG than Control (4.9 ± 1.2 ng/mL) and COeCG (4.6 ± 1.1 ng/mL). In addition, eCGCR (11.7 ± 1.6 pg/mL) had greater (P = 0.03) concentrations of E at 44 h after PG compared with Control (6.9 ± 1.7 pg/mL), COCR (7.1 ± 1.5 pg/mL), and COeCG (7.5 ± 1.7 pg/mL) treatments (Figure 4-4).

Concentrations of P on d 10 tended (P = 0.08) to be greater for cows exposed to CR (2.8 ± 0.3 ng/mL) compared with cows not exposed to CR (2.1 ± 0.3 ng/mL) and for cows exposed to eCG (2.8 ± 0.3 ng/mL) compared with cows not exposed to eCG (2.1 ± 0.3 ng/mL; Table 4-1).

Experiment 2

Pregnancy rates at d 35 after timed AI did not differ (P > 0.10) between Control (49.8%) and COeCG (42.9%; Table 4-2). There was no statistical significance (P > 0.10) on the effect of cycling status or the interaction treatment

× cycling status on pregnancy rates (Table 4-2). Pregnancy rates differed (P < 0.05) by location and ranged from 34 (location 1) to 59% (Location 2).

Concentrations of P were similar on d 35 of pregnancy (6.0 ± 0.3 and 6.4 ± 0.4) and on d 67 (6.6 ± 0.4 and 6.4 ± 0.3) for COeCG and Control groups, respectively (Table 4-2).

Pretreatment cycling status differed (P < 0.01) among locations (Location 1 = 76.5%; location 2 = 54.3%; and location 3 = 27.4% cycling; Table 4-3). For cows with elevated (>1 ng/mL) P at CIDR insert removal, 97.4% experienced luteolysis by timed AI, and 17.3% of cows had low (<1 ng/mL) P at CIDR removal and at timed AI, and 1.2% with increasing P from insert removal to timed AI.

Herd, cycling status, technician, and treatment influenced PR. Cycling cows were 1.5 times more likely (P = 0.046; 95% CI = 1.01-2.27) to conceive compared with noncycling cows. Control cows were 1.5 times more (P = 0.036; CI = 1.03-2.13) likely to conceive than those treated with eCG. Cows at location 3 were 1.8 to 3.5 times more (P = 0.004; CI = 1.1-5.6) likely to conceive than cows at other locations. Pregnancy loss to d 67 did not differ between treatments (3.7 vs. 2.3% for eCG vs. Control), respectively.

Discussion

Numerous reports have indicated that CR before timed AI improved fertility in beef cows (Meneghetti et al., 2001; Baruselli et al., 2004; Duffy et al., 2004; Pinheiro et al., 2009; Small et al., 2009; Sá Filho et al., 2009; 2010). Endorphins are known to reduce GnRH neuronal activity thus decreasing the secretion of GnRH into the portal vessels that impact anterior pituitary function (Cox and Britt, 1982; Malven and Hudgens, 1987; Myers et al., 1989; Rund et al., 1989). Calf

removal and the temporary cessation of the suckling stimulation reduce the release of endorphins into the hypothalamus, and consequently, GnRH pulses are elevated (Edwards, 1985; Shively and Williams, 1989), resulting in increased LH pulse frequency. Increasing LH pulses stimulate DF growth, ovulation, and luteinization (Schallenberger et al., 1984; Walters et al., 1984), thereby increasing the opportunity for conception to occur.

Shively and Williams (1989) demonstrated that pulses of LH increase gradually from the time of CR to 48 h and remain elevated for as long as 144 h if CR is maintained. Calf removal before timed AI in estrus- or ovulation-synchronization protocols improved conception in most (Kiser et al., 1980; Smith et al., 1979; Yelich et al., 1995; Geary et al., 2001a; Sá Filho et al., 2009; Peres et al., 2009), but not all reports (Geary et al., 2001b; Pinheiro et al., 2009). In Exp. 1, CR increased PR (41 vs. 12%, for CR and no CR, respectively), perhaps because a greater percentage of cows exposed to CR had an LH surge before timed AI (52 vs. 16%) and ovulation by 24 h after timed AI (64 vs. 27%). Every cow (12 of 35 cows) that had an LH surge by 72 h after PG, ovulated between 72 and 96 h after PG, and 62% of those cows conceived. It has been reported that fertilization was improved when the time of ovulation and AI were synchronized (Waberski et al., 1994; Roelofs et al., 2006). In addition, embryo quality was improved when AI was performed 24 to 12 h before ovulation in dairy cattle (Roelofs et al., 2006). Meneghetti et al. (2001) reported an increase in the diameter of the DF and incidence of ovulation when CR was performed before timed AI. Our data indicate that follicle diameter on d 3 did not differ between

cows either exposed or not exposed to CR. However, in cows where follicles had not ovulated by d 4, follicles of cows receiving CR had a greater diameter than those not exposed to CR (data not shown).

Equine chorionic gonadotropin, which binds to FSH and LH receptors of follicular cells to stimulate follicular growth (Soumano and Price, 1997), has been used as a complementary hormone to improve fertility in beef cows; however, reported results have been inconsistent. The inconsistent data on improvement of fertility by eCG application may be attributed to several factors, such as malnutrition, estrous cyclic status, and stage of the estrous cycle when the treatment was applied. Each of these conditions has been reported to result in different fertility outcomes. Cows that were nutritionally stressed or in low BCS had greater PR when treated with eCG, but eCG did not improve PR in cows with good BCS (Bó et al., 2003; Souza et al., 2009).

Previous report indicated that follicle growth rate and PR was improved in cows receiving eCG and the effect was more pronounced in anestrous cows and cows in low BCS (Baruselli et al., 2004; Sá Filho et al., 2010; Sales et al., 2011). In addition, the treatment with eCG may stimulate more than one ovulation when cows were treated in the absence of a DF, plus stimulates the growth and ovulation of a single DF (Duffy et al., 2004). Moreover, the treatment with eCG increased concentrations of P after timed AI, despite similar diameter of the pre-ovulatory follicle (Baruselli et al., 2004). In Exp. 1, eCG increased growth rate and the diameter of the DF, but did not increase the proportion of cows that ovulated assessed on d 10 compared with cows that did not receive eCG.

Concentrations of P 7 d after timed AI tended to be greater when eCG was administered, which concurs with results reported in dairy cattle (Souza et al., 2009). Our data concur with the report from Sales et al. (2011) that indicated that eCG increased follicle growth rate, diameter of the DF, and conception rates in *Bos indicus* beef cattle. In contrast, Pegorer et al. (2011) reported no improvements in follicle diameter, proportion of ovulation or PR in *Bos indicus* beef heifers treated with eCG 2 d before timed AI. In a review, Bó et al. (2003) reported that eCG had an additive effect with estradiol benzoate on PR, especially in cows with small follicles. In Exp. 1, an additive effect of eCG and CR occurred for an increase in concentrations of E, but not in follicle diameter, percentage of cows with peak of LH before second GnRH, proportion of ovulation, or PR. In Exp. 2, the overall PR was not improved in suckled beef cows treated with 200 IU of eCG at the time of PG injection. Treatment with eCG in cows with BCS ≤ 4 seemed to have a positive impact on PR (35 vs. 47% for control and eCG, respectively; data not shown) but an insufficient number of cows with low BCS precluded the possibility to detect a difference. Failure of eCG to improve PR could have occurred because an inadequate dose (200 IU) was applied in Exp. 2. Previous reports indicate that 300 (Pegorer et al., 2011; Sales et al., 2011) or 400 IU (Baruselli et al., 2004; Sá Filho et al., 2009, 2010; Small et al., 2009; Souza et al., 2009) of eCG were effective in enhancing PR in suckled beef cows. In addition, Bó et al. (2003) and Souza et al. (2009) reported that eCG improved PR in cows with poor BCS and short postpartum intervals;

however, cows in Exp. 2 were in good BCS (mean = 5.7 ± 0.7) with relatively long postpartum intervals (mean = 71 ± 16 d) at timed AI.

The variability in results among reports indicating various pregnancy outcomes after administration of eCG likely may arise from the use of different breeds and biotypes. It seems that eCG had greater effects in cows in anestrous, in poor BCS, with small follicles, or nursing their first calf. A majority of these reports were conducted using *Bos indicus* cattle (Baruselli et al., 2004; Sá Filho et al., 2009, 2010; Pegorer et al., 2011). *Bos indicus* cattle tend to have a greater incidence of anestrous and poorer BCS at initiation of the breeding season (Sá Filho et al., 2009) and generally have smaller follicles compared with *Bos taurus* cattle (Figueiredo et al., 1997; Sartori and Barros, 2011). Therefore, the impact of eCG may be greater in *Bos indicus* than in *Bos taurus* cattle. In contrast, eCG may have an impact in *Bos taurus* cattle exposed to sub-optimal nutritional environments (Baruselli et al., 2004; Small et al., 2009; Souza et al., 2009).

Conclusion

Equine chorionic gonadotropin is viable alternative to increase follicle growth rate and diameter before timed AI. Calf removal was more effective at stimulating an LH surge and ovulation than eCG, and the combination of both treatments did not have a positive additive effect on fertility. The variability of pregnancy outcomes in response to eCG may be related to the dose and timing of eCG treatment, BCS, and days postpartum of the cows. More research is warranted to clarify the effect of eCG in low BCS cows.

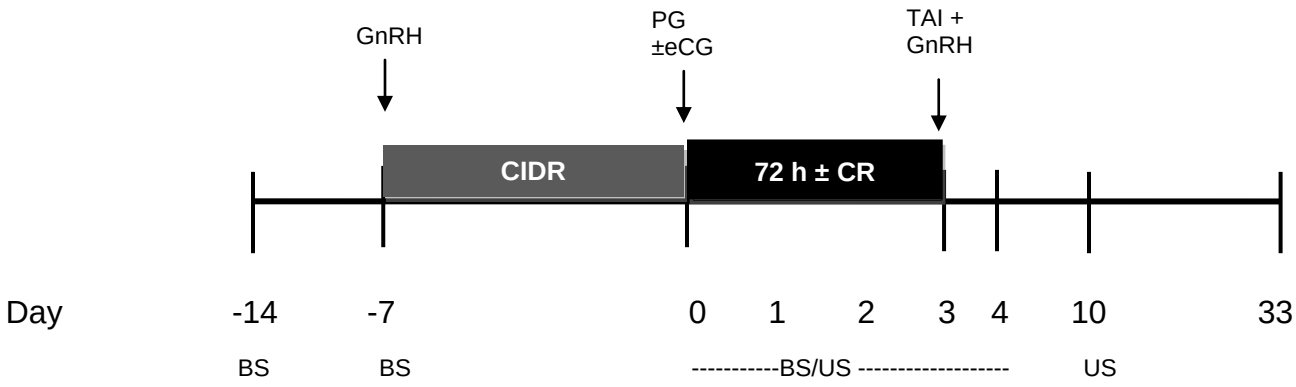

Figure 4-1. Experimental protocol: Cows received a 7-d CO-Synch + CIDR protocol (100 µg of GnRH i.m. [d -7] and a CIDR insert followed in 7 d by 25 mg PG [d 0] and CIDR removal, followed in 72 h [d 3] by TAI and an injection of GnRH). In Exp.1 cows were assigned to 1 of 4 treatment: 1) Control (regular protocol); 2) regular protocol plus calf removal (CR) between d 0 and 3 (COCR); 3) regular protocol plus an injection of 400 IU of eCG on d 0 (COeCG); 4) regular protocol plus an injection of 400 IU of eCG on d 0 and CR between d 0 and 3 (eCGCR). In Exp. 2 cows received the Control and COeCG treatments, however AI was performed at 66 h after PG and 200 IU of eCG were administered on d 0. In Exp. 1 blood sample (BS) were collected on d -14, -7, every four hours between d 0 and 3, every 2 h between d 3 and 4, and once on d 10. In Exp. 2, BS were collected on d -17, -7, 0, 3, and at both pregnancy diagnoses. CIDR = controlled internal device release; PG F2α = Prostaglandin F2α ; GnRH = gonadotropin releasing hormone; TAI = fixed-time artificial insemination.

Table 4-1. Incidences of ovulation, percentage of cows experiencing LH peak, overall PR, and concentrations of progesterone in Exp. 1.

	Treatment[a]				Main effects[b]			
Item	Control	COCR	COeCG	eCGCR	No CR	CR	No eCG	eCG
	------- no./no. (%) -------				------- no./no. (%) -------			
Ovulation to first GnRH[c]	4/9 (44)	3/9 (33)	3/9 (33)	2/8 (25)	7/18 (38)	5/17 (29)	7/18 (38)	5/17 (29)
Ovulation between d 3 and 4	1/9 (11)	6/9 (66)	3/9 (33)	5/8 (62)	5/18 (27)^{x}	11/17 (64)^{y}	8/18 (44)	8/17 (47)
LH peak < 72 h[d]	1/9 (11)	5/9 (55)	2/9 (22)	4/8 (50)	3/18 (16)^{x}	9/17 (52)^{y}	6/18 (33)	6/17 (36)
Ovulation by d 10[e]	6/9 (67)	9/9 (100)	7/9 (78)	8/8 (100)	13/18 (72)^{x}	17/17 (100)^{y}	15/18 (83)	15/17 (88)
Overall PR[f]	0/9 (0)	4/9 (44)	2/8 (25)	3/8 (37)	2/18 (12)^{x}	7/17 (41)^{y}	4/18 (22)	5/17 (29)
		---------- ng/mL ----------				---------- ng/mL ----------		
P on d 10[g]	1.4 ± 0.4	2.7 ± 0.4	2.7 ± 0.4	2.8 ± 0.4	2.1 ± 0.3^{w}	2.8 ± 0.3^{z}	2.1 ± 0.3^{w}	2.8 ± 0.3^{z}

[a]Cows were assigned on d 0 to the following treatments; Control, COCR, COeCG, and eCGCR.
[b]Main effects of calf removal (CR; COCR and COeCG) or no CR (Control and COeCG treatments); and eCG (COeCG and eCGCR) or no eCG (Control and COCR treatments).
[c]Cows that experienced ovulation to the first GnRH injection assessed on d 0 by ultrasonography.
[d]Cows experiencing peak of LH within 72 h after prostaglandin injection.
[e]Percentage of cows that ovulated between d 3 and 10 assessed on d 10
[g]Pregnancy rate
[xy]Means within a row differ ($P < 0.05$).
[wz]Means within a row tend to differ ($0.05 < P < 0.10$).

Table 4-2. Pregnancy rates and concentrations of progesterone (P) in pregnant cows in Exp. 2.

Item	[a]Treatment	
	Control	eCG
Location pregnancy rates	---------- no./no. (%) ----------	
Location 1	36/75 (48)	35/76 (46)
Location 2	33/96 (34)	28/88 (31)
Location 3	56/91 (65)	45/89 (50)
Total	130/262 (50)	108/253 (43)
Cycling status PR[b]		
Anestrus	63/125 (50)	50/117 (43)
Cyclic	67/136 (49)	58/135 (43)
P[c]	---------- ng/mL (n) ----------	
Day 35	6.4 ± 0.3 (126)	6.0 ± 0.4 (100)
Day 67	6.4 ± 0.3 (123)	6.6 ± 0.3 (97)

[a]Cows were assigned to receive eCG (COeCR) or no eCG (Control) at the day of PG.
[b]PR = Pregnancy rates.
[c]Concentrations of progesterone

Table 4-3. Ovarian function and pregnancy outcomes by location in Exp. 2.

		Pattern of progesterone concentrations[a]						
Location	n	Pretreatment cycling status[b]	CL lysis[c]	Premature CL lysis[d]	Anestrus[e]	Metestrus[f]	Pregnancy rate per AI[g]	Pregnancy loss[h]
1	183	76.5^{y}	91.2	5.0	3.3^{y}	0.6	33.9^{y}	6.5^{x}
2	151	54.3^{y}	88.5	0.7	3.4^{y}	0.0	47.0^{y}	4.2^{x}
3	179	27.4^{x}	62.3^{x}	6.3^{x}	28.6^{x}	1.7	58.7^{x}	0.0^{y}

[a] Proportion of cows having high (≥ 1 ng/ mL) or low (< 1 ng/mL) progesterone on d −14, −7, 0, or 3.
[b] Proportion of cows cycling before placement of CIDR insert on d −7.
[c] Proportion of cows having high-low concentrations of progesterone on d 0 and 3, respectively.
[d] Proportion of cows having low concentrations of progesterone on d 0 and 3.
[e] Proportion of cows having low progesterone on d −17, −7, 0, and 3.
[f] Proportion of cows having low-high concentrations of progesterone on d 0 and 3, respectively.
[g] Detected on d 35 post-timed AI.
[h] Between d 35 and 67 of pregnancy.
[yx] Means within columns with different letter superscript differ ($P \leq 0.05$).

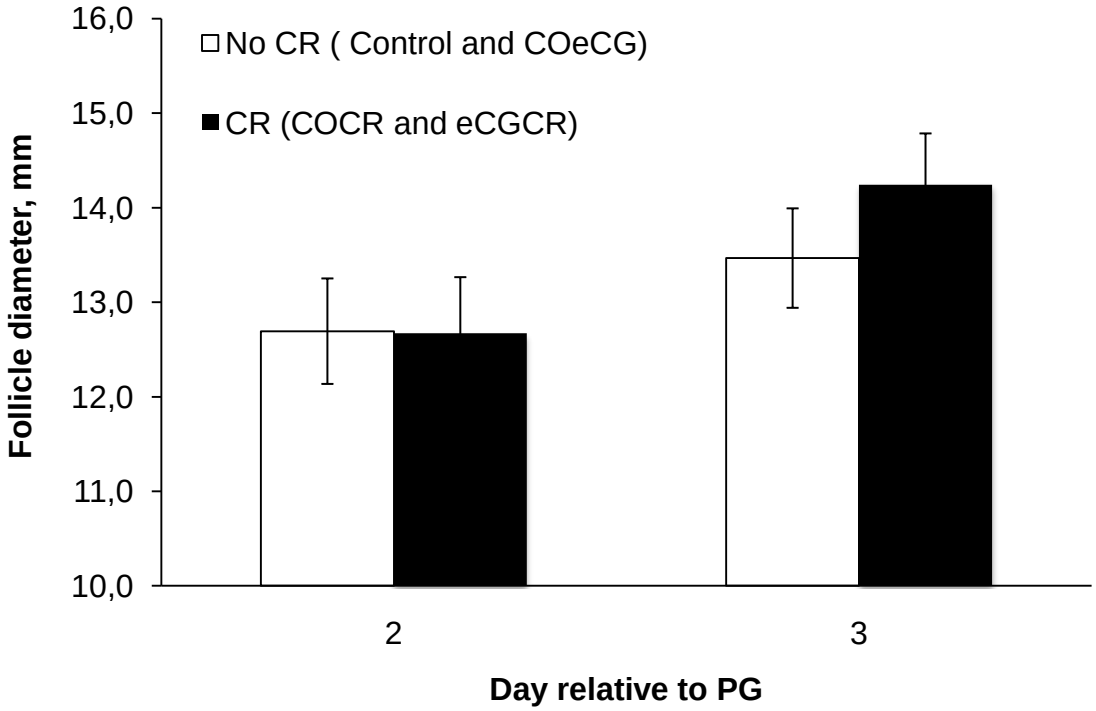

Figure 4-2. Exp. 1: Diameter of the largest follicle present on the ovary associated with cows receiving calf removal (CR) or no CR on d 0.

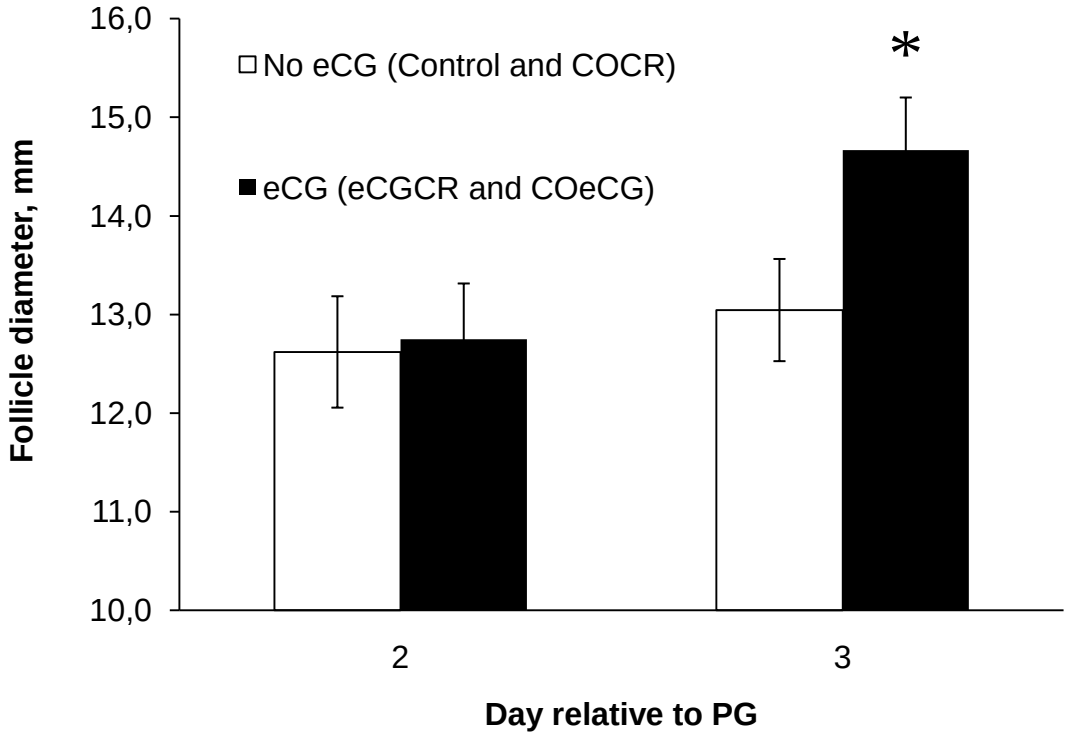

Figure 4-3. Exp. 1: Diameter of the largest follicle present on the ovary associated with cows receiving 400 IU of eCG or Control treatments on d 0. *Treatment with eCG differs from control on d 3 (P = 0.03).

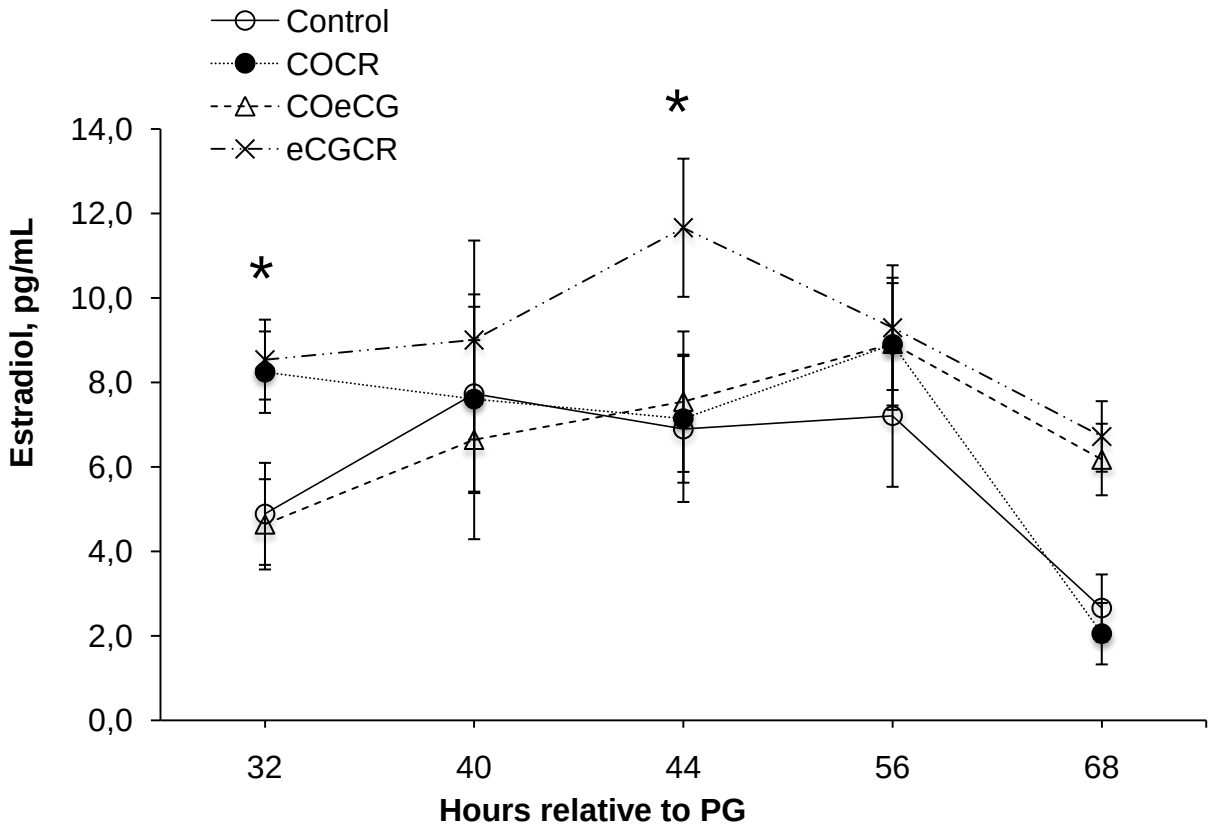

Figure 4-4. Experiment 1: Diameter of the largest follicle present on the ovary associated with Control, COCR, COeCG, and eCGCR on h 0. Least square means were adjusted based on h 28 used as covariate. *Treatment differs on h 32 and 44 (P = 0.03).

CHAPTER 5
CAPABILITY OF A NEW OR ONCE-USED CONTROLLED INTERNAL DRUG RELEASING DEVICE (CIDR) TO DEVELOP PERSISTENT FOLLICLES AND INCIDENCE OF INDUCED FOLLICLE TURNOVER IN RESPONSE TO INCREASED PROGESTERONE IN REPLACEMENT BEEF HEIFERS

Two experiments were designed to evaluate the pattern of follicular growth following CIDR insertion in pre-pubertal heifers. Exp. 1 evaluated the incidence of a persistent follicle when a once-used or new CIDR was inserted, as well as the incidence of follicle turnover following insertion of a second new CIDR 8 d later. Exp. 2 evaluated whether an additional new CIDR induced turnover of a growing follicle. In Exp. 1, 59 Brangus pre-pubertal heifers were randomly assigned to a 2 $\times$ 3 factorial arrangement of treatment. On d -9 all heifers received prostaglandin $F_{2\alpha}$ (PG) and on d -8 heifers were randomly assigned to receive one of six treatments; 1) heifers received either a new (NewSal; n = 9) or once-used (for 7 d; UsedSal; n = 10) controlled internal drug releasing device (CIDR; Pfizer Animal Health; New York, NY) containing 1.38 g of progesterone (P) inserted on d -8 and 2 mL i.m. of saline on d 0; 2) heifers received either a new (NewAsp; n = 10) or once-used (UsedAsp; n = 10) CIDR on d -8 and aspiration of all follicles ≥ 5mm on d 0; and, 3) heifers received either a new (NewCIDR; n = 10) or once-used (UsedCIDR; n = 10) CIDR on d -8 and a second additional new CIDR on d 0. All CIDR were removed on d 3. Transrectal ultrasonography was used to scan the ovaries of all heifers on d -8, -5, -3, -1, 0, 2, 3, and 5 for assessment of follicle and corpus luteum (CL) development. Blood samples were collected on d -19, -9, -8, -5, -3, -1, 0, 2, 3, and 5 for analysis of P. A follicle was deemed persistent when the same follicle was observed on d -8 and d 0. Follicle turnover was

defined as the largest follicle on d 0 that regressed by d 5 and new follicular wave was initiated by d 5. New (10 of 25; 40%) and once-used (11 of 27; 40%) CIDR were equally (P = 0.95) effective at inducing persistent follicles. Follicular aspiration (18 of 18; 100%) had a greater (P < 0.0004) capacity to induce follicle turnover compared to those heifers receiving an additional new CIDR (12 of 19; 63%) or saline (8 of 18; 44%). In Exp. 2, 41 Brangus peripubertal heifers with a mean age of 395 ± 12 d and mean body weight (BW) of 485 ± 69 kg received an injection of PG on d -12. On d -10 all follicles ≥ 5 mm were aspirated from all heifers and each heifer received a once-used CIDR. On d 0, heifers were randomly assigned to one of two treatments in a completely randomized design; 1) heifers received a 2 mL i.m. injection of saline (UsedSal; n = 21) or 2) heifers received an additional new CIDR (UsedCIDR; n = 20). All CIDR were removed on d 3. Transrectal ultrasonography of ovaries was performed on d -10, 0, 2, and 4 for assessment of follicle and CL development. Blood samples were collected on d -22, -12, -10, -9, -8, 0 (at 0800, 1200, and 1800 h), 1, 3, 4, and 10 for analysis of P. On d 1 concentrations of P were greater (P < 0.001) for UsedCIDR (7.3 ± 0.5 ng/mL) compared to UsedSal (2.6 ± 0.5 ng/mL). Similarly, on d 3, concentrations of P were greater (P < 0.001) for UsedCIDR (5.1 ± 0.4 ng/mL) compared to UsedSal (1.6 ± 0.4 ng/mL). However, the incidence of follicle turnover was similar (P = 0.12) between UsedCIDR (10 of 18; 55%) and UsedSal (15 of 19; 78%). We conclude that new or once-used CIDR were equally effective at inducing persistent follicles and the additional CIDR or saline were equally effective at inducing follicle turnover of a growing follicle.

Important Remarks

Inclusion of exogenous progesterone (P) in cattle estrous synchronization protocols improves conception rates to artificial insemination (Macmillan and Peterson. 1993; Stevenson et al., 2003; Lamb et al., 2006, 2010). Progestins may be used as an ovulatory inhibitor during an estrous synchronization protocol (Lamb et al., 2001, 2006). However, persistent follicles may result with the insertion of a controlled internal drug releasing device (CIDR) in the absence of a corpus luteum (CL; Sirois and Fortune, 1990). Persistent follicles result in compromised fertility as a result of compromised oocyte quality (Savio et al., 1993; Anderson and Day, 1994; Mihm et al., 1994; Ahmad et al., 1995; Cerri et al., 2009). During the 7-d CO-Synch + CIDR estrous synchronization protocol (Lamb et al., 2006) GnRH is administered at CIDR insertion to induce ovulation of a dominant follicle (DF) and initiate a new follicular wave (Pursely et al., 1995; Lamb et al., 2006; Larson et al., 2006). Follicles that fail to ovulate to the first GnRH may persist in the presence of low concentrations of (P) provided by the CIDR. However, increased concentrations of P may turnover persistent follicles and initiate a new follicular wave (Adams et al., 1992). Therefore, fertility of current estrous synchronization protocols that utilize a CIDR for supplementation of P may be compromised.

Two experiments were designed to evaluate the pattern of follicular growth following CIDR insertion in pre-pubertal heifers. Exp. 1 evaluated the incidence of persistent follicles when a new or once-used (for 7 d) CIDR was inserted and the incidence of follicle turnover following insertion of a second new CIDR 8 d after receiving a new or used CIDR. Exp. 2 evaluated the ability of a new CIDR to

induce follicular turnover of a growing follicle. Our hypotheses were: 1) a used CIDR induces a greater percentage of persistent follicles than a new CIDR; and 2) the insertion of a new CIDR elevates concentrations of P sufficient to induce follicular turnover.

Materials and Methods

Experiments were conducted at the North Florida Research and Education Center (NFREC) in Marianna, FL. Exp. 1 was conducted in the spring of 2009, and Exp. 2 in the spring of 2010. Both experiments were performed in compliance with the University of Florida Institutional Animal Care and Use Committee (IACUC) approval.

Animals and treatments

Experiment 1. In Exp. 1, 59 Brangus pre-pubertal heifers at a mean age of 351 ± 29 d (mean ± SD) and a mean weight of 339 ± 42 kg (mean ± SD) were randomly assigned to a 2 × 3 factorial arrengements of treatments (Figure 5-1). On d -9 all heifers received prostaglandin $F_{2\alpha}$ (PG; 5 mL of Lutalyse; Pfizer Animal Health; New York, NY) and on d -8 heifers were randomly assigned to receive one of six treatments; 1) heifers received a new (NewSal; n = 9) or once-used (UsedSal; n = 10) CIDR (Pfizer Animal Health; New York, NY) containing 1.38 g of P for 7 d on d -8, and 2 mL i.m. of saline on d 0; 2) heifers received a new (NewAsp; n = 10) or once-used (UsedAsp; n = 10) CIDR on d -8 and aspiration of all follicles ≥ 5mm on d 0; 3) heifers received a new (NewCIDR; n = 10) or once-used (UsedCIDR; n = 10) CIDR on d -8 and a second additional new CIDR on d 0. All CIDR were removed on d 3.

Experiment 2. In Exp. 2, 41 peripubertal Brangus heifers at a mean age of 395 ± 12 d (mean ± SD) and mean weight of 485 ± 69 kg (mean ± SD) received an injection of PG on d -12. On d -10, follicles ≥ 5 mm were aspirated and heifers received a once-used CIDR. On d 0, heifers were randomly assigned to one of two treatments in a completely randomized design (Figure 5-1): 1) a 2 mL i.m. injection of saline (UsedSal; n = 21); and 2) insertion of an additional new CIDR (UsedCIDR; n = 20). All CIDR were removed on d 3.

All once-used CIDR inserts had been obtained from cows involved in a previous estrous synchronization of ovulation experiment, and had been inserted for 7 d. Immediately after removal, CIDR inserts were placed in an empty bucket, washed thoroughly in a chlorhexidine gluconate solution (0.03%), to be disinfected and to remove mucus and debris that had accumulated in empty spaces between the silicon layer and the T-shaped body. Inserts were rinsed thoroughly with water and allowed to air-dry and then autoclaved at 121°C and 724mmHg for 20 min, allowed to cool and placed in zip-lock bags for storage before use.

For heifers on d 0 in Exp. 1 assigned to the NewAsp and UsedAsp treatments and all heifers on d -10 in Exp. 2, follicular aspiration of follicles ≥ 5 mm in diameter were performed. An intravaginal 7.5-MHz convex-array transducer (Aloka SSD-500, Aloka Co. Ltd, Wallingford, CT) was used to guide the transvaginal placement of the aspiration needle. An epidural anesthesia with 5 mL of 2% lidocaine (Lidocaine HCL 2%, AgriLab, St Joseph, MO) was performed before intravaginal insertion of a 17-gauge single lumen needle and

the transducer into the vaginal fornix. Follicles ≥ 5 mm were localized in the ultrasound display and the needle was carefully inserted into the follicles. The follicular fluid was aspirated using a 10 mL syringe connected to the needle. The follicular fluid was discharged after the aspiration. Follicular aspiration was confirmed by the disappearance of the follicle and the appearance of the follicular fluid into the syringe.

Ultrasonography and blood sample collection

In Exp. 1, transrectal ultrasonography of ovaries of all heifers occurred on d -8, -5, -3, -1, 0, 2, 3, and 5, and blood samples were collected on d -19, -9, -8, -5, -3, -1, 0, 2, 3, and 5. In Exp. 2, transrectal ultrasonography of ovaries of all heifers occurred on d -10, 0, 2, and 4; and blood samples were collected on d -22, -12, -10, -9, 0 (at 800, 1200, and 1800 h), 1, 3, 4, and 10.

Transrectal ultrasonography of all ovaries was conducted with a 5.0-MHz linear array transducer (Aloka SSD-500). Ovaries were mapped to monitor changes in CL characteristics and follicular dynamics. The vertical and horizontal diameters of antral follicles ≥ 6 mm in diameter on each ovary and all CL present were measured and recorded. The diameter of the follicles was calculated by the average of the vertical and horizontal measurements. Volume of CL tissue was calculated using the formula $V = 4/3\pi r^3$ where r = ½ of the average value for vertical and horizontal CL measurements. In cases where CL had fluid filled cavities, the volume of the cavity was subtracted from the total volume of the CL.

A persistent follicle was defined as the largest follicle present on d -8 that was also present on d 0 in the absence of initiation of a new follicular wave (Sirois and Fortune, 1990; Anderson and Day, 1994). In Exp. 1, follicle turnover

were defined as the largest follicle present on d 0 that had decreased in diameter by d 3 and a new follicular wave was initiated by d 5. In Exp. 2, follicle turnover was defined as the largest follicle present on d 0 that decreased in diameter by d 4, with initiation of a new follicular wave.

Blood samples were collected via jugular vein using 7.5 mL Vacutainer tubes containing heparin (Becton & Dickinson Vacutainer Systems; Rutherford, NJ). Blood remained on ice for 2 h after collection and then centrifuged for 15 min at 4,000 × *g* at 4° C. After centrifugation a pipette was used to siphon plasma into polypropylene vials (12mm × 75mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and stored at -80° C until analyses.

Hormone assay

Concentrations of plasma P were analyzed in duplicate by RIA using progesterone kits (Coat-A-Count; Diagnostic Products Corp. Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 μl. Assay tubes for the standard curve contained 0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, and 20 ng/ml. Assay sensitivity for a 100-μl sample was 0.1 ng/mL. The intra and inter-assay CV were 6 and 14%, respectively. Heifers were considered to be cycling at the initiation of treatments if at least 1 of 2 blood samples on d -19 and -9 (Exp. 1) or -22 and -12 (Exp. 2) had concentrations of P ≥ 1 ng/mL (Perry et al., 1991), or if a CL was present on the ovary on d -8 (Exp. 1) or d -10 (Exp. 2).

Statistical analyses

Experiment 1. Exp. 1 was designed as a 2 × 3 factorial arrengments of treatments. The main effects of type of CIDR (inserted on -8) and treatments (d 0) were analyzed. The effect of P source from the CIDR (new or once-used) on inducing persistent follicles and treatments on inducing follicular turnover were analyzed using the PROC GLIMMIX procedure of SAS (version 9.2; SAS Inst. Inc.; Cary, NC), specified as binary distribution. Because no significant effect of P source was detected for induction of a persistent follicle, the data from used and new CIDR were pooled to evaluate the effect of treatments on d 0 on follicle turnover. Concentrations of P from d -8 to 5 and follicle diameter (changes in diameter of the same follicle) were analyzed by ANOVA for repeated measures using the PROC MIXED procedure of SAS, where the day was the repeated factor and heifer was the subject.

Experiment 2. Exp. 2 was designed as a completely randomized design. Follicle turnover and percentage of heifers having concentrations of P > 1ng/mL on d 10 were analyzed using the PROC GLIMMIX procedure of SAS (version 9.2; SAS Inst. Inc.; Cary, NC), specified as binary distribution. Concentrations of P and follicle diameter from d 0 to 4 were analyzed by analysis of variance for repeated measures using the PROC MIXED procedure of SAS, where day was the repeated factor and heifer was the subject.

Percentages of incidence of persistent follicles, follicle turnover and heifers with concentrations of P >1 ng/mL on d 10 are reported as real means, however

all remaining values are reported as LSM ± SE. Significance was established at $P < 0.05$ and a tendency was considered when $P = 0.06$ to $P = 0.10$.

Results

Experiment 1

Three heifers were excluded from the trial because, one (in NewCIDR treatment) did not develop a follicle > 5 mm, and two (one each from NewSal and UsedAsp treatments) because they developed a CL after receiving PG. Four heifers (one each from NewAsp, NewSal, UsedAsp, and UsedCIDR treatments) did not have follicle ≥ 6 mm on d -8 and were not included in the persistent follicle analysis. One heifer (exposed to UsedAsp treatment) did not have a DF on d 0 and was not included in the analysis for follicle turnover. Concentrations of P were greater ($P = 0.03$) in heifers exposed to a new CIDR compared to heifers receiving a once-used CIDR on d -5, -3, 0, 2, and 3 (Figure 5-2). In addition, concentrations of P were greater ($P < 0.001$) on d 3 in heifers receiving an additional CIDR (3.3 ± 0.2 ng/mL) on d 0 compared to those heifers exposed to saline (1.6 ± 0.2 ng/mL) or aspiration (1.2 ± 0.2 ng/mL; Figure 5-3).

Induction of a persistent follicle from d -8 to 0 did not differ ($P = 0.95$) for heifers receiving a new (10 of 25; 40%) or once-used (11 of 27; 40%) CIDR. The mean diameter of persisted follicles (12.4 ± 0.4 mm) on d 0 was greater ($P = 0.002$) than follicles that were deemed to be non-persistent (10.7 ± 0.3 mm).

No effects ($P = 0.89$) on the incidence of new or once-used CIDR to induce follicle turnover after d 0 was detected. Therefore, data from heifers receiving a new or once-used CIDR were pooled to evaluate the main effect of an additional CIDR (NewCIDR and UsedCIDR treatments), saline (NewSal and UsedSal

treatments), or aspiration (NewAsp and UsedAsp treatments) on follicle turnover. Aspiration (18 of 18; 100%) induced a greater (P = 0.0004) percentage of follicles to turnover than an additional CIDR (12 of 19; 63%) or saline (8 of 18; 44%; Table 5-1). No treatment × incidence of persistent follicle × follicle turnover rate interaction existed (P = 0.26). The mean follicle diameter on d 0 of follicles that were turned over (11.9 ± 0.3 mm) was greater (P = 0.01) than follicles that failed to turnover (10.4 ± 0.5 mm). Follicle diameter on d 3 did not differ (P = 0.11) between treatments (10.8 ± 0.5, 10.2 ± 0.4, 9.0 ± 0.6 mm for heifers exposed to new additional CIDR, saline, and aspiration, respectively; Figure 5-4).

Experiment 2

Five heifers (two in the Control and three in the CIDR treatment) had follicles ≤ 6 mm in diameter on d 0, and were not included in the follicle turnover or follicle diameter analyses. Concentrations of P were greater (P < 0.001) in heifers exposed to UsedCIDR compared to heifers in the UsedSal treatment at 6 (6.9 ± 0.4 vs. 2.5 ± 0.4 ng/ml), 12 (6.8 ± 0.4 vs. 2.5 ± 0.4 ng/ml), 24 (7.3 ± 0.5 vs. 2.6 ± 0.5 ng/ml) and 72 h after treatment (5.1 ± 0.4 vs. 1.6 ± 0.4 ng/ml; Table 5-1).

There was no difference (P = 0.12) in the ability of the additional new CIDR (10 of 18; 55%) or saline (15 of 19; 78%) to induce follicle turnover. The mean diameter of follicles on d 0 that were deemed to have turned over (10.9 ± 0.3 mm) did not differ (P = 0.91) from follicles that did not turnover (10.8 ± 0.5 mm). Follicles greater than 10 mm tended (P = 0.08) to be more likely to turnover than follicles smaller than 10 mm (odds ratio = 3.8) regardless of treatment. The

number of heifers having concentrations of P ≥ 1 ng/mL on d 10 did not differ (*P* = 0.22) between treatments (8 of 20; 40% vs. 12 of 21; 57% for additional new CIDR and Saline, respectively). In addition, follicle turnover did not affect (*P* = 0.47) the number of heifers having concentrations of P ≥1 ng/mL on d 10 (11 of 25; 44% vs. 6 of 12; 50% for heifers that had or did not have follicle turned over, respectively).

Discussion

Exogenous sources of P have been extensively used in cattle as an aid to suppress estrus and ovulation in estrous synchronization protocols. Intravaginal or oral progestins are included in many protocols with the intention to prevent ovulation and synchronize follicular waves (Lamb et al., 2001, 2006; Larson et al., 2006). The effects of endogenous or exogenous sources of P may inhibit or stimulate LH secretion depending on whether concentrations of P are high or low, respectively (Roberson et al., 1989; Kojima et al., 1995; Bergfeld et al., 1996). Using a progesterone releasing internal device (PRID), Roberson et al., (1989) introduced 2 or 0.5 PRID to simulate normal and low concentration of P in blood, respectively. They observed that cows with low concentrations of P had a greater number of LH pulses during 24 h of blood sample collection on d 10 of the estrous cycle compared to cows with normal concentrations of P. It was observed that the change from high to low and low to high concentrations of P caused an increase and decrease in the number of LH pulses, respectively (Bergfeld et al., 1996). This effect may be similar to physiological P released by the CL during the estrous cycle.

During mid-diestrus the CL releases significant quantities of P and follicle ovulation is inhibited as a result of low frequency pulses of LH (Short et al., 1979); however, in proestrus and estrous, concentrations of P are low and frequency of pulses of LH increase, thereby inducing ovulation (Rahe et al., 1980; Ireland and Roche, 1982). When cows received an exogenous source of P to establish high or low concentrations of P during follicle growth of the first follicular wave, follicle growth was inhibited in cows with high concentrations of P, whereas follicle growth persisted in cows treated with low concentrations of P source (Adams et al., 1992). Sirois and Fortune (1988) induced the formation of persistent follicles (follicles that have a prolonged lifespan; Sirois and Fortune 1988) by treating cows with external device releasing low concentration of P. In addition, Anderson and Day (1994) observed the development of persistent follicle when cows were treated with progestogen-like compound melengestrol acetate (MGA) for 10 d. However, fertility was compromised as a result of poor oocyte quality of those oocytes originating from a persistent follicle (Savio et al., 1993; Anderson and Day, 1994; Ahmad et al., 1995; Cerri et al., 2009).

In Exp. 1 we hypothesized that a once-used CIDR would induce a greater percentage of persistent follicles than a new CIDR when inserted into prepubertal heifers for 8 d. The results indicates that the induction of persistent follicles was similar between heifers receiving a new (40%) or once-used (40%) CIDR, although concentrations of P in heifers receiving a new (3.1 ± 0.2 ng/mL) or once-used CIDR (2.5 ± 0.2 ng/mL) 3 d after device insertion differed between treatments. When P was maintained between 1 and 2 ng/mL persistent follicles

were induced and follicle recruitment was absent (Sirois and Fortune, 1990). In addition, when females received physiological (6 to 8 ng/mL) or high (12 to 16 ng/mL) exogenous doses of P, follicle turnover was induced and a new follicular wave was initiated (Adams et al., 1992; Kojima et al., 1995). In Exp. 1, concentrations of P in heifers receiving either a new or once-used CIDR remained greater than 2.5 ng/mL during the first 3 d following insertion of the CIDR. In addition, in Exp. 2, concentrations of P exceeded 5 ng/mL after two days following once-used CIDR insertion. Concentrations of P during this period may have been sufficiently high to allow induction of follicle turnover.

High concentrations of P deplete both frequency and amplitude of LH pulses, which alters secretion of estradiol (E) released by the follicle (Kojima et al., 1995; Sanchez et al., 1995; Bergfeld et al., 1996). A negative correlation ($r = -0.42$ and -0.39 for exogenous and endogenous source of P, respectively) exists between P and E, indicating that P may indirectly alter E secretion (Bergfeld et al., 1996). Persistent follicles were induced when one but not 2 CIDR were inserted on d 14 of the estrous cycle (Sirois and Fortune, 1990). However, even when a single CIDR was inserted, two heifers developed a persistent follicle and 4 out of 6 heifers had follicles that regressed (Sirois and Fortune, 1990). The P released by the CIDR in addition to the P from the CL increased concentrations of P to exceed 12 ng/mL, which may have been sufficient to reduce secretion of LH and hinder follicle growth. In addition, on d 14 of the estrous cycle, a percentage of follicles may be in a static (not growing) or regressing phase, which may be more susceptible to turnover when exposed to increased P

(Adams et al., 1992; Kojima et al., 1995; Bergfeld et al., 1996). In Exp. 1, follicles that failed to persist did regress with a concomitant initiation of a new follicular wave between d -8 and 0, indicating that P from the CIDR during 3 d after insertion may have been sufficient to induce follicle turnover.

In the current study the mean diameter of persistent follicles on d 0 was greater than that of non-persistent follicles, which concurs with previous reports (Stock and Fortune, 1993; Adams et al., 1992). Persistent follicle diameter is increased as a result of increased steroidogenesis (synthesis of E) by follicular cells, increased intra-follicular concentrations of IGF-I, decreased intra-follicular concentrations of IGBP-2 (Kojima el al., 2003), and increased pulsatility of LH (Roberson et al., 1989; Sirois and Fortune, 1990; Kojima et al., 1995, 2003).

In Exp. 2 once-used CIDR were inserted for 10 d before treatments were applied on d 0 (heifers received additional new CIDR or saline). A once-used CIDR was used because heifers exposed to a once-used CIDR in Exp. 1 had decreased concentrations of P, approaching 1 ng/mL from d -3 to 0. Therefore, a once-used CIDR was more suited to inducing persistent follicles than a new CIDR (Roberson et al., 1989; Adams et al., 1992; Kojima et al., 2003). In addition, the interval from CIDR insertion to treatment was extended from eight to 10 d to ensure adequate follicle development prior to treatments and a gradual decline in concentrations of P after insertion (MacMillan and Peterson, 1993). For Exp. 2 we expected concentrations of P to remain low (≈1 ng/mL) for the 10 d between aspiration and treatment. In addition, all follicles ≥ 5 mm were aspirated to initiate a new follicular wave on d -10. However, mean concentrations of P two

days after once-used CIDR insertion was 5.0 ± 0.4 ng/mL, and on d 0 concentrations of P remained greater than expected (mean = 2.7 ng/mL). Therefore, the greater concentration of P from the once-used CIDR was likely the primary reason for no differences between the CIDR and saline treatments in their ability to induce follicular turnover. Of the heifers receiving saline, 78% of the follicles regressed, which indicated that a once-used CIDR for 13 d was capable of inducing turnover of follicles.

Follicles that turned over following treatment on d 0 were greater on d 0 than those that failed to regress, regardless of treatment in Exp. 1 but not in Exp. 2. The mean diameter of follicles on d 0 (12.0 and 10.8 mm for Exp.1 and Exp. 2, respectively), were significantly smaller than the diameter of persistent follicles reported in previous studies (means exceeding 20.0 mm; Stock and Fortune, 1993; Anderson and Day, 1994). However, in these studies follicles were exposed to extended exposure and lower concentrations to P, whereas in this study exposure to P was for a short duration and concentration of P was higher. In addition, in Exp. 2 aspiration of follicles on d -10 coincident with high P from the CIDR may have compromised follicle growth of the new follicular wave.

Persistent follicles in cows and heifers 11 d after receiving MGA were induced to turnover by injection of P (Anderson and Day, 1994, 1998). In addition, the authors indicated that prepubertal heifers and anestrus cows receiving P that had their follicle regressed, had improved fertility. The quality of oocytes from persisted follicles are compromised (Wehrman et al., 1993; Mihm et al., 1994; Cerri et al., 2009), therefore the turnover of persistent follicles and

development of a new follicular wave with healthy oocytes may improve fertility. Current estrous synchronization protocols using CIDR are shorter than protocols using MGA; however, if ovulation or regression of follicle does not occur within 3 d, synchrony of the follicular wave may be compromised. In Exp. 1 a majority of the new follicular waves from heifers that did not developed persistent follicles were initiated between 3 and 5 d after CIDR insertion. Therefore, the CIDR may disrupt the synchrony of the follicular wave resulting in reduced fertility in heifers that do not respond to the GnRH injection administered at the time of CIDR insertion in the 7-day CO-Synch + CIDR protocol (Lamb et al., 2006).

Conclusion

The insertion of new or once-used CIDR at random stages of the follicular phase induces persistent follicles in some but not all heifers, however the CIDR may also result in asynchrony of the new follicular wave when a follicle does not persist. Fertility of heifers synchronized with the 7-day CO-Synch + CIDR protocol that do not respond to the first GnRH injection may be compromised due to the asynchrony caused by the high P released by CIDR during the first 3 d of insertion.

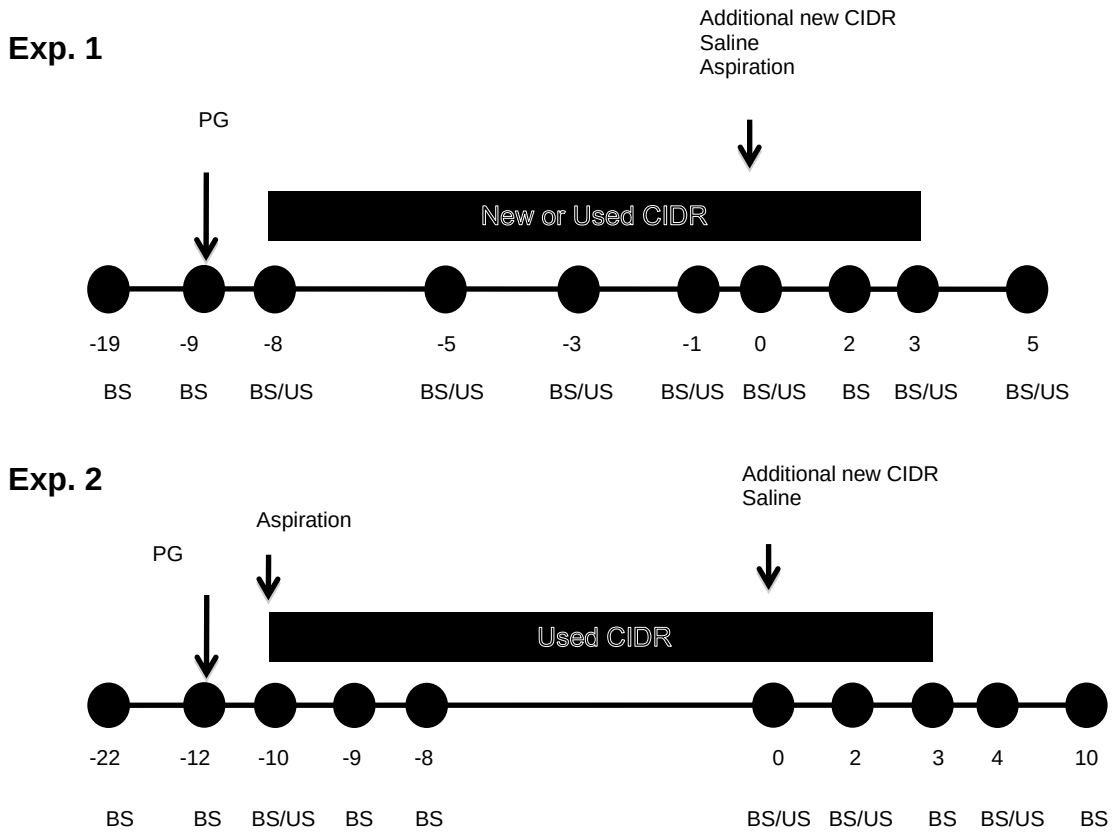

Figure 5-1. Experimental protocol for treatments in Exp.1 (NewCIDR, NewSal, NewAsp, UsedCIDR, UsedSal, and UsedAsp) and Exp. 2 (UsedCIDR and UsedSal). Blood (BS) samples were collected on d -19, -9, -8, -5, -3, 0, 2, 3, and 5 in Exp. 1 and on d -22, -12, -10, 0, 2, 3, 4, and 10 in Exp. 2. Ultrasonography (US) of ovarian follicles was performed on d -8, -5, -3, -1, 0, 3, and 5 in Exp. 1, and on d -10, 0, 2, and 4 in Exp. 2. CIDR = controlled internal device release; PG = Prostaglandin $F_{2\alpha}$.

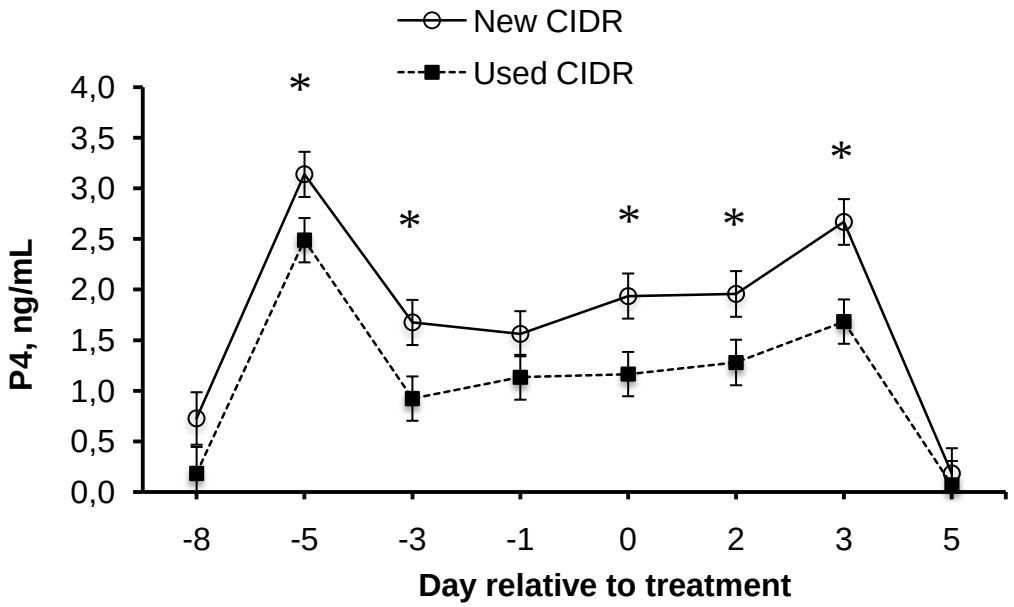

Figure 5-2. Exp. 1, concentrations of P associated with heifers receiving a new or once-used CIDR on d -8. *Concentrations of P differ on d -5, -3, 0, 2, and 3 ($P < 0.01$).

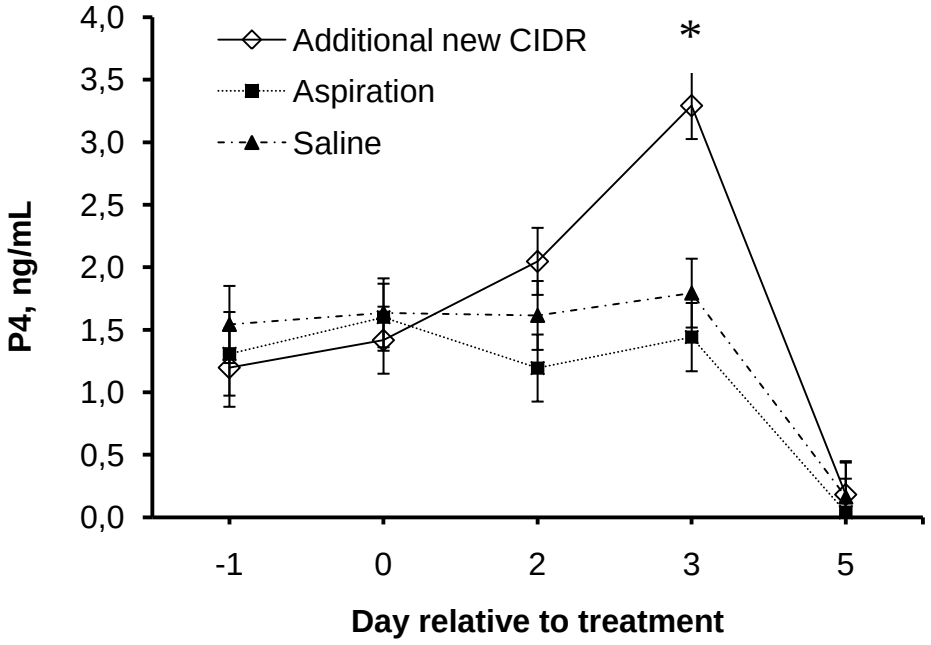

Figure 5-3. Exp. 1, concentrations of P associated with heifers receiving an additional CIDR, aspiration or saline on d 0. *Concentrations of P differ on d 3 ($P < 0.01$).

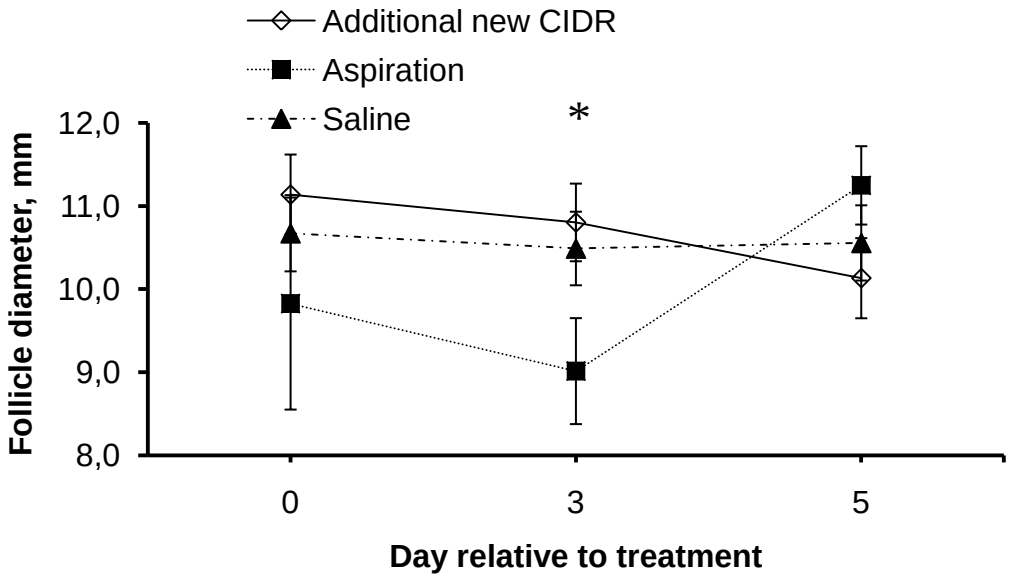

Figure 5-4. Exp. 1, diameter of the largest follicle present on the ovary associated with heifers receiving an additional CIDR, aspiration or saline on d 0. *Follicle diameter differs ($P < 0.01$).

Table 5-1. Concentrations of P and follicle diameter in heifers treated with additional CIDR, saline, or aspiration (Exp. 2)

	Treatments[a]	
Item	CIDR	Saline
Exp. 2	---------- ng/mL ----------	
P concentration[b]		
h 0	2.7 ± 0.4	2.7 ± 0.4
h 6	6.9 ± 0.4^{x}	2.5 ± 0.4^{y}
h 12	6.8 ± 0.4^{x}	2.5 ± 0.4^{y}
h 24	7.3 ± 0.5^{x}	2.6 ± 0.5^{y}
h 72	5.1 ± 0.4^{x}	1.6 ± 0.4^{y}
Follicle diameter	---------- mm ----------	
d 0	10.8 ± 0.5	10.8 ± 0.5
d 2	11.0 ± 0.5^{x}	10.2 ± 0.5^{y}
d 4	9.6 ± 0.5	9.9 ± 0.5
d 6	8.7 ± 0.5	9.5 ± 0.7
Follicle turnover[c], %	10/18 (55)	15/19 (78)

[a]Heifers were assigned to treatments and received an additional new CIDR follicular aspiration or saline on d 0.
bConcentration of progesterone measured at 0, 6, 12, 24 and 72 h after treatment on d 0.
[c]Follicle turnover was defined as the largest follicle present on d 0 that had decreased in diameter by d 3 and a new follicular wave was initiated by d 5 in Exp. 1 and if largest follicle present on d 0 that decreased in diameter by d 4, with the initiation of a new follicular wave in Exp. 2.
^{xy} Means with different superscript letters within a row differ ($P \leq 0.05$).

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSION

There is little doubt that research on cattle reproductive physiology, genetics and management has resulted in benefits to cattle producers. Prior to the development of frozen-thawed semen, the dissemination of genetics was difficult and slow, because breeding depended on natural service between a cow and bull. The development of the frozen-thawed semen technique enabled artificial insemination (AI) of large groups of cows to be inseminated from a single ejaculate, with pregnancy rates greater than 50% to synchronized fixed-time AI (Larson et al. 2006; Lamb et al., 2010). However, the intensive labor/time and high cost required to perform AI, are some reasons for the low acceptance by cattle producers, resulting in ≈ 8% of the cows in US being exposed to AI each year (NAHMS, 2011).

Attempting to minimize cost and labor, and improve pregnancy rates (PR) outcomes to TAI programs, several researchers have been focusing on the physiology of follicle and corpus luteum development during the estrous synchronization programs (Geary et al., 2001; Lamb et al., 2001; Larson et al., 2006; Bridges et al., 2008). The most current and utilized synchronization program recommended for beef cows is the CO-Synch + CIDR protocol. This program relies on three important outcomes; 1) synchronizing the follicular wave with an injection of GnRH at a random d of the estrous cycle and inducing emergence of a new follicular wave 1.5 to 2 d later; 2) regressing any CL present in the ovary with an injection of PG, 7 d after the GnRH treatment; 3) growth and ovulation of the newly emerged follicle after PG injection. Therefore, cows may have compromised fertility if they fail to respond to the first GnRH or to the PG, or if the dominant follicle has compromised development prior to AI (Vasconcelos et al.,

1999). In the current first and second study our objective was to evaluate the effect an eCG treatment concomitant with a PG injection and/or temporary calf removal (CR) between PG and TAI in the CoSynch + CIDR protocol, on follicle development, hormonal pattern release and pregnancy rates to TAI in beef cows. It has been reported that eCG (Roche et al., 1992; Baruselli et al., 2004; Sá Filho et al., 2010) and CR (Geary et al., 2001; Baruselli et al., 2004; Duffy et al., 2004) applied to estrous synchronization programs have a positive effect on fertility. In the current experiments, we observed inconsistent results; however both eCG and CR were able to improve some characteristics positively correlated with fertility. Cows treated with eCG had greater follicle growth rate, follicle diameter at TAI, and concentration of P 7 d after TAI, compared to cows not treated with eCG. Calf removal treatments increased follicle growth rate, the percentage of cows experiencing an LH peak prior to TAI, percentage of cows ovulating around TAI, and (although inconsistent) improved PR at TAI. In contrast, CR had a negative effect on calf performance during the first 63 d after temporary weaning. In the current experiment, we were unable to follow calf performance at later stages of life. Previous report observed similar negative effects on calf performance when CR was applied (Odde et al., 1986; McCartney et al., 1990; Sá Filho et al., 2009d); however, there is evidence that calves exposed to CR between d 39 and 68 d of life were lighter at 240 d, but reached similar weight at 420 d of age (Sá Filho et al., 2009d). In addition, the current experiment indicates that temporary calf removal did not affect subsequent cow milk production.

The third study was designed to better understand the follicular dynamics following a once-used or new CIDR inserted at random phases of the estrous cycle in beef

heifers. Progestin devices have been used between the first GnRH injection and PG, in estrous synchronization protocols to prevent estrous from occurring during this period (Kojima et al., 2000; Lamb et al., 2001; Lucy et al., 2001). It has been demonstrated that cows, when exposed to low concentration of P, number of LH pulses increased and a persistent follicles developed (Roberson et al., 1989; Anderson and Day, 1994). In contrast, exposing cows with high concentrations of P caused a decrease in frequency of LH pulses, inducing dominant follicle turnover (Adams et al., 1992; Anderson and Day, 1994; Kojima et al., 1995). When a CIDR was inserted in heifers in our study, concentration of P reached 5 ng/mL the d after insertion and remained close to 3 ng/mL for at least 3 d. The high P concentrations released by a CIDR may disrupt the synchrony of the new follicular induced by the first GnRH injection in the CO-Synch + CIDR protocol. The effect of high progesterone may induce a delay in turnover of follicles that did not ovulate to the first GnRH injection. In addition, high concentration of P may interfere in the natural development of the new growing follicle. Even though, the use of an external releasing P device may improve pregnancy rates (Macmillan and Peterson. 1993; Stevenson et al., 2003; Lamb et al., 2006, 2010), the effects of high P exposure on follicle development may be one factor impairing further improvement on fertility.

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BIOGRAPHICAL SKETCH

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