



Postpartum interval to estrus and patterns of luteinizing hormone (LH) concentrations in first-calf suckled beef cows exposed to mature bulls
by Edward Earl Custer

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

The objectives of this study were to determine if: 1) exposure to mature bulls, initiated during the early postpartum period, hastens the onset of estrus and uterine involution in first-calf suckled beef cows; 2) patterns of LH secretion in first-calf suckled beef cows were altered due to presence of mature bulls throughout the postpartum period; and 3) other factors associated with postpartum cows, such as calving difficulty, weight change, condition score change and progesterone concentrations may be associated with the influence of bulls on postpartum reproductive activity of cows.

Fifty-two Angus x Hereford cows were assigned randomly, in a pairwise manner, by calving date to one of two treatments: exposure to mature bulls (BE; n = 25) or isolated from bulls (NE; n = 24). Male to female ratio was maintained at 1:13 throughout the study. Eight cows from each treatment were fitted with indwelling jugular catheters 5 to 9 d postpartum and blood samples were collected for assay of LH at 15-min intervals for 6 h beginning on D 10 postpartum and at weekly intervals until a cow exhibited estrus. Blood samples for progesterone were collected at weekly intervals from each cow and ovaries of each cow were examined for the presence of a corpus luteum. Cows were observed for estrus twice daily (am:pm) beginning 10 d postpartum. Postpartum weight change, dystocia score, condition score change and time to uterine involution did not differ ($P > .10$) between treatments. A greater percentage ($P < .05$) of BE cows exhibited estrus by 60 and 90 d postpartum compared to NE cows (44 and 88 % for BE cows and 25 and 46 % for NE cows). However, there was no interaction between percent of cows exhibiting estrus and days postpartum. Interval to estrus was shorter ($P < .05$) for BE cows (62 ± 3.7 d) compared to NE cows (77 ± 3.8 d). Changes in mean and baseline LH concentrations, amplitude, frequency and duration of LH pulses throughout the postpartum period were not altered ($P > .10$) by bull exposure. In addition, mean and baseline LH concentrations, amplitude, frequency and duration of LH pulses during the six weeks before estrus were not altered ($P > .10$) by bull exposure. There was no difference ($P > .10$) between treatments in the proportion of cows that showed an increase in progesterone prior to first estrus. In conclusion, exposure of first-calf suckled beef cows to mature bulls throughout the postpartum period hastens the resumption of ovarian cycling activity. However, the mechanism by which bulls cause this response does not appear to involve alterations in postpartum patterns of LH secretion.

POSTPARTUM INTERVAL TO ESTRUS AND PATTERNS OF LUTEINIZING
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by

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TABLE OF CONTENTS

	Page
LIST OF TABLES	viii
LIST OF FIGURES.	ix
ABSTRACT	x
INTRODUCTION	1
REVIEW OF LITERATURE	3
Factors Influencing Postpartum Reproduction.	3
Breeds.	3
Parity.	3
Dystocia.	4
Sex of Calf	4
Uterine Involution	5
Suckling Stimulus	6
Nutrition and Body Condition.	10
Endocrine and Neuroendocrine Factors Associated with Postpartum Cows.	15
Anterior Pituitary.	15
Gonadotropins.	15
Ovarian Steroids and Follicular Growth.	20
Estrogen	20
Progesterone	21
Follicular Development	23
Gonadotropin Releasing Hormone.	24
Social Interaction and Reproductive Function	27
Summary.	35
STATEMENT OF PROBLEM	37
MATERIALS AND METHODS.	39
Intensive Blood Sampling for LH.	41
Assays for LH and Progesterone	42
Statistical Analyses	43
RESULTS.	45
Postpartum Characteristics	45
Proportions (%) Exhibiting Estrus at Different Times	
Postpartum	48
Hormone Patterns	49
Progesterone.	49
Luteinizing Hormone	49

	Page
DISCUSSION	54
PRACTICAL APPLICATION AND FUTURE RESEARCH.	61
LITERATURE CITED	63
APPENDIX	77
Radioimmunoassay for Bovine Luteinizing Hormone.	77
Reagents and Solutions.	77
Principle of Assay.	78
Radioiodination	78
Separation.	79
% Incorporation and Specific Activity	79
Immunoprecipitate Test.	81
Validation	81
Specificity	81
Accuracy, Parallelism, Sensitivity and Precision. . .	82

LIST OF TABLES

Table	Page
1. Least-squares means and pooled standard errors (SE) for calving difficulty (CD), birth weight (BW), calf sex ratio (CSR), condition score change (CSC), body weight change (WC), postpartum interval to estrus (PPIE) and postpartum interval to uterine involution (PPIUI) for first-calf cows exposed (BE) or not exposed (NE) to mature bulls . .	46
2. Least-square means for effects of calving difficulty (CD) and sex of calf (CS) on postpartum interval to estrus (PPIE) and uterine involution (PPIUI) for first-calf cows.	47
3. Proportion (%) of first-calf cows exposed (BE) or not exposed (NE) to mature bulls which showed an increase in progesterone prior to first estrus.	49
4. Least-squares means and pooled standard errors (SE) of estimated regression coefficients for characteristics of luteinizing hormone patterns of first-calf cows exposed (BE) or not exposed (NE) to mature bulls.	50
5. Least-squares means and pooled standard errors (SE) of estimated regression coefficients for characteristics of luteinizing hormone patterns of first-calf cows exposed (BE) or not exposed (NE) to mature bulls that exhibited estrus.	51
6. Least-squares means and pooled standard errors (SE) of estimated regression coefficients for characteristics of luteinizing hormone patterns for six weeks before first estrus in first-calf cows exposed (BE) or not exposed (NE) to mature bulls.	52
7. Percentage of 125-I incorporated with protein collected from the "broad peak" of a Sephadex G-75 column (52.2 x 1.76 cm).	80
8. Percent recovery of bovine IH in standards and 50, 100 and 200 ul of ovariectomized cow serum.	82

LIST OF FIGURES

Figure	Page
1. Cumulative distribution of percentages of first-calf cows exposed (BE) or not exposed (NE) to mature bulls that exhibited estrus by 45, 60, 90 and 108 d postpartum. Letters above bars indicate difference between treatments at $P < .05$	48
2. Illustration of weekly patterns of IH concentrations collected at 15-min intervals for 6 h. Triangles represent pulses of IH in a 6-h period per week. The straight line represents the regression of IH pulses $\cdot 6 \text{ h}^{-1}$ on week. Units for regression coefficients (b_1) for mean, baseline (b_l) and amplitude (ampl) are $(\text{ng} \cdot \text{ml}^{-1} \cdot 6 \text{ h}^{-1}) \cdot \text{wk}^{-1}$ and for frequency (freq) and duration (dura) are $(\text{pulses} \cdot 6 \text{ h}^{-1}) \cdot \text{wk}^{-1}$ and $(\text{min} \cdot 6 \text{ h}^{-1}) \cdot \text{wk}^{-1}$, respectively .	53
3. Typical elution pattern of ^{125}I incorporated with protein and free ^{125}I from an iodination of bIH on a Sephadex G-75 column (25.6 x .7 cm). Tube number represents .5 ml fractions	85
4. Typical elution pattern of protein-bound fractions collected from a 25.6 x .7 cm Sephadex G-75 column eluted on a 52.2 x 1.76 cm Sephadex G-75 column. Tube number represents 1.0 ml fractions.	86

ABSTRACT

The objectives of this study were to determine if: 1) exposure to mature bulls, initiated during the early postpartum period, hastens the onset of estrus and uterine involution in first-calf suckled beef cows; 2) patterns of LH secretion in first-calf suckled beef cows were altered due to presence of mature bulls throughout the postpartum period; and 3) other factors associated with postpartum cows, such as calving difficulty, weight change, condition score change and progesterone concentrations may be associated with the influence of bulls on postpartum reproductive activity of cows.

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INTRODUCTION

A major goal in beef cattle production is to increase the number of calves produced each year. To accomplish this goal and maximize profits, producers must have an understanding of reproductive processes of the bovine.

Reproductive efficiency in farm animals, especially beef cattle, can be increased by decreasing the interval from parturition to conception. It is well known that failure of females to display estrus early in the breeding season is a primary cause of decreased reproductive performance in beef cattle (Wiltbank, 1970). Furthermore, it is generally accepted that in order to produce a calf each year a cow should conceive and maintain pregnancy by Day 85 postpartum. Therefore it is essential to have at least one normal ovulation before this time. A "normal" postpartum cow has been defined as one which resumes ovarian activity by Day 50 postpartum (Lamming et al., 1981). Thus, it is necessary to understand the physiological mechanisms involved in the restoration of ovarian activity in postpartum cows so this knowledge can be applied to beef cattle production.

Genetic and environmental factors can influence the prepartum and postpartum cow to alter resumption of ovarian activity after parturition. Some of these factors are: breed, age, nutritional and(or) metabolic status, lactation and social interactions. One or a combination of these factors may result in extending the postpartum interval to estrus in beef cows.

The following review identifies and discusses factors which affect the postpartum interval in cattle with emphasis on effects of suckling, nutrition and social interaction.

REVIEW OF LITERATURE

Factors Influencing Postpartum ReproductionBreeds

Wettemann (1980) stated that interval from parturition until onset of luteal activity is usually longer in beef cattle than in dairy cattle. Postpartum anestrous periods, intervals from parturition to behavioral estrus, range from 30 to 72 d in dairy cows (Graves et al., 1968) and from 46 to 104 d in beef cows (Lauderdale et al., 1968). Similarly, Casida (1968) and Marion and Gier (1968) reported that average interval from calving until first ovulation ranged from 14 to 45 d in dairy cows and from 36 to 71 d in beef cows. Postpartum interval can also be influenced by breed within beef cattle: Angus cows exhibited estrus earlier after calving than Brahman-cross cows (Reynolds et al., 1967) and Hereford and Shorthorn cows (Wiltbank et al., 1961).

Parity

Parity and(or) age may play a role in determining length of postpartum intervals in cattle. Wiltbank (1970) concluded that older cows have shorter postpartum anestrous intervals than younger cows. He reported that average postpartum intervals were 53.4 d for 5-year-old cows, 69.2 d for 4-year-old cows, 66.8 d for 3-year-old cows and 91.6 d for 2-year-old cows. In agreement with Wiltbank (1970), Bellows et al. (1982) reported that cows demonstrated better postpartum reproductive performance than heifers bred to calve at 2 years of age, as measured

by: day of the year when first estrus occurred (154.1 and 181.1 d, respectively), postpartum interval to estrus (59.1 and 88.9 d, respectively) and percentage in estrus by the beginning of the breeding season (90.6 and 31.6 %, respectively). Izaike et al. (1984) showed a relationship between the number of calvings and postpartum interval in which they reported as parity increased, postpartum interval decreased in Japanese black cows. In contrast to these studies, Smith and Vincent (1972) failed to show any affect of age on the postpartum interval to estrus in cows, while Stevenson and Britt (1979) reported that the interval to first ovulation tended to be longer for pluriparous than for primiparous dairy cows (18.7 and 16.3 d, respectively), but interval to estrus (26.1 and 27.7 d, respectively) was not different between parities.

Dystocia

Several researchers have shown that dystocia (calving difficulty) increases postpartum interval to estrus and lowers subsequent fertility in beef cows (Wiltbank et al., 1961; Brinks et al., 1973; Iaster et al., 1973).

Sex of Calf

Bellows et al. (1982) reported that sex of calf can influence time to estrus in postpartum beef heifers and cows. The authors concluded that dams nursing bull calves returned to estrus more slowly than dams nursing heifer calves, as measured by day of the year when first estrus occurred, (171.5 and 164.4 d for dams nursing bull and heifer calves, respectively). However, there was no difference in postpartum

intervals of dams nursing bull calves and dams nursing heifer calves (77.8 and 70.1 d, respectively). It would appear that the influence of sex of calf on postpartum reproductive activity in beef cows requires further investigation before any generalizations can be made.

Uterine Involution

The uterus is usually considered involuted when it has returned to its normal, non-pregnant position and when both horns are similar in diameter and show normal consistency and tone (Casida, 1968).

Several factors influence the time to uterine involution in the cow. Suckling has been reported to hasten time to uterine involution in cows (Casida, 1968; Izaïke et al., 1985; Izaïke et al. 1986). Bastidas et al. (1984) reported that uterine involution was influenced by age in Brahman cows and Izaïke et al. (1986) found that the time required for uterine involution increased with increasing parity in Japanese Black cows. Contrary to these studies Tennant et al. (1967) and El-Fouly et al. (1976) reported that age or parity had no effect on time to uterine involution in beef and dairy cows or Egyptian buffaloes.

Dunn and Kaltenbach (1980) stated that uteri of cows fed high energy diets after calving involuted three days sooner than cows fed moderate energy diets. However, Kiracofe et al. (1969) found no difference in uterine involution rates in cows fed high or low levels of energy or protein. Based on these studies one can conclude that energy and(or) protein in the diet has minimal effects on time to uterine involution in cows.

Numerous studies have found no relationship between uterine involution and interval from calving to first estrus of normal cows (Perkins and Kidder, 1963; Tennant and Peddicord, 1968). Therefore, based on the data presented in these studies it would seem that time to uterine involution is not a limiting factor in determining duration of postpartum anestrous in cattle.

Suckling Stimulus

In cattle, as in other domestic livestock, postnatal support of existing offspring via lactation is in a large degree inhibitory to further procreation (Edgerton, 1980). Clapp (1937) first reported the inhibitory effect of suckling on reproductive function in cows. Since then numerous investigators have reported that postpartum intervals of non-suckled cows are shorter than those of suckled cows (Saiduddin et al., 1968; Oxenreider and Wagner, 1971; Short et al., 1972; Bellows et al., 1974; Carruthers and Hafs, 1980; Lavoie et al., 1981; Acosta et al., 1983; Garcia-Winder et al., 1984; Dunn et al., 1985; Faltys, 1985). Graves et al. (1968) reported postpartum intervals for five studies with a total of 87 suckled cows and 88 cows which were neither suckled nor milked. Mean intervals to first estrus in these studies ranged from 18 to 41 d in the non-lactating cows and 53 to 93 d for suckled cows. Oxenreider and Wagner (1971) found that either twice daily milking or suckling by two calves doubled the postpartum interval to ovulation of Holstein cows. Wettemann et al. (1976) demonstrated that suckling intensity influenced the length of the postpartum interval of range cows. Cows nursing one calf had a shorter interval

from parturition to first estrus than cows nursing two calves (67 and 94 d, respectively).

Several investigators have attempted to reduce postpartum interval to estrus in anestrous cows suckling calves by manipulating the suckling stimulus. Bellows et al. (1974) reported that weaning calves at three days of age resulted in an average postpartum interval of 19.6 d compared with 39.1 d in dams nursing calves to Day 35 postpartum, which suggest that weaning, initiated during the early postpartum period, is an effective method to hasten the onset of estrus in postpartum cows. Also, it was reported that early weaning resulted in a shortened postpartum interval to estrus in dams that gave birth to one or more calves. Laster et al. (1973) demonstrated that weaning calves at an average of 55 d after calving had a much greater effect on increasing the number of cows exhibiting estrus from calving to the end of the breeding season in 2- and 3-year-olds (29 and 27 %, respectively) compared to mature cows (16.3 %). Reeves and Gaskins (1981) reported that Angus cows suckling calves once daily for 30 min, beginning either 21 or 30 d postpartum, exhibited estrus 20 d earlier than suckled cows. Similarly, Randel (1981) reported that Brahman x Hereford first-calf heifers suckled once daily for 30 min beginning 30 d after calving reduced postpartum intervals from 168 to 68.9 d, without affecting calf weights at weaning. In agreement with these data, several researchers have reported that short-term calf removal reduced the time from calving to first estrus in postpartum beef cows (Beck et al., 1979; Smith et al., 1979; Odde et al., 1982).

Short et al. (1972) reported that first estrus cycles of cows induced by weaning was of shorter duration than subsequent cycles. More recently, Reeves and Gaskins (1981) reported that more Angus cows that were nursed once daily for 30 min had shorter first estrous cycles (< 11 d in length) than did normally nursed cows. Ramirez-Godinez et al. (1982) reported that all of the Hereford cows that had their calves weaned at approximately 35 d postpartum exhibited first estrous cycles of less than 10 d. In agreement with these studies, Ramirez-Godinez et al. (1981), Ward et al. (1979) and Odde et al. (1980) reported that approximately 80 % of postpartum anestrous cows that exhibited estrus within 10 d after weaning calves had estrous cycles of 7 to 12 d in length. Short-term calf removal, early weaning and once daily suckling are effective in reducing the postpartum interval to estrus without having any detrimental effects on calf performance. However, using such manipulation dramatically increased the incidence of short estrous cycles and there was no reduction in postpartum interval to conception. These aforementioned observations have led investigators to examine mechanisms involved in the inhibitory role of suckling on postpartum interval to estrous.

Short et al. (1972) examined the influence of the udder on postpartum interval to estrus and found that the interval from calving to first estrus was longer for suckled and nonsuckled cows (65 and 25 d, respectively) than for mastectomized cows (12 d), however treatments had no effect on the interval from calving to conception. Therefore the presence of the udder seems to enhance the inhibitory effect of suckling on postpartum interval to estrus in beef cows. In a follow-

up study, Short et al. (1976) reported that mammary denervation failed to decrease the interval from calving to estrus in fall-calving suckled beef cows, which indicates that something unique to the calf, other than the stimulus of suckling alone, acts to inhibit the onset of estrus in postpartum cows. What the stimulus(i) might be is unknown, however, Williams et al. (1984) examined the effect of chronic manual teat stimulation in ovariectomized non-lactating beef cows on the release patterns of IH and found that neither concentration nor pulse frequency of IH were affected by treatment. These results were unexpected, since it has been reported that suckling acts to extend the postpartum interval in beef cows by inhibiting the pulsatile release of IH. Although mechanical teat stimulation was not effective in altering the release patterns of IH this does not imply that a suckling calf or some component associated with a suckling calf does not affect gonadotropin secretion in the cow. In a more recent study, Williams et al. (1987) attempted to show that chronic milking and(or) the physical presence of the calf would have an effect on the postweaning rise of tonic IH secretion in postpartum cows. They found that physical presence of a calf or milking eight times daily did not prevent the postweaning rise in IH from occurring in nonsuckled beef cows, and both factors together did not combine to simulate the physiological state of a suckled cow. The authors suggested that neural input, unique to the calf and transmitted from the level of the teat, seems to be required for the suckling effect to manifest itself in the form of suppressed IH release. The evidence presented in these studies supports the theory that suckling prolongs the postpartum interval of the cow, and it would

seem that a combination of factors including the somatosensory stimulus by the calf at the level of the teat and the physical presence of the calf act to inhibit the onset of reproductive activity in the postpartum beef cow.

Nutrition and Body Condition

Several studies have shown that reproductive performance in beef cattle is influenced by nutrition (Dunn and Kaltenbach., 1980). One of the major areas of research emphasis has been on the effect of pre-calving nutrition on subsequent postpartum reproduction in beef and dairy cattle. Falk et al. (1975) reported that interval from parturition to first estrus in Hereford heifers assigned to three energy intakes of 100, 85 and 75 % of the National Research Council (NRC) requirement for beef cattle, approximately 150 d prepartum, were 63, 67 and 78 d for the 100, 85 and 75 % intakes, respectively. Bellows and Short (1978) reported that heifers receiving high feed levels 90 d prior to calving (6.3 or 6.4 kg of total digestible nutrients, TDN) had shorter postpartum intervals and, a greater number of heifers exhibited estrus before the breeding season compared with heifers fed a low level of feed (3.2 or 3.4 kg TDN) for 90 d prior to calving. In a more recent study, Henricks et al. (1986) reported that the number of heifers ovulating before Day 70 postpartum was higher if they were fed to gain 1 kg per day prepartum and postpartum than if they were fed to gain .4 kg per day prepartum and 0 kg per day postpartum. In agreement with these studies, several investigators have reported that low levels of prepartum nutrition prolonged the

postpartum interval in beef cattle (Dunn et al., 1969; Clemente et al., 1978; Echternkamp et al., 1982; Ducker et al., 1985). Contrary to these studies, Doornbos et al. (1984) reported no effect of precalving feed level on the interval from parturition to first observed estrus. The contradictory results in this study could be a result of the moderate and high prepartum feed levels of 110 and 135 % of the NRC (1976) requirements for TDN, which is considerably higher than levels used in the previous studies. Based on these studies one can conclude that prepartum level of nutrition, especially low energy intake, has a significant influence on the postpartum interval to estrus of beef cattle.

It has been well established that the postpartum interval can also be influenced by postcalving level of nutrition. Wiltbank (1970) reported that the proportion of cows exhibiting estrus by 100 d postpartum was greater for cows fed a high (22 lb) or medium (13 lb) level of TDN postcalving than cows fed a low level (7 lb) of TDN postcalving (98, 97 and 81 %, respectively). In agreement with this study, Bartle et al. (1984) found that increasing postcalving energy intake shortened the postpartum interval of 2- to 6-year-old cows. Bellows and Short (1978) found that the effect of precalving feed level on subsequent postpartum reproduction is dependent on postcalving feed level. They reported that high postcalving feed level tended to be advantageous when the precalving feed level was high but was detrimental to postpartum intervals when the precalving feed level was low. Low levels of feed postcalving prolong the postpartum interval

and high levels of feed postcalving cannot overcome the detrimental effects of low precalving feed levels.

The information on precalving and postcalving feeding levels on postpartum interval to estrus raises questions as to the effect of body condition or composition on postpartum reproductive function. Walters (1981) stated that body condition was the ratio of the amount of fat to the amount of non-fatty matter in the body of the living animal. Rutter and Randel (1984) classified females according to whether or not they were able to maintain body condition after calving, regardless of dietary nutrient level, and reported a dramatic decrease in the postpartum interval of females that maintained body condition compared with females that lost body condition after calving, (31 and 60 d, respectively). Also, they reported that 88 % of the females that were able to maintain body condition after parturition were observed in estrus within 42 d postpartum compared with only 36 % of females that were unable to maintain body condition after calving. Richards et al. (1986) reported that cows calving with a body condition score less than or equal to 4 or more than or equal to 5, had mean postpartum intervals to estrus of 61 and 49 d, respectively. Humphrey et al. (1983) reported that reduced prepartum energy intake did not seem to prolong the postpartum interval of cows in their study, however, backfat thickness for the six suckled beef cows in their study was 8 ± 3 mm at the beginning of the study, 5 ± 3 mm at parturition and 4 ± 2 mm just before first postpartum estrus. They suggested that cows that are in relatively good body condition precalving can loose some weight without having a dramatic effect on the length of the postpartum

interval. In agreement with these studies, several researchers have found that postpartum intervals of cows in good body condition were shorter than cows in poor body condition. Body condition at calving, prepartum feed level and to a lesser degree postcalving nutritional level may act with body composition to effect resumption of ovarian activity in the postpartum cow.

Restriction of dietary energy intake and subsequent loss of body condition are inhibitory to postpartum reproductive function in cattle. Several studies have attempted to determine the site of action of this inhibition and its effect on endocrine patterns of postpartum cows. Gombe and Hansel (1973) reported a progressive increase in both basal levels and peaks of IH from the first to third estrous cycles in heifers fed a low level of dietary energy compared to those fed a high level of dietary energy. Also, they found that during the first cycle, plasma progesterone was slightly higher in the low energy group, but became progressively lower in the subsequent cycles. Similarly, Beal et al. (1978) reported that peripheral concentrations of progesterone tended to be reduced when dietary energy was restricted in heifers and cows. However, Spitzer et al. (1978) reported no differences in systemic concentrations of progesterone or IH in yearling beef heifers fed either a ration meeting NRC recommendations for all nutrients or a ration with only one third of the recommended energy. Several studies have reported that low levels of dietary energy either decreased (Echternkamp et al., 1982; Gauthier et al., 1983; Whisnant et al., 1985a) or did not effect the concentration, pulse frequency or pulse amplitude of IH in postpartum cows (Rutter and Randel, 1984; Easdon et

al., 1985; Wright et al., 1987).. However, Rutter and Randel (1984) reported marked differences in LH characteristics between females that lost and those that maintained body condition after calving, regardless of the level of nutrient intake. Brangus females that were able to maintain body condition after calving released more endogenous LH and more LH in response to an exogenous gonadotropin releasing hormone (GnRH) challenge, than females that lost body condition after calving. Lishman et al. (1979) reported a delayed and reduced response of LH to GnRH in postpartum cows receiving inadequate dietary energy during late gestation and early lactation. However, Beal et al. (1978) found that low dietary energy increased the LH response after a GnRH injection in intact heifers and spayed cows but not in intact cows. In a more recent study, Whisnant et al. (1985b) reported that the LH response to GnRH was greater in postpartum cows fed a low energy diet compared to postpartum cows fed a high energy diet. Rutter and Randel (1984) stated that comparisons of systemic LH levels and pituitary responsiveness between postpartum and normally cycling cows may be misleading due to the possibility that different mechanisms may be involved in regulating onset of cycling activity in the postpartum cow compared with maintenance of cycling in the normal cycling animal.

Echternkamp et al. (1982) demonstrated that release of LH was greater in response to an injection of estradiol benzoate (which could act both at the pituitary and the hypothalamus) in cows fed a high energy diet as opposed to cows fed a low energy diet. Restricting dietary energy intake in the postpartum cow acts at the level of the pituitary by increasing the responsiveness to GnRH and possibly

decreasing the sensitivity of the hypothalamus to increased levels of estrogen or increasing the inhibitory effects of low levels of estrogen.

Endocrine and Neuroendocrine Factors Associated with Postpartum Cows

The endocrine and neuroendocrine systems are major physiological regulatory systems involved with resumption of estrous cycles in postpartum suckled beef cows, however, their exact role is not fully understood. The early postpartum period is usually characterized by ovarian inactivity and the absence of estrus in suckled beef cows. The following sections review three major regulatory components of the reproductive system (pituitary, hypothalamus and ovaries) and their relationships in the suckled beef cow.

Anterior Pituitary

Gonadotropins. The anterior pituitary gland synthesizes and secretes glycoprotein hormones that stimulate ovarian activity in females. These are luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL). Pituitary content of LH of suckled cows is low at parturition and increases during the first 30 d of the postpartum period (Graves et al., 1968; Wagner et al., 1969; Cermak et al., 1983). A more recent study indicated that content of LH in the anterior pituitary gland was lowest on Day 1 postpartum and remained low through Day 15 postpartum, however by Day 30 postpartum there was a more than six fold increase in pituitary content of LH compared with that observed on Day 1 (Nett et al. 1988). Carruthers et al. (1980) and

Faltys (1985) reported that concentrations of LH in the anterior pituitary increased after calving independent of suckling. LH synthesis by the anterior pituitary increases during the early postpartum period and does not appear to be limited by the suckling stimulus.

Saiduddin et al. (1968) reported that pituitary FSH content in postpartum cows decreased during the first 20 d postpartum and was lower on Day 20 than on Days 10 or 30. However, Cermak et al. (1983) and Nett et al. (1988) reported that pituitary content of FSH in postpartum cows showed no change during the postpartum period. Carruthers et al. (1980) reported that suckling did not alter the pituitary concentration of FSH. Therefore, pituitary content of FSH does not appear to be a limiting factor in the resumption of reproductive activity in the postpartum cow.

Riesen et al. (1968) reported that pituitary content of PRL in suckled and nonsuckled dairy cows did not change as time postpartum increased from 1 to 30 d, although suckled cows had a tendency to have a lower PRL contents than nonsuckled cows. In contrast, Carruthers et al. (1980) reported that suckling did not alter pituitary content of PRL in dairy cows. PRL synthesis by the anterior pituitary does not increase during the early postpartum period and is not dramatically effected by suckling.

Several researchers have reported that serum concentrations of LH are low at parturition and during the early postpartum period then gradually increase by 30 d after calving in suckled beef cows (Arije et al., 1974; Kesler et al., 1977; Carruthers et al., 1980; Rawlings et

al., 1980; Humphrey et al., 1983). Echterkamp and Hansel (1973) reported that LH concentrations in plasma were low ($1.0 \text{ ng}\cdot\text{ml}^{-1}$) at parturition and remained low (.5 to $3.0 \text{ ng}\cdot\text{ml}^{-1}$) except on the day of estrus at which time large increases were observed. However, the absence of changes in LH in their study was a consequence of infrequent sampling since LH appears to be secreted into the blood in a pulsatile manner (Rawlings et al., 1980; Humphrey et al., 1983). Frequency and amplitude of pulsatile LH release increases prior to the first postpartum ovulatory surge of LH in suckled and nonsuckled beef and dairy cows (Short et al., 1972; Forrest et al., 1979; Goodale et al., 1978; Carruthers and Hafs, 1980; Carruthers et al., 1980; Rawlings et al., 1980; Garcia-Winder et al., 1984).

Many investigations have been performed to examine the nature of postpartum LH release and its physiological significance on resumption of ovulatory cycles. Edwards (1985) examined the influence of short-term calf removal on serum LH concentrations and found that mean LH concentration and LH pulse frequency increased by 48 to 56 h following calf removal and that returning the calf decreased both LH concentrations and LH pulse frequency within 8 h. Furthermore, several researchers have reported that suckling decreased serum LH concentrations and pulse frequencies in postpartum beef and dairy cows (Carruthers and Hafs, 1980; Dunlap et al., 1981; Peters et al., 1981; Walters et al., 1982a; 1982b; Garcia-Winder et al., 1984; Faltys, 1985; Whisnant et al., 1985b; Garcia-Winder et al., 1986). Carruthers and Hafs (1980) and Carruthers et al. (1980) reported that suckling decreased the amplitude of episodic peaks of LH in the postpartum cow.

Taken together data support the hypothesis that suckling inhibits the release of LH in the postpartum cow primarily by a reduction in the frequency and amplitude of pulsatile LH release which results in delaying return to estrus.

Available information on serum concentrations of FSH during postpartum anestrous in cattle is limited. Gauthier et al. (1982) reported that serum concentrations of FSH were higher on Day 50 postpartum than on Day 5 postpartum in anovulatory beef cows. In agreement with this study, Webb et al. (1980) demonstrated that circulating FSH levels remained low during the first two weeks postpartum then increased in two of four dairy cows. However, Dobson (1978) reported that serum FSH concentrations between 21 to 48 d postpartum in dairy cows were not different from concentrations between 0 to 20 d postpartum.

Information on the effect of suckling on serum concentrations of FSH is limited. Rothchild (1960) reported that suckling suppresses FSH secretion in rats and this suppression is directly proportional to litter size. Walters et al. (1982b) reported that serum FSH concentrations of weaned cows were greater than that of suckled cows. However, Carruthers et al. (1980) found that suckling did not affect serum concentrations of FSH in dairy cows during the first 14 d postpartum. The contradictory nature of the data on FSH makes it difficult to determine the role that FSH plays in resumption of cyclic activity in postpartum cows.

Humphrey et al. (1983) reported that serum concentrations of prolactin tended to decrease as time postpartum increased in four of

six suckled beef cows. However, the two animals with the shortest postpartum intervals were found to have the highest serum concentrations of prolactin just prior to first estrus. On the other hand Carruthers et al. (1980) reported that ad libitum suckling did not alter basal or milking-induced serum concentrations of prolactin and that suckling by two calves did not increase prolactin secretion in comparison to controls. Similar results have been reported for first-calf beef heifers suckling either one or two calves (Gimenez et al., 1980).

The physiological significance of data for prolactin during the postpartum period in cattle is not well understood, however Cummins et al. (1977) tested whether or not lowering prolactin levels by injecting CB-154 (ergocryptine, a dopamine agonist), effected interval to estrus or conception in first-calf suckled beef heifers. They found that suppression of prolactin with CB-154 did not effect postpartum interval to estrus or conception in these cows. Furthermore, Clemente et al. (1978) reported similar results in mature cows, and Montgomery (1982) reported that injection of bromocryptine was not effective in reducing the interval from calving to first estrus in crossbred beef cows. However, Short et al. (1978) demonstrated that injection of a prolactin secretion inhibitor (CB-154) was effective in reducing the interval from calving to estrus in beef cows. Except for the results of Short et al. (1978), most evidence tends to support the hypothesis that prolactin does not appear to be a limiting factor in the resumption of estrous cycles in the postpartum cow.

Ovarian Steroids and Follicular Growth

Estrogen. At parturition, estradiol- 17β levels are elevated and then decline rapidly during the early postpartum period and remain low until just prior to first estrus (Arije et al., 1974; Stevenson and Britt., 1979; Rawlings et al., 1980; Humphrey et al., 1983). Several investigators have reported that serum concentrations of estrogen do not differ between suckled and nonsuckled dairy (Carruthers and Hafs, 1980; Carruthers et al., 1980) or beef cows (Chang et al., 1981) throughout the postpartum period.

Pituitary responsiveness to exogenous estrogen, assessed by inducing a preovulatory-like release of IH, increases as time postpartum increases. Forrest et al. (1981) reported that a single injection of estradiol benzoate (EB) failed to induce a release of IH in 100 percent (6 of 6) of the cows at 2 to 3 d postpartum. However, 4 of 6 and 2 of 5 cows responded to an injection of EB on Days 9 to 10 and 16 to 17 postpartum, respectively. These findings are in agreement with Cermak et al. (1983) who reported that anterior pituitary receptors for estrogen were lowest immediately following parturition and increased to their highest concentration by Day 15 postpartum.

Several researchers have reported that the positive feedback effect of estrogen on IH secretion is delayed by suckling until 2 to 4 wk postpartum in beef (Radford et al., 1978; Short et al., 1979) and dairy cows (Stevenson et al., 1983). Furthermore, Peters (1984) reported that responsiveness of the hypothalamo-pituitary axis to the positive feedback effects of estrogen is functional by Day 10 postpartum and

this responsiveness increases as time postpartum increases through Day 17 in suckled postpartum beef cows.

Acosta et al. (1983) reported no significant changes in LH concentrations or number of LH pulses in response to estrogen treatment during the first three weeks postpartum in ovariectomized suckled beef cows. When cows were weaned at 21 d postpartum they found no difference in the frequency of LH pulses between weaned and suckled ovariectomized cows, however, in estrogen-implanted cows, suckling suppressed the frequency of LH release compared to the weaned cows. The authors proposed the hypothesis that suckling contributes to lactational anestrus in the postpartum cow by increasing the sensitivity of the hypothalamus to the negative feedback of low, relatively constant circulating estrogen concentrations which result in a reduction in pulsatile release of GnRH which in turn decreases pulsatile release of LH from the anterior pituitary gland.

Progesterone. Circulating concentrations of progesterone decline after parturition remained at these low basal concentrations until just prior to estrus in suckled beef (Arije et al., 1974; Rawlings et al., 1980; Humphrey et al., 1983) and dairy cows (Stevenson and Britt, 1979). Several investigators have reported a rise in progesterone concentrations 2 to 5 d prior to the first postpartum estrus (Arije et al., 1974; Corah et al., 1974; Stevenson and Britt, 1979; Rawlings et al., 1980; Lavoie et al., 1981; Humphrey et al., 1983). The source of the preestrus progesterone increase in postpartum cows has not been clearly established. Corah et al. (1974) suggested that since there

are no apparent functional corpora lutea present, that either luteinized follicles or adrenal glands are responsible for the pre-estrual progesterone rise. Berardinelli et al. (1979) observed a similar progesterone rise prior to first estrus in prepuberal beef heifers and concluded that the source of progesterone was from nonpalpable luteal tissue embedded in the ovary. Castenson et al. (1976) reported that the rise in serum progesterone which precedes first estrus in postpartum cows is frequently due to ovulation and subsequent corpus luteum formation, without expression of estrus.

The relationship of the increase in progesterone before postpartum estrus and the onset of regular cycling activity in postpartum cows is not well understood. However, Lavoie et al. (1981) reported that plasma progesterone levels were greater for nonsuckled than for cows suckled twice daily or suckled ad libitum throughout the postpartum period. In addition, the magnitude of the preestrus progesterone peak was greater in cows suckled ad libitum than in those suckled twice daily or nonsuckled. Furthermore, only two of six cows from the nonsuckled group had normal first estrous cycles preceded by a preestrus rise in progesterone compared to nine of twelve in the two suckled groups. The authors suggested that progesterone is involved in the reestablishment of estrous cycles in the postpartum cow. Faltys (1985) hypothesized that the increase in IH (discussed in previous section) and progesterone that is seen prior to normal estrus cycles in postpartum cows may act to prime an endocrine system that has been acyclic during gestation and the postpartum anestrous period.

Follicular Development. Ovarian follicular development increases with time postpartum. Saiduddin et al. (1968) reported that the number of ovarian follicles greater than or equal to 5 mm and total follicular weight increased from Days 1 to 10 postpartum in suckled dairy cows. Spicer (1985) found that the number of medium follicles increased two-fold between Days 7 and 14 postpartum and again between Days 28 and 56 postpartum. The author suggested that the increase in number of medium follicles may provide a large pool of follicles from which ovulatory follicles can be selected prior to the first postpartum ovulation.

Carter et al. (1980) reported that ovaries of nonsuckled beef cows had greater follicular volumes on Day 5 postpartum than did ovaries of suckled beef cows, primarily because of a greater number of medium and large follicles. In support of these data, Bellin et al. (1984) reported that suckled beef cows had smaller and fewer follicles than nonsuckled beef cows on Day 5 postpartum. However, Saiduddin et al. (1968) found no difference due to suckling on follicular volume or number by Day 10 postpartum in suckled dairy cows. Therefore, it would seem that suckling inhibits follicular growth in cows during the early postpartum period and the inhibitory effect is no longer evident by Day 10 postpartum. It would appear that the ovaries contain follicles of sufficient size and volume for estrus activity to occur during the early postpartum period, because of the inappropriate levels of LH and possibly FSH and estrogen these follicles are not yet competent at this time to ovulate. However, once LH pulses begin to increase follicles begin to "see" appropriate levels of LH and begin to synthesize and

secrete estrogen which leads to a preovulatory increase in LH ovulation.

Carter et al. (1980) reported that follicular concentrations of estrogen were not different between suckled and nonsuckled beef cows on Day 5 postpartum. However, Bellin et al. (1984) reported that follicular estrogen concentrations were lower in suckled than nonsuckled cows (14.7 and 27.8 ng·ml⁻¹, respectively) on Day 5 postpartum. The conflicting results are not readily reconcilable and this makes it difficult to assess the importance of follicular estrogen on physiological changes of postpartum cows at least during the early period of postpartum anestrous.

Gonadotropin Releasing Hormone

The hypothalamus is an integral component of the neuroendocrine system which synthesizes factors, such as gonadotropin releasing hormone (GnRH), which are secreted into the blood and transported by way of the hypophyseal portal system to the anterior pituitary gland. These hypothalamic factors regulate the release of pituitary hormones, such as LH and FSH, which in turn mediate reproductive function at the level of the ovary.

It is well established that GnRH secreted from the hypothalamus mediates the pulsatile release of LH and probably FSH from the anterior pituitary gland. However, the mechanism by which GnRH regulates pituitary gonadotropin secretion, and the factors that modulate the activity of GnRH in the postpartum cow are not clearly understood. Cermak et al. (1983) observed no changes in hypothalamic content of

GnRH through 45 d postpartum, indicating that hypothalamic content of GnRH is not a limiting factor in the resumption of cyclic activity in the postpartum cow. In agreement with this result, Carruthers et al. (1980) found no difference in the content of hypothalamic GnRH of suckled and nonsuckled cows. However, in contrast to this study, Minaguchi and Meites (1967) reported that frequent suckling by litters of postpartum lactating rats resulted in a significant decrease in hypothalamic content of GnRH, compared to nonlactating rats. The contradiction of the two studies could be due to the intensity of the suckling stimulus or species differences in the regulation of GnRH release during the postpartum period.

The pattern of GnRH secretion in the postpartum cow has yet to be established. However, Levine et al. (1982) reported that GnRH is released in a pulsatile fashion in unanesthetized ovariectomized ewes and that each GnRH pulse either accompanied or directly preceded an episodic pulse of IH. In a more recent study, Rodriguez and Wise (1987) demonstrated the presence of pulsatile secretion of GnRH during the infantile period of development (birth to six weeks of age) in the dairy bull calf. Several investigators have reported that intermittent small dosages of GnRH induced episodic IH release in the postpartum cow (Riley et al., 1981; Walters et al., 1982c; Peters et al., 1985; Jagger et al., 1987; Wildeus et al., 1987). These data support the hypothesis that GnRH is released in a pulsatile fashion and probably causes the pulsatile rhythm of IH secretion observed in postpartum cows.

The ability of the anterior pituitary gland to respond to pulsatile release of GnRH with a corresponding episodic release of LH appears to be effected by time postpartum and suckling. Several investigators have reported that the ability of the pituitary to release LH in response to GnRH increases with time postpartum and is fully restored by at least Day 10 postpartum (Kesler et al., 1977; Fernandez et al., 1978; Forrest et al., 1981). In support of these studies, a recent study by Moss et al. (1985) demonstrated that anterior pituitary cells prepared from postpartum cows obtained at 5, 10, 20 and 30 d post-calving, did not differ in the amount of LH released in response to GnRH. This result may not be surprising, since Cermak et al. (1983) reported that the concentration of anterior pituitary receptors for GnRH were lowest immediately following parturition and increased to their highest concentration by Day 15 postpartum. The authors suggested that the increase in GnRH receptors possibly acts to increase the sensitivity of the anterior pituitary gland to GnRH during the early postpartum period. From these data it seems clear that pituitary sensitivity to GnRH for release of LH is regained early postpartum and that pituitary responsiveness to GnRH is not a limiting factor in the reestablishment of reproductive activity in the postpartum cow.

Suckling increases time to first postpartum estrus in cattle and presumably does so by decreasing or inhibiting pulsatile release of LH. Numerous researchers have reported that suckling decreases the responsiveness of the anterior pituitary gland to GnRH in vivo (Carter et al., 1980; Walters et al., 1982b; Walters et al., 1982c; Dunn et

al., 1985) and in vitro (Carruthers et al., 1980). These studies support the hypothesis proposed by Carruthers et al. (1980) and Walters et al. (1982b; 1982c) that suckling prolongs the postpartum interval of the cow by reducing the frequency of GnRH release from the hypothalamus.

Social Interaction and Reproductive Function

Animals communicate information concerning reproduction to conspecifics in order to coordinate reproductive activities. One means of transmitting such information is by chemical communication with pheromones (Vandenbergh, 1983).

Izard (1983) stated that in 1959 Karlson and Butenandt coined the word "pheromone" to designate substances that are secreted externally by an animal and cause a specific reaction in a receiving individual of the same species; the reaction involves either the release of a specific behavior or a physiological change in the recipient's endocrine or reproductive system.

Wilson and Bossert (1963; cited in Izard, 1983) classified pheromones into distinct categories based on pheromonal communication in insects. Releasing pheromones were defined as pheromones which cause an immediate but reversible change in behavior, whereas, priming pheromones were defined as pheromones which initiate a change of physiological events, either through inhibition or stimulation, in which endocrine, reproductive and possibly other systems could be altered.

The way in which males interact with females to alter the reproductive function of the female has been extensively researched in mice. Whitten (1956) demonstrated that anestrous female mice exposed to a male were induced to resume normal ovarian cyclicity. Similarly, Vandenberg (1967) reported that sexually immature females housed in the absence of males reached puberty later than females housed in the presence of males. Bronson and Whitten (1968) and Colby and Vandenberg (1974) demonstrated that the effect of the male on female reproductive function can be mimicked by the contact of females with urine from mature males.

An endocrine basis for the "male effect" has been described in the mouse and appears to involve mediation via the neuroendocrine system. Bronson (1976) demonstrated that ovariectomized adults, implanted with estradiol showed an increase in LH release when exposed to urine of mature males. Subsequently, Bronson and Desjardin (1974) found that exposure of peripuberal females to a mature male results in an immediate elevation in LH secretion, followed shortly thereafter by a profound increase in serum estradiol levels. The activation by the male of the LH-estradiol pathway induces ovulation or puberty in young female mice.

The pheromonal effect of males in rodents appears to involve male sex steroids. Bronson and Whitten (1968) demonstrated that orchietomized males lose their ability to activate estrus in anestrous females, however, the ability to induce estrus is restored when males are treated with androgens. These studies demonstrate clearly that the adult male has a profound effect on reproductive

function of female mice by increasing the release of IH from the pituitary and that this effect is androgen-dependent and transmitted via a chemical signal to the neuroendocrine system of the female, probably via the vomer-nasal-olfactory pathway.

Recent studies have demonstrated the effect of exposure to mature males on the activation of estrus in female gray opossums (Fadem, 1985). In a more recent study, Fadem (1987) reported that pheromonal cues provided by intact males in the absence of any other exteroceptive stimuli, were sufficient to induce estrus reliably in 75 to 100 % of anestrus females and this effect was at least partially androgen-dependent since pheromonal cues provided by males castrated for an extended period or other intact females induced estrus in only 25 % of anestrus females.

Numerous studies have implicated the "male effect" as a stimulator of reproductive function in domestic livestock. Brooks and Cole (1970) demonstrated that puberty was attained at an earlier age by gilts having contact with a boar than by those kept in the absence of a boar in a nonconfinement system. Similar results were obtained for gilts reared in confinement (Thompson and Savage, 1978). Furthermore, it has been reported that ovulation rates were increased in gilts exposed to boars during the prepuberal period (Patterson and Lindsay, 1980). Thus, it would seem that the male has a profound effect on induction of puberty in swine as it does in some laboratory and "wild" species.

A question arises: does the boar affect the anestrus postpartum interval of sows? Ovarian activity following farrowing is usually inhibited in sows due to the effects of lactation. Rowlinson et al.

(1975) grouped Landrace sows and their litters at three weeks postpartum and subsequent exposure to a boar resulted in lactational estrus by all 180 sows in the study. The interval from grouping until lactational estrus was 11.5 d, with a conception rate of 84.9 %. In a more recent study, Walton (1986) reported that boar exposure, either before or after weaning, was effective in reducing the number of anestrus and anovulatory sows to between 15 and 30 %. Furthermore, boar exposure before or after weaning resulted in 95 % of sows in estrus and ovulating within 20 d of weaning compared to 45 and 38 % of those not exposed to boars. These results demonstrate the effectiveness of boar exposure on hastening the time to puberty in gilts and reducing the inhibitory affect of lactation on estrus in sows. At the present time there is no information on the "pheromone" or endocrine mechanism by which the boar effects prepuberal or postpartum anestrus swine.

It has been well established that exposure to a mature male is effective in terminating the seasonal anestrus period of sheep and goats. Underwood et al. (1944) reported that introduction of rams could interrupt seasonal anestrus in ewes. More recently, it was shown that introduction of rams at the beginning of the breeding season stimulates noncycling ewes to ovulate within three days of ram exposure (Knight et al., 1978; Martin et al., 1980; Poindron et al., 1980). Shelton (1960) found that introduction of a buck to a group of Angora does just before the start of the breeding season resulted in initiation of synchronized estrus 5 to 10 d after the onset of exposure to the buck.

Endocrine patterns associated with the exposure of the ram to anestrus ewes has been examined. Pulse frequency of LH in anestrus ewes has been observed to increase within 10 (Martin et al., 1980) to 40 min (Poindron et al., 1980) subsequent to introduction of a ram. Oldham and Pearce (1984) reported increases in pulse frequency of LH of ewes exposed to rams compared to isolated ewes (.30 and .07 pulses·h⁻¹, respectively). Chesworth and Tait (1974) reported that exposure of rams to anestrus ewes in the middle of the nonbreeding season resulted in increases of plasma LH shortly after exposure. Knight et al. (1978) reported an LH peak, similar to the preovulatory peak, occurred on average 35 h following introduction of the ram to anestrus ewes. Furthermore, Knight et al. (1978) reported that estradiol-17 β concentrations were unchanged as a result of exposure of ewes to mature rams, which led the authors to suggest that rams may have stimulated a direct release of LH which was not dependent on stimulation of the hypothalamus by estradiol-17 β . From these studies it is clear that the primary effect of exposing anestrus ewes to mature rams is to increase the pulse frequency of LH or preovulatory-like LH surges which leads to induction of cycling activity in anestrus ewes.

The red deer (*Cervus elaphus*) is similar to our domestic cattle in that they exhibit estrus for 18 to 24 h and have an estrous cycle length of approximately 20 d. In a recent study, McComb (1987) reported that vocalization by red deer stags affected the timing of ovulation in hinds. Exposure to a vasectomized stag or a recording of a stag roaring resulted in a higher percentage of hinds calving during the first three weeks of the breeding season compared to hinds isolated

from stags (77, 56 and 45 %, respectively). The author concluded that something peculiar to the male, other than roaring alone, affected the time of ovulation and estrus in hinds since the presence of the vasectomized stag resulted in the greatest percentage of calving during the first three weeks of the breeding season. It would appear that in light of these results a probable chemical signal "pheromone" is involved.

Until recently, the "male effect" in cattle has received little attention. Several investigators have reported that exposure to mature bulls failed to hasten the onset of puberty in beef heifers (Berardinelli et al., 1978; MacMillan et al., 1979; Roberson et al., 1987). Contrary to these studies, Izard and Vandenberg (1982) found that a larger percentage of heifers treated oronasally with bull urine at weekly intervals during the experimental period reached puberty than water-treated heifers (67 and 32 %, respectively). This result must be interpreted with caution, since there was no difference in age at puberty between the urine- and water-treated groups. Bull exposure is not an effective method in reducing the age at puberty in beef heifers.

Some of the first evidence that the bull has an effect on the postpartum reproductive function of cows was reported by Petropavlovskii and Rykova (1958; cited in Izard, 1983). They found that short-term daily exposure to vasectomized bulls during the early postpartum period resulted in reducing the time to conception in comparison to controls. Nersesjans (1959; cited Izard, 1983) also demonstrated that exposure to vasectomized bulls during the postpartum period increased the number of cows that conceived prior to Day 60

postpartum. In agreement with these studies, Skinner and Bonsma (1964) reported that running cows with vasectomized bulls 30 d prior to the start of the breeding season resulted in 100 percent conception during the first three weeks of the breeding season whereas seven weeks were required for 100 percent conception in the controls. Macmillan et al. (1979) reported that exposure of spring-calving cows to vasectomized bulls during the pre-mating period was associated with a significant increase in the percentage of cows detected in estrus during a 19-d spring insemination program (69 and 40 % of the bull-exposed and isolated cows, respectively).

Recent studies have examined the effect of bull exposure on resumption of estrous activity in the postpartum cow. In a two-year study, Zalesky et al. (1984) found that cows exposed to mature bulls during the early postpartum period initiated estrous cycles earlier than those not exposed to bulls (41 and 62 d, respectively). Naasz and Miller (1987) reported similar results in a 1985 study in which beef cows exposed to bulls had a shorter postpartum interval to estrous than those not exposed (46.6 and 70.8 d, respectively). However, they reported no difference between treatments in a subsequent 1986 study (45.8 and 52.2 d, respectively). Alberio et al. (1987) also failed to show a difference in postpartum interval when cows were initially exposed to bulls during the early postpartum period or isolated from bulls throughout the postpartum period (55.6 and 67.3 d, respectively). However, there were only nine animals used in this study which could account for the lack of a statistical difference. Lastly, Spitzer et al. (1987) reported that cows not exposed to bulls throughout the

postpartum period had shorter intervals to estrus than those exposed to bulls (38 and 56 d, respectively).

The influence of mature bulls may depend upon the time postpartum when bulls are introduced to anestrous cows. Alberio et al. (1987) reported that exposing cows to vasectomized bulls at 58 d postpartum resulted in a higher percentage exhibiting estrus within 28 d of exposure in comparison to controls (67.9 and 32.7 %, respectively). However, Scott and Montgomery (1987) reported no difference in the time from calving until estrus when bulls were introduced 21 d prior to the start of the breeding season (69 and 73 d for exposed and non-exposed cows, respectively).

In an attempt to determine if the "male effect" is androgen dependent, Spitzer et al. (1987) exposed cows to a mature bull or to testosterone-treated cows throughout the postpartum period and found no difference in the interval from calving to first estrus between treatments (45 and 43 d, respectively). However, no control or non-exposed treatment was employed in the study which makes an interpretation of these quite data difficult.

Stumpf et al. (1987) reported that level of nutrition during the precalving period effects the ability of the bull to alter the reproductive activity of the postpartum cow. They found that postpartum interval to estrus in cows fed a high level of nutrition was not effected by the presence of the bull. However, postpartum intervals to estrus in cows fed the low level of nutrition and exposed to the bull had a shorter interval to estrus than those not exposed (43.6 and 57.7 d, respectively). Based on these data it would seem

that high levels of nutrition counteracts the positive effect of the bull, however, the bull seems to override the negative effects of low precalving nutrition on postpartum reproductive function in the cow.

At the present time only one study has looked at the effect of bull exposure on postpartum reproductive activity of first-calf heifers. Berardinelli et al. (1987) reported that first-calf heifers exposed to mature bulls had shorter postpartum intervals than those not exposed to bulls (56 and 73 d, respectively). Furthermore there is no information in the literature on the effect of bull exposure on the endocrine physiology in the postpartum cow.

Summary

This review of literature indicates clearly that suckling, pre- and postpartum nutritional level, body condition and social interaction can have a profound effect on the resumption of postpartum reproductive activity of first-calf heifers and cows. The mechanism by which some of these factors elicit their effects appears to be mediated through the neuroendocrine and reproductive endocrine system.

Mechanisms presented in the literature indicate that as the onset of estrus approaches the suckled cow escapes the negative effects of estrogen on the hypothalamus which ultimately leads to an increase in the pulsatile release of LH from the anterior pituitary gland, probably due to an increase in GnRH release. Subsequent to a preovulatory release of LH, circulating concentrations of estrogen increase to threshold levels which have a positive effect on the preovulatory and

behavioral centers in the brain. This cascade of events initiates the LH surge which results in ovulation.

Furthermore, suckling, body condition and social interaction in some species, mice and sheep, mediate their effects on reproductive function through this mechanism. However, there are no data available to define the mechanism by which mature bulls hasten the onset of estrous activity after calving in first-calf heifers and cows.

STATEMENT OF PROBLEM

Reducing the interval from calving to first observed estrus in first-calf cows enhances the opportunity for the producer to maximize the reproductive capability of a cow throughout her lifetime. As stated in the literature review, exposure of mature bulls to cows throughout the postpartum period was effective in reducing the interval from parturition to first estrus by approximately 20 d. This would be of particular importance in first-calf cows since it is not unusual to have postpartum intervals in excess of 85 d.

It has been reported that the introduction of the male to seasonal anestrous ewes and noncycling female mice results in a dramatic increase in the pulsatile release of LH. Furthermore, there is quite a bit of data supporting the notion that regulation of the postpartum interval to estrus is related to changes in the neuroendocrine and endocrine systems which regulate ovarian activity. In addition, there is a void in the literature as to the physiological mechanisms by which the bull elicits his effect on postpartum reproductive function of the cow.

Therefore, the objectives of this study were to determine: 1) if exposure to mature bulls initiated during the early postpartum period hastens the onset of estrus and uterine involution in first-calf suckled beef cows; 2) if the patterns of LH secretion in first-calf suckled beef cows were altered due to the presence of mature bulls throughout the postpartum period, and, 3) if other factors associated with postpartum cows, such as calving difficulty, weight change,

condition score change and progesterone concentrations may be related to the influence of presence of bulls on postpartum reproductive activity of cows.

MATERIALS AND METHODS

Sixty four, two-year-old Angus x Hereford crossbred first-calf cows and two mature Red Angus x Simmental penile-blocked bulls were used in the following study conducted at the Bozeman Livestock Center, Montana State University, Bozeman. Fifty-two cows were assigned randomly at calving to one of two treatments: 1) exposure to mature bulls (BE; n = 25) or 2) not exposed to mature bulls during the postpartum period (NE; n = 24). Randomization of the cows was accomplished by a pairwise procedure which involved randomly assigning the first cow to calve either to the BE or NE group. The next cow to calve went into the treatment opposite the first. This procedure continued for each successive pair until all 52 cows were assigned to one of the two treatments.

Female to male ratio was maintained at 13:1 using 12 extra cows and calves. The first cow assigned to the BE treatment was placed into the pasture with the bull and the 12 extra cows. When the second cow was added to the BE treatment one of the extra cows was removed at random. This procedure continued until the 12 extra cows were removed from the BE group. When the 14th cow was added to the BE treatment the second bull and the 12 extra cows were also added to maintain the ratio at 13:1. The procedure for maintaining the male to female ratio was repeated until 26 cows were assigned to the BE treatment.

Two adjacent pastures in the northern and southern portion of the Livestock Center were used in the study. The distance between pastures was approximately .7 km with prevailing winds West to East. The BE

treatment was initially in the northern pastures while the NE treatment was in the southern pastures. Midway through the study the treatments were rotated into the adjacent pastures in their respective areas. The BE treatment was moved to the southern pastures and the NE treatment to the northern pastures during the final three weeks of the study.

Cows in both treatments were fed alfalfa hay ad libitum and supplemented with approximately 1.4 kg of cracked barley $\cdot \text{head}^{-1} \cdot \text{d}^{-1}$ throughout the study. Water and a mineral supplement were available ad libitum for the duration (108 d) of the study.

Cows were weighed prepartum and on Day 3 postpartum before being placed into their respective treatment. At calving, each cow was assigned a calving difficulty score of 1 to 7 based on the following criteria: (1) no assistance, (2) easy hand assistance, (3) difficult hand assistance, (4) easy mechanical assistance, (5) difficult mechanical assistance, (6) abnormal presentation and (7) cesarian section. Birth weight of each calf was obtained within 24 hours after calving. A final weight for each cow and calf was obtained on the last day of the experiment.

Ovaries and uteri of each cow were examined rectally at weekly intervals for the presence of a corpus luteum and for obtaining uterine involution scores. Uterine involution scores ranged from 0 to 5 based on the following criteria: (0) cycling uterine characteristics and tones, (1) non-cycling uterus in normal position, cervix in posterior half of pelvis, (2) uterus mostly in pelvic canal, one horn slightly larger than the other, cervix in near normal position, (3) uterus over the pelvis, one horn larger than the other and external bifurcation

readily palpable, (4) uterus swollen, usually one horn extremely oversized and cervix pulled well past the middle of the pelvis and (5) pathological condition. In addition, cows were assigned a body condition score ranging from 1 to 9, with 1 being emaciated and 9 being obese.

A jugular-venous blood sample (10 ml) was collected at weekly intervals for assay of progesterone. This schedule continued for a two week period after a cow was observed in estrus.

Postpartum estrous detection was initiated 10 d after the first cow was assigned to the BE group. Cows were observed for estrus twice daily (am:pm) for approximately 1 h, for the duration of the study.

Cows were bred by artificial insemination after synchronization of estrus with prostaglandin $F_{2\alpha}$ for a 10-d interval then exposed to fertile bulls an additional 40 d. Pregnancy rates were determined by rectal palpation approximately 60 d after the breeding season.

Intensive Blood Sampling for IH. The first four cows that calved and four cows that calved approximately mid-way through the calving season, from each treatment, were bled intensively at weekly intervals beginning approximately one week after each cow had calved. Cows assigned to the intensive bleeding groups were surgically fitted with an indwelling jugular catheter (50 cm in length, 1.27 mm i.d., 2.29 mm o.d.; Microrenanthane) on the day prior to the initiation of the sampling. The angled end of the catheter was inserted into the jugular vein with the aid of a 6.0 cm stainless steel luer-type needle (10-1 ga) through a 3 mm incision over the top of one jugular vein.

Catheters were sutured into place. Lidocaine, HCL (2 % solution) was the local anesthetic. Once inserted the tubing was flushed with 10 ml of sterile, heparinized saline (20 IU·ml⁻¹) and a "test" blood sample was drawn and discarded to insure that the catheter was functional.

Blood samples were collected at 15-min intervals for 6 h beginning at 1200 h every Tuesday and Thursday for cows in the BE and NE treatments, respectively, for the duration of the study. Intensive blood samples were collected at the Livestock Center AI facility which required transporting the cows from the BE treatment approximately .4 km by trailer. To insure similar transportation stress, cows in the NE treatment were also transported approximately 1 km by trailer on the day of their scheduled intensive bleedings. On the day before each intensive bleeding, catheters were checked for patency. If a catheter had become non-patent or unusable, it was replaced according to the procedure outlined above.

Blood samples were allowed to clot at 22 C for approximately 4 h, and then they were centrifuged at 1000 x g at 4 C for 30 min. Serum was decanted and stored at -25 C until assayed for IH and progesterone.

The following criteria were used to characterize resumption of estrous cycles postpartum: 1) display of behavioral estrous; 2) presence of a palpable corpus luteum; and, 3) an increase in progesterone concentration greater than 1 ng·ml⁻¹ for two consecutive weekly samples after estrous activity.

Assays for IH and Progesterone. Bovine IH was quantified by a double antibody radioimmunoassay. Validation and procedures for this assay

are given in the Appendix. Progesterone was quantified in two assays using a solid-phase radioimmunoassay kit obtained from Diagnostic Products Corporation (5700 West 96th Street, Los Angeles, CA.). The procedure for the progesterone assay was modified by using standards prepared in ovariectomized-dexamethasone-blocked cow serum. The sensitivity and intra- and inter-assay coefficients of variation for serum samples of cow serum that inhibited binding at approximately 50 % were .31 ng per tube, and 7.4 and 8.2 %, respectively.

Statistical Analyses

For statistical purposes, postpartum intervals for cows that had not exhibited estrus by the end of the 108-d experimental period were calculated by subtracting calving date from 167 (Julian date corresponding to the last day of the study). Data for postpartum interval to estrus, uterine involution and condition score change were analyzed by analysis of variance for a factorial arrangement of treatments in a completely random design using the General Linear Model (GLM) procedure of SAS (SAS, 1985). Treatment, calving difficulty, sex of calf and all interactions were independent variables. Calf birth weight was used as a covariate in the model. Condition score at calving, postpartum weight change and calving difficulty scores were analyzed using t-tests (Mendenhall, 1971). Proportion of cows that exhibited estrus by 45, 60, 90 and 108 d postpartum were analyzed by a log-linear procedure (MSUSTAT, 1986).

Mean ($\text{ng}\cdot\text{ml}^{-1}$), baseline ($\text{ng}\cdot\text{ml}^{-1}$), pulse frequency ($\text{pulses}\cdot 6\text{ h}^{-1}$), amplitude ($\text{ng}\cdot\text{ml}^{-1}$) and duration (pulse length in min) were determined

for frequent samples collected at weekly intervals to characterize patterns of LH for each cow. The baseline was defined as any value that was not associated with a peak. A peak was defined as a value that was at least two standard deviations above the baseline with at least one value increasing to or decreasing from the peak. Amplitude was calculated by subtracting the highest concentration of LH in a peak from the baseline. Regression coefficients were calculated for each of the LH characteristics using the Proc Regress procedure of SAS (SAS, 1985). To test the hypothesis that bull exposure did not contribute to the variation in the LH patterns over time, regression coefficients were analyzed by analysis of variance for a completely random design using GLM procedure of SAS (SAS, 1985). The model included regression coefficients for mean and baseline LH concentrations, pulse frequency, amplitude and duration of LH pulses as dependent variables. Treatment, calving date and interaction of treatment and calving date were independent variables. Two additional analyses of regression coefficients of LH characteristics was performed for cows observed in estrus. The first considered all sampling periods postcalving while the second considered only sampling periods for six weeks preceding estrus. The model included regression coefficients for mean and baseline LH concentrations, pulse frequency, amplitude and duration of LH pulses as the dependent variables with treatment as the independent variable.

The proportion of cows that exhibited an increase in progesterone prior to their first observed estrus and pregnancy rates were analyzed by chi square analyses (Mendenhall, 1971).

RESULTS

Three cows were removed from the data set. One was diagnosed as having a cervical tumor, one was subject to chronic bloat and one failed to accept her calf.

Postpartum Characteristics

Degree of calving difficulty, calf birth weight, calf sex ratio, body condition score change and body weight change throughout the postpartum period did not differ ($P > .10$) between treatments (Table 1) and body condition score at approximately Day 10 postpartum did not differ ($P > .10$) between BE and NE cows (5.26 and 5.25, respectively). Postpartum interval from calving to first estrus was approximately 19 d shorter ($P < .05$) for BE cows compared with NE cows (Table 1). Similar results were obtained for intensively bled cows that were exposed or not exposed to mature bulls (mean \pm SE = 72 ± 13 and 88 ± 13 d, respectively). However, interval from calving to uterine involution did not differ ($P > .10$) between BE and NE cows (Table 1).

Calving difficulty had no effect ($P > .10$) on interval to estrus (Table 2). However, cows suckling bull calves had shorter ($P < .06$) intervals to estrus than those suckling heifer calves (Table 2). Furthermore, calving difficulty and sex of calf had no effect on interval to uterine involution ($P > .10$; Table 2). Calf birth weight used as a covariate had no influence on interval to estrus or involution ($P > .10$). There were no interactions ($P > .10$) between treatment and calving difficulty, treatment and sex of calf or calving

difficulty and sex of calf or the three-way interaction of these variables for postpartum interval to estrus or uterine involution.

Table 1. Least-squares means and pooled standard errors (SE) for calving difficulty (CD), birth weight (BW), calf sex ratio (CSR), condition score change (CSC), body weight change (WC), postpartum interval to estrus (PPIE) and postpartum interval to uterine involution (PPIUI) for first-calf cows exposed (BE) or not exposed (NE) to mature bulls

Item	Treatment		
	BE	NE	SE
n	25	24	
CD ^a	3.6	3.6	1.2
BW (kg)	75.9	75.1	6.7
CSR	.48	.30	
CSC	.76	.81	.54
WC (kg)	16	9	22
PPIE (d)	62*	81	18
PPIUI (d)	32	34	8

^aSee Materials and Methods for CD criteria and scoring.

*P<.05.

Table 2. Least-squares means for effects of calving difficulty (CD) and sex of calf (CS) on postpartum interval to estrus (PPIE) and uterine involution (PPIUI) for first-calf cows

Effects	n	PPIE ^C	PPIUI ^C
Calving Difficult ^a			
AS	19	72	35
NA	30	71	31
Sex of Calf ^b			
HC	20	77	33
BC	29	66*	33

^aAS = Assistance; NA = No Assistance.

^bHC = Heifer calf; BC = Bull calf.

^cPooled standard errors for PPIE and PPIUI equaled 17.4 and 7.7d, respectively.

*P<.06 for sex of calf.

Proportions Exhibiting Estrus at Different
Times Postpartum

Proportion of BE cows that exhibited estrus by Day 45 postpartum did not differ ($P > .10$) from NE cows (4 of 24 and 4 of 25, respectively; Figure 1). However, a greater proportion ($P < .05$) of BE cows had exhibited estrus by 60 and 90 d postpartum compared with NE cows (11 of 25 and 20 of 25 for BE cows, respectively and 6 of 24 and 11 of 24 for NE cows, respectively; Figure 1). Cows exhibiting estrus by 108 d postpartum did not differ ($P > .10$) between BE and NE treatments (22 of 25 and 18 of 25, respectively; Figure 1). There was no interaction ($P > .10$) between treatment and days postpartum for proportions of cows that exhibited estrus.

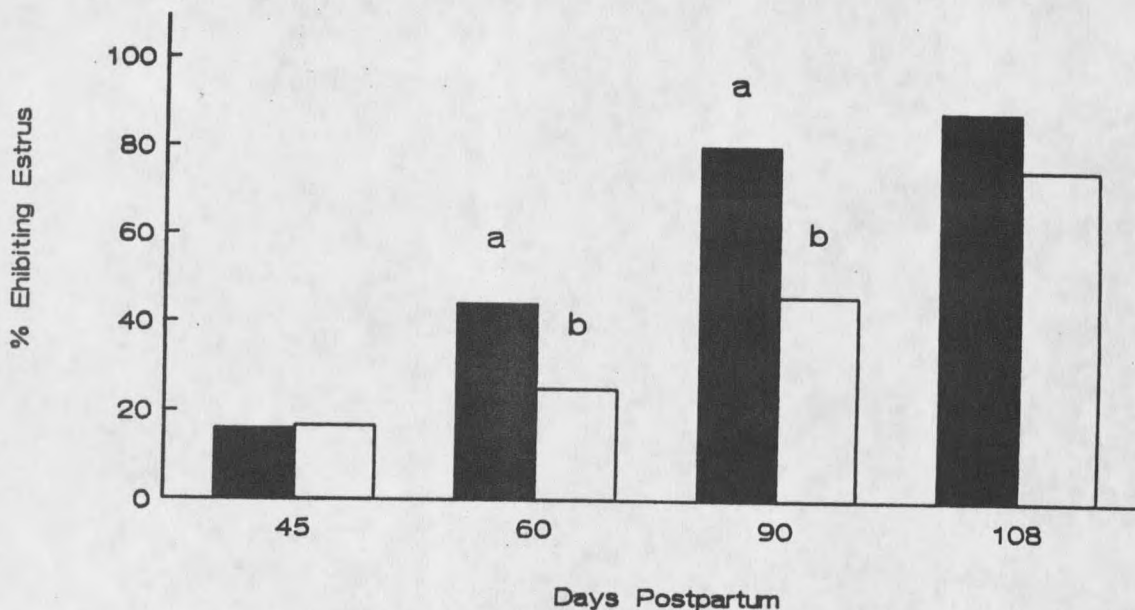


Figure 1. Cumulative distribution of percentages of first-calf cows exposed (BE; ■) or not exposed (NE; □) to mature bulls that exhibited estrus by 45, 60, 90 and 108 d postpartum. Letters above bars indicate difference between treatments at $P < .05$.

Hormone Patterns

Progesterone. There was no difference ($P > .10$) in the proportion of cows that showed an increase in progesterone prior to exhibiting first postpartum estrus between treatments (Table 3).

Luteinizing Hormone. Figure 2 illustrates the method employed for derivation of regression coefficients. The example is for frequency of IH pulses from original data. Regression coefficients for mean and baseline concentrations of IH, frequency, amplitude and duration of episodic IH pulses did not differ ($P > .10$) between BE and NE cows throughout the study (Table 4). There were no differences ($P > .10$) in regression coefficients for characteristics of pulsatile IH patterns between BE and NE cows that were observed in estrus (Table 5) and there were no differences ($P > .10$) in the regression coefficients for mean and baseline concentrations of IH, frequency, amplitude and duration of episodic IH pulses between BE and NE cows for the six weeks before estrus (Table 6).

Table 3. Proportion (%) of first-calf cows exposed (BE) or not exposed (NE) to mature bulls which showed an increase in progesterone prior to first estrus

Treatment	Proportion(%)		χ^2
BE	11/22	(50)	2.03*
NE	5/18	(27)	

* $P = .16$.

Table 4. Least-squares means and pooled standard errors (SE) of estimated regression coefficients for characteristics of luteinizing hormone patterns of first-calf cows exposed (BE) or not exposed (NE) to mature bulls

Item	Estimates of Regressions for Treatments		
	BE	NE	SE
n	25	24	
Mean IH ^a	.039	.025	.031
Baseline IH ^a	.032	.011	.026
Frequency of IH pulses ^b	.158	.167	.073
Amplitude of IH pulses ^a	.031	.044	.039
Duration of IH pulses ^c	-.006	.373	.477

^a(ng·ml⁻¹)·wk⁻¹.

^b(pulses·6 h⁻¹)·wk⁻¹.

^c(min·pulse⁻¹)·wk⁻¹.

Table 5. Least-squares means and pooled standard errors (SE) of estimated regression coefficients for characteristics of luteinizing hormone patterns of first-calf cows exposed (BE) or not exposed (NE) to mature bulls that exhibited estrus

Item	Estimates of Regressions for Treatment		
	BE	NE	SE
n	7	4	
Mean IH ^a	.052	.009	.11
Baseline ^a	.044	-.002	.09
Frequency of IH pulses ^b	.195	.104	.16
Amplitude of IH pulses ^a	.047	.021	.13
Duration of IH pulses ^c	-.318	.016	1.12

^a(ng·ml⁻¹)·wk⁻¹.

^b(pulses·6 h⁻¹)·wk⁻¹.

^c(min·pulse⁻¹)·wk⁻¹.

Table 6. Least-squares means and pooled standard errors (SE) of estimated regression coefficients for characteristics of luteinizing hormone patterns for the six weeks before first estrus, of first-calf cows exposed (BE) or not exposed (NE) to mature bulls

Item	Estimates of Regression for Treatment		
	BE	NE	SE
n	7	4	
Mean LH ^a	.065	.057	.15
Baseline LH ^a	.052	.054	.12
Frequency of LH pulses ^b	.243	.054	.20
Amplitude of LH pulses ^a	.023	.058	.25
Duration of LH pulses ^c	-2.530	-1.720	3.17

^a(ng·ml⁻¹)·wk⁻¹.

^b(pulses·6 h⁻¹)·wk⁻¹.

^c(min·pulse⁻¹)·wk⁻¹.

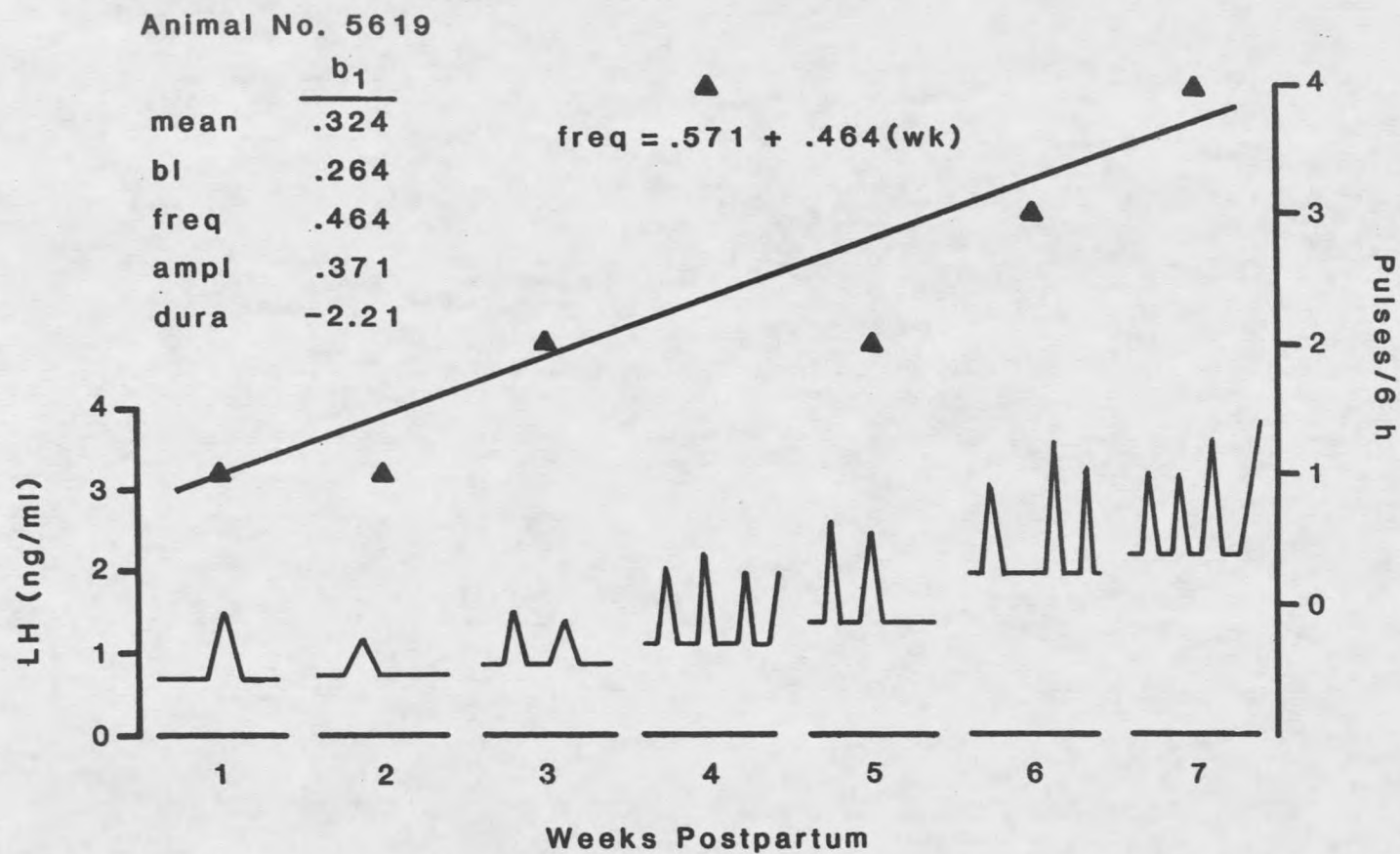


Figure 2. Illustration of weekly patterns of LH concentrations collected at 15-min intervals for 6 h. Triangles represent pulses of LH in a 6 h period per week. The straight line represents the regression of LH pulses $\cdot 6 \text{ h}^{-1}$ on week. Units for regression coefficients (b_1) for mean, baseline (bl) and amplitude (ampl) are $(\text{ng} \cdot \text{ml}^{-1} \cdot 6 \text{ h}^{-1}) \cdot \text{wk}^{-1}$ and for frequency (freq) and duration (dura) are $(\text{pulses} \cdot 6 \text{ h}^{-1}) \cdot \text{wk}$ and $(\text{min} \cdot 6 \text{ h}^{-1}) \cdot \text{wk}^{-1}$, respectively.

DISCUSSION

The interval from calving until the onset of estrus in the beef cow has been reported to be influenced by many factors: level of nutrition, body condition at calving, degree of calving difficulty, suckling, sex of calf and social interactions. In the present experiment, nutritional level, reflected by body weight change throughout the postpartum period, did not differ between treatments. Body condition at calving, degree of calving difficulty, sex ratio of calves and condition score change throughout the study did not differ between treatments. Since these factors, which are known to influence the postpartum interval to estrus, did not differ between treatments, any difference in the interval to estrus in these first-calf cows should have been primarily a result of treatment.

There was no difference in the time to uterine involution due to treatment, calving difficulty or sex of calf or their interactions in this experiment. These findings support the conclusion that any difference in the interval to estrus was due to the treatment employed. These data are in agreement with earlier studies (Perkins and Kidder., 1963; Tennant and Peddicord., 1968) in which the authors concluded that time to uterine involution is not a limiting factor in determining the onset of estrus after calving.

We found that calving difficulty did not influence the interval from calving to estrus in first-calf suckled beef cows in this study, however, cows with bull calves exhibited estrus earlier than those with heifer calves. This was unexpected, since Bellows et al. (1982)

reported that dams nursing bull calves tended to return to estrus more slowly than dams nursing heifer calves. An explanation for the contradictory results of the present study eludes us, however, the hypothesis that bull calves suckle more aggressively enhancing the inhibitory effect of suckling may not be applicable to first-calf cows.

Cows exposed to mature bulls resumed cycling activity approximately 19 d earlier than cows not exposed to bulls. The difference in interval to estrus between BE and NE cows was directly related to exposure to a mature bull throughout the postpartum period. Furthermore, progesterone concentrations subsequent to estrus and the presence of a palpable corpus luteum confirmed that cows that had exhibited estrus during the experimental period had also ovulated in conjunction with estrus. These results are similar to those reported for mature cows exposed to bulls during the postpartum period (Zalesky et al., 1984; Alberio et al., 1987; Naasz and Miller., 1987).

By Day 45 postpartum, the proportion of cows that had exhibited estrus did not differ between BE and NE cows. However, by Day 60 and 90 postpartum, a greater proportion of BE than NE cows had exhibited estrus, but by Day 108 postpartum there was no difference in the proportion of BE and NE cows that had exhibited estrus. There was no interaction between the proportion of cows exhibiting estrus and days postpartum. The effect of bull exposure on resumption of cycling activity in first-calf suckled beef cows does not seem to be immediate, but rather appears to require some time to alter physiological systems required in resumption of ovarian cycling activity. In support of this hypothesis, Alberio et al. (1987) reported that a greater percentage of

mature cows exposed to bulls at 58 d postpartum exhibited estrus and ovulation by the end of the experimental period. Similar results were reported when mature bulls were introduced to spring-calving cows 21 d prior to the beginning of the breeding season (Scott and Montgomery, 1987). In a study similar to ours, Zalesky et al. (1984) initiated bull exposure at approximately 3 d postpartum and concluded that the bull elicits his effect on mature cows sometime before Day 58 postpartum.

We found no difference in proportions of BE and NE cows that had a preestrus progesterone rise (11/22 and 5/18, respectively). However, the lack of a statistical difference may be a result of the small number of animals from each treatment that actually had a preestrus progesterone rise. Lavoie et al. (1981) reported a rapid 1 d increase in progesterone prior to first estrus and suggested that the rise in progesterone may be of such short duration that it could be missed in an infrequent sampling regimen, such as was carried out in the present study. This may explain why the preestrus progesterone rise was not detected in a larger percentage of our cows. In agreement with our findings, several studies have reported a preestrus rise of progesterone in postpartum suckled beef cows (Corah et al., 1974; Lavoie et al., 1981; Humphrey et al., 1983). Faltys (1985) suggested that the increase in progesterone seen prior to normal estrus cycles in postpartum suckled beef cows may act to prime an endocrine system that has been acyclic during gestation and the early postpartum period. If this is indeed so, then the proportion of cows that had a preestrus

progesterone rise, which tended to be greater in the BE cows may shed some light on the mechanism of the "bull effect" in postpartum cows.

The patterns of IH release in weekly sampling intervals, which include mean, baseline, frequency, amplitude and duration of episodic pulses, tended to increase with time postpartum in the present study. This is in agreement with early studies which reported increases in the patterns of IH release as time to first postpartum estrus decreased (Arije et al., 1974; Rawlings et al., 1980; Humphrey et al., 1983). However, the rate with which these IH patterns increased, evaluated by estimates of the regression coefficients, did not differ between BE and NE cows in this experiment. This was unexpected, since it has been shown in mice that exposure of peripuberal or adult females to a mature male results in an immediate elevation in IH secretion (Bronson., 1976; Bronson and Desjardins, 1974). Also it has been reported that exposure of anestrus ewes to a mature ram during the middle of the non-breeding season increased plasma IH levels (Chesworth and Tait., 1974). Since then, Martin et al. (1980) and Poindron et al. (1980) reported that IH pulse frequency increased within 10 to 40 min following the introduction of mature rams to seasonally anestrus ewes. In a more recent study, Oldham and Pearce (1984) reported an increase in the pulse frequency of IH in ewes exposed to mature rams compared to isolated ewes.

The physiological and(or) endocrine bases of the "bull effect" are unclear. Exposure of a mature bull to first-calf cows throughout the postpartum period did not result in an increase in either the concentration or pulse frequency of IH as time postpartum increased or

during the six weeks before first estrus in this study. However, it would seem that the presence of a bull, initiated at 3 d postpartum had a tendency to increase the proportion of cows that showed a preestrus progesterone rise.

Although we did not see any difference in the pattern of IH release throughout the postpartum period between treatments, this does not preclude the possibility that the effect of the bull on the resumption of cycling activity is mediated via the central nervous system by stimulating IH release immediately following exposure. Our sampling regimen for IH would not have enabled us to detect such an immediate change. Studies in sheep and mice, cited earlier, would support the idea that exposure to the bull would result in an increase in the plasma levels and pulse frequency of IH within minutes or hours following introduction. If in fact IH levels were increased shortly after introduction of the bull, it would seem that estrogen synthesis would increase and this may enhance the ability of the ovary to maintain follicles that might otherwise have become atretic. These follicles might then have had an opportunity to mature at an early time postpartum and could account for the reduction in the postpartum interval that we detected in the BE cows.

In a study with anestrous ewes, Knight et al. (1978) reported that there was no change in estradiol-17 β concentrations associated with the increase in IH following the introduction of the ram. These observations led the authors to suggest that the rams may have stimulated a direct release of IH which was not dependent on the stimulation of the pre-ovulatory center within the hypothalamus by

estradiol-17 β . Based on these data one might hypothesize that the "bull effect" acts to increase the sensitivity of the pre-ovulatory center within the hypothalamus to estradiol-17 β without having any dramatic effect on the secretory patterns of LH or estradiol-17 β prior to the pre-ovulatory LH surge. This would account for the fact that we failed to see any difference in the pattern of LH secretion between the treatment groups, but could explain the difference in the interval to estrus between the BE and NE cows.

Another possible explanation for the mechanism by which the presence of the bull hastened the onset of estrus in these first-calf cows may be by acting directly on the sensitivity of the ovary to circulating levels of LH via some chemical unique to bulls and passed to the female. Inskeep et al. (1988) reported that suckled beef cows which received an implant of norgestomet for 9 d at approximately 23 d postpartum had a greater number of LH receptors than did controls. In the present study, there appeared to be a tendency for a greater proportion of cows exposed to bulls to have an increase in progesterone prior to their first estrus. This increase in progesterone may have increased the sensitivity of the ovary to LH by increasing the number of LH receptors. This could account for the differences observed in the interval to estrus between the BE and NE cows and also further support the fact that there were no difference in the LH patterns between the treatments.

We did not look at FSH levels in this study, however if FSH levels increased in the BE cows this might act to increase the quantity of LH receptors on follicles which would increase the sensitivity of the

follicles to IH. Ramirez-Godinez et al. (1982) reported that FSH levels were greater during a 4-d period preceding the pre-ovulatory surge of a normal estrous cycle induced by early weaning, but found no difference in the levels of IH. If indeed the presence of the bull acts to override the inhibitory effect of suckling we might have observed an increase in FSH in cows exposed to bulls. The increase in estrogen associated with the increasing sensitivity of the ovary to IH would enable the postpartum cow to escape from the inhibitory effects of low levels of estrogen on the hypothalamus as was hypothesized by Acosta et al. (1983). If this event occurred just prior to estrus we would not have been able to detect any changes in the pattern of IH secretion in the BE cows. It is evident from this study that the way in which the bull acts to hasten the onset of ovarian activity in the postpartum cow remains elusive, but many possibilities exist.

In conclusion, exposure of first-calf suckled beef cows to mature bulls throughout the postpartum period was effective in reducing the time from calving until the onset of estrus. It is evident from the results of this experiment that the presence of the bull does not hasten the onset of estrus in postpartum first-calf suckled beef cows by altering the pattern of IH release throughout the postpartum period. However, the presence of a mature bull tended to increase the proportion of cows that showed a preestrous progesterone rise and it would seem that this may be an important event in determining the mechanism by which bull exposure hastens the resumption of reproductive activity in the postpartum cow.

PRACTICAL APPLICATION AND FUTURE RESEARCH

Maintaining calving intervals of less than one year in beef cattle herds requires conception in cows by at least 85 d postpartum. It is not unusual for first-calf suckled beef cows to have postpartum intervals in excess of 90 d, which can decrease the reproductive efficiency of the cow throughout her lifetime. Based on the data from this study, it was evident that the presence of mature bulls throughout the postpartum period was effective in reducing the interval from calving to first estrus by 19 d. This reduction in time to first postpartum estrus could have a dramatic impact on the reproductive capabilities of cows. By exhibiting estrus during the early part of the postpartum period, cows will have gone through at least one complete cycle before the breeding season. This should enhance a cow's opportunity to become pregnant during the early part of the breeding season. The advantage gained during the early part of a cow's life should result in an increase in the reproductive efficiency of the cow throughout her lifetime.

Pregnancy rates associated with the first postpartum estrus were not obtained in the present study due to management constraints on the experimental herd. However, Zalesky et al. (1984) reported that mature cows exposed to bulls had a conception rate associated with their first postpartum estrus of approximately 50 %. Conception rate associated with first postpartum estrus was lower than the authors had expected. However, Reeves and Gaskins (1981) reported that conception rates in beef cows tended to be lower if estrous cycles were induced by once

daily suckling early in the postpartum period. Inducing postpartum estrus may result in lower conception rates associated with first postpartum estrus regardless of the method employed for inducing early resumption of cycling activity. Therefore it is essential to determine whether pregnancy rates of estrous cycles induced by mature bulls are lower than those not exposed to bulls.

The experimental evidence from this study leads one to the conclusion that bull-cow interactions influence reproductive activity in first-calf suckled beef cows, however, the physiological basis of this effect warrants further investigation. Experiments designed to answer the following questions may enable researchers to determine the mechanisms underlying the "bull effect". Does exposure to a mature bull throughout the postpartum period: 1) alter concentrations and(or) pulse frequency of IH soon after exposure; 2) alter concentrations of FSH; 3) increase the sensitivity of the hypothalamic preovulatory center to the positive feedback effects of estradiol-17 β ; and 4) increase the sensitivity of the ovaries to IH by increasing the number of IH receptors in follicles.

Finally, from an economical viewpoint, it appears that the male to female ratio used in this study would not be feasible to beef cattle producers. Therefore a study designed to examine the minimum male to female ratio that would be effective in reducing the interval from calving to first postpartum estrus is essential.

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APPENDIX

Radioimmunoassay for Bovine Luteinizing Hormone

Reagents and Solutions. The following reagents and solutions were used in the radioimmunoassay of bovine luteinizing hormone (bLH).

Reagents: all of the following were obtained from Sigma Chemical Company, St. Louis, MO 63178.

Sodium Phosphate, Monobasic
 Sodium Phosphate, Dibasic
 Sodium Chloride
 Phenol Red (pH indicator)
 Thimerosal (bacteriostat)
 Ethylenediaminetetraacetic Acid (EDTA)
 Polyethylene glycol (PEG)
 Trichloroacetic Acid (TCA)
 Bovine Serum Albumin, Fraction V (BSA)

Solutions: (all solutions except sera were prepared in glass-distilled water)

Phosphate Buffered Saline, PBS ($\text{PO}_4 = .01 \text{ M}$; $\text{NaCl} = .14 \text{ M}$; Thimerosal = .01 %; phenol red = .005 % ; Ph = 7.0)
 Phosphate Buffer (.5 M, pH = 7.0)
 EDTA-PBS (EDTA = .05 M in PBS)
 PBS-BSA (BSA = .1 % in PBS)
 Normal Rabbit Serum (NRS)^a
 NRS-EDTA-PBS (NRS:EDTA-PBS = 1:400, v/v)
 Sheep Anti Rabbit Gammaglobulin (SARGG)^a
 SARGG-PBS (SARGG:PBS = 1:20, v/v)
 Bovine LH anti-serum (Ab-bLH, rabbit #16)^a
 Ab-bLH-NRS-EDTA-PBS (Ab-bLH:NRS-EDTA-PBS = 1:40,000, v/v)
 PEG-PBS (6 and 22.5 % in PBS)
 TCA (10 % in distilled water)

^aGenerously provided by Dr. R.B. Staigmilller, USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301.

Principle of Assay. The radioiodination procedure used in this study were based on the following principles. Unknown concentrations of hormone can be determined since radiolabeled hormone molecules compete physiochemically with unlabeled hormone for limited numbers of binding sites on an antibody. It is essential that the standard and unknown antigen be equal in their ability to displace labeled antigens from a labeled antigen-antibody complex. As one increases the amount of unlabeled hormone, the limited binding sites on the antibody become progressively saturated, therefore the antibody can bind less of the radiolabeled hormone. The assay antiserum is usually diluted to insure about 50 % binding of the labeled hormone in the absence of unlabeled hormone. If there is limited non-specific binding, a diminished binding of labeled hormone indicates the presence of unlabeled hormone. Following the incubation of the labeled hormone, unlabeled hormone and antibody the bound hormones are separated from the unbound hormones and the radioactivity of the bound hormone was determined.

Radioiodination. Bovine IH (LER-1072-1) obtained from Dr. L.E. Reichert Jr. was used for iodination. Radioiodination of bIH was accomplished in the following manner. Sixty ul of .01 M phosphate buffer (pH = 7.0) was added to a 12 x 75 mm glass culture tube (reaction vessel) which contained 2 ug of Iodo-Gen (Pierce Chemical Company, Rockford, IL 61105). Twenty-five ul of bIH (LER-1072-1; 5 ug IH·25 ul⁻¹ distilled water) was added to the reaction vessel and mixed thoroughly. Approximately 9.6 ul of .5 M phosphate buffer was added to the vial containing NaI¹²⁵ (pH = 8-10; New England Nuclear, catalog no.

NEZ033L) and this solution was added to the reaction vessel and the reaction was allowed to continue for 3 min while being agitated to insure adequate incorporation of I^{125} to bIH.

Separation. Free I^{125} was separated from I^{125} that was incorporated onto the protein, by gel chromatography. The entire solution from the reaction vessel was pipetted onto the top of a Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, NJ 08854) column (25.6 x .7 cm) and eluted with BSA-PBS. Thirty-five fractions of approximately .5 ml were collected from this column. The elution pattern in counts per minute (cpm) of the I^{125} from this column is shown in Figure 3. Two peaks are readily observed, the first representing I^{125} incorporated with protein and the second free I^{125} based on molecular weight and characteristics of Sephadex G-75. Fractions, representing I^{125} incorporated protein, were pooled for subsequent purification on a Sephadex G-75 column (52.2 x 1.76 cm). The elutant was BSA-PBS and the first 25 1 ml fractions, which represented the void volume, was discarded. An additional 100 fractions were collected and the elution pattern is shown in Figure 4.

% Incorporation and Specific Activity. The large broad peak shown in Figure 3 was subjected to procedures to determine the specific and immunologic activity of the iodinated bIH. One hundred ul from fractions collected at the beginning, middle and end of the radioactive peak were diluted 1:20 in BSA-PBS, then 100 ul from these diluted fractions was added to 100 ul of BSA-PBS and 200 ul of a 10 % solution of TCA in glass culture tubes (12 x 75 mm) for precipitation of

protein. Tubes were vortexed and placed into a centrifuge (4 C) for 10 minutes. After 10 min, tubes were centrifuged for 30 minutes at 1000 x g. Following centrifugation the supernatant was decanted and the radioactivity (cpm) was determined for the pellet and supernatant on a Packard Auto-Gamma Scintillation Spectrometer, which had an efficiency of 62 %. Percent incorporation of I^{125} with protein (bLH) was determined by dividing the sum of the cpm of the supernatant and pellet into the cpm of the pellet and expressing the quantity as a percentage. Table 7 gives data for % incorporation of a typical elution pattern.

Table 7. Percentage of I^{125} incorporated with protein collected from the "broad peak" of a Sephadex G-75 column (52.2 x 1.76 cm)

Fractions	% Incorporated
30	83.9
36	87.4
42	77.2
50	92.4
59	78.9
68	82.5
77	79.1
mean = 83.1	

The specific activity of the protein contained in representative fractions was determined by taking the average % incorporation, multiplying it by the uCi of I^{125} used for iodination and dividing this quantity by the amount of bLH added to the reaction vessel. The specific activity for a typical radioiodination with percent incorporation of 83.1 was $166.2 \text{ uCi} \cdot \mu\text{gLH}^{-1}$.

Immunoprecipitate Test. After determining % incorporation, fractions from the beginning, middle and end of the broad peak from the Sephadex column were evaluated for immunoreactivity with (R #16) Anti-bLH. Two hundred ul of "raw" antiserum antibody and 200 ul of BSA-PBS were added to 100 ul of fractions to approximately 10,000 cpm · 100 ul. Tubes were vortexed and incubated at room temperature (23 C) for 24 hours. Following incubation, 100 ul of a 1:1 NRS-PBS (v/v) and 1 ml of ice-cold 22.5 % PEG was added to each tube. Tubes were incubated for 10 min at 4 C, then centrifuged for 30 min at 1000 x g. Pellets and supernatants were separated as previously described and radioactivity in these were determined. Percent immunoreactivity of fractions were determined using the same formula for determining % incorporation. The average percent immunoreactivity of a typical iodination was approximately 81 %. Seventy percent immunoreactivity was determined to be acceptable. Fractions containing this or higher percent immunoreactivity were pooled and diluted to approximately 30,000 cpm · 100 ul⁻¹ in BSA-PBS for use in radioimmunoassays.

Validation

Specificity. The antiserum to bLH used in this radioimmunoassay cross-reacted negligibly with other purified pituitary hormones (Dr. R.B. Staigmilller, personal communication). The antiserum (R #16 Anti-bLH) was used at a titer of 1:40,000 in 1:400 (v/v) NRS-EDTA-PBS, which had a percent binding of approximately 40 %.

Accuracy, Parallelism, Sensitivity and Precision. Accuracy or the ability to measure known quantities of bLH in serum was evaluated by adding 100 ul of each standard (prepared from NIADDK bLH-4, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Washington D.C.), which ranged from .15625 to 10 ng per tube, to a known quantity of bLH in 50, 100 and 200 ul of ovariectomized (ovx) bovine serum. Percent recovery was determined by dividing the amount of bLH recovered by the total quantity of bLH added to each sample and the percent recoveries for 50, 100 and 200 ul of ovx serum are shown in Table 8.

Table 8. Percent recovery of bovine LH in standards and 50, 100 and 200 ul of ovariectomized cow serum

Standards ^a	% Recovery in ovx serum		
	50ul	100ul	200ul
.15625	92	89	101
.31250	92	90	98
.62500	99	91	93
1.2500	104	99	99
2.5000	114	103	103
5.0000	116	123	123
10.000	154	158	183

^angLH·tube⁻¹

A volume dilution test for parallelism was carried out with 50, 100 and 200 ul in triplicate aliquants of ovariectomized bLH serum using an antiserum dilution of 1:40,000. The parallelism between assay standards and ovariectomized bovine serum samples indicated that we

were measuring the same molecule that was being detected in the standards.

Sensitivity, the smallest amount of unlabeled antigen that can be distinguished from no antigen, was determined in ovariectomized bovine serum and was found to be $.11 \text{ ng} \cdot \text{tube}^{-1}$ which corresponded to 50 ul of serum.

Precision was defined as the amount of variation in the estimation of unlabeled antigen in the assay system. The precision of this assay was measured by coefficients of variation for serum that yielded a percent binding of approximately 60 % and the inter- and intra-assay variations were 11.9 and 9.8 %, respectively.

From these results we concluded that the radioimmunoassay was sufficiently specific, sensitive, accurate and precise to reliably measure from .15 to 5 ng of IH in samples of bovine serum.

Serum samples collected in this study were analyzed using the following procedure. Duplicates of serum samples, standards, ranging from .15 ng to 10 ng per tube, total count tubes, non-specific binding tubes (NSB), ovariectomized bovine serum samples and triplicates of serum samples with high and low concentrations of IH were assayed in each assay. One-hundred percent binding tubes (tubes which contained no unlabeled antigen) were assayed in duplicate at the beginning and end of each standard curve. Standard curves were placed at the beginning and end of each assay. The volume of each sample, ovariectomized, high and low pool of serum was 200 ul. The volume of the standards and the 1:400 v/v NRS-EDTA that was used for the NSB tubes was 100 ul. Each reaction tube received 100 ul of R #16 Anti-

bovine-IH except for the total count and NSB tubes. All reaction tubes in the assay received 100 ul of bIH-I¹²⁵. BSA-PBS was added to each reaction tube to maintain the assay volume at 700 ul. All reaction tubes were vortexed and incubated at 4 C for 72 h. After the primary incubation, 200 ul of SARGG was added to all reaction tubes except for the total count tubes and incubated at 4 C for 48 h. Then 1 ml of ice cold 6 % PEG was added to each reaction tube and immediately placed into the centrifuge for a period of 10 min. Reaction tubes were centrifuged for 30 min at 1000 x g at 4 C. After centrifugation the supernatant of each reaction tube was decanted and dried for approximately 20 min and then measured for radioactivity in the Packard Auto-Gamma Scintillation Spectrometer for 1 min.

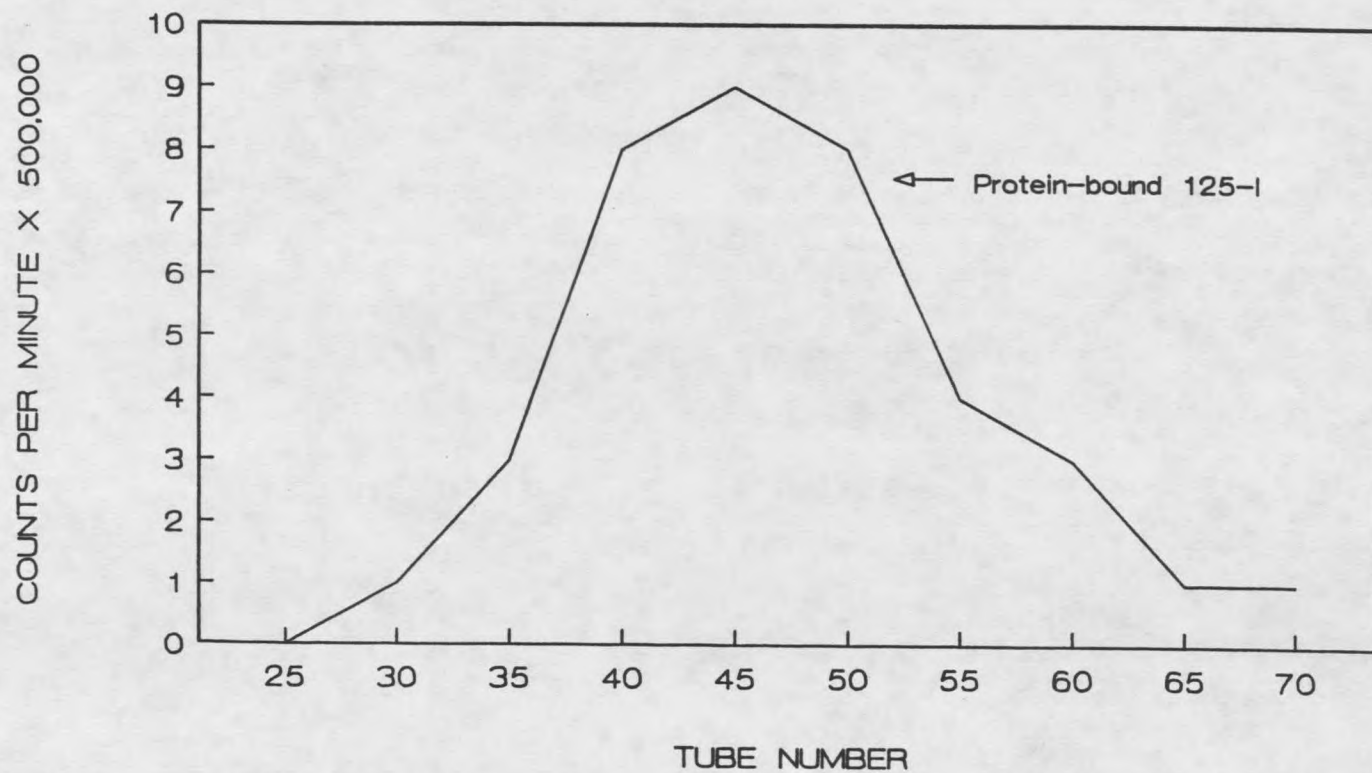
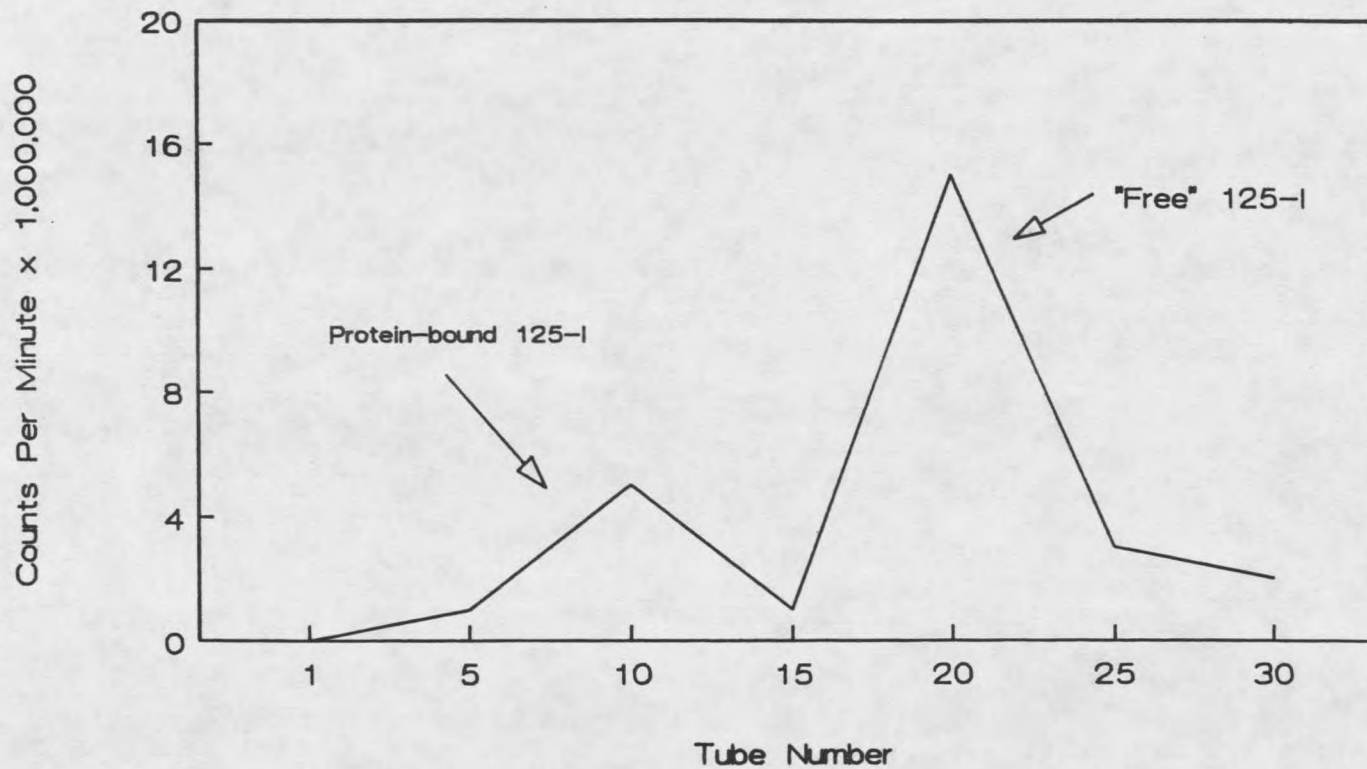


Figure 3. Typical elution pattern of ¹²⁵I incorporated with protein and free ¹²⁵I from an iodination of bIH on a Sephadex G-75 column (25.6 x .7 cm). Tube number represents .5 ml fractions.



98

Figure 4. Typical elution pattern of protein-bound fractions collected from a 25.6 x .7 cm Sephadex G-75 column eluted on a 52.2 x 1.76 cm Sephadex G-75 column. Tube number represents 1.0 ml fractions.

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