INFLUENCE OF NUTRITION ON PREPARTUM ENDOCRINE FUNCTION OF BEEF COWS

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CHAPTER I

INTRODUCTION

Reproductive performance is one of the most important aspects of beef cattle production. It is economically important for beef cows to produce a calf at yearly intervals. Consequently, a cow must become pregnant within 85 days following calving. However, one of the most common problems associated with reproduction in beef cattle is an abnormally long period from parturition to first estrus, commonly referred to as the postpartum anestrous interval. Several factors are known to influence the length of the postpartum interval including breed of cow, suckling intensity, age of cow and nutrition.

Nutritional intake, as reflected in body condition, during the last trimester of gestation has been demonstrated to be one of the most important factors affecting the postpartum reproductive performance of beef cows. Cows in moderate or thin body condition that lose body condition during pregnancy generally have longer intervals from parturition to estrus than those that maintain body condition throughout pregnancy. However, the specific mechanism by which prepartum nutrition influences reproductive performance has not been elucidated.

During the last three months of gestation, the fetus and placenta are growing rapidly and producing various hormones associated with reproduction. Dynamic changes are evident in plasma concentrations of progestrone, estrone, estradiol and estrone sulfate during late pregnancy. Therefore, it seems likely that at least part of the influence of nutrition on postpartum ovarian function is mediated through the endocrine system. However, little information is available on the influence of nutrition on prepartum endocrine function. An evaluation of endocrine responses to changes in body condition during pregnancy would aid in the understanding of the physiological mechanism by which nutrition influences postpartum reproduction. The purpose of this study was to examine the effects of nutrition, as reflected by body condition, on endocrine function during late gestation in beef cows.

CHAPTER II

LITERATURE REVIEW

Postpartum Reproduction in Cows

The postpartum interval is the period from parturition to some designated event. This event may be the first ovulation, the first estrus, the completion of uterine involution, the first breeding or conception (Casida et al., 1968). However, in beef cows the most commonly studied postpartum interval is the period from calving to first estrus.

The length of the postpartum interval to first estrus varies between species. In the ewe, the postpartum anestrous period usually extends from lambing in the spring until the resumption of estrous activity the next autumn. In sows, there is usually complete anestrous during lactation. The postpartum anestrous interval of the cow is probably the most variable. For example, Casida et al. (1968) summarized data that demonstrated that the average anestrous interval for dairy cows ranged from 30 to 72 days after calving compared to 46 to 104 days for beef cows. However, due to differences in management practices, comparisons of estrous activity between beef and dairy cows

are difficult.

Ovarian Function Postpartum

The corpus luteum of pregnancy begins to regress by two to four days after parturition (Oxenreider, 1968) and has degenerated by seven days postpartum (Wagner and Hansel, 1969). Consequently, postpartum anestrous is not a result of the maintenance of the corpus luteum of pregnancy (Labhsetwar et al., 1964). Moreover, uterine involution occurs rapidly after parturition (Wagner and Hansel, 1969; Kiracofe, 1980).

Follicular activity can begin soon after parturition (Morrow, 1969; Wagner and Hansel, 1969; Moller, 1970) resulting in the presence of small corpora lutea (Moller, 1970); however, these corpora lutea are inactive and the ovulations that lead to their development are usually infertile. In dairy cows, ovulation and luteal development increases during the postpartum interval as evidenced by increases in average progesterone concentrations in the plasma (Edgerton and Hafs, 1973; Ferrandes et al., 1978).

The average interval from parturition to first ovulation in beef cows is between 36 and 71 days (Casida et al., 1968). However, the first ovulation may or may not be preceeded by behavioral estrus (Graves et al., 1968; Wettemann et al., 1978). For example, Kiracofe (1980) summarized data that revealed the first ovulation occurred between 35 and 60 days postpartum while the first estrus

occurred between 45 and 85 days. The occurrence of ovarian activity before the first estrus is confirmed by increases in the concentration of progesterone in the plasma before the first estrus (Donaldson et al., 1970; Arije et al., 1974; Corah et al., 1974; LaVoie et al., 1981). The increase in progesterone may come from a corpus luteum formed from the first postpartum ovulation (Ward et al., 1979). Connor et al. (1974) speculated that the lutenization of follicles may be the source of the increase in progesterone which preceeds the first postpartum estrus.

Endocrine Function During the Prepartum and Postpartum Periods in Cows

Significant changes occur in the endocrine function of cows during the prepartum and postpartum periods. During pregnancy, the placenta, ovary and adrenals are involved in steriod hormone production. Consequently, dramatic changes occur in endocrine function following parturition, with the loss of the placenta and the corpus luteum of pregnancy. In addition, the ovary is relatively inactive during the early postpartum period. Therefore, it is likely that prepartum endocrine function may regulate postpartum ovarian activity (Wettemann, 1980). An evaluation of pre- and postpartum endocrine function will clarify the interrelationship of the two.

Progesterone

During pregnancy the corpus luteum is the major source of progesterone in the cow (Gorski et al., 1958). However, the placenta as well as the adrenal glands also produce significant amounts of progesterone during pregnancy (Balfour et al., 1957; Ainsworth and Ryan, 1967; Wendorf and First, 1977). For example, removal of the ovaries or the adrenal glands in late pregnancy does not lead to abortion; however, removal of both ovaries and adrenals does result in abortion (Wendorf and First, 1977).

Concentrations of progesterone in the plasma during early and mid gestation average about 10 ng/ml (Donaldson et al., 1970; Arije et al., 1974). One day prior to parturition, progesterone concentrations begin to decrease to less than 1 ng/ml (Donaldson et al., 1970; Edquist et al., 1973; Smith et al., 1973; Arije et al., 1974; Wise et al., 1975). The regression of the corpus luteum of pregnancy as well as the expulsion of the placenta are the primary causes for the reduction in progesterone concentrations at parturition (Ainsworth and Ryan, 1967; Oxenreider, 1968; Wagner and Hansel, 1969).

Following parturition, progesterone concentrations remain below 1 ng/ml through the majority of the postpartum anestrous interval (Donaldson et al., 1970; Arije et al., 1974; Kesler et al., 1977; Webb et al., 1977; Edquist et al., 1978; Kesler et al., 1980). Concentrations of

progesterone in plasma equal to or greater than 1 ng/ml are usually associated with the presence of a lutenized follicle or corpus luteum and consequently progesterone in plasma is a good indicator of ovarian activity (Stabenfeldt et al., 1969; Swanson et al., 1972). Prior to the first postpartum estrus concentrations of progestrone usually increase from less than 1 ng/ml to 2.0 ng/ml. This increase in progesterone usually occur 2 to 4 days before the beginning of normal estrus activity (Pope et al., 1969; Donaldson et al., 1970; Arije et al., 1974; Corah et al., 1974; LaVoie and Moody, 1976; Dobson, 1978). In addition, Rawlings et al. (1980) reported that progesterone concentrations were low until 55 days postpartum when they increased to .5 ng/ml for 4 days then declined for 5 days and rose again to normal luteal-phase levels following the first estrus.

Estrogen

The placenta is the major source of estrogens in pregnant cows (Gorski, 1956; Veenhuizen, 1960). Estrone is the principal unconjugated estrogen in pregnant cows (Kesler et al., 1976; Chew et al., 1977). For example, at 26 days prepartum concentrations of estrone in plasma are 250 - 300 pg/ml compared to 50 pg/ml for estradiol 17-β (Smith et al., 1973). Robertson (1974) found estrone concentrations of 2 ng/ml compared to 150 pg/ml for estradiol 17-β eight days before parturition and the ratio for the concentration of estrone to the concentration of estradiol 17-β remained

constant at 10:1 during the last 40 days of gestation.

Concentrations of estrone sulfate, the principal conjugated estrogen in cows, increase dramatically in plasma during the last trimester of pregnancy (Eley et al., 1979). Moreover, concentrations of estrone sulfate are greater than concentrations of free estrone (Eley et al., 1979; Thatcher et al., 1980; Collier et al., 1981). For example, at day 111 of pregnancy, plasma estrone sulfate concentrations were 581.0 pg/ml compared to 11.5 pg/ml for free estrone and 4.9 pg/ml for estradiol 17-β (Eley et al., 1979).

Dramatic increases in estrogen in the plasma occur just prior to parturition (Smith et al., 1973; Arije et al., 1974; Robertson, 1974; Kesler et al., 1976). Between day 4 prepartum and the day of parturition, concentrations of estradiol increase by 150 pg/ml (50%) and similar, although not as large percentage wise, increases in estrone (Robertson, 1974) and estrone sulfate occurred (Thatcher et al., 1980; Collier et al., 1981).

Following parturition, plasma estrogens decrease rapidly (Mellin, 1966; Smith et al., 1973; Robertson, 1974; Arije et al., 1974). By four days postpartum, the ratio of estrone to estradiol 17-β decreases and estradiol 17-β becomes the major estrogen. Concentrations of estradiol 17-β at day 4 postpartum average 28 pg/ml compared to 14 pg/ml for estrone (Smith et al., 1973). Plasma estradiol concentrations vary during the early postpartum

period but are generally less than 10 pg/ml (Echternkamp and Hansel, 1973). For example, Kesler (1980) reported estradiol 17-β during the early postpartum period and are usually less than 5 pg/ml. Walters et al. (1982) reported concentrations of estrone were at 3.6 pg/ml in suckled cows compared to estradiol 17-β levels of 8.5 pg/ml at 22-25 days postpartum.

Concentrations of estrogens in plasma begin to increase about two to three days prior to the first postpartum estrus and this increase is due to follicular growth (Hendricks et al., 1972; Echternkamp and Hansel, 1973; Arije et al., 1974). Concentrations of estradiol increased to 16 pg/ml on the day of the first estrus and concentrations of estrone increased, but by only a third of that which occurred for estradiol (Echternkamp and Hansel, 1973). In contrast, Raminez and Godinex (1982) reported no increase in estradiol at the first postpartum estrus but an increase from 1.6 pg/ml 12 days before the second estrus to 5 pg/ml on the day of the second estrus. However, the data were highly variable and from only a small number of cows.

Luteinizing Hormone

Concentrations of luteinizing hormone (LH) in serum are relatively constant throughout pregnancy and remain unchanged at parturition (Schams et al., 1972; Ingalls et al., 1973;). Arije et al. (1974) reported that the concentration of LH in the serumum of three beef cows from

three weeks before until the day of parturition ranged between 0.4 and 1.4 ng/ml. Schams et al. (1972) demonstrated transitory increases in LH in serum on day 94 and 103 of pregnancy; however, this observation was made in only one animal.

Following parturition, basal LH concentrations begin to increase to around 2 ng/ml by day 30 postpartum (Edgerton and Hafs, 1973; Arije et al., 1974; Kesler et al., 1977; Ferrandes et al., 1978). Moreover, transitory increases of 3 to 5 ng/ml occur in LH concentrations from day 30 postpartum to just prior to the first postpartum estrus (Echternkamp and Hansel, 1973; Arije et al., 1974; Humphrey et al., 1976; Goodale et al., 1978; Carruthers et al., 1980; Walters et al., 1982) and the frequency of the episodic changes increase during the two weeks prior to the first estrus. Randel et al. (1981) reported episodic increases in LH concentrations with maximum values of 6.1 to 6.8 ng/ml occurring during the last 30 days before estrus; Stevenson and Britt (1979) observed increasing elevations in LH as the first ovulation approached. On the day of the first postpartum estrus, LH in serum is dramatically elevated and concentrations exceed 10 ng/ml (Echternkamp and Hansel, 1973; Arije et al. 1974; Stevenson and Britt, 1979). Following estrus, concentrations of LH return to less than 2 ng/ml (Echternkamp and Hansel, 1973).

Prolactin

Concentrations of prolactin in the serum of cows are greatly influenced by daylength and ambient temperature (Wettemann and Tucker, 1978). Consequently, these factors must be considered and they account for much of the variation in prolactin concentrations during pregnancy and the postpartum period.

Prolactin concentrations averaged about 15 ng/ml during late pregnancy in beef cows (Arije et al., 1974) and increased to a maximum of 348 ng/ml before parturition and decreased to 250 ng/ml at parturition. Furthermore, prolactin concentrations fluctuated around 150 ng/ml during the postpartum period (Arije et al., 1974). Similarly, Walters et al. (1982) reported prolactin concentrations of 143 ng/ml between day 22 and 25 postpartum. Concentrations of prolactin increased to 200 ng/ml three days before the first estrus (Arije et al., 1974) and subsequently decreased to less than 100 ng/ml. This increase in prolactin at estrus agrees with other reports in cows (Sevanson et al., 1970).

Adrenal Corticosteriods

The adrenal glands have a role in reproduction in most mammals (Wagner et al., 1974). Corticoids may reduce gonadotropin secretion in cattle (Wagner and Oxenreider, 1972; Wagner et al., 1977). Concentrations of

corticoids in serum range between 10 and 70 ng/ml in cows during late pregnancy (Adams and Wagner, 1970). Concentrations increased about five days prepartum and attain a maximum of 100 ng/ml at calving (Adams and Wagner, 1970; Arije et al., 1974). Similarly, Smith et al. (1973) reported that glucocorticoid concentrations in dairy cows averaged about 5 ng/ml from 26 days prepartum to the day before calving and increased to 10.3 ng/ml at 12 hours before parturition and to 17 ng/ml at parturition. Then concentration of corticoids decreased to 5 ng/ml by 3 days after calving. Dunlap et al. (1981) found that basal serum levels of cortisol during the first 28 days postpartum averaged 19.0 ng/ml in suckled beef cows. Although Arije et al. (1974) observed elevated concentrations of corticoids at the first postpartum estrus, Erb et al. (1971) did not detect an increase in corticosteriods in plasma at estrus. If cows are suckled or milked after parturition, concentrations of corticoids in plasma are usually increased compared to non-lactating cows (Wagner and Oxenreider, 1971; Smith et al., 1972; Dunlap et al., 1981).

Influence of Prepartum Nutrition on Postpartum Reproduction in Beef Cows

Prepartum nutrition influences reproductive activity in sows (Christian and Nofziger, 1952; Self et al., 1955; Gossett and Sorensen, 1959; Haines et al., 1959; Zimmerman

et al., 1960), ewes (Clark, 1934; El-Sheikh et al., 1955; Foote et al., 1959), and dairy cows (Joubert, 1954; Gardner, 1969). In beef cows, reduced energy intake before parturition, increases the interval from calving to first estrus (Joubert, 1954; Wiltbank et al., 1962; Wiltbank et al., 1964; Bellows and Short, 1978). For example, when the N.R.C. recommended energy intake was reduced by one-half before calving and the suggested amount of energy was fed after calving, the postpartum interval was increased by an average of 22 days compared to cows fed according to N.R.C. requirements throughout gestation (Wiltbank et al., 1962). Similar effects of reduced energy intake prepartum have been observed in heifers (Turman et al., 1964; McClure et al., 1968; Dunn et al., 1969). For example, 69% of heifers fed a high precalving energy level exhibited estrus by 60 days postpartum while only 44% of those heifers fed a reduced amount of energy were in estrus by 60 days postpartum (Dunn et al., 1969). Furthermore, heifers fed a low level of supplemental feed before and after calving had an average postpartum interval of 93 days, while heifers fed a high intake of energy before calving with intake reduced to one-half following parturition exhibited an average postpartum interval of 64 days (Turman et al., 1964). In contrast, Corah et al. (1975) found that prepartum nutrition did not significantly influence the interval from parturition to first estrus in either heifers or cows. However, the

animals used in the study were in good body condition, as measured by fat cover at the beginning of treatment, which may have influenced the response to nutritional deprivation.

In many studies, body weight change has been used as an indicator of body condition at calving. However, weight change does not always accurately reflect body condition. The postpartum reproductive response of an animal to a prepartum nutritional stress appears to depend on the body condition of the cow at parturition (Dunn and Kaltenbach, 1980). Cows that are in good to moderate body condition at calving are affected little by postpartum weight decreases, but a significant decrease in reproductive performance occurs when cows lose body weight and condition prior to calving (Whitman, 1975; Dunn and Kaltenbach, 1980). For example, in mature, spring calving cows the percentage decrease in body weight from mid-pregnancy until just prior to calving was correlated ($r = .58$, $P < .01$) with days to first estrus (Wettemann et al., 1982). Moreover, for each 10% of body weight lost before calving the period to first estrus was delayed by about 19 days. A similar relationship between percentage change in body condition score from mid-pregnancy to calving and days to first estrus was also demonstrated.

Influence of Postpartum Nutrition on Reproduction

Low energy intake following calving delays the onset of

estrus in dairy cows (Reid, 1960; McClure et al., 1968; Gardner et al., 1969; Oxenreider and Wagner, 1971). A similar response occurs in beef cows (Wiltbank et al., 1962; Wiltbank et al., 1964; Randel and Walker, 1977; Dunn and Kaltenbach, 1980). However, the response to postpartum energy intake appears dependent on prepartum energy level and body condition at calving (Wiltbank et al., 1962). Cows fed a high level of energy before calving, followed by one-half the amount of energy postpartum, had similar intervals to first estrus as cows fed high amounts of energy both before and after calving (48 days and 43 days, respectively) (Wiltbank et al., 1962). In contrast, if cows were fed low energy diets before calving, the period from calving to first estrus was prolonged irregardless of postpartum nutritional intake (Wiltbank et al., 1962).

Wiltbank et al. (1964) evaluated cows that were fed one-half the N.R.C. recommended level of energy before calving and various amounts of energy postpartum. Cows fed the recommended intake of energy postpartum averaged 49 days from calving to first estrus compared with 73 days for cows fed 75% of the recommended amount. In contrast, Morris et al. (1978) found no difference in reproductive performance of mature Angus cows fed either a high or low amount of supplemental feed postpartum. However, the Angus cows were in good body condition at the time of calving, which may have influenced the response to the postpartum nutritional

deprivation.

Postpartum nutritional intake influences reproductive performance of heifers and the response is similar to that which occurs for cows. Dunn et al. (1969) fed heifers either a low amount of digestible energy (8.7 mcal/day) or a high amount (17.3 mcal/day) before parturition. Following calving, the heifers were allotted to three energy intakes: low (14.2 mcal/day), moderate (27.3 mcal/day) or high (48.2 mcal/day). By 80 days postpartum 90% of the heifers that received the high level postpartum were estrus while 80 and 82% of the low and moderate groups had exhibited estrus. Furthermore, 19% of the heifers fed the low level of energy postpartum failed to show estrus during the study. Pregnancy rates were also influenced by postpartum nutrition. Heifers that lost weight after calving had significantly lower conception rates; 64% of the heifers on low energy postpartum conceived compared to 87% and 72% for the high and moderate groups, respectively, by 120 days after calving (Dunn et al., 1969).

In conclusion, postpartum nutritional intake appears to be a major factor that influences reproductive performance in beef heifers and cows; however, the effects of postpartum nutrition are influenced by the nutritional status of the animal before calving.

Influence of Nutrition on Endocrine Function in Beef Cows

Undernutrition has been recognized as a cause of reproductive malfunction for many years (Lamming, 1966; Leathem, 1966; Lamond, 1970). However, the exact mechanism by which nutrition influences reproductive performance is not understood. The effects of nutritional stress may involve the endocrine system (Mulinos and Pomerantz, 1940; Lamming, 1966; Leathem, 1966). Information on the effect of nutrition on endocrine function of beef cows is limited but an examination of recent work reveals evidence for nutritionally induced changes in endocrine function.

Progesterone

Restriction of energy intake may cause an increase in concentrations of progesterone in plasma of prepartum beef cows (Donaldson et al., 1970; Gauthier et al., 1981). Cows that were fed 25% of their normal energy intake (5g/kg body weight) had significantly greater concentrations of progesterone in plasma during mid and late gestation than those fed a normal diet (20g/kg body weight). In mid-pregnancy, cows on a restricted diet had 10.0 ng/ml of progesterone compared to 6.0 ng/ml for control cows (Donaldson et al., 1970). Moreover, similar effects of nutrition on plasma progesterone were observed in late pregnancy (Donaldson et al., 1970; Gauthier et al., 1981).

In contrast, no significant effect of nutrition on plasma progesterone occurred in pregnant heifers fed 11.4 mcal of digestible energy per day compared to control heifers fed 17.8 mcal digestible energy per day. Progestrone in the plasma averaged 2.5 ng/ml for both groups of heifers at 13 days prepartum (Corah et al., 1975).

In cycling cows and heifers, undernutrition appears to decrease progesterone in the plasma during the luteal phase of the estrous cycle (Donaldson et al., 1970; Hill et al., 1970; Gombe and Hansel, 1973; Beal et al., 1978). Mature cows fed 25% of their normal intake exhibited an initial increase in concentrations of progesterone during the luteal phase of the first cycle after feed was restricted. Then progesterone in the plasma was decreased during the following two cycles (8.2 ng/ml versus 6.1 ng/ml and 4.3 ng/ml) (Donaldson et al., 1970). Beal et al. (1978) observed similar responses in beef heifers. In contrast, Dunn et al. (1974) found that mature Hereford cows on a restricted energy diet had greater concentrations of progesterone in plasma than control cows during the luteal phase of the cycle; however, the number of animals used was limited (n = 4).

Estrogens

Information on the effect of nutrition on concentration of estrogens in the plasma of cows is limited. Corah et al. (1975) reported no significant effect of undernutrition on

estradiol concentrations in heifers fed either 17.6 mcal of digestible energy per day or 11.4 mcal. Plasma estradiol averaged 35 pg/ml for heifers on both diets 13 days before calving and increased to 62 pg/ml on the day of parturition. However, the lack of a response may have been due to a limited number of animals (n = 6), and a short sampling period of only 14 days prepartum or the body condition of the animals before and during treatment.

In mature beef cows, animals fed low levels of energy (12 mcal of metabolizable energy per day) had similar concentrations of total estrogens (conjugated and nonconjugated) as cows fed adequate amounts of energy (21 mcal M.E./day) from days 45 to 14 prepartum (Gauthier et al., 1980). However, underfed cows had slightly greater concentrations of estrogens from day 14 prepartum to parturition (15 ng/ml versus 13 ng/ml). Dunn et al. (1974), using four cows per group, found no difference in concentration of estradiol in daily plasma samples obtained from cows fed either energy restricted or control diets during a normal estrous cycle.

Boyd et al. (1982) fed two groups of mature pregnant Angus cows from 50 days prepartum to parturition, so that one group lost an average of 1.8 kg body weight by day 10 prepartum while the other group gained a total of 22.7 kg body weight. Blood samples taken at days 10, 20, 30 and 50 prepartum revealed a slightly lower concentration of estrone

sulfate in cows losing weight. However, estrone concentrations in the plasma were not altered by nutritional treatment. Since the body condition of the cows and the interval from calving to first estrus were not reported, it is difficult to determine if this is a response of thin and moderate cows at parturition or moderate and fat cows.

Gonadotropins

Information on the effect of nutrition on gonadotropin concentrations in cows is contradictory. Beal et al. (1978) found that cycling heifers fed a low energy ration for 64 days had greater maximum concentrations of lutenizing hormone (40 ng/ml versus 25 ng/ml) than control heifers after treatment with GnRH. In contrast, heifers fed 85% of the estimated daily maintenance requirements for energy and protein beginning on day 5 of the estrous cycle had similar concentrations of lutenizing hormone in the plasma as control heifers (Hill et al., 1970). However, the nutritional restriction may not have been severe enough or may not have been imposed for a long enough duration to adequately test the response.

Mature Hereford cows, on a restricted diet (3.6 kg cubed alfalfa hay/day) for one estrous cycle had greater peak concentrations of LH than control cows (36.8 ng/ml versus 23.8 ng/ml, respectively; Dunn et al., 1974). Follicle stimulating hormone concentrations in serum were not significantly different between cows on the two diets.

However, only four cows per group were used in this study, which makes interpretation difficult.

Other Factors Influencing Postpartum Reproduction

Suckling

Beef cows that are suckled have longer intervals from parturition to first estrus than cows that have had their calves weaned (Casida et al., 1968; Graves et al, 1968; Oxenreider, 1968; Saiduddin et al., 1968; Short et al., 1972; Laster et al., 1973; Bellows, 1974; Radford et al., 1978; LaVoie et al., 1981). For example, with cows that have had their calves removed at parturition the average interval from calving to first estrus was 25 days compared to 65 days for cows that suckled a calf (Short et al., 1972). Similarly, Graves et al. (1968) performed five studies using 87 suckled and 88 non-suckled cows and found that mean intervals from calving to first estrus ranged from 18 to 41 days in nonlactating cows compared to 53 to 93 days for suckled cows. LaVoie et al. (1981) reported that cows which had their calves weaned at 3 days postpartum had an average postpartum interval of 20 days compared to 34 and 38 days for cows suckling calves either once or twice daily.

Intensity of suckling also influences postpartum reproduction (Wettemann et al., 1978; Randel et al., 1981). Under range conditions, cows suckling two calves had

significantly longer postpartum anestrous periods than cows suckling only one calf (95 days versus 67 days; Wettemann et al., 1978). Gimenez et al. (1980) reported that cows suckling one calf demonstrated estrus between days 39 and 93 postpartum compared to days 76 and 97 for cows suckling two calves.

Once daily suckling of calves from 30 days of age until the dam exhibited her first estrus reduced the postpartum interval in first calf heifers (Randel, 1981). By 100 days postpartum only 15% of normally suckled cows had exhibited estrus while 80% of once daily suckled cows were estrus (Randel, 1981). In contrast, LaVoie et al. (1981) reported that cows suckled twice daily had similar intervals from calving to first estrus as cows suckled only once daily (34 versus 38 days); but the number of animals in each group was limited.

Age

Postpartum reproduction is influenced by age of the cow (Inskeep, 1981; Laster et al., 1973; Davis et al., 1977; Teruit et al., 1977). It is commonly accepted that two-year-old first calf heifers have longer intervals from parturition to first estrus than older cows. Tervit et al. (1977) reported that the average postpartum interval for two-year-old beef heifers was 85 days compared to 63 days and 58 days for three-year-old and four-year-old cows,

respectively. Early weaning of calves increased the percentage of cows exhibiting estrus during the breeding season by 29% in two-year-olds and 27% in three-year-olds compared to 16.3% in mature cows (Laster et al., 1973). Increasing energy intake either pre- or postpartum can decrease the interval from parturition to first estrus in two-year-old heifers (Dunn et al., 1969). Consequently, age of cow does influence reproductive function but the effect of age can be influenced by nutritional and lactational status.

Breed

Dairy cows ovulate earlier postpartum than beef cows (Graves et al., 1968); however, comparisons between dairy and beef cows are complicated by management differences. Inskeep et al. (1979) using data collected from 1164 cows in 24 purebred herds in West Virginia, reported that two-year-old and mature Angus cows and exotic crosses were more likely to have a corpus luteum at any given stage postpartum than Herefords or various crosses of the British breeds. Casida et al. (1968) summarized data that revealed a 4 day advantage for Angus over Herefords in interval from parturition to first estrus. Laster et al. (1973) reported that Angus cows suckling calves had an advantage of 4 and 7 days less in the interval to first estrus for two and four-year-olds, respectively, when compared to Hereford cows. Similarly, when Hereford and Angus two-year-old heifers were

maintained on a low energy ration following calving, 30% of the Hereford heifers failed to show estrus compared to only 9% of the Angus heifers (Dunn et al., 1969). Consequently, it appears that breed of cow may influence postpartum reproduction.

CHAPTER III

INFLUENCE OF NUTRITION ON PREPARTUM ENDOCRINE FUNCTION OF BEEF COWS[1,2,3]

Summary

Seventy-nine spring calving Hereford cows were used to determine the effect of prepartum nutrition on endocrine function. About 120 d before calving (November) 60 cows were assigned to a low level of supplemental feed so as to lose 10% of the November body weight by calving and 19 cows were fed a moderate level of supplement to maintain body weight (M). Low cows were divided into 3 groups about 60 d before calving, one-third of the cows remained on low (LL), one-third received moderate (LM) and one-third of the cows were increased to a high level of supplemental feed (160% of M; LH). Body weights, body condition scores (BCS) and plasma samples were obtained every 2 w starting 60 d

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prepartum. Percentage body weight changes from November until just prior to calving were +1, -11, -6, -5 for M, LL, LH, and LM, respectively. Average BCS (scale of 1 to 9) was 6.5 for all treatment groups in November and were 5.3, 3.9, 4.3, 5.0 prior to calving for cows on the M, LL, LM, and LH treatments, respectively. Polynomial response curves were used to describe plasma progesterone (P), estrone (E_1), estradiol (E_2) and E_1SO_4 concentrations in plasma. Concentrations of progesterone for M cows were significantly different from LL, LM and LH ($p < .05$) and LL P concentrations were significantly different from LM and LH. Concentrations of P were greater for M from day 30 to day 9 prepartum. P concentrations in LL cows were lower from day 30 prepartum to day 20 prepartum compared to LM and LH. LL had lower concentrations of E_1 from day 15 prepartum to day 1 compared to LM and LH. M cows had lower E_2 concentrations from day 18 to parturition compared to LL, LM and LH. M cows had lower E_1SO_4 concentrations compared to LL and LH but larger concentrations than LM during the last 30 days of pregnancy. This study demonstrates that nutrition influences endocrine function during the last 30 days of gestation.

Introduction

Prepartum energy intake influences postpartum reproductive performance of beef cows (Wiltbank et al., 1962; Wiltbank et al., 1964; Dunn et al., 1969; Bellows et

al., 1982). Cows and heifers losing body condition during gestation have longer intervals from parturition to first estrus (Wiltbank et al., 1962; Wiltbank et al., 1964; Dunn et al., 1969). The influence of nutrition on reproductive performance may be mediated by the endocrine system. Restriction of energy intake to cows during pregnancy may result in greater concentrations of progestrone than in cows given adequate nutrition (Donaldson et al., 1970; Gauthier et al., 1981); however, other studies demonstrated no effect of nutrition on concentrations of progesterone in plasma (Corah et al., 1974; Boyd et al., 1982). Concentrations of estrone and estradiol in plasma are not influenced by nutrition (Corah et al., 1974; Gauthier et al., 1980; Boyd et al., 1982). In contrast, estrone sulfate concentrations may be reduced during late gestation in cows that are losing body weight (Boyd et al., 1982). However, the influence of prepartum nutrition on endocrine function may be related to body condition instead of current weight change.

The objective of this study was to examine the effect of nutrition and body condition during late gestation on plasma concentrations of progestrone, estrone, estradiol and estrone sulfate in mature beef cows.

Materials and Methods

Seventy-nine mature pregnant Hereford cows were maintained under tall-grass native range conditions. Cows

were assigned to four nutritional treatments and blocked according to weight, body condition, age and expected calving date.

Cows on the moderate treatment (M) were fed an amount of supplemental protein and energy necessary to maintain daily body weight from November 19 (fall) to parturition (March). Cows on the low-low treatment (LL) were supplemented so that they would lose 15% of their fall weight before calving. Cows on the low-moderate (LM) treatment were fed similarly to low-low cows until January 21 then they were changed to the amount of ration fed to moderate cows. Low-high (LH) cows were fed similarly to low-low cows until January 21 then additional supplement (160% of M) was fed so that cows would gain additional weight before calving. It was anticipated that moderate, low-low, low-moderate and low-high cows would lose 0%, 10%, 7% and 0%, respectively, of their November 19 body weight by calving.

Blood plasma samples were collected at biweekly intervals by jugular puncture during the third trimester of gestation. Blood samples (30 ml) were collected in tubes containing 32 mg oxalic acid, cooled to 5C, centrifuged (5,000^{x} g for 15 min) and plasma was decanted and stored at -10C.

Body weights were obtained after animals were removed from feed and water for 16 hr on December 20, January 10, January 24, February 7, February 21, March 6 and March 20.

On days that blood samples were taken, the samples were collected after weighing.

Body condition scores were determined by three individuals at the initiation of treatment (November 19), at the change of nutrition (January 21) and before calving (March 6). With the body condition scoring system used, a score of 9 is an obese cow and a score of 1 indicates an emaciated cow.

Following parturition all cows were maintained on the same pasture and fed supplemental protein and energy necessary to maintain the body weight of cows on the moderate treatment. The nutritional program for the four treatments is summarized in Table I.

Plasma progesterone was quantified by a double antibody radioimmunoassay similar to that described by Convey et al. (1977). The specificity and validation of the assay in our laboratory has been described (Lusby et al., 1981). In this experiment, the between assay coefficient of variation was 6.3% and the within assay coefficient of variation was 0.7%. When 10 ng of progesterone was added to 1 ml samples of plasma from steers 92% (n = 12) was recovered.

Plasma estrone was quantified by a radioimmunoassay, similar to that described by Eley et al. (1981). To validate the assay in our laboratory, plasma (n = 27) was extracted with ethyl ether and values for the same samples were compared before and after estrone was isolated from

TABLE I

NUTRITIONAL TREATMENTS FOR COWS DURING THE WINTER FEEDING PERIOD (NOVEMBER 19, UNTIL CALVING IN MARCH)

Treatment Group	Interval	Nutritional Program[1]
Moderate	Nov. 19 to calving[3]	9.5 kg of csm[2] per cow per week
Low-Low	Nov. 19 to Feb. 20	2.7 kg of csm per cow per week
	Feb. 21 to calving[3]	5.4 kg of csm per cow per week
Low-Moderate	Nov. 19 to Jan. 23	2.7 kg of csm per cow per week
	Jan. 21 to calving[3]	9.5 kg of csm per cow per week
Low-High	Nov. 19 to Jan. 20	2.7 kg of csm per cow per week
	Jan. 21 to calving[3]	15.9 kg of csm per cow per week

Sephadex LH-20 columns using benzene:methanol (9:1) as the solvent system. The amount of estrone in ether extracts (510 pg/ml) differed from that determined after chromatographic isolation (728 pg/ml; r = 0.97). This difference suggests that some component in plasma inhibited the binding of estrone to the antibody. Consequently, estrone was isolated from all samples by column chromatography. When 250 pg/ml of estrone was added to 1 ml of plasma from a steer, 106% was recovered (n = 15). The between assay coefficient of variation was 19% and the within assay coefficient of variation was 5%.

Plasma estradiol was quantified by a radioimmunoassay, similar to that described by Hallford et al. (1979). To validate the assay for plasma samples from cows during late pregnancy, plasma was extracted with ethyl ether and values for the same samples (n = 22) were compared before and after estradiol was isolated from Sephadex LH-20 columns using benzene:methanol (9:1) as the solvent system. The amount of estradiol in ether extracts (102 pg/ml) differed from that determined after chromatographic isolation (131 pg/ml; r = 0.52). This difference suggests that the antibody to estradiol was not specific for estradiol in bovine plasma during late pregnancy, consequently estradiol was isolated from all samples by chromatography. When 100 pg/ml of estradiol was added to 1 ml plasma samples from a steer, 84% was recovered (n = 16). The between assay coefficient of

variation was 9% and the within coefficient of variation was 6%.

Plasma estrone sulfate was quantified by the radioimmunoassay as described above after cleavage of sulfate from estrone. Free estrone and estradiol were removed by extraction with ethyl ether. Following treatment with sulfatase enzyme, samples were extracted twice with ethyl ether. The ether extracts were concentrated and subjected to radioimmunoassay. When 2 ng/ml of exogenous estrone sulfate was added to steer plasma, an average of 91.4% (n = 9) was recovered. Further details of estrogen assays are described in appendix.

Polynomial response curves were used to describe plasma estrone, estradiol, estrone sulfate and progesterone concentrations in cows on the four treatments. Tests of heterogeneity of regression coefficients were used to determine if time trends between treatments were not parallel. Body weight and body condition score changes were analyzed by split plot analysis of variance.

Results

Body weights on November 17 were similar for cows on all treatments and averaged 407±13 kg, 409±12 kg, 417±7 kg and 404±14 kg for treatments M, LL, LM and LH, respectively. Winter nutritional treatment influenced body weight changes ($P < .001$), and on March 6 the percentage body weight changes were +0.8±0.5%, -11±1.1%, -6±1% and -5±0.8% for cows

on the M, LL, LM and LH, treatments respectively. Body weight changes are depicted in Table II. Body condition score changes were in agreement with body weight changes (Table III). Body condition scores were affected by treatment ($P < .001$). Postpartum reproductive performance is summarized in Table IV.

Concentrations of progestrone in plasma of all cows during the last 30 days of gestation were best described by a second order polynomial regression equations (Figure 1). Concentrations of progesterone during the last 30 days of pregnancy were similar for cows on the LM and LH treatments. However, progesterone concentrations for M cows were significantly different from those for cows on the LL, LM and LH treatments (heterogeneity of regression; $P < .05$). Moreover, the response for cows on the LL treatments was significantly different from that for LM and LH cows ($P < 0.05$).

Least-square means for plasma concentrations of progesterone on day 30 prepartum were 12.8 ng/ml for M cows compared to 10.0, 10.8 and 10.6 ng/ml for cows on the LL, LM and LH, respectively. By day 15 prepartum, progesterone in the plasma of M cows averaged 8.9 ng/ml, compared to 7.8, 7.9 and 7.6 ng/ml for LL, LM and LH cows, respectively. On the day before parturition progesterone concentrations were similar for all groups and averaged 3.4, 4.9, 3.6 and 4.4 ng/ml for cows on the M, LL, LM and LH treatments, respectively.

TABLE II

PREPARTUM BODY WEIGHTS OF COWS FROM NOVEMBER 1979 TO MARCH 1980

Date	Treatment			
	M	LL	LM	LH
11-19-79	$407\pm13^{a,b}$	409 ± 12^{c}	414 ± 7^{b}	404 ± 14^{b}
12-20-79	426 ± 9^{b}	401 ± 12^{c}	404 ± 7^{c}	406 ± 10^{bc}
1-10-80	426 ± 9^{b}	380 ± 10^{c}	381 ± 6^{c}	394 ± 10^{c}
1-24-80	424 ± 9^{b}	363 ± 10^{c}	362 ± 6^{c}	367 ± 9^{c}
2-7-80	429 ± 9^{b}	363 ± 10^{c}	392 ± 7^{d}	392 ± 11^{d}
2-21-80	413 ± 8^{b}	342 ± 9^{c}	379 ± 7^{d}	381 ± 12^{d}
3-6-80	412 ± 8^{b}	354 ± 13^{c}	386 ± 13^{d}	389 ± 14^{d}
3-20-80	412 ± 10^{b}	347 ± 16^{c}	352 ± 17^{c}	406 ± 27^{d}

[a] $\bar{x}\pm$S.E. kg.

[b,c,d] Means in a row which do not have the same superscript are significantly different ($p < 0.05$).

TABLE III

BODY CONDITION SCORES OF COWS FROM NOVEMBER 1979 TO JUNE 1980

Date	Treatment			
	M	LL	LM	LH
11-19-79	$6.5 \pm 0.1^{a,b}$	6.3 ± 0.2^{b}	6.4 ± 0.2^{b}	6.4 ± 0.1^{b}
1-24-80	6.0 ± 0.2^{b}	4.9 ± 0.1^{c}	4.9 ± 0.1^{c}	5.1 ± 0.1^{c}
3-13-80	5.3 ± 0.2^{b}	3.9 ± 0.2^{c}	4.3 ± 0.2^{c}	5.0 ± 0.2^{b}
6-19-80	5.7 ± 0.1^{b}	5.3 ± 0.1^{b}	5.5 ± 0.1^{b}	5.6 ± 0.2^{b}

[a] 1 = very thin, 9 = very fat, $\bar{x} \pm$ S.E.

[b,c] Means in a row which do not have the same superscript are significantly different ($p < 0.05$).

TABLE IV

INFLUENCE OF PREPARTUM NUTRITION ON POSTPARTUM REPRODUCTIVE PERFORMANCE

Criteria	Treatment			
	M	LL	LM	LH
No. of Cows	19	19	22	19
Ovarian Activity By 85 Days Postpartum (%)	10.5[a]	0[b]	9.1[a]	15.8[a]
Days to Onset of Ovarian Activity For Cows With Activity By 85 Days Postpartum ($\bar{x}$ ± S.E.)	68±35[a]	-	56±2[a]	59±25[a]
Conception Rate (%)	58[a]	21[b]	50[a]	39[a]
Days Postpartum to Conception ($\bar{x}$ ± S.E.)	115±28[a]	135±28[a]	120±25[a]	91±16[a]

[a,b]Values in a row which do not have the same superscript are significantly different ($p < 0.05$).

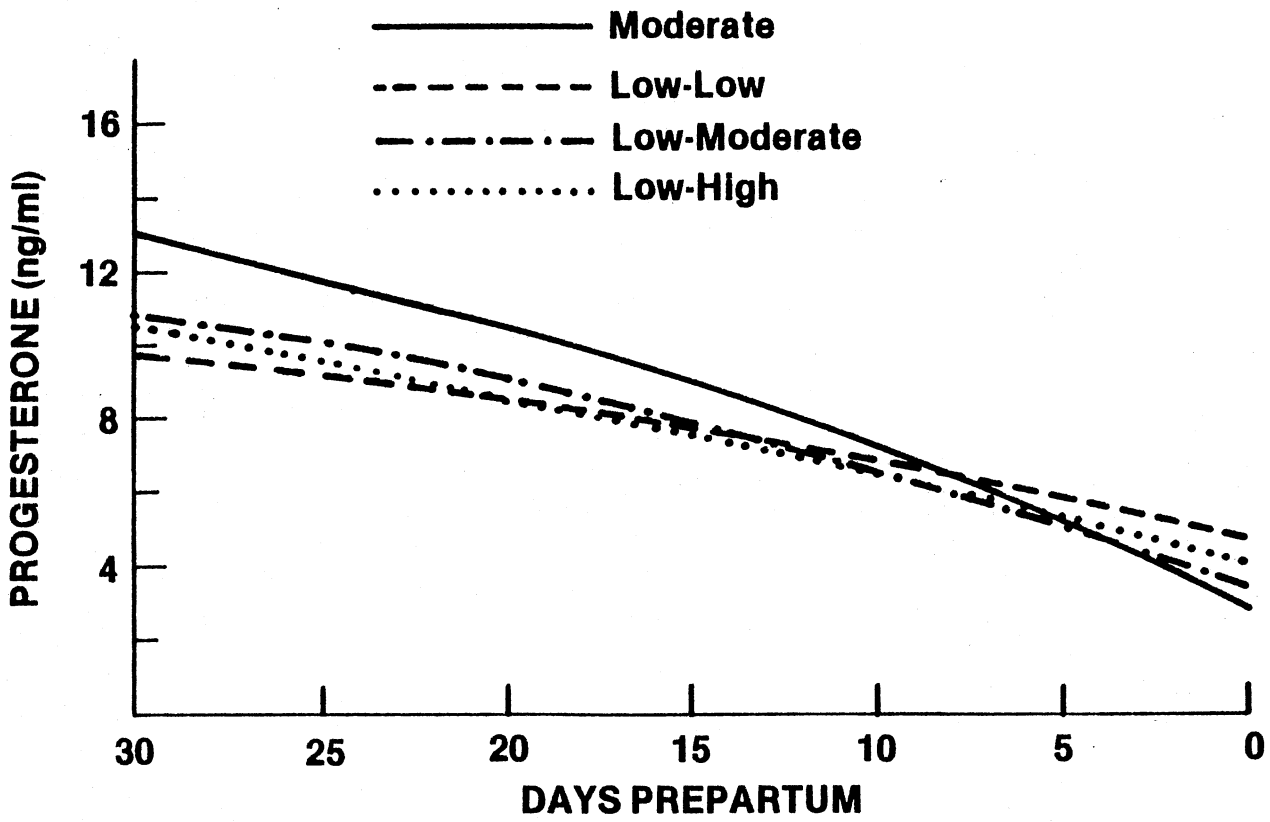

Figure 1. Least Squares Regression of Progesterone Response to Four Nutritional Treatments

Concentrations of estrone in plasma during the last 30 days of pregnancy were best described by a second order polynomial regression equation (Figure 2). Estrone concentrations for cows on the M treatment were not significantly different from cows on the LL, LM and LH treatments. In addition, estrone concentrations were not significantly different between cows on LM and LH treatments; however, cows on the LL treatment had a significantly different response curve from LM and LH ($P < .05$).

At day 30 prepartum, plasma estrone concentrations were 412 pg/ml for LL compared to 306, 81 and 377 pg/ml for cows on M, LM and LH, respectively. On day 15 prepartum, LL estrone concentrations were the lowest at 1,020 pg/ml in contrast to 1,080, 1,280 and 1,450 pg/ml for cows on the LM, M and LH treatments, respectively. The prepartum increase in estrone was reduced in cows on the LL treatment and one day before parturition estrone concentrations for LL cows were 1,890 pg/ml compared to 2,973, 2,554 and 2,957 pg/ml for M, LM and LH, respectively.

A second order polynomial regression equation best described plasma estradiol concentrations during the final 30 days of gestation (Figure 3). Cows on the moderate level of nutrition throughout pregnancy had different ($P < .05$) concentrations of estradiol during the last 30 days of gestation compared to cows on the LL, LM and LH treatments. However, there were no significant differences between the

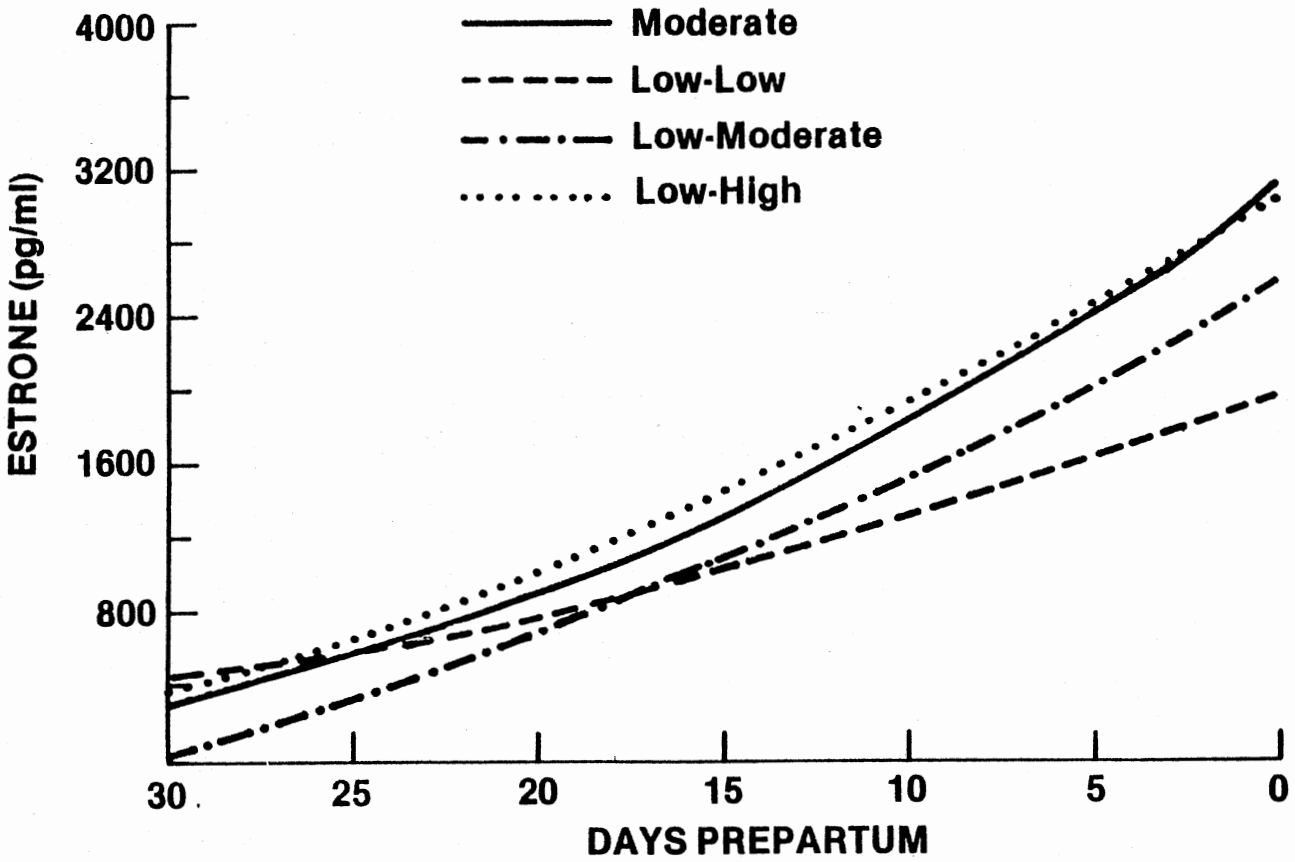

Figure 2. Least Squares Regression of Estrone Response to Four Nutritional Treatments

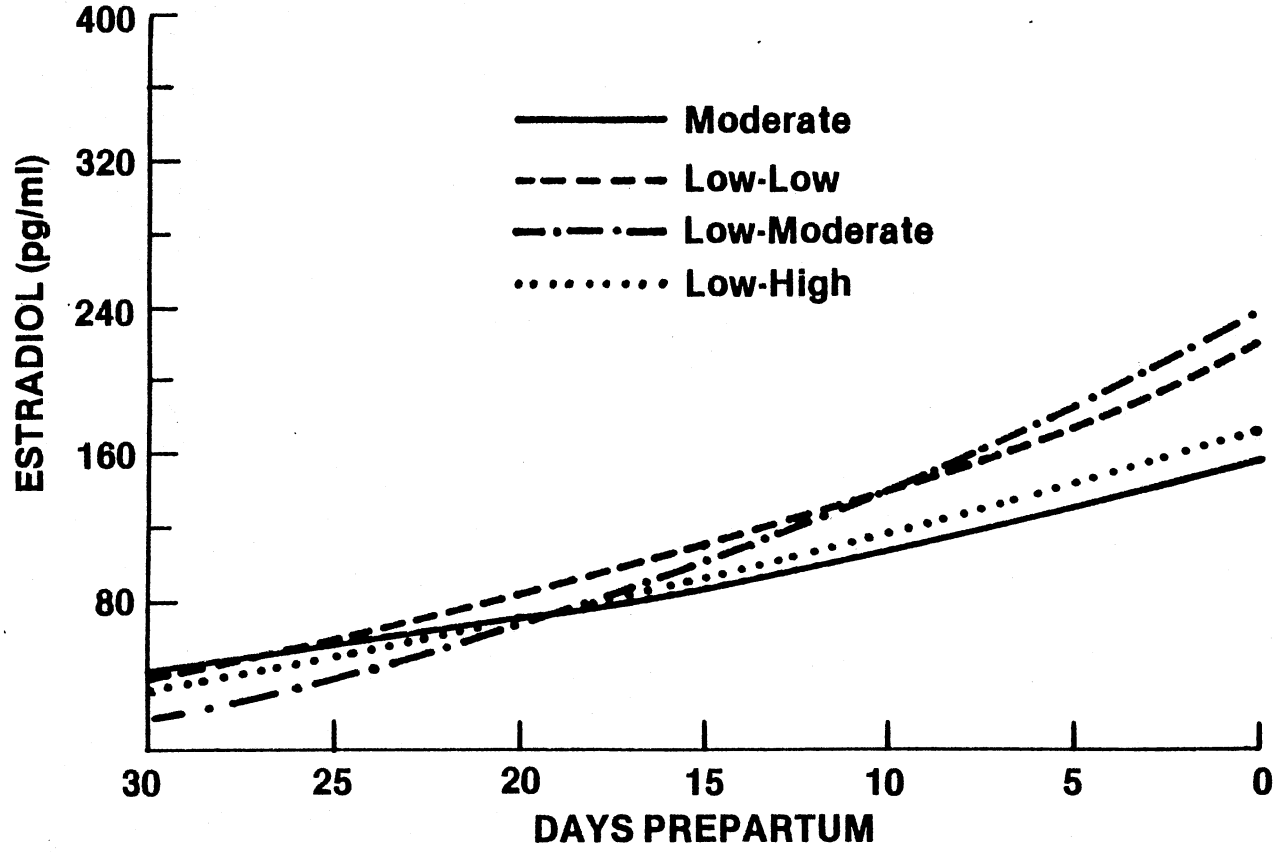

Figure 3. Least Squares Regression of Estradiol Response to Four Nutritional Treatments

response curves for cows on the LL, LM and LH treatments.

At 30 days prepartum estradiol concentrations were 45 pg/ml for M cows compared to 39, 19 and 35 pg/ml for cows on the LL, LM and LH treatments, respectively. By day 15 prepartum M cows had 88 pg/ml of estradiol in contrast to 113, 101 and 92 pg/ml for LL, LM and LH cows, respectively. On the day before calving estradiol concentrations were 154 pg/ml for M cows compared to 217, 228 and 168 pg/ml for cows on the LL, LM and LH treatments, respectively.

A linear regression equation best described the concentrations of estrone sulfate in the plasma of cows during the last 30 days of gestation (Figure 4). Cows on the M treatment had a significantly different ($P < .0005$) response curve than cows on the LL, LM and LH treatments. In addition, the response for LL cows was significantly different ($P < .05$) from that for cows on the LM and LH treatments. The response curves for cows on LM and LH were parallel so there was no significant difference between the two.

At 30 days prepartum, estrone sulfate concentration in the plasma of M cows was 5,400 pg/ml, compared to 6,300, 3,300 and 6,300 pg/ml for cows on the LL, LM and LH treatments, respectively. Similar differences were present at day 15 prepartum with estrone sulfate in the plasma of cows averaging 8,900 pg/ml for cows on the M treatment as opposed to 9,500, 7,600 and 10,400 pg/ml for cows on the LL,

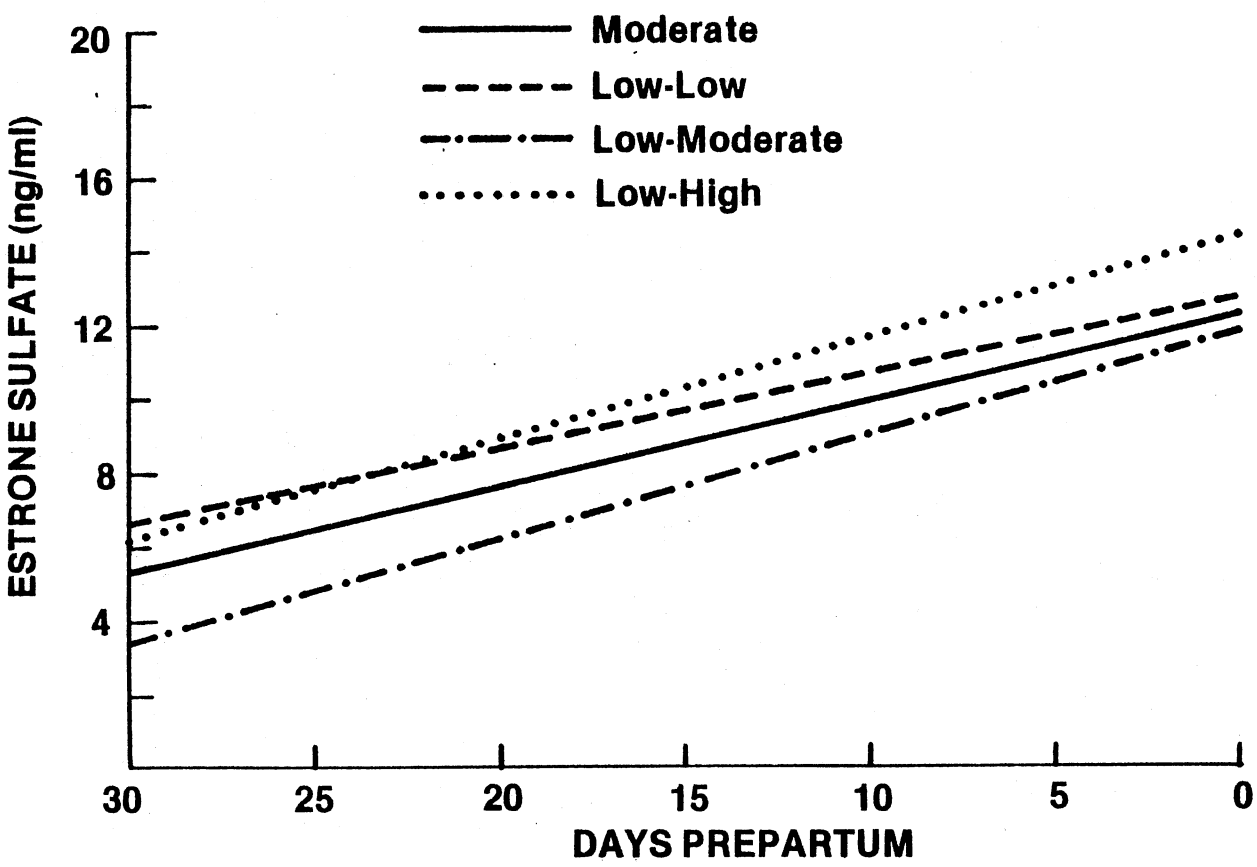

Figure 4. Least Squares Regression of Estrone Sulfate Response to Four Nutritional Treatments

LM and LH treatments, respectively. On the day before parturition cows on LL had 12,400 pg/ml, cows on M had 12,200 pg/ml while cows on LM and LH had 11,500 and 14,700 pg/ml, respectively.

Discussion

The decreasing plasma concentration of progesterone during late gestation agrees with previous investigations (Donaldson et al., 1970; Arije et al., 1974; Boyd et al., 1982). Donaldson et al. (1970) found increased concentrations of progesterone in the plasma of pregnant cows on restricted energy diets as opposed to cows receiving normal energy intake. Similar results were observed by Gauthier (1980). However, others indicate that nutrition has no effect on plasma progesterone concentrations (Corah et al., 1975; Boyd et al., 1982). Our research indicates greater concentrations of progesterone from day 30 prepartum to day 10 prepartum in cows that maintain body weight.

In previous studies, body condition was not reported. This makes interpretation of data difficult since there could be a considerable difference in the response of an obese cow to nutritional deprivation compared to the response of a cow in moderate body condition. In our experiment, body condition scores of cows on the moderate treatment averaged 5.3±.2 at parturition with cows on the low-low treatment averaging 3.9±.2. Body condition scores of cows on the low-moderate and low-high treatments were

intermediate between those extremes.

Estrone, estradiol and estrone sulfate concentrations during the last 30 days of gestation were similar to previously reported data (Smith et al., 1973; Robertson et al., 1974; Eley et al., 1979; Boyd et al., 1982). Previous work indicates that prepartum nutrition has no effect on estrone or estradiol concentrations (Corah et al., 1975; Boyd et al., 1982); however, the small numbers of animals and lack of body condition information in those studies makes interpretation difficult. Our data suggest that cows losing body weight and condition throughout pregnancy have lower concentrations of estrone when compared to cows that lose weight through mid-gestation and gain weight during the last 60 days of pregnancy. Gauthier et al. (1980) reported lower concentrations of total estrogens during the last 14 days of pregnancy in cows losing body weight, which is in agreement with our data for concentrations of estrone in plasma. In contrast, in the present study cows that maintained body weight throughout gestation had reduced estradiol concentrations in plasma during the last 15 days of pregnancy. This could be due to conversion of estradiol to estrone by placental tissue resulting in the increased concentration of estrone in cows on the moderate treatment. Boyd et al. (1982) found slightly lower concentrations of estrone sulfate in cows gaining weight during the last 50 days of pregnancy. In the present study, cows maintaining

body weight during pregnancy had similar concentrations of estrone sulfate to those reported by Boyd et al. (1982). In addition, a significantly different response in estrone sulfate concentrations for cows maintaining weight as opposed to those losing body weight through all or part of gestation was noted.

In conclusion, this study indicates that prepartum nutrition may influence the concentrations of progesterone, estrone, estradiol and estrone sulfate in plasma of mature beef cows.

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSIONS

Prepartum nutrition influences the concentration of progesterone, estrone, estradiol and estrone sulfate in plasma of cows during late gestation. The alteration in concentrations of reproductive hormones may be a major factor in the regulation of the effect of reduced nutrition, as expressed by reduction in body weight and body condition, on postpartum reproductive performance. Postpartum reproductive activity was reduced for cows losing the most weight during the last half of pregnancy (Table IV). It seems logical that alterations in hormone concentrations during the last 30 days of gestation may be involved in regulating the length of the postpartum anestrous interval. For example, for each of the four hormones studied either the cows losing weight throughout the study (low-low) or those maintaining weight during the experiment (moderate) had significantly different response curves for concentrations of hormones in the plasma than cows on the other treatments.

These results lead us to the question, if prepartum endocrine function regulates postpartum reproduction, what is the mechanism involved? Changes in concentrations of

reproductive steriod hormones influence the synthesis and secretion of hormones by the pituitary or hypothalamus. Therefore, following parturition, the initiation of cyclic activity by the pituitary may be impaired. It is important to note that in cows on the moderate treatment, concentrations of progesterone and estrone were generally greater than those cows on the low-low treatment. In contrast, concentrations of estradiol and estrone sulfate were greater in cows on the low-low treatment. Consequently, it seems reasonable to assume that nutritional intake is influencing the steriodogenic pathway in the synthesis of estrogens (i.e progesterone -- estradiol -- estrone -- estrone sulfate). For example, if cows gained or maintained weight during late pregnancy (M, LM and LH) estrone sulfate concentrations were reduced compared to cows losing weight. This would suggest that when cows are on reduced nutrition and lose body condition there is increased conversion of estrone to estrone sulfate.

The shift in concentrations of steriods may be the factor that influences production and secretion of gonadotropins by the pituitary. Since cows on the low-low treatment had reduced plasma concentrations of estrone, LH and FSH release could be increased compared to moderate cows resulting in depletion of pituitary reserves of gonadotropins. In converse, reduced concentrations of estrone in plasma during late pregnancy could be associated

with less synthesis of gonadotropins and thus less release aftr parturition. It is also possible that changes in the ratio of estrone to estradiol during late pregnancy may change the sensitivity of the pituitary to feedback control. More frequent blood sampling coupled with measurements of gonadotropin concentrations would clarify these relationships.

Another important question brought up by this study is, how does nutrition change concentrations of the steriod hormones. One possible mechanism would be by an alteration in the transport or supply of precursor molecules such as cholesterol to the placental cells. This theory is substantiated by the decreased concentrations of progesterone in cows on the low-low treatment compared to cows on the moderate treatment.

Another possible mechanism would be that nutrition, by changing availability of energy and protein, alters the enzyme pathways involved in steriod biosynthesis. This would result in increased concentrations of some hormones (estradiol, estrone sulfate) and decreased concentrations of others (progesterone, estrone) in cows on the low-low treatment.

These data demonstrate that nutritional intake of beef cows during late pregnancy influences the fetal placental unit, based on alterations in plasma concentrations of steriod hormones synthesized by the placenta. Thus, our findings support the hypothesis that prepartum nutrition may

exert its influence on postpartum reproductive performance by altering the endocrine function of the fetal placental unit.

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APPENDIX

ESTROGEN ASSAY

Extraction

All glassware and caps used were washed in soap water, rinsed four times with distilled water and rinsed with freshly distilled methanol before use. Approximately 3,000 cpm/10 ul each of E_1 (2,4,6,7-$^3HE_1$) and E_2 (2,4,6,7-$^3HE_1$) supplied by New England Nuclear Inc., were pipetted into round bottom extraction tubes (50 ml) fitted with Teflon caps to determine extraction efficiency. Concurrently, 10 ul of the E_1 recovery solution was added to each of three scintillation vials (total count vials) and 10 ul of the E_2 recovery solution was added to another series of three scintillation vials. The recovery solution was allowed to air dry in the extraction vials before samples were added.

Two ml of stripped steer plasma, reference plasma (stripped steer plasma plus 250 pg/ml E_1 and 250 pg/ml E_2) or unknown plasma samples were added to each extraction tube. Tubes were vortexed for 10 seconds after addition of sample. Then tubes were incubated at room temperature for 30 minutes.

Each sample was extracted twice with a freshly opened can of ethyl ether at the ratio of 5 ml of ether per ml of plasma. Samples were shaken slowly for 5 minutes. Tubes

were placed in a freezer (-10 C) until extracted plasma was frozen (>1 hour). Extracts were decanted into 40 ml conical tubes and the ether was totally evaporated on a drying block (45 C) under nitrogen. After the second extract was evaporated to dryness, 200 ul of benzene:methanol (9:1) was added and vortexed to rinse the tube before chromatography.

Chromatography

Sephadex LH-20 (Sigma Chemical Co.) was soaked for at least 4 hours in freshly distilled solvent (benzene:methanol, 9:1). After 3.0 ml glass syringe columns were rinsed with solvent (1 ml/column), a small filter paper disc was placed at the bottom of each syringe. Using a Pasteur pipette, the Sephadex LH-20 slurry (in benzene:methanol, 9:1) was poured into the syringe until it reached the 2.8 ml mark. The Sephadex expanded to reach the 3.0 ml volume. The sides of the column were washed with solvent and a second small filter paper disc was placed on top of the column. The column was rinsed with 10 ml of solvent and the elution pattern was determined after the addition of H^3-estrone (2,000 cpm/10 ul) and H^3-estradial (2,000 cpm/10 ul). The elution pattern was redetermined before each set of samples was chromatographed.

To isolate E_1 from E_2 in the plasma samples, the extract dissolved in 200 ul of solvent was transferred to the column using a Pasteur pipette. Daily elution patterns were used to determine the fractions to be collected which

contained the following compounds: pre-estrone, estrone, post-estrone, pre-estradial, estradial. The elute was collected in 10 X 75 mm disposable culture tubes and the columns were washed with at least 20 ml of solvent between samples.

Estrone Radioimmunoassay

A typical assay contained 48 12 X 75 mm disposable culture tubes. The elute from the tubes containing the estrone fraction was pipetted, using a 500 ul Hamilton syringe, into each of two duplicate tubes. Usual aloquot sizes were 200 and 300 ul; however, variation in aloquot size was necessary when samples contained greater concentrations of estrone. One ml of the estrone fraction was added to a scintillation vial to determine procedural losses. Solvents in the vials used to determine procedural losses and the total count vials were allowed to air dry before addition of 5.0 ml of liquid scintillation cocktail.

Standard estrone was prepared in redistilled ethanol so that 100 ul contained 0, 4, 8, 16, 32, 64, 128, 256 pg. Two standard curves were included in each assay of 48 tubes.

After evaporation of the solvent from samples and standards, 200 ul of antisera (1:100,000 dilution in phosphate buffered saline plus 0.1% gelatin) was added to each tube. The antisera was supplied to us by Dr. David Guthrie (antisera WII Barc #4). Tubes were then greatly

vortexed and incubated at room temperature for 30 minutes. Approximately 20,000 cpm of ^{3}H-2,4,6,7-estrone (in 100 ul PBS plus 0.1% gelatin) was added to each tube and tubes were vortexed and allowed to incubate for 6 hours at 5 C.

Following incubation, tubes were placed in an ice water bath (4 C) for 10 minutes, then 1 ml of dextran-coated charcoal solution (2.5 g activated neutral Norit and 0.25 g dextran T-150 per liter of distilled water at 4 C) was added to each tube. The charcoal solution was added to all 48 tubes in an assay rack within two minutes. Tubes were vortexed immediately, allowed to incubate 10 minutes at 4 C and then centrifuged at 2,555 g at 4 C for 10 minutes. A 500 ul aliquot of the supernate from each sample was diluted with 4.5 ml of scintillation cocktail and radioactivity was quantified.

Estradial Radioimmunoassay

The elute from the tubes containing the estradial was pipetted into each of two duplicate tubes (500 or 700 ul). Variation in aloquot size was necessary when samples contained greater concentrations of estradial. One ml of the estradial fraction was added to a scintillation vial to determine procedural losses.

Standard estradial was prepared in redistilled ethanol so that 100 ul contained 0, 1, 2, 4, 8, 16, 32, 64 pg. Two standard curves were included in each assay of 48 tubes.

Solvent was evaporated from samples and standards and

200 ul of antisera (1:100,000 dilution in phosphate buffered saline plus 0.1% gelatin) was added to each tube. Antibody was prepared against 6 -succinyl-estradial conjugated to bovine serum albumin (antisera #244) and was supplied by Dr. G. D. Niswender. Following addition of antisera, tubes were gently vortexed and incubated at room temperature for 30 minutes. Approximately 20,000 cpm of ^{3}H-2,4,6,7-estradial (in 100 ul PBS plus 0.1% gelatin) was added to each tube. Tubes were then vortexed and allowed to incubate for 4 hours at 5 C.

Following incubation, tubes were placed in an ice water bath (4 C) for 10 minutes, then 1 ml of a dextran-coated charcoal solution (2.5 g activated neutral Norit and 2.5 g dextran T-150 per liter of distilled water at 4 C) was added to each tube. The charcoal solution was added to all 48 tubes in an assay within two minutes. Tubes were vortexed immediately, allowed to incubate 10 minutes in the ice water bath and then centrifuged at 2,500 g at 4 C for 10 minutes. A 500 ul aliquot of the supernate from each sample was diluted with 4.5 of aqueous scintillation fluid and radioactivity was quantified.

$\underline{E_1SO_4}$ Assay

Round bottom extraction tubes (50 ml) fitted with Teflon lined caps were rinsed with freshly distilled methanol. Then 1,800-2,000 cpm/10 ul (in ethanol) of a 2-

labelled recovery solution (6,7-^{3}H-E_1SO_4, 40-60 Ci/mmol, New England Nuclear, #NET 203) was added to the bottom of each extraction tube and to each of 3 scintillation vials for recovery references. Extraction tubes were allowed to air dry. Two ml of plasma were added to each extraction tube. A reference sample and stripped steer sample were included in each assay. Each sample was vortexed gently an incubated for 30 minutes in a 45 C water bath. Following incubation, 0.1 ml of 0.1N NaOH was added to each tube and vortexed. Free estrogens were extracted twice by adding 10 ml of ethyl ether (freshly opened can) to each tube, shaking gently and freezing for at least 1 hour at -10 C. Ether was decanted and discarded. Residual ether was evaporated on a dry block at 45 C for 10 minutes. Acetate buffer (5.0 ml, pH = 4.0) were added to each sample and vortexed for 10 seconds. Sulfatase was from Helix pomatia type H-2 obtained from Sigma Chemical Co. (St. Louis, Missouri). A clean amber bottle was wrapped in foil and placed in an ice bath. Acetate buffer (2.5 ml) was pipetted into the amber bottle and 100 ul of sulfatase stock was added. Stock enzyme was diluted to concentration of 100 u/ml although vials of enzyme varied in specific activity. Ten units of the enzyme solution was added to each sample and the tube was gently vortexed for 10 seconds. Samples were then immediately placed in a 37 C water bath in an oven and incubated for a minimum of 6 hours and a maximum of 12 hours. After incubation, 2.5 ml of Tris buffer (0.2 m of trizma base, pH

= 10.5) was pipetted into each vial and vials were vortexed gently. Samples were then extracted twice with ethyl ether (5 ml ether/1 ml plasma). The extracts were decanted in conical test tubes (40 ml) and estrone was quantified by radioimmunoassay.

Recovery of Mass for E_1SO_4

To determine the recovery of added mass, the potassium salt of E_1SO_4 (estra-1,3,5(10)-triene-17-one-3-sulfate) in 10% potassium acetate stabilizer (Sigma Chemical Co.) was used. A stock solution was prepared by adding 492.0 ug of E_1SO_4 K salt to 4.920 ml of freshly distilled ethanol. Then 40 ul of the stock solution was added to a flask and the ethanol was evaporated under nitrogen. Next, 20 ml of stripped steer plasma was added, vortexed and the sample was allowed to equilibrate overnight at 4 C. After adjustment for stabilizer content, each ml of plasma contained 835 pg of E_1SO_4.

TABLE V

ORTHOGONAL COMPARISONS FOR PROGESTERONE RESPONSE CURVES

M versus LL, LM, LH

Error	D. F.	S. S.	M. S.	F
LL, LM, LH	100	457,651,110		
M	34	269,628,610		
total	134	727,279,720	5,427,461	
combined	136	766,063,012		
difference	2	38,703,292	19,391,646	3.57*

LL versus LM, LH

Error	D. F.	S. S.	M. S.	F
LM, LH	65	222,311,639		
LL	33	204,869,271		
total	98	427,180,910	4,358,989	
LL, LM, LH	100	457,651,110		
difference	2	30,470,200	15,235,100	3.5*

LM versus LH

Error	D. F.	S. S.	M. S.	F
LH	34	89,218,000		
LM	29	115,863,125		
total	63	205,081,125	3,255,256	
LM, LH	65	222,311,639		
difference	2	17,230,514	8,615,257	2.6

*$P < .05$

TABLE VI

ORTHOGONAL COMPARIOSN FOR ESTRONE RESPONSE CURVES

M versus LL, LM, LH

Error	D. F.	S. S.	M. S.	F
LL, LM, LH	105	46,190,471		
M	35	34,969,784		
total	140	81,160,255	579,116	
combined	142	82,396,256		
difference	2	1,236,001	618,001	1.06

LL versus LM, LH

Error	D. F.	S. S.	M. S.	F
LM, LH	69	34,723,751		
LL	34	7,501,949		
total	103	42,225,700	409,958	
LL, LM, LH	105	46,190,471		
difference	2	3,964,771	1,982,386	4.8*

LM versus LH

Error	D. F.	S. S.	M. S.	F
LH	36	21,891,204		
LM	31	12,699,877		
total	67	34,591,081	516,285	
LM, LH	69	34,723,751		
difference	2	132,670	66,335	.13

*$P < .05$

TABLE VII

ORTHOGONAL COMPARIOSNS FOR ESTRADIOL RESPONSE CURVES

M versus LL, LM, LH

Error	D. F.	S. S.	M. S.	F
LL, LM, LH	106	332,853		
M	33	14,015		
total	139	346,868	2,495	
combined	141	367,069		
difference	2	20,201	10,100	4.1*

LL versus LM, LH

Error	D. F.	S. S.	M. S.	F
LM, LH	68	217,893		
LL	36	114,372		
total	104	332,264	3,195	
LL, LM, LH	106	332,853		
difference	2	588	294	.09

LM versus LH

Error	D. F.	S. S.	M. S.	F
LH	37	41,030		
LM	29	165,443		
total	66	206,473	3,128	
LM, LH	68	217,893		
difference	2	11,420	5,710	1.8

*$P < .05$

TABLE VIII

ORTHOGONAL COMPARIOSNS FOR ESTRONE SULFATE RESPONSE CURVES

M versus LL, LM, LH

Error	D. F.	S. S.	M. S.	F
LL, LM, LH	97	684,992,828		
M	33	137,549,363		
total	130	684,992,828	5,269,176	
combined	131	826,637,778		
difference	1	141,644,950	141,644,950	26.9**

LL versus LM, LH

Error	D. F.	S. S.	M. S.	F
LM, LH	62	472,401,629		
LL	34	178,892,647		
total	96	651,294,276	6,784,315	
LL, LM, LH	97	684,992,828		
difference	1	33,698,552	33,698,552	4.9*

LM versus LH

Error	D. F.	S. S.	M. S.	F
LH	27	165,432,929		
LM	34	306,961,198		
total	61	472,394,128	7,744,166	
LM, LH	62	472,401,629		
difference	1	7,501	7,501	.000096

**P < .001
*P < .05

TABLE IX

BIRTH WEIGHT OF CALVES FROM COWS ON FOUR TREATMENTS

Treatment	Birth Weight
M[b]	33±4[a]
L-L[b]	31±4
L-M[b]	33±4
L-H[b]	33±5

[a] $\bar{x}$ ± S.E. kg.

[b] No significant effect of treatment on birth weight.

TABLE X

R^2 AND PROBABILITY LEVELS OF REGRESSION ANALYSIS OF HORMONE CONCENTRATIONS

Order	Hormone			
	P	E_1	E_3	E_1SO_4
Linear	0.708[a] <0.0001[b]	0.580 <0.0001	0.610 <0.0001	0.840 <0.0001
Quadratic	0.730 <0.0004	0.660 <0.0001	0.680 <0.0001	0.840 <0.08
Cubic	0.737 <0.19	0.670 <0.027	0.696 <0.008	0.847 <0.150
Quartic	0.746 <0.04	0.675 <0.629	0.697 <0.66	0.847 <0.936

[a]R^2 value.

[b]Probability level.

TABLE XI

MEAN PROGESTERONE CONCENTRATIONS

Days Prepartum	Treatment[a]			
	M	LL	LM	LH
1-10	6.1±0.5[b] 13[c]	6.4±1.0 11	5.3±0.4 10	6.5±0.7 14
11-17	7.3±1.3 7	7.6±1.4 7	7.4±0.8 10	8.6±2.7 3
18-24	10.9±1.2 9	8.6±0.6 7	9.4±1.0 9	8.7±0.7 13
25-31	11.8±0.9 8	8.8±1.1 8	12.3±0.7 7	8.2±2.3 4
32-38	14.6±1.7 9	11.5±0.7 6	10.5±0.4 7	12.0±0.8 10
39-45	12.5±1.5 3	9.8±1.6 5	10.5±1.1 4	7.8±2.3 3
46-52	14.5±2.0 4	11.3±1.8 3	11.6±1.3 4	13.0±0.4 3

[a]No significant treatment effect.

[b]$\bar{x}$ ± S.E. ng/ml.

[c]Number of cows.

TABLE XII

MEAN ESTRONE CONCENTRATIONS

Days Prepartum	Treatment[a]			
	M	LL	LM	LH
1-10	2608±520[b] 13[c]	1615±244 11	1877±389 12	5820±360 15
11-17	694±113 7	1031±289 7	1256±270 10	707±234 4
18-24	672±159 10	739±211 8	549±121 9	613±82 12
25-31	369±82 8	425±88 7	591±150 8	1137±594 4
32-38	522±244 9	281±81 7	223±41 7	245±50 10
39-45	222±104 3	399±172 4	272±97 4	374±52 3
46-52	400±145 4	268±93 3	136±24 3	371±32 3

[a]No significant treatment effect.

[b]$\bar{x}$ ± S.E. pg/ml.

[c]Number of cows.

TABLE XIII

MEAN ESTRADIOL CONCENTRATIONS

Days Prepartum	Treatment[a]			
	M	LL	LM	LH
1-10	131±11[b] 13[c]	200±32 11	179±40 12	138±15 16
11-17	87±12 7	105±16 8	101±22 10	53±26 3
18-24	50±7 10	55±11 8	46±9 8	63±82 13
25-31	46±8 8	46±10 8	48±8 8	83±27 4
32-38	38±6 9	41±5 7	51±16 6	22±2 10
39-45	22±5 3	35±7 5	36±14 4	37±18 3
46-52	36±14 4	19±4 3	20±6 3	25±6 3

[a]No significant treatment effect.

[b]$\bar{x}$ ± S.E. pg/ml.

[c]Number of cows.

TABLE XIV

MEAN ESTRONE SULFATE CONCENTRATIONS

Days Prepartum	Treatment[a]			
	M	LL	LM	LH
1-10	10395±956[b] 12[c]	12129±1488 11	9766±1436 9	12426±1390 16
11-17	8301±918 5	8844±1762 7	7548±1589 7	12667±3585 3
18-24	7092±1704 9	9492±1960 8	6324±1203 9	6744±1212 12
25-31	6121±2505 7	5140±1391 7	3617±1613 6	11729±783 4
32-38	3622±1274 9	6475±1648 7	4479±1107 7	3351±953 10
39-45	2025±799 3	4215±1311 4	1523±343 3	6534±1388 3
46-52	3019±1608 4	3888±2388 3	1384±211 4	3517±1309 3

[a]No significant treatment effect.

[b]$\bar{x}$ ± S.E. pg/ml.

[c]Number of cows.

VITA

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