

THE BIOSTIMULATORY EFFECT OF BULLS ON POSTPARTUM
FOLLICULAR WAVE DEVELOPMENT IN, POSTPARTUM,
ANESTROUS, SUCKLED BEEF COWS

by

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ABSTRACT

The objective of this experiment was to determine if bull exposure influences follicular wave dynamics in primiparous, postpartum, anestrous, suckled, beef cows exposed to bulls. In Experiment 1, cows were exposed (continuously 24 h/d), (EB; n = 5) to bulls or not exposed to bulls (NE; n = 5) throughout the experimental period. In Experiment 2, cows were exposed to bulls for either 12 h, (EB12; n = 15), 6 h, (EB6; n = 14) or not exposed to bulls (NE; n = 10) from the start to the end of the experimental period. In Experiments 1 and 2, cows were $67 \text{ d} \pm 3.8$ (mean $\pm$ SE) and 51.5 ± 2.3 d postpartum at the start of the experiment. Follicular characteristics of each cow were examined by transrectal ultrasonography. In Experiment 1, interwave interval for wave 3 was shorter in EB than NE cows. Maximum dominant follicle (MDF) diameter tended to be greater during wave 2 for EB than NE cows, while wave 3 was greater for EB than NE cows. However, MDF diameter for wave 6 was greater for NE than EB cows. In Experiment 2, EB12 cows had fewer follicular waves to the resumption of luteal activity (RLA) than NE cows, while the number of waves to RLA for EB6 cows did not differ from that of EB12 or NE cows. Normalizing follicular waves to the time of RLA for cows within the EB12 and EB6 indicated that those cows at RLA had larger MDF diameters for the wave that produced the ovulatory follicle than cows that did not RLA. These data show the effects of bull exposure in altering follicular growth and developmental patterns, shortening the inter-wave interval and increasing the MDF diameter. Though the mechanism through which bull exposure alters postpartum follicular development is not entirely understood, these data provide new understanding.

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CHAPTER 1

INTRODUCTION

A major problem facing cow calf producers is the failure of cows to rebreed after calving. The failure of cows to rebreed after calving decreases reproductive efficiency and limits individual animal lifetime productivity (Lesmiester et. al., 1973).

Reproductive efficiency is determined by the proportion of cows within a herd that become pregnant, give birth, and produce calves each year. Postpartum anestrous is the period after calving during which cows do not exhibit estrus, fail to ovulate dominant follicles, and cannot become pregnant. Unfortunately, this problem is most pronounced in primiparous, suckled, beef cows that require 15 to 25 d longer to resume ovarian cycling activity than multiparous cows (Short et al., 1994). A variety of management strategies have been developed and implemented to overcome problems associated with postpartum anestrus. These strategies may include early weaning of calves, increasing feed intake of cows pre- and postpartum, and hormonal therapies. However, any management strategy implemented needs to be cost effective, efficient, and socially acceptable. Unfortunately, many of these strategies are costly, labor intensive, and unsustainable.

There are many factors that influence the interval from calving to resumption of ovarian cycling activity in postpartum, anestrous cows. One of these is the biostimulatory effect of bulls. Biostimulation is the stimulatory effect of males on estrous and ovulatory activity in females (Chenoweth, 1983). The physiological

mechanism of the biostimulatory effect of bulls appears to involve a change in the hypothalamic-pituitary-ovarian (HPO) axis that results in an increase in the frequency of luteinizing hormone (LH) pulses (Fernandez et al., 1996; Roelofs et al., 2007), which in turn, stimulates resumption of ovarian cycling activity.

Ovarian follicular development is the process of the growth and development of an immature oocyte to a mature oocyte (Aerts and Bols, 2008). During this period the growth and development of a mature oocyte relies on the growth and regression of primordial follicles. Primordial follicle growth is dependent on a variety of endocrine signals to trigger antral follicle development and the growth of a dominant follicle capable of ovulating a mature oocyte. The hormone most responsible for final growth of a dominant follicle and release of the mature oocyte is LH. Increases in the frequency of LH pulse release trigger the final growth and ovulation of the selected dominant follicle (Aerts and Bols, 2008; Ginther et al., 2001). Thus, one could speculate that an increase in the frequency of LH pulses may have an effect to produce larger dominant follicles capable of maturation and ovulation sooner in cows exposed to bulls than cows not exposed to bulls. Experiment 1 of this thesis, focuses on how the biostimulatory effect of bulls influences postpartum follicular wave dynamics in primiparous, postpartum, anestrous, suckled beef cows, and in it, we investigated the ability of cows to respond to continuous bull exposure before the breeding season. In Experiment 2, we investigated the possibility that the duration of daily bull exposure may affect postpartum follicular wave dynamics in primiparous, postpartum, anestrous, suckled beef cows. The review of literature focuses primarily on reproductive processes of female bovids and encompasses:

1) overview of the endocrinology of postpartum cows and factors that influence the postpartum interval to resumption of ovarian cycling activity; 2) review of the biostimulatory effect of bulls on the resumption of ovarian cycling activity of postpartum cows; 3) a review of ovarian follicular growth and development; and 4) postpartum ovarian follicular growth and development.

CHAPTER 2

LITERATURE REVIEW

Endocrinology of the Postpartum Anestrous CowGonadotropin Releasing Hormone

Gonadotropin releasing hormone (GnRH) is a neurohormone produced in a pulsatile rhythm by neurosecretory neurons located in the median eminence to stimulate the anterior pituitary (Moenter et al., 1992). The main function of this decapeptide is to stimulate the release of the gonadotropins (follicle stimulating hormone (FSH) and LH) from the anterior pituitary. After parturition, the hypothalamic GnRH pulse generator is very sensitive to the negative feedback of estradiol-17 β (E₂). This sensitivity results in low frequency, high amplitude release of GnRH from the hypothalamus. The following is a synopsis of the concepts involved with the neuroendocrine endocrine regulation of postpartum anestrus given by Williams (1990). The tonic release of GnRH is insufficient to release low amplitude, high frequency pulses of LH; which is the signal to the ovary to resume ovulatory activity. Therefore, postpartum anestrous/anovulation is endocrnologically characterized by inadequate LH release. As time progresses after calving, the sensitivity of the GnRH pulse generator to the negative feedback effect of E₂ decreases causing low amplitude, high frequency release of LH from the anterior pituitary.

Gonadotropins

Follicle Stimulating Hormone (FSH). Follicle stimulating hormone is a dimeric glycoprotein secreted by the gonadotrophs of the anterior pituitary (Hafez and Hafez, 2000). It should be noted that FSH and LH are produced by the same gonadotrophs. However, both gonadotrophs have a common α -subunit they differ molecularly from one another in their β -subunit. In the postpartum cow, temporal concentration patterns of FSH change very soon after calving. Concentrations of FSH increase within 5 to 10 d after calving and remain elevated for several weeks (Crowe et al., 1998; Moss et al., 1985). Webb et al. (1980) found that plasma FSH concentrations increased after the first 15 d and peaked in concert with increasing LH pulses. Crowe et al. (1998) reported that peripheral concentrations of FSH increased during the first 10 d after calving and were associated with the first appearance of an ovarian, dominant follicle (DF). Similar results were reported by Murphy et al. (1990) who found that the first postpartum DF was detected by 10 d after calving. However, the appearance of a DF does not necessarily mean that ovulation will occur because final maturation of the DF requires an appropriate change in LH pulse frequency. Thus, DFs developed soon after calving fail to ovulate due to inappropriate release of LH (for reviews see, Yavas and Walton, 2005; Williams, 1990). Furthermore, these so-called non-ovulatory DFs produced during this period grow to 12 mm only; smaller than that of an ovulatory DF (Stagg et al., 1994).

Luteinizing Hormone (LH). Luteinizing hormone is a dimeric glycoprotein produced by the gonadotrophs of the adenohypophysis and is responsible for final follicle growth, maturation, and ovulation. During postpartum anestrus temporal LH concentration patterns are insufficient to cause follicle maturation and ovulation. Low concentrations of E_2 inhibit hypothalamic release of GnRH which decreases the frequency of LH secretion from the pituitary (for review see Williams, 1990). Luteinizing hormone concentrations and concentration patterns gradually increase as time postpartum increases. Prior to the resumption of ovarian cycling activity in anestrus cows there is an increase in LH pulse frequency (Walters et al., 1982; Humphrey et al., 1983; Peters and Lamming, 1990). Furthermore, LH pulse frequency, amplitude (Rawlings et al., 1980; Garcia-Winder et al., 1984; Garcia-Winder et al., 1986; Savio et al., 1990; Wright et al., 1990), and average concentrations (Walters et al., 1982; Humphrey et al., 1983; Garcia-Winder et al., 1984; Garcia-Winder et al., 1986; Nett et al., 1988) also increase as the time postpartum increases.

Ovarian Steroids

Progesterone (P_4). Progesterone is a 21-carbon ovarian steroid hormone produced by the theca interna of antral follicles and the corpus luteum (CL) (for review, see Hafez and Hafez, 2000). Progesterone is known as the hormone of pregnancy, it is also the dominant hormone of the luteal phase of the estrous cycle. Progesterone acts primarily to prepare the reproductive tract for implantation and pregnancy. However, P_4 has a negative effect on the reproductive centers of the brain, suppressing GnRH release from

the hypothalamus. Progesterone concentrations fall rapidly prior to parturition. The first postpartum ovulation in both beef and dairy cows is generally “silent” (Kyle et al., 1992; Yavas and Walton, 2000) and ovulation is not accompanied by outward signs of behavioral estrus. This most likely occurs because no preovulatory increase in estrogen is present to trigger a behavioral response. The postpartum cow exhibits an increase in P_4 3 to 7 d before the first postpartum ovulatory cycle (Arije et al., 1974; Stevenson and Britt, 1979; Rawlings et al., 1980; Humphrey et al., 1983; Werth et al., 1996). This rise in P_4 is short-lived and associated with a small CL (Perry et al, 1991), and is referred to as a “short cycle”. Most, but not all (88 to 92%), cows express a “short cycle” before resumption of normal ovarian cycling activity (for review see, Hafez and Hafez, 2000); this is usually true for primiparous beef cows. Short cycles are due to ovulation and formation of a CL (Castenson et al., 1976; Stevenson and Britt, 1979); however, the CL formed after this ovulation is short-lived and produces small amounts of progesterone (Corah et al., 1974). Perry et al. (1991) found that short cycles do not result from ovulation of smaller or inferior follicles. However, the lifespan of this CL is shortened because of a premature release of $PGF_{2\alpha}$ soon after uterine exposure to progesterone, thus shortening the luteal phase of this cycle (Zollers et al., 1988). Furthermore, in beef cows that are uterectomized before first estrus, CLs are maintained and regress only after injection of $PGF_{2\alpha}$ (Copelin et al., 1987). These data indicate that premature luteal regression resulting in short cycles occurs as a response to premature $PGF_{2\alpha}$ secretion by the uterus. Garverick et al. (1998) found that E_2 concentrations were higher in cows with normal luteal function than cows with subnormal luteal function. Decreased

concentrations of E_2 in cows that exhibit short cycles or subnormal luteal function lead researchers to postulate that inadequate E_2 concentrations at estrus may inadequately stimulate P_4 receptors in the endometrium. It is postulated that because of the decreased E_2 concentration, P_4 receptors synthesis is decreased allowing an increase in the concentration of oxytocin receptors in the endometrium; this in turn, induces a positive feedback loop between $PGF_{2\alpha}$ and oxytocin causing early $PGF_{2\alpha}$ secretion resulting in premature luteolysis (Zollers et al., 1993).

Estrogen. The dominant form of ovarian estrogen in females is estradiol-17 β . Estradiol-17 β is synthesized primarily by the granulosa cells of ovarian antral follicles. Due to the low frequency, high amplitude pulsatile pattern of LH release early after parturition, ovarian follicles produced during this time fail to mature and ovulate. As a result, E_2 concentrations are very low (Humphrey et al., 1983; Stagg et al., 1995; Crowe et al., 1998). However, follicular waves begin to develop between 5 to 11 d after parturition (Crowe et al., 1998). As time postpartum increases maximum diameter of non-ovulatory follicles increase (Stagg et al., 1996; Spicer et al., 1986). Similarly, E_2 concentrations remain relatively constant and low throughout postpartum anestrous (Humphrey et al., 1983; Crowe et al., 1998). A change in E_2 occurs only when the dominant follicle is stimulated by the appropriate LH signal; the follicle becomes larger and secretes high concentrations of E_2 (Humphrey et al., 1983). These changes lead to behavioral estrus and signal the preovulatory release of LH in normal cycling cows.

Postpartum Anestrus and the Negative Feedback Effect of Estrogen

Estrogen concentrations in the blood do not change dramatically throughout postpartum anestrus (Carruthers et al., 1980; Chang et al., 1981). Neurosecretory neurons responsible for the tonic release of GnRH are quite sensitive to low concentrations of E_2 (Acosta et al., 1983). Sensitivity of tonic GnRH release to E_2 suppresses the pulsatile release of LH from the adenohypophysis (Acosta et al., 1983). This effect is called the negative feedback effect of estrogen. As time progresses after parturition sensitivity of neurosecretory neurons to estrogen decreases, allowing a gradual increase in GnRH pulses to occur. This change in temporal GnRH release stimulates low amplitude, high frequency LH secretion necessary for ovulation and resumption of ovarian cycling activity.

Summary of Neuroendocrine-endocrine Control of Postpartum Resumption of Ovulatory Cycles

After parturition, the hypothalamic GnRH pulse generator is very sensitive to the negative feedback of E_2 . This sensitivity causes the anterior pituitary to release high amplitude, low frequency pulses of LH; which is a signal to the ovary to remain quiescent or anovular. As time increases postpartum, the sensitivity of the GnRH pulse generator to the negative feedback effect of estrogen decreases releasing LH from the adenohypophysis in a low amplitude, high frequency pulsatile manner. Low amplitude, high frequency LH release causes an immature DF to ovulate and secrete progesterone for a short period of time allowing a new DF to develop. As this DF matures it

synthesizes and secretes estrogen. High concentrations of estrogen trigger the episodic release of LH, final maturation of DFs, behavioral estrus, ovulation of the DF and formation of the CL, i.e., resumption of ovarian cycling activity.

Factors Affecting the Postpartum Anestrous Interval of the Bovine

The length of postpartum anestrous is the result of a variety of factors acting independently or synergistically to lengthen the interval from calving to the resumption of ovulatory activity. Major factors that affect the length of time from calving to the resumption of ovulatory activity are nutrition, lactation, suckling, and parity. Other factors that influence this interval are breed, dystocia, and social factors (for review see, Short et al., 1990; Yavas and Walton, 2000).

Nutrition

The primiparous cow is faced with a difficult task of maintaining pregnancy while continuing to grow and mature. As a result the effects of inadequate nutrition and heightened metabolic demands during their first pregnancy and parturition adversely effect the ability of primiparous cows to rebreed earlier in the following breeding season. Essential bodily functions like metabolic homeostasis, growth, and development of vital energy reserves take precedence over reproductive functions (Grimard et al., 1997; Guedon et al., 1999). The effect of nutrient partitioning has been illustrated by research that has shown that low energy intake pre- and post-calving increases the interval from calving to resumption of ovarian cycling activity (Dunn et al., 1969; 1985; Wiltbank, 1970; Falk et al., 1975). The effects of adequate nutrition during this period are not only

beneficial to increase calf birth weights, but also to shortening the postpartum interval. Bellows and Short (1978) reported that increased pre- and post-calving feed energy levels of suckled cows decreased the postpartum interval and increased the percentage of cows that showed estrus compared to those cows that received a low pre-calving feed energy level. Similar results were reported by Houghton et al. (1990), who found that postpartum intervals to ovulatory activity decreased and the percentages of cows in estrus increased within 60 d after calving when cows were fed a maintenance diet prepartum and a low or high energy intake postpartum. Undoubtedly, feeding higher levels of nutrition has a benefit of shortening the postpartum interval to resumption of ovulatory activity.

If level of nutrition has an effect to alter the postpartum interval and cows fed at high energy levels exhibit changes in LH concentrations, follicular wave dynamics should change in cows fed higher energy diets. This idea was tested by Stagg et al. (1994) who reported that cows fed a high energy diet had a shorter postpartum interval and exhibited a decrease in the total number of follicle waves to ovulation than cows fed a low energy diet. Also, cows on the high energy ration exhibited a decrease in the total number of follicle waves to ovulation. Cows fed a high energy diet had greater LH release in response to an injection of estradiol benzoate than cows fed a low energy diet (Echternkamp et al., 1982). Henricks and Rone (1986) reported that cows fed a high energy ration had more medium- and small-sized follicles than cows fed a low energy ration. Thus, it would appear that changes in nutrition, intake or quality, influences follicular wave dynamics in postpartum, anestrous cows.

Body condition score (BCS) is an easy and effective measure of energy reserves. Evaluating pre- and postpartum BCS can help identify cows with negative energy balances and indicate cows that might have prolonged postpartum intervals to the resumption of ovulatory activity. Body condition scores at calving of ≤ 4.5 result in a postpartum interval of approximately 40 d (Short et al., 1990). Lents et al, (2008) reported similar results to those of Short et al. (1980) where they found that cows with $BCS \geq 5$ had shorter postpartum interval and larger dominant follicle diameters (15.0 mm) than cows with $BCS < 5$ (13.4) mm.

Suckling Stimuli

Negative energy balances and low BCS pre- and post-partum can be responsible for increasing the length of postpartum anestrous, although, they are not the only reason. Daily suckling intensity has been shown to influence the length of postpartum anestrous when adequate nutrition is available (Short et al., 1990; Stagg et al., 1998). Several studies have indicated that postpartum anestrous is longer in suckled than non-suckled beef cows (Carruthers et al., 1980; Crowe et. al., 1980). The majority of work shows that complete weaning is not effective in initiating LH pulses and ovulation until around day 13 after parturition in dairy cows (Carruthers et al., 1980ab) and after day 20 in beef cows (Short et al., 1972). As time postpartum increases, the effects of nutrition and suckling stimuli decrease as a result of decreased hypothalamic sensitivity to the negative feedback effect of E_2 . This was shown by Walters et al. (1982b), who demonstrated that if calves are weaned after birth or before the resumption of ovarian cycling activity (20 to 40 d after calving), cows will return to estrus within a few days.

Cows whose calves have been restricted to one suckling session daily show a sustained increase in LH concentrations within 20 days after calving, while cows subjected to intensive suckling do not show a sustained increase until 48 days after calving (Garcia-Winder et al., 1984). Furthermore, cows whose calves have been weaned exhibited greater concentrations of LH than cows with suckling calves (Walters et al., 1982; Carter et al., 1980). In cows that have been weaned, mean concentrations and concentration patterns of LH increase and change, respectively, within 48 h of weaning compared to suckled cows. Mean concentration, peak frequency, and amplitude (Carruthers and Hafs, 1980; Chang et al., 1981) of LH rise significantly higher within 48 h after weaning compared to that of non-weaned, suckled cows. Therefore, suckling may affect the hypothalamus suppressing neurosecretory release of GnRH, reducing the tonic release of LH which results in the postponement of ovulation.

Constant, low concentrations of estrogen secretion after calving have a negative feedback effect that delays estrus and ovulation. Acosta et al. (1983) showed that cows implanted with E_2 , that had their calves weaned early, exhibited estrus 23 d sooner than normal suckled cows. However, there was no difference in LH concentrations during the first 3 wk after calving between any of the treatments. Thereafter, cows that had their calves weaned early had increased LH pulse frequencies and increased mean concentrations of LH compared to E_2 -implanted, suckling cows. Thus, as time after calving increases the effect of suckling and the negative feedback effects of estrogen wane. The waning of these inhibitory factors induces hypothalamic activation of the

GnRH pulse generator; stimulating high frequency, low amplitude release of LH, that culminate in ovulation.

The inhibitory effect of suckling stimuli on LH release appears to involve more than mammary-somatosensory pathways. The physical presence of the calf is an integral component of suppressed LH release during suckling-mediated anestrus. Short et al, (1972) found that non-suckled and mastectomized cows had shorter intervals from calving to first estrus than suckled cows; 25 d, 12 d and 65 d, respectively. Therefore, this shows the inhibitory effect of the udder on the postpartum interval. To further test the effects of the udder on the interval from calving to estrus, Short et al. (1976) tested the effect of mammary denervation. Mammary denervation failed to decrease the interval from calving to estrus. Similarly, Williams et al. (1993) found that mammary denervation did not increase the LH pulse frequency. Therefore, it appears that the calf must act in some manner to create a psycho-physical “bond” with the dam. Cows who suckle their own calves as opposed to nursing a foster calf exhibit longer intervals to the resumption of ovulatory activity (Wetteman et al., 1978; Williams et al., 1991). Most interesting is that mammary denervation had no effect on the pituitary to increase LH concentrations and concentration patterns to shorten the interval to the resumption of ovulatory activity. The bond formed between the dam and calf acts to suppress the hypothalamic GnRH pulse generator inhibiting release of LH to stimulate the resumption of ovulatory activity.

The data presented thus far provide a basis for the role of suckling and the presence of the calf to influence postpartum anestrus and how it fits into the

endocrinology of postpartum anestrus. As the calf matures, its dependence on the dam for nutrition and nurturing decreases; greatly reducing the amount of mammary stimulation and the cow-calf bond. As a result, the negative feedback placed on the hypothalamus by constant low level E_2 production is slowly removed. This stimulates the release of LH in high frequency low amplitude pulses; triggering the resumption of ovarian cycling activity.

Parity

The primiparous cow is faced with a difficult task of maintaining pregnancy while continuing to grow and mature. As a result this places additional stress on an already stressed system. Generally primiparous cows have an interval to the resumption of ovulatory activity 30 d or longer than that of multiparous cows (Short et al., 1994). Similarly, Wiltbank (1970) found that older cows have shorter postpartum interval than younger cows.

Effect of Bull Exposure on the Postpartum Anestrous Cow

Several early reports indicated an increase in the number of cows expressing estrus during periods of artificial insemination in cows exposed to teaser bulls than in cows not exposed to bulls (Nersesjan, 1962; Sipilov, 1966; Ebert et al., 1972). Ebert et al. (1972) suggested that the effect of teaser bulls may be to aid in better estrous detection rather than in directly stimulating estrus. Recent research has solidified and eliminated any uncertainty as to the effect of bull exposure in primiparous and multiparous cows to decrease the length of postpartum anestrus and increase the percentage of cows that

show estrus after calving (Macmillan et. al., 1979; Zalesky et al., 1984; Berardinelli et. al., 1987; Custer et. al., 1990; Peres-Hernandez et al., 2002; Landaeta-Hernandez et al., 2004; 2006).

Biostimulation describes the stimulatory effect of males on the resumption of estrous and ovulatory events in females (Chenoweth, 1983). A major component of the biostimulatory effect of bulls is the duration and type of contact among animals. Several studies have indicated that the continuous presence of bulls for longer than 60 d reduces the interval from parturition to the resumption of ovarian cycling activity (Macmillan et. al., 1979; Zalesky et al., 1984; Berardinelli et. al., 1987; Custer et. al., 1990; Fernandez et al., 1996). Research from our laboratory indicated that cows exposed continuously to bulls from 3 or 30 d after parturition had shorter postpartum intervals than cows not exposed to bulls (Fernandez et al., 1993). In a later study, Fernandez et al. (1996) tested the durational component of bull exposure by restricting the physical presence of the bull to cows for only 2 h every third day. In this experiment bull exposure failed to hasten the interval from calving to first postpartum estrus. Thus, bull exposure for 2 h or less, every third day, for 18 d was inadequate to trigger resumption of ovarian cycling activity.

These findings left many questions to be answered in regard to how the time after calving would effect the biostimulatory response of cows to bulls. Berardinelli and Joshi (2005) tested whether the response of cows to bulls varied with time after calving. In that study, cows were exposed to bulls at 3 different days after calving, 15, 35, and 55 d. They reported that cows not exposed to the biostimulatory effect of bulls had longer intervals to the resumption of luteal activity than cows exposed to bulls. Interestingly, a

greater number of cows exposed to bulls starting 55 d after calving responded than cows exposed on days 15 or 35 after calving. These findings indicate that more cows respond sooner to bulls as the time after calving increases.

The aforementioned conclusion is interesting in that as time after calving increases, suckling intensity decreases, and the negative feedback effects of E_2 on the hypothalamus wane. As a result, there is an increase in the frequency of GnRH and LH pulse release (Yavas and Walton, 2000). Similarly, changes in LH concentrations and concentration patterns of cows are associated with bull exposure. Custer et al. (1990) was the first to postulate that the effect of bull exposure to hasten the resumption of ovulatory activity of postpartum anestrous cows involved increasing LH pulse frequency earlier after calving, accelerating final follicle maturation and ovulation. They reported that LH baseline concentration, mean concentration, pulse duration, pulse amplitude, and pulse frequency did not differ between cows exposed to bulls and cows not exposed to bulls. In that study intensive blood sampling was performed weekly after the start of bull exposure. The authors concluded that any immediate changes in LH concentrations and concentration patterns would not have been detected using this sampling regimen. To further evaluate LH concentrations and concentration patterns in suckled cows exposed to bulls, Fernandez et al. (1996) intensively collected blood samples every 10 min for 4 h every three days from cows exposed continuously to bulls, intermittently exposed to bulls for 2 h every third day, or not exposed to bulls. They found that suckled beef cows exposed intermittently or continuously to bulls showed increased mean LH concentrations and pulse frequencies compared to cows not exposed to bulls.

Identifying and understanding the mechanism whereby the biostimulatory effect of bulls acts on the hypothalamic-pituitary-ovarian axis to shorten the postpartum interval has proved to be difficult. Nonetheless, changes in LH concentrations and concentration patterns provide insight into how the physical presence of the bull stimulates the neuroendocrine-endocrine centers of the brain to reduce the interval to the resumption of ovulatory activity. Furthermore, one could conclude that the hastening of the postpartum interval in cows exposed to bulls works by somehow altering follicular wave dynamics. Cows exposed to bulls show increased LH concentrations. Changes induced by bull exposure appear to mimic those associated with “normal” resumption of ovulatory activity.

Folliculogenesis

Estrous Cycle

The estrous cycle in the bovine is approximately 21 d in length and consists of two phases. The first phase is the luteal phase which occurs directly after ovulation, while the second phase known as the follicular phase begins shortly after luteolysis. The purpose of the estrous cycle is to provide females with an opportunity to coordinate ovulation with copulation and become pregnant. There are a variety of factors known to influence the occurrence of estrus and estrous cycles. These include pregnancy, parturition, suckling, nutrition and the presence of bulls. In the “normal” cycling cow there is a series of complex anatomical and physiological transitions leading to estrus and ovulation. The follicular phase of the estrous cycle is dominated by large antral follicles

and increasing E_2 concentrations and LH, while the luteal phase is dominated by the presence of the corpus luteum and elevated P_4 .

In mammalian species, females are born with a limited number of primordial follicles arrested in prophase I of meiosis. In cattle, the number of primordial follicles present in an ovary is approximately 150,000 (Bao and Garverick, 1998; Lucy, 2007). Primordial follicles continuously enter the growing pool of follicles throughout a female's lifetime; however, very few follicles actually ovulate (Lucy, 2007). Ovarian follicular growth and development, in a monovulate like the cow, occurs in a wave-like pattern. A majority of cows exhibit 2 or 3 ovarian follicular waves during an estrous cycle (Savio et al., 1988; Sirios and Fortune 1988; Lucy, 2007). Each follicular wave involves: the recruitment of a cohort of follicles from the growing pool of follicles; selection of a follicle from the cohort; and so-called dominance by the selected follicle. Endocrine conditions during the time of follicular divergence and dominance dictate the fate of the dominant follicle to either ovulate or regress. The majority of ovarian follicles never ovulate and subsequently regress (Lucy, 2007).

Follicular Phase

In the cow, the follicular phase is approximately 4 d in length and can be further divided into two periods: proestrus and estrus. Proestrus is the period preceding estrus and ovulation wherein follicle recruitment and pituitary secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) is elevated. Elevated concentrations of FSH and LH promote follicle maturation, LH-dependency, and ovarian secretion of inhibin and E_2 . The multiple-follicle coupling theory states that once a dominant follicle

(DF) is selected it becomes LH-dependant and begins producing small quantities of inhibin and E_2 (for a review see, Ginther, 2001). Once divergence has taken place the selected follicle acquires the ability to respond to LH, no longer being dependant on FSH for growth (Ginther et al., 2001). This shift from FSH dependency to LH dependence shows the importance of FSH during the initial growing phase of follicular development. This was tested by ablating the largest follicle at expected deviation. Ablation caused an increase in FSH concentrations within hours after ablation (Ginther et al., 2000). Once a large follicle achieves LH-dependency there occurs an autocrine effect of the DF to suppress the growth of non selected follicles in the cohort while promoting its own growth (single-follicle coupling). Estradiol- 17β production by the DF is also responsible for the preparation of the reproductive tract for reproduction. The estrous phase lasts about 15 h and is the time during which final maturation and ovulation of the DF and copulation occurs. The dominant hormone during estrus is the ovarian steroid E_2 . Estradiol- 17β is necessary for the priming of the reproductive tract for implantation and pregnancy, as well as for muscular contractions and epithelial secretion that promote movement of gametes, fertilization and behavioral estrus, and triggering the preovulatory surge of LH needed to cause ovulation.

Luteal Phase

The luteal phase comprises 80% of the estrous cycle and lasts 17 to 18 d. The luteal phase is dominated by the suppressive effects of P_4 acting on the hypothalamus. The luteal phase consists of two separate stages: metestrus and diestrus. During metestrus, concentrations of LH and E_2 rapidly decrease. The decline in E_2

concentrations stops the production of inhibin causing an increase in FSH concentrations. This brief increase in FSH promotes the recruitment and growth of antral follicles 2 to 4 d before follicular selection and divergence (Ginther et al., 1996; Mihm and Austin, 2002). During ovulation the DF ruptures mixing granulosa and thecal cells of the DF. The mixing of granulosa and thecal cells forms a mass of glandular tissue capable of steroidogenesis. This mass of tissue is known as the corpus luteum (CL) or yellow body. Progesterone produced by the CL has a negative effect on the reproductive centers of the brain (Spicer and Echternkamp, 1986), in particular, the hypothalamus; suppressing GnRH release into a tonic pattern. Tonic GnRH secretion is a pulsatile pattern of low frequency and high amplitude. Tonic GnRH secretion is insufficient in stimulating the anterior pituitary to release the necessary high frequency, low amplitude pulses of LH needed to promote final follicle maturation and ovulation (Gong et al., 1995). During metestrus and part of diestrus, low concentrations of E_2 inhibit hypothalamic release of GnRH, which decreases the frequency of LH secretion from the pituitary (Williams, 1990). Follicles produced during the metestrus and diestrus periods of the luteal phase fail to ovulate, become atretic, and regress. However, as time after ovulation increases the suppressive effect of P_4 begins to wane during the end of diestrus and beginning of proestrus. This decrease in P_4 is a result of luteolysis or the breakdown or regression of the CL. The hormone responsible for regression of the CL is prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Diaz et al., 2002). Prostaglandin $F_{2\alpha}$ is produced by the endometrium of the uterus and is transported to the ovary containing the CL via a vascular countercurrent exchange system (Berisha and Schams, 2005). However, recent research has indicated the potential

for intra-luteal lysis or auto-regulation of luteal function. Niswender et al. (2007) reported that ewes treated with 10 mg of indomethacin had greater concentrations of P_4 from D 13 to 16 of the estrous cycles. Further findings indicated that luteal weights were greater in ewes receiving indomethacin than control ewes. As the CL regresses P_4 concentrations begin to decrease during the late period of diestrus and the beginning of proestrus. Concomitant with decreasing P_4 is a change in the pulsatile release of GnRH secretion from the hypothalamus. This change from low frequency, high amplitude pulsatile release of GnRH to one of high frequency, low amplitude marks the start of increased LH release from the anterior pituitary stimulating the next follicular phase and wave (Aerts and Bols, 2008).

Hormones of the Estrous Cycle of Cows that are Essential for Proper Follicular Growth and Development

Gonadotropin Releasing Hormone

The frequency of GnRH appears to be important to the release of FSH and LH. Less frequent pulses of GnRH lead to greater secretion of FSH while more frequent pulses of GnRH results in increased LH release (Aerts and Bols, 2008). While intermittent GnRH administration results in episodic LH secretion (Walters et al. 1982c), chronic GnRH treatment suppressed follicle growth and LH pulse frequency inhibiting follicle growth beyond 9 mm (Gong et al., 1996).

Pituitary Gonadotropins

Follicle Stimulating Hormone (FSH). Follicle stimulating hormone causes the recruitment and development of antral (secondary and tertiary) follicles of the ovary (for review see, Sanger, 2003). Furthermore, the process of follicular selection and dominance is dependent on FSH (Hafez and Hafez, 2000). The recruitment and growth of follicles in the cohort is marked by an increase in FSH 2 to 4 d before follicular selection and divergence (Ginther et al., 1996; 2001; Mihm and Austin, 2002).

Luteinizing Hormone (LH). Temporal release of LH is characterized by low frequency and high amplitude pulse patterns (Walters et al., 1982; Peters and Lamming, 1990). However, release of LH in high frequency low amplitude pulses during the follicular phase of the estrous cycle stimulate final maturation and ovulation of ovarian DF (Mihm and Austin, 2002). In the normal cycling cow concentrations of P_4 decrease during late diestrus and early proestrus of the estrous cycle. The decrease in P_4 lifts the negative feedback on the GnRH neurons ending the low frequency, high amplitude pulses of GnRH from the hypothalamus allowing for high frequency, low amplitude pulses necessary to trigger the pre-ovulatory surge of LH (Hafez and Hafez, 2000). The importance of LH on the various growth stages of follicles was further explained by Gong et al, (1996) who reported that follicles did not grow beyond 7 to 9 mm when LH pulse frequency was suppressed. These data can be further supported by the work of Bao and Garverick (1998), who reported that LHr are detected in only one healthy follicle > 8 mm in diameter.

Ovarian Steroids

Progesterone (P₄). Progesterone acts primarily to prepare the reproductive tract for implantation and pregnancy. However, P₄ has a negative effect on reproductive and behavioral centers of the brain; suppressing GnRH release from the hypothalamus. As a result, P₄ suppression of the hypothalamus results in low frequency, high amplitude pulse release of GnRH (Mihm and Austin, 2002).

Estrogen. A change in E₂ occurs only when the dominant follicle is stimulated by the appropriate LH signal; the follicle becomes larger and secretes high levels of E₂ (Fortune, 1994). These findings coincide with the detection of increased E₂ concentrations at deviation (Ginther et al., 2001). In the absence of P₄ this leads to behavioral estrus and signals the preovulatory release of LH in normal cycling cows.

Inhibin (INH): Inhibin is a dimeric glycoprotein secreted from the granulosa cells to inhibit the secretion of FSH from the anterior lobe of the pituitary. However, there are a variety of dimeric and monomeric INHs found in bovine follicular fluid (Mihm and Austin, 2002). Dimeric INHs are critical to the endocrine regulation of follicular development. The main function of INH is to serve as an endocrine signal to the pituitary gland as to the number of growing ovarian follicles (Hafez and Hafez, 2000). During follicular growth estrogen-active antral follicles have a greater number of the larger (>160 kDa) INHs (Mihm and Austin, 2000; Hopko Ireland et al., 1994). Within 33 h of the FSH peak, increases in the number of larger molecular weight (MW) INHs are

seen in the largest of follicles making up the cohort (Mihm and Austin, 2000). These studies indicate the role of estrogen-active follicles and INHs to act as a regulator of follicle selection and atresia.

Oxytocin (OT). Oxytocin is a protein hormone produced in the hypothalamus and stored in the posterior portion of the pituitary gland. Furthermore, OT is produced by large luteal cells of the CL. Oxytocin plays a critical role during the late luteal phase and early follicular phase to stimulate endometrial production of the luteolytic agent $\text{PGF}_{2\alpha}$ (for review see, Hafez and Hafez, 2000).

Uterine Hormones

Prostaglandin ($\text{PGF}_{2\alpha}$). Prostaglandin $\text{F}_{2\alpha}$ is a fatty acid derivative that functions as the main luteolytic agent. Prostaglandin $\text{F}_{2\alpha}$ is produced by the uterine endometrium and luteal cells to initiate luteal regression and ovulation, respectively (Hafez and Hafez, 2000). Prostaglandin $\text{F}_{2\alpha}$ is transported from the uterus to the ipsilateral ovary via a vascular countercurrent exchange mechanism (for review see, Sanger, 2003). Soon after the pre-ovulatory surge of LH, $\text{PGF}_{2\alpha}$ is synthesized and secreted by the ovary. However, the exact role of $\text{PGF}_{2\alpha}$ in the ovulatory processes is unknown. Data indicates that $\text{PGF}_{2\alpha}$ may cause the contraction of the myoid layer and rupture of lysosomes causing connective tissue deterioration (Algire et al., 1992; Senger, 2003). Nonetheless, research has shown an increase in the follicular fluid concentration of $\text{PGF}_{2\alpha}$ in superovulated heifers 24 to 25 h following the pre-ovulatory LH surge (Algire et al., 1992).

Neuroendocrine-endocrine Control of Reproduction

Hypothalamic-Pituitary-Ovarian Axis

The hypothalamic-pituitary-ovarian (HPO) axis is a complex neuroendocrine-endocrine system connecting the brain to the gonads. The process of folliculogenesis and ovulation is directly related to hypothalamic release of GnRH and ovarian E_2 release triggering the necessary pattern of pituitary LH release.

The hypothalamus consists of a series of nerve cell bodies (hypothalamic nuclei) that are arranged in clusters. There are two portions of the hypothalamus that are specific to reproduction; these include the tonic and surge center. Within these regions neurons produce GnRH. Gonadotropin releasing hormone is secreted in a basal pattern from the tonic center. The GnRH surge center of the hypothalamus is responsible for the pre-ovulatory surge of GnRH in response to elevated (threshold) levels of E_2 . The pre-ovulatory surge of GnRH is responsible for triggering the surge of LH that causes ovulation. After traveling down the hypothalamic-hypophyseal portal vascular system GnRH reaches the anterior pituitary, stimulating gonadotropin release from the gonadotrophs into the blood stream. The function of the pituitary gland is to produce and release the gonadotropins, FSH and LH that are responsible for ovarian follicular growth and ovulation, respectively. These gonadotropins stimulate the ovary, in particular ovarian follicles, to secrete E_2 and elicit a positive feedback on the neurosecretory neurons of the hypothalamus. During the early proestrous phase of the estrous cycle, as P_4 begins to fall, these neurons release GnRH more frequently, causing release of LH

from the anterior pituitary in a low amplitude, high frequency manner (Rawlings et al., 1980; Humphery et al., 1983; Garcia-Winder et al., 1984; Garcia-Winder et al., 1986; Savio et al., 1990; Wright et al., 1990). The temporal release of LH in this manner stimulates the continued growth and development of the DF in the ovary, causing the synthesis and secretion of E_2 . When E_2 concentrations reach a threshold, the pre-optic area of the brain, located above the optic chiasm, stimulates the episodic or preovulatory surge of LH. This episodic release of LH assures final follicle growth, maturation and ovulation of the DF.

Postpartum Follicular Growth and Development

Immediately after parturition P_4 and E_2 concentrations decline to a nadir (Crowe, 2008). At this time the hypothalamic GnRH pulse generator becomes highly sensitive to E_2 at these low concentrations. This sensitivity to E_2 causes a decrease in the frequency of GnRH release from the hypothalamus. Release of GnRH in a tonic manner is insufficient in increasing the pulse rate of LH; which results in the resumption of ovulatory activity. Therefore, postpartum anestrous/anovulation is endocrinologically characterized by inadequate LH release.

In the postpartum cow, temporal concentration patterns in FSH change very early after calving. Concentrations of FSH increase within 5 to 10 d after calving and remain elevated for several weeks (Crowe et al., 1998; Moss et al., 1985). Crowe et al. (1998) reported that peripheral concentrations of FSH increased during the first 10 d after calving and were associated with the first appearance of a DF. This was further validated

by the work of Murphy et al. (1990) who found that the first postpartum DF was detected by 10 d after calving. Unfortunately, LH concentrations and concentration patterns are insufficient to trigger ovulation (Yavas and Walton, 2005; Williams, 1990) during this period. Therefore, the appearance of a DF does not necessarily mean that ovulation will occur. These so-called non-ovulatory DFs produced during this period grow to 12 mm only, much smaller than that of ovulatory DF observed during estrous cycles (Stagg et al., 1994).

As time after calving increases, LH concentrations and concentration patterns gradually change (Stagg et al., 1998; Savio et al., 1990; Crowe, 2008). As a result the DF is stimulated by the appropriate LH signal; the follicle becomes larger and secretes increasing concentrations of E_2 (Humphrey et al., 1983). This increase in E_2 concentrations changes the negative feedback effect associated with low E_2 concentrations on the hypothalamus to one of positive feedback effect. This change in feedback allows GnRH pulses to occur more frequently; resulting in the low amplitude, high frequency secretion of LH necessary for ovulation and resumption of ovarian cycling activity.

Summary

Control of resumption of ovulatory activity in the bovine is under the neuroendocrine-endocrine control of the hypothalamic-pituitary-ovarian axis. Gonadotropin releasing hormone released from the hypothalamus in high frequency, low amplitude temporal release pattern stimulates the adenohypophysis to release high

frequency, low amplitude pulses of LH. High frequency, low amplitude release of LH is necessary to stimulate follicle maturation, ovulation and the resumption of ovarian cycling activity.

There are a multitude of factors that act upon the hypothalamic-pituitary-ovarian axis to lengthen postpartum anestrus. Nutrition is the primary factor affecting the postpartum interval. Negative energy balances pre- and post-calving as a result of inadequate nutrition can be the sole reason cows fail to rebreed after calving.

Unfortunately, as mentioned earlier, primiparous cows are faced with greater metabolic demands than multiparous cows who have already reached maturity. Another factor is the frequency of suckling stimuli and the physical presence of the calf can have an effect to lengthen the postpartum interval. Postpartum anestrus is longer in nursing cows than cows that are partial-weaned or not suckled.

The physical presence of bulls reduces the interval from calving to resumption of ovarian cycling activity in primiparous and multiparous cows. This effect is termed biostimulation. The ability of the cow to respond to the biostimulatory effect of bulls increases with time postpartum. Similarly, cows exposed to bulls show increased LH concentrations and concentration patterns compared to cows not exposed to bulls. Therefore, cows exposed to bulls may be under an endocrinological advantage to produce larger LH dependant DF capable of resuming ovulatory activity sooner after calving than cows not exposed to bulls.

Development of a DF is a prerequisite for ovulation and changes in LH pulse frequency are related to the final maturation of DF. Therefore, it would be reasonable to

postulate that since the physical presence of bulls to cows increases pulse frequency of LH in postpartum cows that the biostimulatory effect of bulls will influence follicular wave dynamics of cows; possibly by functioning through the hypothalamic-pituitary-ovarian axis to produce larger DFs that reach LH-dependence sooner causing accelerated growth and ovulation earlier after exposing cows to bulls.

CHAPTER 3

EXPERIMENTS 1 & 2: BIOSTIMULATORY EFFECT OF BULLS ON THE
POSTPARTUM FOLLICULAR DEVELOPMENT IN ANOVULAR, PRIMIPAROUS,
SUCKLED BEEF COWSIntroduction

Reproductive efficiency is a major factor influencing the profitability of cow calf production. Failure of cows to rebreed after calving reduces reproductive efficiency (Williams, 1990; Short et. al., 1990). The factor that most affects the ability of cows to rebreed after calving is prolonged intervals from calving to the onset of ovarian cycling activity (Short et. al., 1990). Postpartum anestrus is a major problem in primiparous, suckled beef cows (Short et. al., 1990). Furthermore, primiparous cows generally become pregnant later in the breeding season and tend to calve later in the next calving season causing a decrease in productivity throughout their lifetime (Lesmiester et. al., 1973).

The physical presence of bulls is known to reduce the interval from calving to the resumption of ovarian cycling activity in primiparous and multiparous cows (Fernandez et al., 1996; Berardinelli and Joshi, 2005). This effect is termed biostimulation. Similarly, cows exposed to bulls show increased LH concentrations and concentration patterns compared to cows not exposed to bulls (Fernandez et al., 1996). Changes in LH pulse frequency are related to ovarian follicular development, maturation of DFs, and ovulation. Therefore, it would be reasonable to postulate that since the physical presence

of bulls to cows increases pulse frequency of LH in postpartum cows, that the biostimulatory effect of bulls may influence follicular wave dynamics of exposed cows.

Materials and Methods

Two experiments were conducted at the Montana State University Livestock Teaching and Research Center, Bozeman. Animal care, handling, and protocols used in these experiments were approved by the Montana State University Institutional Agricultural Animal Care and Use Committee. Experiments 1 and 2 were performed in 2007 and 2008, respectively.

Animals and Treatments

Experiment 1. Ten Angus X Hereford, primiparous, suckled beef cows and 2 mature, epididectomized Angus X Hereford bulls were used in this experiment. Cows and calves before the start of the Experiment were maintained in a single pasture from calving to 67 ± 3.8 d (mean $\pm$ SD) after calving and had no contact with bulls or their excretory products for at least 9 mo until D 0 (April 18, 2007). One wk before the start of the experiment cows were stratified by body weight, BCS, calf birth weight, calving date, sex of calf, dystocia score, and assigned randomly to two treatments: exposed to bulls (EB; $n = 5$) or not exposed to bulls (NE; $n = 5$). Table 1 shows the number of cows per treatment and least square means for days from calving to start of the experiment, calf birth weight (BW), dystocia score, cow BW at the start of treatment, BCS, calf sex ratio

for primiparous, suckled, beef cows either exposed to bulls (EB) or not exposed to bulls (NE).

Table 1. Number of cows per treatment and least square means for days from calving to start of the experiment, calf birth weight (BW), dystocia score, cow BW at the start of treatment, BCS, calf sex ratio for primiparous, suckled, beef cows either exposed to bulls (EB) or not exposed to bulls (NE)

Variable	Treatment		SEM ^a	P value
	EB	NE		
n	5	5		
Calving date	36.4	36	3.83	0.87
Calf BW (Kg)	33.7	34.7	4.30	0.70
Dystocia Score ^b	1.2	1.0	0.32	0.35
Cow BW (Kg)	471.3	448.6	24.8	0.19
BCS ^c	4.8	4.7	0.27	0.58
Calf Sex Ratio ^d	0.20	0.40	0.48 ^e	0.54

^aSEM = Standard error of the mean.

^bDystocia Score: 0 = No assistance to 5 = Caesarean section.

^cBCS; 1 = Emaciated, 9 = Obese.

^dCalf sex ratio = Ratio of male to female calves, 1 = male and 0 = female. Tested with χ^2 analysis.

^e χ^2 value.

Experiment 2. Thirty-nine, primiparous, Angus X Hereford, suckled, beef cows and four Angus X Hereford epididectomized bulls were used. Cows and calves had no contact with bulls or their excretory products until the start of the experiment (D 0). Before the start of the experiment cows and calves were maintained in a single pasture

from calving to 51 days after calving (March 9, 2008). Two days before the start of the experiment cows were stratified by body weight, BCS, calf birth weight, calving date, sex of calf, dystocia score, and assigned randomly to three treatments: exposed to bulls for 12 h/d (EB12; n = 15), exposed to bulls for 6 h/d (EB6; n = 14), or not exposed to bulls (NE, n = 10) for 45 d (Table 2).

Table 2. Number of cows per treatment and least square means for days from calving to start of the experiment, calf birth weight (BW), dystocia score, cow BW at the start of treatment, BCS, calf sex ratio for primiparous, suckled, beef cows either exposed to bulls (EB) for 12 hr (EB12) and 6 hr (EB6) or not exposed to bulls (NE)

Variable	Treatment			SEM ^a	P value
	EB12	EB6	NE		
n	15	14	10		
Calving date	52.8	51.1	43.5	14.0	0.25
Calf BW (Kg)	35.1	36.4	37.4	4.16	0.41
Dystocia Score ^b	1.1	1.2	1.2	0.57	0.92
Cow BW (Kg)	462.6	474.1	465.9	37.7	0.71
BCS ^c	4.3	4.1	4.1	0.29	0.33
Calf Sex Ratio ^d	0.47	0.57	0.50	0.33 ^e	0.85

^aSEM = Standard error of the mean.

^bDystocia Score: 0 = No assistance to 5 = Caesarean section.

^cBCS; 1 = Emaciated, 9 = Obese.

^dCalf sex ratio = Ratio of male to female calves, 1 = male and 0 = female. Tested with χ^2 analysis.

^e χ^2 value.

Animal Housing Areas (Experiments 1 and 2)

Experiment 1: Cows were housed within pens in separate lot areas at the Bozeman Area Research and Teaching Farm. Pens within the north lot were used for maintaining EB cows while pens within the south lot were used for maintaining NE cows. During ultrasonography examination of the ovaries and blood sample collection NE cows were first processed through handling facilities, followed by the EB cows.

Experiment 2. Similar to Experiment 1, cows were housed within pens in separate lot areas. However, in Experiment 2, bull-exposed cows were housed within the south lot pens while cows not exposed to bulls were housed in the north lot pen. Bulls were housed in a separate pen (bull pen) that was out of visual and olfactory range for cows in treatments. This pen was approximately 0.25 km from the north lot and 30 m from the south lot. Cows in the EB treatments were exposed to bulls beginning at 0700 h each d. Bulls were removed from EB6 and EB12 cows at 1300 and 1900 h each day, respectively, during the 45-d exposure period (D0 = first d of the exposure period). Bull to cow ratio for EB6 and EB12 treatments were, 1:7.5, and 1:7, respectively. Figure 1, shows diagrammatic representation of bull exposure and rotation for experiment 2.

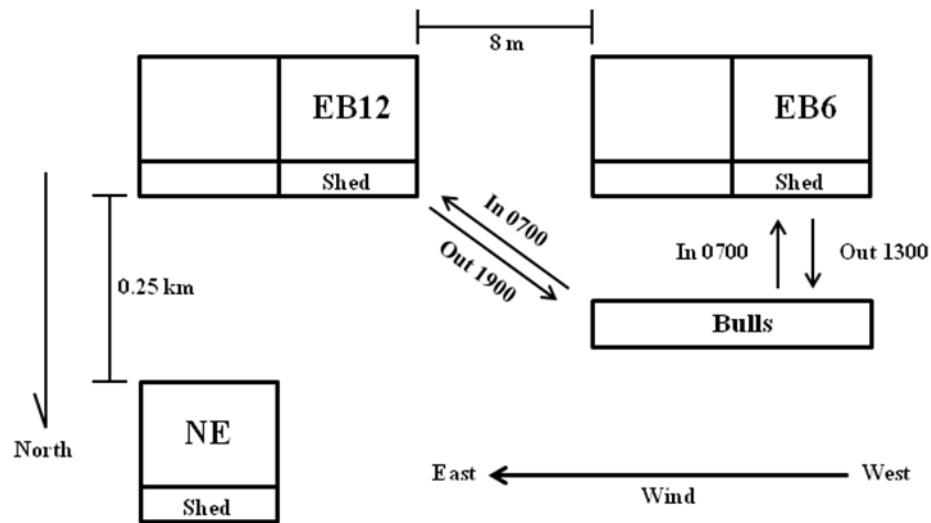


Figure 1. Diagrammatic representation of bull exposure and rotation for experiment 2.

Nutrition (Experiments 1 and 2)

In both experiments, cows and calves had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available before (d 0) the start of the experiment. Once cows and calves in both experiments were moved into their respective treatment pens they were given free access to the same hay (chopped), water, and a mineral-salt supplement. This diet was 97% of the NRC's requirements for a 533 Kg lactating beef cow (NRC, 1996). Bulls were allowed free access to water and mineral-salt supplement and were fed the same ration in both experiments.

Ultrasonography (Experiments 1 and 2)

Each ovary of each cow was examined by transrectal ultrasonography. In Experiment 1, ovaries of each cow were scanned every other day starting D -5 to 0, and then performed daily from D 0 to 42. However, based on the results of Experiment 1 it was concluded that ultrasound examinations in Experiment 2 needed to be performed every other day only. In both experiments, a patient file was created for each cow and used to track follicular measurements for both right and left ovaries throughout the duration of both experiments. Upon completion of both experiments all data for both ovaries was compiled and evaluated separately for each experiment. For both experiments, a Titan Ultrasound Imaging System (Sonosite, Bothell, WA) ultrasound machine equipped with a selectable 5 to 10 MHz transducer was used. Before each ultrasound examination fecal material was evacuated from the rectum and then the transducer was inserted transrectally. The transducer was set at a scan depth of 4.9 cm, approximately 8.0 MHz. The right ovary was scanned first in a right to left motion, then the left ovary scanned in the same motion. Once the ovary was located and scanned, the image was “frozen” and all 177 auto-saved frames were reviewed and selected frames from the scan containing follicular images were saved for later evaluations. Selected frames from each scan that were saved for later evaluation contained each follicle to be measured in its entirety. Non-bull exposed cows were examined first each day throughout the experiment. Excretory products of the EB cows were removed from the handling and squeeze chute areas after each ultrasonography examination using a 1200

psi Briggs & Stratton (Briggs & Stratton., Milwaukee, WI) pressure washer to eliminate any possible exposure of bulls throughout the experiment.

Morphometric Analyses of Ultrasound Images(Experiments 1 and 2)

After each ovary was scanned, all 177 of the auto-saved frames were reviewed and those images depicting follicle(s) in their entirety were saved on the Sonosites' image card for computer download at the end of each day. Follicular images were downloaded to a computer using Site Link Manager Software (Sonosite, Bothell, WA). Once images were down-loaded they were opened using Sigma Plot Graphic Software (Systat Software, INC., San Jose, CA). Each image was calibrated using the on-screen calibration scale in Sigma Plot. Once image calibration was completed, size of individual follicles was determined by averaging follicle diameter at its widest point and at a right angle to the first measurement and then at a point bisecting the right angle. The average of these measurements was placed in its respective patient profile for that day and that follicle. This process was repeated for all visible follicles in an ovary. At the end of the experiment all follicle measurements were analyzed and evaluated for patterns associated with follicle wave emergence and ovulation.

Definitions Used in Follicle Measurements

In order to identify, measure, and evaluate follicular class and size, we used the following definitions outlined by Stagg et al. (1995) with modifications.

1. Dominant follicle: the largest follicle present on either ovary, that grew to a diameter of at least 8.5 mm and was at least 2 mm larger than other follicles present on either ovary.
2. Follicle wave: the emergence of a dominant or medium-dominant follicle and a number of smaller follicles that apparently originate from the same follicular pool.
3. Ovulation: the disappearance of a dominant follicle followed by the development of a corpus luteum in the position previously occupied by the ovulatory dominant follicle.
4. Ovarian cycle: the interval elapsing between two consecutive ovulations, which may or may not be associated with an observed estrus.
5. Normal cycle: an ovarian cycle of 18-24 d duration. Short cycle: an ovarian cycle of < 17 d duration. Long cycle: an ovarian cycle of > 24 d duration.
6. Inter-wave interval: the number of d between the first measurement of a follicle of > 4 mm and the appearance of a > 4 mm follicle at the beginning of the next follicular wave.
7. Dominant inter-wave interval: the number of d between the first measurement of a dominant follicle of > 8.5 mm and the appearance of a > 8.5 mm follicle at the beginning of the next follicular wave.
8. Subordinate follicle: any follicle < 8.5 mm and smaller than the dominant follicle.
9. Persistent follicle: any dominant follicle lasting > 9 d in length.
10. Short-cycle follicle loss interval: the number of d from the short-cycle ovulation to next ovulation (real ovulation) defining the return to normal ovarian function.

11. Total waves to ovulation: the number of follicle waves counted from D 1 of the experiment to the first real ovulation signaling the resumption of luteal activity.
12. Total waves after ovulation: the number of follicular waves after ovulation resulting in the resumption of luteal activity.
13. Days from start of bull exposure to first true ovulation: the number of d from the start of bull exposure (D0) to the resumption of luteal activity.

Blood Sampling for Progesterone(Experiments 1 and 2)

Jugular venous blood samples were obtained from each cow every 3 d beginning on D -1 to 42 in Experiment 1 and from D 0 to 45 in Experiment 2. Progesterone concentrations were assayed using solid phase RIA (Diagnostic Products Corp., Los Angeles, CA). Intra- and interassay coefficient of variation (CV) in Experiment 1, for a serum pool that contained 2.5 ng/mL of progesterone were 0.4 and 18%, respectively; and less than 10% for both intra- and interassay variation for a pool that contained 5.5 ng/mL. In Experiment 2, intra- and inter-assay coefficient of variation for serum pools containing 2.2 ng/mL of progesterone were 10.2 and 15.4%, respectively, and 8.9 and 11.8%, respectively, for a pool that contained 5.75 ng/mL.

Resumption of Luteal Activity

Resumption of luteal activity was identified by evaluating systemic progesterone concentrations. Progesterone concentrations > 1.0 ng/mL for three consecutive samples coupled with the visual presence of a CL detected by ultrasonography was used to identify the resumption of luteal activity.

Statistical Analyses Experiments (1 and 2)

Mean maximum dominant follicle (MDF) diameter, and the number of subordinate follicles were analyzed by ANOVA using repeated measure of SAS (SAS Inst. Inc., Cary, NC). The model included treatment, animal within treatment, and follicular wave number. The covariance structure was compound symmetry. Means were separated by Bonferroni multiple comparison tests. Inter-wave interval, follicle persistence length, total number of waves to resumption of luteal activity, number of days from D 0 to the resumption of luteal activity, and the interval from calving to the resumption of luteal activity were analyzed using separate ANOVA for a completely random design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment, and animal within treatment. Animal within treatment variance component was used to test the effect of treatment. Means were separated by Bonferroni multiple comparison tests. Proportions of cows resuming luteal activity during the exposure period were analyzed using the chi-square method of SAS (SAS Inst. Inc., Cary, NC).

Results

Experiment 1. The number of subordinate follicles, persistent follicle length, total number of follicle waves to ovulation, and interval from calving to the resumption of luteal activity (RLA) in days, and the proportion of cows that had RLA did not differ ($P > 0.5$) between cows exposed to bulls and those cows not exposed to bulls (Table 3).

Table 3. Number of cows per treatment and least square means for the total number of subordinate follicles, persistent follicle length in d, total follicular waves to resumption of luteal activity (RLA), and the interval from calving to RLA for primiparous, suckled, beef cows either exposed to bulls (EB) or not exposed to bulls (NE)

Variable	Treatment		SEM ^a	P value
	EB	NE		
n	4	4		
Total number of subordinate follicles	0.98	1.01	1.2	0.94
Persistent follicle length, d	12.3	12.5	2.4	0.88
Total follicle waves to RLA	1.8	1.2	0.9	0.39
Interval calving to RLA, d	83.8	85.5	6.9	0.73
% that Resumed ^d	100.0	80.0	1.11 ^d	> 0.10

^aSEM = standard error of the mean.

^bRows that lack common superscript differ at $P < 0.05$.

^cIntervals for cows that did resume luteal activity of the total number of days from calving to the end of the experiment.

^d χ^2 value.

Similarly, inter-wave interval for waves 2, 4, and 5 did not differ between cows exposed continuously to bulls or not exposed to bulls. However, inter-wave interval for wave 3 was shorter ($P < 0.05$) for EB (7.3 ± 0.86 d) than NE (12.8 ± 0.86 d) cows (Figure 2).

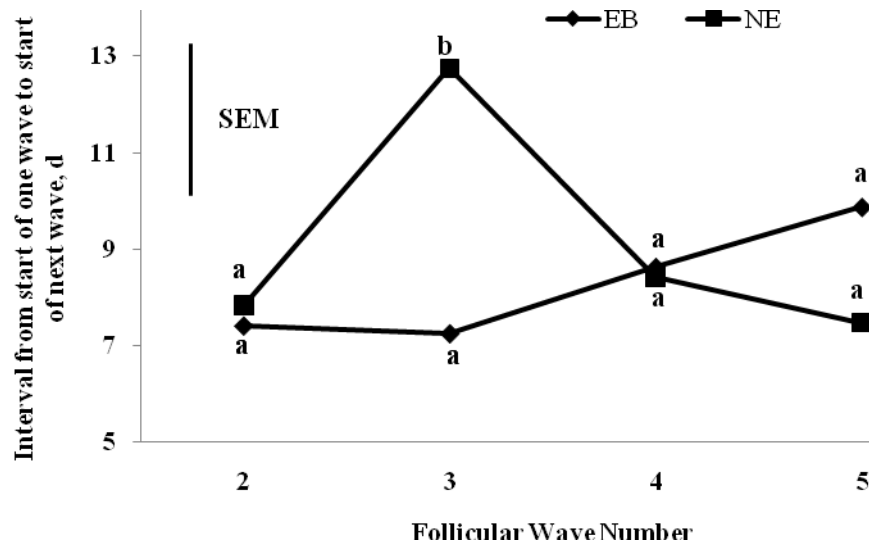


Figure 2. Least squares means for the inter-wave interval in d, for follicular waves 2 to 5, of cows exposed to bulls for 24 hr/d (EB) or not exposed (NE) to bulls during the 42-d exposure period. The vertical bar represents the pooled standard error of the mean (SEM).

Maximum dominant follicle diameter tended to be greater ($P < 0.08$) during wave 2 for EB (18.9 mm) than for NE (15.0 mm) cows, while MDF diameter of wave 3 was greater ($P < 0.05$) for EB (20.3 mm) than for NE (15.1 mm) cows. However, MDF diameter for wave 6 was greater ($P < 0.05$) for NE (21.5 mm) than for EB (13.7 mm) cows (Figure 3).

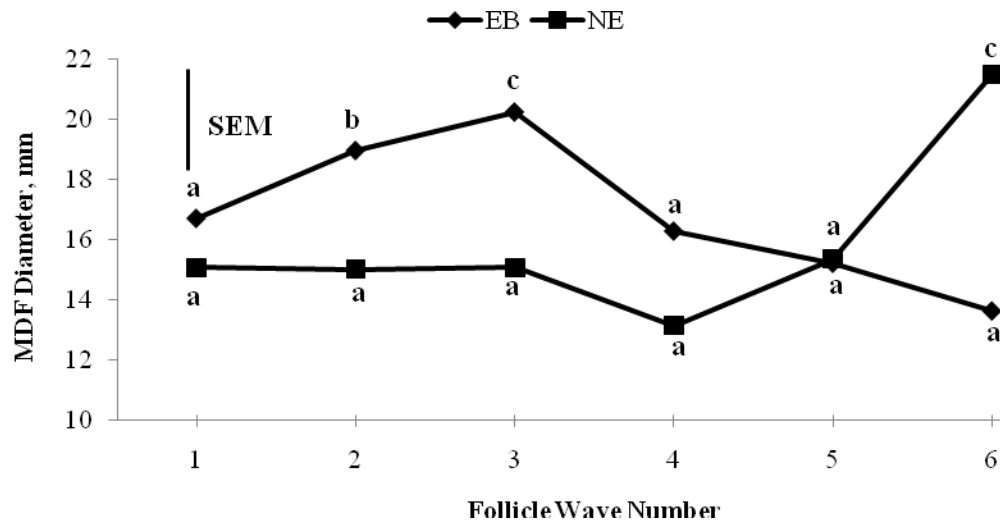


Figure 3. Least squares means for the maximum dominant follicular (MDF) diameter in mm, for follicular waves 1-6, of cows exposed to bulls for 24 hr/d (EB) or not exposed (NE) to bulls for the 42 D exposure period. The vertical bar represents the pooled standard error of the mean (SEM).

Experiment 2. In Experiment 2, NE cows had more ($P < 0.05$) subordinate follicles (1.6) than either cows exposed to bulls for 12 and 6 hours daily (1.1) and (1.0), respectively. Cows exposed to bulls for 12 h daily had fewer ($P < 0.05$) follicular waves (4.3) to the resumption of luteal activity (RLA) than NE (5.9) cows, while the number of waves to RLA for EB6 (4.6) cows did not differ from that of EB12 or NE cows. However, persistent follicle length and interval from calving in days to the resumption of luteal activity did not differ among cows in each treatment groups. Whereas, the proportions of EB12 and EB6 cows that had RLA were greater ($P < 0.05$) than that of NE cows (Table 4). The proportion of EB6 cows that RLA did not differ from that of EB12 cows (Table 4).

Table 4. Number of cows per treatment and least square means for the total number of subordinate follicles, persistent follicle length in d, total follicular waves to resumption of luteal activity (RLA), and the interval from calving to RLA for primiparous, suckled, beef cows either exposed to bulls (EB) or not exposed to bulls (NE)

Variable	Treatment			SEM ^a	<i>P</i> value
	EB12	EB6	NE		
n	15	14	10		
Total number of subordinate follicles	1.1 ^b	1.0 ^b	1.6 ^c	0.9	< 0.05
Persistent follicle length, d	9.0	12	16	3.2	0.48
Total follicle waves to RLA	4.3 ^b	4.6 ^b	6 ^c	1.5	< 0.05
Interval calving to RLA, d	84.9 ^a	90.3 ^{a,b}	101.5 ^a	13.5	< 0.05
% that Resumed ^d	60.0 ^a	64.3 ^{a,b}	10.0 ^a	6.23 ^d	0.04

^aSEM = standard error of the mean.

^bRows that lack common superscript differ at $P < 0.05$.

^cIntervals for cows that did resume luteal activity of the total number of days from calving to the end of the experiment.

^d χ^2 value.

The inter-wave interval and maximum dominant follicle diameter did not differ among treatments (Table 5 and 6).

Table 5. Inter-wave intervals (d) for cows exposed to bulls for 12 h/d (EB12), 6 h/d (EB6) or not exposed (NE) to bulls

Variable	Treatment			SEM ^a	<i>P</i> value
	EB12	EB6	NE		
n	15	14	10		
Wave 2	8.4	8.8	8.1	2.3	0.78
Wave 3	9.7	9.9	8.3	2.6	0.28
Wave 4	9.3	9.2	9.5	2.8	0.97
Wave 5	8.5	9.6	9.9	2.4	0.48
Wave 6	9.3	9.7	6.7	2.0	0.21

^aSEM = standard error of the mean.

Table 6. Maximum dominant follicle diameter (mm) for cows exposed to bulls for 12 h/d (EB12), 6 h/d (EB6) or not exposed (NE) to bulls

Variable	Treatment			SEM ^a	<i>P</i> value
	EB12	EB6	NE		
n	15	14	10		
Wave 1	12.0	12.5	11.5	1.6	0.32
Wave 2	12.4	12.9	12.5	1.9	0.75
Wave 3	13.8	13.6	12.9	2.3	0.65
Wave 4	13.6	13.9	13.7	2.1	0.89
Wave 5	12.8	13.1	13.1	1.9	0.85
Wave 6	12.5	13.0	12.7	2.2	0.88

^aSEM = standard error of the mean.

Normalizing follicular waves to the time of RLA for cows within the EB12 and EB6 treatments indicated that those cows that RLA had larger ($P < 0.05$) MDF diameters (15.0 mm) for the wave that produced the ovulatory follicle than cows that did not RLA (13.1 mm) (Figure 4).

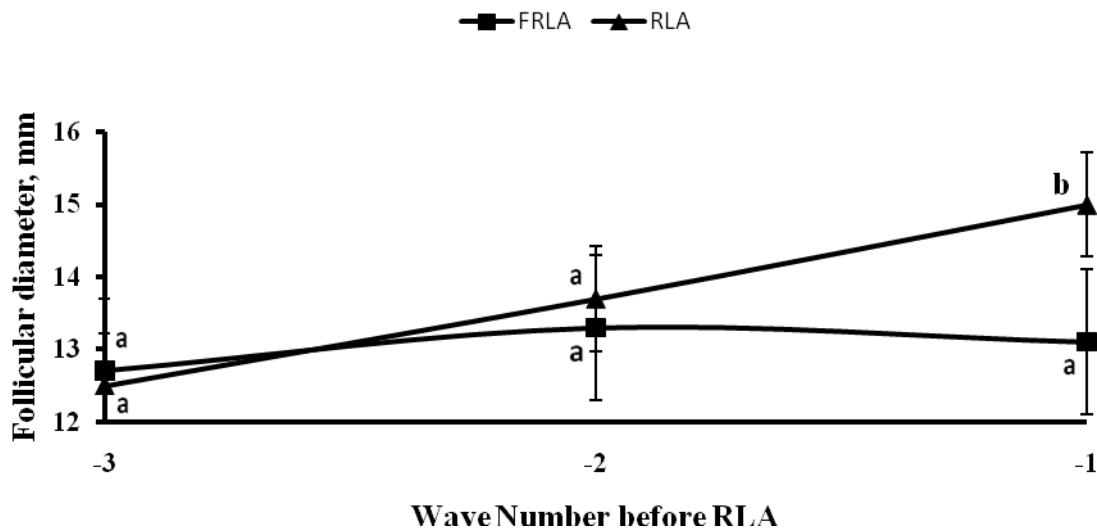


Figure 4. Least squares means for the maximum dominant follicular (MDF) diameter in mm, three follicular waves before the resumption of luteal activity (RLA) for cows exposed to bulls that RLA during the 45 d exposure period and those cows exposed to bulls that failed to RLA (FRLA) during the exposure period and were assigned the end of the exposure period as their RLA date. The vertical bars represent the pooled standard error of the mean (SEM).

CHAPTER 4

GENERAL DISCUSSION

This is the first study wherein the characteristics of postpartum follicular development in cows exposed to bulls have been evaluated. Results of Experiment 1 indicate that bull exposure reduces postpartum anestrus by producing larger dominant follicles that are capable of ovulating sooner in cows exposed to bulls than cows not exposed to bulls. Unfortunately, in Experiment 2, only one of the NE (control) cows resumed luteal activity. Because of this we were forced to perform a retrospective analysis of the data. However, the retrospective analysis provided similar findings to that of Experiment 1, wherein cows exposed to bulls produced larger dominant follicles sooner after parturition than cows not exposed to bulls. Two experiments were necessary to accomplish this objective because the number of primiparous cows available for experimentation and the facilities required, insuring that treatments did not interact in any one year limited our ability to conduct one single experiment. Nonetheless, the genetic base of cows, calves and bulls, the facilities and environmental conditions, the forage quality for rations, and the personnel that conducted the experiments and collected the data were the same for both experiments. The only major differences between experiments (yr) were that cows in Experiment 1 calved 14 d earlier (Feb. 5) than cows in Experiment 2 (Feb. 19) and cows in Experiment 1 were exposed to bull stimuli 10 d later after calving than cows in Experiment 2.

In both experiments, cows were exposed to bulls and biostimulatory stimuli of bulls; however, the duration of biostimulatory stimuli of bulls was limited to 6 and 12 h of exposure stimuli in Experiment 2. In Experiment 1, cows had unlimited access to biostimulatory stimuli of bulls throughout the exposure period. In both experiments there was an increase in the size of the maximal diameter of the dominant follicle in cows exposed continuously to bulls and a decrease in the inter-wave interval for Experiment 1, but not in Experiment 2. These results are similar to those reported in a variety of species indicating that the presence of males can influence reproductive events in females and conspecifics on estrous, ovulation and postpartum anestrous in females. In mice, ovulation can occur sooner in female mice when exposed to male mice (Hau et al., 2007). In swine, sows exposed to boars show an increase in the number of individuals that ovulated as well as an increase in follicle diameter from D 0 to 4 (Langendijk et al., 2000). Similarly in sheep, anestrous ewes exposed to rams at the beginning of the breeding season show an increase in size and number of ovarian follicles (Atkinson & Williamson, 1985). Furthermore, ram exposure increases LH concentrations leading to estrus and ovulation sooner in ewes (Evans et al., 2004).

One interpretation of the results is that bull biostimulation works via the hypothalamic-pituitary-ovarian (HPO) axis by stimulating dominant follicles capable of producing LH receptors allowing them to secure LH dependence sooner and ovulate earlier under appropriate endocrine signals. Recent research in sheep has shown a direct relationship of ram exposure increasing pregnancy rates in ewes. Lucid et al (2001), reported proestrus ewes exposed to rams showed an increase in LH within 2 h similar to

that seen in the preovulatory surge of LH prior to ovulation and increased pregnancy rates of exposed ewes by 20% compared with ewes that were not exposed to rams. Moreover, this interpretation follows that given by Custer et al. (1990), who proposed that bull biostimulation decreased the effects of postpartum anestrus by increasing LH pulse frequency earlier after calving, accelerating final follicle maturation and ovulation in those individuals exposed to bulls. Unfortunately, this doesn't explain the variation in days to the resumption of luteal activity among cows exposed to bulls. Fernandez et al. (1996) showed that cows exposed to bulls show increased LH concentrations and concentration patterns than cows not exposed to bulls. Because bull exposure only changes (increases) LH pulse frequency and concentrations patterns; the stage of follicular wave development must explain the variation in the resumption to luteal activity among cows exposed to bulls. It is very likely that where the cows are in the stage of their follicular wave development when exposed to bulls may explain why some animals take longer to resume luteal activity and ovulate on their second and third follicular waves after the start of bull exposure.

Follicular development may involve a signaling pathway modulated by the oocyte. Mitchell et al. (2003) reported that granulosa and theca cell function and signaling appears to be under the functional control of the oocyte and stem cell factors. In that study, porcine granulosa and theca cells were co-cultured and independently cultured. Oocyte secreted factors (OSF) stimulated estradiol production and cell proliferation of granulosa cells, respectively. Similar results were seen with an increase in theca cells numbers when exposed to OSF along with a decrease in P_4 synthesis.

Recent research has identified the earliest noted change in the future dominant follicle. Fortune et al. (2004), reported an increase in the IGF binding protein-4 (IGFBP-4) degrading protease pregnancy-associated plasma protein-A (PAPP-A). Acquisition of PAPP-A results in the decrease of inhibitory IGFBPs (-4 and -5) while causing an increase in free IGF and E_2 synthesis. Free IGF has the ability to act with FSH to promote follicular growth by amplifying the signaling of FSH. This change in FSH signaling causes up-regulation of E_2 in the future dominant follicle, suppressing the growth of subordinate follicles.

More recently, researchers identified an inhibitory role of the cocaine and amphetamine-regulated transcript (CART) on granulosa cell estradiol production by disrupting FSH-induced estradiol production. The role of CART as an appetite suppressant is well known, but its role as an intrafollicular regulator of estradiol production is less known. Kobayashi et al. (2006) identified greater concentrations of CART and the expression of CART mRNA in the follicular fluid of subordinate follicles than in healthy estrogen-active dominant follicles. Therefore, the expression of CART mRNA and concentrations of CART appear to be valid indicators of the health status of follicles. To further test the effects of the CART peptide Kobayashi et al. (2006) tested the effects of increased CART peptide concentrations on bovine granulosa cells from dominant follicles collected at random stages of the follicular wave and treated them for 18 h with increasing concentrations of the CART peptide. Interestingly they found that the treatment with CART decreased the production of granulosa cell estradiol in cells with high estradiol producing capacity at the time of collection and had no effect on

granulosa cells with a low estradiol producing capacity at the time of collection. These findings provide potential understanding into the regulation of subordinate follicle atresia and why only one dominant follicle is present on the ovary of monovulatory species. Again, this data may help explain the presence of larger dominant follicles in cows stimulated by bulls than those cows not exposed or stimulated by bulls. Future work must look toward identifying changes in the follicular microenvironment and oocyte secreted factors to help identify whether or not the bull is having a direct or indirect effect on follicular growth and development to produce larger dominant follicles, capable of ovulation sooner in those cows stimulated by the bull.

In conclusion, primiparous, postpartum, anestrous, beef cows exposed to bulls tend to exhibit a shorter inter-wave interval while producing larger dominant follicles. This is the first study wherein the characteristics of postpartum follicular development in cows exposed to bulls have been evaluated. Both experiments indicate that bull exposure reduces postpartum anestrus by producing larger dominant follicles that are capable of ovulating sooner compared to dominant follicles of cows not exposed to bulls. It is possible that bull exposure works via the hypothalamic pituitary ovarian axis (HPOA) by producing larger dominant follicles capable of producing LH receptors allowing them to secure LH dependence sooner and ovulate earlier under appropriate endocrine signals. Further research is needed to elucidate the physiologic mechanism associated with accelerated follicular growth and ovulatory competence in cows exposed to bulls.

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