INFLUENCE OF RESTRICTED NUTRIENT INTAKE ON OVARIAN AND PITUITARY FUNCTION IN BEEF COWS

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"PEOPLE OF GREAT ACHIEVEMENTS ARE PEOPLE
OF SPECIAL ATTITUDES."

- Stuart Briscoe-

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CHAPTER I

INTRODUCTION

A primary goal of the cow-calf producer is to wean as many pounds of calf as possible while expending minimal resources. Sub-maximal calf crops and lengthy calving intervals are the major causes of reduced net income in a beef operation. Estimates indicate that only about 70% of the cows in herds in the United States wean a calf each year (Dziuk and Bellows, 1983). This reduction of net calf crop (calves weaned/cows exposed to fertile bulls) is primarily due to failure of cows to become pregnant during the breeding season.

Research with laboratory animals between 1917 and the middle 1930's implicated poor nutrition as a major cause of reproductive failure (Reid, 1960). Understanding the effects of nutrient insufficiency imposed at different stages of the production cycle is important when considering management for a 12-month calving interval.

The precise mechanisms by which altered nutritional status affects reproduction have not been elucidated. However, it is clear that reduced nutrient intake can influence reproduction. Body condition, suckling, age, metabolic status, and breed interact with nutrient intake to

alter reproductive characteristics in healthy beef cows. In particular, endocrine status, interval from calving to first estrus, and pregnancy rate are altered significantly when cows are subjected to reduced nutrient intake (Wiltbank et al., 1962, 1964; Whitman, 1975; Dunn and Kaltenbach, 1980; Wettemann, 1980; Dziuk and Bellows, 1983; Richards et al., 1986; McCann and Hansel, 1986).

These studies were designed to evaluate the inter-relationships between reduced nutrient intake and endocrine function associated with the bovine ovary and pituitary gland. Our experiments will help to elucidate the mechanisms by which nutrition regulates the hypothalamic-pituitary-ovarian axis in beef cows. Ultimately, this should provide methods to increase efficiency in animal production.

CHAPTER II

REVIEW OF LITERATURE

Non-pregnant domestic animals exhibit behavioral estrous cycles at regular intervals. The estrous cycle is described by four distinct periods: proestrus, estrus, metestrus, and diestrus. In adult female animals the ovarian structures and function of the ovary undergo continual change during the estrous cycle. Thus, ovarian cycles that consist of a follicular, ovulatory, and luteal phase can be characterized. Central nervous system, hypothalamic, pituitary, and ovarian cues work in concert to regulate estrous cycles.

Reproduction in primate females centers on ovarian cycles and an endometrial cycle called the menstrual cycle. Menstrual cycles are described by three distinct phases: menstrual, proliferative, and secretory. Ovulation occurs in the middle of the menstrual cycle. Hormonal changes produce physiological alterations that result in sloughing of the endometrium which is called menstruation.

The period when females, of the farm animal species, are receptive to mating with a male is referred to as estrus. Estrous behavior is not expressed in primates. In non-primates the onset of estrus is characterized by

behavioral as well as histological and endocrinological changes that facilitate mating and subsequent fertilization of an ovulated ovum. When mating does not result in fertilization, estrus will occur at regular intervals in healthy females. Social and physical factors have major roles in governing sexual receptivity in primate females. If mating does not result in pregnancy in healthy female primates, menstruation will occur at regular intervals until menopause.

Cows fail to become pregnant because they do not exhibit estrus or ovulate, fertilization fails to occur, or fertilization occurs and embryonic death ensues. Anestrus is a period when ovarian activity is minimal or absent and when animals are not receptive to mating. This phenomenon is a major cause of reduced reproductive performance and sub-maximal calf crops in beef cattle (Wiltbank, 1970; Dziuk and Bellows, 1983).

The events that occur prior to the onset of estrus and those prior to menarche are similar. Physiological and endocrinological events that occur during prepubertal anestrus (the period prior to the onset of regularly occurring estrous cycles) have been studied extensively in many species (Wiltbank et al., 1966; Wiltbank et al., 1969; Arije and Wiltbank, 1971; Gonzalez-Padilla, 1975a,b,c; Grass et al., 1982; Foster et al., 1985; Deutscher et al., 1986; Rutter and Randel, 1986). Frisch (1973, 1983) described the physiological and body compositional changes that occur

before the onset of menarche in humans.

Postpartum anestrus has been extensively investigated (Zemjanis, 1961; Wiltbank et al., 1962, 1965; Casida et al., 1968; Whitman, 1975; Holness et al., 1978; Bellows et al., 1979; Dunn and Kaltenbach, 1980; Hansen et al., 1982; Moore and da Rocha, 1983; Rutter and Randel, 1984; Richards et al., 1986; Bartlett et al., 1987; Wettemann et al., 1986) and has been reviewed extensively (Dunn and Kaltenbach, 1980; Wettemann, 1980; Dzuik and Bellows, 1983). Understanding the mechanisms that regulate the incidence and epidemiology of the anestrous condition in domestic species and amenorrhea in human females is complicated by factors such as age, lactation, and level of nutrition. In laboratory and domestic animals other confounding factors such as breed and season can also influence onset of anestrus. The objective of this section is to review the major factors associated with acyclicity in domestic species and humans.

Initiation of Puberty

Dorland (1985) defines puberty as "the period during which the secondary sex characteristics begin to develop and the capability of sexual reproduction is attained". Females that have not reached puberty can be classified as anestrus or, in the case of primates, amenorrheic. Vaginal canalization, first cornified vaginal smear, first mating, and onset of pregnancy are criteria that have been used to

describe the onset of puberty in rodents (Kennedy and Mitra, 1963; Howland, 1971). Onset of puberty in bovine females is commonly associated with the presence of the first corpus luteum (Plasse et al., 1969, Brooks et al., 1985). First estrus has been used as a criterion for onset of puberty in heifers (Wiltbank et al., 1969). However, Rutter and Randel (1986) indicated that due to non-pubertal estrus, caution should be exercised when considering estrus as the sole criterion for puberty. Achievement of menarche is considered to be indicative of puberty in female primates (Frisch et al., 1973; Frisch, 1983).

Factors that influence attainment of puberty can be assigned to genetic or environmental categories and the interactions that exist between the two.

Genetic Components

Genetic parameters that affect puberty are not well understood. Based on evidence derived from studies with beef heifers (Wiltbank et al., 1966; Arije and Wiltbank, 1971), gilts (Zimmerman et al., 1960; Hixon et al., 1987), humans (Damon et al., 1969) and mice (Eisen, 1973), nonadditive genetic variance affects onset of puberty. In general, these effects are related to characteristics such as age, weight, and skeletal size. Attempts to estimate phenotypic and genetic associations between body fat and sexual maturity have been inconclusive.

Heritability estimates for traits associated with

puberty vary. Arije and Wiltbank (1971) reported heritabilities (h^2) in heifers of .20 $\pm$ .16 and 1.09 $\pm$ .27 for age and weight, respectively, at puberty. Cunningham et al. (1974) estimated the h^2 for age of gilts at puberty to be .64, whereas Hixon et al. (1987) reported h^2 to be .11 or .24 in gilts depending on the method used for calculation. Damon et al. (1969) calculated phenotypic correlation coefficients between human mothers and their daughters for age and stature at first menses to be .24 and .41, respectively. Heritability estimates for age and weight of mice at puberty (first appearance of a vaginal plug) are .13 and .35, respectively, (Eisen, 1973).

Heterosis which results from crossbreeding in cattle is thought to hasten the onset of puberty, in part, through increased average daily gain (Wiltbank et al., 1966, Wiltbank et al., 1969). Heifers which are heavier for their age reach puberty earlier than lighter contemporaries (Wiltbank et al., 1969), therefore, weight is thought to be more important than age when considering onset of puberty. However, only one-half to one-fourth of the heterotic effects could be accounted for when adjustments were made for weight. Nelsen et al. (1982) found that heterosis for age at puberty in heifers was -3.6%, whereas heterosis for weight and height at puberty were 8.2 and 2.1%, respectively. These studies indicate that genetics can play an important role in timing the onset of puberty.

Environmental Components

Influence of Nutrition. Nutrient intake is an environmental factor often focused on when considering onset of puberty in domestic species. A deficiency of nutrients in the young animal is a more serious problem than in the mature animal because malnutrition prevents orderly development of the reproductive organs as a physiologically integrated system (Asdell, 1949; Allden, 1970). It is obvious that dietary restriction will alter body weight and composition. The mechanism(s) whereby reduced amounts of nutrients delay puberty in humans and domestic species is not fully understood.

Post-weaning energy restriction can delay the onset of puberty in cattle (Reid, 1960; McClure, 1968) regardless of season of birth (Grass et al. 1982). Joubert (1954) reported that Friesian, Jersey, Africander, and Shorthorn heifers reached puberty earlier when they were supplemented with additional feed in the winter. Arije and Wiltbank (1971) demonstrated that beef heifers raised on a high plane of nutrition initiated estrous cycles at younger ages than those raised on a low plane of nutrition. Deutscher et al. (1986) implanted beef heifers with zeranol and raised them on a high or low level of nutrition. Irrespective of implantation, more heifers raised on a high nutritional regimen reached puberty and became pregnant when compared with contemporaries raised on low nutrition. The high plane of nutrition tended to overcome the detrimental effects of

implantation on ages at puberty and conception rates.

Day et al. (1986a) suggested that the delay in puberty in heifers related to dietary energy restriction was due to impaired LH secretion. Their results indicated that the pituitary became less responsive to LHRH under conditions of nutrient restriction and supported the concept that cattle (Schillo et al., 1982; Day et al., 1984) and rats (Piascek, 1985) fed low energy diets are more susceptible to the inhibitory effects of estradiol.

Prolonged undernutrition can delay puberty in the ewe (Foster et al., 1985, Foster and Olster, 1985). Lambs fed low energy diets reached puberty at a later age than those fed adequately (Foster and Olster, 1985; Fitzgerald et al., 1982). The tonic LH center of lambs, that received reduced energy, secreted fewer pulses of LH with lower amplitudes.

Undernutrition may reduce activity of the LHRH pulse generator that controls LH secretion. Without adequate stimulation, the gonads are unable to respond with adequate follicular development and produce concentrations of estradiol necessary to elicit estrus and ovulation. In the absence of ovarian steroids, nutrition appears to have a direct effect on the hypothalamic oscillator (Foster et al., 1983).

The influence of nutrition on puberty in swine, humans and rodents is less clear than in cattle. Puberty in gilts appears to be influenced more by age than weight unless feed is severely restricted (Zimmerman et al., 1960). Studies

with humans (Frisch, et al., 1972; Frisch et al., 1973; Frisch and McArthur, 1974; Frisch, 1977; Frisch, 1983;) and rodents (Kennedy and Mitra, 1963; Frisch et al., 1975; Frisch et al., 1977) support the concept that a critical weight and/or body composition is necessary for onset of menarche and cyclicity, respectively. In any event, malnutrition delays puberty in humans (Frisch, 1983).

Influence of Body Weight and Body Composition. The relationship between body weight (fat plus non-fatty material) and sexual maturation is a well established phenomenon in cattle, rodents and primates. In cattle, attainment of a critical body weight, varying with breed, is necessary for puberty (Arije and Wiltbank, 1971; Dunn, 1980). Kennedy and Mitra (1963) found that rats fed for optimal, retarded, or very retarded growth exhibited first estrus and mated at similar weights. These authors suggested that growth altered feedback between the ovaries and hypothalamus, and hypothesized that attainment of a critical body weight caused an increase in metabolic rate, thus, increasing the output of FSH and LH. Since dietary restriction and weight loss alter body composition (Robinson et al., 1975), altered feedback may be mediated by body compositional changes. Girls have first menses at varying weights and heights, which correspond to a specific percent fat or body composition (Frisch and McArthur, 1974; Frisch, 1983). Amount of body fat is an accurate predictor of onset of menarche in humans.

Compositional changes that occur during body growth in meat animals have been reviewed by Trenkle and Marple (1983). Little information is available on body composition at puberty in female farm animals. However, several clinical studies regarding associations between body composition and achievement of menarche in humans have been conducted (Frisch, 1983).

Rats fed high (HF) and low fat (LF) diets had similar body composition at first estrus (Frisch et al., 1977). Although HF rats showed estrus at an earlier age, carcass fat content was the same (15.6 g $\pm$ .95) for HF and LF rats when first estrus occurred. At estrus, total body water and percentage protein were similar for HF and LF rats.

Frisch et al. (1973) and Frisch (1976) studied anthropometric and radiographic measurements of fat content in human females. During the adolescent growth spurt, just prior to the onset of menarche, body fat increased 120% (5 to 11 kg), whereas lean body weight only increased 44%. The ratio of fat to lean body weight shifted from 1:5 at initiation of the growth spurt to 1:3 at menarche. At sexual maturity (between the ages of 16 and 18 years) well nourished women were about 165 cm tall, weighed 57 kg, and were composed of 26-28% fat and about 52% water (Frisch, 1983). In contrast, males were approximately 14% fat and 61% water. Frisch (1983) hypothesized that the higher proportion of fat in women provides energy for pregnancy, delivery of viable young and lactation. Alterations in

lean to fat ratio may be natures way of synchronizing hypothalamic control of reproduction.

Wilen and Naftolin (1977) stated that the critical body mass and critical metabolic rate theories were "manifestations of the process of maturation without either of them having a level which could be called critical". However, these hypotheses have not been proven false and are probably important concepts when considering body condition as it relates to reproduction in the bovine.

Influence of Season and Breed. Seasonal effects on the initiation of sexual cycles can be a combination of the effects of nutrition, temperature, and/or photoperiod. However, there are substantial data indicating that independent of any other variable, season can have an influence on reproductive cycles in certain species.

Reports of seasonal influences on reproduction in cattle and swine are contradictory. Grass et al. (1982), from a limited number of observations, suggested that attainment of puberty in beef heifers is a complex interaction among season of birth, genotype, and level of nutrition. They observed that puberty was delayed in winter-born heifers when compared with spring-born heifers, yet winter-born heifers were fatter, heavier, and had a greater daily feed intake. These results agree with results of Menge et al. (1960) who found that dairy heifers born in the spring reached puberty earlier than those born in other seasons, but are in contrast to Plasse et al. (1968) who

found that season of birth had no effect on age at puberty. Bolton et al. (1987) reported that spring-born crossbred heifers were younger at puberty and had higher pregnancy rates than fall-born contemporaries. Randel (1984) indicated that Bos Indicus cattle were seasonal breeders and that the mechanism involved with seasonal reproduction in cattle is related to altered LH secretion.

Bolton et al. (1987) also found that as percentage of Brahman breeding increased form 0 to 1/4 to 1/2, the magnitude of reduction in estrous and pregnancy rates increased. In that study, percentage of Brahman breeding did not affect age or weight at puberty which is in contrast to Gregory et al. (1979) and Morgan (1981) who found that Brahman type cattle were older and heavier at puberty than other breeds. Breed is generally considered to influence attainment of puberty in cattle through its influence on growth rate.

Ewes of most breeds of sheep become sexually receptive in the fall. Photoperiod controls the timing of puberty in lambs through its influence on LH secretion (Goodman and Karsch, 1981; Foster et al., 1985). Fall born lambs had a prolonged period of hyper-responsiveness to estradiol's negative inhibition of LH secretion, which resulted in a 20 week delay in initiation of ovulation (Foster et al., 1985).

The pineal gland, through its' production of melatonin, has been implicated as the zeitgeber that synchronizes photoperiodic timing of puberty in the female lamb. Puberty

can be prevented in lambs by denervation of the pineal gland (Yellon and Foster, 1984; Foster et al., 1985). Melatonin is thought to entrain the release of LH by exerting control on the LHRH pulse generator. It is not known when or how the female lamb becomes sensitive to photoperiodic cues.

It is not clear why rats raised during two seasons and exposed to 14 h of light daily at a constant temperature of 23 C reached puberty at different times (Ramaley and Bunn, 1972). Vaginal opening and ovulation occurred significantly earlier in rats born in the summer than those born in the winter.

Integration of the central nervous system, hypothalamus, pituitary and gonads controls the onset of puberty. Studies that lend insight to the mechanisms involved with initiation of cyclicity in prepuberal females may enhance our understanding of the anestrous condition.

Effects of Nutrition and Body Condition on Reproduction

Influence of Prepartum Nutrition

Onset of the first postpartum estrus is delayed in cattle by low energy intake in late pregnancy (Joubert, 1954; Zimmerman et al., 1961; Wiltbank et al., 1962, 1964, 1965; Reynolds et al., 1964; Turman et al., 1964; Hight, 1966; Dunn et al., 1969; McClure, 1970; Corah et al., 1974, 1975; Bellows and Short, 1978; Hansen et al., 1982; Bellows et al., 1982; Rasby et al., 1982; Wettemann et al., 1982;

Rasby, 1986), thus, increasing days to conception (Dunn et al., 1969; Richards et al., 1986). Reduced body condition at calving below a critical point results in fewer cows showing estrus early in the breeding season (Whitman, 1975; Richards et al., 1986; Selk, 1986). Rasby (1986) suggested that increased concentrations of estrogens in cows in thin condition prepartum may influence postpartum reproduction. However, this effect was most likely related to amount of available body energy reserves rather than concentrations of estrogens.

The effects of low body condition at calving are not readily overcome by a high level of nutrition after calving (Wiltbank et al., 1962; Dunn et al., 1969; Richards et al., 1986). Pre-calving energy exerts its greatest influence early in the postpartum period. By 40 and 60 days post partum, 25 and 60 percent, respectively, of the cows fed a high energy ration pre-partum had exhibited estrus compared with only 6 and 44 percent, respectively, of the cows receiving a low energy diet pre-partum (Dunn et al., 1969). As the postpartum period increased to 80 days, the effect of a low prepartum energy level was less pronounced. By 100 days, which is 10 to 15 days beyond the time at which cows must be re-bred to maintain a 12 month calving interval, prepartum energy intake had no influence on reproduction. In other studies, postpartum reproductive performance was not impaired by low prepartum energy intake provided cows were maintained on a high plane of nutrition after calving

(Bellows, 1966; Hight, 1966; Bellows et al.,1982).

Decreased prepartum energy has many effects that relate to subsequent producing ability. Reduced levels of prepartum energy decreases prepartum body weight, condition score, and pelvic area of the dam (Bellows and Short, 1978) and decreases the birth weight of the calf, yet not the incidence of dystocia (Hight, 1966; Corah et al., 1975; Bellows and Short, 1978). Turman et al. (1964) found that prepartum nutrition had a marked effect on subsequent performance of the beef heifer. Heifers allowed to lose 20 percent or more of their fall weight, through calving, raised calves 14.5 kg lighter than those who lost less than five percent of their fall weight through calving. Feed intake of heifers prior to calving was more critical than feeding level after calving. Richards and co-workers (1986) studying postpartum cows corroborated these conclusions.

Pre-calving nutrition per se has little effect on interval from calving to first estrus or other reproductive parameters provided cows remain in good body condition at parturition (McGinty and Ray, 1973; Whitman, 1975; Lowman, 1982; Richards et al., 1986; Selk, 1986, Boyd et al., 1987). Spring calving cows can withstand underfeeding and live weight loss during the early postpartum period without affecting reproductive performance, as long as cows calve in good body condition (Whitman, 1975; Holness et al., 1978; Dunn and Kaltenbach, 1980; Lowman, 1982; Humphrey et al., 1983; Richards et al., 1986). However, fall calving range

cows must maintain weight to ensure optimal postpartum reproductive efficiency (Rakestraw et al., 1986). Although, all of the ramifications of prepartum energy level have not been elucidated, it is evident that starvation will not yield dividends.

Postpartum Nutrition and Body Weight

The effects of nutrition on reproduction during the postpartum period in suckled beef cows and dairy cows have been studied and reviewed extensively (Wiltbank et al., 1964; McClure, 1965; Wagner and Hansel, 1969; Lammond, 1970; Oxenreider and Wagner, 1971; Whitman, 1975; Topps, 1977; Wettemann et al., 1978; Bellows et al., 1979; Dunn and Kaltenbach, 1980; Hansen et al., 1982; Dzuik and Bellows, 1983; Moore and da Rocha, 1983; Rutter and Randel, 1984; Richards et al., 1986). Unlike the level of prepartum nutrition, there is controversy with respect to the optimal postpartum energy level that ensures early return to estrus and rebreeding (Rutter and Randel, 1984). These inconsistencies may be due to the region of the country where experiments were conducted (Wettemann et al., 1986) and/or result from disregarding the confounding effects of body condition at calving with nutritional management.

A major concern is that pregnancy rate is decreased by feeding reduced amounts of energy after calving (Wiltbank et al., 1964, 1965; McClure, 1965; Dunn et al., 1969; Whitman, 1975; Spitzer et al., 1978; Bellows and Short, 1978; Dunn

and Kaltenbach, 1980; Dzuik and Bellows, 1983; Richards et al., 1986). Richards and co-workers (1986) found that if cows calved with body condition scores (1=emaciated to 9=fat) $\geq$ 5, first service pregnancy rate was not influenced by postpartum nutritional management. However, within body condition score $\leq$ 4 only 54% of the cows on a low level of nutrition became pregnant compared with 75% of those fed a flushing ration.

Topps (1977) concluded, from a study of Richardson et al. (1976a), that the most important factor influencing conception was actual body weight. However, in that study, there was a tendency for cows gaining weight to have the greatest pregnancy rates. Youdan and King (1977) associated body weight change with number of successful services in dairy cattle. They reported a positive association between weight gain and fertility. Cows gaining weight after parturition had 1.63 services per conception, while cows losing weight had 2.11 services per conception.

Although body weight per se does not offer an accurate assessment of the level of body energy reserves (body condition) or reproductive capacity, change in body weight may be a way to estimate body condition (Broster, 1973; Whitman, 1975; Wettemann et al., 1982) and evaluate the capability to reproduce. Clearly defining body condition score is imperative before evaluating response to pre- and postpartum diets.

Flushing (feeding additional energy)

Thomson and Aitkin (1959) defined flushing as "the practice of giving animals which are in poor condition an improved diet for a few weeks before mating so that they are in rapidly rising in body condition when they are bred". Flushing (feeding additional energy) has been used in an attempt to shorten the postpartum anestrous interval and enhance reproductive performance in cows (Bellows et al., 1968; Loyacano et al., 1974; Richards et al., 1986; Wettemann et al., 1986), ewes (Marshall and Peel, 1910; Thomas et al., 1987), and sows (Zimmerman et al., 1960; Bazer et al., 1968; Davis et al., 1987).

Neither Bellows et al. (1968), Loyacano et al. (1974) or Wettemann et al. (1986) found that flushing benefited pregnancy rates of range cows. In contrast, Richards et al. (1986) found that cows that calved in low body condition, lost weight early post partum, and were flushed with energy 2 weeks prior to and through breeding had similar pregnancy rates as cows fed adequately from calving through breeding (92 and 85%, respectively). Differences in response to flushing between range cattle and cattle fed a controlled diet are most likely due to variation in range conditions, research location, and/or body energy reserves of cows. Animals with adequate energy reserve are not likely to benefit from nutrients beyond what is adequate for maintenance (Rutter and Randel, 1984; Wettemann et al., 1986).

Ewes (Reid, 1960; Thomas et al.,1987) and sows (Zimmerman et al,. 1960; Davis et al., 1987) respond to flushing diets. Reid (1960) reported a 10 to 20 percent increase in lamb crop and a 40 percent increase in twinning, when nutritionally stressed ewes were flushed at various times prior to mating. Increased ovulation rate is commonly associated with energy flushing in swine (Zimmerman et al., 1960; Bazer et al., 1968). Futhermore, flushing with glucose (one percent of body weight) and lard (0.66 percent of body weight) for two or three weeks prior to breeding produced similar ovulation rates to gilts flushed with energy over an extended period of time (Zimmerman et al., 1960).

Until recently, the mechanism(s) associated with the flushing response was not clear. Thomas et al. (1987) measured changes in concentrations of liver enzymes associated with flushing and administration of phenobarbital to ewes. Flushing and phenobarbital increased concentrations of liver cytochrome b_5 oxidase and NADPH cytochrome c reductase. The hypothesis is that an increase in mixed function oxidases will increase steroid hormone metabolism which will result in reduced hypothalamic-pituitary inhibition of LH secretion. Greater gonadotropin release will then enhance ovulation rate. These data are a promising step toward elucidation of the mechanism(s) that elicit the flushing response in ewes.

Effects of Nutrition on Endocrine Function

Mulinos and Pomerantz (1940) described the influence of undernutrition on ovarian function in the rat as "pseudohypophysectomy". Low levels of nutrition somehow alter the delicate interrelationship among the hypothalamus, pituitary, and ovaries. Endocrine and ovarian changes leading to resumption of ovarian cycles after parturition in adequately fed animals are only partially understood. The lack of postpartum ovarian activity in most species is thought to be caused by reduced gonadotropin secretion. This concept seems plausible since follicular growth can be induced in anestrous postpartum cows, ewes and sows by treatment with exogenous gonadotropins (for review see Wettemann, 1980).

Gonadotropin secretion

The mechanisms whereby undernutrition affects endocrine status and function of the bovine are not well established. Hansel and Siefart (1967) demonstrated that LH and FSH are the primary gonadotropins controlling reproduction in the bovine. Luteinizing hormone stimulates conversion of a steroidogenic pool of cholesterol to pregnenolone in ovarian theca interna cells (Hansel and Convey, 1983). Although its mechanism of action is not understood, FSH is involved with progesterone synthesis and release in the bovine (Schallenberger et al., 1985).

The effects of varying amounts of energy on concentrations of LH in the cow are not clear. Gombe and Hansel (1973) and Beal et al. (1978) suggested that the ability of the ovary to respond to gonadotropins may be reduced by inadequate feeding. Rutter and Randel (1984) found that regardless of energy intake, cows that maintained body condition from calving through 20 days postpartum had greater concentrations of LH than those that lost body condition. Yet, pituitary weight, an indicator of pituitary synthetic activity, is not influenced by nutritional regime or body condition (Beal et al., 1978; Moss et al., 1982; Rasby, 1986). Spitzer et al. (1978) reported that heifers on diets restricted in energy had systemic blood concentrations of LH similar to those fed to meet 1964 NRC requirements. These results are agreement with Hill et al. (1970), but not with Lishman et al. (1979) or Echternkamp et al. (1982) who found that increased dietary energy increased LH secretion. Similarly, Dunn et al. (1974) found that peak LH concentrations were greater for cows fed diets with restricted energy contents. Cows fed restricted energy diets release more LH in response to GnRH challenges (Whisnant et al., 1985; Rasby, 1986). This introduces the question," How is LH storage altered by undernutrition? ".

Increasing dietary energy level results in increased ovulation rates in ewes and gilts. Feed intake and body condition altered ovulation rate and LH secretion during the early follicular phase, but not during any other phase of

the estrous cycle in Greyface ewes (Rhind and McNeilly, 1986). Greater LH pulse frequency but not amplitude, in ewes on high nutrition with good body condition was associated with enhanced ovulation rates (3.36 vs 2.33, high and low body condition, respectively). Although the influence of nutrition and body condition were confounded in that study, the results are consistent with a study of Rhind et al. (1985) who found that dietary intake influenced LH pulse frequency. Cox et al. (1987b) reported that improvement in ovulation rate in gilts is not necessarily accompanied by enhanced gonadotropin secretion. However, gilts on a high energy-insulin treatment had 3.3 pulses of LH per 4 hr versus only 2.6 pulses per 4 hr in gilts on a maintenance ration without insulin treatment. Despite improved ovulation rates, both ewes and gilts fed supplemental energy have reduced embryonic survival per female mated (Rhind and McNeilly, 1986; Cox et al., 1987b).

Reports on the influence of dietary protein on gonadotropin secretion are also conflicting. Dairy cows fed a ration containing 16.3 or 19.3% crude protein (CP) had reduced serum concentrations of LH when compared with those fed a diet containing 12.3% CP (Jordan and Swanson, 1979). By contrast, reproductive performance was not altered in dairy cows fed a 20% CP diet when compared with those fed a diet containing 15% CP (Howard et al., 1987) and limiting protein affected the ability of the pituitary to release or store LH in postpartum beef cows (Nolan et al., 1984).

Undernutrition in women which results in loss of between 10 and 15% of their body weight causes amenorrhea (Frisch, 1983). Endocrine changes associated with undernutrition mainly revolve around a deficiency in the secretion of LH. This deficiency is caused by lack of normal episodic GnRH secretion, which is necessary for normal cyclicity in human females (Warren, 1983). Warren (1983) also suggested that the hypothalamus of women with low body weight is more susceptible to sustained suppression by gonadal hormones.

Studies on the effects of energy restriction on follicle stimulating hormone (FSH) concentrations are limited. In cows, follicle stimulating hormone is responsible for follicular growth and development, has greatest blood concentrations at parturition, and decreases in the early postpartum period (Saiduddin et al., 1968, Moss et al., 1985). Concentrations of FSH in peripheral blood during days 0 to 20 postpartum are similar to those present on days 21 and 48 postpartum in the dairy cow (Dobson, 1978). Plane of nutrition does not impair FSH secretion or follicular growth in heifers (Spitzer et al., 1978), postpartum cows (Lishman et al., 1979)., ewes (Rhind et al., 1985), gilts (Cox et al., 1987) or rats (Meredith and Butcher, 1985). In contrast, Warren (1983) indicated that endocrine changes in human females in response to undernutrition include reduced concentrations of FSH. Although Oxenreider and Wagner (1971) did not measure FSH

directly, they found that postpartum Holstein cows on restricted energy diets had a longer interval from parturition to the presence of 10mm follicles when compared with contemporaries fed greater amounts of energy. This finding implicates nutrition as a mediator of changes in concentrations of FSH in humans. The significance of nutrition on FSH secretion in domestic species is equivocal.

Gonadal hormones

The effects of undernutrition on circulating concentrations of progesterone are not well established. The corpus luteum is the major source of progesterone in the cow (Gorski and Erb, 1959). Systemic concentrations of progesterone greater than 1 ng/ml blood are associated with the presence of a functional corpus luteum in the bovine (Stabenfeldt et al., 1969; Wettemann et al., 1972; Arije et al., 1974; Humphrey et al., 1983; Imakawa et al., 1987). Nutrition had no significant effect on peripheral concentrations of progesterone in postpartum dairy cows (Folman et al., 1973) or in beef cows and heifers (Corah et al., 1974; Spitzer et al., 1978; Harrison and Randel, 1986). In contrast, Hill et al. (1970), Gombe and Hansel (1973), and Beal et al. (1978), found that heifers fed restricted energy diets had reduced concentrations of progesterone. Mobley et al. (1983) reported that cows in late gestation losing weight and body condition had reduced plasma concentrations of progesterone. Lishman et al. (1979)

observed that cows losing weight prior to a GnRH induced LH surge had reduced concentrations of progesterone early post partum. Apgar et al. (1975) demonstrated that in vitro progesterone production by corpora lutea of cows fed restricted energy diets was reduced when compared with that of cows fed adequately. In contrast, Donaldson et al. (1970) and Dunn et al. (1974) observed that cows fed energy restricted diets had greater concentrations of progesterone than those fed adequate diets. In ewes, greatly elevated concentrations of progesterone have been associated with underfeeding (Cummings et al., 1971). It appears from results of these studies that other factors confound the influence of nutrition on blood progesterone concentrations.

Plane of nutrition can influence the concentrations of estrogens in peripheral blood of cows in certain reproductive states. In cows, concentrations of estradiol 17-β, estrone and estrone sulphate are elevated just prior to parturition (Henricks et al., 1972; Corah et al., 1974; Wettemann, 1980; Humphrey et al., 1983; Guilbault et al., 1985; Boyd et al., 1987) and decline sharply at parturition (Corah et al., 1974; Humphrey et al., 1983; Guilbault et al., 1985; Boyd et al., 1987). Nutrition does not appear to alter concentrations of estradiol 17-β in postpartum cows (Corah et al., 1974; Lishman et al., 1979) or sows (Armstrong et al., 1986; Cox et al., 1987). However, increased prepartum nutrition was associated with reduced serum concentrations of estrogen sulphate in late gestation

(Mobley, 1983; Boyd et al., 1987). Cows that are thin during late gestation have increased placental weight (Rasby, 1986) and larger placental weight would allow greater nutrient extraction efficiency to sustain feto-placental symbiosis (Guilbault et al., 1985).

In normal cows, by 5 to 10 days after parturition, follicular development is initiated (Saiduddin et al., 1968; Moss et al., 1985). Plasma concentrations of estradiol increase with days postpartum (Kesler et al., 1977; Spicer et al., 1986). In the early postpartum period, follicles develop to a large size, but the enzyme systems required for adequate production of estrogen may be lacking (Spicer and Echternkamp, 1986).

Although undernutrition does not appear to alter concentrations of estradiol in postpartum anestrous cows, tissue sensitivity could be changed or metabolites of estradiol, that could act as local mediators of the message of primary estrogens, could be produced (Ball et al., 1983) when food is limited in animals. Usually estradiol 17-β is metabolized quickly by the ruminant liver and is excreted in bile as estradiol 17-β glucuronide or sulfate (Rico, 1983). By contrast, swine and horses excrete free estrone in the urine (Rico, 1983). Fishman and Bradlow (1976) demonstrated that when human females lose weight the metabolism of estradiol, which normally proceeds with 16-hydroxylation, is altered in favor of 2-hydroxylation and catechol estrogen or 2-methoxy estrone is formed. This estrone has no intrinsic

activity and may be anti-estrogenic (Gordon et al., 1976). Other studies indicate that catechol estrogens may effect pituitary LH release (Warren, 1983).

Since body condition was not monitored in many studies it is difficult to assess the effects of nutritional stress on hormone profiles. The influence of nutrition on concentrations of gonadal hormones probably depends on amounts of body energy reserve available and reproductive state at the time the nutritional restriction is imposed.

Thyroid hormones

Nutritional status of an animal can influence thyroid function. General inanition results in involution of the thyroid gland with a decrease in the size of the epithelial cells and of the blood supply together with an increase in the colloid and in iodine storage (Hoskins, 1941). There is a paucity of information dealing with the relationship between nutrition, thyroid function, and reproduction; however, if undernutrition results in hypothyroidism, degenerative changes in the reproductive organs could ensue.

Clinically healthy male and female goats were fed thiourea for 90 days to induce hypothyroidism (Reddi and Rajan., 1986). Protein-iodine was significantly reduced within three weeks in treated does and bucks. Hypothyroid bucks had a decreased volume of semen in the ejaculate and decreased sperm concentration, motility and viability with

an increased incidence of abnormal spermatozoa. Hypothyroid does became anestrus by 6 wk. When treatment was discontinued, both male and female reproductive characteristics returned to normal.

Undernutrition may influence thyroid stimulating hormone (TSH) secretion from the pituitary. Since LH and TSH have the same α chain, reduced concentrations of LH could be due to partitioning of precursors away from LH producing basophils towards TSH producing cells. Louw et al. (1964) found that LH and TSH had an inverse relationship which implies that low concentrations of LH are limiting reproduction in cows with high concentrations of TSH. In contrast, Wagner et al. (1969) found that thyroprotein-fed anestrous cows had adequate concentrations of pituitary LH and concluded that the LH releasing mechanism could be defective in hypothyroid animals. Rasby (1986) observed that thin cows, despite releasing more LH in response to GnRH than moderate and fat conditioned cows, had similar serum concentrations of thyroxine after a TRH challenge. However, basal thyroxine tended to be reduced in thin cows. To date, relating LH and thyroid function is equivocal.

Blood metabolites and insulin

Under normal conditions the central nervous system depends exclusively upon a minute to minute supply of glucose (Steinberg, 1985). The largest proportion of circulating glucose in the ruminant is produced from

conversion of propionate via the gluconeogenesis pathway (Bassett et al., 1970). Nutritionally induced changes in amino acid and lipid concentrations in animals alter glucose production in the liver and kidneys (Bergman, 1982) which could influence reproductive capacity. Loss of ovarian activity, resulting form depressed hypothalamic activity, could result from hypoglycemia (Oxenreider and Wagner, 1971).

Gonadotropin secretion may be influenced by altered concentrations of glucose at the hypothalamus or pituitary gonadotropes. Although glucose distribution is not affected by stage of the estrous cycle in ewes (Dunn et al., 1972), glucose appears to be an essential nutrient for GnRH-induced LH release in the rat (Sen et al., 1979). Lynn et al. (1965) found that in vivo and in vitro progesterone production by luteal tissue was greater in the presence of increased glucose concentrations. These results suggest that the glycemic state of an animal may be involved in determining gonadotropin synthesis and/or secretion and rate of steroidogenesis. McCann and Reimers (1986) found that estrus enhanced the acute insulin response to glucose and decreased the glucoregulatory effects of insulin in obese compared with lean heifers. This indicates that estrogens may modulate the effects of insulin, which may, in turn, alter gonadotropin secretion. It follows that reproductive hormone changes associated with reduced nutrient intake may be secondary to changes in concentrations of glucose and/or

insulin in the blood.

Rutter et al. (1983) increased concentrations of glucose from 79.6 to 86.9 mg% in Brangus heifers by infusing propionate into the abomasum for 21 days. After 24 hours of propionate infusion, both control and propionate treated heifers had similar responses to a GnRH challenge, at which time concentrations of glucose tended to be reduced in treated heifers. When concentrations of glucose were greater in treated heifers (after 21 days of propionate infusion), propionate treated heifers released more LH in response to GnRH than control heifers. These results provide evidence that support metabolic involvement, namely glucose concentrations, in the capacity of the pituitary to respond to stimulation by GnRH. Rutter et al. (1983) proposed that this effect could be mediated via an increased number of GnRH receptors on gonadotropes.

No direct relationships between increased concentrations of glucose and improved fertility have been established in cows (McCaughey et al., 1985; Selk, 1986; Rutter and Manns, 1987) or ewes (Rutter and Manns, 1986). Yet, hypoglycemia has been associated with reduced fertility in beef and dairy cows (McClure, 1968; Oxenreider and Wagner, 1971; Patil and Deshpande, 1979; McCann and Reimers, 1985 a,b). Oxenreider and Wagner (1971) reported significant negative correlations between plasma concentrations of glucose and interval to occurrence of 10 mm follicles (r=-.50) and ovulation (r=-.62). Patil and

Deshphande (1979) found that cows with long postpartum anestrous intervals had reduced blood glucose concentrations when compared with cows with short anestrous intervals. Rasby et al. (1982) supplemented cows at different rates and demonstrated differences in plasma concentrations of glucose which were related to reproductive performance. Continuous infusion of glucose for 12 d increased the number of LH peaks, mean concentrations of LH and total response area during an LHRH infusion (Garmendia, 1986). McCann and Hansel (1986) found that concentrations of insulin and glucose in dairy heifers were decreased in response to short-term fasting and did not return to control values until fasting ended. In addition, serum LH concentrations were reduced in fasted heifers when compared with control animals. When feeding was increased, concentrations of LH increased abruptly in the fasted group. Although glucose does not appear to regulate reproduction, reduced concentrations of glucose probably reflect a reduced capability of becoming pregnant.

Rutter and Manns (1987) used a Na^+-dependent glucose transport protein blocker (phlorizin) and infusions of glucose (500g) to study the influence of glucose on the postpartum interval in beef cows. Glucose infusion increased concentrations of glucose to 131 $\pm$ 8 mg/dl and insulin to 67 $\pm$ 16.8 µU/ml. During the first day of phlorizin treatment, concentrations of glucose in plasma of treated cows were reduced. Glucose returned to pre-infusion

concentrations by three days after phlorizin treatment. Although increased glucose clearance, due to phlorizin, did not affect concentrations of FSH, LH pulse amplitude was reduced by treatment. Since phlorizin or weaning did not influence the number of cows ovulating within 10 days, it is possible that the reproductive capacity of cows used in that study was not limited by nutrition, body energy reserves, or glucose and supports the contention that increasing nutrient availability beyond what is adequate does not enhance reproduction (Rutter and Randel, 1984).

Insulin enhances utilization of glucose and amino acids in all body tissues except the brain and has been implicated as a component of the network that regulates reproductive parameters in animals (Davis et al., 1947; Kirchick et al., 1982; McCann and Reimers, 1985a,b; Harrison and Randel, 1986; Armstrong et al., 1986; Cox et al., 1987b). However, to what extent insulin influences reproductive parameters is not known. Basset et al. (1974) and Gill and Hart (1981) reported that reduced concentrations of insulin were involved with irregular estrous cycles, low conception rates, and reduced viability of young. Concentrations of insulin are reduced in ovariectomized and intact gilts fed diets restricted to 10% of NRC recommendations (Armstrong and Britt, 1987). Selk (1986) observed that insulin response curves were different for cows fed to maintain or lose weight that became pregnant when compared with contemporaries that did not conceive.

Harrison and Randel (1986) evaluated the effects of nutrition and insulin on reproductive traits using Brangus heifers. Heifers were fed 75 or 180% of NRC energy and dry matter requirements, infused with 40 IU of insulin or saline and treated with FSH. Energy restriction did not affect concentrations of LH or progesterone in these heifers. Infusion of pharmacological doses of insulin increased concentrations of insulin in low energy heifers to 163.1 ng/ml, whereas concentrations of insulin in the high energy heifers were only 115.3 ng/ml after infusion. Control heifers had mean values for insulin of 16.7 and 48.8 ng/ml (low and high energy, respectively). Ovulation rate in the group deprived of energy was enhanced by insulin treatment (7.8 vs 1.3 corpora lutea, low and high energy, respectively). These findings are similar to Jones et al. (1983) and Cox et al. (1987b) who reported that administration of insulin prior to estrus increased ovulation rates in gilts.

Kasuga et al. (1977) demonstrated that metabolic parameters could be altered by fasting and then refeeding rats. Rats fasted for two days had reduced body weight, fat cell diameter, and plasma concentrations of glucose and insulin when compared with control and rats fed ad libitum for two days. Concentrations of glucose were 120, 75, and 123 mg% for control, fasted, and re-fed rats, respectively. Fasting resulted in greater insulin binding, which were induced by a greater number of insulin receptors. These

authors postulated that concentrations of insulin regulated its own receptor population. Hexokinase activity was decreased, thereby, glucose oxidation was decreased in fasted rats which suggested that fasted rats became insulin resistant. All metabolic parameters returned to control levels after refeeding. Although reproductive traits were not evaluated, these data display evidence for interaction between metabolic components and adipocytes which can be implicated in reproductive function through their relationship with body condition.

Vernon et al. (1985) found that when adipose tissue was removed from the bovine a transient refractoriness to insulin ensued. Bovine adipocytes are very sensitive to insulin in short-term incubations (Etherton and Evock, 1986). Etherton and Evock (1986) indicated that insulin was a predominant factor in maintaining lipogenic capacity of cultured bovine adipose tissue.

The availability of blood metabolites and partitioning of nutrients may influence synthesis and/or secretion of reproductive hormones. A better understanding of the relationships between hormone secretory patterns and the interactions among various reproductive and metabolic constituents in cows with different amounts of body energy reserves would be beneficial. Furthermore, identification of the specific hormone(s), metabolite(s), or product(s) that controls gonadotropin secretion and thus, regulate cyclic ovarian activity is necessary.

Factors Associated with Anestrus

Nutritional Induction of Anestrus

Anestrus has been induced by restricting the amount of nutrients offered to cattle (Bond et al., 1958; Imakawa et al., 1986a; Johnson et al., 1987), gilts (Armstrong and Britt, 1985,1987) and hamsters (Howland and Skinner, 1973). Cattle (Imakawa et al., 1986a; Johnson et al., 1987) and pigs (Armstrong and Britt, 1987) that become anestrus because of severe nutrient restriction can be re-fed to cause resumption of normal estrous cycles.

It took 100 days and a 20% loss of initial body weight for dairy heifers to become anestrus (Johnson et al., 1987). Heifers lost .73 kg per day when fed 40% NE_m. After termination of the study, the heifers were placed on a regaining plane of nutrition. They returned to estrus and conceived at 45 and 72 days, respectively. Imakawa and coworkers (1986a) observed that when beef heifers were fed 50% of the energy required for maintenance it took 186 days and a 20% loss of initial body weight for them to have concentrations of progesterone (<1 ng/ml) indicative of anestrus. The anestrous heifers re-initiated estrus cycles 49 days after initiation of a high energy diet.

It has been proposed that energy restriction affects reproduction in cows and pigs by altering GnRH secretion and/or decreases pituitary sensitivity to GnRH which results in altered gonadotropin secretion (Beal et al., 1978;

Carruthers et al., 1980b; Walters et al., 1982; Armstrong and Britt, 1987). Rasby (1986) and Whisnant et al. (1985) found that cows that lost weight and cows that maintained weight responded differently to a GnRH challenge. This lends additional credence to the hypothesis that reduced energy reserves impairs GnRH secretion or limits is action on pituitary cells.

Influence of Estradiol on LH Secretion

The ovary itself, does not limit initiation of cyclic ovarian activity in the postpartum cow. If appropriate gonadotropic stimulation is provided by the pituitary during postpartum anestrus, beef females have an adequate number of FSH and LH receptors to initiate steroidogenesis (Moss et al., 1985; Braden et al., 1986). Episodic release of LH seems to be the key to the initiation of the first postpartum estrus in nonlactating (Dunlap et al., 1981), milked (Stevenson and Britt, 1979) and suckled cows (Carruthers and Hafs, 1980: Rawlings et al., 1980; Walters et al., 1982; Humphrey et al., 1983).

England et al. (1973) and Garcia-Winder et al. (1984) ovariectomized suckled cows and found that their is a mechanism independent of suckling that inhibits LH secretion. In fact, they pointed to suppressants secreted by the ovary.

Ovarian secretions suppressed concentrations of LH in prepuberal Holstein heifers (Anderson et al., 1985) and

postpartum dairy cows (Azzazi et al., 1983). Imakawa et al. (1986a) have shown that estradiol suppresses the secretion of LH in nutritionally anestrous heifers. It appears that estradiol causes a reduction in the frequency of LH pulses in the prepubertal heifer (Day et al., 1986b), in anestrous cows (Imakawa, 1986a,b), in acutely ovariectomized nulliparous beef cows (Kinder et al., 1983), and in mature ovariectomized cows (Day et al., 1986a; Acosta et al., 1983; Hinshelwood et al., 1985). In addition, underfeeding has been reported to increase the sensitivity of the hypothalamus and/or pituitary to the negative effects of estradiol in cows (Imakawa et al., 1987) and ovariectomized prepubertal rats (Piascek, 1985).

Recently, Hinshelwood et al. (1986) demonstrated that estradiol acts at the pituitary to cause reduced concentrations of LH in response to a GnRH challenge. However, this work did not rule out the possibility of hypothalamic mediated alterations in LH secretion due to estradiol negative feedback. It is not known if the inhibition of LH secretion by estradiol subsides with attainment of puberty or with onset of estrous cycles.

Ovariectomized sows respond to exogenous estrogen treatment by expressing estrus and releasing a preovulatory surge of LH (Cox et al., 1987a). In addition, long term anestrous sows will ovulate in response to exogenous estrogen (Cox et al., 1983). These results demonstrate that positive estrogen feedback exists for LH secretion. In

addition, it appears that seasonal influences on reproduction in ewes and sows are mediated by interactions between negative estrogen feedback and the hypothalamic-pituitary axis (Karsch, 1987). Goodman et al. (1982) reported that the concentrations of LH were greater and the LH pulse frequency was greater in ewes during the breeding season when compared with the non-breeding season. They indicated that season altered the sensitivity of the hypothalamic-hypophyseal unit to negative feedback by estradiol. In sows, the frequency of pulsatile LH secretion is not affected by season, but the average amplitude and baseline of LH is lower in fall, winter and spring when compared with the summer months (Cox et al., 1987a).

In humans, estradiol can interact with micronutrients to alter reproduction. Deuster et al. (1986) found that women runners who were amenorrheic had reduced serum concentrations of estradiol, dietary fat intake and zinc density when compared with their eumenorrheic counterparts. Papronty et al. (1981) found that concentrations of zinc in plasma varied during the menstrual cycle and were maximal in ovulatory women at ovulation, while zinc concentrations were static in anovulatory women. Habib et al. (1980) indicated progesterone binding to plasma proteins and endometrial cytosolic proteins was associated with a critical concentration of zinc. Not only zinc, but other micronutrients could alter concentrations of steroids and, in particular, estradiol binding to tissues. Since much

variation exists in clinically oriented retrospective studies, caution should be taken when interpreting results of these studies. The implications of dietary intake on endocrine and/or reproductive function remain to be determined.

Relationships Among Body Condition, Body Composition and Anestrus

Body condition has been defined as "the ratio of the amount of fat to the amount of non-fatty matter in the living animal" (Murray, 1919). Reproductive performance of beef cows varies according to the total amount of body energy reserves (fat) available, or body condition, at specific stages of the production cycle (Wiltbank et al., 1962; Donaldson et al., 1967, 1969; Baker, 1969; Croxton and Stollard, 1976; Stollard et al., 1976; Lowman et al., 1976; Dunn and Kaltenbach, 1980; Dzuik and Bellows, 1983; Richards et al., 1986, Selk, 1986). Wiltbank et al. (1962) first proposed a 1 to 9 system to evaluate body energy reserves in cows. Since that time, the system has been modified, validated and used extensively by researchers (Whitman, 1975; Spitzer, 1986; Wagner et al., 1988).

Body composition as it relates to puberty was discussed previously in this review. Studies that associate total body components and anestrus in adult animals are lacking in number. Body condition score and body fat determined by slaughter data (Dunn et al., 1983; Wagner et al., 1988),

chemical analysis (Wright and Russel, 1984), and percent body lipid (Johnson et al., 1987) are highly correlated. Correlations between body fat and body condition score range from .86 (Dunn et al., 1983) to .91 (Wagner et al., 1988). It is tempting to speculate that acyclicity in cattle is associated with a critical, minimum amount of fatness or carcass energy content as is the case in human females (Frisch, 1983).

Carcass energy and body condition have been associated with reproductive characteristics. Ferrell et al. (1976) evaluated relationships between pregnancy status and carcass density, empty body weight, and body composition. Non-pregnant and pregnant cows had similar body composition characteristics. For example, energy concentrations in the empty body were 3.10 $\pm$ .14 and 3.21 $\pm$ .12 mcal/kg for non-pregnant and pregnant animals, respectively. Wagner et al. (1988) estimated carcass composition of cows with varying body condition scores (BCS) using regression equations derived from slaughter data. He predicted total mcal of carcass energy and carcass fat content per kilogram of live weight with the following equations: carcass fat = -53.84 + 6.987(BCS) + .115(LW); carcass energy = -487.2 + 78.38 (BCS) + 1.30(LW). On a per unit basis, BCS was superior to live weight, weight to height ratio, carcass water, carcass protein, and water space as a predictor of carcass composition.

Reduced nutrient intake adversely affects reproductive

capacity. Anestrus is the major cause of cows not becoming pregnant by the end of a breeding season. The precise mechanisms whereby nutritional status controls anestrus in cows have been only partially elucidated. To date, there are no results that demonstrate the level of fatness or condition domestic animals cease to have normal ovarian cycles or at what level of fatness normal cyclicity resumes after cessation has been induced by reduced nutrient intake. The experiments described in this manuscript were designed to answer questions regarding the influence of nutrition on ovarian and pituitary function in an effort to devise methods to control the reproductive problem of anestrus in beef cows.

CHAPTER III

NUTRITIONAL ANESTRUS IN BEEF COWS I. ASSOCIATIONS AMONG BODY WEIGHT, BODY CONDITION AND REPRODUCTIVE TRAITS

Abstract

Multiparous Hereford cows (n=22) with moderate to good body condition scores (BCS) were randomly allotted to maintenance (M) or restricted (R) diets under drylot conditions. Cows on M diets received adequate feed to maintain initial body weight and R cows were fed to lose 1% of their initial weight weekly until ovarian luteal activity ceased. When most of the R cows became anestrus, their diet was increased to allow weight gain and resumption of ovarian cyclicity. Body weights and BCS were recorded weekly and ovarian luteal activity was assessed by weekly determination of progesterone in plasma. At four times, concentrations of LH in serum were quantified at frequent intervals and LH was quantified in weekly samples. Restricted cows had reduced weight ($P<.01$) by 5 wk and BCS ($P<.01$) by 15 wk, when compared with M cows. Ovarian luteal activity ceased after 26 $\pm$ 1 wk of reduced nutrient intake in 91% of the R cows; R

cows had lost 24.0 ± .9% of their initial weight and had a BCS of 3.5 ± .3. Lack of ovarian luteal activity was associated with absence of behavioral estrus. Estrous cycles resumed 9 ± 2 wk after the beginning of increased nutrient intake, at which time, R cows weighed 12 ± 3% less than at the start of the trial and had a BCS of 4.6 ± .2. Concentrations of LH in serum samples obtained weekly were reduced ($P<.01$) in R cows when compared with M cows. In addition, LH pulse frequency was reduced ($P<.05$) when R cows were initiating anestrus. Pulse amplitude was not influenced significantly by feed restriction. We conclude that anestrus occurs when nonlactating, Hereford cows lose weight and have a BCS of approximately 3.5. Anestrus is associated with a decrease in the frequency of LH pulses. Increased nutrient intake after a period of severe restriction will result in resumption of estrous cycles and normal pregnancy rates.

(Key Words: Anestrus, Beef Cow, Body Condition, LH, Nutrition)

Introduction

The length of time from parturition until first observed estrus in beef cows is dictated by interactions among nutrient intake, weight change, body condition at calving, and suckling (Whitman, 1975; Dunn and Kaltenbach, 1980; Edgerton, 1980; Dziuk and Bellows, 1983; Richards et al., 1986). Pre- and postpartum weight changes per se are

not as important as body condition at calving when considering the postpartum interval in beef cows (Dunn and Kaltenbach, 1980; Richards et al., 1986; Selk, 1986). Inadequate nutrition alters the intricate balance among the hypothalamus, pituitary, and ovaries. Nutritional anestrus (lack of regularly occurring estrous cycles) has been induced in cattle (Bond et al., 1958; Imakawa et al., 1986; Johnson et al.; 1987) and pigs (Armstrong and Britt, 1987) by offering diets that contained $\leq$ 50 and 10%, respectively, of the NRC energy requirement for maintenance. Severe energy restrictions, resulting in cessation of cyclic ovarian activity, were overcome by re-feeding.

The physiological mechanisms whereby undernutrition and reduced body energy reserves cause anestrus in cattle are only partially understood. Mulinos and Pomerantz (1940) described the influence of undernutrition on ovarian function in rats as "pseudohypophysectomy". In domestic species and primates, lack of ovarian activity has been attributed to reduced gonadotropin secretion (Wettemann, 1980; Lamming et al., 1981; Humphrey et al., 1983; Warren, 1983; Imakawa et al., 1986). Reduced gonadotropin secretion results in decreased follicular development, absence of estrous activity, ovulation failure, and subsequent lack of ovarian luteal activity. It has been proposed that energy restriction affects reproductive characteristics by altering GnRH secretion and/or decreasing pituitary sensitivity to GnRH which results in reduced gonadotropin secretion (Beal

et al., 1978; Carruthers et al., 1980: Walters et al., 1982; Armstrong and Britt, 1987). Selection of the preovulatory follicle may be associated with the increased LH pulse frequency observed before pre-ovulatory gonadotropin surges in cattle (Schallenberger et al., 1985; Spicer and Echternkamp, 1986).

The objectives of this study were 1) to determine the associations between BCS and the cessation and resumption of estrous cycles and ovarian luteal activity and 2) to evaluate concentrations of LH associated with nutritional anestrus and reproductive performance after resumption of estrous cycles in beef cows.

Materials and Methods

Multiparous, nonlactating, Hereford cows (n=22) with good to moderate body condition score (BCS = 5.5 $\pm$ .1); were used in this study. Two people independently assessed BCS using the system where 1=emaciated and 9=obese (Spitzer, 1986; Wagner et al., 1988). All cows exhibited normal estrous cycles and weighed 420 $\pm$ 10 kg at the initiation of the study. One half of the cows were randomly assigned to maintain (M) their initial body weight (BW) and BCS and the remaining cows were fed a restricted diet (R) to lose 1% of their body weight (BW) weekly until they became anestrus (absence of ovarian luteal activity and behavioral estrus). After a majority of the R cows were anestrus, they were offered approximately 160% of the diet given M cows until

they re-initiated estrous cycles.

Cows in both treatment groups were kept in a drylot and individually fed a complete ration (12% CP and 2.25 Mcal ME/kg) each day for 41 wk. All cows were then exposed to fertile bulls for 42 d of natural service. During breeding cows were maintained on dry native range and supplemented with 1 $kg^{-1}hd^{-1}d$ of a 41% protein supplement.

Androgenized cows (Kiser et al., 1977) fitted with chin-ball markers, weekly chalking of the tail of experimental animals, and visual observation were used to monitor estrous activity. Pregnancy was diagnosed via rectal palpation 60 d after the end of the breeding season.

Weights and BCS were determined weekly for 41 wk. Prior to weighing and scoring, cows were denied water and feed for 16 h. Percentage weight change was calculated as a deviation from a cow's initial weight.

Blood samples (plasma, 40 ml; serum, 10 ml) were collected by venipuncture from all animals at weekly intervals for 41 wk. In addition, blood samples were collected every 3 to 4 days during the regain period to monitor concentrations of progesterone. Upon collection, 31.2 mg of oxalic acid were added to the 40 ml blood tubes and they were placed on ice. Samples were centrifuged at 4,800 x g within 4 h and plasma was decanted and stored at -20 C until assayed for hormones. Ten ml samples were allowed to clot (24 h), serum was separated by centrifugation (3,500 x g) and stored at -20 C until

analyzed.

Serum was collected every 10 min for 8 h from six M and five R cows to assess LH secretion at 4 times during the study. Cows were sampled when R cows were exhibiting normal estrous cycles (after 5 wk of feed restriction), at the initiation of anestrus (after 20 wk of feed restriction), during anestrus (after 25 wk of feed restriction), and at the re-initiation of estrous cycles (after 6 wk of increased nutrient intake). Two injections of prostaglandin $F_2\alpha$ (25 mg; i.m.) were used 11 d apart to synchronize estrus. On D 9 to 11 (D 0 = estrus) polyvinyl cannulae[1] were inserted into jugular veins and cows were confined to metabolism stalls.

Concentrations of progesterone, estradiol, and LH in blood samples were quantified by radioimmunoassays. Plasma concentrations of progesterone were determined using techniques described by Lusby et al. (1981). Antiserum to bovine progesterone (OSU B1-1) was used at a dilution of 1:32,000. Intra- and inter-assay coefficients of variation (n=87 assays) were 5.1 and 19.8%, respectively. The procedure used to quantify estradiol was validated by Hallford et al. (1979). Antiserum to bovine estradiol (no. 224 from Dr. G. Niswender) was used at a dilution of 1:100,000. Intra- and inter-coefficients of variation (n=8 assays) were 11.8 and 19.5%, respectively. The assay used

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[1]Bolab Inc., BB 317-v/10, id .157cm, od 2.083cm; Lake Havasu City, Az 86403.

to quantify concentrations of LH in weekly serum samples was described by Hallford et al. (1979). Antiserum to ovine LH (#15 from Dr. G. Niswender) was used as the primary antibody at a dilution of 1:80,000 and ovine anti-rabbit serum was used as the second antibody (1:150). Intra- and inter-assay coefficients of variation (n=4 assays) were 2.0 and 4.0%, respectively. Concentrations of LH in frequent serum samples were quantified by procedures described by Wettemann et al. (1988). Antiserum to bovine LH (Rabbit OSU B-4) was used as the primary antibody at a dilution of 1:160,000 and ovine anti-rabbit serum was used at a dilution of 1:40 as the second antibody.

Analyses of variance were used to evaluate weight and BCS change, ovarian activity, pregnancy response and LH pulse amplitude and frequency (Steel and Torrie, 1980). During 8 h sampling periods, LH pulses were defined using a modified version of the criteria established by Goodman and Karsch (1980). Any value of LH larger than 2 standard deviations above the mean for a cow on a day, that was followed by at least 2 values of lesser concentration, was considered a pulse. The amplitude of an LH pulse was the difference between the highest value during a pulse and the nadir within 30 min before the pulse. Concentrations of estradiol were analyzed as a split-plot in time, with repeated measurements from the same cow over time (Gill and Hafs, 1971). Concentrations of LH in weekly samples were analyzed by regression analysis. When a significant

treatment by time effect existed, polynomial response curves of concentrations of LH were tested for heterogeneity of regression. Standard errors at the mean of the continuous independent variable are reported as estimates of variation for each regression curve.

Results and Discussion

Reduced nutrient intake resulted in loss of BW and BCS, decreased ovarian function, and cessation of normal estrous behavior. Close agreement existed between changes in BW and BCS (Table 1; Figure 1). Restricted cows lost more weight ($P<.01$) by 5 wk and BCS ($P<.01$) by 15 wk of the study when compared with M cows.

Figures 2 and 3 depict associations between BCS and plasma concentrations of progesterone in a M and R cow, respectively. The profile for the M cow was typical for 11/11 M cows and that of the R cow typical of 10/11 R cows. Cyclic ovarian luteal activity ceased (progesterone concentrations < 1 ng/ml for 3 consecutive wk) in 10 of 11 cows in the R group by 30 wk (mean = 26 $\pm$ 1 wk) of restricted nutrient intake, while 100% of the M cows had normal ovarian function at wk 30 (Table 2). Restricted cows had lost 24.0 $\pm$ .9% of their initial weight and had a BCS of 3.5 $\pm$ .1 at the onset of luteal inactivity. Estrous behavior was not exhibited when ovarian luteal activity ceased.

Imakawa et al. (1986) found that anestrus occurred in

beef heifers 186 ± 28 d after initiation of a diet that provided 50% of the energy required for maintenance (NRC, 1976). Lack of ovarian luteal activity as determined by concentrations of progesterone was associated with a 20% loss of initial BW. Similarly, Johnson et al. (1987) observed that 100 d of feeding a diet consisting of 40% NE_m to dairy heifers, resulted in a 20% BW loss and anestrus. Estrous cycles in gilts stopped after 46 ± 9 d of feed restriction, at which point, they had lost 14.5 ± 2.0 and 25.0 ± 6.9% of their initial BW and back fat, respectively (Armstrong and Britt, 1987).

Undernutrition of human females and a 10 to 15% weight loss causes amenorrhea (Frisch et al., 1983). A critical body mass (fat to lean ratio) may be necessary for normal reproductive function in women (Frisch et al., 1973; Frisch and McArthur, 1974) and rats (Kennedy and Mitra, 1963). Well-nourished women had 26-28% body fat (Frisch, 1983), whereas, amenorrheic women runners only had about 11% body fat (Deuster et al., 1986). Thin cows in our study became anestrus when the estimated amount of carcass fat[2] decreased to 4.1% (total carcass energy = 197 mcal) from a pre-treatment amount of 14% (total carcass energy = 490 mcal). At resumption of normal estrous cycles carcasses of R cows contained 10.4% estimated fat (total carcass energy content = 349 mcal).

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[2]Carcass fat = -53.84 + 6.987(BCS) + .115(BW); Carcass energy = -487.2 + 78.38(BCS) + 1.30(BW); From regression equations derived from slaughter data reported by Wagner, 1985.

After 17 wk on a diet containing 160% of the energy required for maintenance, BW and BCS of R cows were still significantly reduced when compared with M cows, although R cows were rapidly gaining weight and increasing in BCS (Table 1). Seventy-three percent of the R cows resumed estrous cycles by 8 wk of the regain period. The interval to estrus for R cows was 9 $\pm$ 2 wk from initiation of increased feeding. At resumption of estrous cycles, R cows had regained 12 $\pm$ 3% of their initial BW and had achieved a BCS of 4.6 $\pm$ .2. Gilts on energy restricted diets resumed estrous cycles after realimentation when they regained about 13% of their initial BW (Armstrong and Britt, 1987).

Plasma progesterone concentrations greater than 1 ng/ml for 2 consecutive wk are an indication of ovarian luteal activity (Stabenfeldt et al., 1969; Wettemann et al., 1972; Humphrey et al., 1983; Lauderdale, 1986; Imakawa et al., 1987). Ovarian luteal activity occurred 10 $\pm$ 2 wk after implementation of increased nutrient intake. At that time, cows had regained 16% of their initial weight and about 6% of their initial estimated fat, and had achieved a body condition of 4.9 $\pm$ .4. Imakawa et al. (1986) observed that 80% of the heifers that were nutritionally induced to become anestrus re-initiated ovarian activity by 41 $\pm$ 5 d of re-feeding. This suggests that greater body energy reserve is required to re-initiate gonadotropin secretion and estrous cycles than to maintain them. Variation in resumption of reproductive cycles is most likely influenced by species,

severity of the initial restriction, daily feed intake, and the physiological and genetic capacity of an individual animal to withstand the imposed stress.

First estrus was associated with the onset of ovarian luteal activity in 8 of the 8 R cows that were positively identified as standing to be mounted by other animals. The length of the first estrous cycles after increased nutrient intake were normal (17-24 d) in 6 of the 8 cows. Of the remaining 3 cows, 2 had cycles that were 42 d long, suggesting that the cows either did not exhibit behavioral estrus or were not observed in estrus. Lauderdale (1986) indicated that formation of corpora lutea without previous detection of estrus occurs in sows, ewes, and cows (incidence ranges from 5 to 14%).

First service pregnancy rate was similar for cows on both treatments (82 vs 86, M and R, respectively). This indicates that fertility is normal when cows gain enough BW and BCS to resume normal estrous cycles. These results agree with studies by Imakawa et al. (1986) and Johnson et al. (1987).

Concentrations of estradiol in plasma of M and R cows were similar when evaluated at 3 weekly intervals during: normal estrous cycles, at initiation of anestrus, during anestrus, and during re-initiation of estrus (Table 3). Within treatment groups, cows were at different stages of the estrous cycle when samples were collected. Therefore, it is unlikely that our sampling schedule was adequate to

accurately evaluate estradiol concentrations. However, these findings are consistent with reports from work with cows (Corah et al., 1974; Lishman et al., 1979) and pigs (Armstrong et al., 1986; Cox et al., 1987a) which indicated that plane of nutrition does not alter peripheral blood concentrations of estradiol. These results suggest that, although ovulation and luteal development do not occur in anestrous cows, follicular growth is not totally impaired by restricted nutrient intake.

Concentrations of LH in weekly serum samples in M and R cows were best described by a second order polynomial regression equation (Figure 4). Analysis of time trends indicated that concentrations of LH were different for M and R cows ($P<.01$; Table 4). Restricted cows had reduced concentrations of LH when compared with M cows during periods of severe nutritional restriction. These results support the concept that nutrient restriction compromises pituitary function (Gombe and Hansel, 1973; Beal et al., 1978; McCann and Hansel, 1986; Imakawa et al., 1987), but are in contrast to studies that indicate no change (Hill et al., 1970; Spitzer et al., 1978; Harrison and Randel, 1982) or increases (Dunn et al., 1974; Lishman et al., 1979; Eckternkcamp et al., 1982) in concentrations of LH due to energy restriction. Differences in the response in LH secretion due to nutrient intake are influenced by body energy reserves, duration of restriction and other environmental factors.

At the time when R cows were initiating anestrus, they had fewer LH pulses when compared to M cows (Table 5). However, once R cows had become anestrus, the negative feedback provided by progesterone was removed and LH pulse frequency was similar to that of M cows. Imakawa et al. (1987) found that ovariectomized heifers fed a low energy diet had fewer LH pulses when compared with heifers fed a high-energy diet. They proposed that nutritional deprivation lowered pulse frequency by initiating hypothalamic inactivity rather than altering pituitary response to hypothalamic secretions.

Luteinizing hormone pulse amplitude was not significantly influenced by feed restriction (Table 5). Johnson et al. (1987) reported that energy restriction delayed the LH response to GnRH, but did not affect the magnitude of an LH response. By contrast, Whisnant et al. (1985) and Rasby (1986) observed that cows on restricted diets released more LH in response to a GnRH challenge. Similarly, ovariectomized, feed restricted gilts released more LH in response to a GnRH challenge than ovariectomized gilts on a maintenance diet (Armstrong and Britt, 1987).

Dietary energy restriction reduces blood concentrations of LH in cows (Beal et al., 1978; McCann and Hansel, 1986; Imakawa et al., 1987), pigs (Armstrong and Britt, 1985, 1987) and humans (Frisch, 1983; Warren, 1983) probably through direct influences which depress the hypothalamic LHRH pulse generator. Infusion of postpartum anestrous beef

cows with glucose increases the pulse frequency of LH release (Garmendia, 1986). Cows that maintain BCS have greater basal LH and concentrations of LH after a GnRH challenge (Rutter and Randel, 1984). Body energy status is in some way perceived by cells that regulate LH secretion.

Receptor populations for LHRH are dynamic and pituitary gonadotropes are sensitive to these changes (Schoenemann et al., 1985; Braden et al., 1986). It is possible that changes in body energy status could mediate LH secretion through effects on LHRH receptor populations. These results enforce the fact that BCS should be monitored in cattle when examining the influence of nutrient intake on hypothalamic and pituitary function.

We conclude that nutrient intake significantly influenced cyclic ovarian activity and estrus in beef cows. Nonlactating, nonpregnant cows that were allowed to deplete body energy reserves to a BCS of 3.5 had reduced LH secretion and ceased to exhibit normal estrous behavior. Ovarian luteal activity was re-initiated by feeding cows to achieve an average body condition score of 4.6.

TABLE 1. LEAST-SQUARES MEANS (± SEM) FOR PERCENTAGE WEIGHT CHANGE AND BODY CONDITION SCORE (BCS) OF BEEF COWS FED MAINTENANCE OR RESTRICTED DIETS

	Weight change (%)		BCS	
Week	Maintain	Restricted	Maintain	Restricted
Restriction period				
0	-	-	5.5 ± .2	5.4 ± .2
5	0.2 ± 1.0[a]	- 5.7 ± 0.6[b]	5.5 ± .2	5.1 ± .2
10	- 0.3 ± 0.9[a]	- 9.0 ± 0.6[b]	5.3 ± .2	4.9 ± .2
15	1.9 ± 1.0[a]	-13.0 ± 0.6[b]	5.7 ± .2[a]	4.8 ± .2[b]
20	0.0 ± 1.1[a]	-21.2 ± 0.6[b]	5.7 ± .2[a]	4.4 ± .1[b]
25	- 2.5 ± 1.2[a]	-23.1 ± 0.6[b]	5.8 ± .2[a]	3.7 ± .2[b]
30	- 1.7 ± 1.1[a]	-25.8 ± 0.7[b]	5.7 ± .1[a]	2.7 ± .3[b]
Regain period				
5	- 2.2 ± 0.9[a]	-19.0 ± 1.2[b]	5.6 ± .1[a]	4.0 ± .2[b]
10	2.0 ± 1.7[a]	-10.4 ± 0.8[b]	5.6 ± .1[a]	4.8 ± .1[b]
15	11.6 ± 2.1[a]	0.7 ± 1.4[b]	5.8 ± .2[a]	5.0 ± .2[b]
17	16.9 ± 2.3[a]	6.5 ± 1.5[b]	5.8 ± .1[c]	5.2 ± .2[d]

[a,b]Means in the same row within a trait with different superscripts differ (P<.01).
[c,d]Means in the same row within a trait with different superscripts differ (P<.05).

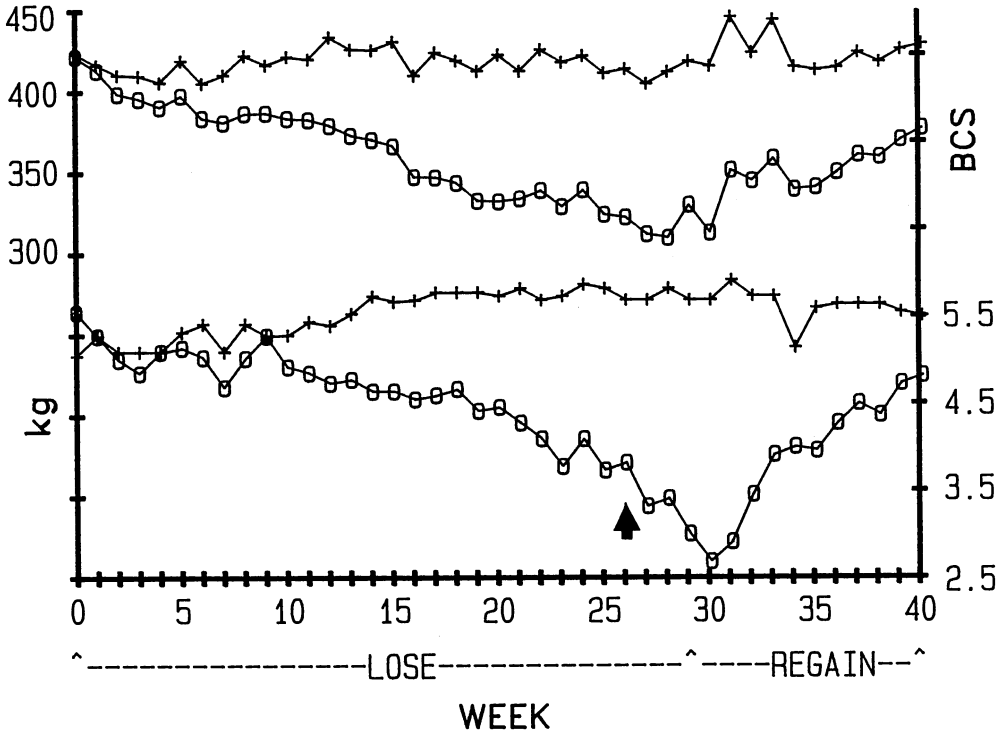

Figure 1. Least-squares means for body weights and body condition scores (BCS) of beef cows fed maintenance (+) or restricted (O) diets. Ninety-one percent of the restricted cows were anestrus by wk 26 (arrow).

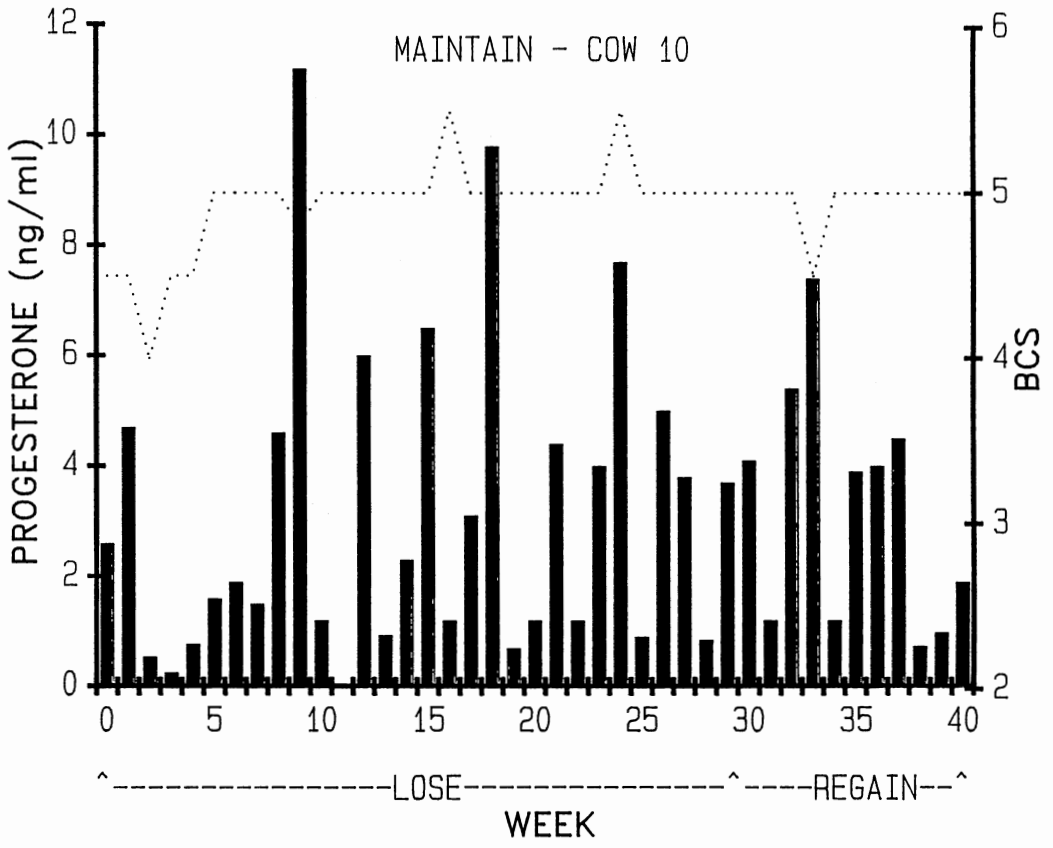

Figure 2. Relationship between body condition score (BCS; dotted line) and concentrations of progesterone (ng/ml; bars) in plasma of a cow fed a maintenance diet.

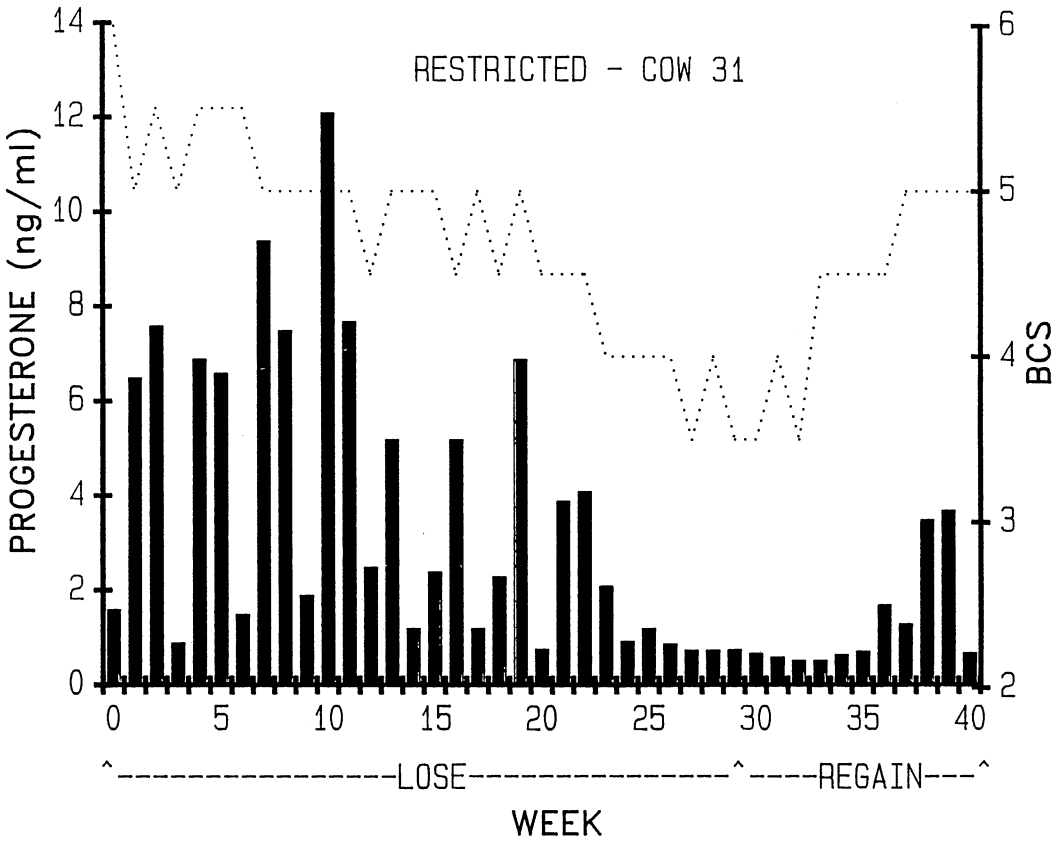

Figure 3. Relationship between body condition score (BCS; dotted line) and concentrations of progesterone (ng/ml; bars) in plasma of a cow fed a restricted diet.

TABLE 2. INFLUENCE OF BODY WEIGHT AND BODY CONDITION LOSS ON OVARIAN LUTEAL ACTIVITY (OLA)[a] IN BEEF COWS

Week	Cows with OLA (%)	
	Maintain (n=11)	Restricted (n=11)
Restriction period		
0	100	100
5	82	100
10	100	100
15	100	100
20	100	100
25	100	91
30	100[b]	9[c]
Regain period		
5	100[b]	27[c]
10	91[d]	64[e]
15	91	82
17	100	91

[a]Ovarian luteal activity determined by concentrations of progesterone in plasma.

[b,c]Least-Squares Means in the same row with different superscripts differ (P<.01).

[d,e]Least-Squares Means in the same row with different superscripts differ (P<.05).

[f]MSE = 13.0.

TABLE 3. LEAST-SQUARES MEANS FOR CONCENTRATIONS OF ESTRADIOL IN PLASMA OF BEEF COWS FED MAINTENANCE OR RESTRICTED DIETS

	Estradiol (pg/ml)	
Time (wk)	Maintain (n=6)	Restricted (n=5)
Restriction period		
Normal estrous cycle[c]		
1	6.1[a]	8.4[a]
2	11.5[b]	11.3[b]
3	7.5[a]	6.5[a]
Initiating anestrus[d]		
1	6.5	5.1
2	7.4	7.1
3	7.2	6.7
Anestrus[e]		
1	6.4	8.4
2	6.9	9.4
3	9.3	8.4
Regain period		
Re-initiating estrus[f]		
1	5.2	5.1
2	5.1	5.3
3	3.6	6.3

[a,b]Means in the same column with different superscripts differ (P<.01).
[c]MSE = 2.91.
[d]MSE = 1.02.
[e]MSE = 3.05.
[f]MSE = 2.48.

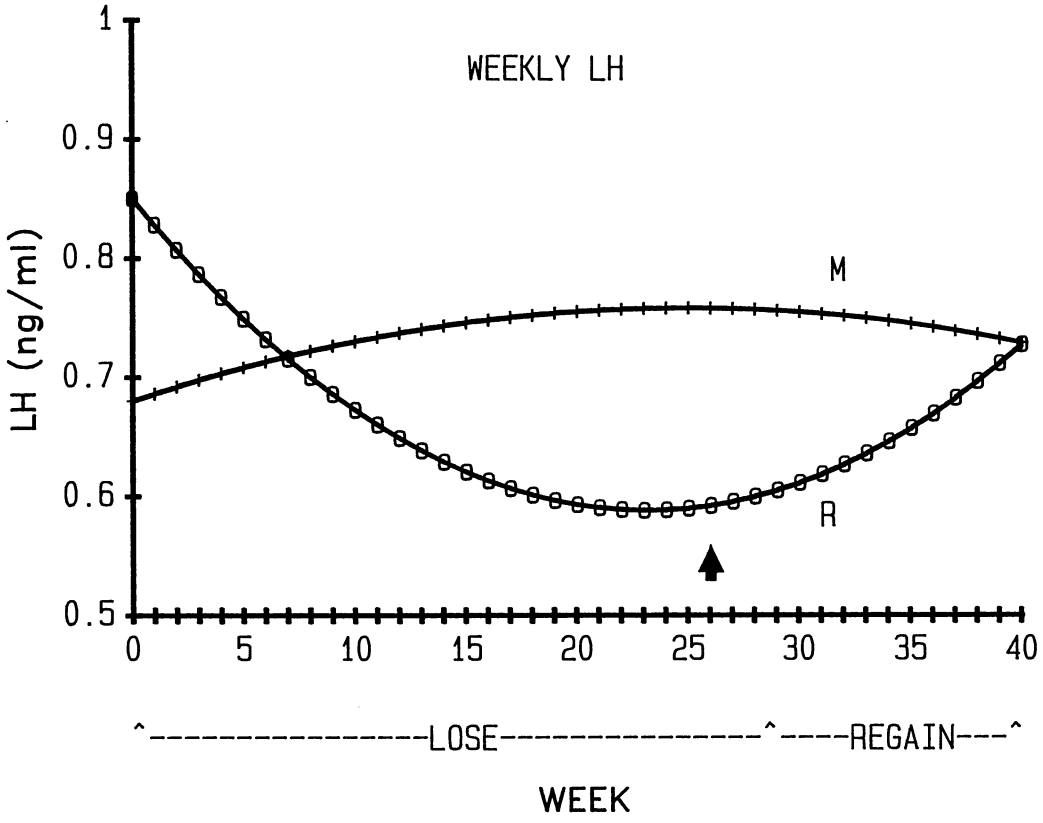

Figure 4. Least-squares regressions (SEM ± .22) for LH concentrations in weekly serum samples of cows fed maintenance (+ = M) or restricted (O = R) diets. Ninety-one percent of the restricted cows were anestrus by wk 26 (arrow).

TABLE 4. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF LH IN WEEKLY SAMPLES

Error	D.F.	S.S.	M.S.	F
Restricted	394	70.32		
Maintain	391	56.22		
Total	787	128.29	.16	
Restricted, Maintain	785	126.54		
Difference	2	1.75	.88	5.50*

*(P<.01).
R^2 = .08 and trt*time = .10.

TABLE 5. LEAST-SQUARES MEANS (±SEM) FOR CONCENTRATIONS OF LH IN SERUM OF BEEF COWS FED MAINTENANCE OR RESTRICTED DIETS

Time (period)	Characteristic: Pulses per 8 h (no.)	Characteristic: Pulse amplitude (ng/ml)
Lose period		
Normal estrous cycle		
Maintain	1.57 ± .27	1.82 ± .56
Restricted	1.00 ± .29	2.48 ± .62
Initiating anestrus		
Maintain	1.09 ± .28[a]	1.53 ± .62
Restricted	.25 ± .29[b]	.92 ± 1.24
Anestrus		
Maintain	1.29 ± .28	2.44 ± .56
Restricted	1.00 ± .29	1.34 ± .88
Regain period		
Re-initiating estrus		
Maintain	1.09 ± .28	2.35 ± .62
Restricted	.50 ± .29	.69 ± .88

[a,b]Means in the same column with different superscripts differ ($P<.05$).

CHAPTER IV

NUTRITIONAL ANESTRUS IN BEEF COWS II. CONCENTRATIONS OF GLUCOSE, INSULIN AND NON-ESTERIFIED FATTY ACIDS IN BLOOD

Abstract

Twenty-two nonlactating Hereford cows were fed maintenance (M) or restricted (R) diets until most of the R cows became anestrus (progesterone < 1 ng/ml for 3 consecutive wk). Restricted cows were then fed 160% of the M diet until estrous cycles resumed. Body weights (BW) and body condition scores (BCS) were monitored weekly for 41 wk. Concentrations of progesterone, glucose (GLU), insulin (INS), and non-esterified fatty acids (NEFA) were determined in weekly blood samples. Blood was collected frequently before and after infusion (300 ml; i.v.) of a 40% GLU solution to evaluate concentrations of GLU and INS when cows were exhibiting normal estrous cycles, when R cows were initiating anestrus, during anestrus and at the re-initiating of estrous cycles. Losses of BW and BCS in R cows were associated with reduced ($P<.01$) concentrations of GLU and INS, and greater ($P<.01$) concentrations of NEFA when compared with M cows. During normal estrous cycles,

disappearance of infused GLU from plasma and concentrations of INS in serum were similar for R and M cows. Disappearance of infused GLU was prolonged and concentrations of INS remained increased longer in blood when R cows were initiating anestrus ($P<.01$) when compared with M cows. Similarly, during anestrus, GLU disappearance was prolonged for R cows ($P<.01$). During refeeding (160% or M diet) of R cows, disappearance of infused GLU was similar for R and M cows. However, mean INS during refeeding was greater (6.0 vs 3.8 ng/ml; R and M, respectively; $P<.08$) in R cows. These results suggest that reduced concentrations of GLU and INS, and increased concentrations of NEFA in blood are associated with nutritional anestrus and the glucoregulatory effects of INS are compromised during nutritional anestrus. Concentrations of GLU and INS, estrous cycles, and ovarian luteal activity were similar in M and R cows when R cows regained adequate BW and body energy reserves.

(Key Words: Beef Cow, Body Condition, Nutrition, Insulin, Glucose)

Introduction

Endocrine status (Wettemann, 1980; McCann and Hansel, 1986), interval from calving to first estrus (Dzuik and Bellows, 1983; Richards et al., 1986), and pregnancy rate (Wiltbank et al., 1962, 1964; Richards et al., 1986) are altered significantly when cows are subjected to reduced

nutrient intake. Results suggest that concentrations of glucose in the blood of animals may determine rate of steroidogenesis and gonadotropin synthesis and/or secretion (Lynn et al., 1965; Sen et al., 1979). Hypoglycemia has been associated with reduced fertility in beef and dairy cattle (McClure, 1968; Oxenreider and Wagner, 1971; Patil and Deshpande, 1979; Chang et al., 1984; McCann and Reimers, 1985 a,b). Although LH secretion in cows is increased by infusion of glucose (Garmendia, 1986), propionate (Rutter et al., 1983) and phlorizin treatment (Rutter and Manns, 1987), the relationship between increased concentrations of glucose and improved fertility has not been established in cows (Chang et al., 1984; Kazmer et al,. 1985; McCaughey et al., 1985; Selk, 1986; Garmendia, 1986; Rutter and Manns, 1987), ewes (Rutter and Manns, 1986) or pigs (Armstrong et al., 1986; Cox et al., 1987b; Armstrong and Britt, 1987).

Insulin enhances the utilization of glucose. Cows (Selk, 1986), goats (Gill and Hart, 1981), and sheep (Basset et al., 1974) with reduced concentrations of insulin may exhibit irregular estrous cycles and have reduced conception rates when compared with animals with normal concentrations of insulin. Also, infusion of insulin enhances ovulation rates in heifers (Harrison and Randel, 1986) and gilts (Jones et al., 1983; Cox et al., 1987b).

This study was conducted to determine the influence of body energy reserves on concentrations of glucose, insulin and NEFA in blood of cows during the cessation and

resumption of normal estrous cycles. Secondly, we infused glucose into cows with different amounts of body energy reserves to evaluate concentrations of glucose and insulin in plasma and serum, respectively, after glucose infusion.

Material and Methods

Multiparous, nonlactating, Hereford cows (n=22) previously described in Chapter III were used in this study. Cows were fed either a maintenance (M) diet which ensured that cows maintained BW, BCS and normal estrous cycles or a restricted (R) diet that caused cows to lose BW, BCS and become anestrus. Weekly blood samples were collected (Chapter III) and quantified for concentrations of glucose (GLU), insulin (INS), and NEFA.

At four times during the study, blood was collected frequently from six M and five R cows to assess disappearance of infused GLU and INS secretion. Cows were sampled when R cows were exhibiting normal estrous cycles (after 5 wk of feed restriction), at the initiation of anestrus (after 20 wk of feed restriction) during anestrus (after 25 wk of feed restriction) and at the re-initiation of estrous cycles (after 6 wk of increased nutrient intake). Two injections of prostaglandin $F_{2\alpha}$ (25 mg; i.m.) were used 11 d apart to synchronize estrus. On D 9 to 11 (D 0 = estrus) polyvinyl cannulae[3] were inserted into jugular veins and

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[3]Bolab Inc., BB 317-v10, id = .157cm od = 2.803cm, Lake Havasu City, Az 86403.

cows were confined to metabolism stalls. Two days later at 0800 h, pre-treatment serum and plasma samples (10 ml) were collected at -60, -30 and 0 min. Cows were then infused (i.v.) with 300 ml of a 40% GLU solution and serum (5 ml) and plasma (5 ml) were obtained every 15 min for the first 2 h and every 30 min for the next 2 h. Plasma and serum were prepared (Chapter III) and stored at -20 C until assayed.

Concentrations of GLU in weekly and acute plasma samples were determined by an enzymatic colorometric procedure[4]. Intra- and inter-assay coefficients of variation (n=28 assays) were 3.1 and 2.6%, respectively. Concentrations of NEFA in plasma were also quantified by a colorimetric procedure (Patterson, 1963). Intra- and inter-assay coefficients of variation (n=34 assays) were 16.5 and 11.0%, respectively.

Concentrations of INS in serum were quantified by a radioimmunoassay described by Selk (1986). Antiserum (guinea pig anti-bovine insulin, lot GP 23; Miles-Yeda, Ltd. Research Products, Elkhart, Indiana) to bovine insulin was used as the primary antibody at a dilution of 1:40,000 and ovine anti-guinea pig serum was used (1:20) as the second antibody. Intra- and inter-assay coefficients of variation (n=6 assays) were 11.9 and 15.4%, respectively.

Analyses of variance were used to evaluate differences in body weight and BCS. Concentrations of GLU, INS and NEFA

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[4]Sigma Chemical Co., Kit No. 510, St. Louis, Mo.

in weekly and GLU and INS in acute samples were analyzed by split-plot analysis. When a significant treatment by time effect existed, polynomial response curves for concentrations of blood constituents were tested for heterogeneity of regression. Standard errors at the mean of the continuous independent variable are reported as estimates of variation for each regression curve.

Results

Percentage BW change, BCS change, ovarian and estrous activity, and pregnancy rates were previously reported (Chapter III). Maintenance and R cows had similar BW and BCS when they were exhibiting normal estrous cycles (after 5 wk of feed restriction; Table 6). When R cows were initiating anestrus (after 20 wk of feed restriction) and when they were anestrus (after 25 wk of feed restriction), they were lighter ($P<.01$) and had reduced ($P<.05$) BCS when compared with M cows. Normal estrous cycles and ovarian luteal activity resumed by 10 wk of realimentation. However, at that time, although R cows were gaining weight and increasing in BCS, M cows were still heavier and had greater ($P<.05$) BCS than R cows.

All R cows displayed cyclic ovarian luteal activity and exhibited normal estrous cycles at the first frequent sampling period. During period 2 (initiation of anestrus), 20% of the R cows had concentrations of progesterone < 1 ng/ml blood for at least 2 wk, whereas during the anestrus

(period 3), 80% had progesterone < 1 ng/ml blood. By period 4 (re-initiation of estrus) 40% of the R cows had resumed normal cyclicity. Maintain cows exhibited typical estrous cycles at all periods.

Concentrations of GLU in weekly plasma samples were best described by a fourth order polynomial regression equation (Figure 5; Table 7). Analysis of time trends indicated that concentrations of GLU were different for M and R cows ($P<.01$; Table 8). Second order polynomial regression equations (Table 7) best described concentrations of INS (Figure 6) and NEFA (Figure 7) in weekly samples. Analysis of time trends indicated that concentrations of INS and NEFA were also different for M and R cows (Table 9 and 10; INS and NEFA, respectively). Concentrations of GLU in plasma and INS in serum were reduced in R cows when they were initiating and during anestrus when compared with M cows. By contrast, concentrations of NEFA in plasma were increased during these periods.

Fifteen min after infusion of GLU, blood concentrations of GLU in plasma averaged 206 $\pm$ 11 mg% and concentrations of INS were 13.2 $\pm$ 4.6 ng/ml for both treatments at the four periods (Table 11). When M and R cows had similar BW and BCS and were exhibiting normal estrous cycles, disappearance of GLU from blood (Figure 8) and concentrations of INS (Figure 9) after GLU infusion were not different ($P>.10$) and best described by third order polynomial regression equations (Table 7).

Regression analyses revealed that disappearance of infused GLU (Figure 10) and concentrations of INS (Figure 11) when R cows were initiating anestrus were best described by third and second order polynomial equations (Table 7), respectively. Nutritional treatment influenced disappearance of GLU (Table 12; $P<.01$) and secretion of INS (Table 13; $P<.01$). Rate of disappearance of infused GLU was decreased and the period of INS secretion was prolonged when R cows had lost enough BW and BCS to initiate anestrus.

During anestrus, concentrations of GLU were increased for a longer interval ($P<.01$; Figure 12) in R cows when compared with M cows. The response was similar to that observed during initiation of anestrus and best described by a third order polynomial regression equation (Table 7). The profiles for concentrations of INS in serum after GLU infusion were not different between R and M cows, and the mean concentrations of INS over time (7.3 vs 4.3 ng/ml; R and M, respectively) were not statistically different (Figure 13).

During the refeeding period there was a treatment by time effect for disappearance of infused GLU (Figure 14; Table 14). Concentrations of INS after glucose infusion were similar for M and R cows (Table 7; Figure 15); however, mean insulin concentrations over time were greater ($P<.08$) in R than M cows (6.0 vs 3.8 ng/ml, respectively).

Discussion

Nutritionally induced decreases in BW and BCS caused cessation of normal estrous cycles and lack of ovarian luteal activity in nonlactating beef cows. These results are comparable to anestrus caused by reduced nutrient intake in cattle (Bond et al., 1958; Imakawa et al., 1986; Johnson et al., 1987), pigs (Armstrong and Britt, 1987), hamsters (Howland and Skinner, 1973), and rats (Howland, 1971, 1972; Knuth and Freisen, 1983), and amenorrhea in women (Frisch, 1983).

Reduced concentrations of GLU and INS in R cows when BW and body energy reserves were being depleted were expected since many investigators have observed similar results in ruminants fed restricted diets (Trenkle, 1978; Rasby et al., 1982; Chang et al., 1984; McCann and Hansel, 1986). By contrast, short-term fasting did not alter concentrations of GLU in plasma of Holstein heifers (Kazmer et al., 1985). Concentrations of NEFA in plasma of R cows were greater compared with M cows, when R cows were losing BW and BCS. Increased concentrations of NEFA in plasma of cows are an indication of negative energy balance and fatty acid release from adipocytes (Fritz, 1961; Bines and Hart, 1982). The observed increase in NEFA was also related to reduced plasma concentrations of INS in R cows which enhanced adipocyte lipolysis (Basset, 1968). The metabolic changes that we observed with weekly blood samples reflect the influence of GLU on INS secretion and the inverse relationship between

GLU utilization and fatty acid release from adipocytes. These metabolic indices were first evident when R cows were initiating anestrus and continued throughout anestrus.

Plasma concentrations of GLU increased from basal concentrations of 65 mg% to about 206 mg% within 15 min after infusion of M and R cows with GLU. Maximal plasma concentrations GLU after infusion were similar in M and R cows at all periods evaluated and were similar to those observed after GLU infusion into cows in other studies (Hove, 1978; Rasby, 1986).

In most species, GLU causes release of stored INS as well as synthesis of INS by pancreatic β-cells (Hove, 1978). Insulin was released and reached maximal values within 15 min after GLU infusion at all periods evaluated. Only when R cows were initiating anestrus, was their maximal INS release less than that for M cows (9.9 vs 15.3 ng/ml, R and M, respectively; $P<.05$). Although it is possible that R cows had less stored INS available for release, the significance of this finding is not clear.

As expected, when R and M cows had adequate body energy reserves and were exhibiting normal estrous cycles, disappearance of infused GLU and concentrations of INS were similar for R and M cows. Glucose and INS response curves were similar to those previously reported for pregnant cows with thin and moderate BCS (Rasby, 1986).

When R cows had depleted body energy reserves (BCS = 3.5) and were initiating anestrus, concentrations of GLU

were increased and concentrations of INS were greater for a longer period after GLU infusion when compared with M cows. Similarly, starved lactating dairy cows (range of days post partum = 57 to 148) had increased concentrations of GLU longer after infusion of GLU when compared with normal cows (281 $\pm$ 47 vs 143 $\pm$ 23 min, starved and normal, respectively; $P<.01$; Hove, 1978). McCann and Reimers (1985a) observed that GLU concentrations returned to pretreatment values more expediently in obese than in lean heifers.

Increased INS concentrations in R cows without increased disappearance of GLU suggests that the glucoregulatory effects of INS may be diminished or that there is insulin resistance as anestrus approaches. Insulin resistance involves both pre- and postreceptor defects and results in the uncoupling of insulin receptor complexes and disturbance of GLU transport and intracellular GLU metabolism (Kasuga et al., 1977; Olefsky, 1982). This effect could be mediated by adipocytes. Kasuga et al. (1977) observed that although adipocytes from fasted rats had a greater number of INS receptors and thus greater INS binding, fasting caused decreased insulin-stimulated glucose oxidation. This supports the concept of INS resistance.

Glucose may not enter body cells as effectively in thin anestrous cows when compared with cows with moderate body condition that exhibit normal estrous cycles. The concentration of GLU at pituitary gonadotrophs could regulate gonadotropin secretion. Sen et al. (1979) found

that glucose was essential for GnRH-induced release of LH in rats. Brangus heifers, with propionate induced increases in blood glucose, released more LH in response to GnRH than control heifers (Rutter et al., 1983) and infusion of GLU into postpartum beef cows enhanced LH secretion (Garmendia, 1986). By contrast, Rutter and Manns (1987) found that infusion of GLU into ewes had no influence on hypothalamic or hypophyseal tissue hormone content or pituitary GnRH receptor concentration. In addition, GLU infusion depressed ovarian activity. Responses to GLU infusion probably are related to body energy reserves.

Realimentation reversed the negative effects of feed restriction on BW, body energy reserves, and regulation of GLU in plasma and caused re-initiation of normal estrous cycles. During re-feeding, concentrations of GLU and INS in weekly samples from R cows were similar to those in M cows. However, concentrations of NEFA decreased in R cows to less than pre-treatment values and remained less than those of M cows. Euglycemia suggests that R cows had overcome their previous inability to reduce concentrations of GLU when they were initiating anestrus and were anestrus. Our results indicate that realimentation will restore metabolic homeostasis and normal reproductive function, similar to findings with heifers (McCann and Hansel, 1986) and pigs (Armstrong and Britt, 1987). Garmendia (1986) observed that infusion of glucose increased the mean number of LH peaks, mean concentrations of LH and total LH secreted during

treatment with LHRH in lactating beef cows. Taken together, our results and those of Garmendia (1986) provide evidence that LH secretion and maintenance of normal ovarian function rely on an adequate concentration of GLU in blood.

In conclusion, loss of sufficient BW to deplete body energy reserves altered concentrations of GLU, INS and NEFA in blood and anestrus occurred in beef cows. Nutritional anestrus was coincident with reduced concentrations of GLU and INS and increased NEFA concentrations in the blood of cows. Since disappearance of infused GLU was prolonged and secretion of INS was altered when R cows initiated anestrus, the entrance of GLU into cells must have been compromised at that time. Concentrations of GLU and INS in R cows were similar to those in M cows when R cows had regained adequate BW and body energy reserves to re-initiate estrous cycles. The mechanism by which nutrient deprivation and subsequent refeeding regulates estrous cycles in cows involves a metabolic signal that modulates LH secretion.

TABLE 6. LEAST-SQUARES MEANS (± SEM) FOR BODY WEIGHT AND BODY CONDITION SCORE (BCS) OF BEEF COWS DURING CHANGES IN REPRODUCTIVE STATUS

	Body weight (kg)		BCS	
Period[+]	Maintain	Restricted	Maintain	Restricted
1	421 ± 22	397 ± 16	5.5 ± .6	5.1 ± .5
2	422 ± 23[a]	331 ± 12[b]	5.7 ± .6[c]	4.4 ± .4[d]
3	411 ± 21[a]	323 ± 12[b]	5.8 ± .5[a]	3.7 ± .6[b]
4	412 ± 20[c]	340 ± 12[d]	5.6 ± .4[c]	4.0 ± .7[d]

[a,b]Means in the same row within a trait with different superscripts differ (P<.01).
[c,d]Means in the same row within a trait with different superscripts differ (P<.05).
[+]1=normal estrous cycles; after 5 wk of feed restriction.
2=initiating anestrus; after 20 wk of feed restriction.
3=anestrus; after 25 wk of feed restriction.
4=re-initiation of estrus; after 6 wk of realimentation.

TABLE 7. R^2 AND PROBABILITY LEVELS OF POLYNOMIAL REGRESSION EQUATIONS FOR CONCENTRATIONS OF GLUCOSE, INSULIN AND NON-ESTERIFIED FATTY ACIDS (NEFA)

TIME AND CONSTITUENT	ORDER OF FIT	R^2	PROBABILITY LEVEL (TRT*TIME)
WEEKLY			
GLUCOSE	QUARTIC	.48	.01
INSULIN	QUADRATIC	.29	.08
NEFA	QUADRATIC	.21	.01
NORMAL ESTROUS CYCLE			
GLUCOSE	CUBIC	.93	.34
INSULIN	CUBIC	.81	.31
INITIATING ANESTRUS			
GLUCOSE	CUBIC	.86	.01
INSULIN	QUADRATIC	.52	.04
ANESTRUS			
GLUCOSE	CUBIC	.93	.01
INSULIN	QUADRATIC	.44	.48
RE-INITIATING NORMAL ESTROUS CYCLES			
GLUCOSE	CUBIC	.94	.01
INSULIN	QUADRATIC	.65	.39

TABLE 8. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF GLUCOSE IN WEEKLY SAMPLES

Error	D.F.	S.S.	M.S.	F
Restricted	475	9403.01		
Maintain	477	11631.96		
Total	952	21034.97	22.09	
Restricted, Maintain	956	21703.00		
Difference	4	668.03	167.01	7.56*

*(P<.01).

TABLE 9. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF INSULIN IN WEEKLY SAMPLES

Error	D.F.	S.S.	M.S.	F
Restricted	436	36.67		
Maintain	435	62.01		
Total	871	98.68	.11	
Restricted, Maintain	873	100.07		
Difference	2	1.39	.70	6.15*

*(P<.01).

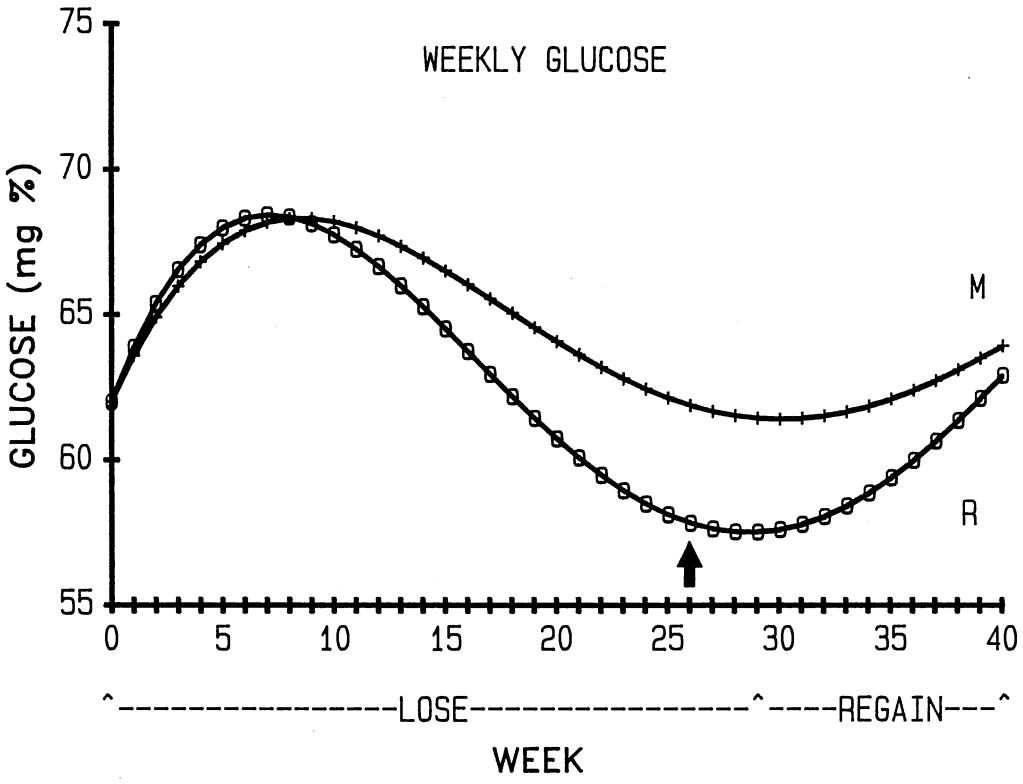

Figure 5. Least-squares regressions (SEM ± 4.4) for concentrations of glucose in weekly plasma samples from cows fed maintenance (+ = M) or restricted (O = R) diets. Ninety-one percent of the restricted cows were anestrus by wk 26 (arrow).

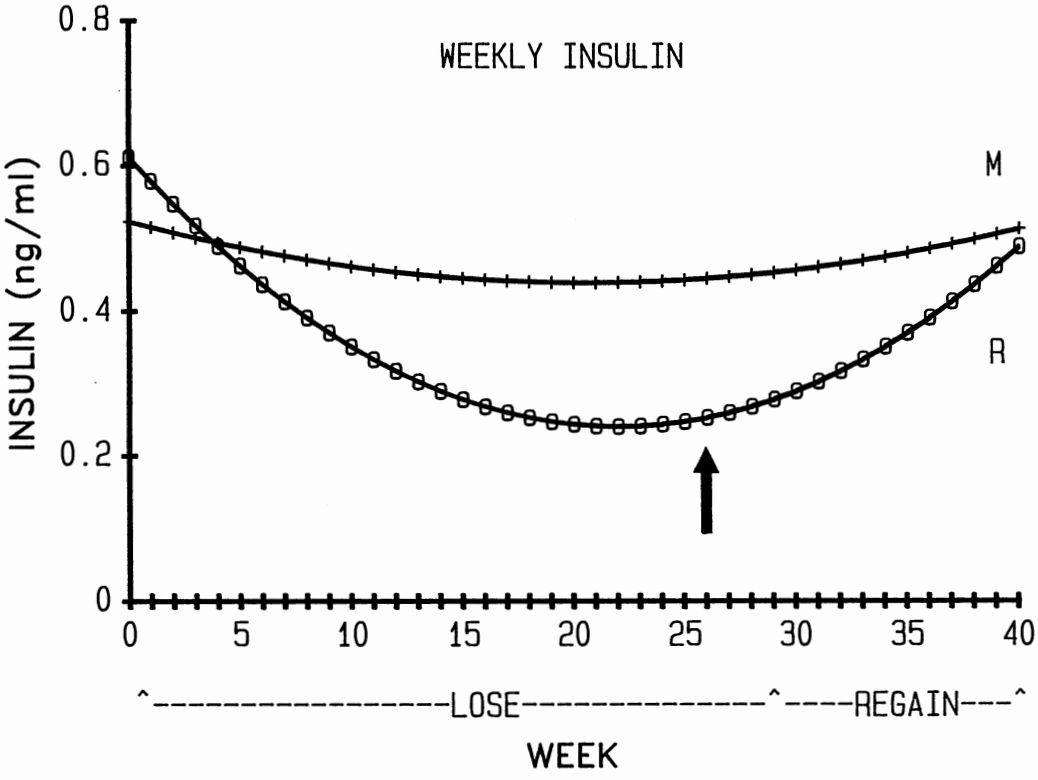

Figure 6. Least-squares regressions (SEM ± .40) for concentrations of insulin in weekly serum samples from cows fed maintenance (+ = M) or restricted (O = R) diets. Ninety-one percent of the restricted cows were anestrus by wk 26 (arrow).

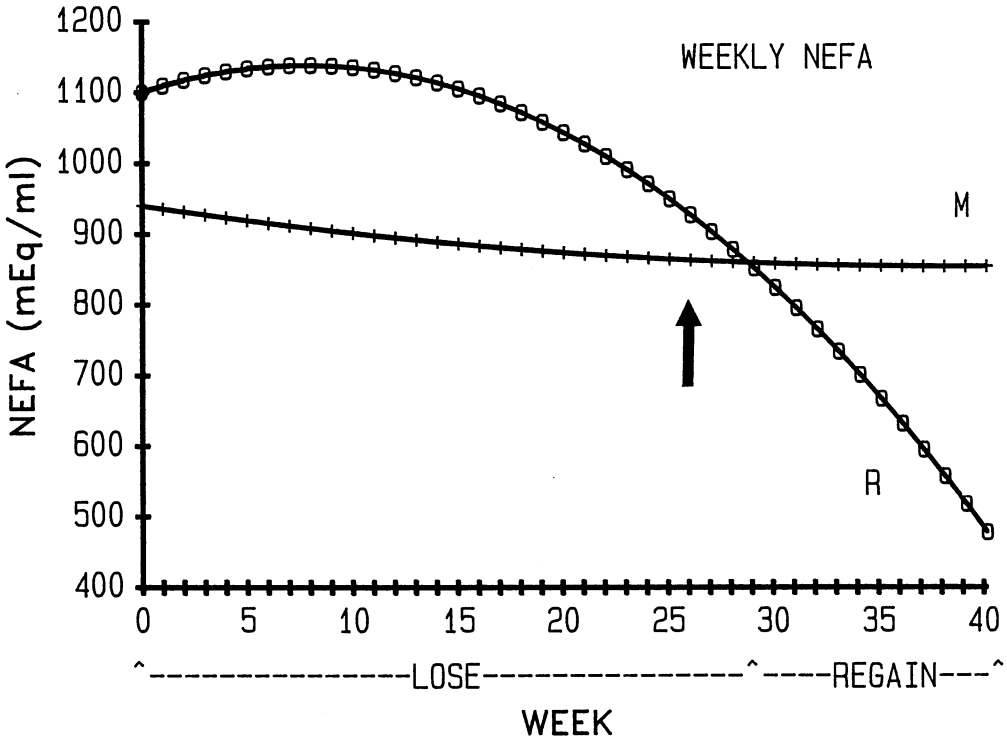

Figure 7. Least-squares regressions (SEM ± 359) for concentrations of NEFA in weekly plasma samples from cows fed maintenance (+ = M) or restricted (O = R) diets. Ninety-one percent of the restricted cows were anestrus by wk 26 (arrow).

TABLE 10. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF NEFA IN WEEKLY SAMPLES

Error	D.F.	S.S.	M.S.	F
Restricted	458	105259315		
Maintain	448	78705443		
Total	906	183964758	203052	
Restricted, Maintain	896	157585555		
Difference	10	26379203	2637920	12.99*

*(P<.01).

TABLE 11. CONCENTRATIONS OF GLUCOSE AND INSULIN AT 15 MINUTES AFTER INFUSION OF A 40% GLUCOSE SOLUTION

TIME AND CONSTITUENT	CONCENTRATION[C] ± SEM
NORMAL ESTROUS CYCLE	
GLUCOSE	
MAINTAIN	194.7 ± 20.5
RESTRICTED	212.8 ± 22.4
INSULIN	
MAINTAIN	11.2 ± 1.8
RESTRICTED	16.0 ± 2.0
INITIATING ANESTRUS	
GLUCOSE	
MAINTAIN	197.2 ± 18.2
RESTRICTED	224.4 ± 20.0
INSULIN	
MAINTAIN	15.3 ± 1.9[a]
RESTRICTED	9.9 ± 2.0[b]
ANESTRUS	
GLUCOSE	
MAINTAIN	203.5 ± 17.5
RESTRICTED	215.2 ± 19.2
INSULIN	
MAINTAIN	7.8 ± 4.4
RESTRICTED	7.5 ± 4.9
RE-INITIATING ESTRUS	
GLUCOSE	
MAINTAIN	195.1 ± 10.5
RESTRICTED	205.2 ± 14.2
INSULIN	
MAINTAIN	18.2 ± 3.2
RESTRICTED	19.3 ± 3.5

a,b ($P<.05$).
[C] Glucose = mg %; insulin = ng/ml.

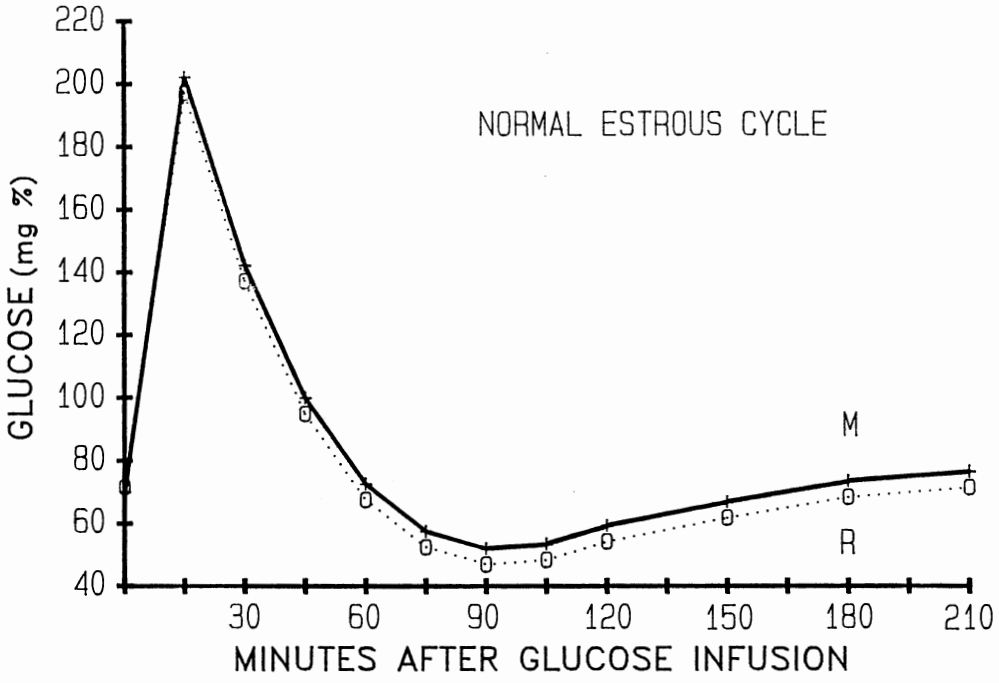

Figure 8. Least-squares regressions (SEM ± 15.2) for concentrations of glucose in plasma after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were exhibiting normal estrous cycles.

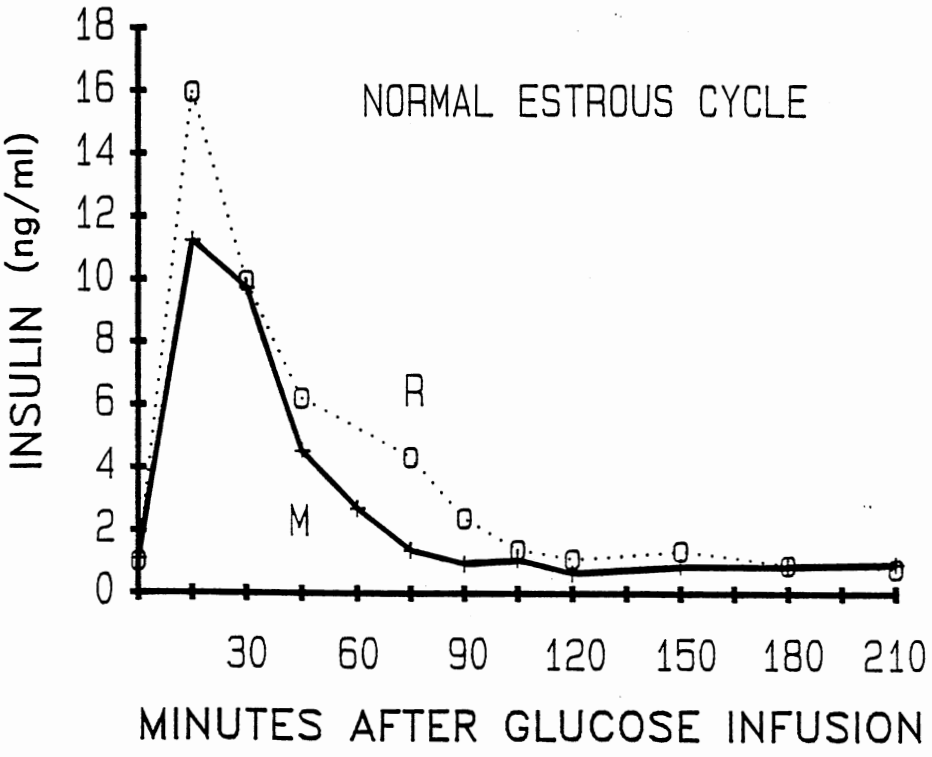

Figure 9. Least-squares means (SEM ± 1.3) for concentrations of insulin in serum after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were exhibiting normal estrous cycles.

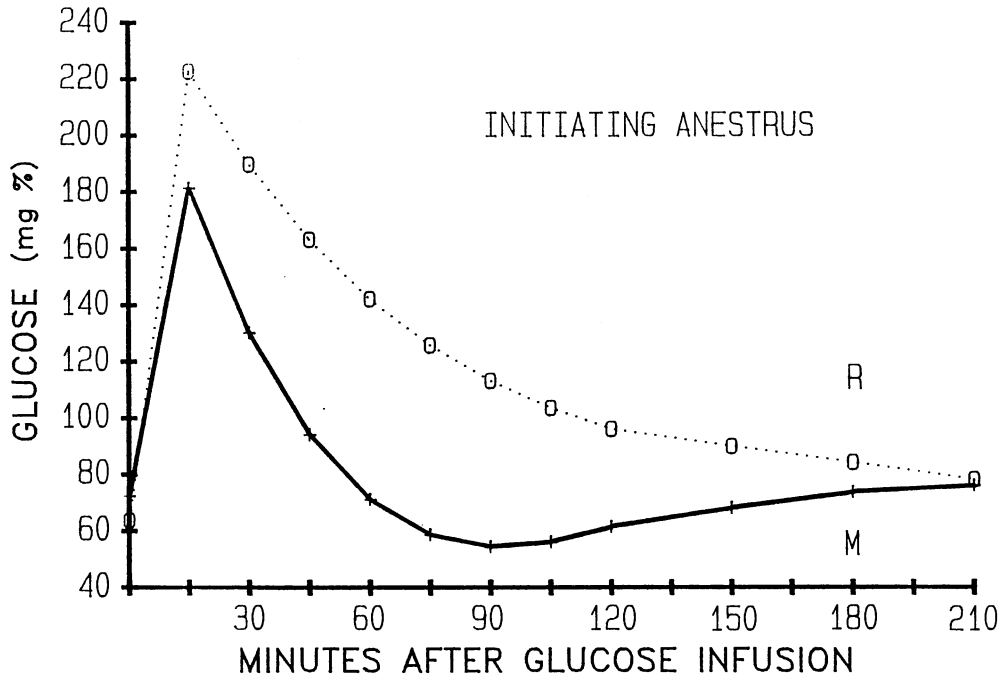

Figure 10. Least-squares regressions (SEM ± 13.5) for concentrations of glucose in plasma after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were initiating anestrus.

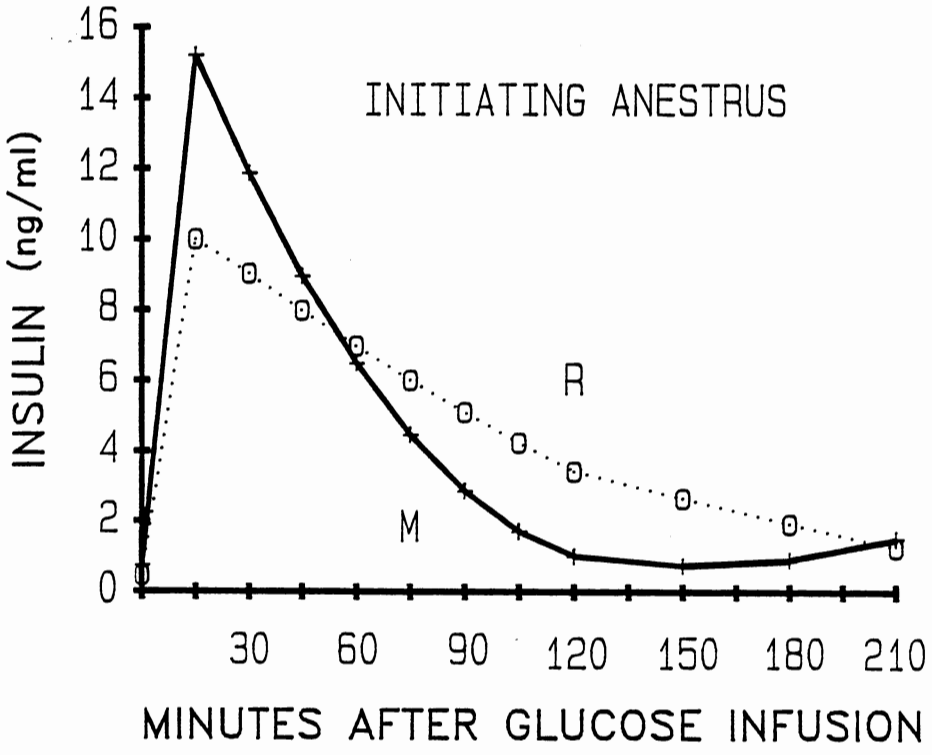

Figure 11. Least-squares regressions (SEM ± 1.4) for concentrations of insulin in serum after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were initiating anestrus.

TABLE 12. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF GLUCOSE WHEN RESTRICTED COWS WERE INITIATING ANESTRUS

Error	D.F.	S.S.	M.S.	F
Restricted	56	7283.49		
Maintain	68	11824.75		
Total	124	19108.24	154.10	
Restricted, Maintain	127	43933.15		
Difference	3	24824.91	8274.97	53.70*

*(P<.01).

TABLE 13. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF INSULIN WHEN RESTRICTED COWS WERE INITIATING ANESTRUS

Error	D.F.	S.S.	M.S.	F
Restricted	58	913.09		
Maintain	70	927.51		
Total	128	1840.60	14.38	
Restricted, Maintain	30	2054.79		
Difference	3	214.17	107.09	7.45*

*(P<.01).

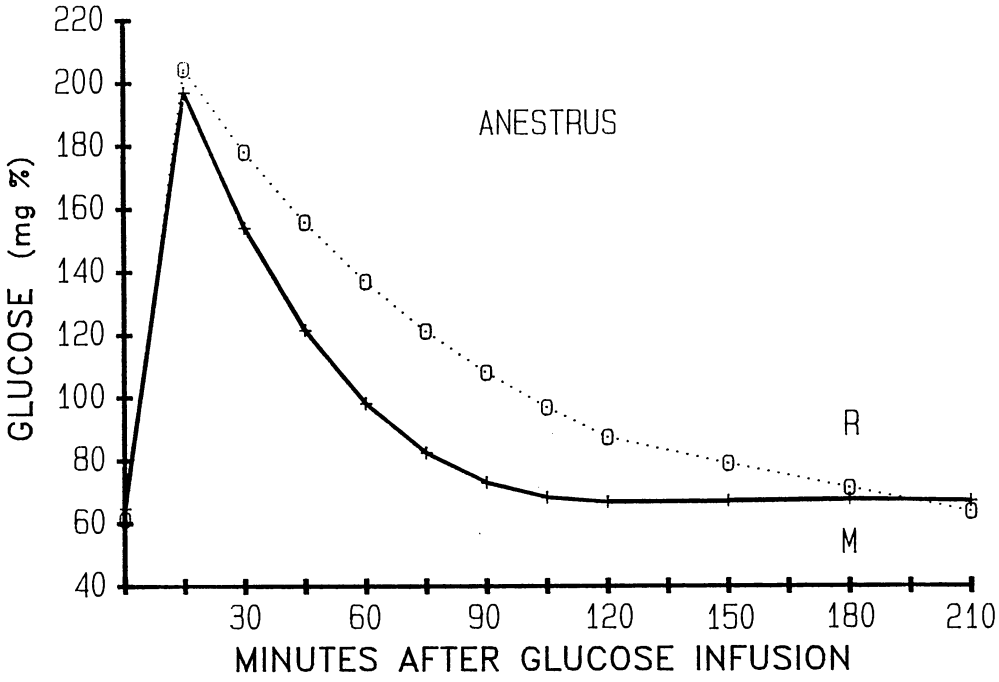

Figure 12. Least-squares regressions (SEM ± 13.0) for concentrations of glucose in plasma after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were anestrus.

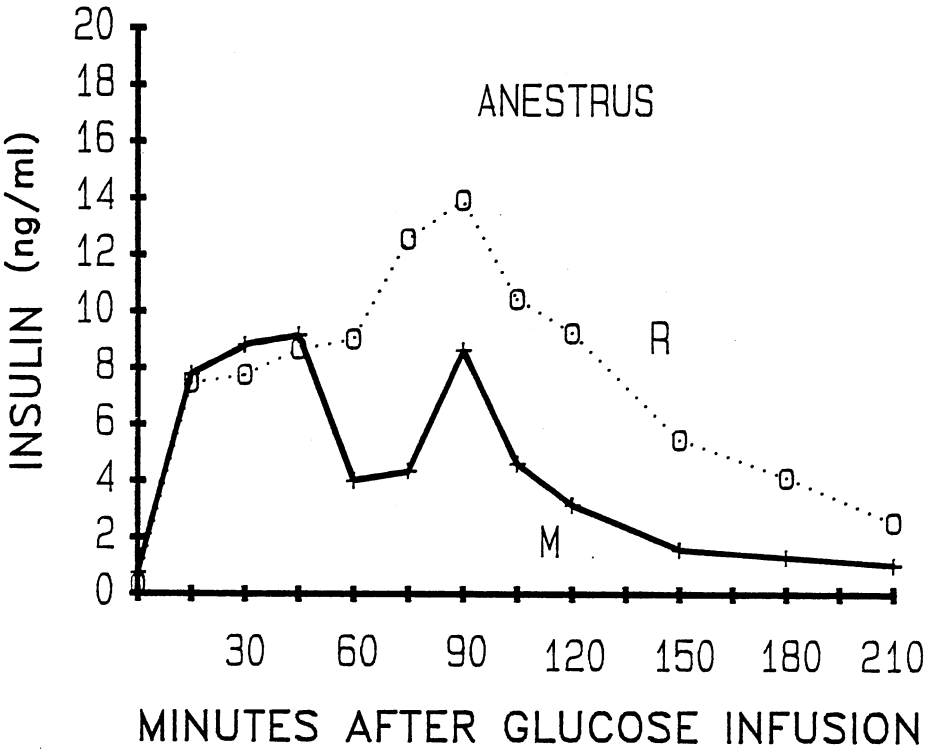

Figure 13. Least-squares means (SEM ± 3.3) for concentrations of insulin in serum after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were anestrus.

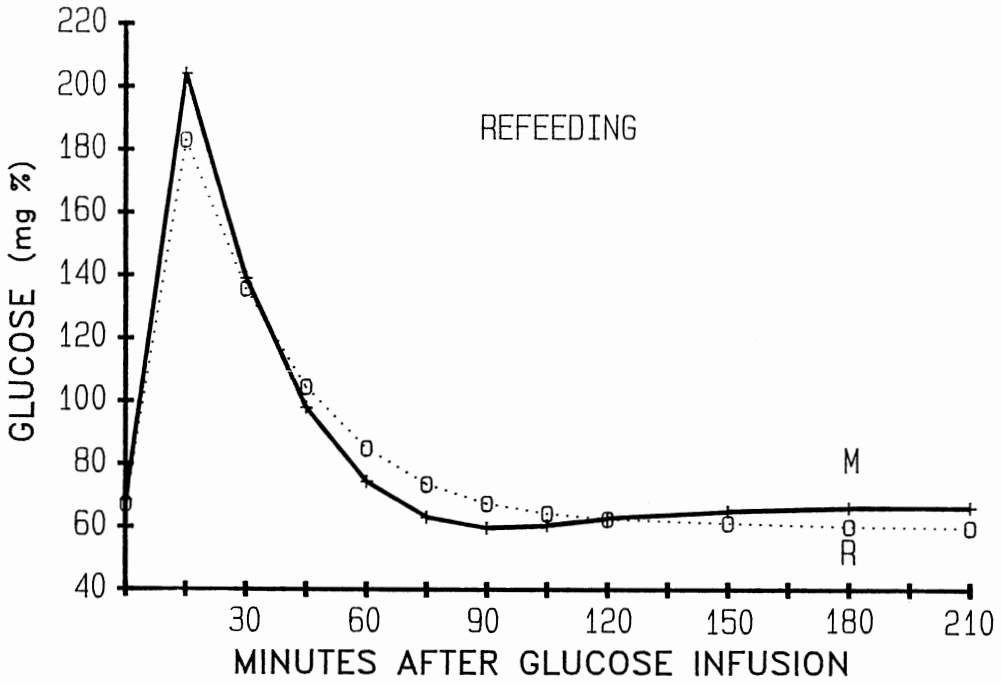

Figure 14. Least-squares regressions (SEM ± 10.5) for concentrations of glucose in plasma after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were re-initiating normal estrous cycles.

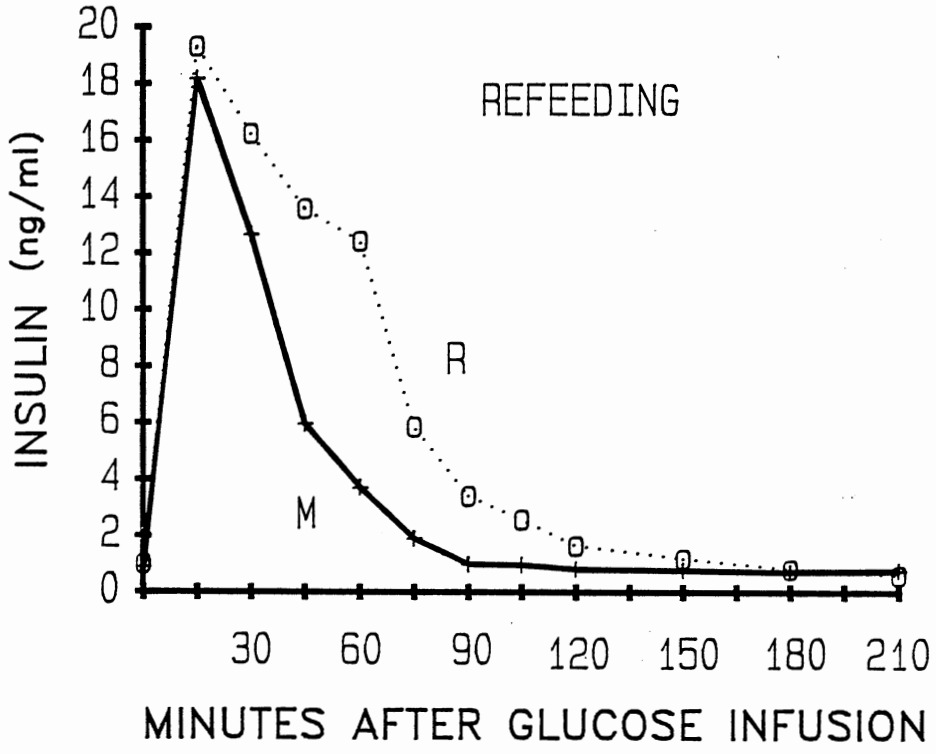

Figure 15. Least-squares means (SEM ± 3.5) for concentrations of insulin in serum after glucose infusion. Cows were fed maintenance (+ = M) or restricted (O = R) diets and were re-initiating normal estrous cycles.

TABLE 14. ANALYSIS OF VARIANCE USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF GLUCOSE IN SAMPLES WHEN RESTRICTED COWS WERE RE-INITIATING ESTRUS

Error	D.F.	S.S.	M.S.	F
Restricted	56	4732.85		
Maintain	68	6424.12		
Total	124	11156.97	89.98	
Restricted, Maintain	127	13747.27		
Difference	3	2590.30	863.43	9.60*

*(P<.01).

CHAPTER V

NUTRITIONAL ANESTRUS IN BEEF COWS III. EFFECT OF BODY CONDITION AND OVARIECTOMY ON SECRETION OF LH

Abstract

Nonpregnant, nonlactating, Hereford cows were fed to maintain (M) body weight (BW), body condition score (BCS), and normal estrous cycles (n=5) or were fed a restricted (R) diet to lose BW and BCS and to become anestrus (n=10). Anestrous R cows were randomly alloted to be ovariectomized (OVX) via flank incision (n=5) or to remain intact (INT) (n=5). All M cows were OVX. Serum was collected at 10 min intervals for 4 h on D -1, +1, +2, +3, +4, +5 and +10 (D 0 = day of OVX) and LH was quantified. Concentrations of progesterone, estradiol and LH were determined in daily samples collected on D -1 through +10. After the 4 h sampling period on D +10, one mg of estradiol was injected (i.m.) into 3 cows from each group. Blood samples were then collected at 15 min intervals for the next 2 h and at 2 h intervals for the subsequent 30 h. After ovariectomy, there was a treatment X day effect ($P<.01$) for mean serum LH concentrations. Concentrations of LH increased ($P<.01$) in

M-OVX cows when compared with R-OVX and R-INT cows, whereas concentrations of LH were similar for restricted OVX and INT cows. The number of LH pulses was similar for M and R cows. Estradiol treatment increased LH secretion in all M cows and some R cows. We conclude that secretion of LH is greater, before and after ovariectomy, in cows with moderate BCS than in thin cows and that the ovary does not inhibit secretion of LH in thin anestrous cows.

(Key Words: Beef Cow, Body Condition, Nutrition, LH Ovariectomy)

Introduction

The negative and positive influences of estradiol on LH secretion are time and dose dependent and vary due to species, reproductive status, body energy reserves and season. Significant increases in both LH pulse frequency and amplitude occur after gonadectomy or inactivation of gonadal steroids by immunoneutralization in domestic animals (Karsch, 1987). In rhesus monkeys, estradiol exerts negative effects on LH secretion by suppressing the response at the pituitary to gonadotropin releasing hormone (GNRH) (Knobil, 1981). Estradiol may also have inhibitory effects on the hypothalamus since micro-injections of estradiol into the hypothalamus will alter pulsatile GnRH secretion (Ferin et al., 1974, 1984). Estradiol causes a reduction in LH pulse frequency in prepubertal heifers (Day et al., 1986b), anestrous cows (Imakawa et al., 1986a, b), acutely

ovariectomized cows (Kinder et al., 1983) and mature ovariectomized cows (Day et al., 1986a; Acosta et al., 1983; Hinshelwood et al., 1985). Underfeeding appears to increase the sensitivity of the hypothalamus and/or pituitary to the negative effects of estradiol in cows (Imakawa et al., 1987) and prepubertal rats (Piaseck, 1985).

The objectives of this study were to determine if a post-castration increase in concentrations of LH occurs in nutritionally anestrous beef cows and to examine the relationships between body energy reserves and the secretion of LH in ovariectomized cows. We also tested the hypothesis that cows with reduced body energy reserves have altered sensitivity to the effects of estradiol when compared with cows with optimal body energy status.

Material and Methods

Fifteen nonpregnant, nonlactating, Hereford cows with moderate to good body condition (BCS = 5.2 $\pm$ .3) were used in this study. Two people independently assessed BCS using the system where 1=emaciated and 9=obese (Spitzer, 1986; Wagner et al., 1988). All cows exhibited normal estrous cycles at the initiation of the study. Five cows were randomly allotted to maintain (M) their initial BW and BCS and the remaining ten cows were fed a restricted (R) diet (approximately 2 kg of prairie hay d^{-1} in a drylot) for approximately 26 wk until they became anestrus (absence of ovarian luteal activity). Within the R group, 5 cows were

assigned to be ovariectomized (OVX) and the other 5 cows were left intact (INT). All M cows were ovariectomized.

Body weights and BCS were recorded monthly until R cows became anestrus. Concentrations of progesterone in plasma samples (Lusby et al., 1981) were used to determine absence of ovarian luteal activity. All cows were given two injections (i.m.) of prostaglandin F2 (25 mg) 11 d apart to synchronize estrus. On days 3 to 5 after estrus polyvinyl cannulae[5] were nonsurgically inserted into jugular veins and cows were confined to metabolism stalls. Two days later, 5 R (R-OVX) and 5 M (M-OVX) cows were ovariectomized[6] through flank incisions. Ovaries were placed on ice until evaluated. The number and size of all follicles on the surface of each ovary that were 2 mm or greater in diameter were recorded. Follicles were classified as small ($\leq$ 3.9 mm), medium (4.0 - 7.9 mm) or large ($\geq$ 8.0 mm) (McNatty et al., 1984; Spicer et al., 1986). Total ovarian weights were determined. Corpora lutea (CL), when present, were dissected free of ovarian stromal tissue and weighed. Ovaries were sliced with a scalpel to section follicles, blotted on a paper towel, and re-weighed to determine follicular fluid weights (Rasby, 1986). Dry ovarian weights were determined after desiccation in an oven at 90 C for 54

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[5]Bolab Inc., BB 317-v10, id = .157cm od = 2.803cm, Lake Havasu City, Az 86403.

[6]Surgeries were performed by Gregor Morgan, DVM, PhD, employed by Oklahoma State University College of Veterinary Medicine, Stillwater, Ok 74078.

h. Percentage dry ovarian weight was defined as dry ovarian weight divided by total ovarian weight minus CL weight.

At 0800 h, on D-1, +1, +2, +3, +4, +5, and +10 (D 0 = day of ovariectomy), serum was collected at 10 min intervals for 4 h. Concentrations of LH in frequent samples were quantified by radioimmunoassays (Wettemann et al., 1988). Antiserum to bovine LH (Rabbit OSU B-4) was used as the primary antibody at a dilution of 1:160,000 and ovine anti-rabbit serum was used at a dilution of 1:40 as the second antibody. Intra- and inter-assay coefficients of variation (n=7 assays) were 5.8 and 15.9 %, respectively.

On D+10, after the 4 h intensive sampling period, three cows on each treatment were injected (i.m.) with 1 mg of estradiol 17-β in corn oil. Following treatment, plasma was collected at 15 min intervals for 2 h and serum was collected at 2 h intervals for 30 h. Estradiol (Hallford et al., 1979) concentrations in frequent samples and progesterone (Lusby et al., 1981) in daily samples were quantified. Antiserum to bovine estradiol 17-β (no. 224 from Dr. G. Niswender) was used at a dilution of 1:100,000. Intra- and inter-coefficients of variation (n=6 assays) were 13.0 and 28.0 %, respectively. Antiserum to bovine progesterone (OSU B1-1) was used at a dilution of 1:32,000. Intra- and inter-assay coefficients of variation (n=14 assays) were 7.8 and 14.1 %, respectively.

The mean LH concentration for a cow on a day was obtained by averaging the LH concentration in the frequent

samples taken on the day during 4 h. Two-way comparisons between means of number of LH pulses, LH pulse amplitude, and mean concentrations of LH within a day were made by Tukey's procedure (Steel and Torie, 1980). During the 4 h sampling periods, LH pulses were defined using a modification of the criteria established by Goodman and Karsch (1980). Any value of LH greater than 2 standard deviations above the mean for a cow on a day, that was followed by at least 2 values of lesser concentration, was considered a pulse. The amplitude of a LH pulse was the difference between the greatest concentration during a pulse and the nadir within 30 min before the pulse.

Analyses of variance were used to evaluate weight changes and ovarian characteristics. Concentrations of estradiol and LH concentrations in daily and frequent samples, and mean LH concentrations at 4 h intervals after estradiol treatment were analyzed by split-plot analysis of variance, with repeated measurements on the same cow over time (Gill and Hafs, 1971). When a significant treatment by time effect was detected, treatment polynomial response curves for concentrations of LH were tested for heterogeneity of regression. Orthogonal contrasts (M-OVX vs R-OVX and R-INT; R-OVX vs R-INT) were used to detect differences between treatments. Standard errors at the mean of the continuous independent variable are reported as estimates of variation for each regression curve.

Results and Discussion

Restricted cows lost BW and BCS and became anestrus after approximately 26 wk on the restricted diet (Table 15). Body weight and BCS were maintained for M cows and they exhibited normal estrous cycles until ovariectomy. At ovariectomy, R cows were 98 kg lighter and had a 3 unit reduction in BCS ($P<.01$) compared with M cows.

Concentrations of progesterone in the plasma of M cows averaged 2.49 $\pm$ .49 ng/ml on the day of ovariectomy (day 6-7 after estrus) and declined to less than 1.0 ng/ml within 72 h. By contrast, concentrations of progesterone in the plasma of R cows averaged .64 $\pm$ .10 ng/ml on the day of ovariectomy and averaged less than 1.0 ng/ml throughout the study.

Maintain cows had heavier total ($P<.01$) and dry ($P<.10$) ovarian weights than did R cows (Table 16). There were no CL present on the ovaries from any of the R cows and one M cow that exhibited estrus did not have a CL. These results are in agreement with previous studies which demonstrated that cows fed diets with reduced energy had lighter ovaries than those fed adequately (Gombe and Hansel, 1973; Beal et al., 1978; Spitzer et al., 1978; Rasby, 1986). Neither follicular fluid weight nor percentage dry ovarian weight were significantly different between M and R cows (Table 16). However, M cows tended ($P<.12$) to have more small follicles than R cows. The numbers of medium and large follicles were similar for M and R cows. These results

demonstrate that when a cows body energy reserves are depleted cyclic ovarian function ceases.

Average daily concentrations of LH increased linearly ($P<.01$) in M cows after ovariectomy; however, R cows did not exhibit a post-castration increase in serum LH (Figure 16). Concentrations of LH in daily samples for cows in all treatments were best described by a linear equation. Analysis of time trends for LH concentrations in serum of M-OVX, and R- OVX and R-INT cows indicated that the response curve for M cows was not parallel (Figure 16; Table 17; $P<.01$) to that of R cows and that the response curves for R OVX and R INT were similar (Table 18).

Examples of the tonic LH secretory pattern of a cow from each treatment group are presented in figures 17, 18 and 19 (M-OVX, R-OVX AND R-INT, respectively). Pulse frequency of LH secretion was not influenced by ovariectomy (Table 19). Since so few pulses existed, it is possible that the sampling duration was not long enough to adequately evaluate pulse frequency. Schallenberger and Peterson (1982) found that pulse frequency increased by as much as 3-fold within the first four days after ovariectomy of cows . Ovariectomized heifers fed a diet low in energy had fewer pulses of LH per 6 h than those fed a diet with adequate energy (Imakawa et al., 1987).

Pulse amplitude of LH was greater for M-OVX than R-INT cows at all times evaluated (Table 19). Restricted-OVX cows had less LH pulse amplitude on D +2, +4 and +5 after

ovariectomy when compared with M-OVX cows. Our results suggest that the hypothalamic-pituitary axis of cows that have adequate body energy reserves and normal estrous cycles prior to ovariectomy are capable of secreting greater quantities of LH once the negative feedback or "brake" (Walters et al., 1982) is removed when compared with thin anestrous cows. These results agree with those of Schallenberger and Peterson (1982) who found that dairy cows in a negative energy balance that were ovariectomized 4 d after parturition had reduced LH pulse frequency and amplitude and reduced mean LH concentrations when compared with those cows that were cyclic prior to ovariectomy. Luteinizing hormone pulse amplitude increased after ovariectomy in prepubertal heifers (Anderson et al., 1985) and cyclic beef cows (Schallenberger and Peterson, 1982).

Concentrations of LH usually increase after ovariectomy in the bovine (Hobson and Hansel, 1973; Convey et al., 1977; Schallenberger and Peterson, 1982; Garcia-Winder et al., 1984; Anderson et al., 1985; Hinshelwood et al., 1985; Imakawa et al., 1987). The linear increase in LH after ovariectomy that we observed is similar to findings of Hobson and Hansel (1972) who found that LH concentrations increased for approximately 28 d after ovariectomy in cows.

Since ovariectomy did not alter concentrations of LH in R cows, reduced body energy reserves suppress LH secretion by a mechanism which is independent of ovarian steroid or other hormonal feedback. This supports the concept that,

although ovarian secretions inhibit LH secretion, reduced nutrient intake has a direct effect on the hypothalamic-pituitary axis (Hinshelwood et al., 1985; Armstrong and Britt, 1987; Imakawa et al., 1987). Rutter and Manns (1987) suggested that the depression in reproductive function associated with metabolic stress was regulated by a mechanism distinct from the mechanism that controls suckling-induced depression of LH secretion.

Concentrations of estradiol in plasma were similar for M and R cows after estradiol injection and were best described by third order polynomial regression equations (Figure 20). Concentrations of estradiol for the three treatments averaged 175 pg/ml within 15 min after estradiol injection. Regardless of BCS, within 90 min after estradiol injection, concentrations of estradiol in plasma averaged 400 pg/ml and began to decrease thereafter.

Estradiol did not affect LH secretion in M or R cows during the first 16 h (range 16-24 h) after treatment (Figure 21). Subsequently, 3/3 of M-OVX, 2/3 of R-OVX, and 1/3 of R-INT exhibited a preovulatory-like surge of LH (Table 20). All cows that responded with a surge of LH did so at similar times and had maximum concentrations of LH of similar amplitude (Table 20). The timing of those surges was similar to that observed in androgenized and prepubertal heifers that release pre-ovulatory like surges of LH with a peak 15 to 21 h after injection of estradiol (Hamernik et al.; 1987).

Pituitaries of thin, anestrous cows are sensitive and capable of releasing LH if stimulated with exogenous LHRH (Whisnant et al., 1985; Rasby, 1986). Since fewer R cows exhibited ovulatory surges of LH after estradiol and the number of pulses of LH was less in R when compared with M cows, the hypothalamus of thin cows probably does not respond to estradiol in the same manner as does that of cows with adequate body energy reserves does.

In conclusion, our results suggest that body energy reserves and/or presence or absence of normal estrous cycles prior to ovariectomy alter LH secretion in beef cows. Cows with reduced body energy reserves secrete less LH than those with adequate amounts of body energy reserves. Since ovariectomized thin cows do not have a post-castration increase in LH secretion, signals from body energy reserves are independent of the ovary and may directly influence hypothalamic and/or pituitary regulation of secretion of LH in beef cows.

TABLE 15. LEAST SQUARE MEANS (± SEM) FOR BODY WEIGHT AND BODY CONDITION SCORE (BCS) OF BEEF COWS FED MAINTENANCE OR RESTRICTED DIETS

	Body weight (kg)+		BCS+	
Time	Maintain	Restricted	Maintain	Restricted
Initiation of treatment	453 ± 9	460 ± 6	5.3 ± .4	5.1 ± .2
Ovariectomy	438 ± 19[a]	340 ± 6[b]	5.7 ± .1[a]	2.7 ± .2[b]

[a,b]Means in the same row within a trait with different superscripts differ (P<.01).

+Weight and BCS of 1 cow from each treatment were not available at initiation of the study.

TABLE 16. LEAST SQUARE MEANS FOR OVARIAN CHARACTERISTICS FOR COWS FED MAINTENANCE OR RESTRICTED DIETS

	Diet		
Criteria[+]	Maintenance	Restricted	SEM
Total ovarian weight, g	8.92[a]	5.86[b]	.74
Dry ovarian weight, g	1.24[c]	.92[d]	.13
Dry ovarian weight, %	15.00	16.00	.01
Corpus luteum weight, g	.87[e]	.00[f]	.29
Follicular fluid weight, g	1.15	.86	.13
No. small follicles ($\leq$ 3.9 mm)	3.90	2.00	.84
No. medium follicles (4.0 to 7.9 mm)	.90	1.10	.33
No. large follicles ($\geq$ 8.0 mm)	.30	.10	.13

[+]Values are a total for both ovaries.
[a,b]Means not having a common superscript differ P<.01.
[c,d]Means not having a common superscript differ P<.10.
[e,f]Means not having a common superscript differ P<.05.

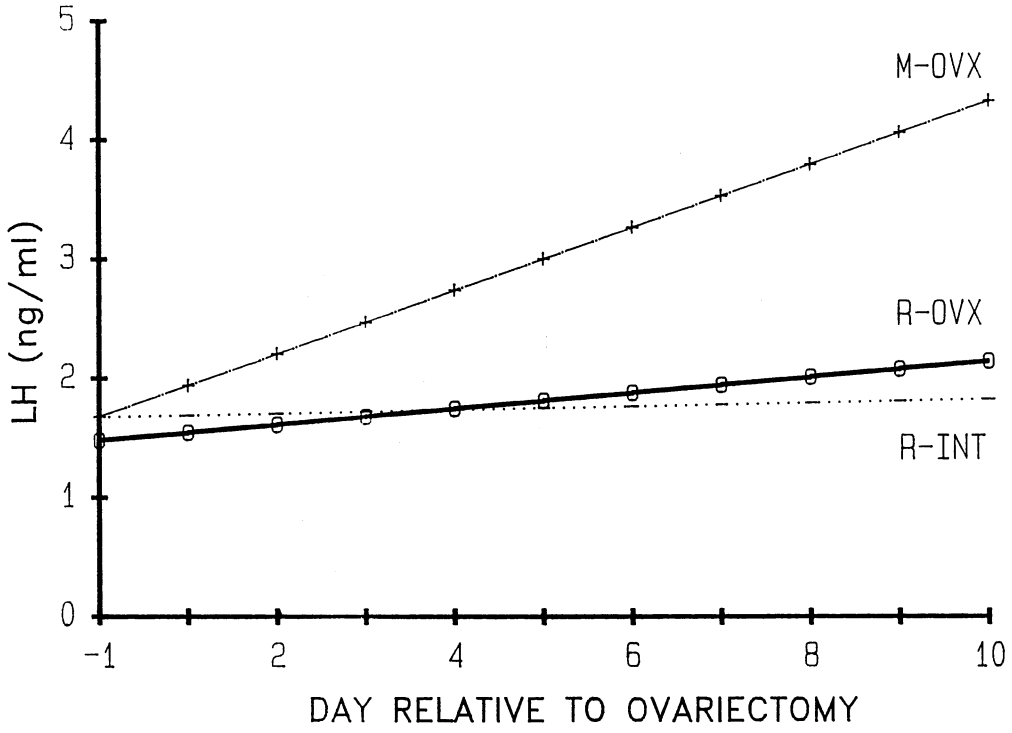

Figure 16. Least-square regressions (SEM ± .25) for concentrations of LH in daily serum samples from cows fed a maintenance (+ = M) diet and ovariectomized (OVX) or a restricted (O = R) diet and OVX or left intact (INT).

TABLE 17. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF LH IN DAILY SAMPLES FROM BEEF COWS FED MAINTENANCE OR RESTRICTED DIETS

Error	D.F.	S.S.	M.S.	F
Maintain	44	63.82		
Restricted	91	31.19		
Total	135	94.38	.70	
Maintain, Restricted	137	112.44		
Difference	2	18.06	9.03	12.90*

*(P<.01).

TABLE 18. ORTHOGONAL COMPARISONS USED TO TEST FOR HETEROGENEITY OF REGRESSION COEFFICIENTS FOR POLYNOMIAL RESPONSE CURVES FOR CONCENTRATIONS OF LH IN DAILY SAMPLES FROM BEEF COWS FED A RESTRICTED DIET AND OVARIECTOMIZED OR LEFT INTACT

Error	D.F.	S.S.	M.S.	F
Ovariectomized	45	18.05		
Intact	46	13.13		
Total	91	31.18	.34	
Ovariectomized, Intact	92	31.89		
Difference	1	.71	.71	2.09*

*(P>.10).

TABLE 19. LEAST-SQUARES MEANS FOR OVARIECTOMIZED (OVX) AND INTACT (INT) COWS FED MAINTENANCE (M) OR RESTRICTED (R) DIETS

Day	Characteristic	Group M-OVX	R-OVX	R-INT	SEM
Pre-ovariectomy					
-1	No. pulse	.20	.40	.60	.29
	Amplitude	2.60[ac]	.41[b]	1.13[d]	.29
	Mean LH	1.24	1.37	1.34	.28
Post-ovariectomy					
+ 1	No. pulse	1.20	1.00	.60	.29
	Amplitude	3.93[e]	2.36	1.67[f]	.89
	Mean	2.22	1.21	1.55	.35
+ 2	No. pulse	1.20	.60	.40	.23
	Amplitude	3.59[a]	1.09[b]	1.00[b]	.70
	Mean LH	2.28	1.21	1.92	.41
+ 3	No. pulse	1.20	.80	1.20	.27
	Amplitude	2.89[a]	2.23	1.16[b]	.46
	Mean LH	2.31	1.72	1.84	.43
+ 4	No. pulse	1.00	.40	.60	.20
	Amplitude	4.54[ce]	2.43[f]	.95[d]	.67
	Mean LH	3.07	2.02	2.02	.39
+ 5	No. pulse	1.00	.80	.60	.26
	Amplitude	4.15[ac]	1.66[b]	.85[d]	.39
	Mean LH	3.44	2.01	2.23	.48
+10	No. pulse	.40	1.00	1.00	.29
	Amplitude	4.61[a]	3.46	2.01[b]	.62
	Mean LH	4.70[a]	2.61[b]	2.17[c]	.46
Average					
	No. pulse	.89	.71	.71	.26
	Amplitude	3.66[c]	1.92[d]	1.29[d]	.52
	Mean LH	2.75	1.74	1.87	.43

[a,b]Means within a characteristic not having a common superscript differ P<.01.
[c,d]Means within a characteristic not having a common superscript differ P<.05.
[e,f]Means within a characteristic not having a common superscript differ P<.10.
[g]No. pulses per 4 h; amplitude and mean LH = ng/ml.
[h]Treatment X Day (P<.01) for mean LH.

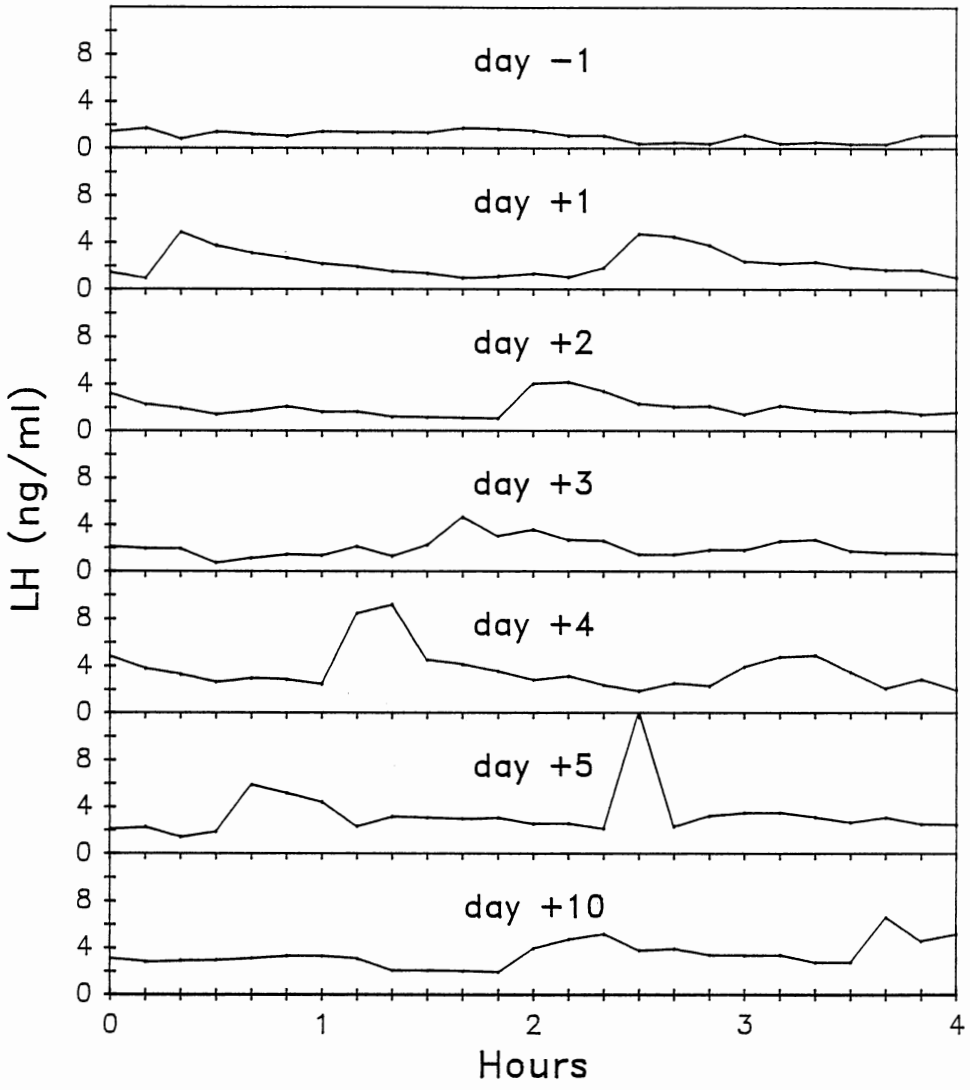

Figure 17. Tonic LH secretion in a bilaterally ovariectomized cyclic cow (BCS = 6). Blood samples were collected at 10 min intervals for 4 h on the days indicated (D 0 = day of ovariectomy).

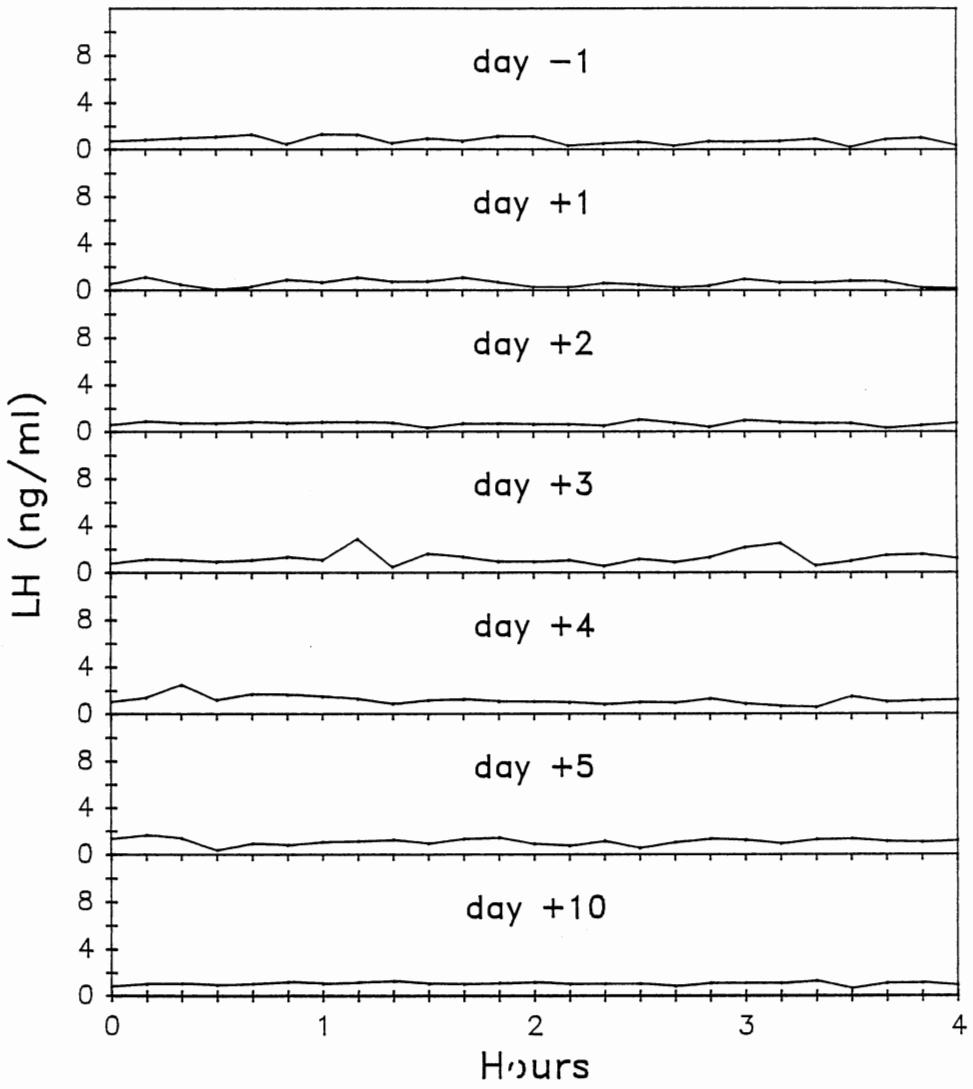

Figure 18. Tonic LH secretion in a bilaterally ovariectomized anestrous cow (BCS = 2.5). Blood samples were collected at 10 min intervals for 4 h on the days indicated (D 0 = day of ovariectomy).

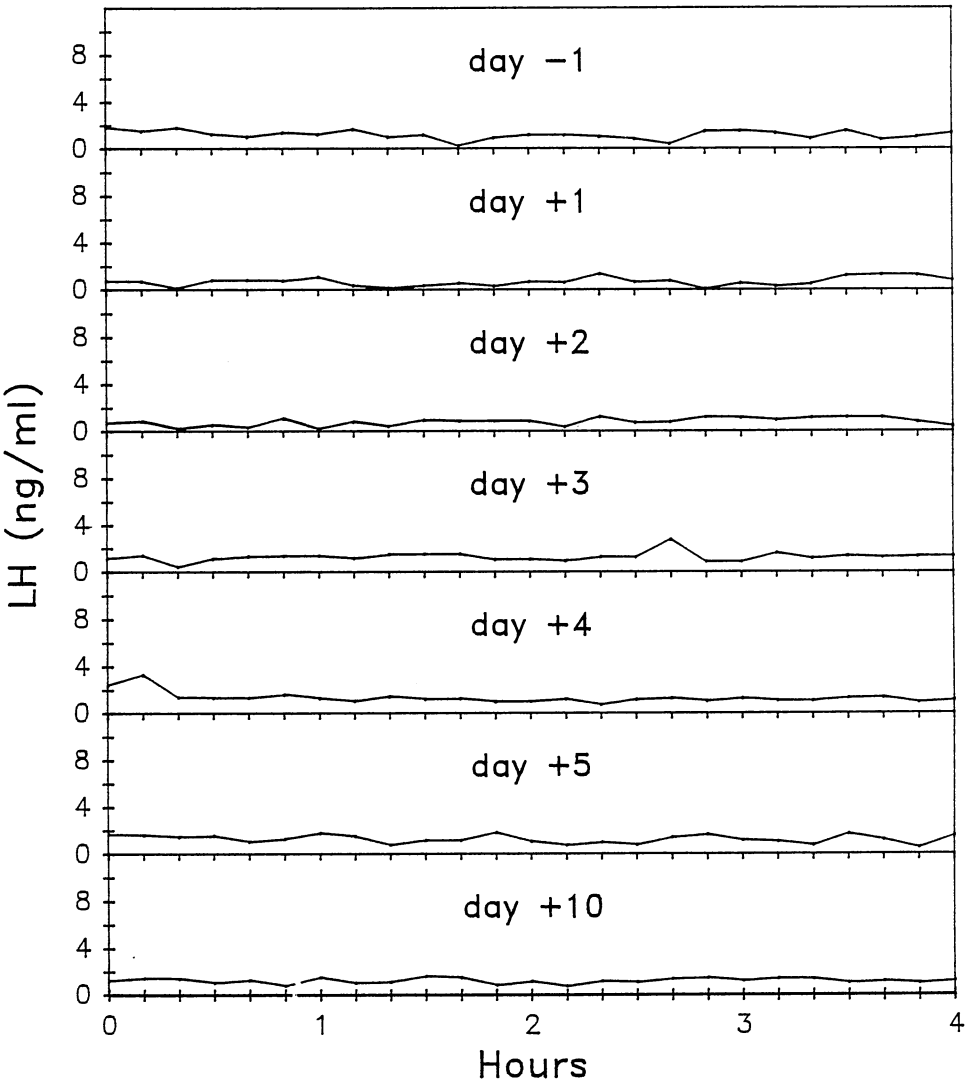

Figure 19. Tonic LH secretion in an intact anestrous cow (BCS = 3). Blood samples were collected at 10 min intervals for 4 h on days indicated (D 0 = day of ovariectomy in OVX groups).

TABLE 20. CHARACTERISTICS OF SERUM LH CONCENTRATIONS OF BEEF COWS FED MAINTENANCE DIETS AND OVARIECTOMIZED (M-OVX) OR FED RESTRICTED DIETS AND OVARIECTOMIZED (R-OVX) OR LEFT INTACT (INT) FOLLOWING TREATMENT WITH ESTRADIOL (E^2)

Treatment	Mean LH concentration before E^2 ng/ml	No. with LH surge	Time to LH surge[a] h	Surge concentration[b] ng/ml
M-OVX	4.7[c]	3/3	19.3	92.0
R-OVX	2.6[d]	2/3	24.0	117.1
R-INT	2.2[d]	1/3	22.0	118.0

[a]MSE = 11.6.
[b]MSE = 289.5.
[c,d]Least-Squares Means in the same column with different superscripts differ ($P<.05$; MSE = 1.1).

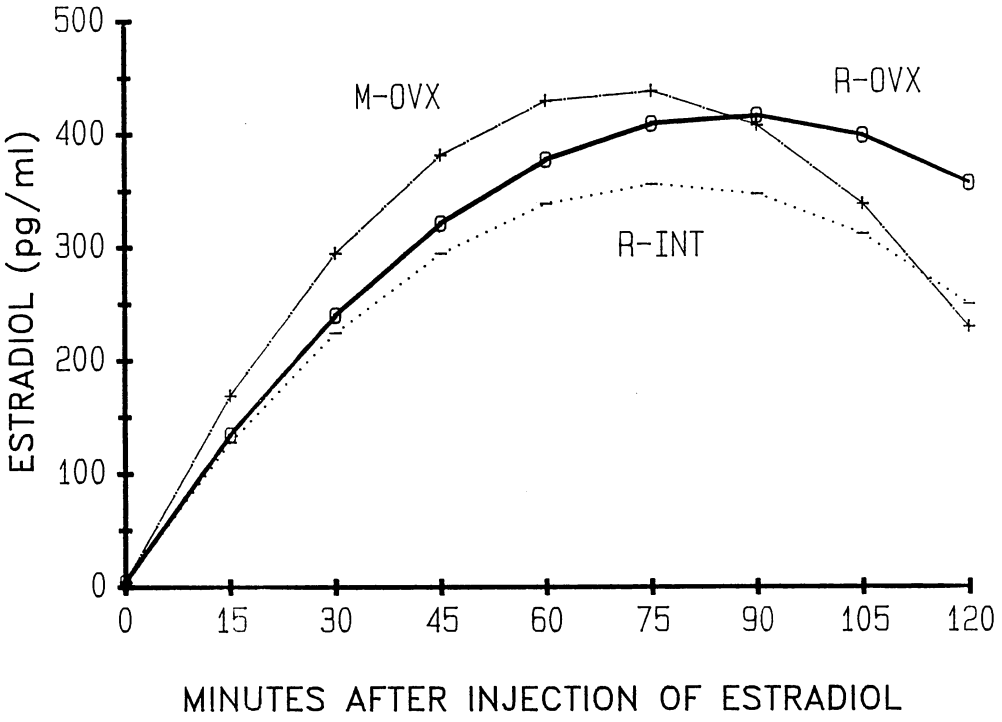

Figure 20. Least-square regressions (SEM ± 124) for concentrations of estradiol in plasma of ovariectomized (OVX) or intact (INT) treated with estradiol. Cows were fed maintenance (+ = M) or restricted (R = O) diets.

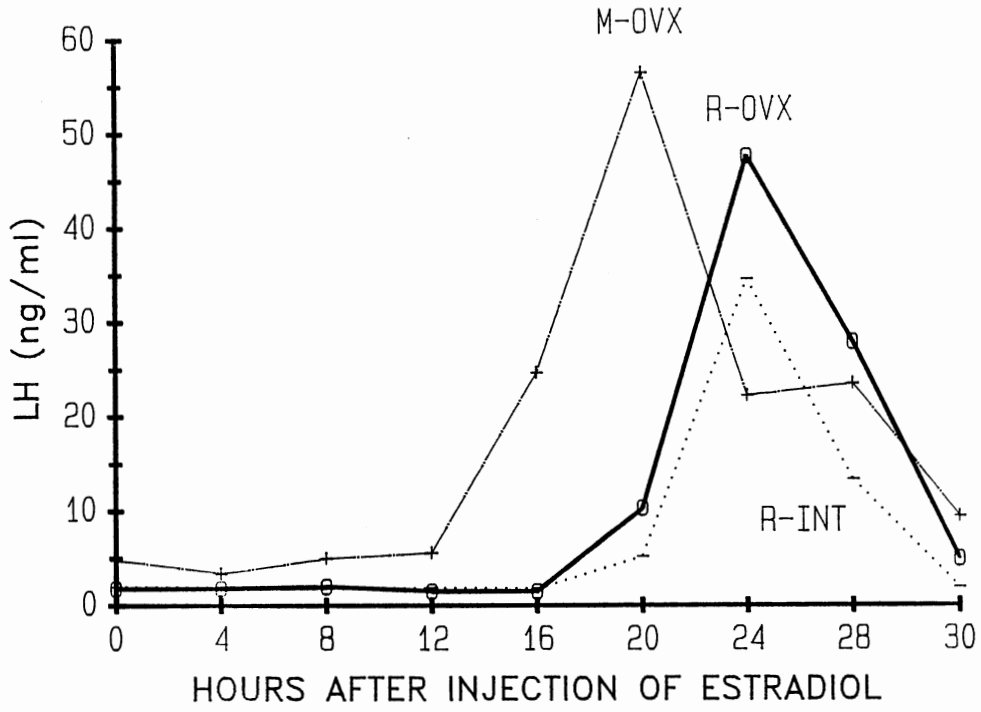

Figure 21. Least-squares means for concentrations of LH in serum of ovariectomized (OVX) or intact (INT) cows treated with estradiol. Cows were fed maintenance (+ = M) or restricted (R = O) diets.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

Twenty-two nonpregnant, nonlactating Hereford cows were monitored for estrous activity and randomly assigned to maintain (M) or lose body weight (R). Cows were maintained in a drylot and individually fed daily a complete ration consisting of 12% crude protein and 2.25 Mcal of metabolizable energy per kilogram of feed. Maintain cows were fed a sufficient quantity of the diet to maintain body condition, and body weight and to continue to have normal estrous cycles. Restricted cows were fed to lose 1% of their initial body weight weekly until approximately cows became anestrus. After anestrous cows were evaluated, they were fed 160% of maintenance until they expressed estrus and cyclic ovarian activity resumed.

Weights and body condition (1=emaciated and 9=obese) were monitored weekly during the experimental period. Blood samples were collected via venipuncture at weekly intervals during the restricted period and twice weekly during regain. Concentrations of progesterone, estradiol, insulin and LH were quantified by radioimmunoassays. Plasma glucose and non-esterified fatty acids (NEFA) were determined by

colorometric procedures. Cows from each treatment (M=6 and R=5) were transported 10 km from the drylot to the Nutrition and Physiology Research Center in Stillwater when all R cows were exhibiting normal estrous cycles (after 5 wk of feed restriction), at the initiation of anestrus (after 20 wk of feed restriction), during anestrus (after 25 wk of feed restriction), and at the re-initiation of estrus cycles (after 6 wk of increased nutrient intake). Cannulae were placed in a jugular vein of all cows. The following day, blood samples were collected at 10 min intervals for 8 h from all cows for quantification of LH. The next day, cows were infused with 300 ml of a 40% glucose solution. Samples were collected at 30 minute intervals prior to glucose infusion, at 15 minute intervals for 2 hours after infusion and at 30 minute intervals for the following 2 hours. Plasma glucose and serum insulin were quantified.

Reduced nutrient intake resulted in loss of body weight (BW) and body condition score (BCS), decreased ovarian function, cessation of normal estrous behavior, and altered concentrations of reproductive and metabolic hormones in blood. Restricted cows had lost 24 $\pm$ .9% of their initial BW and had a BCS of 3.5 $\pm$ .1 at the onset of luteal inactivity. At anestrus, estimated carcass fat content had decreased to 4.1% from a pretreatment amount of 14%.

Concentrations of LH, glucose and insulin were reduced and concentrations of NEFA were increased in weekly blood samples when R cows were initiating anestrus and during

anestrus. Estradiol was not altered by nutrient restriction.

Analysis of frequently collected blood samples revealed that LH pulse frequency and amplitude were reduced by nutrient restriction. In addition, disappearance of infused glucose and insulin was prolonged in R cows when they were initiating anestrus.

Cows returned to estrus 9 $\pm$ 1 wk after increased feeding. At resumption of estrous cycles, R cows had regained 12 $\pm$ 3% of their initial BW and had achieved a BCS of 4.6 $\pm$.2. First estrus was associated with onset of luteal activity and first service pregnancy rate was similar for M and R cows. Concentrations of LH, glucose and insulin were similar for M and R cows when normal cycles resumed.

In a second experiment, fifteen nonpregnant, nonlactating Hereford cows were fed to achieve a moderate to good body condition score (BCS) (1=emaciated to 9=obese). Cows were kept at the range cow reserve, monitored for estrous activity, and randomly assigned to maintenance (M; n=5) or restricted diets (R; n=10).

Body condition and weights were monitored monthly during the experiment. Estrous activity was observed daily and blood samples were collected every two months. Progesterone was quantified by radioimmunoassays to confirm ovarian luteal activity.

When all R cows became anestrus (approximate BCS = 3.5), both treatment groups received two injections (i.m.)

of prostaglandin F2α (25 mg) 11 days apart. Four days after the second injection, cows were transported to the Nutrition and Physiology Research Center (NPRC) in Stillwater and began a 5-day acclimation period and were fed a maintenance ration. Nine days from the second prostaglandin injection, a cannula was placed in one jugular vein of all cows. The following day (D -1), blood samples were collected at 10 minute intervals for 4 hours. On D 0 5, M and 5 R cows were ovariectomized through a flank incision. Ovaries were cooled immediately after collection and kept at 4C until evaluated. The following assessments were made: 1) ovarian weight 2) corpus luteum weight 3) number of follicles greater than 2 mm 4) size of follicles 5) follicular fluid weight.

On D+1, +2, +3, +4, +5, and +10 after ovariectomy, blood samples were collected at 10 minute intervals for 4 hours each day. After the 4 h period on D+10, estradiol (1 mg i.m.) was injected into each cow and samples were collected at 15 min intervals for the next 2 h and at 2 h intervals for the following 30 h. Concentrations of LH, progesterone and estradiol were quantified in frequent and daily samples.

Maintain cows had heavier wet and dry ovarian weights when compared with R cows and ovaries from R cows were void of corpora lutea. Follicular fluid weight, and number of medium and large follicles were similar for M and R cows, however, M cows tended to have more small follicles.

Concentrations of LH in M cows increased linearly within 4 d after ovariectomy, whereas R cows did not exhibit a post-castration increase in LH. Luteinizing hormone pulse frequency was not influenced by treatment; however, LH pulse amplitude was greater for M-OVX than R-OVX cows. Injection of 1 mg of estradiol 17-β caused all M and some R cows to exhibit a pre-ovulatory like surge of LH. All cows that responded to estradiol had surges of LH of similar amplitude at approximately the same time (range 16-24 h).

Conclusions

Our results indicate that nutrient intake significantly influenced cyclic ovarian activity and estrus in beef cows. Nonlactating, nonpregnant cows that were allowed to deplete body energy reserves to a BCS of 3.5 had reduced secretion of LH and ceased to exhibit normal estrous behavior.

Loss of sufficient body weight to deplete body energy reserves altered concentrations of glucose, insulin, LH, and NEFA in blood. Nutritional anestrus was coincident with reduced secretion of glucose, insulin, and LH, and increased concentrations of NEFA. Ovarian luteal activity was re-initiated by feeding cows to achieve an average BCS of 4.6; at that time, R and M cows had similar concentrations of glucose and insulin in their blood and LH pulse frequency and mean concentrations had returned to pre-treatment values.

Our results further suggest that presence or absence of

normal estrous cycles prior to ovariectomy alters LH secretion in beef cows. Since ovariectomized thin cows did not have a post-castration increase in LH secretion, signals from body energy reserves are independent of the ovary and directly influence hypothalamic and/or pituitary regulation of secretion of LH.

The precise mechanisms by which altered nutrient intake affects reproduction remain to be elucidated. These experiments have provided further evidence that the inter-relationships between body energy reserves and reproductive characteristics are complex and multifaceted.

Since LH pulse frequency is reduced when thin cows are initiating anestrus, it seems plausible that signals from adipocytes of these cows are altered when cows lose body energy reserves. It may be that "starved" adipocytes send fewer stimulatory or more inhibitory signals to the LHRH pulse generator in the hypothalamus when compared to "energized" adipocytes. The hypothalamus then reduces LHRH output and fewer pulses of LHRH are perceived at the pituitary. Since estradiol can cause the anterior pituitary of thin anestrous cows to release a pre-ovulatory like LH surge, the anterior pituitary may be relatively unaffected. Comparison of adipocytes from cows with reduced body energy reserves with those of cows with adequate body condition is a logical approach to more closely identifying the mechanisms that regulate nutritional anestrus in beef cows.

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