Biostimulatory effect of bulls : exposure type and day of exposure on resumption of postpartum ovarian cycling activity in first-calf suckled beef cows
by Pramod Shrinivas Joshi

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Montana State University
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Abstract:
Experiment 1. The hypothesis tested was that proportions of first-calf cows cycling did not differ among cows exposed (BE) or not exposed (NE) to bulls, and suckled continuously (CS) or restricted to suckling twice daily (RS) on d 15, 35, or 55 postpartum. A rise in progesterone of > 0.5 ng/mL was used as evidence of resumption of cycling activity. Proportions of cows that resumed cycling activity was independent (P >0.10) of suckling type. Proportions of BE cows cycling that were exposed to bulls on d 15, 35, or 55 increased sooner (P < 0.05) than NE cows. More (P < 0.05) BE cows exposed on d 55 resumed cycling activity than BE cows exposed to bulls on either d 15 or 35. Cows exposed to bulls on d 55 postpartum appeared to respond more rapidly to the biostimulatory effect of bulls than if they were exposed earlier in the postpartum period.

Experiment 2. The hypotheses tested were that postpartum interval to ovarian cycling activity, proportions of cows cycling, and proportions of cows showing short luteal phase before a normal-length luteal phase did not differ among first-calf restricted suckled beef cows exposed continuously to presence of a bull (BE), exposed to excretory products of bulls (EPB), not exposed to a bull (NE), or exposed to excretory products of cows (EPC). Interval to resumption of cycling activity was shorter (P < 0.05) for BE, EPB, and EPC cows than NE cows. Proportions of cows cycling by d 50 did not differ (P > 0.10) between NE and EPC cows; however, proportions of EPB and BE cows were higher (P < 0.05) than NE and EPC cows. By d 70, proportions of EPC cows cycling did not differ (P >0.10) from those of BE and EPB cows. Proportions of cows that showed short luteal phase before a normal-length luteal phase did not differ (P >0.10) among BE, EPB, NE, and EPC cows. We conclude that exposing first-calf restricted suckled beef cows to excretory products of bulls hastened the resumption of postpartum cycling activity. Therefore, the biostimulatory role of bulls appears to be mediated by pheromones present in their excretory products.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

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I would like to dedicate this thesis to my father Mr. Shrinivas Shankar Joshi and my mother Sucheta Shrinivas Joshi for all the love, support, and encouragement they gave to me during my postgraduate studies at Montana State University. While my research was going on I received very sad news that my beloved mother passed away. Her blessings encouraged me to be more determined in pursuit of this degree and ultimately achieve my goal of continuing my education towards my Ph.D. degree.

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ABSTRACT

Experiment 1. The hypothesis tested was that proportions of first-calf cows cycling did not differ among cows exposed (**BE**) or not exposed (**NE**) to bulls, and suckled continuously (**CS**) or restricted to suckling twice daily (**RS**) on d 15, 35, or 55 postpartum. A rise in progesterone of > 0.5 ng/mL was used as evidence of resumption of cycling activity. Proportions of cows that resumed cycling activity was independent ($P > 0.10$) of suckling type. Proportions of BE cows cycling that were exposed to bulls on d 15, 35, or 55 increased sooner ($P < 0.05$) than NE cows. More ($P < 0.05$) BE cows exposed on d 55 resumed cycling activity than BE cows exposed to bulls on either d 15 or 35. Cows exposed to bulls on d 55 postpartum appeared to respond more rapidly to the biostimulatory effect of bulls than if they were exposed earlier in the postpartum period.

Experiment 2. The hypotheses tested were that postpartum interval to ovarian cycling activity, proportions of cows cycling, and proportions of cows showing short luteal phase before a normal-length luteal phase did not differ among first-calf restricted suckled beef cows exposed continuously to presence of a bull (**BE**), exposed to excretory products of bulls (**EPB**), not exposed to a bull (**NE**), or exposed to excretory products of cows (**EPC**). Interval to resumption of cycling activity was shorter ($P < 0.05$) for BE, EPB, and EPC cows than NE cows. Proportions of cows cycling by d 50 did not differ ($P > 0.10$) between NE and EPC cows; however, proportions of EPB and BE cows were higher ($P < 0.05$) than NE and EPC cows. By d 70, proportions of EPC cows cycling did not differ ($P > 0.10$) from those of BE and EPB cows. Proportions of cows that showed short luteal phase before a normal-length luteal phase did not differ ($P > 0.10$) among BE, EPB, NE, and EPC cows. We conclude that exposing first-calf restricted suckled beef cows to excretory products of bulls hastened the resumption of postpartum cycling activity. Therefore, the biostimulatory role of bulls appears to be mediated by pheromones present in their excretory products.

INTRODUCTION

The primary goal of beef cattle producers is to obtain and wean one calf per cow per year. To attain this goal, beef cows must become pregnant within 82 d after calving due to the relatively long gestation interval of 283 d. Satisfying this requirement is difficult because the reproductive endocrine system must recover from pregnancy and the reproductive tract must be remodeled to accommodate fertilization and embryonic development. The period required for re-setting the reproductive endocrine system and uterine remodeling is approximately 30 d after calving, and is generally referred to as postpartum anestrous (Short et al., 1990). This period is characterized generally by the absence of overt signs of sexual and reproductive activity, ovulation and corpus luteum (CL) function, and low levels of progesterone concentrations.

Ovulatory activity usually resumes within 2 to 3 wk postpartum in milked dairy cows; however, it does not resume for 35 to 60 d or even longer in beef cows (for review see Yavas et al., 2000) because of the negative effect of suckling. On an average, 30 to 50 % of beef cows in a herd that do not return to cycling activity by this time are culled (Beal et al., 1984; Wiltbank et al., 1978). Prolonged postpartum anestrus in suckled beef cows is a major factor that reduces the efficiency of reproduction in a beef cattle herd and contributes to a reduction in calf crop. This is especially evident in first-calf suckled cows (Lamming et al., 1981, Malven, 1984, McNeilly, 1988, Nett, 1987, Peters and Lamming, 1990, Short et al., 1990, Williams, 1990).

The primary factors that affect the postpartum anestrous period are suckling, nutrition, and age (parity). Other factors can also affect this interval and include breed,

individual genetic variation, stress, presence of bulls, disease, multiple births, dystocia, and retained placenta (Edgerton, 1980; Short et al., 1990).

Of particular interest to this thesis is the observation that reproductive processes of many female mammals can be altered by the presence of males. Exposing primiparous or multiparous cows to presence of yearling or mature and epididymectomized or vasectomized bulls during the postpartum anestrous period accelerates the resumption of ovarian cycling activity compared to cows isolated from bulls (Macmillan et al., 1979; Zalesky et al., 1984; Berardinelli et al., 1987; Scott and Montgomery, 1987; Custer et al., 1990; Naasz and Miller, 1990; Stumpf et al., 1992; Cupp et al., 1993; Soto Belloso et al., 1997; Rekwot et al., 2000a). However, there is no general consensus among the scientific community regarding the nature of the mechanism of action whereby presence of bulls affects the resumption of postpartum ovarian cycling activity. It has been postulated that this effect, generally referred to as a biostimulatory effect, is mediated through pheromonal cues produced by bulls. However, there is the possibility that the biostimulatory effect of bulls is meditated through some putative social bonding between bulls and cows that involves behavioral cues, without pheromonal communication. The following literature review will discuss the major factors (nutrition, suckling, physical presence of a calf) that influence the postpartum anestrous interval in beef cows; and physiological changes associated with them. Special emphasis is given to the biostimulatory effect of bulls.

LITERATURE REVIEW

Factors Affecting Postpartum Reproductive Processes in Cows

Endocrine and Neuroendocrine Factors Associated with Postpartum Cows

Role of Hypothalamo-Hypophyseal-Ovarian Axis. Previous studies attempted to establish relationship between the postpartum anestrous interval and the physiological mechanisms responsible for its occurrence and subsidence (for reviews see Peters and Lamming, 1986; Short et al., 1990; Williams, 1990). The following is an overview of characterization of these important relationships.

Physiological controls of the postpartum period are located in the complex of neuroendocrine-endocrine relationships within the hypothalamo-hypophyseal-ovarian axis. Ovarian follicular wave development is reduced during the periparturient period in cattle (Savio et al., 1990). However, definitive follicular waves begin to appear within 10 d after calving; with the appearance of a dominant follicle present on the ovary by d 11. The appearance of the initial postpartum follicular wave coincided with a rise in pituitary secretion of follicular stimulating hormone (FSH) between d 5 and 10 after calving. Thereafter, emergence and subsidence of follicle waves are accompanied by fluctuations in FSH concentrations (Crowe et al., 1998). However, dominant follicles (DF) of these waves usually fail to ovulate (Crowe et al., 1998). So the question is, "why morphologically sound DF fails to ovulate during the early postpartum anestrous period?" One possible answer to this question may be a lack of hypothalamic gonadotropin releasing hormone (GnRH), and/or pituitary GnRH and estradiol-17β (E_2)

receptors during this period. Nett et al. (1988) reported that by d 15 postpartum, content of the GnRH receptors and E_2 receptors were elevated in beef cows. The probable increase in the GnRH and E_2 receptors from d 1 through 15 is probably attributed to the rising functional competence of the hypothalamo-hypophysial axis which was lowered for first 15 d postpartum due to lack of GnRH synthesis and pituitary LH synthesis suppressed by pregnancy concentrations of progesterone, estrogens, and glucocorticoids (Moss et al. 1981). The inability of the pituitary to respond to GnRH due to lack of GnRH and E_2 receptors is not the only cause because it has been observed that pituitary release of LH in response to GnRH challenge increases by d10 postpartum (Williams, 1990). Another possible cause would be the inability of the positive feedback center in the hypothalamus to respond to the positive feedback effect of E_2. However, this is not the case because an ovulatory challenge of E_2 evokes pre-ovulatory like LH surges in both dairy and beef cows during this time (Schallenberger and Prokopp, 1985; Peters and Lamming, 1986).

The inability of DFs to ovulate during the postpartum anestrous period is not due to lack of hypothalamic GnRH, lack of FSH support, inability of pituitary to respond to ovulatory signals, or inability of hypothalamic positive feedback center to respond to E_2. The cause of this may be related to ovarian follicular incompetence to respond to ovulatory stimuli. That this is not the case is supported by reports of Carter et al. (1980) and Dunn et al. (1985) who reported that 50 % of postpartum cows formed luteal tissue after treatment with ovulatory dosages of GnRH 5 d after calving.

In the context of cattle, dominant follicle development in the postpartum period is driven in part by luteinizing hormone (LH) as evidenced from the following observations: 1) follicles did not grow beyond 7 to 9 mm when LH pulse frequency was suppressed (Gong et al., 1995, 1996); 2) the growth phase of the dominant follicle was associated with higher frequency LH pulses than for the plateau phase (Rhodes et al., 1995); and, 3) the life span of dominant follicle can be extended by increasing the LH-pulse frequency (Fortune et al., 1991; Savio et al., 1993). Data summarized by Williams (1990), Humphrey et al. (1983), and Custer et al. (1990) indicated that during the early postpartum anestrous period LH patterns are characterized by low mean concentrations of LH due to low LH pulse frequency and high amplitude. But as the postpartum anestrous interval advances, LH concentrations progressively rise, primarily due to an increase in pulse frequency in response to hypothalamic GnRH secretions. Therefore failure of a DF to ovulate is due to lack of appropriate and sufficient LH signals that are necessary for its final maturation followed by ovulation.

Gonadotropin and Ovarian Steroids

Luteinizing Hormone. The most obvious factor consistently reported to precede the postpartum return to ovarian cycling activity is the onset and increase in LH pulsatility (Walters et al., 1982a; Peters and Lamming, 1990). LH is a glycoprotein hormone secreted by the gonadotropic cells of the anterior pituitary. Many scientists have documented that peripheral LH concentrations increase with time postpartum (Walters et al., 1982b; Humphrey et al., 1983; Garcia-Winder et al., 1984; Garcia-Winder et al., 1986; Nett et al., 1988). Moreover, the change in tonic LH concentrations of

weaned cows was greater than the change in LH concentrations of suckled cows (Walters et al., 1982b). In addition, LH pulse frequency (Rawlings et al., 1980; Humphrey et al., 1983; Garcia-Winder et al., 1984; Garcia-Winder et al., 1986; Savio et al., 1990) and amplitude (Rawlings et al., 1980; Garcia-Winder et al., 1986; Wright et al., 1990) have been shown to increase postpartum. However, returning calves to dams after temporary calf removal decreased LH concentrations and pulse frequency within 8 h in anestrous cows, but no changes were seen in cyclic cows (Edwards, 1985). Therefore, suckling strongly reduces the pulsatile secretion of LH during the postpartum anestrous period; however, after commencement of ovarian cycling activity, LH concentrations are not affected by suckling.

Humphrey et al. (1983) reported that the frequency of LH peaks in serum tended to be greater during the second week preceding estrus than any other time during the postpartum period. Authors have suggested that increased mean values for LH were a result of increased frequency and magnitude of LH peaks during episodes of LH secretion that seem to be a prerequisite for the occurrence of first postpartum estrus and ovulation.

The past evidence strongly suggests that ovarian steroids, in particular estradiol, and opioid peptides influence inhibition of pulsatile LH secretion during the puerperium of cows. Garcia-Winder et al. (1984) indicated that OVX suckled cows showed lower LH pulse frequency from d 6 through d 55 postpartum compared to OVX non-suckled cows. These researchers have thus interpreted that suckling and ovarian factors act together during the postpartum period to suppress LH levels and pulse frequency.

Furthermore, postpartum nursing stimulus increases the sensitivity of the tonic center of the hypothalamus to the negative feedback of low and constant levels of estrogen which decreases GnRH release. However, Hinshelwood et al. (1985) reported that cows ovariectomized at d 5 postpartum had suppressed LH concentrations in both OVX suckled and non-suckled cows but concentrations of LH were lower in suckled than non-suckled cows at d 10 postpartum. Thus suckling might have had a direct effect on the hypothalamic-pituitary axis independent of the ovarian secretions. And it is possible that suckling may be acting through some other mechanism than facilitating the sensitivity of the hypothalamus to the negative feedback effects of estradiol, such as inhibiting the pituitary LH release in postpartum anestrous cows. Nevertheless, decreased pulse frequency and amplitude of LH release as well as reduced ability to respond to GnRH may be the cause of suckling induced anestrus (Carruthers et al., 1980).

Malven et al. (1986) provided evidence that opioids have a role in LH inhibition during postpartum anestrus and reported that hypothalamic concentrations of opioid peptides are affected by suckling. Opioid suppression of LH release in postpartum cows was demonstrated by Whisnant et al. (1986) and Rund et al. (1989) by subjecting postpartum anestrous beef cows to naloxone challenge at d 14 and 28 postpartum. Administration of GnRH 3 h after naloxone treatment resulted in a rise in mean LH concentrations with OVX non-suckled cows showing highest levels compared to intact non-suckled or OVX suckled groups. Investigators thus interpreted that apart from ovarian secretions and suckling stimulus, naloxone induced LH rise shows opioid inhibition of LH. Moreover, presence of other factors such as a carryover effect of the

steroids of pregnancy (parturition) during the early postpartum period of cows may mimic the opioid inhibition of LH secretions at pituitary level. Thus it would appear that obtaining an optimum LH concentration and frequency required for return to normal cycling activity, the opioid tone and the effects of estradiol and other steroids thereafter must subside.

Follicle Stimulating Hormone (FSH). It is a glycoprotein hormone secreted by the gonadotropic cells of the anterior pituitary. It has been observed that after parturition the negative feedback effect of steroids of pregnancy on FSH secretions usually wanes; with anterior pituitary FSH content either not changing (Moss et al., 1985; Nett et al., 1988) or decreasing (Labhsetwar et al., 1964; Lamming et al., 1981) from d 20 through 45 postpartum.

Rothchild (1960) reported that suckling suppresses FSH secretion in rats and that the level of suppression was directly proportional to litter size. Walters et al. (1982b) indicated that duration of weaning had no effect on FSH concentrations in cows, but weaned cows had a greater change in FSH concentrations than suckled cows. Plasma FSH concentrations increased after the first 15 d postpartum and peaked at LH peaks in addition to other times (Webb et al., 1980). Moss et al. (1985) reported that serum concentrations of FSH were high on d 5 and remained high through d 30 postpartum. Mean FSH concentrations were not affected by body condition and there was a three-fold increase in FSH on day 3 post-ovariectomy and a further two-fold increase from day 3 to 15 post-ovariectomy (Wright et al., 1990). Regulation of FSH concentrations during the postpartum anestrous period in cattle has not been studied extensively. Recently, 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. Thereafter, FSH concentrations varied with onset and regression of follicular wave development. So it may be that regulation of FSH concentration during postpartum anestrus in cows may be similar to that observed in cycling cows. In cycling cows, FSH concentrations are regulated by follicular secretion of inhibin and its negative feedback effects on gonadotropes (Hafez et al., 2000).

Estrogen. Estrogens are steroid hormones (18 carbons) formed primarily from cholesterol. The primary ovarian estrogen of the ovary of females is estradiol-17β. This steroid is synthesized primarily in the granulosal cells of antral follicles. Following parturition, systemic concentrations of estradiol-17β are very low during the early postpartum period because follicles are largely absent at parturition (Arije et al., 1974; Humphrey et al., 1983;Crowe et al., 1998). Some studies indicated that serum concentrations of estrogen throughout the postpartum period do not differ over time or among suckled and non-suckled beef (Chang et al., 1981) or dairy cows (Carruthers et al., 1980).

Pituitary responsiveness to exogenous estrogen, assessed by inducing a preovulatory-like LH release, increases as the time postpartum increases. Forrest et al. (1981) reported that a single injection of estradiol benzoate failed to induce a release of LH on d 2 to 3 postpartum, however cows responded on d 9 to 10 and 16 to 17 postpartum. Nett et al. (1988) demonstrated on d 15 postpartum that pituitary content of

the GnRH receptors and E_2 receptors were elevated in beef cows, thus LH release was dependent on their number and functionality.

Several researchers tested the influence of synthetic estradiol on formation and function of initial postpartum corpus luteum. Nephew et al. (1989) reported luteal function was extended in beef cows weaned on d 1 (d 0 = parturition) and was associated with enhanced PGFM release compared to control cows after a catecholestradiol challenge on d 5 to 9, before the appearance of the first CL postpartum. It appears that an increase in estradiol before the first ovulation postpartum may be necessary to prevent the occurrence of short luteal cycles associated with the resumption of cycling activity. However, data reported by Day et al. (1993) do not support this notion, because their results indicated that postpartum interval (60 ± 3.4 d) and incidence of short luteal phases (77%) were similar among suckled beef cows either untreated or treated with intrauterine infusions of catecholestradiol or vehicle from d 15 to 22 postpartum.

The positive feedback effect of estrogen on LH secretion is delayed by suckling until 2 to 4 wk postpartum (Short et al., 1979). The hypothalamo-hypophyseal axis response to this effect is functional by d 10 postpartum and it increases as time postpartum increases through d 17 in suckled beef cows (Peters, 1984).

Acosta et al. (1983) reported no significant changes in LH concentrations or number of LH pulses in response to estrogen treatment during the first 3 wk postpartum in ovariectomized suckled beef cows. The authors proposed the hypothesis that postpartum nursing stimulus increases the sensitivity of the tonic center of the hypothalamus to the negative feedback of low and constant levels of the estrogen which

result in a reduction in pulsatile release of GnRH which in turn causes decrease in pulsatile release of LH from the anterior pituitary gland.

Progesterone. Progesterone is a steroid hormone (21 carbons) formed primarily from cholesterol. Progesterone is synthesized primarily in the theca interna of antral follicles and the corpus luteum. It has been observed that progesterone concentrations fall rapidly after calving and remain low throughout the postpartum period. A short-term progesterone rise from 3 to 7 d before first postpartum estrus has been observed, however this increase was followed by decline to baseline concentrations at estrus (Arije et al., 1974; Stevenson and Britt, 1979; Rawlings et al., 1980; Humphrey et al., 1983; Werth et al., 1996). It has been observed that the rise in peripheral progesterone preceding the first estrus is frequently due to ovulation and corpus luteum formation without exhibition of behavioral estrus (Castenson et al., 1976; Stevenson and Britt, 1979); however; luteinized ovarian follicles may produce small amounts of progesterone (Corah et al., 1974). The reason for the short lifespan of the first postpartum corpus luteum appears to involve a rise in $PGF_{2\alpha}$ immediatley after uterine exposure to progesterone of the short luteal phase (Dailey et al., 1992). However one of the probable causes of short-lived corpora lutea may be sub-optimal levels of preovulatory estradiol secretion that precedes their formation (Garcia-Winder et al., 1986). The recent study by Mann and Lamming (2000) supports this notion of short luteal cycles in first-calf postpartum cows as an outcome of low levels of preovulatory estradiol that cause impaired inhibition of oxytocin receptors in endometrium of uterus, allowing increased $PGF_{2\alpha}$ release and premature luteolysis. Hunter (1991) in his review documented that induced corpora lutea regressed rapidly

after d 4, unless the animals (anestrous ewes) had been primed with progesterone before GnRH therapy or hysterectomy. It appears that presence of oxytocin receptors could be a crucial factor in influencing lifespan of CL which are in turn controlled by the steroid environment to which the uterus has previously been exposed.

The role of progesterone during the luteal phase is to inhibit the development of the endometrial oxytocin receptors until the appropriate time, while at the same time inducing endometrial ability to synthesize and release $PGF_{2\alpha}$ in response to oxytocin (Lamming and Mann, 1995). Exposure to progesterone for only 6 d is sufficient to maximize ability to release $PGF_{2\alpha}$ in response to oxytocin (Lamming and Mann, 1995). Therefore, it appears that progesterone influenced control of luteolysis may be mediated through regulation of oxytocin receptors. Nevertheless, the timing of the early luteal phase progesterone increase appears to be independent of the luteolytic signal in OVX cows and there may be involvement of some other factor to program luteolysis by a given time (Mann et al., 1998). Indeed occurrence of short cycles may be advantageous to the postpartum cow because they are generally followed by normal cycles, thus fertility after a short cycle may improve chances of embryonic survival. Indeed, Short et al. (1990) suggested that the short-term rise in progesterone before the first luteal phase may act as a primer, controlled by the ovary, for the endocrine system and the reproductive tract. Chang et al. (1981) documented that plasma estradiol concentrations were higher after progesterone releasing intravaginal device (PRID) removal for PRID administered postpartum cows than control cows. Results by Fike et al. (1997) support the concept of priming effect of progesterone who reported that exogenous progesterone administered

via an intravaginal device in combination with estradiol benzoate (EB) enhanced the expression of estrus and increased the proportion of cows that formed CL with a typical lifespan in suckled beef cows.

Nutrition

Low levels of energy before (Dunn et al., 1969; Falk et al., 1975) or after (Dunn et al., 1969; Wiltbank, 1970) calving increased the postpartum interval to resumption of ovarian cycling activity (Perry et al., 1991). In fact, a majority of pluriparous cows on a low plane of nutrition failed to display estrous behavior during the next breeding season (Wiltbank, 1970). On the other hand, cows fed high energy rations had shorter postpartum intervals to estrus (Bellows and Short, 1978; Henricks and Rone, 1986), and ovulation than cows fed a low or moderate energy ration (Ducker et al., 1985). Feeding postpartum suckled cows a high energy diet increased proportions of cows that exhibited estrus before the breeding season (Bellows and Short, 1978; Henricks and Rone, 1986), increased the number of cows that ovulated by 150 d after calving (Perry et al., 1991), and increased pregnancy rates compared to cows fed a low energy diet (Bellows and Short, 1978; Henricks and Rone, 1986; DeRouen et al., 1993). Indeed, the consequence of feeding a low energy diet results in a negative energy balance which in turn reduces the availability of glucose and increases the mobilization of body reserves (Grimard et al., 1995). Under this condition, basal metabolism decreases, requirements for growth and basic energy reserves take priority over reproductive processes such as onset of cycling activity, and pregnancy (Short et al., 1990; Grimard et al., 1997; Guedon et al., 1999).

There are some conflicting reports regarding the prepartum and postpartum nutrition regimens affecting postpartum ovarian cycling activity. For instance, Houghton et al. (1990) reported that cows fed a low energy diet prepartum and switched to a high energy diet postpartum had shorter postpartum intervals to estrus and more cows cycling than cows fed the low energy diet Prepartum and low energy diet postpartum. Contrary to these results, Dunn and Kaltenbach (1980) concluded that prepartum nutrition was more important than postpartum nutrition in reducing the postpartum interval. On the other hand Doornbos et al. (1984) determined that the effect of prepartum nutrition on pre-calving, calving, and postpartum data was not significant. The lack of difference found by Doornbos et al. (1984) may have been due to a relatively high plane of nutrition fed to cows in both treatments (135% and 110% of NRC requirements for high and low energy treatments, respectively).

One question that arises from these data is how do nutritional manipulations affect ovarian cycling activity during postpartum anestrus? It has been observed that increasing the energy intake beginning either two wk before parturition (Lammoglia et al., 1996), at parturition (Beam and Butler, 1997; DeFries et al., 1998), or at four wk after calving (Khireddine et al., 1998) increased the number of antral follicles in the ovaries. Furthermore, increasing the energy intake beginning at parturition prolonged the life span of CL induced by weaning and exogenous GnRH between days 21 and 26 postpartum (Williams, 1989). On the other hand, Gombe and Hansel (1973) and Beal et al. (1978) found that plasma progesterone concentrations were lower for first-calf cows fed a restricted energy diet. The low levels of progesterone in these cows could have been due

in part to the low rate of follicular development caused by poor nutrition documented by Henricks and Rone (1986) and Perry et al. (1991). Also, energy-restricted cows had smaller corpora lutea with lower progesterone contents on d 10 of the third postpartum cycle (Gombe and Hansel, 1973). However, Gauthier et al. (1983) reported that underfeeding for the first 45 days postpartum increased progesterone levels. These equivocal results may be due to the physiological status of the cows at the time of implementation of diets. Cows in varying body condition may respond to dietary treatments differently, and this interaction may affect how diet influences reproductive processes (for review see Wettemann, 1994).

Wright et al. (1992) reported that postpartum OVX beef cows subjected to treatments like thin or fat body condition showed significant differences in mean concentration, basal concentration, pulse frequency, and pulse amplitude of LH with cows in fat body condition certainly on the higher side at 35 and 63 d postpartum. Estradiol implantations in both groups resulted in reduction of above mentioned LH characteristics. Hence based on the data obtained, investigators have suggested that absence of interaction between body condition and presence or absence of E_2 implies the independent mechanisms through which body condition and E_2 (negative feed back) operate to affect the LH release.

Most cows with good body condition score at parturition resumed cycling activity (Vizcarra et al., 1998) and conceived (DeRouen et al., 1994; Spitzer et al., 1995) by the end of the breeding season. Body condition score at parturition was positively correlated with follicular development early postpartum (Ryan et al., 1994), pituitary LH content at

30 d postpartum (Connor et al., 1990), LH pulse frequency, and postpartum interval after weaning (Bishop et al., 1994). It appears that the body condition score, a measure of metabolic reserves, in concert with diet composition is an important factor affecting duration of postpartum anestrus in beef cattle. Thus, these factors should be taken into account when designing experiments that address the physiological mechanism related to the resumption of ovarian cycling activity in postpartum beef cows.

Suckling Stimulus

The most obvious factor that accounts for extended postpartum anestrus in beef cows is suckling. Suckling stimuli are the primary reason for extended anestrus in postpartum beef cows, if nutrition is not a limiting factor (Short et al., 1990; Williams, 1990; Stagg et al., 1998). Calf removal (weaning) at birth significantly reduces the postpartum anestrous interval compared to cows suckling calves (Williams, 1990; Short et al., 1990). Several studies have been performed to evaluate the effect of either short- or long-term weaning at different days postpartum on postpartum interval to resumption of ovarian cycling activity. Walters et al. (1982b) showed that weaning of calves between 20 and 40 d postpartum and before onset of estrous cycles accelerated resumption of ovulatory cycles; this response occurred within a wk after weaning. Similarly, either short-term weaning for 24 to 72 h or restricting the number of suckling bouts of calves can reduce postpartum anestrous interval in beef cows (Randel, 1981). On the other hand, intensive suckling prolongs the anestrus (Wettemann et al., 1986; McNeilly, 1988). Contrary to the results of these studies is the report by Bonavera et al. (1990) who reported that short-term calf removal for 72 h on 33 d after calving had no

effect on proportions of cows exhibiting estrus. The reason for this negative result was probably due to the fact that 95% of the control cows in this study showed estrus by 38 d postpartum. Therefore, weaning or removal of suckling stimuli for short or long periods accelerates resumption of ovarian cycling activity, whereas intensive suckling prolongs the postpartum anestrous interval in postpartum beef cows.

What is the physiological consequence of weaning on the reproductive neuroendocrine-endocrine system that allows for resumption of ovarian cycling activity? Garcia-Winder et al. (1984) evaluated intervals to initiation of the ovarian cycling activity and luteinizing hormone (LH) concentrations and in intact pluriparous beef cows that were either suckled *ad libitum* by two calves, suckled once daily by one calf, or not suckled. They found that interval to resumption of cycling activity was shortest for non-suckled cows, intermediate for cows suckled once daily, and longest for cows suckled *ad libitum* by two calves. Mean LH concentrations and LH pulse frequency increased in cows suckled *ad libitum* by two calves, cows suckled once daily by one calf and non-suckled cows in the same manner as they approached ovulation. This means that neuroendocrine-endocrine mechanism regulating the resumption of cycling activity is independent of suckling. In other words, cows that are weaned show the same LH response as those suckled intensively however, the effect happens to occurs at different times. Thus, suckling merely prolongs the initiation of the neuroendocrine-endocrine mechanism regulating resumption of postpartum ovarian cycling activity.

How does suckling prolong the initiation of ovulatory activity in postpartum beef cows? Suckling appeared to decrease the ability of the hypothalamus to respond to the

positive feedback effect of E_2 within the first 3 wk after calving (Short et al., 1979). Thus, tonic LH secretion is suppressed which results in lower estrogen production by the ovarian follicles thereby suppressing the preovulatory LH surge center in the hypothalamus that in turn delays ovulation early in the postpartum anestrous period.

Acosta et al. (1983) reported that postpartum OVX cows exposed to early weaning and exogenous progesterone had a rapid increase in LH pulse frequency and mean LH concentration than cows which were normally nursed or normally nursed with progesterone implant, or early weaned without progesterone implant. They suggested that the postpartum nursing stimulus increases the sensitivity of the tonic center of the hypothalamus to the negative feedback of low and constant levels of the estrogen. In a subsequent study, Garcia-Winder et al. (1984) compared mean LH concentrations and LH pulse frequency in ovariectomized (OVX) cows that were either suckled *ad libitum* by two calves, suckled once daily by one calf, or not suckled. Mean LH was different in OVX suckled cows compared to OVX non-suckled cows on d 13, 20, and 27 postpartum. Pulse frequency of LH was lower in suckled OVX cows compared to non-suckled OVX cows from d 6 through 55 postpartum. They concluded that suckling and ovarian factors interact during the postpartum period to suppress LH secretion and pulse frequency. Based upon the increase in mean LH concentration and frequency on different days postpartum, the authors suggested that suckling stimuli becomes less potent an inhibitor as time postpartum progresses and this allows the hypothalamo-hypophyseal axis to increase GnRH and LH secretion. The increase in LH pulse frequency in cows suckling two calves was delayed extensively until d 55 postpartum; when they began to exhibit

ovulation (Walters et al., 1982b). A similar concept was postulated earlier in lactating rats (Hammons et al., 1973; Smith and Neill, 1977).

It would appear that suckling interacts with ovarian steroid inhibition to delay resumption of cycling activity in postpartum cows. It is well known that estradiol-17β (E_2) in both males and females inhibits LH secretion from the pituitary by inhibiting the pulsatile or tonic secretion of gonadotropin releasing hormone (GnRH) from the median eminence region of the hypothalamus (Hafez et al., 2000). The results of the study by Acosta et al. (1983) and Garcia-Winder et al. (1984) seem to support the notion that suckling interacts with E_2 to inhibit LH secretions in postpartum cows. There have been a few studies which have addressed this specific interaction. Hinshelwood et al. (1985) reported that mean concentrations of LH in OVX cows at d 10 after calving were lower in suckled than non-suckled cows following removal of ovaries on d 5 postpartum. This indicated that suckling mechanism operates independent of the negative feedback effect of ovarian steroid. In support for these data, Zalesky et al. (1990) attempted to show that suckling has a direct inhibitory effect on hypothalamic GnRH release independent of negative feedback effect of E_2. Using OVX cows that were either non-suckled or suckled by three calves, and intact non-suckled cows, they showed that between d 31 and 37 after calving mean LH concentrations were greater for OVX non-suckled cows relative to LH concentrations than either of the other treatments. Furthermore, content of GnRH in median eminence OVX non-suckled cows was lower than that found in either intact suckled or OVX suckled cows. Thus OVX or removal of the source of E_2 caused depletion of GnRH stores in median eminence in postpartum cows. However; suckling

appeared to inhibit GnRH depletion from median eminence into hypophysial portal circulation in OVX suckled cows. These data show that intact non-suckled cows had a normal functioning of the negative feedback mechanism exerted by the estrogen on the median eminence of the hypothalamus as well as pituitary gland while in case of OVX suckled cows suckling stimulus is apparently playing the major role in inhibiting the GnRH followed by LH release from pituitary gland and it acts at the level of hypothalamic region to suppress GnRH release in response to ovariectomy. Therefore, suckling may have a direct effect on the hypothalamic-pituitary axis independent of ovarian hormones. It is possible that suckling may be acting through some other mechanism than facilitating the sensitivity of the hypothalamus to the negative feedback effects of E_2 which is more important in inhibiting the pituitary LH release in postpartum anestrus cows.

Physical Presence of Calf

Indeed it appears that suckling stimuli play a major role in delaying cycling activity in postpartum cows, but how? It is obvious that the intimate association between the calf and the mammary gland would have a major role in inhibiting LH release and delay the onset of cycling activity. Sensory information associated with the calf suckling the teat and nuzzling the inguinal region is transmitted to the hypothalamus and exerts the inhibitory action on GnRH release (McNeilly, 1988). However, it may not be the case that this type of somato-sensory information is the primary factor for the negative action of suckling on hypothalamic GnRH release.

Short et al., (1972) found that the postpartum interval to first estrus in non-suckled mastectomized cows was shorter than that for non-suckled intact cows. These results were confirmed by Grass and Hauser (1981), who performed mastectomies when heifers were two months of age. In three consecutive parities as mature cows, postpartum intervals to ovulation were shorter in mastectomized cows maintained with no calves than in udder-intact suckled cows. These data indicate that the presence of a functional mammary gland independent of suckling may contribute to a delay in return to cycling activity in postpartum cows. However, studies by Viker et al (1989, 1993), Mukasa-Mugerwa et al. (1991) and Stevenson et al. (1994), using the mastectomized cows or nose plated calves, indicated that the continual presence of the cow's calf independent of suckling or presence of mammary gland inhibits resumption of estrus cycles after calving. Their findings were supported by Williams et al. (1993) who failed to simulate the effects of suckling using mechanical, electrical, and thermal hyperstimulation of sensory neurons in teat; these treatments had the same effect as placing a latex mask over the teats and udder of the cow (McVey and Williams, 1991). Furthermore, Hoffman et al. (1996) found that restricting contact of the calf to only the head and the neck of the dam delayed resumption of ovulatory cycles compared to cows suckling calves. Here the delay for cows whose calves were restricted was not as long as that for those cows whose calves were allowed to suckle *ad libitum*. Hence, it appears that although lactating mammary gland contributes some to the anestrous condition, a more important factor may be physical presence of calf.

What is it about the physical presence of calf that impedes the hypothalamo-hypophyseal axis from re-establishing proestrous-like patterns of LH secretions after parturition? Williams et al. (1993) using total denervation of the mammary gland tested the hypothesis that mammary somatosensory pathways by were not required for the presence of the calf to inhibit pulsatile LH secretions and delay resumption of ovarian cycles. They found that weaning of non-denervated cows increased LH pulse frequency within 9 to 13 d and decreased interval to onset of ovulatory cycles compared to non-denervated suckled cows. However, mammary denervation in either suckled cows failed to increase LH pulse frequency and interval to the onset of ovulatory cycles. They concluded that mammary somatosensory pathways are not a major factor for a transfer of cues that regulate the hypothalamo-hypophyseal axis during postpartum anestrus in cows. They suggested that a maternal-offspring interaction might be an important component in mediating the neuroendocrine-endocrine regulation of postpartum anestrus in cows.

Silveira et al. (1993) showed that neither LH pulse frequency nor interval to first ovulation after imposition of treatment differed between cows forced to suckle alien calves or cows whose calves were weaned. Both were significantly shorter than those associated with cows suckling their own calves. Williams and Griffith (1995) in their review emphasized the key role played by the suckling calf on the resumption of postpartum ovarian cycles in cattle through inhibitory influence on central regulatory elements controlling the release of GnRH through median eminence. They suggested LH secretion during postpartum anestrus is associated with the exteroceptive cues of the dam's own calf and not with the somatosensory stimulation of mammary gland. They

referred to this relationship as the "maternal-calf bond". This bond appears to evolve during sometime after d 2 of calving because if unrelated calves were transferred to foster dams within 2 d of birth, the percentage exhibiting estrus by 60 or 90 d postpartum did not differ from that of cows nursing their own calf (Wettemann et al., 1978).

Thereafter, Griffith and Williams (1996) evaluated specific exteroceptive stimuli and their importance in this bonding relationship between the dam and calf. They found that LH pulse frequency increased in cows that were blinded, anosmic or both and suckling either their own or alien calf, to the same extent as weaned cows. Therefore, one might conclude that the effect of suckling on prolonging the postpartum anestrus in cows involves a social bonding between the cow and calf. This bond is established via visual and olfactory cues of the calf. These studies established that a cow-calf bond dictated by exteroceptive cues is an essential component in regulating the anestrous interval to resumption of ovulatory cycles in postpartum cows.

There are two recently reported studies that indicate that there is an additional component to the cow-calf bond hypothesis that involves stimulation of the mammary gland. Lamb et al. (1997) reported that interval to first increase in progesterone (ovulation) was shorter for cows that were weaned (15 d) or had their calf present but restricted from udder contact (20 d) compared to cows suckling their own calves (36 d), cows suckling unrelated calf (38 d), or cows suckling an unrelated calf with own calf present continuously but restricted from udder contact (38 d). Treatments were initiated between d 13 and 18 after calving. These results appear to contradict the results of Williams et al. (1993), Silveira et al. (1993), Griffith and Williams (1996). Recently,

Lamb et al. (1999) indicated that twice daily suckling had the same effect to prolong postpartum anestrus as suckling *ad libitum,* however, milking two or five times daily, even in the presence of a cow's non-suckling calf did not prolong postpartum anovulation. Thus, it appears that milking does not mimic the perception of suckling, even when the cow's own calf is present to reinforce the cow-calf bond. Although cow's recognition of her calf is requisite for prolonging the anestrus, further inhibition will be enforced only when suckling but not milking follows the cow-calf recognition. These researchers have emphasized the important role of *ad libitum* suckling together with maternal bonding in prolonging the process of anovulation.

Social Interaction and Reproductive Function

Animals communicate information concerning reproduction to conspecifics in order to coordinate reproductive activities. However, synchronization of mating is one of the problems associated with sexual reproduction in mammals. One way by which reproductive efficiency in farm animals can be increased is by decreasing the interval from parturition to conception. Hormonal treatments have resulted in marginal success in decreasing the same. Hence this interval may be reduced by methods developed from research to elucidate the factors and mechanisms that regulate gonadotropin secretion (for review see Wettemann, 1980).

Chemical communication with pheromones is one way of transmitting such information as suggested and speculated by some earlier researchers. In mammals, signaling is thought to take place through either olfaction, auditory, visual (sight) or tactile stimuli, or combinations thereof (for review see Rekwot et al., 2000b).

Pheromones are airborne chemical substances (signals) released in the urine or feces of animals or secreted from cutaneous glands that are perceived by the olfactory system and that elicit both behavioral and endocrine responses in conspecifics (for review see Rekwot et al., 2000b). Pheromones can be classified into two types, signaling and priming pheromones. Signaling pheromones are biochemical substances that cause an immediate behavioral response, invoking a classical stimulus-response pattern mediated by the central nervous system (Izard, 1983). Responses of conspecifics are generally behavioral and the effect is reversible and short-term. On the other hand, priming pheromones are biochemical substances that induce physiological events through inhibition or stimulation to alter the neuroendocrine-endocrine system that regulates reproductive activity (Izard, 1983). This class of pheromones is a major component involved in mediating the phenomenon of biostimulation. Priming pheromones initiate a chain of endocrine, reproductive events, which are generally long-lasting and irreversible (Izard, 1983).

In the following section, the term biostimulation will be used to describe male and female interactions that may be mutually beneficial for successful reproduction. Originally, biostimulation was defined as any stimulatory effect of a male on estrus and ovulation in females through genital stimulation, priming pheromones, or other less defined external cues (Chenoweth, 1983a). However, there are cases where females may have a biostimulatory effect on males or females; thus, this term is not mutually exclusive for a given sex in mammals.

Rodents. The presence of a male hastens the onset of puberty in rodents (Rattus norvegicus; Vandenberg 1976). Female ovarian function can also be influenced by cues originating from other females and age at puberty in young females is increased by priming pheromones produced by grouped females (Izard, 1983).

Pheromonally accelerated puberty in mice appears to involve a hypothalamic-pituitary pathway (Izard and Vandenberg, 1982). Bronson and Desjardins (1974) showed that exposing prepubertal female mice to males caused a rapid (within 1 h) increase in plasma LH concentrations, which was maintained for several hours. FSH levels did not change. Preovulatory surges of LH and FSH were detected on d 3 after exposure to males.

Exposure to male mice and housing of females in cages containing soiled bedding from males modified the peak incidence of mating when males were finally allowed to mix with females (Whitten, 1956). Ovariectomized mice implanted with .5 μg of estradiol exposed to male mouse urine had significantly elevated serum LH concentrations (Bronson, 1976).

Perhaps the most profound effect of males on the reproductive activity of females is the Bruce effect in mice (Bruce, 1959). In this process, exposure of a pregnant female to an unfamiliar male within 72 h after mating to a familiar male results in loss of that pregnancy and resumption of estrous activity. This effect is mediated by a priming pheromone produced by intact male mice that acts through the vomeronasal organ located in the nasal passages of mice. The pheromonal signal is transduced into a nervous signal and relayed to the hypothalamus. This pathway increases dopamine release from the

hypothalamus which in turn inhibits prolactin secretion from the pituitary gland. In mice prolactin is a luteotropic hormone. The reduction in prolactin secretion results in luteolysis and loss of progesterone secretion which causes abortion and return to estrus (Marchlewska-Koj, 1983).

It appears that in rodents chemical communication through pheromones is the main mode of communication among conspecifics for altering reproductive processes. Male pheromones influence age at puberty or ovarian function in females accompanied by appropriate changes in concentrations of gonadotropins. Furthermore, the vomeronasal organ is predominantly involved in conveying the pheromonal signals to hypothalamus to bring about alterations in reproductive processes in female rodents.

Swine. Puberty in gilts has been shown to be accelerated by the presence of a boar in domestic pig (Brooks and Cole, 1970). Paterson and Lindsay (1980) showed that gilts raised with contemporary intact males reached puberty earlier than gilts raised with castrated male contemporaries. However age at puberty did not differ between groups of gilts either exposed to mature intact boars or contemporary boars (Paterson and Lindsay, 1980).

Rowlinson et al. (1975) conducted observations at commercial swine unit and reported that all sows exposed to boars exhibited lactational estrus, and conception rate at lactational estrus was 84.9 %. In a study by Walton (1986) the proportion of sows ovulating or exhibiting estrus within 10 d of weaning increased as time of exposure to boars increased. It appears that presence of boar influences the age of puberty and return

to estrus or ovulation in sows. Moreover, the biostimulatory effect of boars operates via pheromonal mechanism and these pheromones appear to be androgen-dependent.

Small Ruminants. The presence of bucks stimulated Angora does to ovulate early in the breeding season (Shelton, 1960). Claus et al. (1990) found that acyclic does exposed to male hair, diethyl extracts of male hair, and polar and nonpolar subfractions of diethyl ether extract for 72 h showed a rapid increase in LH and estradiol concentrations, and resulted in exhibition of estrus in seasonally anestrous does. Moreover, it was observed that though anosmic does exposed to bucks exhibited increased LH pulses, interval to increased LH and onset of estrus was similar to control does, the percentage of anosmic does exhibiting estrus was lower than control females after exposure. These results suggest that olfaction is not responsible for the buck effect and the possibility that pheromones may be absorbed directly across the surface of respiratory alveoli and carried via blood to hypothalamus, anterior pituitary or ovary to cause their effect in goats.

Introduction of rams to ewes early in the breeding season stimulated a varying proportion of ewes to ovulate at 37 to 41, 61 to 65, 70 to 72, 84 to 88 and 94 to 96 h after exposure (Knight et al., 1978). The stimulated ovulations in ewes following ram introduction were preceded by LH peaks (Knight et al., 1978). Moreover, a higher percentage of ewes ovulated if exposed to rams for 72 h than ewes exposed only for 6 to 24 h (Oldham and Pearce., 1984). This means that seasonally anestrous ewes must be exposed for at least 24 h to rams to receive sufficient pheromonal stimuli to induce the ovulatory cascade and resumption of cycling activity.

It is clear from the results that ram induces an ovulatory response in the ewe. The question thus arises concerning the physiological changes that occur to accelerate ovarian cycling activity. Serum LH concentrations rose within 2 h of exposure to a peak (Knight et al., 1978; Martin et al., 1980; Cohen-Tannoudji and Signoret, 1987). Frequency of LH pulse increased and baseline concentrations were elevated within 18 h of exposing ewes to rams (Martin et al., 1980; Poindron et al., 1980; Oldham and Pearce, 1984). Oldham and Pearce (1984) suggested that the LH surge that occurs within 18 h of the introduction of rams to anovular ewes could not be induced by the release from estradiol negative feedback alone. Ewes re-exposed to rams 1, 5, or 15 d later exhibited the same LH response as at the first exposure. Thus, continuous presence of male is necessary to maintain pulsatile pattern of LH, which decreases rapidly after removal of rams (Cohen-Tannoudji and Signoret, 1987). However, a marked and sustained increase in LH pulses was not essential to induce ovulation (Oldham and Pearce, 1984).

It appears that continuous presence of rams may be necessary to initiate high frequency and low amplitude pattern of LH necessary for ovulation in seasonally anestrus ewes; however it may not be necessary and sufficient condition to cause ovulation.

Bovine

Cow Effect. Wright et al. (1994) reported that cows exposed to a cow in estrus or her cervical mucus discharge have differential intervals of postpartum anestrus compared to cows that were not exposed to a cow in estrus or exposed to distilled water. These authors have suggested that cervical mucus from cows in estrus appears to contain a compound (s) which may be helpful in reducing the postpartum anestrous interval in

cows particularly, cows with extended anestrus. It is a question of speculation as to whether or not these compounds act as priming pheromones to reduce the postpartum anestrous interval in cows.

Bolanos et al. (1998) reported that exposure of suckled postpartum Zebu cows at parturition to progesterone-treated cows did not give significant proportion of cows (19%) resuming cycling activity compared to proportion of cows (57%) exposed to progesterone-treated cows and a bull within first 21 d after start of treatments. It appears that progesterone-treated cows are not effective in accelerating the onset of ovarian cycling activity in anestrous cows during the first 21 d after calving. However progesterone-treated cows in combination with bull exposure can influence the early onset of cycling activity during the first 21 d after calving. It appears that effect of pheromones by estrual cows on the reproductive activity of anestrous cows may be a possible factor involved in affecting postpartum anestrus.

In support of the idea that cows produce an excretory pheromone, Ramesh Kumar et al. (2000) reported that bovine estrual urine was distinguished biochemically by the presence of two specific compounds namely, di-n-propyl phthalate and 1-iodoundecane, that elicit signals to allow a bull to detect the estrous odor. There is a possibility that urinary signaling pheromones of cows in estrus may increase the efficiency of estrous detection by bulls.

Bull Effect on Puberty. The presence of mature bulls had no effect on accelerating the onset of puberty in beef heifers (Berardinelli et al., 1978; Roberson et al., 1987). However, Roberson et al. (1991) found that although prepubertal heifers exposed

to bulls in the first yr of a two-yr experiment did not attain puberty earlier than heifers isolated from bulls, in the second yr, heifers exposed to bulls attained puberty sooner and at lighter weights than heifers not exposed to bulls. The effect of bulls was greater in the high growth rate treatment than in the moderate growth rate treatment. Thus, biostimulatory effect of bulls may not directly reduce the age at puberty in heifers; however, other factors, like nutrition and body condition, may interact with the biostimulatory effect of bulls to alter the age at puberty in heifers.

Bull Effect on Postpartum Anestrus. Early observational studies indicated that the presence of bulls increased the percentages of postpartum anestrous cows that showed estrus compared to cows not exposed to bulls (Nersesjan, 1962; Sipilov, 1964, Ebert et al., 1972; Foote, 1975). However, these studies were not designed to specifically address the effect of the presence of bulls on resumption of ovarian cycling activity, and the observational differences may have been due to differences in more efficient estrus detection by bulls.

Skinner and Bonsma (1964) are the earliest researchers to provide evidence of a bull effect on postpartum ovarian activity in cows. They found that if a vasectomized bull was introduced to a breeding herd of females 30 d before the breeding season, postpartum ovarian activity increased. Thereafter, several researchers reported that cows exposed to yearling or mature intact, epididectomized, or vasectomized bulls during the postpartum period had shorter postpartum intervals to resumption of ovarian cycling activity than cows isolated from bulls (Macmillan et al., 1979; Zalesky et al., 1984; Berardinelli et al., 1987; Scott and Montgomery, 1987; Custer et al., 1990; Naasz and

Miller, 1990; Stumpf et al., 1992; Cupp et al., 1993; Soto Belloso et al., 1997; Rekwot et al., 2000a). Of particular interest is the study by Soto Belloso et al. (1997) who reported that introduction of bulls to first-calf or mature Brahman postpartum cows two wk after calving reduced the anestrus period by 29 and 19 d respectively relative to cows not exposed to bulls, which provides an economical and a practical option to tropical cattle producers.

The biostimulatory effect of bulls may interact with other factors known to influence postpartum anestrus such as, nutrition, body condition of cows, and season. Hornbuckle et al. (1995) evaluated the interaction of feeding two different grasses and exposing cows to bulls. Exposing cows to bulls accelerated resumption of cycling activity in cows independent of grass type. However, the effect of bulls was more pronounced in cows on fescue pastures than for cows on bluestem pastures.

Stumpf et al. (1992) reported that postpartum cows in poor body condition exposed to bulls initiated estrus cycle 14 d earlier than the cows in poor body condition not exposed to the bulls. For cows with good body condition this difference was smaller (6 d). Cows that lose body weight and are in poor condition at parturition would be expected to have long intervals of the postpartum anestrus (Monje et al., 1983), and the duration of the postpartum anestrus would not be expected to be affected by the presence of the bulls. The authors suggested that the presence of bulls may have stimulated ovarian function by stimulating the secretion of GnRH to a greater degree in cows in poor body condition than in cows with good body condition. They speculated that the

biostimulatory effect of bulls may be used to offset high maintenance costs during the winter and maintain reproductive efficiency of mature cows.

There are a few reports in which presence of bulls failed to reduce the anestrous interval in postpartum cows. Macmillan et al. (1979) reported that the bulls were effective in reducing the anestrous interval in spring-calving cows but not in fall-calving cows. Perry et al. (1993) reported similar results. These results are difficult to explain but there might be a possible interaction between the effect of bulls and season, such that fall-calving cows would be refractory to bull stimuli during the winter months. The other possible reason for failure of fall calving cows to resume cycling activity earlier in breeding season may be to ensure the survival of calf during next calving season.

Bonavera et al. (1990) reported that the bull exposure beginning 33 d postpartum and/or short-term calf removal at 33 d postpartum for 72 h had no effect on proportions of cows exhibiting estrus. The reason for this result was that 95% of control cows resumed ovarian cycling activity by d 38 after calving.

Recently, Shipka and Ellis (1998) reported that either fence-line contact or close proximity to the bulls did not affect the onset of estrus in postpartum high-producing dairy cows. They concluded that fence-line exposure or close proximity to bulls limited direct physical contact with cows, thus reducing the biostimulatory effect of bulls. In a second experiment, Shipka and Ellis (1999) reported that cows not exposed to bulls had a shorter interval to progesterone rise than cows either exposed to fence-line contact twice daily or housed in close proximity to a pen containing bulls. The results of these two experiments are quite different from those obtained in beef cows but not unexpected.

One explanation for these results may be that fence-line contact or close proximity of milking cows to bulls may be insufficient for the biostimulatory actions of bulls, i.e., direct physical contact is essential. More importantly is the fact that calves were weaned at calving so that one would expect that the effect of weaning would supercede the biostimulatory effect of bulls. Furthermore, Fernandez et al. (1993) reported that interval to resumption of cycling activity did not differ between cows exposed to bulls 72 h after calving or at 30 d after calving; indicating that the biostimulatory effect of bulls during at least the first 20 d after calving is relatively ineffective compared to its effect later in the postpartum anestrous period.

It is interesting to note that Fike et al. (1996) reported that fence-line contact of primiparous cows with bulls reduced the postpartum interval to resumption of cycling activity. However, this type of exposure in multiparous cows was not effective in reducing the interval to resumption of cycling activity. The reasons for the ineffectiveness of fence-line contact to reduce the interval to cycling activity in multiparous cows may indicate that multiparous cows require more intensive exposure to bulls for bulls to accelerate resumption of cycling activity, or perhaps multiparous cows had a shorter period to respond to the biostimulatory effect of bulls because the interval to resumption of cycling activity is considerably shorter in multiparous cows than primiparous cows.

From the data presented to this point, bull exposure accelerates resumption of cycling activity in postpartum suckled *Bos indicus* and *Bos taurus* cows. The response to bulls may be affected by parity, season, and plane of nutrition and/or body condition.

The presence of bulls decreases postpartum interval to the resumption of cycling activity. An important question regarding this effect is, "when and for how long cows must be exposed to bulls to take advantage of the biostimulatory effect of bulls?' There are no reports in the literature which have focused upon this question. However, there are four studies that shed some light on this question. Fernandez et al. (1993) hypothesized that exposing first-calf suckled cows to bulls continuously beginning on d 3 or on d 30, or from d 3 to 30 only after calving would not affect resumption of cycling activity. They found that exposing cows to bulls on either d 3 or 30, or for 30 d only after calving accelerated resumption of cycling activity compared to cows not exposed to bulls. Interestingly, they found intervals to resumption of cycling activity did not differ among cows exposed to bull 3 d after calving, exposed to bull on d 30 after calving, or cows exposed to bull from d 3 to 30 only after calving. This means that at least 30 d of exposure is necessary for the bull to influence cycling activity in first-calf suckled cows early in the postpartum period. More importantly, the interval from bull exposure to resumption of cycling activity in cows exposed continuously to bull beginning on d 30 was shorter that for cows exposed to bulls continuously from d 3 or from d 3 to 30 after calving. Zalesky et al. (1984) reported that a greater proportion of multiparous cows exhibited estrus by d 53 after calving when bulls were introduced to cows on d 3 postpartum than when bulls were introduced to cows on d 53 postpartum. However, we examined the rate of change in the proportion of cows that showed estrus after exposure to bulls from either d 3 or 53. We found that cows exposed to bull after d 53 postpartum responded more rapidly to bull exposure than cows exposed on d 3 postpartum. It

appears that Scott and Montgomery (1987) may have obtained a similar pattern and rate of increase in cycling activity of cows after mixing and exposing herds of cows early exposed to bulls and cows not exposed to bulls to four mature bulls on d 56 approximately.

Fernandez et al. (1996) evaluated the intensity of bull exposure on resumption of cycling activity in first-calf suckled beef cows. Exposure of cows to bulls on d 30 continuously was more effective than intermittently exposing cows for 2 h once daily on every third d for 18 d. One interpretation of these data is that continuous but not intermittent exposure to bull stimuli is necessary to sustain the action by which bulls cause a reduction in postpartum anestrus.

The conclusions from the results of the aforementioned studies are: 1) postpartum cows exposed to bulls appear to respond to the biostimulatory effect of bulls more rapidly after d 30 postpartum than cows exposed shortly after calving, 2) continuous exposure of cows to bulls is a requirement, whether it is in direct physical contact or direct fence-line contact 3) the biostimulatory effect of bulls requires a period of at least 20 to 30 d to manifest its action, and, 4) there appears to be no biostimulatory effect of bulls if calves are weaned early after calving.

The question is by what mechanism bull effect acts to reduce the postpartum anoestrus interval? There are few reports which have attempted to address the mechanism by which the biostimulatory effect of bulls acts upon the postpartum physiology of cows. Custer et al. (1990) tested the hypothesis that bull exposure altered characteristics in LH concentrations. Exposure to bulls reduced the postpartum interval

to resumption of cycling activity in primiparous cows. However, there was no difference in LH basal concentrations, amplitude, frequency, and duration of LH pulses between bull exposed or not exposed cows. The authors conceded that the sampling regimen used in this study was such that the immediate effect of bull exposure on LH concentrations might have remained undetected.

Fernandez et al. (1996) tested the hypothesis that the presence of the bull induces a rapid response in pulsatile LH patterns and that this is maintained until cows exhibit estrus and/or ovulation. Intensive samples were collected within 45 min at 15-min intervals for 6 h beginning on d 30 postpartum and every third d until d 48 postpartum in cows exposed to bulls either continuously from d 30 postpartum or intermittently for 2 h daily every third d until d 48 postpartum. Mean LH concentrations and LH pulse frequencies were maintained in cows exposed continuously to bulls after d 30 postpartum. Surprisingly, mean LH concentrations and LH pulse frequency were increased at every 3-d sampling period in cows exposed to bulls for 2 h daily. However, postpartum intervals to estrus for cows exposed continuously to bulls from d 30 postpartum were significantly shorter than for cows exposed to bulls intermittently over an 18-d period (64 and 88 d, respectively). In fact, postpartum interval to estrus for cows exposed intermittently to bulls was not different from that of cows not exposed to bulls (88 and 79 d, respectively).

Baruah and Kanchev (1993) concluded that oronasal treatment with bull urine increased concentrations of LH and FSH within 80 min. in dairy cows treated on d 7 after calving. Previously, Izard and Vandenberg (1982) found that a higher proportion of

prepubertal heifers treated oro-nasally daily with bull urine attained puberty earlier than heifers treated with water. These studies support the hypothesis that bull urine may contain a priming pheromone that hastens the puberty in heifers and alters LH and FSH secretion in postpartum cows.

Exposing postpartum cows to bulls acutely increases mean concentrations of LH and LH pulse frequency. However, if bulls are not continuously present with cows for at least 18 d then the presence of bulls fails to reduce the postpartum anestrus in suckled beef cows between d 30 and 48 postpartum. Furthermore, the effect of the bull on reducing the postpartum anestrous interval is mediated through the hypothalamic-pituitary-ovarian axis. This must involve a change in the GnRH pulse generator which alters the frequency of LH pulses in such a manner as to induce final maturation of dominant follicles, which in turn increases estradiol secretion to trigger the LH surge mechanism. The mechanism whereby the bull affects a change in the hypothalamo-hypophysial axis remains unknown.

Of particular relevance to the experiments reported in this thesis is the study by Burns and Spitzer (1992) in which they focused upon defining the biostimulatory effect of bulls. They showed that multiparous beef cows exposed to either bulls or testosterone-treated cows (TTC) early in postpartum had similar and short postpartum anestrous intervals than cows isolated from either bulls or TTC. From the study the authors suggested that androgenized cows and/or bulls elicit or induce similar biostimulatory effects in reducing the postpartum interval to estrus in suckled postpartum beef cows. The possible conclusion one can draw is either androgenization has induced the cows to

produce androgen dependent pheromones, that affected the anestrous interval, or the androgenization has resulted in noticeable change in their behavior, which mimics to that of bulls. Therefore the androgenized cows produced the exteroceptive cues through their behavior, which facilitated the early resumption of the cycling activity in suckled anestrous cows. It appears that biostimulatory effects of bulls may be a combination of pheromonal and behavioral cues. For the bull effect to affect reproductive activity of a postpartum cow these two cues may have to act together to make biostimulatory effect of bulls practically viable.

It appears that bull urine or possibly other body secretions of a bull may contain some signaling or priming pheromones that cause an acute change in gonadotropin secretions in anestrus cows which, may or may not be manifested by early resumption of ovarian cycling activity by beginning of breeding season. However, it is not known whether these kinds of pheromonal cues need be reinforced by behavioral interaction or social interaction or social bonding between a bull and a cow to culminate ultimately in remodeling the sexual behavior and cycling activity of postpartum primiparous or multiparous cow.