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INFLUENCE OF SUCKLING AND DIET ON ENDOCRINE PHYSIOLOGY OF POSTPARTUM BEEF COWS

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ABSTRACT

INFLUENCE OF SUCKLING AND DIET ON ENDOCRINE PHYSIOLOGY OF POSTPARTUM BEEF COWS

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Two experiments were conducted to determine the influence of suckling and dietary energy on concentration of gonadotropins and steroids in the serum and estradiol-17β (E_2) receptors in the anterior pituitary and hypothalamus of postpartum beef cows.

Experiment I

Twenty-six cows in postpartum anestrus were either allowed to nurse their calves ad libitum or were separated from their calves at d 35 postpartum (PP). Serial samples of blood were collected twice daily between d 35 and 41 PP. Baseline and pulse frequency of LH in serum increased ($P<.01$) following weaning while the amplitude of the pulses remained unchanged. The secretion of cortisol did not differ between treatment groups within 72 h postweaning.

Experiment II

Sixty-nine multiparous crossbred cows were fed either a low (60% NRC) or high (120% NRC) energy ration 85 d before calving and during the PP period. One-half of the cows in each group were allowed to nurse a calf during the PP period while the remaining

cows were separated from their calves at 21 d PP. Cows were slaughtered on d 14, 23 and 25 PP and the pituitaries and hypothalami removed. Neither calf removal nor diet increased E receptors in the pituitaries or hypothalami. Concentrations of LH in the anterior pituitaries increased ($P<.01$) from d 14 to 25 PP independent of diet or suckling.

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Gary L. Faltys

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CHAPTER I

INTRODUCTION

INTRODUCTION

A goal for the cattle industry is to produce a healthy calf every year from each cow inseminated. However, actual calf crops are approximately 70% of expectations and the major cause of the lowered calf crop is the failure of the cow to become pregnant (Bellows et al., 1979). A prolonged period of time from parturition to first postpartum ovulation (postpartum interval) may cause a large percentage of cows to be anovulatory during the breeding season and, therefore, may reduce the number of cows that are pregnant (Symington, 1969; Wiltbank, 1970).

The length of the postpartum interval varies in dairy and beef cows. Postpartum intervals less than 20 d and longer than 100 d have been reported in cattle (Wiltbank, 1970; Short et al., 1972; Dunn and Kaltenbach, 1980). A variety of factors contribute to the length of the postpartum interval and include suckling stimuli, nutrition, age of dam, dystocia, breed and season. Identifying all of the factors affecting postpartum intervals and determining associated costs are formidable tasks for scientists, economists and livestock producers.

The intent of the following review is to identify and discuss factors which affect duration of postpartum anovulation in cattle with emphasis on regarding effects of suckling and nutrition on postpartum endocrine and ovarian events.

CHAPTER II

LITERATURE REVIEW

LITERATURE REVIEW

Influence of Suckling on Postpartum Reproduction

The inhibitory effect of suckling and(or) presence of a calf on reproductive function was first reported by Clapp (1937). Similar observations have been reported in numerous studies since that time (Graves et al., 1968; Oxenreider, 1968; Wagner and Oxenreider, 1971; Short et al., 1972; Bellows et al., 1974).

Wettemann et al. (1976; 1978) reported that cows nursing two calves had a longer interval from calving to first estrus than cows with only one calf (94 vs 67 d). Milking delayed postpartum ovulation but delays were less than observed with suckling. Oxenreider and Wagner (1971) showed that Holstein cows milked twice daily had an extended postpartum interval as compared to nonlactating cows (46 vs 24 d) but a shorter postpartum interval than suckled cows (46 vs 52 d). These studies demonstrate that removal of milk from the udder or stimuli associated with this event inhibit reproductive function. Furthermore, the intensity of the stimulus influences the length of the postpartum interval.

Short et al. (1972) reported that postpartum intervals were longer for suckled and nonsuckled cows than for mastectomized cows (65 and 25 vs 12 d, respectively) suggesting that the presence of a lactating gland is sufficient stimulus to extend postpartum intervals. However, mammary denervation did not reduce the interval from calving to estrus indicating that the lactating gland is extending postpartum intervals by means other than sensory stimuli from the udder (Short et al., 1976). The possibility exists that inhibition of reproductive function by suckling, lactation and(or) presence of a calf is mediated through endocrine organs. This will be discussed in subsequent sections.

Attempts have been made to shorten the postpartum interval by permanently weaning calves, controlling the frequency of suckling or temporarily separating calves from cows. Weaning calves (<60 d of age) shortens postpartum anovulation of cows (Smith and Vincent, 1972; Laster et al., 1973; Bellows et al., 1974; Lusby and Parra, 1981). Removing calves from dams at parturition reduced the interval from calving to first estrus by 40 d (Short et al., 1972) while calf removal on d 10 postpartum reduced postpartum intervals by 33 d (Clemente et al., 1978) when compared to suckled cows. Suckling once daily (30 min) beginning at 21 d postpartum induced cows to exhibit estrus earlier (20 d) than suckled cows (Reeves and Gaskins, 1981). Similar results were reported when once-daily suckling was initiated at 30 d postpartum (Randel and Welker, 1976; Randel, 1981; Bluntzer, 1982). Cows temporarily separated from their calves for 48 h (d 21 to 80 postpartum) exhibited estrus earlier than suckled cows (Beck et al., 1977; Beck et al., 1979; Odde et al., 1982; Tervit et al., 1982). Calves that nursed once daily or were temporarily separated from their dams had daily rates of gain that were comparable to calves that suckled continuously. These management schemes offer the benefits of reducing the postpartum interval in the cow as well as a continued supply of milk for the calf.

An increased incidence of short first estrous cycles (<11 d) among cows nursed once daily was observed by Reeves and Gaskins (1981). Odde et al. (1980) reported that 80% of early-weaned cows showed short estrous cycles (7 to 10 d) compared with 1% normally nursed. These observations indicate that, even though postpartum interval can be shortened by once-daily suckling or temporary calf removal, the subsequent estrous cycle is abnormal.

Influence of Nutrition on Postpartum Reproduction

Optimum reproductive performance of cows depends on adequate nutrition (Parr and Klemmedson, 1924; Hafez, 1959; Bellows, 1966). Cows receiving prepartum diets that are low in energy have longer intervals from calving to first estrus than cows fed high prepartum levels of energy (Wiltbank et al., 1962; Dunn et al., 1969; Falk et al., 1975). The percentage of heifers exhibiting estrus 80 d after calving was greater (92 vs 73%) if the cattle were fed 3.6 kg of total digestible nutrients (TDN) before calving (120 d) rather than 2 kg TDN (Dunn et al., 1969; Wiltbank, 1970). Bellows and Short (1978) reported that cows fed a high (6.4 kg TDN) daily feed level for 90 d before calving exhibited estrus earlier postpartum than cows fed a low (3.3 kg TDN) daily feed level (50 vs 60 d). Contrary to these studies, Corah et al. (1974) observed that prepartum nutrition did not signficantly influence the interval to first estrus in heifers or cows. These authors suggested that the results may be explained by the fact that the cattle were in excellent body condition.

Level of energy in the diet after calving also affects the expression of postpartum estrus. Wiltbank (1970) reported that the percentage of cows exhibiting estrus at 80 d postpartum was greater if the cows received 10 or 6 kg TDN following calving rather than 3.2 kg TDN (93 and 92 vs 81%, respectively). High energy diets fed following calving, however, do not overcome effects of low prepartum dietary energy (Wiltbank et al., 1962; 1964; Bellows and Short, 1978).

Supplemental protein has been shown to decrease postpartum intervals. Williams et al. (1980) reported that heifers fed isocaloric diets that were high in crude protein (.96 kg, CP) had a shorter interval to first estrus than animals receiving a low crude protein diet (75 vs 86 d). Dairy cows fed diets that were high in protein (19.3% CP) had shorter postpartum intervals than cows receiving medium (16.3% CP) or low (12.7% CP) protein diets (Jordan and Swanson, 1979). Treacher et al. (1976) reported that dairy cows fed 75% of the recommended protein requirement for 8 wk before and 8 wk following parturition had longer intervals to first estrus than cows fed the recommended level of protein (46 vs 35 d).

Influence of Body Condition and Weight on Postpartum Reproduction

Body condition and weight before parturition influence the reproductive performance of postpartum cattle (Langley and McCarthy, 1981; Graham et al., 1982; Post et al., 1982). Wettemann et al. (1981) reported that postpartum intervals were correlated with the percentage decrease in body condition ($r=.61$) and body weight ($r=.58$). These findings suggested to the authors that body weight or condition score could be used to estimate the length of the postpartum interval.

Sows that lose large quantities of weight and backfat during lactation have a higher incidence of anestrus through 70 d after weaning than those that experience minimal losses (Reese et al., 1982a; b). Contrary to these results, Lishman et al. (1974) found that weight loss in Merino and Merino-cross ewes during an 84-d lactation period had little effect on the length of the postpartum interval (range 39 to 53 d).

Whether body condition and weight change affect the length of the postpartum interval independent of nutrition or whether these effects interact remain to be determined. Furthermore, research is needed to determine how a loss in body condition and weight affect the endocrine system of postpartum cows.

Factors Influencing Postpartum Reproduction

Young cows have a longer interval from calving to first estrus than older cows. The average postpartum interval in a breeding season was 53.4 d in cows which were 5 yr or older, 69.2 d in 4-yr-old cows, 66.8 d in 3-yr-old cows and 91.6 d in cows calving at 2 yr of age (Wiltbank, 1970). Inskeep and Lishman (1979) reported that a greater percentage of mature cows (>4 yr) had a palpable CL between d 30 and 99 postpartum than 2- and 3-yr-old cows. Other researchers were unable to demonstrate a difference in postpartum interval among age groups (Warnick, 1955; Smith and Vincent, 1972). Body condition and(or) nutrition of cows used in the above studies were not described and, therefore, may account for differences within and among reports.

Dystocia not only increases calf mortality but also increases the postpartum interval and lowers subsequent fertility of the dam (Wiltbank et al., 1961; Anderson and Bellows, 1967; Brinks et al., 1973; Laster et al., 1973). Doornbos (1978) reported that increasing the duration of labor by 10 min increased postpartum interval by 2 d and decreased pregnancy by 0.6%.

The interval from parturition to estrus may be affected by breed. Angus cows exhibited estrus earlier postpartum than Hereford and Shorthorn cows (Wiltbank et al., 1961) and Brahman-cross cows (Reynolds, 1967). Contrary to these reports, Casida et al. (1968) found no difference in the interval from parturition to first estrus between Angus and Hereford cows. Similarly, Laster et al. (1973) found no effect of breed on interval to the first postpartum estrus in Hereford-, Angus- and Charolais-cross cows. Body condition, nutrition and(or) age of cows used in the above studies were not described and, therefore, may account for differences within and among reports.

Cows calving in the spring had a shorter interval to first estrus than cows calving in the fall (Loyacano et al., 1972). Hot temperature and high humidity were considered to be major factors contributing to the seasonal effects on postpartum reproduction in this study. Monty and Wolff (1974) reported that Holsteins calving during the hot summer had longer intervals from parturition to pregnancy than cows calving during cool ambient temperatures. Duration of estrus was reduced in postpartum dairy cows exposed to hot environmental temperatures and may be a contributing factor to lowered fertility in cows calving during the summer months (Monty and Wolff, 1974; Wolff and Monty, 1974).

Effect of Suckling on Endocrine Systems During Postpartum Period

I. Gonadotropins and gonadotropin-releasing hormone (GnRH).

Reproductive endocrine function in the cow becomes operative as the postpartum period progresses. Pituitary content and plasma concentrations of luteinizing hormone (LH) are low at parturition and increase concomitant with the postpartum period (Labhsetwar et al., 1964; Saiduddin et al., 1968; Wagner et al., 1969; Erb et al., 1971; Edgerton and Hafs, 1973). Anterior pituitary content of LH was reported by Cermak et al. (1983) to be low through d 15 postpartum (<.4 mg/pituitary) but increased by d 30 postpartum (1.2 to 1.6 mg/pituitary). Serum LH concentration increases within the first 2 wk postpartum. Kesler et al. (1977) reported that serum concentrations of LH increased from 1.1 ng/ml on d 2 postpartum to 3.5 ng/ml by d 18 postpartum in anovulatory dairy cows. Similarly, Goodale et al. (1978) reported an increase in plasma LH concentrations from d 2 to 13 postpartum. These observations suggest that pituitary content and serum concentrations of LH during the early postpartum period (<15 d) may be a limiting factor in the return to estrous cycles in postpartum cows.

Available information on pituitary content and serum concentrations of follicle-stimulating hormone (FSH) is limited and contradictory. Cermak et al. (1983) reported that anterior pituitary content of FSH did not change significantly during the postpartum period and was comparable to the content in cycling beef cows (1.2 to 2.5 mg/pituitary). In contrast, others have reported that pituitary FSH content was high at parturition and decreased with time in postpartum dairy cows (Labhsetwar et al., 1964; Saiduddin et al., 1968). Cermak et al. (1983) utilized beef cows as an animal model while the latter authors used dairy cows. Differences in body condition, weight and nutrition between breeds may explain differences between the studies. Plasma concentrations of FSH were higher on d 50 postpartum (139±22 ng/ml) than on d 5 postpartum (128±22 ng/ml) in anovulatory beef cows (Gauthier et al., 1982). Webb et al. (1980) found that concentrations of FSH remained low during the first 2 wk postpartum and then increased in two of four dairy cows. Dobson et al. (1978) have shown that serum concentrations of FSH declined from early postpartum (0 to 20 d) to late postpartum (21 to 48 d) in 50% of the dairy cows (110±37 to 84 ±34 ng/ml). Concentrations of FSH in the remaining cows did not change over time. Large variation in FSH concentrations reported in these studies make interpretation of the results difficult if not impossible. An experiment designed to examine the preovulatory secretion of FSH during the postpartum period has not been conducted.

Suckling may not influence the content of gonadotropins in the anterior pituitary of cows. Carruthers et al. (1980) reported that the pituitary content of LH and FSH on d 14 postpartum of suckled dairy cows were comparable to levels observed in milked dairy cows. Pituitary content of LH and FSH in beef cows were not changed within 96 h following weaning (Walters, 1981). Contrary to these results, Cox and Britt (1982) have reported that anterior pituitary content of LH in sows increased 2.5-fold during the first 60 h postweaning. These authors reported that anterior pituitary content of FSH remained unchanged following weaning (60 h).

Weaning calves from cows increased serum concentrations of LH and FSH during the 96-h postweaning period (Smith et al., 1977; Forrest et al., 1979; Walters et al., 1981). Increased serum LH following weaning is primarily a result of increased frequency of LH pulses (Walters et al., 1981). Similarly, an increase in number of LH pulses occurs before the first ovulation in suckled beef cows (Humphrey et al., 1976; Rawlings et al., 1980) but occurrence is delayed relative to nonsuckled cows. Amplitude of episodic peaks of LH increased following separation of calves from cows (Carruthers and Hafs, 1980; Carruthers et al., 1980). These studies indicate that suckling reduces serum concentration of LH by reducing the frequency and amplitude of LH pulses.

Information regarding change in the pulsatile release of FSH during the postpartum period is limited. Schallenberger (1977) observed peaks of FSH every 12 to 14 d during the postpartum period of dairy cows. Others have suggested that pulsatile release of FSH during the postpartum period is absent in beef cows (Garcia-Windsor and Inskeep, personal communication).

Release of gonadotropins from the anterior pituitary is controlled by GnRH, a releasing hormone isolated in the hypothalamus (Schally et al., 1971; Zolman and Convey, 1972; Kaltenbach et al., 1974; Estes et al., 1977; Carruthers et al., 1980). Recent experiments have demonstrated GnRH pulsatile release in rats during the estrous cycle and in ovariectomized sheep utilizing a push-pull perfusion cannula (Levine and Ramirez, 1982; Levine et al., 1982). Information regarding the pulsatile release of GnRH from the hypothalamus of cows during the postpartum period is unavailable. The push-pull perfusion cannula will offer scientists a way of measuring GnRH release in postpartum cows.

Cermak et al. (1983) found that the content of GnRH in the total hypothalamus proper and preoptic area of the hypothalamus in suckled beef cows did not change from 1 to 45 d postpartum. Moss et al. (1980) and Carruthers et al. (1980) reported that hypothalamic GnRH content did not differ between nonsuckled and suckled ewes and cows (one offspring per dam). Contrary to these studies, Minaguchi and Meites (1967) demonstrated that the hypothalamus of suckled rats had less GnRH content than nonsuckled rats. Difference between observations of Minaguchi and Meites (1967) and those of Moss et al. (1980) and Carruthers et al. (1980) may be due to species differences or a difference in suckling stimuli caused by nursing of a litter vs single offspring.

II. Pituitary responsiveness

The ability of the anterior pituitary of cows to respond to exogenous GnRH with a release of LH increases with time postpartum. A single injection of GnRH (100 to 500 μg) induced an LH release in cows during the first 10 d postpartum but maximal response was not obtained until d 20 postpartum (Irvin et al., 1977; Kesler et al., 1977; Webb et al., 1977; Fernandes et al., 1978; Schallenberger et al., 1978).

Suckling stimuli reduce ability of the pituitary to release LH in response to GnRH. Administration of GnRH (250 μg) following calf removal induced an LH release that was greater than the LH release observed in suckled cows (Troxel et al., 1980; Carter et al., 1980). Smith (1977) reported that milked postpartum dairy cows released more LH in response to GnRH than dairy cows that were suckling four calves. Carruthers et al. (1980) observed that pituitaries from nonsuckled cows released more LH in vitro in response to GnRH than pituitaries from suckled cows.

The ability of the anterior pitutiary to respond to exogenous estradiol-17β with a release of LH increases with time postpartum. A

single injection of estradiol benzoate (1 mg) did not cause a release of LH on d 2 postpartum in dairy cows but induced a release of LH (>20 ng/ml) in 66.6 and 40.0% of the cows on d 9 and 16 postpartum (Forrest et al., 1981). An increase in the number of estradiol receptors in the anterior pituitary from d 1 to 15 postpartum (Cermak et al., 1983) may account for the acquired responsiveness of the anterior pituitary to exogenous estradiol. The observations of Forrest et al., (1981) may also be due to an increase in the amount of LH that can be released over time from the pituitary in response to exogenous hormone treatment.

Suckling stimuli reduce the ability of the pituitary to release LH in response to estradiol-17β. Short et al. (1979) reported that estradiol-induced LH release was greater in nonsuckled than suckled cows during the first 2 wk postpartum. However, peak LH concentrations following estrogen administration was not affected by suckling stimuli from wk 3 through 6 postpartum. Therefore, the inhibitory effect of suckling on estrogen-induced release of LH is not a limiting factor in initiation and maintenance of estrous cycles beyond 3 wk postpartum.

III. Ovarian activity and steroids

Ovarian follicular development is positively correlated with time postpartum in cows. Spicer et al. (1983) reported that the number of follicles 4 to 7.9 mm in diameter increased between 7 and 42 d postpartum. Saiduddin et al. (1968) observed that the number of follicles

(>5 mm) on d 1 was less than the mean number on d 10, 20 and 30 postpartum (1.1 vs 4.4). Percent of nonatretic follicles in dairy cows belonging to the class of small diameter follicles (<.22 mm) decreased between 15 and 35 d postpartum; whereas, percent of medium (.29 to .67 mm) and large follicles increased from 37.4 to 47.2% and from 11.5 to 17.3% (Dufour and Roy, 1983). Increase in size and number of follicles between parturition and first ovulation reflect variable follicular growth as well as atresia during the preovulatory postpartum period (Morrow, 1969).

Weaning increases follicular growth and development during the early postpartum period. Bellin et al. (1982) reported that the number and average diameter of follicles on d 5 postpartum were greater in nonsuckled than suckled beef cows. Similarly, Carter et al. (1980) reported that the number of medium and large follicles and follicular volume on d 5 postpartum were greater in nonsuckled than suckled beef cows. In contrast, weaning did not affect follicle numbers, volume of largest follicle or total follicular fluid weight on d 10 and 20 postpartum (Saiduddin et al., 1968). Weaning may stimulate an acute increase in follicular growth during the early postpartum period. However, increased follicular growth in response to weaning does not continue beyond d 10 postpartum.

Serum concentrations of estrogen in cows decrease following parturition and remain low until the preovulatory increase in estrogen (Henricks et al., 1972; Arije et al., 1974; Stevensen and Britt, 1979). Variation in serum estradiol between parturition and first

ovulation reported by Echternkamp and Hansel (1973) may reflect variable follicular growth and atresia during the preovulatory postpartum period.

Weaning calves within 24 h of birth did not increase serum concentrations of estrogen between parturition and d 14 postpartum (Carruthers and Hafs, 1980; Carruthers et al., 1980). Estrogen concentrations of follicular fluid on d 5 postpartum in suckled cows were not significantly different from levels in cows that had their calves weaned following parturition (Carter et al., 1980). In contrast, Bellin et al. (1982) reported that concentrations of follicular estrogens on d 5 postpartum were greater in nonsuckled cows than suckled cows. An explanation for the differences in concentrations in follicular estrogens between the studies is unavailable.

Serum concentrations of progesterone in cows decline following parturition to levels that are less than 1 ng/ml (Edqvist et al., 1974; 1978; Smith et al., 1973; Wise et al., 1975). This reduction in serum progesterone is a result of no luteal function by 7 d postpartum. Serum concentrations of progesterone have been observed to increase in beef cows 2 to 4 d before the onset of estrous cycles (Donaldson et al., 1970; Arije et al., 1974; Humphrey et al., 1976; Lavoie and Moody, 1976; Dobson, 1978). This increase in progesterone is believed to be secreted by ovarian luteal tissue (Donaldson et al., 1970; Castenson et al., 1976) and has been shown to be preceded by a release of LH (Webb et al., 1980). A similar increase in LH followed by an increase in progesterone have been observed in prepubertal heifers before the onset of estrous cycles (Gonzalez-Padilla et al., 1975). These authors suggested that the increase in LH and(or) progesterone before puberty serves as a "primer for further maturation" of the hypothalamo-pituitary-ovarian axis. Increases in LH and progesterone before normal estrous cycles (21 d) in postpartum cows may act to prime an endocrine system that has been noncyclic during gestation and the postpartum period.

IV. Glucocorticoids and cortisol-binding globulin (CBG).

Concentrations of glucocorticoids decrease after parturition and remain low (<.10 ng/ml) through 85 d postpartum (Edgerton and Hafs, 1973; Ellicott et al., 1981). Synthesis of cortisol does not appear to change during the postpartum interval because adrenal weight and content of cortisol remain unchanged through 30 d postpartum (Wagner et al., 1969). Variation in serum cortisol concentrations during the postpartum period may be influenced by milk removal and(or) suckling. Milking stimulated a twofold or greater transient increase in serum glucocorticoids (Koprowski and Tucker, 1973; Smith et al., 1973; Carruthers and Hafs, 1980). Wagner and Oxenreider (1972) showed that glucocorticoids increased in serum collected frequently (5-min intervals for 45 min after initiation of suckling) from dairy cows that had been separated from their calves. Dunlap et al. (1981) reported that cortisol in serum collected (15-min intervals for 45 min) from beef cows that had been separated from their calves only tended to increase

($P>.06$) after the initiation of suckling. Concentrations of glucocorticoids in serum collected (1-h intervals for 8 h on d 13 postpartum) from dairy cows suckled ad libitum and milked (2X daily) did not differ from cows that were milked (Carruthers et al., 1980). Differences among the studies may be explained by the following: (1) Blood collection frequency. Glucocorticoid concentrations fluctuate episodically (two to three pulses/6 h, personal observation) in suckled beef cows and sampling may have to be frequent and prolonged to resolve differences in concentration or patterns of secretion; (2) How the suckling stimulus was administered. Serum glucocorticoids have been measured in blood collected following a period during which the calves were separated from their dams (Wagner and Oxenreider, 1971; 1972; Ellicott et al., 1981). Therefore, it is likely that the observed increase in glucocorticoids is due to stimuli associated with reuniting calf and dam; (3) Animal model. The use of beef or dairy cows and whether the dairy cows were milked and frequency of milking may change concentrations of cortisol.

Glucocorticoids inhibit reproductive function by inhibiting the secretion and(or) action of gonadotropins (Selye, 1939). Administration of glucocorticoids inhibit the preovulatory gonadotropin surges in cycling rats (Baldwin and Sawyer, 1974), the estrogen-induced release of LH in ovariectomized rats (Baldwin, 1979) and the GnRH-induced release of LH from cultured pituitary cells (Padmanabhan et al., 1982). Smith et al. (1971) observed that cortisol implanted into the medial preoptic area of rats inhibited ovulation induced by electrical stimulation of that site. This study leads to the hypothesis that the inhibitory effect of glucocorticoids on gonadotropin release may be mediated via effects on the brain.

A specific binding protein with an affinity for cortisol (CBG) is found in serum of cattle (Seal and Doe, 1966). Cortisol, which is bound to CBG, is biologically inactive and is protected from catabolism (Sandberg et al., 1966). Keech et al. (1983) found that CBG-binding capacity (ng cortisol/ml) was higher in serum of nonsuckled than suckled cows (34.9 vs 25.8). Gala and Westphal (1965) reported that concentrations of CBG in rats were greater after weaning than during lactation. These data support the hypothesis that suckling inhibits secretion of CBG. Increase in concentrations of CBG following weaning would decrease the amount of cortisol capable of interferring with gonadotropin secretion.

V. Prolactin.

Serum concentrations of prolactin decrease in suckled cows from peak concentrations during parturition (245 ng/ml) to levels that remain low (<30 ng/ml) during an 85-d postpartum period (Edgerton and Hafs, 1973; Peters et al., 1979; Kodagli et al., 1980; Humphrey et al., 1983). Prolactin secretion between parturition and first ovulation is pulsatile with a few pulses of prolactin attaining concentrations in excess of 200 ng/ml (Chang et al., 1981; Humphrey et al., 1983). Prolactin is released in a daily biphasic pattern in cattle with low concentrations of prolactin observed between 0900 and 1300 h and significantly higher concentrations between 1900 and 2300 h (Webb and Lamming, 1981). Chang et al. (1981) reported that the number of pulses of prolactin observed during a 24-h period did not change from d 45 to 73 postpartum (range .75 to .91 pulses/h) in suckled cows but amplitude of pulses increased during the postpartum interval (239 vs 333 ng/ml).

Suckling has been reported to increase serum prolactin concentrations in beef cows (Johke, 1969; Falk et al., 1971; Karg and Schams, 1974) and sows (Mulloy and Malven, 1979). Convey et al. (1983) reported that prolactin increase in beef cows accompanied suckling 67, 96 and 95% of the time on d 14, 28 and 42 postpartum, respectively. The increase in the percentage of suckling-associated release of prolactin with time postpartum may represent specific development of the mechanism by which prolactin is released by suckling. Convey et al. (1983) suggested that prolactin release is frequently coincidental with suckling events but can also be released spontaneously at other times. Baseline and suckling-induced release of prolactin were the same for cows suckling one or two calves indicating that the amount of prolactin released in response to suckling is not associated with the amount of stimulation.

Stimuli associated with milking initiate a release of prolactin in dairy cows. Carruthers and Hafs (1980) found that prolactin concentrations increased from low baseline levels (<12 ng/ml) to peak concentrations on d 7 (20.5 ng/ml) and 14 (43.9 ng/ml) prepartum within 13.6 min after premilking udder wash. Release of prolactin in response to milking was largest at 8 wk of lactation and then gradually decreased as lactation advanced (Koprowski and Tucker, 1973).

Goodman et al. (1979) reported that the presence of a calf (suckling not permitted) decreased basal milking-induced and TRH-induced prolactin release (68, 69 and 29%, respectively) in dairy cows (2 to 3 d postpartum) as compared to cows separated from calves. Similarly, Akers and Lefourt (1984) observed that the release of prolactin in response to milking during the first week postpartum was greater in dairy cows separated from their calves than when calves were present. Thus, tactile stimulation of the teats increases secretion of prolactin but presence of a calf immediately adjacent to their dam but without suckling is inhibitory to secretion of prolactin.

Suppression of prolactin secretion by administration of CB-154, an ergot alkaloid, had no effect on the length of the postpartum interval (Cummins et al. 1977; Montgomery, 1982). Contrary to these studies, Short et al. (1978) reported that administration of CB-154 shortened the postpartum interval in beef cows. An explanation for the difference in results among studies is unavailable. Williams and Ray (1980) showed that injections of CB-154 decreased serum prolactin concentrations in postpartum beef cows but did not affect serum concentrations of LH, estradiol-17β or progesterone.

Effect of Nutrition on Endocrine Systems

I. Gonadotropins and GnRH

Terqui et al. (1982) reported that underfeeding cows 2 mo before calving reduced body weight at calving and lowered mean plasma concentrations of LH and FSH on d 30 postpartum compared to cows fed ad libitum. Cows fed a high energy diet (150% National Research Council [NRC]) had greater mean concentrations of LH on d 14 and 50 postpartum than cows receiving a diet containing 100% of the recommended energy requirements (Echternkamp et al., 1982). Pulse frequency of LH in cows fed excess energy (150% NRC) was greater than in cows fed low energy (100% NRC) between d 24 and 84 postpartum. Furthermore, magnitude of LH pulses differed between treatment groups on d 60 and 84 postpartum. These results show that energy intake acts to shorten the postpartum period by increasing LH secretion.

Concentrations of LH and FSH in the pituitary were less in cows fed high energy diets than cows receiving medium or low energy diets (Moss et al., 1982). Pituitary LH and FSH was similar for cows receiving medium or low energy diets. A possible explanation for lower LH in pituitaries of high energy group is that release of gonadotropins from the pituitary is enhanced. Moss et al. (1980) also reported that the content of GnRH in the stalk median eminence, preoptic area and hypothalamus proper did not differ among the three treatment groups. This suggests that GnRH concentrations in the hypothalamus is not a limiting factor to reproductive functions. However, effects of diet on secretion of GnRH is unknown.

II. Steroids

Corah et al. (1974) reported that heifers fed high energy (17.6 mCal digestible energy, DE/d) or low energy (11.4 mCal DE/d) diets had similar levels of progesterone during the first 21 d postpartum. Progesterone levels among dairy cows fed high energy or maintenance rations were not different during any day of the estrous cycle following parturition (Folman et al., 1973). Contrary to these reports, Gombe and Hansel (1973) reported that concentrations of progesterone in serum and CL and response of CL to LH was reduced during the second and third estrous cycle of heifers fed 70% of Morrison's requirements compared to heifers fed an adequate diet. Imakawa et al. (1983) fed heifers either low (6.8 mCal metabolizable energy [ME/d]), moderate (12.7 mCal ME/d) or high (19.2 mCal ME/d) energy for six estrous cycles. Serum progesterone concentrations increased linearly as energy levels increased when analyzed for the six estrous cycles. These authors reported that in vitro progesterone production by corpora lutea cells, when incubated with human chorionic gonadotropin, was less in heifers fed low energy than moderate or high energy rations. Hill et al. (1970) showed an immediate decline in progesterone concentrations occurring within 5 d after a reduction in feed intake by heifers. Others have reported that serum progesterone concentrations in cows increase (Dunn et al., 1974) or remain the same (Apgar et al., 1975) when diets are restricted for successive estrous cycles. An explanation for differences among studies is not immediately apparent but could possibly be due to diets that were not isonitrogenous, duration that cattle were fed diets or condition of animals.

Energy restricted the last trimester of pregnancy and during lactation of cattle did not affect size of ovarian follicles (Lishman et al., 1979) or serum concentrations of estradiol-17β (Corah et al., 1974; Dunn et al., 1974; Lishman et al., 1979). Therefore, it is unlikely that energy restriction delays estrous cycles during the postpartum period by decreasing serum concentrations of estrogens.

During periods of energy restriction, corticosteroids are important in catabolism of muscle protein into amino acids which are used as a substrate for gluconogenesis (Trenkle, 1978). One might hypothesize that animals in negative energy balance or animals fed diets restricted in energy would have greater serum concentrations of glucocorticoids than animals adequately fed. Hall et al. (1984) reported that cortisol concentrations in serum tended to be greater in postpartum cows fed a low energy than a high energy diet. A highly significant negative correlation between weight change of lactating ewes and free cortisol in serum was observed by Barnett and Star (1981). Whether an increase in serum concentrations of cortisol during negative energy balance is due to an increase in secretion of cortisol by adrenals, decrease in the secretion of CBG by liver, or a combination of the two events is unknown.

Summary

Weaning calves markedly reduces the interval from parturition to first ovulation. Weaning calves at 21 d postpartum results in an increase in basal concentration and pulse frequency of LH within 96 h postweaning followed by a preovulatory LH surge. Thus, the endocrine changes that precede, and presumably result in first ovulation postpartum occur during a short period of time (4 d). An understanding of how weaning acutely alters LH secretion is not well understood. A possible explanation is that inhibitory effects of suckling are mediated by a hormone that inhibits the secretion of gonadotropins (e.g., cortisol) and that weaning causes a decrease in concentration or effects of the hormone. However, it is uncertain if cortisol is released in response to sucking in beef cows or if concentrations of cortisol decrease following weaning. An alternative hypothesis is that suckling prevents an increase in LH secretion by inhibiting estrogen receptors in the hypothalamus and pituitary. Weaning likely increases the number of estradiol receptors in the hypothalamo-hypophyseal axis enabling low concentrations of estradiol to increase secretion of LH. Effects of weaning on pattern of secretion of FSH in postpartum cows have not been elucidated.

Experiments were conducted to examine the following remaining questions: (1) Does weaning alter the release of gonadotropins and steroids in postpartum cows? (2) Do concentrations of cortisol decrease postweaning and, if so, does this decrease precede changes in secretion of gonadotropins? (3) Do the number of hypothalamic and hypophyseal estrogen receptors increase in response to weaning and high energy diets?

CHAPTER III

RELATIONSHIP BETWEEN SUCKLING AND THE SECRETION OF LH, CORTISOL AND CORTISOL-BINDING GLOBULIN IN BEEF COWS

Summary

The relationship between suckling and the secretion of luteinizing hormone (LH), cortisol and CBG was investigated in multiparous, crossbred (Hereford x Angus x Simmental) cows. Twenty-six postpartum anovulatory cows were suckled ad libitum by their calves or were separated from their calves at d 35 postpartum (PP). Serial samples of blood (15-min intervals for 6 h) were collected twice daily between d 35 and 41 PP. Cortisol, LH, progesterone and CBG were assayed in harvested serum. Baseline and pulse frequency of LH increased ($P<.01$) following weaning while the amplitude of the pulses remained unchanged. Cortisol concentrations did not change following the initiation of the suckling event. The secretion of cortisol did not differ between treatment groups within 72 h postweaning. Cortisol baseline concentrations decreased in both the nonsuckled and suckled cows between d 35 and 38 PP. CBG-binding affinity and capacity were the same for both treatment groups and d 35 and 38 PP. Data are interpreted to suggest that secretion of cortisol is not changed by suckling or weaning and it is unlikely that cortisol is an inhibitor of LH secretion during the postpartum period in beef cows.

Introduction

The interval of postpartum anovulation is extended by suckling in dairy (Mollar, 1970) and beef (Wiltbank and Cook, 1958;

Oxenreider, 1968; Short et al., 1972) cows; however, the mechanism involved is unclear. Weaning calves increases LH (Forrest et al., 1979; Smith, 1977) and follicle stimulating hormone (Walters et al., 1981) in serum of beef cows during the 96-h postweaning period. Elevated mean levels of serum LH may be a consequence of increased numbers of pulses of LH following weaning of calves (Walters et al., 1981).

Wagner and Oxenreider (1972) reported higher concentrations of glucocorticoids in serum of suckled cows than nonsuckled or milked cows. In contrast, others (Carruthers and Hafs, 1980; Dunlap et al., 1981) could not demonstrate differences in glucocorticoid concentrations between suckled and nonsuckled cows. Whether suckling alters glucocorticoid and LH concentrations independently or whether observed changes in glucocorticoids and LH are associated remains to be determined.

A specific binding protein with a high affinity for cortisol (CBG) has been observed (Seal and Doe, 1966). Cortisol, which is bound to CBG, is biologically inactive. Therefore, only unbound cortisol (free) is able to interact with its receptor in target cells. In rats, total corticosteroids are increased during lactation while CBG concentrations are decreased (Gala and Westphal, 1965). Following weaning, there is an acute increase in CBG while total corticosteroids decrease. These shifts in concentrations of corticosteroids and CBG after weaning precede and may account for resumption of estrous cycles.

The following study was conducted in postpartum beef cows: (1) to determine the secretory pattern of LH and cortisol and binding of CBG for cortisol from weaning to the preovulatory LH surge; and (2) to determine whether concentrations of cortisol increase following suckling.

Materials and Methods

Twenty-six multiparous crossbred (Hereford x Angus x Simmental) anovulatory cows were assigned randomly on d 34 PP to be suckled (S) or nonsuckled (NS). Calves from S cows (n=8) were allowed to nurse ad libitum while calves from NS cows (n=12) were removed from their dams at 35 d PP. Jugular blood was collected daily via cannula at 15-min intervals between 0600 to 1200 h and 1800 to 2400 h on d 35 to 41 PP. In addition, a single blood sample was collected at 0200, 0400, 1400 and 1600 h on d 35 to 41 PP. Serum was stored at -4°C. Concentrations of LH, cortisol and CBG were determined by validated radioimmunoassay techniques.

Ovaries of all cows were examined rectally for presence of a corpus luteum (CL) on d 34 PP. Six cows with a palpable CL were removed from this experiment. Samples of blood were collected and ovaries were examined every third day from d 42 PP until a palpable CL was present. Concentrations of progesterone in serum greater than 1 ng/ml were interpreted to indicate presence of a CL. Interval to ovulation was reported as the number of days from calving to presence of a CL.

Cows were fed 15.5 kg of a balanced ration consisting of corn silage and high moisture corn from calving to the completion of the experiment. The ration was balanced to maintain the weight of lactating cows (475 kg) and provided 11.5 MCal of net energy (100% NRC). Body condition was independently scored from 1 (thin) to 10 (obese) on d 41 PP by two technicians using techniques described by Bellows et al., 1971. The cows were acclimated to surroundings and restrained 2 wk before the intensive bleeding period. Animals were maintained as a group until d 34 PP when each cow and her calf were individually housed in adjacent pens. Suckling events (time of initiation and completion) were recorded for each suckled cow between 0600 to 1200 and 1800 to 2400 on d 35 through 41 PP.

Assay Procedures. Serum concentrations of LH were quantified by double antibody radioimmunoassay (RIA) previously described by Convey et al. (1976). All samples collected were analyzed in duplicate using bovine LH (NIH-LH-B8) as a standard. Intraassay and interassay coefficients of variation were 10.8 and 14.3%. Concentrations of serum cortisol were determined by RIA using procedures validated by Purchas (1985). Diurnal variation in cortisol release was not observed on d 35 and 38 PP. Therefore, only samples collected between 0600 and 1200 h of remaining days were assayed. Intraassay and interassay coefficients of variation were 4.5 and 12.2%.

Cortisol-binding capacity of serum samples collected at 1000 h on d 35 and 38 was determined using a modification of a procedure described by Pegg and Keane (1969), which is based on the principle of the competitive protein-binding assay of Murphy (1967). These samples represent CBG-binding and affinity characteristics following calf removal and preceding the preovulatory LH surge and are assumed to reflect any acute change in CBG in response to weaning. A standard curve was established utilizing cortisol concentrations ranging from .25 to 200 ng/ml. Serum to be analyzed for CBG was diluted with H_2O and tritiated cortisol added such that the resulting plasma-isotope solution contained .04 ml plasma plus .4 ng tritiated cortisol. Serum isotope solution was added to tubes containing standards, contents of these tubes were mixed then allowed to stand for 5 min at 45°C. The tubes were then placed in an ice bath, allowed to stand for 10 min and then .5 ml dextran-coated charcoal was added to each tube. Tubes were vortexed, centrifuged (400xg) and 1 ml of the supernatant counted in a liquid scintillation counter. Binding affinity and capacity were determined from plots and calculations described by Scatchard (1949). Coefficients of variation among and within assays were less than 10%.

Serum concentrations of progesterone were quantified by RIA as described by Convey et al. (1977). All blood samples collected following palpation were analyzed in the same assay. Progesterone concentrations (>1 ng/ml) were used to determine the occurrence of ovulation.

Statistical Analyses. Cortisol and LH data were plotted and baseline (ng/ml), pulse frequency (#/6 h) and amplitude (ng/ml) were determined for each 6-h sampling interval. A peak is defined as a value that is greater than the preceding value by an amount equal to the 95% confidence interval for the assay. Decay of a hormone pulse (peak to baseline) must be consistent with the known half-life of the hormone in question. Baseline is the lowest point between peaks and amplitude is the peak height minus the baseline.

Daily mean baseline, pulse frequency, amplitude, binding affinity and capacity of measured hormones were calculated for both suckled and nonsuckled cows on d 35 and 38 PP. Differences in the secretory characteristics between treatment groups and between d 35 and 38 PP were determined by student t test (Steel and Torrie, 1960). To test the hypothesis that weaning contributes to variation in presurge LH and cortisol release over time, the slope of linear regression lines were compared to lines with zero slope (McClave and Dietrich, 1982).

Results and Discussion

Weaning calves at 35 d PP shortened the interval from calving to ovulation in beef cows an average of 12.7 d (table 1). This supports other studies that have shown that suckling prolongs the postpartum anestrous period (Graves et al., 1968; Oxenreider, 1968; Wagner and Oxenreider, 1971; Short et al., 1972). Body condition did not differ ($P>.1$) between NS ($\bar{x} = 4.5$) and S ($\bar{x} = 4.0$) cows and likely did not influence the interval from calving to ovulation. The mechanism by

TABLE 1. INFLUENCE OF WEANING ON OCCURRENCE OF LH SURGES(a) AND POSTPARTUM INTERVAL TO OVULATION IN LACTATING BEEF COWS

Treatment	Proportion exhibiting LH surge	Interval to ovulation (days) $\bar{x}$ ± S.E.
Nonsuckled	11/12	45 ± .6
Suckled	1/8	58 ± 1.2

[a] Day 35 to 42 d postpartum.

which suckling affects duration of the postpartum interval is not completely known. The proportion of nonsuckled cows exhibiting an LH surge within 7 d after weaning was greater ($P<.01$) than contemporary suckled cows (table 1). The nonsuckled cow that did not exhibit an LH surge and the suckled cow that did were not considered in further analysis of preovulatory secretory patterns of hormones.

Baseline LH concentrations and the number of LH pulses observed (within a 6-h interval) increased ($P<.05$) within 3 d of weaning while amplitude of LH pulses remained unchanged (table 2). These data agree with reports that LH concentrations and pulse frequency increase 96-h after weaning (Forrest et al., 1979; Smith et al., 1977; Walters et al., 1981).

Changes in the secretory pattern of LH before the LH surge in nonsuckled cows were best described by a linear regression line ($R^2>.85$). A regression of either presurge baseline or pulse frequency of LH plotted against time exhibited a positive slope ($P<.01$, figure 1). Less than 63% of the variation of pulse amplitude measured over time is accounted for by either a first, second or third order polynomial (figure 1). The linear regression line formed by LH amplitude tended to increase ($P<.1$) over time. The pattern of secretion of LH in suckled cows (table 2) did not change over time and, therefore, the slope of the regression lines (data not shown) did not differ ($P>.1$) from zero slope. These data are interpreted to show that following weaning baseline concentrations increase in response to an increase in pulse frequency but are not influenced by

TABLE 2. INFLUENCE OF WEANING ON SECRETORY CHARACTERISTICS OF LH[a]

Treatment	Day postpartum	Baseline (ng/ml)	Pulses/6 h	Amplitude (ng/ml)
Nonsuckled	35	$.7^b$	2.5^d	2.0^f
	38	1.0^c	3.6^e	1.7^f
Suckled	35	$.7^b$	1.9^d	2.5^f
	38	$.6^b$	1.9^d	2.1^f
		(.09)	(.28)	(.19)

[a] Data from cows exhibiting an LH surge by d 38 PP were not used in these comparisons. Value within parentheses is a pooled standard error of secretory characteristics.

[b,c,d,e,f] Means within column with different superscripts differ ($P<.05$).

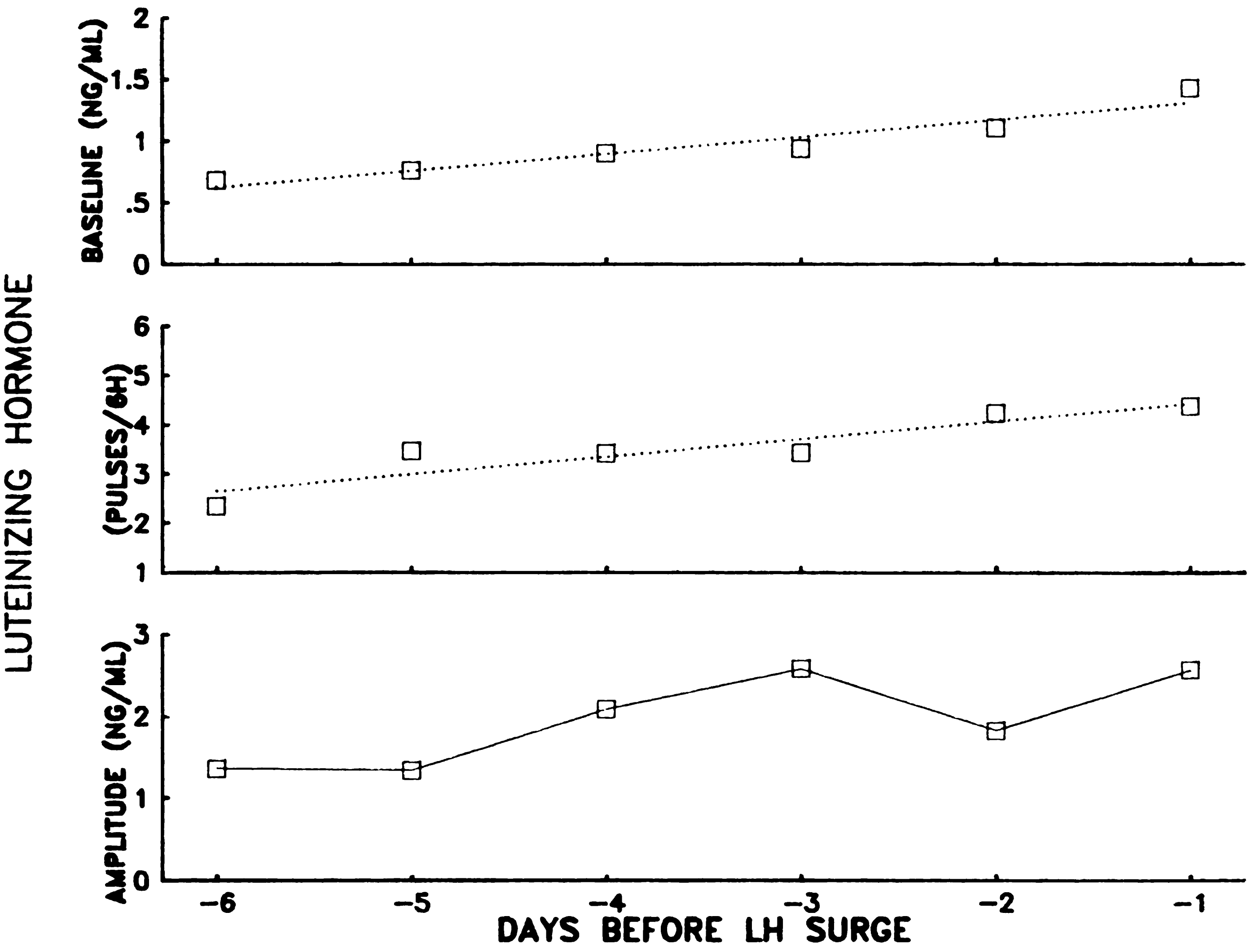

Figure 1. Secretion of luteinizing hormone in nonsuckled cows (calves weaned d 35 postpartum).

amplitude of the pulses. Increases in pulse frequency of LH are likely a result of an increase in the release of GnRH.

These results have led to the hypothesis that suckling inhibits LH release either directly (i.e., neural inhibition due to udder manipulation or presence of calf) or indirectly via suckling-induced release of hormones that would suppress the pulsatile release of LH. Short et al. (1976) reported that mammary denervation did not reduce the postpartum interval from calving to estrus, indicating that sensory stimuli from the udder associated with lactation may not be the cause of postpartum anestrus. In order for suckling to inhibit LH release through the biological actions of other hormones, concentrations of the inhibitory hormone would need to be sufficiently elevated in response to suckling to have a biological effect. Removal of suckling stimuli would presumably decrease the concentration of the inhibitory hormone. Cortisol was released in response to milking and nursing in dairy cows (Kaprowski and Tucker, 1973; Wagner and Oxenreider, 1971; 1972) and may be the hormone that inhibits reproductive function during the postpartum period. This concept is strengthened by the observation that administration of glucocorticoids prolong the reproductive cycle in cows (Kanchev et al., 1976) and inhibit the preovulatory gonadotropin surge in cycling rats (Baldwin and Sawyer, 1974).

From the current study, concentrations of cortisol within 60 min after initiation of suckling did not differ ($P>.1$) with levels measured before initiation of suckling (60 min) or levels observed after

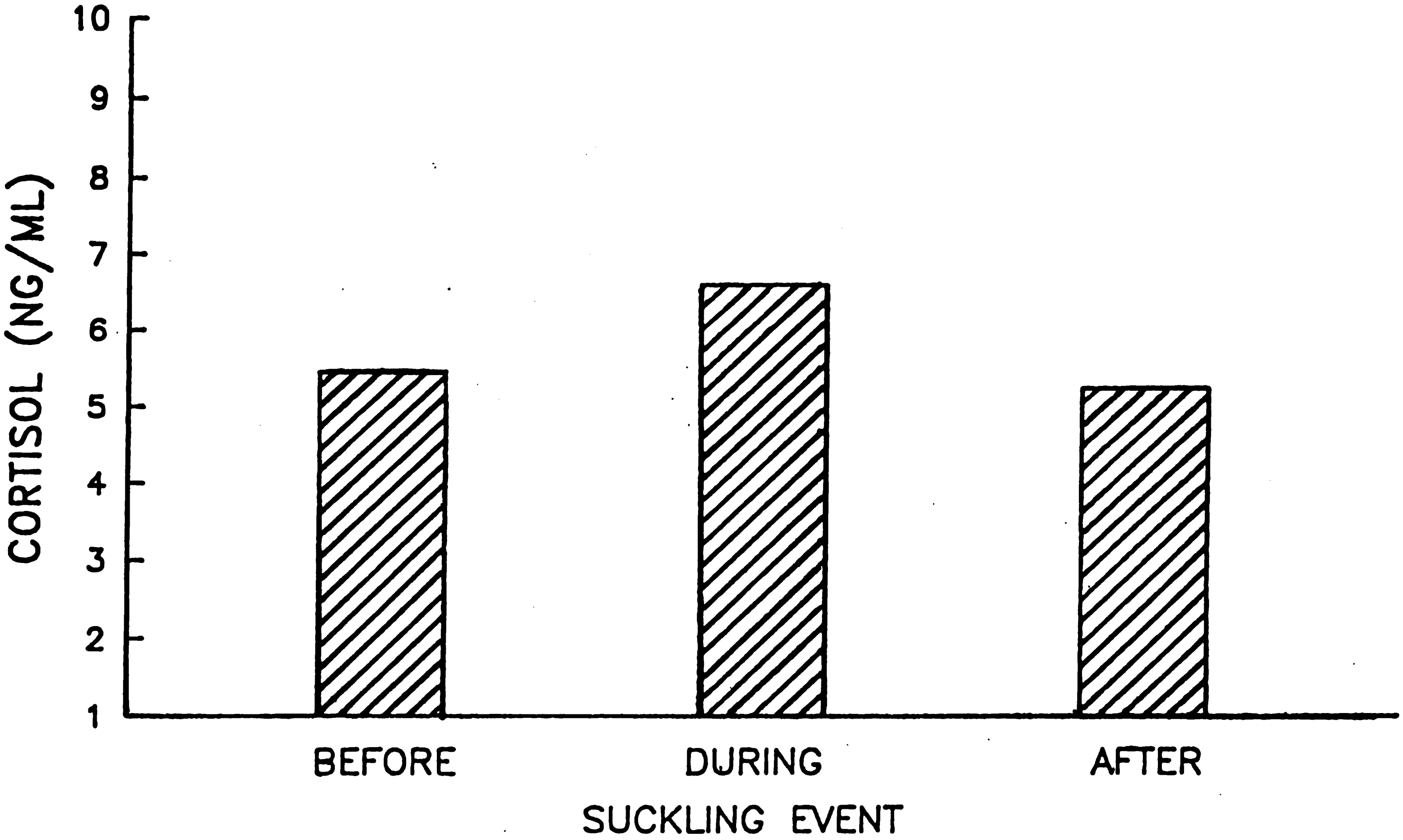

Figure 2. Concentration of cortisol in serum of suckled cows before (60 min), during (60 min) and after (60 min) a suckling event. Pooled standard error for each period was .48, .58 and .44, respectively.

the suckling event (60 min after the suckling period; figure 2). These data support the findings of Carruthers et al. (1980) and Dunlap et al. (1981) but contradict studies of Wagner and Oxenreider (1971; 1972). Serum concentrations of cortisol reported here were collected from beef cows nursing a calf ad libitum while Wagner and Oxenreider (1971; 1972) measured glucocorticoids in blood collected from dairy cows following a period during which the calves were separated from their dams. Reuniting the calf with her dam and the use of dairy cows as an animal model may account for elevated glucocorticoid concentrations observed in the latter study and might explain differences between the two studies.

Baseline concentrations of cortisol decreased ($P<.05$) between d 35 and 38 PP in both nonsuckled and suckled cows, while pulse frequency and amplitude of cortisol remained unchanged (table 3). A regression of cortisol baseline concentrations of nonsuckled cows plotted against time was best described by a first order polynomial ($R^2=.67$) and exhibited ($P<.01$) a negative slope (figure 3). Regression lines formed by cortisol pulse amplitude and pulse frequency of nonsuckled cows measured over time were described by second $R^2=.93$) and third ($R^2=.70$) order polynomials, respectively (figure 3). The slope of the linear regression lines did not differ significantly ($P>.1$) from zero slope.

The secretion of cortisol in suckled cows plotted over time was described by third order polynomials ($R^2>.70$, figure 4). The slope of the linear regression lines formed by cortisol baseline and pulse

TABLE 3. INFLUENCE OF WEANING ON SECRETORY CHARACTERISTICS OF CORTISOL[a]

Treatment	Day postpartum	Baseline (ng/ml)	Pulses/6 h	Amplitude (ng/ml)
Nonsuckled	35	6.72^b	3.14^d	11.54^e
	38	2.04^c	2.57^d	8.68^e
Suckled	35	5.19^b	2.28^d	13.38^e
	38	2.84^c	3.14^d	9.89^e
		(1.10)	(.27)	(.39)

[a] Data from cows exhibiting an LH surge by d 38 PP were not used in these comparisons. Value within parentheses is a pooled standard error of secretory characteristics.

[b,c,d,e] Means within column with different superscripts differ ($P<.05$).

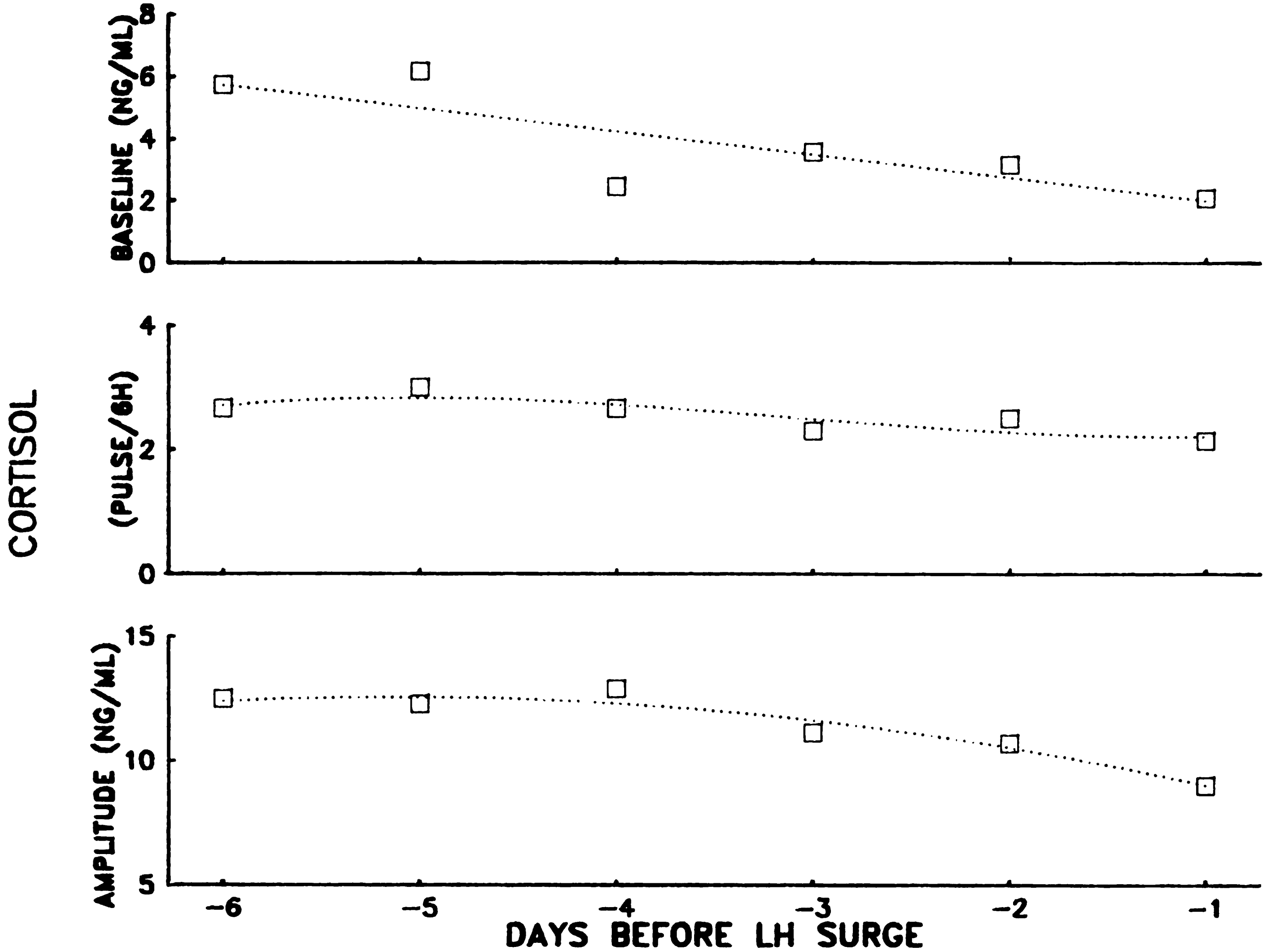

Figure 3. Secretion of cortisol in nonsuckled cows (calves weaned d 35 postpartum)

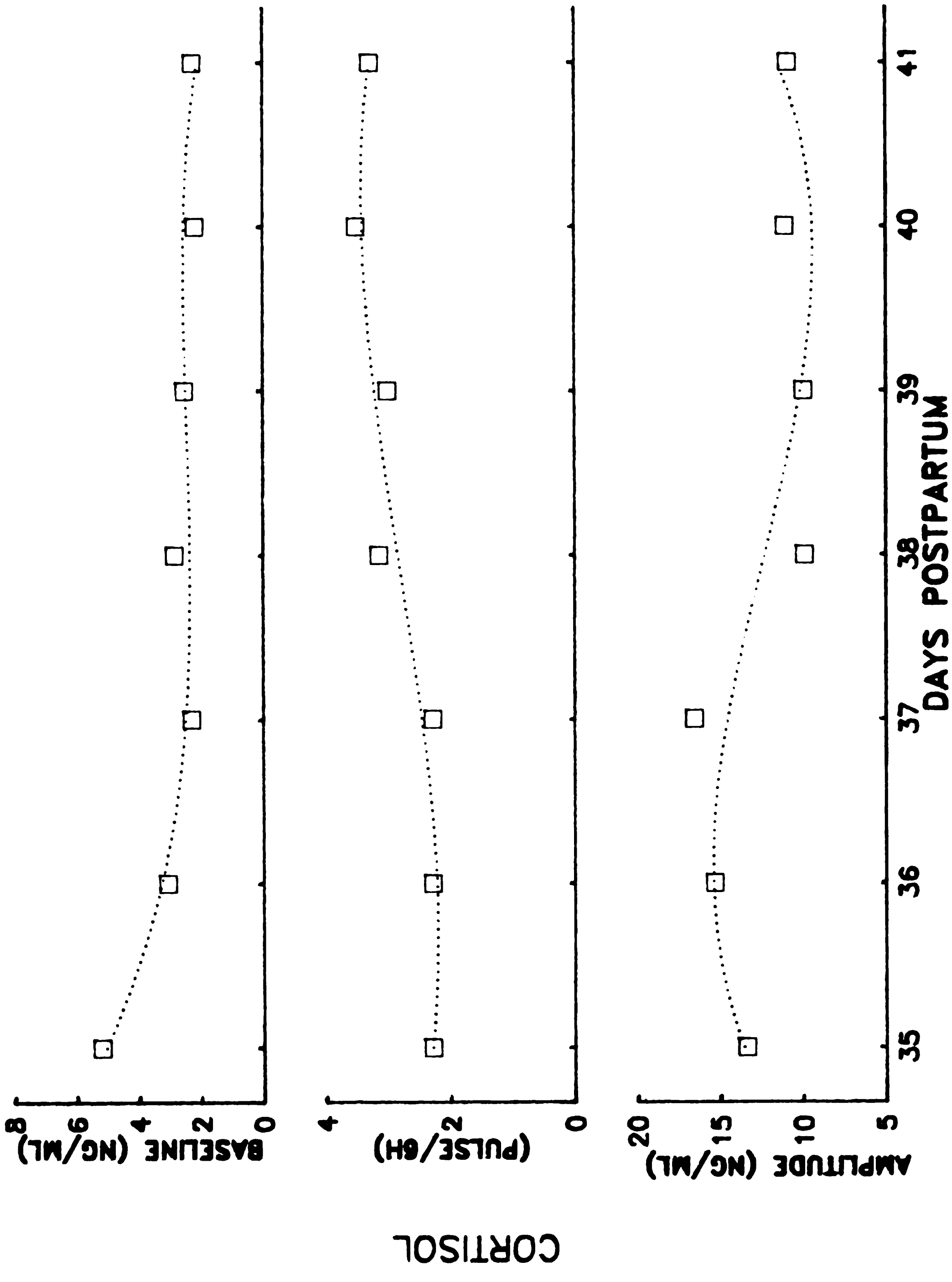

Figure 4. Secretion of cortisol in suckled cows from d 35 to 41 postpartum.

frequency over time differed from zero slope ($P<.01$). Pulse amplitude decreased over time but the slope of the linear regression line tended to differ ($P<.1$) from zero slope. The decrease in the secretion of cortisol in suckled cows from d 35 to 41 PP may be due to an acclimation to stress associated with experimental procedures. Although we attempted to minimize stress, release of cortisol on d 35 PP may have been caused by placing cows in individual pens on d 34 PP, initiation of the bleeding regime and(or) increased activity associated with collecting blood samples. This may also explain why baseline concentrations of cortisol on d 35 were elevated in both suckled and nonsucked cows relative to levels of cortisol on d 38 PP.

Changes in baseline and pulse amplitude of cortisol in nonsuckled cows following weaning paralleled those of suckled cows. The pulse frequency of cortisol on d 35 and 38 PP were the same for both treatment groups. Therefore, it is unlikely that cortisol influences the observed differences in LH secretion between nonsuckled and suckled cows.

Concentrations of systemic CBG regulate the amount of free cortisol (Sandberg et al., 1966). Because only free cortisol is biologically active, variation in CBG concentration could serve as an important regulator of the amount of cortisol available to interact with receptors. CBG is destroyed in the cortisol assay procedure and, therefore, only total cortisol concentrations in serum have been reported. The possibility remains that the amount of free cortisol could change in response to weaning, even though differences in

TABLE 4. INFLUENCE OF CALF REMOVAL ON BINDING AFFINITY AND CAPACITY OF CBG FOR CORTISOL[a]

Treatment	Days postpartum	Affinity ($x10^9 m^{-1}$)	Capacity (ng/ml)
Nonsuckled	35	1.371[b]	22.13[c]
	38	1.08[b]	21.63[c]
Suckled	35	1.21[b]	15.05[d]
	38	1.08[b]	19.91[c]
		(.13)	(.53)

[a] Value within parenthesis is a pooled standard error.

[b] Means in the same column with the same superscripts do not differ (P>.1).

[c,d] Means in the same column with different superscripts differ (P<.1).

cortisol concentrations between treatments were not observed. CBG-binding affinity did not differ (P>.1) between treatment groups on d 35 and 38 PP (table 4). CBG-binding capacity (ng/ml) tended to be lower (P<.1) in S cows on d 35 PP than S cows on d 38 PP or NS cows. Others (Keech et al., 1983) have reported that CBG-binding affinity did not differ between cows nursing calves and cows that had their calves removed at 21 d. However, Keech et al. (1983) also reported that binding capacity was greater in nonsuckled cows than suckled cows (34.9 vs 25.8 ng/ml).

In the present study, baseline and pulse frequency of LH increased following calf removal at 35 d PP. Amplitude of pulses did not change after weaning. Cortisol baseline concentrations decreased in both the nonsuckled and suckled cows. Secretion of cortisol did not differ between treatment groups for 72 h after weaning and was not influenced by suckling events. CBG-binding affinity and capacity did not differ between treatment groups or d 35 and 38 PP suggesting that the amount of free cortisol does not change within 72 h of weaning. These data do not support the hypothesis that suckling increases cortisol concentrations or that cortisol is an inhibitor of LH secretion.

CHAPTER IV

INFLUENCE OF SUCKLING AND DIET ON ESTRADIOL-17β RECEPTORS AND PITUITARY LH IN BEEF COWS

Summary

Influence of suckling and dietary energy on estradiol-17β (E) receptors and concentrations of LH in the pituitary of beef cows was examined in a 2 x 2 factorial experiment. Main effects were: (1) dietary energy (60 and 120% NRC); and (2) suckling (suckled and nonsuckled). Sixty-nine multiparous crossbred cows were fed either a low (60% NRC) or high (120% NRC) energy ration 85 d before calving and during the postpartum (PP) period. One-half of the cows in each dietary group were allowed to nurse a calf during the PP period while the remaining cows had their calves weaned at 21 d PP. Jugular blood was collected every 6 h on d 12, 13, 21, 22, 23 and 24 PP. Cows were slaughtered on d 14, 23 and 25 PP and pituitaries and hypothalami were collected. Ovaries were inspected at slaughter for evidence of an ovulation. An interaction ($P=.02$) of suckling and time after weaning affected the number of E receptors in the posterior hypothalamus (PH). Concentration of estradiol-17β receptors was not different ($P>.1$) between d 23 and 25 in the anterior hypothalamus (AH) and anterior pituitary (AP) due to either suckling or diet. However, concentration of E receptors in the AH was influenced by day postpartum showing an increase ($P=.01$) from d 23 to 25 irrespective of treatment. Concentration of estradiol-17β receptors in the AH, PH and AP on d 14 did not differ ($P>.1$) from E receptors on d 23 and 25 PP. Concentration of LH in the AP increased ($P=.01$) from d 14 to 25 PP. Serum concentrations of E were below the sensitivity of the assay (<2 pg/ml) for anovulatory cows in all treatment groups on all

days. In summary, concentration of E receptors in the PH is affected by an interaction of suckling and time after weaning and concentration of E receptors in the AH and AP are unaffected by suckling or dietary energy.

Introduction

The interval of postpartum anestrus and anovulation is extended by suckling (Clapp, 1937; Oxenreider, 1968; Short et al., 1972) and diets low in energy (Wiltbank et al., 1962; Dunn et al., 1969; Bellows and Short, 1978). Recent reports have demonstrated that concentrations and release of luteinizing hormone (LH) were increased by calf removal (Carruthers et al., 1980) and by feeding high energy rations (Echternkamp et al., 1982). Increases in concentration and release of LH culminate in an LH surge and ovulation (Walters et al., 1982; Faltys et al., 1983). The mechanisms associated with the inhibitory influence of suckling and nutrition on the release of LH have yet to be elucidated.

Ability of the anterior pituitary to release LH in response to estradiol increases between d 2 and 10 postpartum (Forrest et al., 1981) and is greater in nonsuckled than suckled cows early postpartum (Short et al., 1979). An increase in responsiveness of the anterior pituitary to estradiol may be due to an increase in the number of estradiol receptors. Cermak et al. (1983) reported that the concentration of pituitary receptors for estradiol increased from d 1 to

15 PP and appeared to precede release of substantial amounts of LH in beef cows.

The increase in release of LH in response to weaning and high energy is possible due to an increase in the ability of the anterior pituitary to release LH and(or) the ability of the hypothalamus to release GnRH in response to low endogenous levels of estradiol. Weaning and high energy may increase E receptors in the hypothalamo-hypophyseal axis enabling low levels of estradiol to release LH from the AP of the postpartum cows. Therefore, the objectives of this study are to determine the effects of suckling and dietary energy on concentration of E receptors in the AH, PH and AP of beef cows and to determine changes in numbers of receptors for estradiol during the postpartum period.

Materials and Methods

Sixty-nine crossbred beef cows were randomly assigned to either a high energy (HE; 120% NRC) or low energy (LE; 60% NRC) treatment group. A ration containing corn silage (IFN 3-28-250) and barley (IFN 4-00-549) was fed to cows in the HE treatment group from 85 d before calving through the PP period. Grass hay (IFN 2-00-262) was fed to cows in the LE treatment group for a comparable period of time. One-half of the cows in each feed group were suckled and their calves were allowed to nurse ad libitum throughout the experiment (S) while the remaining cows had their calves weaned 21 d PP (NS).

Animals were weighed every 14 d from the beginning of the experiment until calving. Body condition was independently scored from 1 (thin) to 10 (obese) by two technicians using techniques described by Bellows et al. (1971).

Jugular blood (10 ml/sample) was collected from six HE and five LE cows at 0600, 1200, 1800 and 2400 h on d 12 and 13 PP. Blood samples were collected from the remaining cows at 0600, 1200, 1800 and 2400 h from d 21 PP to slaughter. Serum was stored at -4°C until concentrations of LH, progesterone and estradiol-17β were determined via validated radioimmunoassay techniques.

Cows were slaughtered on d 14, 23 or 25 PP (table 1). Pituitaries and hypothalami were collected and placed on ice within 20 min of slaughter. Tissues were dissected, homogenized and centrifuged as described by Glass et al. (1984). The resulting supernatants were frozen in 5% dimethyl sulfoxide (DMSO) until E receptor numbers were determined by radioreceptorassay.

Ovaries were inspected for corpora lutea (CL) at the time of slaughter. Animals were eliminated from statistical analysis if a CL was detected or if elevated serum concentrations of LH (>25 ng/ml) or progesterone (>1 ng/ml) were detected.

Assay Procedures

Concentrations of LH in serum and anterior pituitaries were quantified by double antibody radioimmunoassay (RIA) previously

TABLE 1. NUMBER OF COWS ASSIGNED TO EXPERIMENT AND SLAUGHTERED[a]

Diet	Days, PP	Nonsuckled	Suckled
Low	14	—	5 (5)
	23	8 (6)	6 (5)
	25	8 (7)	8 (7)
High	14	—	6 (6)
	23	5 (3)	6 (5)
	25	10 (7)	7 (4)

[a] Number of cows slaughtered and used in statistical analysis appear in parentheses.

described by Niswender et al. (1969) and modified by Staigmiller et al. (1979). All serum samples collected were analyzed in duplicate. Intraassay and interassay coefficients of variation of a serum pool from cyclic cows were 5.1% and 9.2%, respectively. Supernatant from the anterior pituitaries was diluted with PBS-Gel and assayed for concentration of LH in duplicate in the same assay. Serum concentrations of estradiol-17β were quantified by procedures described by England et al. (1974) and modified by Anthony (1983). Intraassay and interassay coefficients of variation were 4.4% and 10.8%, respectively.

Cytosolic E receptors were determined by radioreceptorassay previously described by Glass et al. (1984). Interassay coefficient of variation of a cytosol pool from anterior pituitaries was 12.5%.

Statistical Analysis

Analysis of variance and Harvey's least-squares analysis (Harvey, 1960) were used to identify differences among treatments in: (1) the number of receptors for E per gram tissue; and (2) differences in concentrations of LH in the anterior pituitary.

Results

Cows fed a diet low in energy for 85 d before calving and throughout the postpartum period lost 6.8% of their initial body weight (figure 1). Cows fed a ration high in energy for a comparable

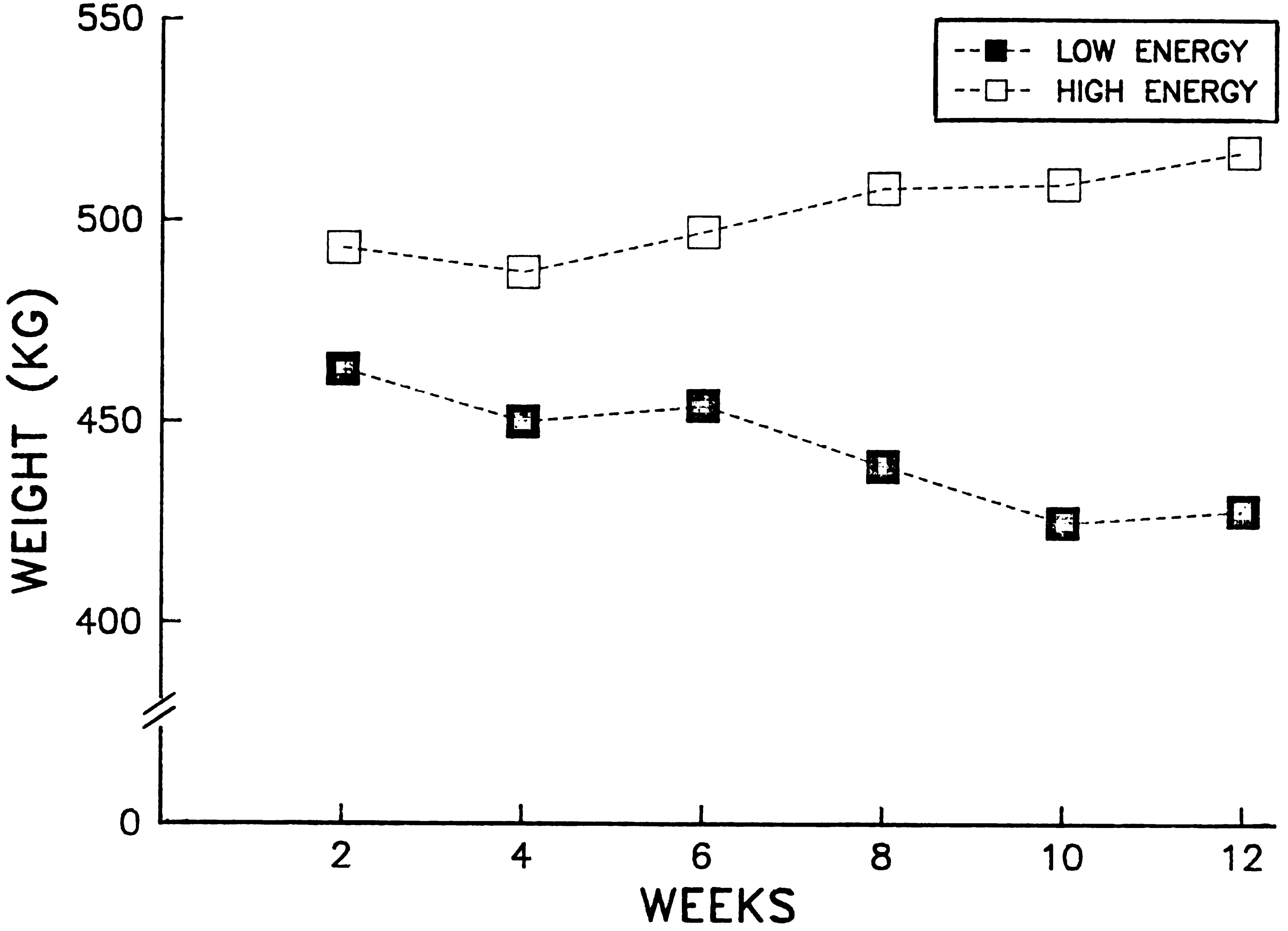

Figure 1. Weight change in cows fed low energy (60% NRC) or high energy (120% NRC) diets.

interval gained 4.2% over their initial body weight. Condition score of cows at slaughter fed the low energy ration averaged 2.7 in comparison with a score of 7.0 for cows fed the high energy ration.

A suckling by d 23 vs 25 interaction ($P=.02$) was observed for concentration of E receptors in the PH (table 2). Concentration of estradiol receptors in the PH of NS cows decreased from d 23 to 25 following weaning but increased from d 23 to 25 in S cows (figure 2). Concentration of E receptors in either AH or AP did not differ ($P>.1$) between nonsuckled and suckled cows on d 23 or 25 PP (figure 3). Concentration of receptors for estradiol on d 14 PP did not differ ($P>.1$) from the number of receptors for estradiol on d 23 and 25 PP in any tissue examined (tables 2, 3 and 4) but concentration of E receptors in the AH increased ($P=.01$) from d 23 to 25 PP (figure 3). Dietary energy did not change ($P>.1$) concentration of receptors for E in any tissues examined (tables 2, 3 and 4).

A suckling by d 23 vs 25 interaction ($P=.04$) was observed for the concentration of LH in the AP (table 5). Concentration of LH in NS cows decreased from d 23 to 25 following weaning but increased from d 23 to 25 in S cows (figure 4). Concentration of LH in the AP of cows did not differ between d 23 and 25 PP but concentrations of LH on both d 23 and 25 were greater ($P=.01$) than measured on d 14 PP (figure 4). Serum concentrations of estradiol-17β in anovulatory cows used in this study were below the sensitivity of the assay (<2 pg/ml) and are not published in this report.

TABLE 2. LEAST-SQUARE ANALYSIS OF ESTRADIOL RECEPTORS (ER) IN POSTERIOR HYPOTHALAMUS

Source	df	ER/g tissue	
		ms	F ratio
Feed (F)	1	3048.71	2.31
Suckling (S)/day (D)	4	2300.83	1.74
F x S/D	4	368.69	.28
Error	45	1323.55	

Contrast	df	P value
Low vs high energy	1	.13
D 14 vs d 23 + d 25	1	.31
D 23 vs d 25	1	.44
F x d 14 vs d 23 + d 25	1	P>.50
F x d 23 vs d 25	1	.47
S	1	P>.50
S x d 23 vs d 25	1	.02
Low vs high energy x S	1	P>.50
F x S x d 23 vs d 25	1	P>.50

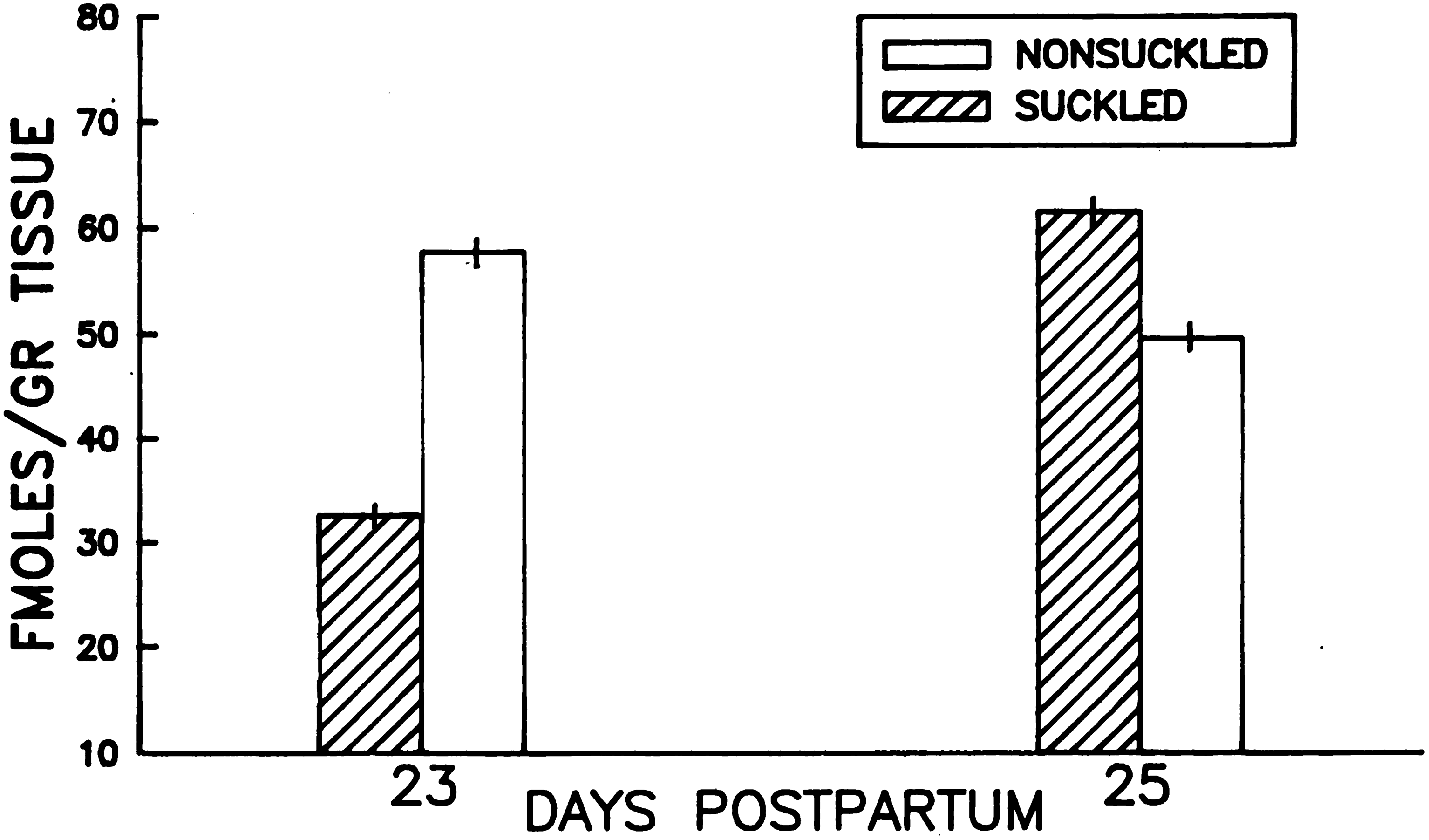

Figure 2. Concentration of estradiol receptors in the posterior hypothalamus of beef cows on d 23 and 25 postpartum.

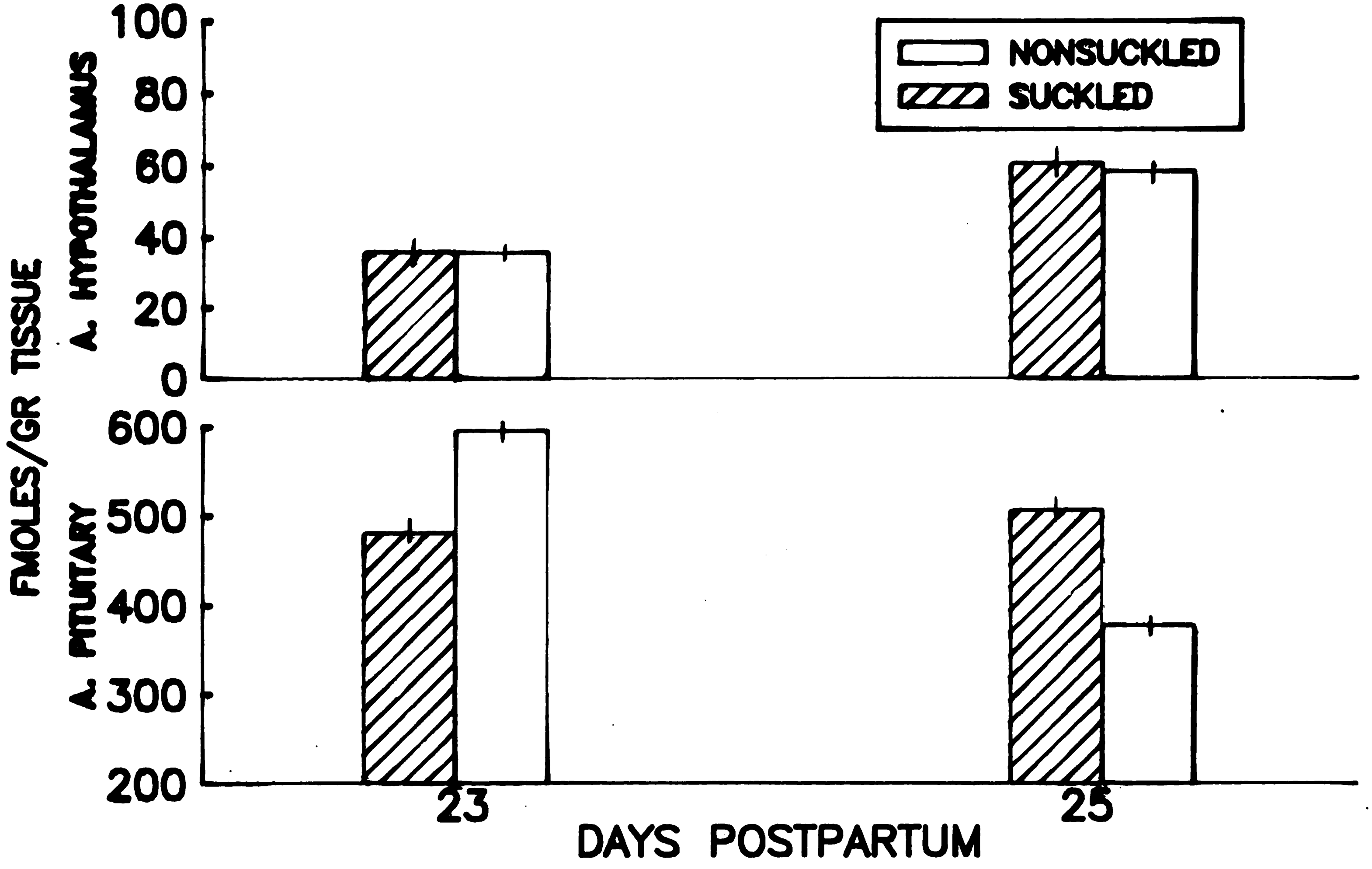

Figure 3. Concentration of estradiol receptors in the anterior hypothalamus and pituitary of beef cows on d 23 and 25 postpartum.

TABLE 3. LEAST-SQUARE ANALYSIS OF ESTRADIOL RECEPTORS (ER) IN ANTERIOR HYPOTHALAMUS

Source	df	ER/g tissue	
		ms	F ratio
Feed (F)	1	683.27	.62
Suckling (S)/day (D)	4	2266.70	2.04
F x S/D	4	1184.08	1.07
Error	45	1108.27	

Contrast	df	P value
Low vs high energy	1	.40
D 14 vs d 23 + d 25	1	.20
D 23 vs d 25	1	.01
F x d 14 vs d 23 + d 25	1	.40
F x d 23 vs d 25	1	.22
S	1	.48
S x d 23 vs d 25	1	.35
Low vs high energy x S	1	P>.5
F x S x d 23 vs d 25	1	.16

TABLE 4. LEAST-SQUARE ANALYSIS OF ESTRADIOL RECEPTORS (ER) IN ANTERIOR PITUITARY

		ER/g tissue	
Source	df	ms	F ratio
Feed (F)	1	234415.88	1.68
Suckling (S) x day (D)	4	104326.67	.75
F x S x D	4	133868.83	.96
Error	45	139437.46	

Contrast	df	P value
Low vs high energy	1	.31
Day 14 vs d 23 + d 25	1	P>.5
Day 23 vs d 25	1	.29
F x d 14 vs d 23 + d 25	1	.12
F x d 23 vs d 25	1	P>.5
S	1	P>.5
S x d 23 vs d 25	1	.35
Low vs high energy x S	1	P>.5
F x S x d 23 vs d 25	1	.39

TABLE 5. LEAST-SQUARE ANALYSIS OF LH IN ANTERIOR PITUITARY

Source	df	ms	F ratio
Feed (F)	1	.16	.16
Suckling (S) x day (D)	1	3.13	3.15
F x S x D	4	1.01	1.01
Error	45	.99	—

Contrast	df	P value
Low vs high energy	1	.42
Day 14 vs d 23 + d 25	1	5.01
Day 23 vs d 25	1	.32
F x d 14 vs d 23 + d 25	1	.20
F x d 23 vs d 25	1	.49
S	1	.06
S x d 23 vs d 25	1	.04
Low vs high energy x S	1	.18
F x S x d 23 vs d 25	1	P>.5

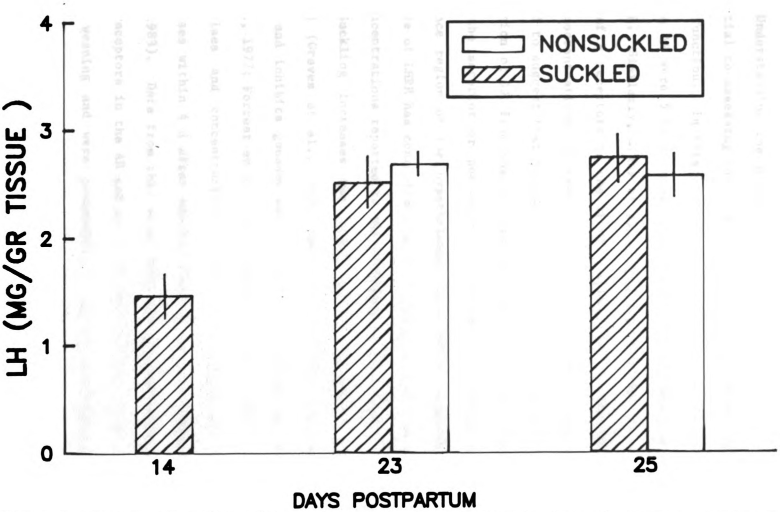

Figure 4. Concentration of luteinizing hormone in the anterior pituitary of postpartum beef cows.

Discussion

Understanding the distribution of E receptors in the brain is essential to assessing the roles of estradiol in regulating reproductive function. In this study, concentration of estradiol receptors in the AP were 5 to 10 times greater than levels observed in the AH and PH. Similarly, Glass et al. (1984) reported that the concentration of E receptors in the AP of castrate ewes in May were greater than concentrations observed in the AH or PH. These data are interpreted to suggest that estrogen has a greater role in controlling the secretion of LH from the AP than controlling the secretion of GnRH from the anterior or posterior hypothalamus. However, the median eminance region of the hypothalamus which has a prominant role in release of LHRH has concentrations of E receptors that are comparable to concentrations reported in the AP (Glass et al., 1984).

Suckling increases the length of the postpartum anovulatory period (Graves et al., 1968; Short et al., 1972; Bellows et al., 1974) and inhibits gonadotropin secretion in postpartum cows (Smith et al., 1977; Forrest et al., 1979; Walters et al., 1982). Number of LH pulses and concentrations of LH in the serum of beef cows increases within 4 d after weaning (Walters et al., 1982; Faltys et al., 1983). Data from this study show that concentration of estradiol receptors in the AH and AP of NS cows did not change within 4 d after weaning and were comparable to levels observed in S cows. These results are interpreted to show that previously observed increases in release of LH in response to weaning are not related to and are likely not a result of changes in estradiol receptors in the AH or AP. An explanation for the suckling by d 23 vs 25 interaction on the number of estradiol receptors in the PH is unavailable.

Cows receiving prepartum diets that are low in energy have longer intervals from calving to first estrus than cows fed high prepartum levels of energy (Wiltbank et al., 1962; Dunn et al., 1969). The mechanism by which nutrition affects the reproductive endocrine system is not well understood. Echternkamp et al. (1982) reported that cows fed a high energy diet (150% NRC) had greater concentrations of LH and numbers of LH pulses during the postpartum period than cows receiving diets containing 100% of the recommended energy requirements. Data from the current study show that concentrations of estradiol receptors did not differ between the two feed groups in any of the tissues examined on d 14, 23 and 25 PP. An interpretation of these data is that effects of dietary energy on postpartum release of gonadotropin is not mediated by changes in concentrations of estradiol receptors.

Concentration of E receptors in the AP on d 14 did not differ from E receptors on d 23 and 25 PP. Cermak et al. (1983) have shown that concentration of E receptors in the AP decreased between d 15 and 45 PP after increasing from d 1 to 15 PP. Serum concentrations of estradiol were not reported by Cermak et al. (1983) and, therefore, changes in estradiol due to follicular growth and atresia could have affected the number of E receptors during the postpartum period. Serum concentrations of estradiol reported in this study remained low

(<2 ng/ml) and, therefore, it is unlikely that numbers of E receptors were changed by varying levels of estradiol.

Concentration of LH in the AP of NS cows decreased from d 23 to 25 in S cows. These results may reflect an increase in release of LH from the AP into the blood of NS cows and a continued increase in concentration of LH in AP of S cows. Concentration of LH in the AP increased between d 14 and 25 PP. Anterior pituitary content of LH was reported by Cermak et al. (1983) to be low through d 15 PP but increased by d 30 PP. Decreased pituitary content of LH during the early postpartum period may be a limiting factor in initiation and maintenance of ovarian activity.

In summary, an interaction of suckling and time after weaning determined the concentration of E receptors in the PH. Suckling did not change E receptors in the AH and AP and dietary energy did not change E receptors in any tissue examined. Concentration of LH in the AP increased from d 14 to 25 PP.

CHAPTER V

GENERAL DISCUSSION

General Discussion

The influence of suckling and dietary energy on endocrine events in postpartum beef cows was examined in two experiments. This section will discuss the results of these experiments in relationship to previous research and will offer possible explanations for the results.

The interval of postpartum anovulation was extended by suckling. Weaning at 35 d PP caused cows to have a shorter postpartum anovulatory period than cows that were suckled ad libitum. Similar observations have been reported in earlier studies (Clapp, 1937; Graves et al., 1968; Oxenreider, 1968; Wagner and Oxenreider, 1971; Short et al., 1972; Bellows et al., 1974). The means by which suckling inhibits reproductive function during the postpartum period remain obscure. It is logical to assume that the influence of suckling on the length of the postpartum interval is mediated by neural stimulation resulting in inhibition of the hypothalamo-hypophyseal-ovarian axis. However, mammary denervation did not reduce the interval from calving to estrus, indicating that postpartum intervals are prolonged by means other than sensory stimuli of the udder (Short et al., 1976). This does not exclude the possibility that neural stimuli associated with presence of a calf (i.e., smell, sight, tactile, etc.) may prolong postpartum intervals.

The possibility exists that inhibition of reproductive function by suckling, lactation and(or) presence of a calf is mediated through endocrine organs. Weaning increased the frequency of LH pulses and nadir concentrations of LH within 7 d after weaning. The increase in nadir concentrations of LH is likely a result of the increase in the number of pulses. The change in the pattern of release and concentration of LH culminate in a preovulatory LH surge within 7 d after calf removal. An increase in pulse frequency of LH in cows following weaning of their calves is likely in response to an increase in the frequency of GnRH released from the hypothalamus (Walters, 1981). If first ovulation postpartum is dependent upon resumption of episodic secretion of frequent pulses of GnRH then ovulation could be induced by giving injections of GnRH at frequent intervals. Short et al. (1981a) were successful in inducing an ovulation (within 8 d of treatment) in suckled beef cows by injections of GnRH every 2 h for 4 d starting on d 20 postpartum. When the study was repeated in cows averaging 90 kg less body weight, exogenous GnRH did not induce ovulation in suckled cows (Short et al., 1981b). The results are interpreted to suggest that suckling prolongs the postpartum interval of beef cows by reducing the frequency of pulsatile GnRH release from the hypothalamus and that cows in poor body condition are unable to respond to pulsatile release of GnRH with an ovulation. Determining the influence of different body conditions on the ability of suckled cows to ovulate in response to intermittent injections of GnRH is an objective for future research.

Further discussion concerning increases in release of LH following weaning is speculative but may offer explanations for endocrine changes that occur in response to weaning. Walters (1981) proposed that for ovulation to occur, pulses of LHRH must be released frequently from the hypothalamus. An increase in the pulsatile release of GnRH following calf removal may "prime" the pituitary and increase the amount of gonadotropin released into the plasma pool. Pituitary "priming" is defined as the increased capacity of the pituitary to release LH in response to GnRH after previous frequent exposures to small quantitites of exogenous GnRH (Padmanabhan et al., 1980). Repeated exposure of the pituitary to GnRH may increase the "releasable pool" of gonadotropins in the pituitary or increase the number of pituitary GnRH receptors or both and may bring about the preovulatory LH surge following weaning. Neurotransmitters of the central nervous system may also be involved in regulating release of LH. Administration of dopamine and seratonin inhibits the pulsatile release of LH in rats. (Kamberi et al., 1970; Schneider and McCann, 1970; Arendash and Gallo, 1978; Gallo, 1978) while administration of norepinephrine has been reported to increase (Porter et al., 1976) or decrease (Gallo and Drouva, 1979; Negro-Vilar and Ojeda, 1978) the release of LH in rats. Future research should test the influence of suckling on concentration of neurotransmitters in the hypothalamus of beef cows.

Concentration of cortisol in cows before initiation of suckling did not differ from levels observed after suckling nor were pattern of release or concentration of cortisol in suckled cows different from nonsuckled cows. These data do not support the hypothesis that suckling induced a release of cortisol from the adrenal glands and that cortisol was inhibiting the release of gonadotropins in suckled cows. Levels of cortisol in suckled and nonsuckled cows were greater on d 35 PP than levels observed on d 38 PP. Although cows were acclimated to their surroundings, stress associated with initiation of blood collection and related handling procedures contributed to high levels of cortisol on d 35 PP. The idea that cortisol is released in response to stress and unfamiliar experimental procedures is not unprecedented. Echternkamp (1984) reported that beef cows that had been stanchioned for the first time had levels of cortisol that were 10 times greater than cows that had been accustomed to the procedure.

The cortisol-CBG complex renders the hormone biologically inactive and incapable of interacting with cortisol receptors (Sandberg et al., 1966). It is possible that changes in binding affinity or capacity of CBG for cortisol could affect the amount of unbound cortisol able to inhibit LH release without any changes in total concentrations of cortisol. Keech et al. (1983) reported that binding capacity of CBG was greater in cows that had their calves weaned than suckled cows suggesting that there was a greater concentration of unbound cortisol available to interact with cortisol receptors in suckled cows than nonsuckled cows. Data reported in the present study showed that CBG-binding affinity and capability did not change following weaning. An explanation for differences in CBG-binding capacity between the two studies is unavailable.

Weaning and diet did not change the concentration of estradiol-17β receptors in the hypothalamus or anterior pituitary. Because cows utilized in the experiment did not exhibit a preovulatory LH surge, it is uncertain if any change in the number of estradol-17β receptors occurred after d 25 PP and before the preovulatory LH surge. It is unknown if an increase in estradiol-17β receptors is a prerequisite for the occurrence of a preovulatory LH surge and is a subject for future research.

Serum concentrations of estradiol-17β reported in experiment 2 remained low (<2 pg/ml) on d 23 and 25 PP. Others have reported that estrogen levels in postpartum cows remain low and increase immediately before ovulation (Henricks et al., 1972; Arije et al., 1974; Stevensen and Britt, 1979). It is likely that cows were slaughtered before any rise in estradiol-17β occurred. It is possible that increases in estrogens before ovulation may increase the concentration of its own receptor. Therefore, an increase in estradiol-17β receptors would be secondary to and dependent on any increase in levels of estradiol that occurred following calf removal.

In summary, reestablishment of estrous cycles in postpartum cows is dependent on an increase in the release of LH. Release of cortisol is not increased by suckling and is not associated with gonadotropin secretion in nonsuckled cows. Concentration of estradiol-17β receptors in the hypothalamus and pituitary of postpartum cows does not appear to be influenced by calf removal or energy in the diet.

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