Effect of exposure to bulls on the postpartum interval to resumption of ovarian cycling activity and estrus, patterns of luteinizing hormone secretion and breeding performance in first-calf suckled beef cows

by David Louis Fernandez

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
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Abstract:

Experiment 1. This experiment was designed to determine whether exposing first-calf suckled beef cows to mature bulls for the first 30 days, after 30 days, or continuously postpartum alters the postpartum anestrous period and first service pregnancy rates. Postpartum, first-calf, suckled, crossbred, beef cows were exposed continuously to mature, epididectomized bulls (BE; n = 18), exposed to bulls for the first 30 days postpartum (BE/NE; n = 17), after the first 30 days postpartum (NE/BE; n = 16), or isolated from bulls (NE; n = 18). Weekly blood samples were assayed fbr. progesterone to determine resumption of ovarian cycling activity. Cows were bred for 21 days by AI and then exposed to fertile bulls for an additional 35 days. Postpartum interval to resumption of ovarian cycling activity did not differ ($P>.10$) among BE, BE/NE, and NE/BE cows but was 15.4 days shorter ($P<.05$) than for NE cows. Overall pregnancy rates did not differ ($P>.10$) among treatments. AI pregnancy rates for BE/NE and NE/BE cows were greater ($P<.05$) than for NE cows. Exposure to bulls for the first 30 days postpartum or after the first 30 days postpartum decreases the postpartum interval. Exposure to bulls may improve breeding performance in first-calf suckled beef cows.

Experiment 2. The objectives of this experiment were to determine if exposure to bulls continuously beginning on Day 30 postpartum or for two hours every third day beginning on Day 30 alters the interval to first estrus, patterns of LH secretion, and AI and total pregnancy rates'. Postpartum, first-calf, crossbred, suckled, beef cows were assigned to exposure to mature epididectomized bulls (BE; n = 20), isolation from bulls (NE; n = 32), continuous exposure to bulls after Day 30 (NEBE, n = 10), or intermittent bull exposure (BEI, n = 21). Blood samples were collected from indwelling jugular catheters every 15 minutes for 6 hours every third day from Day 30 to 48 postpartum and assayed for LH. Cows were observed twice daily for estrus. On June 1, cows were combined in one pasture and bred by AI for 21 days. More ($P < .10$) BE and NEBE cows exhibited estrus before the AI breeding period than BEI cows. Day of first estrus occurred earlier ($P < .05$) for BE and NEBE cows than BEI and NE cows. AI pregnancy rates were higher ($P < .05$) for NEBE cows than for BE, BEI, and NE cows, which were not different ($P > .10$). Overall pregnancy rates were highest ($P < .05$) for BEI cows. Mean LH concentrations and pulse frequency were higher ($P < .05$) for BEI and NEBE cows than for NE cows. BEI cows had higher ($P < .10$) LH pulse frequency than NEBE or NE cows on the first day of treatment. Exposing cows to bulls increases mean LH concentrations and pulse frequency, but other factors appear to be involved in reducing postpartum anestrus.

EFFECT OF EXPOSURE TO BULLS ON THE POSTPARTUM INTERVAL TO RESUMPTION OF OVARIAN CYCLING ACTIVITY AND ESTRUS, PATTERNS OF LUTEINIZING HORMONE SECRETION AND BREEDING PERFORMANCE IN FIRST-CALF SUCKLED BEEF COWS

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ABSTRACT

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INTRODUCTION

The primary goal of beef cattle producers is to produce a live calf from each cow every year. It is well established that long postpartum anestrus periods are a major obstacle in the attainment of this goal (Wiltbank, 1970). Cows must conceive by 85 days postpartum in order to maintain a one year calving interval. Cows with long postpartum intervals have lighter calves at weaning and are less likely to conceive during the breeding season the following year. Therefore, it is imperative to know and understand the factors controlling postpartum anestrus and the mechanisms whereby they exert their effects.

Many factors have been shown to affect postpartum interval. Dairy breeds have been shown to return to estrus by 14 to 17 days postpartum (Marion and Gier, 1968; Webb et al., 1980), whereas suckled beef cows have been shown to require from 54 to 93 or more days to return to estrus (Wiltbank, 1970). Bellows et al. (1982) reported that first-calf cows had poorer postpartum reproductive performance as measured by day of first estrus, postpartum interval, percentage in estrus by the beginning of the breeding season and pregnancy rate following a 45 day AI breeding season than older cows. Data presented by Patterson et al. (1991) indicate that postpartum interval to first estrus increased by 3.9 ± 0.2 days for each unit increase in calving difficulty score. Furthermore, Bellows et al. (1982) reported that cows nursing female calves had higher pregnancy rates than cows nursing male calves. However, Tennant and Peddicord (1968) reported that first service conception rates did not differ between involuted and non-involuted cows. Peters and Riley (1982) reported that there is a highly significant negative correlation (r = -.737) between the daily photoperiod one month before calving and the length of the postpartum anestrous period. Poor nutrition (Randel, 1990) and suckling (Williams, 1990) have both been shown to increase the postpartum anestrous period.

The reproductive processes of females can be altered by the presence of males. This effect, generally referred to as biostimulation, has been established in many laboratory and domestic animal species. In domestic animal species, biostimulation can alter the postpartum interval to estrus, ovulation and pregnancy rates. Since 1984 it has been known that bulls can influence the postpartum interval to estrus in cows. However, the physiological mechanisms involved in this effect are not known. The following review of the literature will discuss the physiological changes associated with nutrition, suckling and biostimulation with particular emphasis on biostimulation.

REVIEW OF THE LITERATURE

Factors Affecting Postpartum Reproduction

Nutrition and Body Condition

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., 1991b). In fact, many pluriparous cows on a low plane of nutrition failed to display estrous behavior during the next breeding season (Wiltbank, 1970). Additionally, first-calf beef cows fed low energy rations prepartum had more dystocia and weaned fewer live calves (Falk et al., 1975). 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 (Ducker et al., 1985), higher frequency of cows exhibiting estrus before the breeding season (Bellows and Short, 1978; Henricks and Rone, 1986), more cows ovulating by Day 150 postpartum (Perry et al., 1991b) and higher pregnancy rates (Bellows and Short, 1978; Henricks and Rone, 1986; DeRouen et al., 1993). Recently, Oss et al. (1993) reported that cows on a high fat diet had longer postpartum intervals to estrus, implying that the source or form of energy may also be important in altering postpartum reproduction. Rutter and Randel (1984) established that cows on a high plane of nutrition had shorter postpartum intervals to estrus than cows on a maintenance diet, which in turn had shorter postpartum intervals to estrus than cows on a low plane of nutrition.

Various combinations of pre- and postpartum nutritional regimens have been shown to have differing effects. Houghton et al. (1990) reported that cows fed a low energy diet prepartum and a high energy diet postpartum had shorter postpartum intervals to estrus than any of the other treatments. Further, they showed that cows fed the Low-High regimen had a greater percentage of cows exhibiting estrus by 60 days postpartum than Low-Low cows (Houghton et al., 1990). These results indicate that postpartum nutrition has more effect on postpartum reproductive performance than prepartum nutririon. Contrary to these results, Dunn and Kaltenbach (1980) concluded that prepartum nutrition was more important than postpartum nutrition in reducing the postpartum interval. However, Doornbos et al. (1984) determined that the effect of prepartum nutrition on pre-calving, calving and postpartum data was non-significant. The lack of differences found by Doornbos et al. (1984) may have been due to a relatively high plane of nutrition (135% and 110% of NRC requirements for high and low energy treatments, respectively) fed to cows in both treatments.

One question that arises from these data is how nutritional manipulation alters ovarian response. Does nutrition alter follicular development and(or) function?

Henricks and Rone (1986) reported that estradiol-17ß concentrations were higher and there were more medium-size follicles on Day 28 and small-size follicles on Day 46 in cows on a high energy ration than in cows on a low energy ration. Perry et al. (1991b) determined that cows receiving lower energy levels prepartum had fewer large follicles; cows receiving low levels of energy postpartum had fewer small and large follicles, while cows receiving low levels of energy both pre- and postpartum could not mature follicles beyond the small stage during the postpartum anestrus period. Cows receiving high energy both pre- and postpartum had fewer medium-size follicles but more large follicles than cows fed any of the other rations. It appears that low energy rations retard follicular growth and development.

Progesterone secreted by the corpus luteum is responsible for preparing the uterus for implantation. Gombe and Hansel (1973) and Beal et al. (1978) found that plasma progesterone levels were lower for heifers fed a restricted energy diet. The low levels of progesterone in restricted-fed animals could have been due in part to the low rate of follicular development caused by poor nutrition reported by Henricks and Rone (1986) and Perry et al. (1991b). Furthermore, energy restricted heifers had smaller corpora lutea with lower progesterone contents on Day 10 of the third postpartum estrous cycle (Gombe and Hansel, 1973). Contrary to these findings, Gauthier et al. (1983) concluded that underfeeding during the first 45 days postpartum increased progesterone levels.

Bartle et al. (1984) showed that the postpartum interval to estrus was related to estimated percent body fat, i.e. body condition and relative energy intake (ME intake ÷ energy requirement). Cows in moderate body condition at parturition returned to estrus regardless of energy intake. However, cows in moderate and thin condition at parturition had higher first service conception rates than cows that were overconditioned at parturition (Houghton et al., 1990). Postpartum intervals could be shortened by increasing energy intake above 100% of requirements (Bartle et al., 1984; Richards et al., 1986). Rutter and Randel (1984) concluded that cows that maintained their body condition regardless of diet had shorter postpartum intervals than cows that lost body condition. Similarly, cows in good body condition can lose some weight postpartum without affecting postpartum interval (Humphrey et al., 1983). Richards et al. (1986) and Osoro and Wright (1992) reported that postpartum nutritional management had no effect on interval to estrus or interval to pregnancy. They stated that body condition score at calving was the most important factor influencing return to estrus and pregnancy. Body condition at start of mating was less important than body condition at parturition, and body condition at the end of mating had no effect on reproductive performance of Hereford X Friesian and Blue-Grey cows (Osoro and Wright, 1992).

Body condition may interact with or affect other factors to influence the postpartum anestrus period. Perry et al. (1991b) reported that correlation coefficients between body condition score and number of small, medium and large follicles were .48, .19 and .66, respectively. Body condition scores were also correlated with mean LH concentrations (Perry et al., 1991b), LH pulse frequency (Wright et al., 1990) and pituitary content of LH on Day 30 postpartum (Connor et al., 1990). However, Wright et al. (1990) reported that mean LH concentrations were not affected by body condition.

Dunn et al. (1969) concluded that pregnancy rate was directly related to postpartum energy intake. Cows fed a high level of energy had higher pregnancy rates than cows fed moderate and low energy levels. Cows fed the high energy ration before calving also had higher pregnancy rates during the first 100 days postpartum than cows fed the lower energy ration prepartum. Dairy heifers fed a high level of energy prepartum and a low level postpartum had higher pregnancy rates to AI (first service) than those fed a High-High or a Low-High diet and had a shorter interval from calving to pregnancy (Ducker et al., 1985). However, Houghton et al. (1990) reported that while high postpartum energy intake resulted in higher pregnancy rates, change in prepartum weight and body condition did not affect postpartum reproductive performance. Reduced pregnancy rates in heifers fed restricted energy diets did not appear to be due to reduced fertilization rates (Spitzer et al., 1978).

Fewer protein restricted cows exhibited estrus, had reduced first service conception rates and overall pregnancy rates and had longer interval to estrus, first service and conception than cows fed an adequate protein diet (Sasser et al., 1988). However, Farthing et al. (1993) reported that cows receiving protein supplements had lower fall pregnancy rates. Regardless of dietary protein content, weight at first estrus, weight at first service and average daily gain were negatively correlated with postpartum intervals to first estrus and first service (Sasser et al., 1988).

The effect of nutrition on serum FSH concentrations is unclear and the reports in the literature are conflicting. Gauthier et al. (1983) reported that underfeeding during the first 45 days postpartum decreased FSH concentrations. However, Perry et al. (1991b) stated that patterns of FSH secretion were not affected by diet at any time. Nolan et al. (1988) found that FSH concentrations were higher in protein deficient cows than in adequately fed cows.

Reports on the effect of nutrition on LH concentrations indicate that LH concentrations increase with proper nutrition. Gauthier et al. (1983) and Hall et al. (1991) found that cows on a low energy diet had lower mean LH concentrations and lower pulse frequency of LH in serum (Perry et al., 1991b) than cows on a high energy diet. Likewise, Echternkamp et al. (1982) and Perry et al. (1991b) reported that cows receiving high levels of energy prepartum had increased mean concentrations of LH postpartum and tonic LH secretion was correlated with daily gain (r = 0.75, Echternkamp et al., 1982; r = 0.58, Gauthier et al., 1983), supporting the conclusion that undernutrition causes low gonadotropin concentrations. Furthermore, LH concentrations increased with time postpartum in cows on a high energy diet (Hall et al., 1991) and pulse frequency increased in cows fed adequate crude protein (Nolan et al., 1988), but not cows fed low energy or low protein diets. By 60 days postpartum, LH pulse frequency in cows fed the adequate protein diet was twice as great as deficient cows (Nolan et al., 1988). However, Gombe and Hansel (1973) reported that dairy heifers on an energy restricted diet had higher mean LH concentrations throughout the experimental period and had higher basal and peak LH concentrations during the third estrous cycle than heifers fed adequate energy.

Echternkamp et al. (1982) reported that first-calf beef cows fed a high (150% NRC prepartum and early postpartum) plane of nutrition released more LH when challenged with 10 mg estradiol benzoate than cows fed 100% NRC rations. The interval from estrogen injection to peak LH release was longer in cows fed the lower energy diet. However, dietary protein did not affect LH response to exogenous estrogen treatment (Nolan et al., 1988). At calf removal, cows on a high plane of nutrition had higher serum LH concentrations than cows on a low plane of nutrition. Serum LH concentrations increased by 24 hours after calf removal for high energy cows but not until 48 hours after calf removal for low energy cows (Whisnant et al., 1985a).

Beal et al. (1978) determined that low energy diets increased LH release after GnRH injection in heifers and spayed cows but not in intact cows or adequate energy spayed cows. However, restricted energy spayed cows had higher pituitary LH content than intact cows. The authors concluded that energy restriction may influence LH release directly at the pituitary as well as indirectly through effects on ovarian steroid production. Another explanation may be that GnRH production or release may be reduced in cows on low energy diets. Contrary to this study, Lishman et al. (1979) determined that serum LH concentrations in underfed (60% NRC prepartum) cows rose less rapidly and to a lower peak value than in well-fed (115% NRC prepartum) cows when challenged with GnRH.

Nolan et al. (1988) determined that cows fed an adequate protein diet had higher LH peaks and total LH under the curve in response to GnRH injection than protein deficient cows. GnRH induced peak height increased linearly in adequately fed cows with time postpartum but not in cows on the deficient protein diet. However, cows on the protein deficient diet responded more rapidly to GnRH injection than cows fed the adequate protein diet. Duration of response was not affected by diet. Anterior pituitary GnRH receptor numbers did not differ among diets. Pituitary concentrations of LH were greatest in cows on the protein deficient diet at 30 days postpartum. Pituitary LH concentrations of cows fed the protein deficient diet declined to concentrations similar to those of cows fed the adequate protein diet with increasing time of protein deficiency.

Suckling Stimulus

Suckling has been shown to affect postpartum anestrus in many experiments. Non-suckled cows and once daily suckled cows had shorter postpartum intervals to estrus than cows suckled twice daily, ad libitum or hypersuckled (Smith and Vincent, 1972; Laster et al., 1973; Carter et al., 1980; Odde et al., 1980; La Voie et al., 1981; Randel, 1981; Reeves and Gaskins, 1981; Ramirez-Godinez et al., 1982; Garcia-Winder et al., 1984; Houghton et al., 1990). Furthermore, Bellows et al. (1974) found that early weaning of calves reduced the postpartum interval to first estrus regardless of whether cows give birth to singles or twins. Cows suckling their own or foster calves had shorter intervals to first estrus than cows suckling two calves (Wettemann et al., 1978). Silveira and Williams (1991) reported that weaned cows and cows nursing calves other than their own had a higher incidence of ovulation than cows nursing their own calves by Day 26 to 29 postpartum. However, Montgomery (1982) reported that while interval to first elevation of progesterone was shorter for cows suckled once daily than for cows suckled twice daily or ad libitum, interval to first observed estrus did not differ among treatments. Carruthers and Hafs (1980) reported that suckled cows had longer postpartum intervals to first ovulation than milked cows. However, Carter et al. (1980) reported interval to first ovulation and to conception were not different for suckled and weaned cows.

Results reported by Odde et al. (1980) and Ramirez-Godinez et al. (1982) indicate that beef cows exhibited a second estrus 8 to 10 days after the first postpartum estrus which resulted from calf removal, and that cows nursed once daily tended to have short cycles (Reeves and Gaskins, 1981). No cows showing short cycles conceived on the first estrus (Odde et al., 1980). Furthermore, Carter et al. (1980) reported that not only did all non-suckled cows demonstrate short cycles, they required more inseminations per conception. Early weaned cows (Houghton et al., 1990) and cows suckled once daily (Reeves and Gaskins, 1981) had lower first service conception rates than normally weaned cows. Similarly, in mastectomized and non-suckled cows, shorter intervals to estrus required more services per conception; thus, interval from calving to conception was not affected (Short et al., 1972). These results indicate that cows with shorter postpartum intervals have a higher incidence of short cycles and lower first service conception rates than cows with longer postpartum intervals. However, in a study involving only suckled cows, Perry et al. (1991a) reported that all cows had short (8.5 day) estrous cycles. Furthermore, Laster et al. (1973) found that weaning increased overall conception and percentage of cows exhibiting estrus from calving to the end of the breeding season and once-daily suckling increased pregnancy rates in Brahman cross cows (Bluntzer et al., 1989).

The presence of mammary tissue itself appears to be sufficient to prolong postpartum anestrus. Short et al. (1972) demonstrated that mastectomized cows had shorter postpartum intervals to first estrus than non-suckled cows, which had shorter postpartum intervals to first estrus than suckled cows. Furthermore, Short et al. (1976) demonstrated that neural input from the udder was not responsible for the increase in the postpartum interval. Williams et al. (1987) suggested that the ability of the suckling calf to suppress tonic LH secretion derives from unique cues that are not fully simulated by frequent milking and the presence of the non-suckling calf, however, both of these factors may modulate behavioral estrus. Viker et al. (1989) and Stevenson et al. (1993) found that mastectomized cows that remained with their calves had longer postpartum intervals to estrus and ovulation than mastectomized cows whose calves were not present. Furthermore, Stevenson et al. (1993) and Viker et al. (1993) reported that stimulation of the inguinal area of mastectomized cows by their calves increased the postpartum interval to ovulation compared to mastectomized cows which could only come into contact with their calves from the neck forward. Suckled control cows and mastectomized cows whose calves had unlimited access to the dam had similar postpartum intervals to ovulation (Stevenson et al., 1993).

Initiation and increase in LH pulsatility is the most consistently reported change preceding the postpartum return to ovarian cycling activity (Peters and Lamming, 1990). Minaguchi and Meites (1967) demonstrated that anterior pituitaries from suckled rats released more prolactin but less LH than anterior pituitaries from cycling rats. Suckling also reduced the weight of pituitaries, ovaries and uteri (Minaguchi and Meites, 1967). However, weaning had no effect on pituitary weights or pituitary LH and FSH concentrations in cows (Walters et al., 1982b). Cows in the weaned treatment were weaned on Day 21 postpartum, but GnRH induced release of LH in vitro was not affected by time after Day 21 to slaughter for weaned, suckled or estrous cows. However, LH release was similar between weaned cows and cows exhibiting estrous cycles and greater for weaned than suckled cows.

Carter et al. (1980) reported that non-suckled cows had higher systemic LH concentrations. LH concentrations rose during the first 20 days postpartum for non-suckled and once daily suckled cows, but not until Day 48 did a sustained increase occur in hypersuckled cows (Garcia-Winder et al., 1984). In fact, mean, peak frequency (Carruthers and Hafs, 1980; Whisnant et al., 1985b) and amplitude (Carruthers and Hafs, 1980; Chang et al., 1981; Whisnant et al., 1985b) of serum LH concentrations in weaned cows rose rapidly within 48 hours of calf removal whereas LH concentrations during the same time period did not change for suckled cows. The reason for depressed serum LH concentrations in suckled cows may be due to a 60% decrease in pulse frequency and a 40% decrease in amplitude reported by Carruthers et al. (1980). In support of these results, Walters et al. (1982a) found that weaned cows had more LH pulses than suckled cows. Additionally, Carruthers and Hafs (1980) stated that suckled cows had lower frequency and amplitude of episodic secretion of LH compared to milked cows. Furthermore, cows nursing their own calves had lower mean LH concentrations on Days 16 to 19 postpartum than weaned cows or cows nursing calves other than their own (Silveira and Williams, 1991). Thus, suckling may delay postpartum ovulation by depressing episodic LH secretion (Carruthers and Hafs, 1980; Dunlap et al. 1981). However, Chang et al. (1981) reported that frequency of LH pulses and basal concentrations did not differ between suckled and non-suckled cows. Likewise, although cows suckled by two calves had a significantly longer postpartum period than those suckling one, average baseline and peak concentrations of LH were not different (Gimenez et al., 1980). The number of suckling episodes did not decrease with time postpartum, but the duration of each episode did decrease (Gimenez et al., 1980). Furthermore, plasma LH concentrations were higher in mastectomized and non-suckled cows by Day 7 than suckled cows (Short et al., 1972). However, mean maximum LH concentrations and LH pulse frequency were higher for the mastectomized, unlimited calf access cows than suckled controls (Stevenson et al., 1993). These results support the hypothesis that suppression of LH secretion is not solely responsible for prolonged postpartum anestrus or that mastectomy per se affects LH release and mastectomized cows may not be appropriate models to evaluate LH secretion during the postpartum anestrus period.

It is possible that cortisol and(or) prolactin have some effect on LH secretion in suckled cows as both have been shown to be elevated during nursing. However, cortisol concentrations remained constant except for a transient rise at 9 to 12 hours after weaning. Thus, cortisol appears to have no effect on LH secretion (Whisnant et al., 1985b; Faltys et al., 1987), and cortisol concentrations did not affect GnRH induced LH response in multiparous Holstein cows (Lefebvre et al., 1990). Alterations in prolactin, total glucocorticoids, progesterone or estradiol-17ß do not appear to mediate directly the effects of suckling on postpartum episodic LH secretion and(or) ovulation (Carruthers and Hafs, 1980). However, in first-calf beef cows, fewer LH peaks were observed during suckling periods and when prolactin was elevated than at other times (Gimenez et al., 1980). Nevertheless, Convey et al. (1983) concluded that suckling does not acutely affect LH or FSH concentrations.

Calf removal for 72 hours (but not calf removal + GnRH at 72 hours) decreased the postpartum interval to estrus and increased pituitary responsiveness to GnRH challenge. It did not, however, alter circulating progesterone concentrations or luteal life span of corpora lutea induced by GnRH injection (Dunn et al., 1985). Smith et al. (1979) reported that use of calf-removal (48 to 60 hours) in combination with Syncro-Mate-B, a synthetic progestin, increased the number of cows detected in estrus and pregnant by 4 days and 21 days after implant removal. Similarly, Ramirez-Godinez et al. (1981) found that all cows treated with 6 mg implants of Norgestomet, a synthetic progestin, 9 days before weaning showed estrus within 10 days although 3 of 10 showed a short cycle. Cows that received implants of Norgestomet after weaning exhibited a normal luteal phase (6 of 10 within 10 days, 10 of 10 within 25 days of weaning), and 6 of 9 cows that were simply weaned showed estrus within 10 days (9 of 9 within 25 days) although 5 of 6 showed a short cycle. All the other cows either had a normal estrous cycle length or conceived at first service. Conception rates at first service were higher for cows treated with Norgestomet than cows that were not.

Acosta et al. (1983) determined that during nursing estradiol-17ß treatment suppressed serum LH concentrations below LH concentrations of non-estradiol treated cows, but after weaning, estradiol treatment stimulated LH release above that of non-estrogen treated cows. The authors conclude that the suckling stimulus increases the sensitivity of the hypothalamus to the negative feedback of estrogen during the postpartum period resulting in reduced LH secretion.

Peters et al. (1981) reported that between Days 13 and 20 postpartum a distinct pulsatile pattern of LH secretion was detected in milked but not suckled cows. Mean concentrations and pulse frequency of LH were not affected by teat stimulation and were not related to prolactin or cortisol release. Mechanical stimulation of the teat is not singly effective in altering the pattern or quantity of tonic LH secretion in ovariectomized cows (Williams et al., 1984).

Endocrine and Neuroendocrine Factors Associated with Postpartum Cows

The endocrine system is one of the two regulatory systems used by organisms to maintain homeostasis and perform the functions associated with survival and reproduction. Reproduction is profoundly affected by the endocrine system because most reproductive functions require long term regulation and endocrine effects are long lasting when compared to neural inputs. The endocrine system cannot, however, act in isolation. The hypothalamus provides the link between the neural and endocrine systems and exerts profound control over the processes involved in reproduction. The following sections attempt to clarify the role of this system regarding the resumption of reproductive function during the postpartum anestrus period.

Anterior Pituitary

<u>Gonadotropins.</u> The change most 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 researchers have established that peripheral LH concentrations increase with time postpartum (Echternkamp and Hansel, 1973; Kesler et al., 1977; Fernandes et al., 1978; Goodale et al, 1978; Peters et al., 1981; Walters et al., 1982b; Cermak et al., 1983; Humphrey et al., 1983; Garcia-Winder et al., 1984; Edwards, 1985; Garcia-Winder et al., 1986; Nett et al., 1988; Wright et al., 1990). Moreover, the change in basal serum 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 (Goodale et al., 1978; Rawlings et al., 1980; Webb et al., 1980; Humphrey et al., 1983; Garcia-Winder, et al., 1984; Edwards, 1985; Garcia-Winder et al., 1986; Savio et al., 1990; Wright et al., 1990) and amplitude (Goodale et al., 1978; Rawlings et al., 1980; Webb et al., 1980; Garcia-Winder et al., 1986; Wright et al., 1990) have been shown to increase

with time postpartum. However, calf return decreased LH concentrations and pulse frequency within 8 hours in acyclic cows but no changes were seen in cyclic cows (Edwards, 1985). It would appear that once ovarian cycling activity has been initiated, LH concentrations are not affected by suckling.

Moss et al. (1985) found that anterior pituitary concentrations of LH were lower at 5 and 10 days postpartum than at 30 days. Concentrations of LH were similar between estrous and 30 day postpartum cows. Only cows slaughtered on Day 5 postpartum released less LH in vitro in response to GnRH challenge than the other cows. In contrast to these studies, Arije et al. (1974) found that serum LH concentrations from 3 weeks before calving to 72 days after calving did not differ. LH peaked approximately 5 hours after estrus and returned to baseline concentrations shortly thereafter.

Garcia-Winder et al. (1984) reported that mean concentrations and LH pulse frequency from Days 6 to 27 and Days 6 to 55 were lower for ovariectomized cows suckled once daily or hypersuckled than ovariectomized cows that were not suckled. In a more recent experiment, Garcia-Winder et al. (1986) found no difference in mean LH concentrations, pulse frequency or amplitude between intact and ovariectomized cows before and after suckling. However, ovariectomized estrogen-treated cows had lower mean LH concentrations after suckling on Days 44 and 58 postpartum. Forrest et al. (1979) reported that injection with .5 mg of estrone or calf removal resulted in elevated serum LH concentrations, but the initial elevation in LH concentrations occurred earlier and lasted longer in cows that had their calves removed than in cows treated with estrone. Maximum serum LH levels did not differ between treatments. There were more peaks on Day 2 for cows that had their calves removed than either controls or estrone treated cows. The results of these experiments indicate that while estrogen interacts with suckling to reduce LH concentrations, the suckling-estrogen interaction is not solely responsible for decreased LH during the postpartum anestrus period. Meyers et al. (1989) concluded that suckling, at least partially, suppresses the release of LH through endogenous opioid mechanisms.

Despite the effect suckling has on serum LH concentrations, suckling appears to have no effect on pituitary LH content (Wagner et al., 1969; Walters et al., 1982b). Wagner et al. (1969) reported that there appeared to be a slight, but non-significant, rise in pituitary LH content from Days 7 to 30 postpartum, but Nett et al. (1988) found that LH increased rapidly from Days 15 to 30. In vitro response of pituitary explants from suckled cows secreted 50% less LH in response to LHRH or K^+ than those from non-suckled cows (Carruthers et al., 1980; Walters et al., 1982b). Decreased pulse frequency and amplitude of LH release as well as reduced ability to respond to LHRH may be the cause of suckling induced anestrus (Carruthers et al., 1980).

The other gonadotropic hormone secreted by the anterior pituitary is FSH. Rothchild (1960) found that suckling suppresses FSH secretion in rats and is directly proportional to litter size. In cattle, however, FSH concentrations did not change during the postpartum anestrus period (Cermak et al., 1983; Nett et al., 1988). Duration of weaning did not affect the change in FSH concentrations of weaned cows, but weaned cows had a greater change in FSH concentrations than suckled cows (Walters et al., 1982b). Plasma FSH concentrations increased after the first 15 days postpartum and peaked at LH peaks but also at other times (Webb et al., 1980). 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). Concentrations of FSH in the anterior pituitary were high on Day 5 and remained high through Day 30 postpartum (Moss et al., 1985).

Prolactin is a pituitary hormone associated with lactation, and several researchers have attempted to establish an association between lactation, prolactin and LH concentrations. Arije et al. (1974) reported that serum prolactin concentrations increase rapidly a few days before parturition and peak (348 ng/ml) the day before calving. This increase may be important for the initiation of lactation. Prolactin concentrations then decrease for the next 3 to 5 days postpartum and increase again 3 days before estrus and decline between 2 and 7 days after estrus. Nett et al. (1988) found that serum prolactin concentrations were high early in each day's bleeding period but fell to basal levels within 1 or 2 hours. Humphrey et al. (1983) stated that prolactin concentrations were not affected by time postpartum. In addition, those cows with the highest mean serum prolactin concentrations and greatest frequency and amplitude of prolactin secretion had the shortest postpartum intervals to estrus. Montgomery (1982) reported that there was no relationship between prolactin concentrations and resumption of ovarian cycling activity.

Prolactin peaks were not all associated with suckling episodes (Gimenez et al., 1980; Nett et al., 1988). Further, serum prolactin concentrations did not differ between weaned and suckled treatments in the study by Walters et al. (1982a). However, follicular fluid prolactin concentrations were higher in weaned and estrous cows than suckled cows (Walters et al., 1982b).

One method by which one could test for whether or not prolactin is involved in resumption of ovarian cycling activity in postpartum suckled beef cows would be to inhibit its secretion. Cummins et al. (1977) reported that suppression of prolactin secretion using CB-154 (ergocryptine; a potent dopaminergic antagonist) did not reduce the postpartum interval and it did not reduce milk production in first-calf cows. However, Short et al. (1978) determined that injections of CB-154 lowered prolactin concentrations, decreased interval from treatment to estrus and decreased interval from calving to estrus. Therefore, lactation induced anestrus is at least partially mediated through mechanisms involved with regulating prolactin secretion.

Gonadotropin Releasing Hormone

Gonadotropin releasing hormone (GnRH) is a hypothalamic neurohormone that has its effect on the anterior pituitary, inducing the pulsatile release of LH and FSH. GnRH is released in a pulsatile fashion beginning at 2 and 5 weeks of age in cattle (Rodriguez and Wise, 1987). However, pulsatile release of LH does not occur before 8 and 12 weeks of age in Holstein bull calves. GnRH pulses increase in frequency at 8 and 12 weeks of age, coincidental with LH pulses. This implies that although GnRH may be present, the pulse frequency of GnRH secretion more important for the induction of pulsatile LH secretion.

There was no change in the content of GnRH in either the hypothalamus or the preoptic area during the postpartum anestrus period (Cermak et al., 1983; Moss et al., 1985). There was no change in GnRH content in estrous cows in a parallel study. However, GnRH content in both the hypothalamus and preoptic area were greater in anestrous cows than in estrous cows (Cermak et al., 1983). These results may indicate that GnRH release during the postpartum anestrus period is suppressed. Connor et al. (1990) reported that high energy postpartum diets and calf removal decreased GnRH content of the preoptic area and the hypothalamus regardless of dietary group. However, Wright et al. (1990) stated that body condition score had no effect on level of LH response. Neither body condition nor time had an effect on FSH release in response to GnRH challenge.

Injection of GnRH caused an increase in circulating LH in all cows, however the amount of LH released over time was greater when preceded by 72 hour calf removal (Carter et al., 1980; Dunn et al., 1985). Moreover, in the GnRH and GnRH + calf removal groups there was no difference in the interval from injection to peak LH release or magnitude of the peak (Dunn et al., 1985). It appears that the pituitary is capable of responding to GnRH stimulation, even though suckling reduces the response, but that GnRH is not present to cause a release of LH.

Riley et al. (1981) reported that injections of GnRH every 2 hours induced pulses of LH in suckled beef cows. However, the LH concentrations always returned to baseline concentrations before the next injection which was given at 2 hour intervals, sometimes decreased during the injection period and even ceased completely in 2 cows before an ovulatory surge. In contrast to this study, Walters et al. (1982c) reported that intermittent injection (every 2 hours) of GnRH increased serum LH concentrations and Wright et al.

(1990) reported that every injection of GnRH was followed by a pulse of LH in the peripheral circulation. Furthermore, GnRH + estradiol-17ß decreased the number of LH pulses released during a 4 hour sampling period (Walters et al., 1982c).

Clearly, exogenous GnRH can induce LH pulses, but the pituitary may not be immediately responsive to GnRH after the long term exposure to progesterone during gestation. Data presented by Peters et al. (1985) demonstrated that injection of GnRH in dairy cows resulted in a pulsatile LH response although the pattern was less uniform in cows injected before Day 7 postpartum, particularly during the last 10 hours of treatment. Amplitude of LH response did not differ between treatment (Peters et al., 1985). However, Kesler et al. (1977) demonstrated that dairy cows injected with GnRH on Days 1 or 2, 3 or 4 or 5 or 6 did not respond with increased plasma LH concentrations. Cows injected on Days 7 or 8, 12 or 13 or 18 or 19 showed an increase in plasma LH concentrations within .5 hours of injection. Cows injected on Day 7 or 8 and on Day 12 or 13 showed an increase followed by a decrease in LH concentrations and a second rise 2 to 3 hours after treatment. Cows injected on Day 18 or 19 did not show a second rise. Plasma LH concentrations returned to baseline concentrations 4 to 6 hours after treatment (Kesler et al., 1977). Fernandes et al. (1978) presented data showing that systemic LH concentrations increased 5 minutes after injection of GnRH and peak values were obtained at about 120 minutes post-injection. Concentrations returned to baseline concentrations by 5 to 6 hours after treatment. Response to GnRH was lower for cows treated on Day 3 postpartum than Day 10. However, the Day 10 response was lower than that on Day 20, indicating that LH release was not fully restored until after Day 10 in dairy cows.

GnRH has also been shown to induce FSH secretion. However, Riley et al. (1981) reported that FSH concentrations appeared to fluctuate randomly after small, repeated doses of GnRH. Peters et al. (1985) reported that the response of FSH after GnRH treatment was irregular in cows milked twice daily with a tendency for 2 peaks to occur between GnRH injections. GnRH injections did not induce discrete FSH peaks; however, mean FSH concentrations were dose dependent, with the high dose eliciting the highest concentrations of FSH (Jagger et al., 1987). The low dose did not cause an increase in FSH from that observed before treatment and the intermediate dose had reduced levels of FSH after the second 12 hours of treatment until the end. On the other hand, Walters et al. (1982c) reported that GnRH injection increased both frequency and amplitude of FSH pulses over suckled control and estrogen treated cows. However, serum concentrations of FSH were not different among treatments. Similarly, Roberge et al. (1992) reported no differences between luteinizing hormone releasing hormone analogue (LHRH-A) treated cows and control cows for

FSH concentrations.

On the day of treatment (Day 5 postpartum), LHRH-A-treated suckled cows had preovulatory-like surges of both LH and FSH throughout the entire 6 hour blood sampling period (Roberge et al., 1992). There was no difference in concentrations of gonadotropins between treatments on Day 10, 20, 30 and 40 postpartum. LHRH-A-treated cows had more LH pulses on Day 10 and 30 than controls and more LHRH-A treated cows (11 of 12) than control cows (6 of 12) exhibited pulses on Day 10. Pulse amplitude did not differ among treatment groups, but there was a steady increase in amplitude with time in the LHRH-A-treated cows. Pulse frequency increased with time in controls but not in treated cows. There were no differences in LH or FSH content on Day 30 postpartum between treatments. Beef cows injected with microencapsulated LHRH-A exhibited pulsatile patterns of LH (Roberts et al., 1989). Cows receiving 6.4 or 25.6 μg LHRH-A per day had more pulses of LH than controls.

LH response to GnRH is related to time postpartum, but, is response to GnRH dose dependent as well? Jagger et al. (1987) reported that the response of LH to GnRH injection was dose dependent in that not all 1 μg injections were followed by an LH pulse; whereas, all 2.5 μg and 5 μg injections were. Injections of 2.5 μg were followed by LH pulses that were not different from pretreatment values whereas 5 μg injections caused a significantly higher LH pulse amplitude. Half of the treated cows in each dose exhibited preovulatory surges of LH at a mean time of 30 hours. Cows receiving continuous infusion of 2.5 μg GnRH exhibited preovulatory surges of LH and FSH from 1 to 6 hours after treatment began. Continuous infusion of 5 μg GnRH resulted in preovulatory surges of LH beginning about 4 hours after treatment began in 3 of 5 cows. Contrary to these results, Wildeus et al. (1987) reported that injection of 5 μg GnRH over 24 hours at 2 hour intervals reduced the number of cows in estrus by 45 days after treatment. However, more cows treated for 6 or 12 hours exhibited estrus than controls. Cows treated for 6 hours displayed estrus sooner than cows treated for 12 hours. Only those cows treated for 6 hours showed a rise in mean concentrations of serum LH, but this was probably due to more LH pulses. Because blood samples were collected 7 hours after treatment, samples were not collected until 19 and 31 hours after the initial injection, respectively for the 12 and 24 hour GnRH treated cows. Therefore, absence of elevated serum LH in the 12 and 24 hour GnRH treatments may have been due to a state of pituitary refractoriness.

GnRH treatment on Day 5 postpartum increased follicular volume whether cows were suckled or non-suckled (Carter et al., 1980). Approximately 50% of cows injected with GnRH formed luteal tissue whether or not calves had been removed for 72 hours (Dunn et al., 1985). Treatment with GnRH and PGF2α may enhance ovarian activity in postpartum dairy cows

(Okuda et al., 1988).

Roberge et al. (1992) reported that days to first postpartum estrus were reduced in beef cows treated with LHRH-A compared to controls. Likewise, Holstein cows injected with GnRH had fewer days to increased progesterone than cows injected with saline (Hussein et al., 1992). However, LHRH-A or GnRH injection did not alter the postpartum interval to first estrus (Roberts et al., 1989; Hussein et al., 1992). Days to conception tended to be less but was not statistically significant (Roberge et al., 1992). Carter et al. (1980) found that suckled GnRH treated cows had shorter postpartum intervals to conception than suckled saline treated cows, but GnRH treatment did not affect any intervals in non-suckled cows.

Concentrations of anterior pituitary receptors for GnRH were lowest at parturition, increased to their highest concentrations by Day 15 and then gradually declined through Day 45 postpartum (Cermak et al., 1983). Receptors for GnRH were high at Days 5 and 10 and appeared to decline from Days 5 to 30 but there was no difference in number of receptors between Day 5 and Day 30. However, when number of GnRH receptors on Day 5 and 10 were combined, there were more receptors than Day 20 and 30 cows combined. Control estrous cycling cows had similar numbers of GnRH receptors to Day 20 and 30 postpartum acyclic cows (Moss et al., 1985). There were no differences in the number of pituitary LHRH receptors, LH content or FSH content on Day 30 postpartum between LHRH-A-treated cows and control cows (Roberge et al., 1992). Decreased responsiveness of the pituitary to LHRH may be part of the cause of suckling induced inhibition of postpartum ovulation in cows (Carruthers et al., 1980). This conclusion is supported by Walters et al. (1982b) who reported that weaned cows secreted more LH in vitro in response to GnRH than suckled cows.

Endogenous Opioid Peptide System

The evidence presented in the previous section support the conclusion that GnRH release is suppressed during the postpartum anestrus period but that the pituitary is still capable of responding to GnRH stimulation, albeit at a reduced rate. As mentioned previously, Meyers et al. (1989) suggested that suckling, at least partially, suppresses the release of LH through endogenous opioid mechanisms. Evidence supporting this hypothesis was presented by Short et al. (1987), who reported that injection of bremazocine, an opioid agonist, during the follicular phase of the estrous cycle depressed LH concentrations. Furthermore, peripheral injection of morphine in the postpartum beef cow decreased LH (Barb, 1988; Peck et al., 1988; Rund et al., 1992) but increased PRL and had no effect on FSH (Barb, 1988; Peck et al., 1989). Morphine infusion decreased frequency (Peck et al., 1988; Rund et al., 1992) but not mean concentrations of LH (Peck et al., 1988).

Average serum LH response to GnRH was reduced in animals receiving morphine infusion. Serum prolactin concentrations increased to a peak within 30 minutes of initiation of infusion of morphine and remained elevated throughout the infusion period (Peck et al., 1988). Area under the LH response curve was greater for non-suckled cows than suckled cows after GnRH even when LH concentrations were depressed due to morphine. However, morphine tended to decrease the response of non-suckled cows to GnRH. It is unclear if morphine attenuated pituitary sensitivity to GnRH (Rund et al., 1992). Barb (1988) proposes that endogenous opioid peptides act to inhibit LH by suppressing GnRH secretion and that endogenous opioid peptide activity is influenced by gonadal steroids.

Simply demonstrating that exogenous opiates suppress LH secretion does not confirm that endogenous opioid peptides act in a similar manner. Naloxone, an opioid antagonist, has been widely used to suppress endogenous opioids to better clarify their effects. Gregg et al. (1986) and Whisnant et al. (1986a; 1986b; 1986c) reported that LH pulse frequency and mean serum LH concentrations (Cross et al., 1987) were greater in naloxone treated cows than in control cows. However, Whisnant et al. (1986b) and Cross et al. (1987) reported that amplitude of LH pulses was not different.

Injection of quadazocine, an opioid antagonist, during the follicular phase of the estrous cycle increased mean LH concentrations during the treatment period, but there was no effect of quadazocine on LH secretion during the luteal phase (Short et al., 1987). All cows produced a large LH peak in response to GnRH; however, cows treated with naloxone and progesterone had a peak with lower average concentration and peak values compared to cows treated with saline or naloxone only (Cross et al., 1987). Since LH concentrations in cows treated with naloxone and progesterone did not increase to the same degree as in cows treated with saline or naloxone only, it appears that progesterone inhibits LH secretion at the level of the pituitary.

In contrast to these studies, naloxone induced LH release during both the follicular and luteal phase of the estrous cycle in yearling heifers but not in multiparous cows (Barb, 1988). Whisnant et al. (1986d) reported serum LH concentrations were increased after 400 and 800 mg of naloxone on all days postpartum but 200 mg of naloxone was effective only after Day 28. Opioid inhibition of LH secretion decreases with time postpartum (Whisnant et al., 1986d). Barb (1988) concludes that only LH secretion is modulated by the endogenous opioid peptide system in the cow.

Leshin et al. (1991) reported that LHRH release was induced from tissue samples from the median eminence and the pre-optic area of the hypothalamus of cows by naloxone. Affinity of 3H-naloxone binding sites in the preoptic area, hypothalamus or basal forebrain was not different between anestrous and cycling cows (Trout and Malven, 1988). However, there were more binding sites for 3H-naloxone in the preoptic area and basal forebrain of anestrous cows than in cycling cows. Whether or not this is a cause of return to cycling activity remains to be determined.

Conversely, naloxone increases serum LH concentrations in anestrous postpartum beef cows after approximately 40 days postpartum (Whisnant et al., 1986b). The lack of effect of naloxone early in the postpartum period may indicate changing brain opioid tone over time postpartum. Naloxone caused a pulse-like increase of LH that began within 15 minutes, peaked approximately 45 minutes after treatment and did not return to baseline concentrations for at least 60 minutes. Intact-suckled cows had the smallest LH response; removal of the suckling stimulus enhanced the response to naloxone (Rund et al., 1989; 1992). Both suckled and non-suckled (75 hour weaning) cows given naloxone had greater LH pulse amplitude and higher mean LH after naloxone treatment. LH pulse frequency and interpulse interval were shorter only in naloxone treated, non-suckled cows (Cross et al., 1987). Naloxone treatment did not influence LH concentrations in non-suckled cows but suckled cows treated with naloxone had higher LH concentrations than suckled saline treated cows (Whisnant et al., 1986c). Mean LH concentrations for non-suckled, non-suckled naloxone-treated and suckled naloxone-treated intact cows were similar (Whisnant et al., 1986c), and the LH response of ovariectomized cows to naloxone treatment was similar for suckled and non-suckled cows (Rund et al., 1992). Serum LH increased after GnRH in all groups within 15 minutes and peaked within 30 minutes. However, the magnitude of the mean LH concentration was less for suckled than non-suckled cows whether they were ovariectomized or not (Rund et al., 1989; 1992). FSH concentrations did not differ among saline-treated control cows, naloxone-treated cows or naloxone and progesterone-treated cows (Cross et al., 1987). Rund et al. (1992) concluded that the suckling stimulus may directly modulate LH secretion at the pituitary independent of the effect of endogenous opioids.

Ovarian Steroids and Follicular Growth

Estrogen. Concentrations of estrogen were high during the last 2 weeks of pregnancy and fell rapidly the day after calving. Concentrations of estrogen during the postpartum period remained low until just before estrus (Arije et al., 1974; Rawlings et al., 1980; Humphrey et al., 1983). However, Stevenson and Britt (1979) reported that serum estradiol concentrations in Holstein cows were higher during the first week postpartum for those cows that ovulated after 20 days postpartum than for those that ovulated before day 20.

Serum concentrations of estradiol-17ß (Carruthers et al., 1980; Chang et al., 1981; Walters et al., 1982a), estrone and progesterone during the postpartum anestrus period were not different between weaned and suckled cows (Walters et al., 1982a). Likewise, neither suckling nor milking had any effect on estradiol-17ß secretion (Carruthers and Hafs, 1980). However, fewer suckled than milked cows respond to estradiol-17ß injection with an increase in serum LH concentrations (Short et al., 1979; Stevenson et al., 1983). Suckled cows injected with cloprostenol and estradiol benzoate at 6 weeks postpartum did not exhibit estrus, formation of a corpus luteum or luteal regression; however, non-suckled cows (5 of 7, the other 2 probably too early in the cycle for estradiol to have an effect) exhibited estrus, luteal regression and formation of a new corpus luteum (Radford et al., 1978). Injection at 14 weeks postpartum resulted in 4 of 5 non-cyclic, suckled cows exhibiting estrus. Suckled cows treated with GnRH on Day 8 postpartum had higher serum estrogen concentrations than suckled saline-treated, non-suckled saline-treated, and non-suckled GnRH-treated cows (Carter et al., 1980). It appears that estrogen interacts with the suckling stimulus to suppress LH secretion at least during the early part of the postpartum period.

In their review, Short et al. (1990) summarized data from authors who 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. Chang et al. (1981) reported that plasma estradiol concentrations were higher after progesterone releasing intravaginal device (PRID) removal for PRID treated postpartum cows than for untreated cows. Stevenson et al. (1983) reported that exogenous progesterone, administered in a subcutaneous implant 2 through 9 days before estradiol injection, did not inhibit estradiol-induced LH release. However, when a corpus luteum was present and endogenous progesterone concentrations were higher than 1 ng/ml, estradiol did not induce an LH release. Estradiol-17ß induced a release of LH in ovariectomized cows and was more effective than estrone. However, ovariectomy did not have any effect on response to estradiol-17ß injection until week 4 when more ovariectomized than intact cows had an estradiol induced LH response. The evidence presented does not appear to support the hypothesis that ovarian steroids prime the endocrine system.

Mean plasma LH and FSH concentrations during a preovulatory surge that may have been induced by estradiol 17-ß treatment were higher in cows receiving 2 implants of estradiol 17-ß, thereby having higher estradiol-17ß concentrations, than those cows receiving only 1 implant (Peters, 1984). Surges occurred between 15 and 79 hours after implant. There was a negative correlation between day of implant postpartum and time at which the LH peak occurred. During nursing, estradiol-17ß treatment suppressed serum LH below that of non-estradiol treated cows, but after weaning estradiol treatment stimulated LH release above that of non-estrogen treated cows (Acosta et al., 1983). It appears that the suckling stimulus increases the sensitivity of the hypothalamus to the negative feedback of estrogen during the postpartum period resulting in reduced LH secretion. In gonadectomized male and female bovines, mean concentrations and amplitude of LH responded in a cubic fashion to increasing doses of estradiol-17ß (Wolfe et al., 1992). Low doses had little effect, whereas high doses had a suppressing effect on LH concentrations. However, as estradiol-17ß increased, frequency of LH pulses decreased.

Concentrations of anterior pituitary receptors for estradiol were lowest immediately following parturition, increased to their highest level by Day 15 and then gradually declined through day 45 (Cermak et al., 1983). Schramm et al. (1991) concluded that enclomiphene, an antiestrogen, reduced the number of total and unoccupied hypothalamic and pituitary estrogen receptors. However, postpartum intervals, mean LH concentrations and pulse frequency of LH were not affected.

Progesterone

Progesterone concentrations remained fairly constant on the day of calving but fell rapidly the day after calving and remained low throughout the postpartum period. A short-term progesterone rise from 3 to 7 days before the first postpartum estrus was observed: this increase was followed by a decline to baseline concentrations at estrus (Arije et al., 1974; Stevenson and Britt, 1979; Rawlings et al., 1980; Humphrey et al., 1983). Magnitude of the pre-estrus progesterone peak was positively correlated with time postpartum (La Voie et al., 1981). Progesterone concentrations immediately before first estrus were higher in those cows that conceived to first service (Corah et al., 1974). Short cycles were observed in 3 of the 12 cows after the first estrus.

The rise in peripheral progesterone preceding the first estrus is frequently due to ovulation and corpus luteum formation without concurrent behavioral estrus (Castenson et al., 1976; Stevenson and Britt, 1979). The reason for the short lifespan of the first postpartum corpus luteum appears to involve a rise in $PGF_{2\alpha}$ immediately after the uterus detects progesterone resulting in a short cycle (Dailey et al., 1992). The second time progesterone increases, however, $PGF_{2\alpha}$ increases at the normal time in the estrous cycle. Marion and Gier (1968) reported that the average interval from first to second ovulation was shorter than the average interval from second to third ovulation. Corpora lutea from the first ovulation were smaller and had shorter lifespans than corpora lutea from subsequent ovulations.

Follicular Development

Spicer (1985) determined that numbers of medium follicles doubled between Days 7 and 14 postpartum and again between Days 28 and 42 to 56 postpartum. In fact, Savio et al. (1990) determined that follicular development was characterized by the detection of several waves of small and medium (< 8 mm) follicles until the detection of the first dominant follicle. During Week 2 postpartum, more cows that ovulated before Day 20 had follicles greater than 10 mm in diameter (Stevenson and Britt, 1979). However, follicular growth patterns, as determined by palpation per rectum, could not be correlated with estradiol-17ß secretion patterns (Carter et al., 1980; Rawlings et al., 1980). Furthermore, ovaries of non-suckled cows had greater follicular volume on Days 5 and 8 postpartum than suckled cows because of the greater number of medium and large follicles (Carter et al., 1980; Bellin et al., 1984). Spicer (1985) suggested that the increase in medium follicles provides a larger pool of follicles from which ovulatory follicles can be selected before first postpartum estrus.

Progesterone concentrations in large follicles increased between Days 7 and 14 and remained high through Days 42 to 56. Estradiol concentrations increased four-fold in large (>8mm) follicles between Days 14 and 28 (Spicer, 1985). Follicles on the ovary containing the corpus luteum of pregnancy were smaller but not fewer in number and had lower estrogen concentrations than follicles on the non-CL ovary (Bellin et al., 1984). Follicles from the ovary bearing the corpus luteum of pregnancy of suckled cows were the smallest with the lowest estrogen content and follicles from the non-CL ovary of non-suckled cows were the largest with the highest estrogen content. Follicular fluid concentrations of LH (Walters et al., 1982a; Walters et al., 1982b), estrone, estradiol-17ß and progesterone were similar, but prolactin concentrations were higher in weaned cows (Walters et al., 1982a).

On Day 8 follicular volume was increased in GnRH treated and non-suckled cows (Carter et al., 1980). Follicles obtained from norgestomet-treated cows were heavier and more variable in weight than controls (Inskeep et al., 1988). Most of the difference was attributable to increased follicular fluid although both the theca and granulosa showed greater variance and tended to be heavier. There was more estradiol in the largest follicle of treated cows than controls.

Changes in follicular development cannot occur without some input from extraovarian stimuli and the follicle must have receptors to receive and transduce the stimuli. Therefore, any changes in the number and(or) type of receptors could have a major impact on follicular development. There were more LH receptors in weaned cows (Walters et al., 1982a) and norgestomet-treated cows (Inskeep et al., 1988) than controls, but the number of FSH receptors in the granulosa did not differ between treatments (Walters et al., 1982a; Inskeep et al., 1988). In fact, follicular LH receptors increased sharply by 36 hours post-progestin implant removal in estrous cows and by 96 hours after weaning, and FSH receptors tended to decrease from 12 to 26 hours post-implant removal and 24 to 72 hours after weaning (Walters et al., 1982b).

Social Interaction and Reproductive Function

One of the problems associated with sexual reproduction in mammals is synchronization of mating so that fertile gametes from each sex are present in the reproductive tract. One of the methods by which one animal can affect the reproductive behavior of another is through pheromones (Izard, 1983). Pheromones can be classified into two types, releasing pheromones and priming pheromones. Wilson and Bossert (1963; cited in Izard, 1983) defined releasing pheromones as pheromones that cause an immediate but reversible behavioral response. Priming pheromones initiate a chain of physiological events which are generally irreversible (Wilson and Bossert, 1963; cited in Izard, 1983).

Laboratory Species

Vandenbergh (1967) reported that sexual maturation of female mice was accelerated by exposure to males. Prepubertal female mice exposed to adult male mouse urine, male rat urine, preputialectomized male mouse urine or sham-operated male mouse urine exhibited first estrus at the same age, and were younger than unexposed female mice and female mice exposed to castrated male mouse urine which were, in turn, younger at first estrus than female mice exposed to female mouse urine (Colby and Vandenbergh, 1974). Moreover, exposing prepubertal female mice to males caused a rapid (within 1 hour) increase in plasma LH concentrations which was maintained for several hours (Bronson and Desjardins, 1974). FSH levels did not change. Preovulatory surges of LH and FSH were detected on Day 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).

A pheromone produced by male mice was shown to be present in urine held within the bladder of intact males and androgenized, spayed females but not in male castrates. Urine was collected directly from the bladder so no accessory gland secretion was added (Bronson and Whitten, 1968). In fact, Vandenbergh et al. (1975) have demonstrated that the pheromone may be a protein or a substance bound to a protein.

Wild Species

Pairing female gray opossums (*Monodelphis domestica*) with males accelerates the occurrence of estrus, ovulation and pregnancy. However, not all of the females responded to exposure (Fadem, 1985; 1987). The pheromone that is involved with this effect is at least partially androgen dependent (Fadem, 1987). Presence of red deer (*Cervus elaphus*) stags advances the onset of estrus in does. However, pheromones are not the only method by which the presence of males may influence female reproduction. Roaring in red deer stags as an isolated stimulus advances ovulation in does, but not to the same extent as male presence (McComb, 1987).

Domestic Livestock

Non-ruminants. Gilts exposed to boars reached puberty at an earlier age than gilts isolated from boars (Brooks and Cole, 1970; Thompson and Savage, 1978; Paterson and Lindsay, 1980). Furthermore, Paterson and Lindsay (1980) reported that gilts raised with intact, contemporary males reached puberty before gilts raised with castrate, contemporary males, and introduction of mature boars into either treatment reduced age at puberty even further. Age at puberty did not differ between the groups introduced to mature boars (Paterson and Lindsay, 1980); however, exposure to boars reduced synchrony of estrus when gilts were removed from confinement (Thompson and Savage, 1978).

Rowlinson et al. (1975) conducted observations at a commercial swine unit and reported that all sows exposed to boars exhibited lactational estrus and conception rate at lactational estrus was 84.9%. Furthermore, the proportion of sows ovulating or in estrus within 10 days of weaning increased as time of exposure to boars increased (Walton, 1986). Sows exposed to boars after weaning returned to estrus or ovulated much sooner than those that were isolated from boars. First-litter sows did not respond to boar exposure before weaning, but older sows had shorter anestrus periods. Exposure to boars both pre- and post-weaning gave maximum results.

Small Ruminants

Shelton (1960) reported that the presence of buck goats stimulated Angora does to ovulate. Claus et al. (1990) determined that acyclic does exposed to male hairs, diethylether extracts of male hair, and polar and nonpolar subfractions of diethylether extract for 72 hours showed a rapid increase in LH and estradiol concentrations and resulted in estrus behavior and ovulation in seasonally anestrous does. Moreover, Chemineau et al. (1986) reported that anosmic (sense of smell destroyed with $ZnSO_4$) does exposed to bucks exhibited increased LH pulses, maximum LH concentrations, interval to increased LH and mean interval to onset of estrus similar to control does. However, the percentages of does exhibiting estrus or ovulation was lower for anosmic females than control females after exposure. These results indicate that the sense of smell is not solely responsible for the buck effect. It is possible that the pheromone is absorbed directly across the surface of the alveoli and carried in the blood to the hypothalamus, anterior pituitary or ovary to affect the reproductive status of the doe.

Martin et al. (1980) demonstrated that introduction of rams to seasonally anovular ewes in crates and in an adjacent pen "caused" ovulation. The proportion of ewes ovulating at 37 to 41, 61 to 65, 70 to 72, 84 to 88 and 94 to 96 hours after exposure to rams was higher for ewes exposed to rams than ewes not exposed to rams (Knight et al., 1978; Poindron et al., 1980). Moreover, Oldham and Pearce (1984) reported that more ewes exposed to rams for 72 hours ovulated than ewes exposed to rams for 6 or 24 hours. Chesworth and Tait (1974) found that time from the beginning of the experiment to estrus did not differ among treatments, but ewes exposed to rams had a higher incidence of twinning than isolated ewes. Isolation of ewes from rams does not appear to be essential to achieve a high response to the presence of rams (Cushwa et al., 1992).

Clearly, the ram induces an ovulatory response in the ewe. The question thus arises concerning the physiological changes that occur to accelerate or enhance ovarian cycling activity. Serum LH concentrations rose within 2 hours of exposure to a peak (Chesworth and Tait, 1974; Knight et al., 1978; Martin et al., 1980; Poindron et al., 1980; Cohen-Tannoudji and Signoret, 1987) whether ewes were raising twins or single lambs (Poindron et al., 1980). LH pulse frequency increased and baseline concentrations were elevated (Martin et al., 1980; Poindron et al., 1980; Oldham and Pearce, 1984). Oldham and Pearce (1984) suggested that the surge of LH that occurs within 18 hours 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 days later exhibited the same LH response as at the first exposure. It would appear that the presence of the male is required to maintain pulsatile LH release, which rapidly decreases after the ram is removed (Cohen-Tannoudji and Signoret, 1987). However, Oldham and Pearce (1984) concluded that a marked and sustained increase in the frequency of LH pulses was not essential for the ram to induce ovulation.

Suppression of prolactin with CB-154 (ergocryptine) had no effect (Poindron et al., 1980). FSH was not affected by exposure to rams on the first day, but on the following day an FSH surge was detected coincidental with an ovulatory surge of LH.

Large Ruminants

Presence of mature bulls had no effect on attainment of puberty in beef heifers (Berardinelli et al., 1978; Macmillan et al., 1979; Roberson et al., 1987). However, Roberson et al. (1991) found that although heifers exposed to bulls in the first year of a two year experiment did not attain puberty earlier than isolated heifers, in the second year, 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. Pregnancy rates to AI were greater in heifers exposed to bulls than in those that were isolated. Izard and Vandenbergh (1982) reported that oronasal application of bull urine to prepubertal heifers increases the number of heifers attaining puberty in a 7 week period. Izard and Vandenbergh (1982) concluded that bull urine contains a priming pheromone that acts in a manner similar to the one in mice.

Colby and Vandenbergh (1974) demonstrated in mice that exposing prepubertal female mice to adult female mice delayed puberty. Contrary to this study, Nelsen et al. (1985) found that exposing prepubertal heifers to mature cows may have reduced the age and weight at puberty. However, the results were confounded with a breed of sire by treatment interaction, and the reductions in age and weight at puberty were only seen in the first year of a two year experiment.

Cows exposed to yearling or mature bulls during the postpartum period had shorter postpartum intervals than cows isolated from bulls (Zalesky et al., 1984; Berardinelli et al., 1987; Custer et al., 1990; Cupp et al., 1993). Skinner and Bonsma (1964; cited in Izard, 1983) found that if a vasectomized bull is introduced to a breeding herd 30 days before the breeding season, postpartum ovarian activity is increased. Furthermore, Berardinelli et al. (1987) reported that more cows exposed to bulls exhibited estrus than cows isolated from bulls. Naasz and Miller (1990) determined that more cows exposed to bulls exhibited estrus before implantation with Synchromate-B than cows isolated from bulls in two separate years (76 and 75% vs 17 and 24%, respectively). However, bull exposure reduced the postpartum interval only in one year and not the second (Naasz and Miller, 1987; Naasz and Miller, 1990). There was no difference in the number of cows detected in estrus during the breeding season between cows exposed to bulls and cows isolated from bulls when calving occurred in the fall (Macmillan et al., 1979). However, more cows exposed to bulls were detected in estrus during the breeding season than controls when calving occurred in the spring. Spitzer et al. (1987) reported that cows exposed to either testosterone treated cows or bulls have similar postpartum anestrus periods. However, cows isolated from bulls had shorter postpartum periods than those exposed to bulls in a second year's experiment. More cows exposed to testosterone treated cows were detected in estrus before the breeding season than control cows (Burns and Spitzer, 1992). Elevated progesterone concentrations indicative of ovarian cycling activity occurred in a higher proportion of cows exposed to bulls during the postpartum period. However, postpartum interval to first estrus, proportion of cows cycling by the beginning of the breeding season and pregnancy rates did not differ (Scott and Montgomery, 1987, Perry et al., 1993). Bull exposure, beginning 33 days postpartum, and(or) 72 hour calf removal had no effect on postpartum interval to first ovulation or first estrus or on proportions of cows showing first ovulation or first estrus (Bonavera et al., 1990).

Berardinelli et al. (1987) found that more cows exposed to bulls were bred by AI during the breeding season than isolated cows. However, overall pregnancy rates were not different. Bull exposure, beginning 33 days postpartum, and(or) 72 hr calf removal had no effect on conception, silent estrus periods, number of first service conceptions or proportions of cows pregnant in multiparous Angus cows (Bonavera et al., 1990). Nersesjans (1959; cited in Izard, 1983) introduced vasectomized bulls to a group of postpartum cows. The number of cows that took less than 60 days to conceive after insemination was greater in cows exposed to bulls than in isolated cows. In addition, more of the cows exposed to bulls conceived from insemination at their first postpartum estrus. Petropavlovskii and Rykova (1958; cited in Izard, 1983) exposed early postpartum cows to a vasectomized bull for 3 to 4 hours twice daily. Cows exposed to the bull conceived earlier than cows that were isolated from the bull. A group of cows exposed to bulls 30 days before the breeding season all mated within 21 days of the start of the breeding season, whereas 52 days were required for the completion of breeding in unteased controls (Skinner and Bonsma, 1964; cited in Izard, 1983). Conception rates to a timed, synchronized estrus and pregnancy rates did not differ between exposed cows and controls (Naasz and Miller, 1987; Naasz and Miller, 1990).

Cows fed a low energy diet and exposed to bulls had similar postpartum intervals to cows fed a high energy diet and exposed to bulls (Stumpf et al., 1987). Cows on lower energy diet were influenced more by the presence of bulls than cows in the higher dietary regimen. The postpartum interval was shorter in cows that were exposed to bulls than those that were isolated from bulls regardless of nutritional regimen and did not differ (Stumpf et al., 1992). The results of this experiment suggest that the effect of the bull may be able to override low levels of nutrition.

Although there is a general concensus that exposure to bulls reduces the postpartum interval, little research conducted to date has focused on the mechanism whereby the bull effect occurs. Custer et al. (1990) reported that baseline LH levels and pulse frequency were not affected by the presence of the bull. However, 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. Baruah and Kanchev (1993) concluded that oronasal treatment with bull urine increases serum concentrations of LH and FSH within 80 min in cows treated after Day 7 postpartum. The results of this experiment support the hypothesis that the bull effect occurs rapidly and may have been missed by Custer et al. (1990).

Summary

Clearly, the postpartum interval in the bovine is affected by nutrition and(or) body condition, suckling, social interactions and a number of other factors. These factors appear to exert their effects through the neuroendocrine system. Ovarian hormones appear to be important intermediaries of at least some of the processes involved.

Throughout the literature, LH appears to be the most important factor involved in the resumption of normal estrous cycling activity. In order to resume normal estrous activity, the hypothalamus and anterior pituitary must overcome the combined effects of suckling and estrogen on the suppression of LH secretion. This suppression seems to be mediated through the endogenous opioid peptide system.

In mice and sheep, the introduction of a male has been shown to induce a rapid release of LH, mating behavior and ovulation. Postpartum anestrus in cattle has been reduced by the presence of bulls. However, the time required for the bull to affect postpartum reproduction and whether LH is involved remain unclear. If, as in mice and sheep, the response to bulls is rapid, the experiment conducted by Custer et al. (1990) does not provide the necessary information. However, Baruah and Kanchev (1993) have presented evidence to support the hypothesis that LH response to the presence of bulls is rapid.

STATEMENT OF PROBLEM

Failure of females to rebreed after calving decreases reproductive efficiency in beef cattle production. One of the most important reasons that cows fail to rebreed, especially first-calf suckled cows, is long postpartum anestrus periods. Many factors affect the length of the postpartum anestrus interval in beef cows. One of these, exposure to mature bulls, shortens the postpartum interval to resumption of ovarian cycling activity in suckled multiparous and primiparous cows. The physiological interactions which induce this response are complex and remain unclear.

In mice and sheep, the introduction of a male has been shown to induce a rapid release of LH, mating behavior and ovulation. Postpartum anestrus in cattle has been reduced by the presence of bulls. If, as in mice and sheep, the response to bulls is rapid, the experiments conducted to study LH involvement in cows exposed to bulls or bull urine do not provide the necessary information.

In order to gain an understanding of the mechanism whereby bull exposure shortens the postpartum anestrus interval we must first determine when and for how long cows need to be exposed to bulls after calving. This knowledge would also allow us to make more specific recommendations about the use of bulls for altering postpartum reproductive performance of first-calf suckled beef cows. It will also allow us to more effectively study the physiological interactions involved in the bull effect, which will in turn provide us with a better understanding of postpartum anestrus and resumption of ovarian cycling activity.

The objectives of Experiment 1 were to determine: 1) if exposing postpartum first-calf suckled beef cows to mature bulls during the first 30 days postpartum, after 30 days postpartum or continuously reduces the postpartum interval to resumption of ovarian cycling activity; 2) if exposure to bulls alters the proportions of cows that are bred by artificial insemination early in the breeding season; and 3) if exposure to bulls alters AI and total pregnancy rates. The objectives of Experiment 2 were to determine: 1) if exposure to bulls continuously beginning on Day 30 postpartum or for two hours every third day beginning on Day 30 postpartum alters the postpartum interval to first estrus; 2) if exposure to bulls alters patterns of LH secretion; and 3) if exposure to bulls alters AI and total pregnancy rates.

MATERIALS AND METHODS

Experiment 1. Sixty-nine first-calf, crossbred Hereford x Angus and Angus x Hereford, suckled beef cows maintained at the Bozeman Livestock Center, Montana State University, were assigned to one of two treatments within 72 hours of calving: 1) exposure to mature epididectomized bulls (BE; n = 35) or 2) isolation from bulls (NE; n = 34). At calving, cows were assigned to treatment in groups of four in the following manner. The first cow to calve was randomly assigned to either the BE or NE treatment. The second and third cows to calve were assigned to the converse treatment, and the fourth cow to calve was assigned to the same treatment as the first cow to obviate the effects of calving date. Body weight and condition score for each cow were obtained within 72 hours of calving and again within 72 hours of being bred by AI at the end of the experiment. Those cows that were not bred by AI were weighed on June 17, 1991. Average body weight and condition score of cows after calving were 454 ± 41 kg and 5.5 ± .1, respectively. Cows calved from February 1 to April 10, 1991, and the average calving date was February 27, 1991. The experiment ended June 18, 1991.

Cows assigned to the BE treatment were placed in a pasture with one bull until the bull to cow ratio reached 1:19, at which time a second bull was introduced. Cows continued to be added until the bull to cow ratio was again

1:19. Cows in the NE treatment were placed into a pasture that was approximately 1 km from the pasture that contained the cows in the BE treatment so that NE cows could not see, smell, hear or otherwise come into contact with a bull. On Day 30 postpartum, cows were randomly assigned to either remain in their initial treatment (BE; n = 18 and NE; n = 18) or to be assigned to the converse treatment BENE (n = 17) or NEBE (n = 16). Cows and their calves that were assigned to the converse treatment were moved by trailer approximately 1 km to the other pasture.

Cows were fed medium quality mixed-grass alfalfa hay until pasture grasses became available and were provided free access to mineralized salt and water.

Blood samples were obtained by caudal venipuncture once each week beginning on February 19. Samples were allowed to clot at 4°C then centrifuged at 1850 x g at 4°C. Serum was harvested and stored at -20°C until it could be assayed for progesterone.

Cows in each treatment were observed visually twice daily (0600 - 0800 and 1700 - 1900) for 40 minutes for behavioral estrus beginning on March 15. Visual observation of behavioral estrus was used as corroborating evidence for progesterone data. Figure 1 shows a representative pattern of progesterone concentration for a cow that showed a transient increase in progesterone and what we considered to be resumption of normal ovarian cycling activity. Resumption of ovarian cycling activity was defined as an increase in systemic progesterone concentrations above .5 ng/ml in two consecutive weekly samples. Resumption was defined as the day of the week before progesterone increased and remained high for two consecutive samples. For this cow resumption of ovarian cycling activity occurred on Tuesday of Week 5.

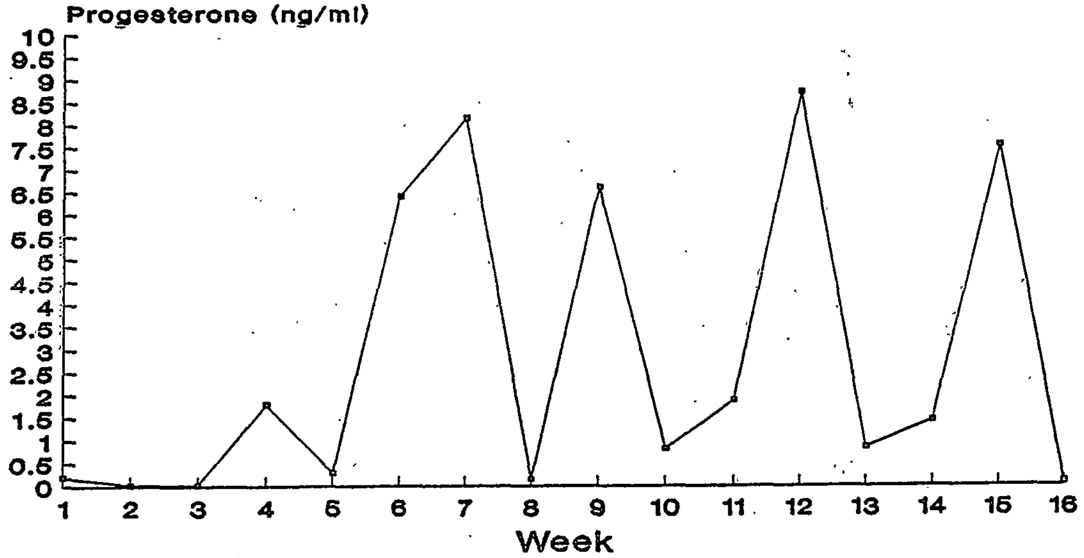

Figure 1. Serum progesterone concentrations during the postpartum period for cow no. 9064. This pattern depicts our definition for resumption of ovarian cycling activity in first-calf suckled beef cows (week = 5) and a "short" luteal phase before the first normal luteal phase (week = 4).

Beginning on June 1 cows in each treatment were bred by AI for 21 days. Cows were bred 12 hours after observation of estrus. Cows remained in their assigned treatments during the

AI period. Breeding date for each cow bred by AI was recorded in order to determine whether the cow maintained pregnancy when bred by AI. On June 22, all cows were exposed to fertile bulls for 35 days. Cows were pregnancy tested by palpation per rectum 46 days after the end of the breeding season.

Progesterone Assay. Progesterone concentrations in serum samples were quantified using solid-phase RIA kits[a]. The assay was modified and validated for use with cow serum instead of human serum in our laboratory by Custer (1988). The sensitivity of this assay was .031 ng/ml, and the inter- and intra-assay CV's were 4.4 and 7.8%, respectively, for a serum pool that contained 1.45 ng/ml.

Statistical Analysis. Data for body weight change from three days after calving to the end of the experiment, postpartum interval to resumption of ovarian cycling activity and number of estrous cycles before each cow was bred or had an opportunity to be bred by AI were analyzed separately by an analysis of variance for a completely randomized design using the General Linear Models procedure of SAS (1987). Opportunity to be bred by AI was defined as the first day of the week that progesterone concentrations in the blood fell below .5 ng/ml during the AI breeding period. Data for postpartum resumption of ovarian cycling activity included all

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[a] Diagnostic Products Corp., Los Angeles, CA

cows in each treatment. Those cows that had not met the definition for resumption of ovarian cycling activity were assigned a postpartum interval equal to the difference between seven days after the end of the experiment and their calving date. Orthogonal contrasts were used to compare means for body weight change, postpartum interval to resumption of ovarian cycling activity and number of estrous cycles before being bred by AI (Lund, 1991). Specific contrasts for each of these variables were: NE vs BE, BENE, NEBE; BE vs BENE, NEBE; and BENE vs NEBE. Proportions of cows that: 1) showed a rise in progesterone greater than .5 ng/ml before the first normal luteal phase, 2) were bred by AI, and 3) were pregnant at 46 days after the breeding season for BE, BENE, NEBE and NE cows were analyzed by chi-square (Lund, 1991).

Experiment 2. Eighty-three first-calf, crossbred Hereford x Angus and Angus x Hereford, suckled beef cows maintained at the Bozeman Livestock Center, Montana State University, were assigned to one of two treatments within 72 hours of calving: 1) exposure to mature epididectomized bulls (BE; n = 20) or 2) isolation from bulls (NE; n = 63). Body weight and condition score for each cow were obtained within 72 hours of calving and at 30 day intervals (BW1, BW2, BW3) until the end of the experiment. Average body weight and condition score of cows after calving were 449.6 ± 41.4 kg and 5.57 ± .5, respectively. Cows calved from January 30 to March

13, 1992, and the average calving date was February 19, 1992. The experiment ended June 21, 1992.

On Day 30 postpartum, NE cows were assigned either to remain in their initial treatment (NE, n = 32), or they were assigned to continuous exposure to bulls (NEBE, n = 10) or intermittent bull exposure (BEI, n = 21). Cows in the BEI treatment were placed in a pen approximately 8 m wide x 25 m long and exposed to a sterile, mature bull for 2 hours on Day 30 postpartum and on every third day thereafter until Day 48 postpartum. BEI cows were placed in a separate pasture from the pastures containing either the BE or NE cows after their first exposure to a bull. The pasture containing the BEI cows was separated from the BE pasture by approximately .7 km and was adjacent to, but not visible from, the NE pasture. Cows assigned to the NEBE treatment were transferred in a trailer from the NE pasture to the BE pasture. The bull to cow ratio was 1:15. Cows were fed a medium quality mixed-grass and legume hay until pasture grasses became available and provided free access to mineralized salt and water.

Cows were observed twice daily (0600 - 0800 and 1700 - 1900) for 40 minutes for behavioral estrus. Only those cows which stood to be mounted by a herdmate or by a sterile bull were considered to be in estrus. Beginning on June 1, cows were combined in one pasture and bred by AI for 21 days. No bulls were present during the AI breeding period. Breeding dates were recorded, and 25 days after AI breeding a blood sample was collected by caudal venipuncture for pregnancy specific protein b_1 ($PSPb_1$) to determine AI pregnancy rates. Cows were exposed to fertile bulls for an additional 35 days after the end of the AI breeding period. Cows were pregnancy tested by palpation per rectum 50 days after the end of the breeding season.

Intensive Blood Sampling for LH. Two days before the initial bleeding, 10 cows from each treatment were non-surgically fitted with an indwelling jugular catheter (Custer, 1988). Cows in each treatment were assigned to be bled by calving date so that their calving dates were separated by no more than 3 days. Beginning on Day 30 postpartum, cows in the BEI treatment were placed into separate breeding stalls for blood sample collection immediately after they had been exposed for 2 hours to a mature, sterile bull. NEBE cows were treated in the same manner on the following day and then placed into the pasture containing the BE cows. On the third day, NE cows were placed immediately into separate stalls for blood sample collection. Blood samples were collected on this rotation until Day 48 postpartum. Calves were placed in the stalls with their dams and could suckle ad libitum. Cows' heads were tied in place and blood samples were collected every 15 minutes for the next 6 hours. Catheters were flushed between samples with 10 mL sterile, heparinized saline. Blood samples were placed on ice, allowed to clot, then centrifuged at 1850 x g for 30 minutes. Serum was harvested and stored at

-20° C until it could be assayed for LH.

Hormone Assays. LH concentrations were quantified using the double antibody RIA method of Custer et al. (1990) using bLH-I-5 as the iodinated hormone and standard. The sensitivity of this assay was .11 ng/mL and the inter- and intra-assay CV's were < 10 and < 15%, respectively. Assay for $PSPb_1$ was performed using the method of Sasser et al. (1986).

Statistical Analysis. The proportion of cows that displayed estrus before the beginning of the breeding season, proportion of cows bred by AI and AI and overall pregnancy rates were analyzed by Chi-square analysis (Lund, 1991). Mean, baseline, frequency, amplitude and interpeak interval for LH concentrations were determined using the PULSAR (Merriam and Wachter, 1982) program and these data were analyzed by an analysis of variance using the GLM procedure of SAS (SAS, 1987) with the repeated measures option with day as the repeated measure. One cow each in both the NE and NEBE treatments had a preovulatory surge of LH during the course of the experiment and data from the day of the surge onward have been excluded from the analysis.

Differences in mean body weight, day of first estrus and postpartum interval were analyzed by an analysis of variance for a completely randomized design using the GLM procedure of SAS (SAS, 1987). Day of first estrus for cows that failed to exhibit estrus was determined by subtracting calving date in 1993 from an average gestation length of 283 days. Means for body weights for BW2, day of first estrus, postpartum interval, mean LH concentrations and LH pulse frequency were compared using orthogonal contrasts (SAS, 1987). Specific contrasts for BW2 were: BEI vs BE, NEBE, NE; NEBE vs BE, NE; and BE vs NE. Specific contrasts for day of first estrus and postpartum interval were: BEI vs BE, NEBE, NE; BEI vs BE, NEBE; and BEI vs NEBE. Specific contrasts for mean LH concentrations and LH pulse frequency were: BEI vs NEBE, NE and BEI vs NEBE.

RESULTS

Experiment 1. Body weight change between three days after calving and the end of the experiment for cows in the BENE and NE treatments did not differ (P > .10; Table 1), and body weight change for cows in the BE treatment did not differ (P > .10) from cows in the BENE or NEBE treatments. However, cows in the BE treatment lost more weight (P < .05) than NE cows (Table 1). Condition scores of cows at the end of the experiment did not differ (P > .10) among treatments and averaged 5.2 ± .1.

All cows in the BE, BENE and NEBE treatments, and 90% (16 out of 18) of cows in the NE treatment met the criterion (Figure 1) for resumption of ovarian cycling activity by the end of the experiment. Postpartum interval to resumption of ovarian cycling activity was shorter (P < .05) for BE, BENE and NEBE cows than for NE cows (Table 1). However, postpartum intervals to resumption of ovarian cycling activity did not differ (P > .10) among BE, BENE and NEBE cows (Table 1).

Proportions of cows in the BENE, NEBE, and NE treatments that exhibited a rise in progesterone greater than .5 ng/ml before the first normal luteal phase, as defined in Figure 1, did not differ (P > .10; Table 2). More (P < .05) cows in the BE treatment exhibited a rise in progesterone before the first normal luteal phase than cows in the NEBE and NE treatments. However, the proportion of cows that showed a rise in progesterone before the first normal luteal phase did not differ (P > .10) between BE and BENE cows (Table 2).

Table 1. Least squares means for postpartum body weight change and interval to resumption of ovarian cycling activity

Treatment[1]	n	Body weight change (kg)[2]	Postpartum interval (days)
BE	18	$-19.4^{a,b}$	46.5^{a}
BENE	17	$4.2^{a,c}$	50.9^{a}
NEBE	16	-27.7^{b}	51.0^{a}
NE	18	18.2^{c}	64.7^{b}
SEM[3]		28.9	17.6

[1] BE = continuous bull exposure, BENE = bull exposed for 30 days post-calving, NEBE = bull exposed after 30 days post-calving and NE = not exposed to bulls.

[2] Final body weight on test minus body weight Day 3 post-calving.

[3] df = 66.

[a,b,c] Means within columns lacking a common superscript letter differ (P < .05).

The number of estrous cycles that had occurred by the time the cows were bred did not differ (P > .10) among cows that were exposed at any time to bulls (Table 2). However, cows not exposed to bulls had approximately .5 fewer (P < .05) estrous cycles before being bred than cows exposed to bulls at any time during the postpartum period (Table 2).

Overall pregnancy rates for the 56-day breeding season did not differ (P > .10) among treatments (Table 3). Fifty-five of the 69 (80%) cows were bred by AI. Proportions of cows that were bred during the 21-day AI period did not differ (P > .10) among treatments (Table 3).

Table 2. Percentages of postpartum first-calf suckled beef cows that exhibited an increase in progesterone before the first normal luteal phase and number of estrous cycles by breeding

Treatment[1]	n	Percentage of cows	No. estrous cycles by breeding[2]
BE	18	72.2^{a}	2.6^{a}
BENE	17	$58.8^{a,b}$	2.5^{a}
NEBE	16	31.3^{b}	2.5^{a}
NE	18	27.8^{b}	2.0^{b}

[1] BE = continuous bull exposure, BENE = bull exposed for 30 days post-calving, NEBE = bull exposed after 30 days post-calving and NE = not exposed to bulls.
[2] SEM = 1.0; df = 65.
[a,b] Percentages and means within columns lacking a common superscript letter differ (P < .05).

Table 3. Overall pregnancy rates, percentages bred by AI and AI pregnancy rates

Treatment[1]	n	Overall Pregnancy Rates (%)	Number (%) bred AI	AI Pregnancy Rates (%)
BE	18	94^{a}	$14\ (78)^{a}$	$57^{a,b}$
BENE	17	100^{a}	$14\ (82)^{a}$	100^{c}
NEBE	16	88^{a}	$13\ (81)^{a}$	$77^{b,c}$
NE	18	83^{a}	$14\ (78)^{a}$	29^{a}
Total	69	91	55 (81)	66

[1] BE = continuous bull exposure, BENE = bull exposed for 30 days post-calving, NEBE = bull exposed after 30 days post-calving and NE = not exposed to bulls.
[a,b,c] Percentages within columns lacking common superscript letters differ (P < .05).

Pregnancy rates for cows bred by AI were lower (P < .05) for cows in the NE treatment than for cows in either the BENE or NEBE treatments. AI pregnancy rates for cows in the BE treatment did not differ (P > .10) from AI pregnancy rates of cows in the NEBE or NE treatments (Table 3). However, AI pregnancy rates were higher (P < .05) for cows in the BENE treatment than cows in the BE treatment (Table 3). There was no difference (P > .10) in AI pregnancy rates between cows in the BENE and NEBE treatments (Table 3).

Experiment 2. Mean cow weights at parturition, BW1 and BW3 did not differ (P > .10) among treatments (Table 4). Cow weights during BW2 were higher (P < .05) for cows in the BEI treatment than cows in the BE, NEBE or NE treatments (Table 4). Cows in the NEBE treatment weighed more (P < .05) during BW2 than cows in the BE and NE treatments (Table 4). Weights for cows in the BE and NE treatments did not differ (P > .10) during BW2 (Table 4). Overall weight changes for the entire experiment were not different (P > .10), and mean cow weights within all treatments increased by the end of the experiment. Body condition scores did not differ (P > .10) among treatments at any time during the experiment and averaged 5.3 ± .5 by the end of the experiment.

The proportion of cows exhibiting estrus before the beginning of the AI breeding period was greater (P < .05) for cows in the BE treatment than in any of the other treatments (Table 5). The proportion of NEBE cows exhibiting estrus before the beginning of the breeding period tended to be higher ($P < .10$) than the proportion of BEI cows exhibiting estrus (Table 5). The proportion of NEBE cows displaying estrus before the beginning of the AI breeding period did not differ ($P > .10$) from the proportion of NE cows displaying estrus by the start of the breeding period (Table 5). The proportion of BEI cows exhibiting estrus by the start of the breeding period did not differ ($P > .10$) from the proportion of NE cows exhibiting estrus by the start of the breeding period (Table 5).

Table 4. Least squares means for cow weights (± SEM) at birth, and Day 30 (BW1), 60 (BW2), and 90 (BW3) of Experiment 2

Treatment[1]	n	Birth (kg)	BW1 (kg)	BW2 (kg)	BW3 (kg)
BE	20	443	439	433^a	480
BEI	21	454	451	462^b	499
NE	32	449	443	424^a	477
NEBE	10	456	434	444^c	482
	SEM[2]	41.9	40.1	35.0	35.8

[1] BE = continuous bull exposure, BEI = intermittent bull exposure, NE = isolation from bulls, NEBE = continuous exposure to bulls after Day 30 postpartum.
[2] df = 76.
[a,b,c] Means within columns lacking common superscript letters differ ($P < .05$).

The proportion of cows that were bred by AI did not differ ($P > .10$) among treatments (Table 5). Pregnancy rates to AI breeding were higher for NEBE cows, which were higher than pregnancy rates for BE, BEI and NE cows (Table 6).

Pregnancy rates to AI breeding did not differ ($P > .10$) among BE, BEI, and NE treatments (Table 6). Overall pregnancy rates were highest ($P < .05$) for cows in the BEI treatment (Table 6). The proportion of cows in the NE treatment that were pregnant was higher ($P < .05$) than the proportion of BE cows that were pregnant (Table 6).

Day of first estrus occurred earlier ($P < .05$) for cows in the BE and NEBE treatments than cows in the BEI and NE treatments (Table 7). Cows in the BEI treatment had the latest ($P < .05$) day of first estrus (Table 7). Likewise, postpartum interval was longest ($P < .05$) for cows in the BEI treatment and shortest for cows in the BE and NEBE treatments (Table 7). Postpartum intervals for cows in the NEBE and NE treatments were not different ($P > .05$; Table 7).

Table 5. Proportion of cows exhibiting estrus by the beginning of the breeding season and proportion bred by AI

Treatment[1]	n	Exhibiting Estrus (%)	AI Bred (%)
BE	20	80^{a}	50
BEI	21	38^{b}	48
NE	33	$58^{b,c}$	61
NEBE	10	70^{c}	70

[1] BE = continuous bull exposure, BEI = intermittent bull exposure, NE = isolation from bulls, NEBE = continuous exposure to bulls after Day 30 postpartum.

[a,b,c] Means within columns lacking common superscript letters differ ($P < .10$).

Baseline, amplitude, number of peaks and peaklength for serum LH did not differ ($P > .10$) among treatments. Mean LH concentrations were higher ($P < .05$) for BEI and NEBE cows than for NE cows (Table 8). LH pulse frequency was higher ($P < .05$) for cows in the BEI and NEBE treatments than cows in the NE treatments (Table 8). Cows isolated from bulls had lower ($P < .10$) mean LH concentrations than cows in either of the other treatments on the first day of treatment (Table 9).

Table 6. Proportion of Cows Pregnant by AI and Total Pregnancy Rates

Treatment[1]	Number (%) AI Pregnant	Total Number (%) Pregnant
BE	6/10 (60)[a]	13/20 (65)[a]
BEI	7/10 (70)[a]	21/21 (100)[b]
NE	12/20 (60)[a]	25/33 (76)[c]
NEBE	6/7 (86)[b]	7/10 (70)[d]

[1] BE = continuous bull exposure, BEI = intermittent bull exposure, NE = isolation from bulls, NEBE = continuous exposure to bulls after Day 30 postpartum.
[a,b,c,d] Means within columns lacking common superscript letters differ ($P < .05$).

Table 7. Least squares means for day of first estrus and postpartum interval to estrus

Treatment[1]	n	Day of First Estrus (Julian Date)[2]	Postpartum Interval (days)[3]
BE	20	120.7[a]	71.3[a]
BEI	21	153.9[b]	101.6[b]
NE	32	141.1[c]	89.3[c]
NEBE	10	122.3[a]	77.8[a,c]

[1] BE = continuous bull exposure, BEI = intermittent bull exposure, NE = isolation from bulls, NEBE = continuous exposure to bulls after Day 30 postpartum.
[2] Pooled SEM = 33.5, df = 75.
[3] Pooled SEM = 33.1, df = 75.
[a,b,c] Means within columns lacking common superscript letters differ ($P < .05$).

Mean LH concentrations on Day 1 of treatment for BEI and NEBE cows did not differ ($P > .10$). Cows in the BEI treatment had higher ($P < .10$) LH pulse frequency than cows in either the NEBE or NE treatments on the first day of treatment (Table 9). LH pulse frequency for NEBE and NE cows on Day 1 of treatment were not different ($P > .10$).

Table 8. Least squares means for mean serum LH concentrations and LH pulse frequency

Treatment[1]	n	Mean LH (ng/ml)[2]	Pulse Frequency (pulses/h)[3]
BEI	10	1.53^a	1.12^a
NEBE	10	1.47^a	$.91^a$
NE	9	1.24^b	$.83^b$

[1] BEI = intermittent bull exposure, NEBE = continuous exposure to bulls after Day 30 postpartum, NE = isolation from bulls.
[2] Pooled SEM = .15, df = 117.
[3] Pooled SEM = .39, df = 117.
[a,b] Means within columns lacking common superscript letters differ ($P < .05$).

Table 9. Least squares means for mean serum LH concentrations and LH pulse frequency on Day 1 of treatment

Treatment[1]	n	Mean LH (ng/ml)[2]	Pulse Frequency (pulses/h)[3]
BEI	10	1.49^a	1.32^a
NEBE	10	1.32^a	$.78^b$
NE	9	1.23^b	$.71^b$

[1] BEI = intermittent bull exposure, NEBE = continuous exposure to bulls after Day 30 postpartum, NE = isolation from bulls.
[2] Pooled SEM = .25, df = 26.
[3] Pooled SEM = .65, df = 26.
[a,b] Means within columns lacking common superscript letters differ ($P < .10$).

DISCUSSION

Experiment 1. Postpartum interval to first estrus is a major factor determining whether cows become pregnant, and in first-calf cows postpartum interval to estrus is 15 to 25 days longer than in multiparous cows (Wiltbank, 1970; Dunn and Kaltenbach, 1980; Short et al., 1990; Williams, 1990). We found that exposing postpartum first-calf suckled beef cows continuously to mature bulls reduced the postpartum interval to resumption of ovarian cycling activity by an average of 18 days in this experiment. These results confirm results obtained in our laboratory in past experiments (Custer et al., 1990) which indicate that exposing first-calf cows to mature bulls decreases the postpartum interval to resumption of ovarian cycling activity. In this experiment we evaluated whether the postpartum interval to resumption of ovarian cycling activity could be altered by exposing cows to bulls for the first 30 days postpartum or by exposing cows to bulls after the first 30 days postpartum. The results show that exposure to bulls for the first 30 days or exposure after 30 days postpartum decreased the postpartum interval to the same extent as continuous exposure. Although cows in the BE and NEBE treatments lost body weight, postpartum intervals to resumption of ovarian cycling activity did not differ from that for cows in the BENE treatment. Cows in the NE treatment gained weight, yet postpartum intervals to resumption of ovarian cycling activity for cows in the BE, BENE, and NEBE treatments were shorter than the postpartum interval to resumption of ovarian cycling activity for NE cows. A practical implication of these results is that cows need not be exposed to bulls immediately after calving, or they may be exposed to bulls for as little as 30 days after calving to achieve the same level of performance. Furthermore, one set of bulls may be used on more than one group of cows over the course of the postpartum anestrus period.

The physiological explanation and the mechanism involved in this phenomenon remain unclear. However, more cows in the BE and BENE treatments showed a short-term rise in progesterone than cows in the NE treatment indicating that during the first 30 days of exposure the bull may be having an effect on ovarian follicular dynamics. Numerically fewer cows that were exposed to bulls at 30 days postpartum displayed a rise in progesterone than BE and BENE cows. Instead, 70% of these cows showed a normal luteal phase within two weeks of exposure to bulls. In their review, Short et al. (1990) summarized data from authors who 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. Whether the rise in progesterone is due to a direct effect of the bull on the ovary or an indirect effect on the ovary mediated by the hypothalamic-hypophyseal axis is unknown. Nevertheless, this narrows the time frame in which investigation of the effect of bulls need be performed.

Overall pregnancy rates were not different among treatments, which is similar to the results reported by Berardinelli et al. (1987) for cows exposed or not exposed to bulls but bred as a single group. However, proportions of cows in the BENE and NEBE treatments that became pregnant after being bred by AI was greater than that for the NE cows. Roberson et al. (1991) have reported that pregnancy rates to AI were greater in heifers exposed to mature bulls prepuberally than in heifers not exposed to bulls. However, total pregnancy rates did not differ. In addition, Zalesky et al. (1984) found that fertility at first breeding increased when cows had been exposed to sterile bulls.

Improvement in AI pregnancy rates for cows in bull exposed treatments appeared to be a result of the number of estrous cycles before the cows were bred. Although the number of estrous cycles before each cow was bred for all cows exposed to bulls (BE, BENE and NEBE) is greater than the number of estrous cycles for NE cows, only 2 of the 11 cows that exhibited only one estrous cycle did not maintain pregnancy when bred by AI.

<u>Experiment 2.</u> In this experiment, cow weights were similar throughout the experiment except for the second weigh period. Stumpf et al. (1992) determined that cows on a low plane of nutrition that were exposed to bulls had similar

postpartum intervals to increased progesterone. Further, cows with body condition scores of ≥ 5 at parturition return to estrus regardless of energy intake (Humphrey et al., 1983; Bartle et al., 1984)

As in Experiment 1, we found that cows exposed to bulls had a postpartum interval that was 18 days shorter than that for cows isolated from bulls, further supporting the results of Custer et al. (1990). In this experiment, we evaluated the effect of short term, intermittent exposure to bulls on the postpartum interval and its physiological effect. We found that intermittent exposure to bulls extended the postpartum anestrus period. These results are puzzling in light of reports in the literature for cattle and other species. Martin et al. (1980) demonstrated that introduction of rams to seasonally anovular ewes in crates and in an adjacent pen "caused" ovulation. The proportion of ewes ovulating was higher for ewes exposed to rams than ewes not exposed to rams (Knight et al., 1978; Poindron et al., 1980; Oldham and Pearce, 1984). Claus et al. (1990) determined that exposing acyclic does to male hairs, diethylether extracts of male hair, and polar and nonpolar subfractions of diethylether extract for 72 hours resulted in estrus behavior and ovulation in seasonally anestrous does. Moreover, Chemineau et al. (1986) reported that anosmic (sense of smell destroyed with $ZnSO_4$) does had similar mean interval to onset of estrus to intact females exposed to bucks. However, the percentages of does exhibiting estrus or ovulation was lower for anosmic females than intact females after exposure (Chemineau et al., 1986). Petropavlovskii and Rykova (1958; cited in Izard, 1983) exposed early postpartum cows to a vasectomized bull for 3 to 4 hours twice daily. Cows exposed to the bull conceived earlier than cows that were isolated from the bull.

Although the postpartum interval was increased in intermittently exposed cows, mean LH and LH pulse frequency increased in cows exposed to bulls continuously or intermittently in a similar manner. These results support studies in prepubertal mice that indicate that exposure to males causes an increase in LH concentrations (Bronson and Desjardins, 1974). In sheep, introduction of a ram to seasonally anovular ewes induces a sharp increase in mean LH concentrations and LH pulse frequency (Chesworth and Tait, 1974; Knight et al., 1978; Martin et al., 1980; Poindron et al., 1980; Oldham and Pearce, 1984; Cohen-Tannoudji and Signoret, 1987). Claus et al. (1990) determined that acyclic does exposed to male hairs, diethylether extracts of male hair and polar and nonpolar subfractions of diethylether extract for 72 hours showed a rapid increase in LH and estradiol in seasonally anestrous does. Moreover, Chemineau et al. (1986) reported that anosmic does had similar percentages of females exhibiting an LH pulse, maximum LH concentrations and interval to increased LH. Recently, Baruah and Kanchev (1993) reported that oronasal treatment of postpartum cows with bull urine resulted in increased LH concentrations within 80 min.

Another puzzling result of this study is that on the first day of exposure to bulls, LH pulse frequency was nearly twice as great for BEI cows as NEBE cows even though there was no difference in the way they were treated on the first day of exposure. An explanation for this result eludes me. The increase in mean LH concentrations and LH pulse frequency taken together with the increase in postpartum anestrum found in BEI cows suggests that there is more involved in the bull effect than simply a pheromone affecting the hypothalamo-hypophyseal axis.

Overall pregnancy rates were different among treatments which conflicts with the results reported in Experiment 1 and by Berardinelli et al. (1987) for cows exposed or not exposed to bulls but bred as a single group and Roberson et al. (1991) for heifers exposed to bulls. Proportions of cows in the NEBE treatment that became pregnant after being bred by AI was greater than that for BE, BEI and NE cows. Roberson et al. (1991) reported that pregnancy rates to AI were greater in heifers exposed to mature bulls prepuberally than in heifers not exposed to bulls. In addition, Zalesky et al. (1984) found that fertility at first breeding increased when cows had been exposed to sterile bulls.

Proportions of cows exhibiting estrus before the breeding season were higher for BE and NEBE cows than BEI cows. Proportions of NE cows exhibiting estrus before the breeding season were intermediate to the proportion of NEBE and BEI cows but not different from either treatment. However, the proportions of cows bred by AI were not different. These results conflict with the results of Experiment 1 which suggest that more estrous cycles result in improved pregnancy rates.

PRACTICAL APPLICATION AND FUTURE RESEARCH

Exposing first-calf cows to bulls reduced the postpartum interval to resumption of ovarian cycling activity. The bull effect can occur either within the first 30 days postpartum or after the first 30 days postpartum and the continued presence of bulls is not necessary for ovarian cycling activity to continue. However, intermittent exposure results in a longer postpartum anestrus period. More estrous cycles occurred before the cows were bred by AI in cows exposed to bulls than in cows not exposed to bulls. Cows exposed to bulls maintained pregnancy more readily when bred by AI than those which were not exposed to bulls in the first experiment but only in cows exposed to bulls after 30 days in the second experiment. Producers can improve reproductive performance in first-calf suckled beef cows by exposing them to mature, sterile bulls for the first 30 days postpartum or after the first 30 days postpartum. The mechanism by which the bull effect occurs involves LH, but other factors appear to be involved as well.

Further experiments should be performed to determine whether the elevated serum LH levels seen in the intermittently exposed cows in this experiment are maintained on days during which they are isolated from the bull or if they are an immediate consequence of exposure to the bull. Samples should be collected either on days when the cows are not exposed to the bull or immediately before bull exposure. Additional studies should also be performed to determine whether the bull effect results from behavioral or pheromonal stimuli. One such study could involve placing a bull in a pen in a pasture so that the cows cannot come into direct, physical contact with the bull. Another study could involve placing cows in a pen in which bulls have been kept so that the cows would be exposed to the urine and feces from the bulls but not the bulls themselves. If the postpartum interval is reduced, then it could be concluded that the cues involved in producing the bull effect are pheromonal rather than behavioral in nature. Should this be the case, the next step would be to determine whether the pheromone is testosterone dependent. By comparing the effect of a steer, cow, bull and testosterone treated steer on the postpartum interval, testosterone dependency could be determined. On the other hand, if the effect appears to be behaviorally mediated, the type of behavior eliciting the response should be determined. As the bull moves through the herd attempting to determine the reproductive status of the females, he licks and(or) sniffs the genital region of the cow or attempts to mount the cow, and clitoral stimulation by the bull or mounting behavior may cause a direct, neural stimulation of the uterus, ovaries and(or) the hypothalamo-hypophyseal axis.

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