EFFECTS OF BODY CONDITION SCORE AND NUTRITION ON ESTROUS BEHAVIOR AND ENDOCRINE FUNCTION IN BEEF HEIFERS AND COWS

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Chapter III

Chapter IV

LIST OF FIGURES

Chapter III

Chapter IV

Figure Page

CHAPTER I

INTRODUCTION

The 2001 U.S. beef cow inventory is 33.4 million cows valued at 24 billion dollars (USDA, 2001). However, the annual calf crop in the U.S. is less than 75% (USDA, 1988). At a cost of $360 to maintain a cow annually (Doye and Northcutt, 1997), producers are spending approximately 3 billion dollars to maintain cows that do not wean calves.

A beef cow must produce and wean calf every 12 mo for beef cattle producers to maintain maximal profitability. To achieve this, a cow must become pregnant during the first 80 d after calving. However, many cows do not become pregnant during the breeding season because they fail to exhibit estrus and do not ovulate. Failure to ovulate is associated with decreased pulses of luteinizing hormone (**LH**) and quiescent ovaries (Dunn and Kaltenbach, 1980; Wettemann, 1980; Randel, 1990). Body energy reserves and nutrient intake are important regulators of the length of the postpartum anestrous period in beef cattle (Rutter and Randel, 1984; Selk et al., 1988; Short et al., 1990), but the exact mechanisms are not fully understood. Further elucidation of how body energy stores and nutrition regulate reproduction will have a two fold benefit: 1) it will aid in the development of management practices that increase the number of cows that become

promote genetic improvement in the nations cow herd. pregnant during the breeding season, and 2) it will increase the use of artificial insemination and

After parturition, cows exhibit a period of anestrous and(or) anovulation, which is caused by insufficient secretion of gonadotropin releasing hormone (**GnRH**) from the hypothalamus of the brain. Inadequate secretion of GnRH results in minimal synthesis and release of LH from the pituitary which does not allow follicles to fully develop or ovulate (Dunn and Kaltenbach, 1980; Short et al., 1990). After an indeterminate period of time, GnRH secretion is increased allowing sufficient pulsatile secretion of LH causing follicles to fully develop and ovulate. If cows that are exhibiting normal estrous cycles lose excessive weight and body condition, they cease to ovulate and become anestrous (Imakawa et al., 1987; Richards et al., 1989). Therefore, the brain must receive signals about nutrient intake and body energy reserves to regulate the secretion of GnRH (Wettemann and Bossis, 2000). The exact mechanisms of this phenomenon have not been fully elucidated. Understanding the mechanisms that control GnRH secretion will allow management of cows that will result in adequate GnRH secretion to resume earlier after parturition so that a greater number of cows will ovulate during the breeding season.

Improvement in beef quality can be made through the use of superior genetics. Artificial insemination (**AI**) is a technology that allows wide spread use of genetically superior bulls, however only 13.3% of U.S. beef producers use AI (USDA, 1997). Accurate detection of estrus is essential to the success of AI programs, however the expense and labor required to detect estrus in beef cattle has limited the use of AI in the beef industry (USDA, 1997). Recently, ovulation synchronization protocols that eliminate estrous detection and incorporate timed breeding have been developed (Pursley

et al., 1997b; Geary and Whitter, 1998) and result in pregnancy rates of 45 to 60% in beef cattle (Pursley et al., 1997a; 1998; Geary and Whittier, 1998; Geary et al., 1998). With these systems, pregnancy rates are limited because many cows are anestrus and do not respond to synchronization protocols. By understanding how anestrus and the secretion of GnRH is controlled, we can develop synchronization protocols that increase the number of cows that ovulate and improve pregnancy rates with timed AI. With improved success of timed AI, the use of AI in the beef industry would increase and result in greater genetic progress in the nations cow herd.

Improving pregnancy rate with AI has additional benefits. A 10% increase in the annual calf crop would allow the production of the same kilograms of beef from 13% fewer cows. This would enable beef producers to maintain production with fewer animal units on the same hectares of land, allowing for conservation of natural resources. In addition, this would allow producers to increase production during periods of additional demand without adversely impacting natural resources.

CHAPTER II

REVIEW OF LITERATURE

Nutrient intake and body condition score (**BCS**) at calving are important regulators of postpartum anestrous (Wettemann, 1980; Rutter and Randel, 1984; Short et al., 1990; Wettemann and Bossis, 2000). The purpose of this paper is to review those factors that influence the interval to first estrus and reproductive performance of beef cows.

The Postpartum Period and Pregnancy in Cattle

In order for a cow to wean a calf each year, she must become pregnant during the first 80 d of the breeding season. However, if cows do not have adequate nutrition, and body energy reserves become minimal, pregnancy rates are decreased. Body energy reserves of cattle are accurately estimated by application of the BCS system (Richard et al., 1986; Wagner et al., 1988). The BCS system uses visual and tactile appraisal of adipose tissue at the vertebral process, ribs, and pin bones of an animal, and is an adequate predictor of carcass fat and body energy reserves in cows (Dunn et al., 1983; Wright and Russel, 1984; Wagner et al., 1988) and heifers (Yelich et al., 1995). The most common BCS system is based on a nine point scale, with 1 being emaciated and 9 being obese (Wagner et al., 1988).

Body condition score at calving is an important factor influencing pregnancy rate in beef cows (Wiltbank et al., 1962; Selk et al., 1988). The interval to first estrus is decreased as BCS at calving increases (Wright et al., 1987; Wright et al., 1992a). Cows that calved with a BCS of 5 or greater had a shorter interval to first estrus and became pregnant earlier than cows calving with a BCS of 4 or less (Richards et al., 1986; DeRouen et al., 1992). Pregnancy rates were also increased when cows calved with a BCS 5 or greater (Selk et al., 1988). In addition, feeding increased amounts of energy after calving decreased the postpartum interval only for cows that calved in thin body condition (Richards et al., 1986; Wright et al., 1987; Wright et al., 1992b). This was also the case for primiparous heifers (Vizcarra et al., 1998). When primiparous heifers calve with a BCS of 5 or greater, more heifers exhibit estrus activity during the breeding season and pregnancy rates are increased (DeRouen et al., 1992; Spitzer et al., 1995). In addition, when nonlactating cows (Richards et al., 1989) or heifers (Vizcarra et al., 1995) become too thin (BCS = 3.5 or less), they fail to exhibit estrus. This anestrous condition is caused by insufficient gonadotropin secretion which causes quiescent ovaries (Wettemann et al., 1980). Therefore, cows that are anestrus during the breeding season fail to become pregnant. A target BCS of 5 or greater at calving will ensure that body energy stores are sufficient for cows to become pregnant during the breeding season (Dzuik and Bellows, 1983; Richards et al., 1986; Selk et al., 1988).

Effects of Nutrition on Reproduction in Cattle

Nutrition is an important regulator of reproduction in beef cattle (Dunn and Kaltenbach, 1980; Wettemann, 1980; Randel, 1990). Heifers fed increased amounts of energy weigh more (Wiltbank et al., 1969; Yelich et al., 1995) and are younger at puberty

(Wiltbank et al, 1969; Arije and Wiltbank, 1974; Schillo et al., 1992). In addition, heifers fed high energy diets exhibited increased frequency of LH pulses earlier (Day et al., 1986). Similarly, supplementation of grazing beef cows is needed to maintain weight and BCS (McCollum and Horn, 1990), and can influence reproduction (Wettemann et al., 1980; Randel, 1990).

Prepartum Nutrition

Prepartum nutrition can influence reproduction of beef cows. Increased energy intake before calving decreases the interval from calving to estrus and ovulation (Wiltbank et al., 1962; 1964; Perry et al., 1991). It can also increase the percentage of cows exhibiting estrus during the breeding season (Corah et al., 1975). Conception rates are increased when cows are fed greater amounts of energy prepartum (Marston et al.; 1995). Cows that were fed to maintain BW and BCS before calving had greater pregnancy rates compared with cows that lost BW and BCS (71.3% vs 41.8%, respectively; Selk et al., 1988). In addition, cows that lost BW and BCS during the middle third of pregnancy, but were then fed to either maintain or gain BW and BCS during the last third of pregnancy, had increased pregnancy rates (Selk et al., 1988). The interval from calving to the appearance of large follicles ($\geq$ 10 mm) is decreased, and the number of large follicles after calving is increased when cows are fed increased energy before calving (Perry et al., 1991).

Postpartum Nutrition

Postpartum nutrition of beef cows has a limited effect on reproduction. The interval from calving to the first estrus maybe decreased when cows calve in thin BCS and are fed

high amounts of energy after calving (Wiltbank et al., 1964). In addition, fewer cows exhibited estrus during the first 70 d after calving when nutrition was reduced (Wiltbank et al., 1964; Rakestraw et al., 1986). However, in those experiments, cows calved in thin BCS. Many reports indicate that feeding increased amounts of energy after calving decreases the postpartum anestrous interval only when cows calve in thin body condition (Richards et al., 1986; Wright et al., 1987; Wright et al., 1992b). When cows were fed increased energy after calving, pregnancy rates were increased (Wiltbank et al., 1962; 1964), but others report that postpartum nutrition did not influence pregnancy rates (Marston et al., 1995). The benefits of increased energy intake after parturition are most apparent when cows calve in thin BCS (Wiltbank et al., 1962; Dunn and Kaltenbach, 1980; Short et al., 1990). Although feeding increased energy to thin cows after calving increases pregnancy rate, it is not possible to achieve the maximal pregnancy rate that occurs with fatter cows (Selk et al., 1988).

Protein Nutrition

The effects of protein supplementation on reproduction of beef cows has been difficult to address because of the inability to separate protein and energy for grazing cows. Most fall calving and early spring calving beef cows graze low quality forage (Waller et al., 1972; Johnson et al., 1998; Lents et al., 2000). Feeding additional protein to cattle consuming low quality forage increases forage intake (Lusby and Wagner, 1986; McCollum and Horn, 1990), thus cows consume more energy. When fall-calving cows were fed additional protein supplement before the breeding season, the interval from calving to first estrus was decreased compared with cows fed less protein (Cantrell et al., 1982). In a similar experiment, Rakestraw et al. (1986) fed fall-calving cows different

amounts of protein supplement so cows would maintain BW from calving to the end of the breeding season, maintain BW until breeding and then lose 10% of their BW during the breeding season, or to lose 10% of their BW after calving until breeding season, then maintain BW. They found that when cows maintained BW both after calving and during the breeding season, they had a decreased interval from calving to first estrus, and a greater percentage of cows had ovarian activity at the beginning of breeding compared with cows that lost BW either before or during the breeding season. In those experiments, increases in energy intake would have been caused by additional protein intake, but whether the effects on reproduction were due to protein or energy per se is unclear. Although days to conception were similar, cows fed additional energy before calving had 11% greater pregnancy rate than cows fed protein (Marston et al., 1995). Sasser et al. (1988) fed postpartum cows diets with different amounts of protein (0.96 kg/d of CP or 0.32 kg/d of CP) that were isocaloric and supplied adequate energy. More cows fed greater amounts of protein exhibited estrus during the breeding season compared with cows fed protein deficient diets (89% vs 63%, respectively). In addition, overall pregnancy rates were decreased for cows deficient in protein (50% vs 82% for adequate protein; Sasser et al., 1988). A second experiment with the same diets determined that it took longer for the frequency of LH pulses to increase after calving in protein deficient cows, and that the GnRH-induced LH release was less compared with cows fed adequate protein (Nolan et al., 1988).

Metabolizable Protein. Cows fed isonitrogenous urea based supplements had decreased estrus and pregnancy rates compared with cows fed a soybean meal supplement (Forero et al., 1980). Traditionally, protein supplements have been

formulated using the CP system (NRC, 1984), which assumes all protein is equally degradable in the rumen. Currently, the metabolizable protein (MP) system is recommended (NRC, 1996). The MP system requires estimates of forage and supplement intake, dietary protein degraded within the rumen (DIP), microbial protein production, and dietary protein that escapes rumen degradation (UIP). The sum of digestible microbial protein and digestible UIP equals the total MP supply to the animal. In the experiment by Forero et al. (1980), urea based supplements had excessive DIP, but were deficient in UIP. Cows may not have had sufficient UIP, causing a deficiency in MP supply, which may have prevented optimal reproductive performance. Results from studies that evaluate the effects of increasing MP supply on reproduction of postpartum cows by altering the amounts of UIP are not conclusive. The postpartum interval was not different for cows supplemented with additional UIP, but first service conception rates were greater (Triplett et al., 1995). Others show that supplementing cows with increased amounts of UIP increased the number of cows exhibiting estrus during the first 21 d of the breeding season (Wiley et al., 1991). However, the interval from calving to luteal function was not different for cows fed additional UIP (Rusche et al., 1993; Lents et al., 2000). In one study, cows returned to estrus later when fed a 50% UIP vs 25% UIP supplement (Dhuyvetter et al., 1993). Rusche et al. (1993) observed that conception rate and calving date were not influenced by additional UIP supplementation. The effects of feeding additional UIP on reproduction in beef cattle is limited. Reasons for differing results could be due to season, breed type, or forage quality.

Nutrition and Primiparous Heifers

Level of nutrition is also important for reproduction in primiparous heifers. Interval from calving to first estrus was longer for heifers fed low energy postpartum compared with heifers fed high energy diets (Ciccioli et al., 2000), and the beneficial effect of feeding primiparous heifers additional energy occurs only when heifers calved in thin BCS (Spitzer et al., 1995; Vizcarra et al., 1998). Wiley et al. (1991) found that prepartum nutrition did not alter the postpartum interval of first-calf heifers, but additional MP after calving decreased the postpartum interval. Others report no effect due to feeding additional MP after calving on the postpartum anestrous interval of primiparous heifers (Triplett et al., 1995; Strauch et al., 2001). The percentage of heifers exhibiting estrus during the breeding season and pregnancy rates are increased when first calf heifers were fed to gain weight after calving (Spitzer et al., 1995; Ciccioli et al., 2000). Primiparous heifers fed greater amounts of energy after calving became pregnant earlier and had greater pregnancy rates (87%) than heifers fed moderate or low energy after calving (72% and 64%, respectively; Dunn et al., 1969). Conflicting reports may be due to differences in breed of cows, as well as type and quality of forage. Because primiparous heifers have not yet reached mature size and are still growing, postpartum nutrition may be more important than for mature beef cows.

In general, feeding supplemental energy before parturition increases body weight, BCS, and pregnancy rates. Although feeding greater amounts of energy after calving does increase reproductive performance of cows in thin condition at calving, performance is less than that for cows calving with adequate BCS. Thus, prepartum nutrition and BCS

at calving are the most important factors influencing reproduction in beef heifers and cows.

Anovulation in Beef Cattle

Beef cows fail to exhibit estrous cycles for a period of time after parturition. The postpartum anestrous period is a result of inadequate gonadotropin secretion and decreased follicular growth (Dunn and Kaltenbach, 1980; Wettemann, 1980; Short et al., 1990). Body energy reserves and nutrient intake are important regulators of the length of the postpartum anestrous period (Wettemann and Bossis, 2000). When nutritionally restricted mature beef cows lost 24% of their body weight, serum concentrations of luteinizing hormone (LH) were decreased and cows failed to have ovarian activity (Richards et al., 1989). Nutritionally restricted beef heifers lost 22% of their body weight before they failed to ovulate (Rhodes et al., 1995; Bossis et al., 1999). Failure to ovulate was associated with reduced concentrations of LH (Rhodes et al., 1996; Bossis et al., 1999). Excessive loss of body weight results in inadequate body energy reserves to maintain sufficient gonadotropin secretion which causes beef cows to fail to ovulate.

Endocrine and metabolic changes during the occurrence of anovulation have been difficult to address with current animal models because of the inability to predict the onset of anovulation. With a recently developed a model, anovulation occurs in primiparous beef heifers with two weeks of feed restriction (Mackey et al., 1999; 2000; White et al., 2000). This model allows the study of acute endocrine and metabolic changes associated with the onset of nutritionally induced anovulation in beef cattle.

Nutritional Regulation of LH

Luteinizing hormone is released from the pituitary in a pulsatile fashion to cause ovulation. Chronic nutrient restriction causes decreased release of LH in rats (McClure and Saunders, 1985; Sisk and Bronson, 1986), monkeys (Dubey et al., 1986), humans (Boyar et al., 1974), sheep (Foster and Olster, 1985), and cattle (Richards et al., 1989; Rhodes et al., 1996; Bossis et al., 1999). For the purposes of this review, only nutritional regulation of gonadotropins in cattle and sheep will be discussed.

Chronic Nutritional Restriction

When nutrient intake is restricted for sufficient time to cause anovulation, LH pulses and plasma concentrations of LH are decreased (Imakawa et al., 1987; Richards et al., 1989). In sheep, chronic nutritional restriction prevented the onset of ovulatory cycles associated with puberty (Foster and Olster, 1985). In addition, nutritional restriction resulted in decreased plasma concentrations of LH and reduced frequency of LH pulses in sheep (Foster and Olster, 1985; Foster et al., 1989). Estradiol treated wethers fed to lose BW for 8 wks had decreased serum concentrations of LH and decreased frequency of LH pulses compared with sheep fed to gain or maintain BW (Beckett et al., 1997b). Chronic nutrient restriction (loss of 1% of BW per wk) decreased plasma concentrations of LH in mature cows (Richards et al., 1989) as well as heifers (Rhodes et al., 1996; Bossis et al., 1999). However, this occurred only after animals had lost approximately 20-25% of their BW.

The mechanism by which nutritional restriction decreases plasma LH appears to be at the level of the hypothalamus, as pituitary responsiveness to GnRH remains intact.

Treating nutritionally restricted, anovulatory ewes with exogenous GnRH resulted in corresponding LH pulses (Foster and Olster, 1985; Foster et al., 1989; Kile et al., 1991). Postpartum anestrous beef cows have sufficient numbers of GnRH receptors in the pituitary to respond to hypothalamic release of GnRH (Moss et al., 1985; Leung et al., 1986). Treating postpartum anestrous beef cows (Echternkamp, 1978; Wettemann et al., 1982; Wright et al., 1990) or dairy cows (Kesler et al., 1977; Fernandes et al., 1978) with exogenous GnRH increases LH secretion. When nutritionally anestrous beef cows were given pulses of GnRH (2μg) every hour, serum concentrations of LH and amplitude of LH pulses were increased (Bishop and Wettemann, 1993; Vizcarra et al., 1997). Cows in thin BCS had greater amounts of GnRH in the infundibular stalk-median eminence (Rasby et al., 1991) and released more LH from the pituitary when treated with GnRH compared with cows in good BCS (Beal et al., 1978; Rasby et al., 1992). This indicates that synthesis of LH is not inhibited, but that less GnRH is released in thin cows than cows with moderate BCS. Together, these results indicate that postpartum or nutritionally induced anestrus and(or) anovulation is caused by decreased GnRH secretion from the hypothalamus which prevents the ovulatory surge of LH.

Acute Nutritional Restriction

Acute nutrient restriction has little effect on LH secretion in post-purbertal ruminants. Plasma concentrations of LH and amplitude and frequency of LH pulses were unaffected when mature ovariectomized beef cows were restricted to 30% of maintenance for 6 d (Looper, 1999). Similarly, removal of 85% of rumen contents from steers for 3 d did not alter LH concentrations, amplitude or frequency of LH pulses compared with control steers (Ojeda et al., 1996). When prepubertal heifers were fasted

for 48 hr, frequency of LH pulses were reduced by 65% (Amstalden et al., 2000), however, plasma concentrations of LH and amplitude of LH pulses were unaffected by fasting. In these studies, animals were restricted for short periods of time. In addition, animals lacked gonadal steroids which alter LH synthesis and secretion. When a limited number of Holstein heifers were fasted for 8 d during the estrous cycle (d 8 to 16 of the cycle), concentrations of LH were reduced (McCann and Hansel, 1986). When beef heifers were fed 40% of maintenance requirements for 14 d, growth rate and maximum size of the dominant follicle were decreased, but LH was not altered (Mackey et al., 2000). However, LH was measured during the luteal phase where progesterone may decrease LH secretion and prevent detection of differences. In a similar study, when heifers were fed 40% of maintenance the ovulatory surge of LH was abolished and heifers failed ovulate (Mackey et al., 1999).

These results indicate that long term nutritional restriction decreases plasma LH and frequency of LH pulses in cattle. This occurs due to decreased release of GnRH from the hypothalamus. Short term nutritional restriction may not alter concentrations of LH in plasma or frequency of LH pulses, but can prevent the surge of LH that causes ovulation. Exact mechanisms for the effects of acute nutritional restriction on LH secretion are unknown.

Nutritional Regulation of FSH

Chronic Nutrient Restriction

Nutritional regulation of FSH differs from that of LH. Serum and pituitary concentrations of FSH in ovariectomized sheep are reduced 63 and 56% respectively with

127 d of nutrient restriction (Kile et al., 1991). Others report that chronic nutrient restriction of intact sheep increases FSH in plasma (McShane and Keisler, 1991; Adam and Findlay, 1997; Adams et al., 1997). Wethers fed to lose BW for 7 wks had increased concentrations of FSH in serum (Beckett et al., 1997). However, nutrition did not alter FSH in ovariectomized ewes (McShane and Keisler, 1991). Feed restriction in sheep may increase the sensitivity to negative feedback of estradiol (Adams et al., 1997; Beckett et al., 1997). Concentrations of FSH were decreased when feed restricted wethers and ovariectomized ewes were treated with estradiol compared with estradiol treated sheep consuming adequate nutrition (Adams et al., 1991; Beckett et al., 1997).

Concentrations of FSH in serum of heifers are increased prior to nutritional anestrus (Rhodes et al., 1996; Bossis et al., 1999). Amplitude and frequency of FSH pulses were also increased (Bossis et al., 1999). Size of the dominant follicle (Rhodes et al., 1995; Bossis et al., 1999) and serum concentrations of estradiol (Rhodes et al., 1996; Bossis et al., 1999) were decreased with nutritional restriction. Presumably, FSH increased with decreasing negative feed back of estradiol and(or) other factors, decreasing the frequency of GnRH pulses. Less frequent pulses of GnRH are associated with increased secretion of FSH in anestrous cows (Vizcarra et al., 1997).

Acute Nutritional Restriction

Restricting nutrient intake of ovariectomized beef cows for 6 d was insufficient to change serum concentrations of FSH (Looper, 1999). Acute nutritional restriction (0.4M for 14 d) causes anovulation and greater serum concentrations of FSH in beef heifers (Mackey et al., 1999; 2000). When ovulation did occur, maximal concentrations of FSH were greater in restricted heifers compared with full fed controls (Mackey et al., 2000).

Metabolic Regulators of Reproduction

Mechanisms by which nutritional status of the animal and the amount of body energy reserves are relayed to the brain are not fully elucidated. Several metabolic and endocrine signals may be important.

Nonesterified Fatty Acids (NEFA)

During times of nutritional restriction, cattle mobilize body energy stores to meet energy demands. Glycerol and nonesterified fatty acids (NEFA) are released during negative energy balance (Lucy et al., 1991). Plasma concentrations of NEFA are increased in nutrient restricted cows (Richards et al., 1989b) and heifers (McShane et al., 1989; Yelich et al., 1995; Bossis et al., 1999). Acute nutritional restriction (3 to 6 d) increased NEFA concentrations in plasma of cows (Looper, 1997) and steers (Ojeda et al., 1996). In prepubertal heifers, a 16 h fast was sufficient to increase concentrations of NEFA in plasma (Lents et al., 1996).

Concentrations of NEFA are positively correlated with body condition of cows on an adequate plane of nutrition (Vizcarra et al., 1998), but are negatively correlated when cows are fed restricted diets (Richards et al., 1989b). Although NEFA are increased prior to nutritionally induced anestrous in cows (Richards et al, 1989b) and heifers (Bossis et al., 1999) concentrations of NEFA are not indicative of luteal activity in cattle (Vizcarra et al., 1998). Peripheral infusion of NEFA to ovariectomized ewes did not alter LH concentrations in serum or frequency and amplitude of LH pulses (Estienne et al., 1990). Thus, concentrations of NEFA are indicative of the metabolic status of the animal, but effects of NEFA on reproduction are not established.

Insulin

Insulin is an important regulator of glucose homeostasis. Feed restriction causes a decrease in plasma concentrations of insulin in cattle (Trenkle, 1978; McCann and Hansel, 1986; Bossis et al., 1999). The roles of insulin in modulating reproduction are not fully understood.

Insulin and Hypothalamic-Pituitary Function. Insulin crosses the blood-brain barrier (Baskin et al., 1983) and receptors for insulin are found in the hypothalamus (Van Houten et al., 1979; Baskin et al., 1983). In diabetic rats, hypothalamic concentrations of GnRH and pituitary concentrations of LH are decreased (Besetti et al., 1985; Besetti et al., 1989; Valdes et al., 1990; Valdes et al., 1991). However, treating diabetic rats with GnRH caused LH release from the pituitary (Valdes et al., 1991) indicating the pituitary of diabetic rats remains responsive to GnRH, and that decreased insulin inhibits GnRH release from the hypothalamus. Insulin did not alter GnRH release from immortalized hypothalamic cells, but did stimulate cell proliferation (Olson et al., 1995). In hypothalamic tissue of rats perifused with insulin (10 mU/L), GnRH secretion was increased (Arias et al., 1992). However, this only occurred in the presence of glucose (at least 100 mg/dL).

Intraventricular infusion of insulin to ovariectomized ewes did not alter plasma LH, or frequency of LH pulses (Hileman et al., 1993). In addition, when ewes were on a high plane of nutrition, insulin infusion decreased plasma LH and frequency of LH pulses (Hileman et al., 1993). In contrast, intravenous infusion of insulin, or insulin plus glucose to rams increased frequency of LH pulses (Miller et al., 1995), but intravenous infusion of insulin did not alter LH in heifers (Harrison and Randel, 1986).

Intraventricular infusion of insulin to ovariectomized ewes increased serum concentrations of LH (Daniel et al., 2000) and frequency of LH pulses (Tanaka et al., 2000).

Concentrations of insulin are decreased in cows (Richards et al., 1989b) and heifers (Bossis et al., 1999) prior to nutritionally induced anestrus. However, injecting postpartum anestrous beef cows twice daily with insulin did not alter LH concentrations or frequency of LH pulses (Garmendia, 1986). Together these data suggest that insulin may modulate hypothalamic or pituitary function through glucose utilization, but that peripheral changes in concentrations of insulin have a limited effect on pituitary function.

Insulin and Ovarian Function. The role insulin plays in ovarian function of cattle, sheep, and pigs has been widely studied (Spicer and Echternkamp, 1995). Receptors for insulin are present in follicular cells (Otani et al., 1985; Spicer et al., 1994), and insulin modulates ovarian function. Diabetic pigs have decreased follicular growth and decreased intrafollicular concentrations of estradiol (Cox et al., 1994; Edwards et al., 1996). Diabetic pigs also have decreased plasma and intrafollicular concentrations of insulin-like growth factor (IGF)-I (White et al., 1993; Cox et al., 1994; Edwards et al., 1996), which potentiates gonadotropin stimulation of ovarian steroidogenesis (Spicer and Echternkamp, 1985). Follicular atresia was reduced when pigs were treated with insulin, but concentrations of gonadotropins were not different from controls (Matamoros et al., 1991; Cox et al., 1994). Treating cows with insulin during superovulation increased size of larger follicles (Simpson et al., 1994). In addition, intrafollicular concentrations of estradiol and IGF-I were also increased (Simpson et al., 1994). Insulin stimulated proliferation and FSH-induced estradiol production by bovine granulosa cells *in vitro*

(Spicer et al., 1993; Spicer et al., 1994), and increased LH stimulated steroidogenesis of cultured bovine thecal cells (Stewart et al., 1995).

Insulin may modulate reproduction both directly and indirectly through glucose utilization of reproductive tissues. Effects at the level of the hypothalamus or pituitary seem to be limited, but stimulatory effects on ovarian function are more evident.

Glucose

When Holstein heifers were fasted for 4 d, plasma concentrations of glucose were decreased (McCann and Hansel, 1986), however restricting beef heifers to 0.4 maintenance energy requirements for 14 d did not alter plasma glucose (White et al., 2000). Chronic nutritional restriction causes cessation of estrous cycles in cows (Richards et al., 1989b) and heifers (Bossis et al., 1999). In both cases, concentrations of glucose in plasma were decreased prior to anovulation (Richards et al., 1989b; Bossis et al., 1999). Concentrations of glucose in plasma was positively correlated with frequency of LH pulses in prepuberal heifers fed two different levels of nutrition (Yelich et al., 1996). Therefore, plasma concentrations of glucose in cattle can be modulated by nutrition and may play a role in altering hypothalamic-pituitary function.

An antagonist of glucose metabolism, 2-deoxy-D-glucose (**2DG**), has been used to explore the possible role of glucose in hypothalamic-pituitary function. When beef heifers were treated with 2DG, estrus and CL formation were inhibited (McClure et al., 1978). Intravenous infusion of 2DG decreased plasma LH in seasonal anestrous ewes (Crump et al., 1982) and ovariectomized ewes (Funston et al., 1995), however FSH was not influenced (Funston et al., 1995). In a similar fashion, insulin-induced hypoglycemia delayed the onset of the estradiol-induced LH surge in anestrous (Crump and Rodway,

1986) or ovariectomized ewes (Medina et al., 1998). When glucose was infused with insulin, timing of the LH surge was restored (Medina et al., 1998). Treating ovariectomized ewes with 2DG did not alter LH secretion (Hileman et al., 1991), but when sheep were treated with 2DG in combination with methyl palmoxirate, an inhibitor of fatty acid oxidation, pulsatile release of LH was blocked (Hileman et al., 1991; Bucholtz et al., 1992). Phlorizin induced diabetic cows had similar concentrations of LH compared with non-diabetic controls (Rutter and Manns, 1987). In that experiment, phlorizin inhibited the uptake of glucose at the brush border of the intestine. However, because ruminants ferment carbohydrates to volatile fatty acids in the rumen, less than 10% of the total glucose required is absorbed from the intestines (Trenkle, 1981).

The means by which glucose alters LH seem to be via a central mechanism in the hypothalamus. Central administration of glucose to ovariectomized ewes increased plasma LH concentrations compared with controls (Daniel et al., 2000). Intracerebroventricular administration of 2DG to sheep decreases plasma LH, however, GnRH-induced LH release was similar to controls (Bucholtz et al., 1996; Ohkura et al., 2000). Excitatory amino acids that cause GnRH secretion were also able to cause LH release similar to controls (Bucholtz et al., 1996). Peripheral infusion of 2DG to anestrous ewes decreased LH, but did not alter the response to exogenous GnRH (Crump et al., 1982). These results suggest that decreased glucose availability decreases LH by preventing GnRH secretion. However, most of these studies evaluated glucose concentrations that were less than normal physiological ranges. Infusion of glucose to cows in good BCS did not alter LH (Garmendia, 1986; McCaughey et al., 1988).

Insulin-Like Growth Factor-I

Insulin-like growth factor (IGF)-I is a metabolic hormone that has positive effects on reproduction. Concentrations of IGF-I increase prior to puberty in beef heifers (Jones et al., 1991; Yelich et al., 1996), and IGF-I increases granulosa cell proliferation and steroidogenesis in cattle, sheep, and pigs (Spicer and Echternkamp, 1995). Insulin-like growth factor-I stimulates GnRH secretion from the median eminence of rats (Hiney et al., 1991), and in vitro secretion of gonadotropins (Kanematsu et al., 1991; Soldani et al., 1995). When GH deficient mice were administered GH, concentrations of IGF-I and LH were increased (Chandrashekar and Bartke, 1993).

Decreased nutrient intake decreases peripheral concentrations of IGF-I in cattle (Houseknecht et al., 1988; Granger et al., 1989; Rutter et al., 1989; Armstrong et al., 1993; Richards et al., 1995). Blood concentrations of IGF-I in response to exogenous GH are reduced during nutrient restriction in cattle (Brier et al., 1988; Ronge and Blum, 1989) due to reduced binding of GH to hepatic membranes (Brier et al., 1988). Decreased concentrations of IGF-I in plasma delays puberty in heifers (Granger et al., 1989), and is associated with increased postpartum anestrous intervals in cows (Nugent et al., 1993). Concentrations of IGF-I are reduced in nutritionally anestrous cows (Richards et al., 1991), and with short term (48 h) fasting (Spicer et al., 1992). However, IGF-I concentrations in follicular fluid were unaltered by fasting. When anestrous cows were pulsed with GnRH (2 μg) every hour, concentrations of IGF-I in follicular fluid were increased (Hamilton et al., 1999) and cows resumed ovarian activity (Vizcarra et al., 1997). Plasma concentrations of IGF-I were increased during the follicular wave before

resumption of ovulation in nutritionally induced anovulatory beef heifers undergoing realimentation (Bossis et al., 2000).

Insulin-Like Growth Factor Binding Proteins

Five proteins that bind IGF-I with high affinity have been identified in the bovine. These IGF binding proteins (IGFBP-1, -2, -3, -4, and -5) act to modulate the function of IGF-I in plasma as well as within the follicle. Each of these IGFBPs have different roles in controlling IGF function and appear to be differentially regulated. These IGFBPs act to modulate IGF-receptor interactions and differentially regulate IGF action at specific tissues. The endocrine or metabolic state of an animal may regulate when and where different IGFBPs are produced. Most IGFBPs are synthesized in the liver but some are also produced locally in specific tissues, such as the ovary (Lee et al., 1993).

Nutritional regulation of IGFBPs. The effects of inadequate nutrition on IGFBPs are dependent on the binding protein and its function. Plasma concentrations of IGFBP-1 (Busby et al., 1988; Baxter, 1993) and IGFBP-2 (Clemmons et al., 1991; Smith et al., 1995) are increased with fasting in humans. Fasting also increases mRNA for IGFBP-1 and -2 in liver (Tseng, et al., 1992) and peripheral concentrations of IGFBP-1 and -2 (Orlowski et al., 1990; Murphy et al., 1991) in the rat. Chronic nutrient deprivation in rats increased mRNA for IGFBP-1 and -2, but decreased mRNA for IGFBP-3 (Donovan et al., 1991). Chronic protein restriction in rats leads to decreased plasma concentrations of IGFBP-3 (Thissen et al., 1991). The effects of nutrition on IGFBP-4 and -5 in rodents has not been determined.

Nutrition alters IGFBPs in the ruminant animal. Restricting heifers to 54% of maintenance for 84 d increased plasma IGFBP-2 by 79%, but plasma IGFBP-3 was not

altered (Vandeharr et al., 1995). Ewes in thin body condition (≤ 3) had decreased plasma concentrations of IGFBP-3 and -4 compared with ewes in good (> 3) body condition (Snyder et al., 1999). At 2 weeks postpartum, plasma concentrations of IGFBP-2 were greater and IGFBP-3 were less in cows that remained anestrous at 20 weeks postpartum compared with cows that resumed ovarian function at 20 weeks (Roberts et al., 1997). In dairy cows, 2 d of feed restriction increased plasma IGFBP-2 but not -3 (McGuire et al., 1995).

IGFBPs and the pituitary. Insulin-like growth factor-binding proteins may influence hypothalamic-pituitary function. Insulin-like growth factor binding protein-2, -3, and -5 have been detected in the anterior pituitary of sheep (Snyder et al., 1999) and cattle (Funston et al., 1995). Ewes in thin body condition had greater amounts of IGFBP-2 in the pituitary than ewes in good condition (Snyder et al., 1999). The exact role of IGFBPs in modulating pituitary function during nutrient restriction has yet to be determined.

Insulin-like growth factor-I is an important metabolic hormone that regulates reproduction. High affinity IGFBPs function to regulate and potentiate the actions of IGF-I. Differential regulation of IGF-I and its binding proteins during decreased nutrient intake acts to alter reproductive function. How the IGF-I and IGFBP systems change with the onset nutritional anestrus and their role in anovulation have yet to be determined.

Neuropeptide-Y

Neuropeptide-Y (NPY) is a 36 amino acid peptide first isolated from porcine brain extracts (Tatemoto et al., 1982). Nuclei within the hypothalamus known to be associated with the feeding response contain NPY neurons (McDonald, 1988; White and Martin, 1997). Intracerebroventricular infusion of NPY increases feed intake in rats (Clark et al.,

1984), rabbits (Pau et al., 1988), pigs (Parrott et al., 1986), and sheep (Miner et al., 1989). Feed restriction increases concentrations of NPY and mRNA for NPY in the hypothalamus (Sahu et al., 1988; Miner, 1992; McShane et al., 1992; 1993).

NPY and reproductive cycles. Neuropeptide-Y changes with reproductive status. In humans, plasma NPY decreases with the onset of menopause (Baranowska et al., 2000; Milewicz et al., 2000). In rats, mRNA for NPY receptor in the hypothalamus increase on the morning of proestrus (Xu et al., 2000). Seasonally anestrous ewes had less NPY in the arcuate nucleus and median eminence of the hypothalamus than cyclic ewes (Barker-Gibb and Clarke, 2000). Hypothalamic concentrations of NPY were also less in ovariectomized ewes during the months of seasonal anestrus (Clarke et al., 2000). Ovarian steroids alter the effects of NPY on GnRH and LH secretion (Kalra and Crowley, 1984; McDonald et al., 1989; Xu et al., 2000). Syrian hamsters exhibited a 91% decrease in the duration of estrus when treated with a NPY agonist (Corp et al., 2001). The effects of season or cyclicity in cattle on NPY and(or) its effects on gonadotropin secretion have not been established.

NPY and gonadotropin secretion. In ruminants, inadequate nutrition inhibits GnRH release from the hypothalamus. This prevents the subsequent ovulatory surge of LH and ovulation. High concentrations of NPY that have been found in the hypophyseal-pituitary portal plasma of rats (McDonald et al., 1987) and castrated monkeys (Terasawa and Gore, 1992). Feed restriction increased mRNA for NPY in the hypothalamus of sheep (McShane et al., 1992; 1993), implicating NPY as a possible mediator of the negative effects of nutrient restriction on LH release. Treating dispersed anterior pituitary cells of ovariectomized rats with NPY caused increased LH (McDonald et al.,

1985; Chabot et al., 1988) and FSH (McDonald et al., 1985) release. However, *in vivo*, intraventricular administration of NPY to ovariectomized rats decreased plasma LH , as well as frequency and amplitude of LH pulses (Kalra and Crowley, 1984; McDonald et al., 1989), but had no effect on FSH (McDonald et al., 1985). Plasma LH was also decreased in ovariectomized rabbits (Khorram et al., 1987) and monkeys (Kaynard et al., 1990) that were treated with NPY. Chronic nutrient restriction of ovariectomized ewes abolished pulsatile secretion of LH and caused an 8.7 fold increase in concentrations of NPY in the median eminence of the hypothalamus (Ober and Malven, 1992; Prasad et al., 1993). Central administration of NPY to sheep decreased LH (McShane et al., 1992; Porter et al., 1993), and concentrations of mRNA for NPY were negatively correlated with concentrations of LH in nutritionally restricted and full fed ewes (McShane et al., 1993). In ovariectomized cattle, central administration of NPY into the third ventricle decreased LH pulses (Gazal et al., 1998; Thomas et al., 1999). When castrated sheep were subjected to energy restriction, mRNA for NPY was increased in the hypothalamus and LH pulse frequency was decreased (Adam et al., 1997).

Neuropeptide-Y inhibits secretion of LH in ovariectomized animals. However, the presence of ovarian steroids augments the effects of NPY on gonadotropin secretion. When ovariectomized rats were pretreated with estradiol and(or) progesterone, intraventricular infusions of NPY increased plasma LH (Kalra and Crowley, 1984; McDonald et al., 1989). Concentrations of LH in plasma were decreased when ovariectomized estradiol primed rats were immunized against NPY (Sutton et al., 1988; Wehremberg et al., 1989), whereas LH concentrations were unchanged in adjuvant treated controls (Wehrenberg et al., 1989). Treating ovariectomized rabbits with NPY

decreased LH concentrations, but had no effect on LH in intact rabbits (Khorram et al., 1987). Intraventricular infusion of NPY to ovariectomized monkeys decreased concentration and frequency of LH pulses in plasma, but had no effect on plasma concentrations of LH in ovariectomized estradiol treated monkeys (Kaynard et al., 1990). However in sheep, central administration of NPY decreased plasma LH regardless of steroid treatment (McShane et al., 1992; Porter et al., 1993). Malven et al. (1995) reported that intraventricular infusion of antibodies to NPY to ovariectomized estradiol treated ewes hastened the onset of the LH surge, however others report that central administration of antibodies to NPY to intact ewes delayed or abolished the LH surge (Advis et al., 1993; Porter et al., 1993).

Ovarian steroids alter NPY effects through modulation of receptors. Treating ovariectomized rats with estradiol and(or) progesterone increased expression of NPY receptors in the hypothalamus (Xu et al., 2000), and treating rats with a progesterone antagonist blocked NPY receptor expression and GnRH release from hypothalamic explants in culture (Xu et al., 2000). These results suggest the effect of NPY on gonadotropin secretion is dependent on the steroid milieu.

NPY and hypothalamic function. Neuropeptide-Y may augment LH secretion by acting at the level of the hypothalamus. Concentrations of NPY (Sahu et al., 1989) and mRNA for NPY (Bauer-Dantoin et al., 1992; Sahu et al., 1995) in the hypothalamus are increased prior to the initiation of the preovulatory LH surge in rats. Colocalization of NPY and GnRH neurons of the hypothalamus have been observed (Gray and Morley, 1986; Madhabananda et al., 1990; Tsuruo et al., 1990). In addition, NPY inhibition of LH release in rats can be overcome by treating them with GnRH (McDonald et al, 1989).

Rat anterior pituitary cells cultured with NPY did not demonstrate an increase in LH release (Crowley et al., 1987), and NPY had no influence on GnRH induced LH release from cultured bovine anterior pituitary cells (Chao et al., 1987), indicating that NPY is not altering pituitary responsiveness to GnRH, but rather limiting hypothalamic secretion of GnRH. However *in vitro*, NPY increased GnRH release from rat hypothalamus tissue (Sabatino et al., 1989; Besecke and Levine, 1994; Parent et al., 2000; Xu et al., 2000), and estrogen enhanced the stimulatory effect (Sabatino et al., 1989). Treating median eminence and arcuate nucleus cultures of rat hypothalmi with an NPY antagonist decreased GnRH secretion (Xu et al., 2000). Neuropeptide-Y had an inhibitory effect on frequency and amplitude of GnRH pulses in ovariectomized rabbits, but had a stimulatory effect on GnRH release in intact rabbits (Khorram et al., 1987). In rats, the NPY stimulated secretion of GnRH is greatest during pro-estrus (Besecke and Levine, 1994), and concentrations of NPY and GnRH increase in a parallel fashion just prior to the ovulatory LH surge in ovariectomized estradiol primed rats (Watanobe and Takebe, 1992). A transient increase in LH was observed in NPY treated intact ewes administered intravenous GnRH (McShane et al., 1992). Neuropeptide-Y containing neurons in the arcuate nucleus of rats have been shown to sequester estradiol (Madhabananda et al., 1990). These data suggest that the pituitary remains responsive to GnRH, but that NPY alters GnRH release. Ovarian steroids may influence the effect of NPY in the hypothalamus.

The exact role that NPY plays in controlling gonadotropin secretion needs further investigation. *In vivo*, many experiments indicate that NPY is inhibitory to gonadotropin secretion. However, many of the studies use ovariectomized animals. Data indicate that

NPY is stimulatory to gonadotropin secretion in rodents and sheep when ovarian steroids are present. The effects that NPY has on feeding behavior may be mediated through different brain centers and may be independent of GnRH secretion.

Leptin

The obese gene product leptin, first identified in mice, is a 17 kDa protein produced and secreted from adipocytes (Zhang et al., 1994). Leptin appears to be a messenger which relays information on body composition and(or) nutritional status from the periphery to the central nervous system. Intraventricular infusion of recombinant leptin decreased feed intake and decreases body weight loss in mice (Campfield et al., 1995; Weigle et al., 1995). Leptin mRNA in fat and plasma leptin are increased with obesity, but decreased with nutrient restriction (Maffei et al., 1995; Tsuchiya et al., 1998; Amstalden et al., 2000).

Leptin and reproduction. Leptin has been implicated as a regulator of reproduction. Exogenous leptin decreased body weight, but hastened the onset of puberty in mice (Ahima et al., 1997; Chehab et al., 1997) and rats (Cheung et al., 1997). In humans, plasma leptin increases with puberty (Blum et al., 1997). Serum concentrations of leptin change with stage of the cycle in women (Teirmaa et al., 1998) and are decreased after menopause (Rosenbaum et al., 1996). The leptin receptor is expressed in the mouse testis (Caprio et al., 1999; El-Hefnawy et al., 2000) and leptin treatment decreased testosterone production by mouse leydig cells *in vitro* (Caprio et all, 1999).

Leptin may be an important signal to the central nervous system in regulation of reproduction. Mice with a deficiency of leptin lack adequate concentrations of LH and are infertile. However, when these mice are administered recombinant leptin,

concentrations of LH are increased and they become fertile again (Mounzih et al., 1997; Yu et al., 1997). In addition, when leptin deficient mice were treated with GnRH, concentrations of LH were increased and fertility was restored. These results indicate that the pituitary of leptin deficient mice is sensitive to GnRH, and that leptin is acting in the area of the hypothalamus to stimulate the production and(or) secretion of GnRH (Yu et al., 1997; Barb et al., 1999).

Receptors for leptin have been isolated in the hypothalamus and pituitary (Dyer et al., 1997; Hakansson et al., 1998), and have been found on NPY neurons in the hypothalamus (Mercer et al., 1996; Finn et al., 1998). In addition, intraventricular administration of leptin decreases NPY in these tissues (Stephens et al., 1995; Schwartz et al., 1996; Xu et al., 1998; Aahima et al., 1999). Since NPY may inhibit GnRH secretion, and central administration of leptin decreases NPY and restores LH secretion, leptin may relay information to the brain about the adequacy of body energy stores to initiate reproduction. Beef cows that were cyclic by 19 wks postpartum had two fold greater plasma concentrations of leptin than cows that were still anestrous (Ciccioli et al, 2001).

Leptin and ovarian function. The role of leptin in directly influencing ovarian function is unclear. The message for leptin receptor has been found in rat and human ovaries (Cioffi et al., 1997; Zamorano et al., 1997). Leptin receptor mRNA has been found in human granulosa and theca cells (Karlsson et al., 1997; Agarwal et al., 1999) as well as the corpora lutea of pigs (Ruiz-Cortez et al., 2000). Leptin also binds to bovine granulosa and theca cells (Spicer et al., 1997; 1998). Infertility of ob/ob mice is associated with decreased serum LH and reduced ovarian weights. Treating ob/ob mice

with leptin results in greater ovarian weights and increased numbers of primary and graafian follicles (Barash et al., 1996). However, treating FSH primed rats with exogenous leptin decreased ovulation rate by 67% (Duggal et al., 2000), and treating perifused rat ovaries with leptin decreased ovulation rate by 77% (Duggal et al., 2000). Kitawaki et al. (1999) determined that leptin increased aromatase activity in cultured luteinized human granulosa cells, however others report that leptin inhibited LH stimulated steroidogenesis in these cells (Karlsson et al, 1997; Agarwal et al., 1999). Treating luteinized human granulosa cells with leptin also inhibited hCG stimulated progesterone production (Brannian et al., 1999). Leptin inhibited gonadotropin stimulated steroid production by bovine granulosa (Spicer and Francisco, 1997; Spicer et al., 2000) and thecal cells (Spicer and Francisco, 1998) as well as rat granulosa cells (Barkan et al, 1999). Glucocorticoid-induced progesterone production in rat granulosa cells was also attenuated by leptin (Barkan et al., 1999). The IGF-I stimulated steroidogenesis of human granulosa and thecal cells was inhibited by leptin (Agarwal et al., 1999), as well as rat granulosa cells (Zachow and Magoffin, 1997). Leptin also attenuated the IGF-I stimulated androstenedione production by bovine thecal cells and progesterone but not estradiol production by bovine granulosa cells (Spicer et al., 2000). These results suggest that leptin is inhibitory to ovarian steroidogenesis in many species, however whether leptin inhibits ovarian function in vivo is unknown.

Leptin and pituitary function. Leptin may have a direct role in modulating gonadotropin secretion from the pituitary. The ob/ob mice do not produce leptin, have decreased concentrations of LH, and are infertile (Yu et al., 1997). Female ob/ob mice treated with leptin had greater serum LH (Barash et al., 1996; Yu et al., 1997) and males

had greater serum FSH (Barash et al., 1996). Leptin receptor mRNA has been found in the pituitaries of mice (Jin et al., 2000), rats (Zamorano et al., 1996; Jin et al., 2000), and monkeys (Finn et al., 2000). Leptin receptors have also been observed in the pituitaries of sheep (Dyer et al., 1997; Daniel et al., 2000) and are localized in corticotropes, somatotropes, and gonadotropes (Iqbal et al., 2000). Intraventricular infusion of leptin to ovariectomized estradiol-primed rats increased plasma LH and amplitude of LH pulses , but plasma FSH and amplitude of FSH pulses were decreased (Yu et al., 1997; Walczewska et al., 1999). Frequency of LH pulses was reduced in rats treated with leptin antiserum (Carro et al., 1997). In vitro, leptin caused the release of LH and FSH from pituitary cultures of rats (Yu et al., 1997) and pigs (Barb, 1999), but inhibited GnRH induced LH release from bovine pituitary cultures (Ridgway, 2001). Thus leptin stimulates secretion of LH from the pituitary.

Leptin and hypothalamic function. Leptin may also have a direct effect on the hypothalamus. There are receptors for leptin in the hypothalamus of mice (Magni et al., 1999), sheep (Dyer et al., 1997), rats (Matsuda et al., 1999; Garcia et al., 2000), and humans (Couce et al., 1997). In monkeys, GnRH containing neurons did not have leptin receptors, but leptin receptors were found on NPY containing neurons which can influence GnRH secretion (Finn et al., 1998). In contrast, immortalized GnRH-secreting cell lines have leptin receptors, and treatment with leptin caused these cells to secret GnRH (Magni et al., 1999). Leptin also caused the release of GnRH from hypothalamic cultures from rats (Yu et al., 1997; Parent et al., 2000), and hypothalamic explants from rats had decreased interpulse intervals of GnRH when treated with leptin (Lebrethon et

al., 2000). However, high doses of leptin (10^{-6} or greater) suppressed GnRH secretion (Yu et al., 1997; Magni et al., 1999; Lebrethon et al., 2000).

Nutritional regulation of leptin. Insulin concentrations are increased in NPY infused rats (Rohner-Jeanrenaud et al., 1996). Insulin increases lipogenesis resulting in increased body fat reserves. Insulin treatment of rats decreased NPY expression in the hypothalamus (Schwartz et al., 1992) and increased leptin gene expression in adipose tissue (MacDougald et al., 1995). Feed restriction for 3 weeks increased mRNA expression of the functional leptin receptor in the ventromedial and arcuate nuclei of the hypothalamus of sheep (Dyer et al., 1997), possibly meaning that concentrations of leptin were decreased allowing upregulation of its receptors. Intraventricular infusion of insulin or insulin plus glucose to feed restricted sheep increased LH in serum and decreased expression of leptin receptors in the hypothalamus (Daniel et al., 2000). In bovine adipose explant cultures, insulin increased leptin mRNA expression (Houseknecht et al., 2000). The addition of GH abolished the stimulatory effects of insulin. Growth hormone treatment increased leptin mRNA in subcutaneous adipose tissue of steers, but only if animals were in a positive energy balance and had adequate IGF-I (Houseknecht et al., 2000). In nutrient restricted ruminants, GH is increased but IGF-I is decreased, suggesting that leptin mRNA would be decreased.

Withholding feed from mature beef cows (Tsuchiya et al., 1998) or prepubertal heifers (Amstalden et al., 2000) for 48 h decreased expression of leptin mRNA in subcutaneous adipose tissue. Leptin mRNA began to increase after 3 h of re-feeding, but not to pre-fasted levels (Tsuchiya et al., 1998). When primiparous beef cows were changed from high to moderate nutrition, plasma concentrations of leptin decreased by

65% within 7 d (Ciccioli et al., 2001). Rapid changes in both leptin and NEFA demonstrate that fat cells respond quickly to acute changes in nutrient availability.

The role of leptin in the regulation of nutritional effects on reproduction. It is clear that leptin has a role in regulation of nutritional augmentation of gonadotropin secretion. In monkeys, a 2 d fast eliminated pulsatile secretion of LH, but treating fasted monkeys with leptin increased plasma concentrations of LH and FSH, as well as frequency and amplitude of LH pulses, to pre-fasted levels (Finn et al., 1998). Intraperitoneal injection of leptin prevented the decrease in LH pulse frequency induced by fasting ovariectomized rats for 48 h (Nagatani et al., 1998). In ovariectomized ewes, central administration of leptin for 3 d decreased feed intake and NPY mRNA in the hypothalamus, but plasma LH and FSH were not changed (Henry et al., 1999). In estradiol-primed wethers, a 78 h fast decreased plasma concentrations leptin and LH as well as frequency of LH pulses (Nagatani et al., 2000). Subcutaneous injections of leptin every 8 h during the 78 h fast prevented the decrease in the concentration of LH in plasma and frequency of LH pulses (Nagatani et al., 2000). However, intraventricular infusion of leptin to feed restricted ovariectomized ewes did not increase serum LH or frequency of LH pulses (Morrison et al., 2001). Whether leptin maintains gonadotropin secretion by acting directly on the pituitary on indirectly via the hypothalamus is still unclear. However, ob/ob mice exhibited normal LH secretion when treated with exogenous GnRH (Yu et al., 1997), indicating that the major effect of leptin regulation of LH secretion is at the hypothalamus.

Leptin may be an important regulatory hormone that has a permissive role in reproduction. However, many *in vitro* studies indicate that leptin may have potential

negative effects on reproduction, especially on ovarian steroidogenesis. Negative effects of leptin may be due to the use of pharmacological rather physiological doses and the down regulation of leptin receptors. In addition, a soluble leptin binding protein has been discovered (Tartaglia, 1997), and its absence from cell culture systems may alter the effects of leptin *in vivo*. To date, most studies would indicate that leptin is permissive for reproduction.

Conclusions

Nutrition and body energy stores can modulate the timing of reproductive events. Chronic nutritional restriction causes decreased gonadotropin secretion and quiescent ovaries. Acute nutritional restriction of beef heifers can also cause anovulation, but the mechanism is not known. Nutrient intake and body energy reserves affects many metabolic hormones and neuroendocrine factors that regulate hypothalamic, pituitary, and ovarian function. A more complete understanding of these interactions will allow development of management strategies to improve reproductive efficiency of beef cattle.

CHAPTER III

EFFECTS OF BODY CONDITION SCORE AT PARTURITION AND POSTPARTUM PROTEIN SUPPLEMENTATION ON ESTROUS BEHAVIOR AND SIZE OF THE DOMINANT FOLLICLE IN BEEF COWS

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ABSTRACT: Multiparous Angus x Hereford cows (n = 45) were fed to calve with a body condition score (BCS; 1 = emaciated, 9 = obese) of thin (< 5) or moderate (≥ 5). Cows were blocked by BCS and calving date (March 22.0 ± 3.2 d) and randomly assigned to receive either low (1.5 kg/d) or high (2.9 kg/d) amounts of a 42% CP supplement. All cows grazed the same native grass pasture with native grass hay free choice, and were fed supplement in individual stalls for 49.2 ± 2.3 d. Beginning 20 d postpartum, blood samples were collected from each cow three times weekly, and estrous behavior was monitored continuously with the HeatWatch® system. Onset of estrus was defined as the first of two mounts within 4 h. Size of the dominant follicle at 4 to 14 h after the onset of estrus was determined by ultrasonography. Duration of luteal phase

before and after estrus was characterized as short (plasma progesterone ≥ 0.5 ng/mL for ≤ 10 d) or normal (plasma progesterone ≥ 0.5 ng/mL for ≥ 11 d). Body condition score of thin cows was less ($P < 0.01$) at calving than moderate cows (4.3 ± 0.1 vs 5.0 ± 0.1, respectively). Cows on high and low nutrition had similar BCS at the end of treatment (4.5 ± 0.1). Weight gains during treatment were not different for cows on high and low nutrition (22.3 ± 3 vs 16.5 ± 3 kg, respectively; $P < 0.13$). Prior to the first estrus, a short luteal phase occurred in 65% of cows, a normal luteal phase occurred in 27% of cows, and luteal activity was not detected in 8% of cows. Duration and number of mounts at the first estrus were not influenced by BCS at calving or postpartum nutrition. Size of the dominant follicle was greater at first estrus ($P < 0.01$) for moderate BCS cows than thin cows (15.3 ± 0.5 vs 13.4 ± 0.4 mm, respectively), and for high vs low postpartum nutrition (15.0 ± 0.4 vs 13.7 ± 0.5 mm, respectively). Concentrations of estradiol in plasma at estrus were not influenced by BCS or nutrition. In conclusion, postpartum nutrient intake and BCS at calving influence the size of the dominant follicle at the first estrus in multiparous beef cows.

Key Words: Nutrition, Estrus, Follicle, Postpartum, Beef Cattle, Body Condition

Introduction

Nutrient intake and body energy reserves are major regulators of reproductive performance of beef cows (Wettemann, 1980; Dunn and Kaltenbach, 1980; Randel, 1990). Increased nutrient intake before and after calving decreases the interval to first estrus and increases pregnancy rates (Wiltbank et al., 1964; Richards et al., 1986; Selk et al., 1988). Cows calving in thing body condition score (BCS = 3 to 4) have longer

intervals to first estrus and decreased pregnancy rates (Wiltbank et al., 1962; Wright et al., 1987; Selk et al., 1988). Prolonged nutritional deprivation of cattle results in loss of body weight and body condition score, decreased growth rate and size of the dominant follicle, and causes anovulation (Rhodes et al., 1995; Bossis et al., 1999).

Most fall calving and early spring calving cows graze low quality forage (Johnson et al., 1998; Lents et al., 2000). Supplemental protein is required to maintain body weight and body condition score (Lusby and Wagner, 1986; McCollum and Horn, 1990), and reduce the interval from calving to first estrus (Rakestraw et al, 1986; Marston et al., 1995). When cows were fed protein deficient diets, they had longer intervals from calving to estrus, decreased conception rates (Sasser et al., 1988), and decreased GnRH-induced LH release (Nolan et al., 1988). Therefore, the objective of this study was to determine the effects of BCS at calving and the amount of postpartum protein supplementation on estrous behavior and follicle development of mature beef cows.

Materials and Methods

Spring calving, multiparous Angus x Hereford cows (n = 45; BW = 478 ± 17 kg; age = 6.6 ± 0.5 yr) were used to determine the effects of body condition score (**BCS**; 1=emaciated and 9=obese; Wagner et al., 1988) at calving and postpartum nutrition on estrous behavior and follicle development. Cows were fed either 0.7 kg/d or 1.4 kg/d of a 42% CP supplement beginning 115 d prepartum so that cows would calve with a BCS of thin (BCS < 5) or moderate (BCS ≥ 5). Cows grazed native tallgrass prairie at the Range Cow Research Center near Stillwater. Predominant forage species were little bluestem (*Andropogon scoparius*) and big bluestem (*Andropogon gerardii*). Following parturition

(March 22 ± 3.2 d), cows were blocked by BCS and calving date and randomly assigned to receive either low (1.2 kg/d) or high (2.5 kg/d) amounts of a 42% CP supplement. The low supplement was formulated to provide a positive balance for degradable intake protein (DIP) of at least 100 g/d using Level I of the Beef Cattle Nutrient Requirements Model (NRC, 1996). Supplement amounts were prorated for 6 d/wk individual feeding in covered stalls. Cows were fed supplement until 60 d after parturition or until May 15 (supplementation averaged 49.2 ± 2.3 d). After calving, all cows grazed the same native grass pasture with native grass hay free choice. Following a 16 h shrink, cow weights and BCS were determined monthly until weaning.

Beginning 20 d after calving, estrous behavior was monitored continuously with a radiotelemetry system (HeatWatch, DDx Inc., Denver, CO). The onset of estrus was defined as the first of two mounts which occur within 4 h. The end of estrus was defined as the last of two mounts that occur within 4 h, with less than 12 h between any two sets of mounts.

Commencing at 20 d postpartum, blood samples were collected from each cow via coccygeal venipuncture three times weekly (Monday, Wednesday, and Friday). Blood was collected into tubes containing EDTA (0.1 mL of a 15% solution) and placed on ice. Plasma was obtained by centrifugation (2000 x g for 10 min at 4 °C) and stored a –20 °C until analyzed for progesterone and insulin-like growth factor-I (**IGF-I**). Concentrations of progesterone in plasma were quantified by solid phase radioimmunoassay (Coat-A-Count® progesterone kit, Diagnostic Products Corp., Los Angeles, CA; Vizcarra et al., 1997). Duration of the luteal phase before and after estrus was characterized as short (plasma progesterone ≥ 0.5 ng/mL for ≤ 4 consecutive samples; ≤ 10 d) or normal

(plasma progesterone ≥ 0.5 ng/mL for ≥ 5 consecutive samples; ≥ 11 d). Concentrations of IGF-I in plasma were determined by RIA with acid-ethanol extraction (Echternkamp et al., 1990). Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used for standards.

Size of the ovulatory follicle was determined by transrectal ultrasonography (7.5 MHz probe; Aloka 500V, Corometrics Medical Systems, Wallingford, CT) at 4 to 14 h after the onset of the first postpartum estrus. Ultrasonography images were recorded with a VHS recorder (Panasonic PV-V4520; Matsushita Electric Corp. of America, Secuucus, NJ, 07094) and later viewed to measure follicle size. Size of follicles was calculated as the mean of the longest and shortest diameter (Pierson and Ginther, 1988). A blood plasma sample was collected at the time of ultrasonography, and plasma obtained for quantification of estradiol. Concentrations of estradiol-17β in plasma were determined by RIA (Serono Estradiol MAIA assay kit, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). At 14 to 20 h after the onset of estrus, and at least 2 h after ultrasonography, cows were artificially inseminated to a single bull by a trained technician. Pregnancy rate to AI at the first estrus was determined from breeding date and subsequent calving date.

To be included in data analyses, cows had to have exhibit first estrus followed by ovulation. Five cows failed to exhibit estrus or luteal activity during the experiment. Three cows exhibited estrus but had no subsequent luteal activity, and were not included in data analyses. Complete estrus data were not available due to an electrical power outage on two separate days for 3 cows and their data were not included in statistical analyses because of uncertainty of when their estrus began. Thus data from 34 cows

were used in statistical analyses. Least squares analyses of variance were used to determine the effects of nutritional treatment on change in BW and BCS. The model included nutritional treatment, with calving date as a block and cow age as a covariant. Least squares analyses of variance for a randomized complete block design in a 2 x 2 factorial arrangement were used to determine the effects of BCS at calving, postpartum protein supplementation, and their interaction on duration of estrus, number of mounts received, and size of the ovulatory follicle. Cow age was included as a covariant. Because cows were assigned to nutritional treatments at calving and supplementation was terminated for all cows on the same day, calving date was included in the model as a block to adjust for variation associated with days on feed. The effects of protein supplementation and BCS on concentrations of estradiol in plasma at estrus were determined by least squares analysis of variance. The model included BCS, protein supplementation, and their interaction. Calving date was included as a block with cow age and the number of hours after the initiation of estrus a sample was collected included as a covariants. Means were compared with Fishers-LSD when a significant ($P < 0.05$) F-test was observed. The effects of BCS and supplementation on concentration of IGF-I before and after treatment, as well as before estrus, were determined by least squares analyses of variance using a mixed model. The model included assay, BCS, supplementation, and week with calving date included as a block effect. If interactions with week were significant, polynomial response curves of appropriate order were fit and tested for heterogeneity of regression (Snedecor and Cochran, 1968) to evaluate BCS and supplementation effects. Chi square analysis was used to determine the effects of postpartum nutrition and BCS on percent of cows exhibiting estrus and pregnancy rate.

Results

Body condition score at calving of thin cows was less ($P < 0.01$) than that for moderate cows (4.3 ± 0.1 vs 5.0 ± 0.1, respectively). Cows on high and low nutrition had similar ($P = 0.80$) BCS at the end of treatment (4.5 ± 0.1; Table 1). Total weight gain during the treatment period was not different ($P < 0.13$) for cows fed high and low amounts of protein (Table 1).

The interactions between BCS and postpartum supplementation on the percentage of cows exhibiting estrus ($P = 0.38$), interval to the first postpartum estrus ($P = 0.84$), the duration of estrus ($P = 0.40$), and the number of mounts at estrus ($P = 0.09$) were not significant. Treatment ($P = 0.11$) and BCS ($P = 0.32$) did not effect the percentage of cows exhibiting estrus (Table 2). The interval from calving to the first postpartum estrus was shorter ($P < 0.01$) for cows calving in moderate BCS compared with cows calving in thin BCS (Table 2). The amount of protein supplementation did not alter ($P = 0.76$) the interval to first estrus. Duration of the first postpartum estrus and the number of mounts received at the first estrus were not influenced by BCS at calving ($P = 0.50$) or protein supplementation ($P = 0.74$; Table 2).

Protein supplementation and BCS did not influence the duration of the luteal phase before or after the first postpartum estrus. Prior to the first postpartum estrus, 65% of cows had a short luteal phase (≤ 10 d). A normal luteal phase (≥ 11 d) occurred in 27% of cows before the first estrus, and luteal activity was not detected in 8% of cows prior to the first estrus after calving. After the first postpartum estrus, 98% of cows had a normal luteal phase.

The interaction between BCS at calving and protein supplementation on size of the ovulatory follicle was not significant ($P = 0.19$). Cows with moderate BCS at calving had larger ($P < 0.01$) ovulatory follicles than cows calving with thin BCS (Table 3). Size of the ovulatory follicle also tended to be greater ($P < 0.07$) for cows fed high protein supplement compared with low cows (Table 3). Neither BCS ($P = 0.82$), or protein supplementation ($P = 0.58$) affected plasma concentrations of estradiol at the first postpartum estrus (Table 3). The pregnancy rate to AI at the first estrus was not affected by protein supplementation ($P = 0.38$) but was increase for cows with moderate BCS at calving ($P < 0.05$; Table 3).

The amount of protein supplement fed after calving did not influence ($P = 0.12$) plasma concentrations of IGF-I three weeks before or three weeks after the end of supplementation. However, there was a tendency ($P < 0.08$) for cows with moderate BCS at calving to have greater plasma IGF-I during this six week period than thin cows (Figure 1). Body condition score at calving ($P = 0.30$) and protein supplementation ($P = 0.39$) did not influence plasma concentrations of IGF-I during the four weeks before estrus. Plasma IGF-I increased ($P < 0.03$) 47% during the 3 wk before the first postpartum estrus (Figure 2).

Discussion

Cows that calved in moderate BCS returned to estrus 30 d sooner after calving than cows calving in thin BCS. However, protein supplementation did not affect the interval to first estrus. The interval to first estrus is decreased as BCS at calving increases (Wright et al., 1987; Richards et al., 1986; DeRouen et al., 1994). Feeding increased

amounts of energy before parturition (Wiltbank et al., 1962; Perry et al., 1991), or protein after parturition (Rakestraw et al., 1986; Sasser et al., 1988) can decrease weight loss and reduce the interval from calving to first estrus. The postpartum interval to estrus is reduced when cows maintain BW after calving (Rakestraw et al., 1986; Selk et al., 1988). In this study, cows fed low nutrition maintained BW during the treatment period.

The duration of estrus averaged 5.7 ± 0.8 h and the number of mounts received average 11.8 ± 2.1. We found that the duration of the first postpartum estrus of primiparous cows averaged 3.9 h with 6.8 mounts (Ciccioli and Wettemann, 2000b). We have previously reported that duration of estrus for non-lactating cows exhibiting normal estrous cycles ranged from 13.9 h to 16.2 h and the number of mounts ranged from 30 to 57 (Floyd et al., 2001; White et al., 2002). Breed, cow age, environment, management, and lactation could influence estrous behavior. Duration of estrus was 14 h and the number of mounts received was 50.1 for crossbred (Angus x Hereford x Brahman) heifers at 12 to 13 mo of age(Stevenson et al., 1996). Mathew et al. (1999) found that cows 5 yr and older were mounted more times than younger cows (≤ 4 yr). Primiparous dairy cows were estrus for almost 50% less time than multiparous cows (7.4 and 13.6 h respectively; Walker et al., 1996). Heat stress reduces the number of mounts per estrus in dairy cows (Gangwar et al., 1965; Pennington et al., 1985). Mature beef cows were estrus longer in summer (17.6 h) than in winter or spring (15.5 h and 13.9 h, respectively), and received more mounts in winter (59) than in summer or spring (43.6 and 38.2, respectively; White et al., 2002). Duration of estrus was shorter and number of mounts received were greater for Brahman heifers compared with Angus or Angus crossbred heifers (Rae et al., 1999). Duration of estrus and the number of mounts

received are increased when more cows are estrus (Hurnik et al., 1975; Floyd et al., 2001). Duration of estrus increased from 11.6 h to 15.4 h and number of mounts received increased from 11 to 27.7 when two or more cows were in estrus at one time compared with only one cow in estrus (Floyd et al., 2001).

Luteal activity was detected in 92% of cows prior to the first postpartum estrus. Protein supplementation and BCS did not effect the percentage of cows with a short or normal luteal phase before estrus. Sixty-five percent of cows had increased plasma concentrations of progesterone ($\geq$ 0.5 ng/mL) for less than 10 d before estrus. Transient increases in plasma concentrations of progesterone occur in beef cattle prior to the first pubertal (Gonzalez-Padilla et al., 1975; Berardinelli et al., 1979) and first postpartum estrus (Rawlings et al., 1980; Humphery et al., 1983; Werth et al., 1996). Previously, we determined that 78% of mature beef cows (Looper, 1999) and 87% of primiparous heifers (Ciccioli and Wettemann, 2000a) had a short luteal phase before the first postpartum estrus. Progesterone during a short luteal phase appears to come from luteal tissue (Berardinelli et al., 1979; Corah et al, 1974; Perry et al., 1991b) associated with ovulation (Odde et al., 1980; Bossis et al., 2000). Concentrations of estradiol in plasma and follicular fluid were less in postpartum beef cattle that formed a short-lived CL compared to those that formed a CL of normal duration (Garverick et al., 1988; Inskeep et al., 1988; Braden et al., 1989). Reduced concentrations of estradiol do not adequately stimulate progesterone receptor synthesis in the uterus (Zollers et al., 1989), which are necessary to inhibit oxytocin from causing the release of PGF2α (Vallet et al., 1990). The lack of behavioral estrous at the first ovulation in beef cows may be attributable to decreased

estrogen levels (Braden et al., 1989). In the current study, 98% of cows had a normal luteal phase after the first postpartum estrus.

Cows that calved in moderate BCS or fed high amounts of protein after calving had larger ovulatory follicles. We previously reported that primiparous heifers fed high amounts of energy after parturition had larger ovulatory follicles at the first estrus than heifers fed moderate amounts of energy (Ciccioli et al., 2001). This may be reason cows with increased nutrient intake and(or) greater BCS have increased conception rates (Dunn and Kaltenbach, 1980; Short et al., 1990; Randel, 1990). Grimard et al. (1995) found that the size of the largest follicle was smaller for suckled beef cows fed low amounts of energy after calving compared with cows fed greater amounts of energy after calving. However, in that study BCS at the time of measurement was confounded with nutrition. Additionally, follicles were measured at predetermined times before ovulation, where as follicles in the current study were measured at estrus and were destined to ovulate. Maximum diameter of dominant follicles was smaller for cyclic heifers when nutrient intake was restricted compared with heifers fed to maintain or gain BW (Murphy et al., 1991).

Nutrition and BCS may regulate ovarian function through mechanisms at the level of the hypothalamus. Nutritional (Richards et al., 1989; Rhodes et al., 1995; Bossis et al., 1999) and postpartum anestrous cows (Wettemann, 1980; Short et al., 1990) lack sufficient LH secretion to develop ovulatory follicles. Treating nutritionally induced (Bishop and Wettemann, 1993; Vizcarra et al., 1997) or anestrous cows postpartum (Echternkamp, 1978; Wettemann et al., 1982; Wright et al., 1990) with exogenous GnRH increases LH secretion and initiates ovarian function. Body condition score of cows is

positively correlated with concentrations of LH in plasma beginning 14 d after calving (Perry et al., 1991a; Rutter and Randel, 1984). Undernutrition decreases BCS and secretion of LH in cows (Perry et al., 1991a; Richards et al., 1989a), and results in fewer large (≥ 10 mm) follicles (Perry et al., 1991; Grimard et al., 1995). Nutritional restriction of heifers (Rhodes et al., 1995; 1996; Bossis et al., 1999) and cows (Imakawa et al., 1987; Richards et al., 1989) decreases plasma LH and reduces follicle size (Murphy et al., 1991; Bergfeld et al., 1994; Stagg et al., 1995). Cows that calved in moderate BCS or fed greater amounts of protein after calving may have greater LH secretion. Greater plasma concentrations of LH could support increased follicular development before the first estrus and may contribute to increased pregnancy rates for cows with greater BCS (Selk et al., 1988).

Protein supplementation and BCS influenced size of the ovulatory follicle at the first postpartum estrus, but not concentrations of estradiol in plasma. Nutritional restriction of beef heifers reduced size of the dominant follicle by 34% before concentrations of estradiol in plasma were decreased (Bossis et al., 1999). The lack of nutritional or BCS effects on concentrations of estradiol may be due to the fact that plasma samples were not collected at the same time after the start of estrus. Estradiol concentrations in blood are maximal shortly before or at the initiation of estrus (Henricks et al., 1971; Shemesh et al., 1972; Wettemann et al., 1972), and then begin to decrease after the onset of estrus (Henricks et al., 1971; Chenault et al., 1975; Glencross et al., 1981). Previously we determined concentrations of estradiol in plasma of beef cows sampled every 4 hr after the start of estrus (White et al., 2002). In that experiment, concentrations of estradiol in plasma did not begin to decline until after 8 h after the onset of estrus, and were not

significantly different until 16 h after the onset of estrus. In the current study, the number of h after the onset of estrus that plasma samples were collected averaged 10.1 ± 0.9 h, and were similar to peak concentrations of estradiol we have previously reported (White et al., 2002). Additionally, time after estrus that a plasma sample was collected was included as a covariant in statistical analyses.

The mechanism(s) by which BCS or nutritional intake regulates follicular development is unclear. Local as well as systemic factors such as IGF-I control ovarian follicular development (Spicer and Echternkamp, 1995; Roche, 1996; Adashi, 1998). Insulin-like growth factor-I has a proliferative effect on granulosa cells (Baranao and Hammond, 1984; Spicer et al., 1993), enhances steroidogenesis of granulosa (Spicer et al., 1993; 1998; Gong et al., 1994) and theca (Stewart et al., 1995) cells. Larger ovulatory follicles of cows fed greater amounts of protein or that calved in moderate BCS might be associated with increased concentrations of IGF-I in plasma and(or) follicular fluid. When nutrition is restricted, BCS (Richards et al., 1989; Rasby et al., 1991) and concentrations of IGF-I in plasma (Granger et al., 1989; Rutter et al., 1989; Richards et al., 1995) are reduced. In the current study, protein supplementation did not alter plasma concentrations of IGF-I during or after the feeding period. Steers fed concentrate diets had greater plasma concentrations of IGF-I when the amount of protein in the diet was increased (Elsasser et al., 1989). Spicer et al. (1991) reported that plasma concentrations of IGF-I were not influenced by level of intake when protein was adequate. Concentrations of IGF-I in plasma were not different when DMI of steers fed a hay based diet was increased above 1.8% of BW (Breier et al., 1986). In this experiment, total protein and energy intake for each treatment may have been sufficient to support

adequate concentrations of IGF-I. However, cows that calved with moderate BCS tended to have greater concentrations of IGF-I in plasma before and after the feeding period. It is possible that cows with moderate BCS had sufficient body energy reserves to maintain increased concentrations of IGF-I which may have stimulated the development of larger ovulatory follicles and reduced the interval to estrus. When nutritionally anovulatory heifers were realimented, plasma concentrations of IGF-I and follicle size were increased prior to the resumption of ovulation (Bossis et al., 2000).

Reduced concentrations of IGF-I in plasma are associated with longer intervals to ovulation and estrus (Rutter et al., 1989; Nugent et al., 1993; Roberts et al., 1997). Although not influenced by nutrition or BCS, concentrations of IGF-I in plasma increased prior to estrus in the current study. Previously, we determined that plasma concentrations of IGF-I were increased during the follicular wave before resumption of ovulation in nutritionally induced anovulatory heifers undergoing realimentation (Bossis et al., 2000). Cows that ovulate have greater concentrations of IGF-I in blood than anovulatory cows (Beam and Butler, 1997). Insulin-like growth factor-I increases LH receptors and enhances LH-induced steroidogenesis of bovine follicular cells in vitro (Spicer and Echternkamp, 1995; Spicer and Stewart, 1996; Stewart et al., 1996). Increasing plasma concentrations of IGF-I in cows prior to estrus may be important for increasing follicular responsiveness to gonadotropins and initiating normal estrous cycles.

A greater percentage of cows calving in moderate BCS became pregnant after AI at the first estrus. Body condition score at calving is the most important factor influencing pregnancy rate (Wright et al., 1987; Selk et al., 1988). Nutrition did not alter the

percentage of cows that became pregnant after AI at the first estrus. When cows calve in thin BCS and are fed inadequate nutrition, fewer cows exhibit estrus (Richards et al., 1986; DeRouen et al., 1994; Spitzer et al., 1995) and pregnancy rates are decreased (Wiltbank et al., 1962; Vizcarra et al., 1998). We previously reported that conception rates of primiparous heifers fed high amounts of energy after calving were 12.3% greater and ovulatory follicles were 10% larger than heifers fed moderate amounts of energy (Ciccioli et al., 2001).

Implications

Intensity of behavior at the first estrus after calving may be limited due to few cows being estrus. Estrous detection aids should be used to help identify estrus cows. Nutritional intake and BCS can directly influence development of the ovulatory follicle at the first postpartum estrus. Reduced conception rate of thin cows may be due to reduced size of the ovulatory follicle. Cows should be managed to calve in moderate BCS and be managed to maintain BW after parturition to decrease the interval to first estrus, increase follicular development, and maximize fertility.

Table 1. Effects of body condition score (BCS) at calving and postpartum protein supplementation on initial and final BW and BCS, and change in BW and BCS during treatment.

	Low[a]		High[a]			P-Value[c]	
Item	Thin[b]	Moderate[b]	Thin[b]	Moderate[b]	SEM	BCS	Supplementation
Initial BW, kg	476.6	496.5	459.2	487.0	15.5	NS	NS
Final BW, kg	492.6	513.6	483.1	507.7	14.7	NS	NS
BW change, kg	15.9	17.0	23.9	20.7	4.7	NS	NS
Initial BCS	4.4	5.0	4.2	5.0	0.05	0.0001	NS
Final BCS	4.3	4.7	4.4	4.6	0.08	0.01	NS
Change in BCS	-0.1	-0.3	0.2	-0.4	0.1	0.01	NS

[a]Low = 1.2 kg/d and high = 2.5 kg/d of a 42% CP supplement.

[b]Thin, BCS < 5; Moderate, BCS ≥ 5.

[c]Nutrition x BCS, $P > 0.1$.

Table 2. Effects of body condition score (BCS) at calving and postpartum protein supplementation on the interval to first estrus after calving, percentage of cows exhibiting estrus, and duration of estrus and number of mounts received at first postpartum estrus.

	Low[a]		High[a]			P-Value[c]	
Item	Thin[b]	Moderate[b]	Thin[b]	Moderate[b]	SEM	BCS	Supplementation
First estrus, d	95.7	63.7	89.9	62.7	9.4	0.01	NS
Percent with estrus	77	86	92	100	-	NS	NS
Duration of estrus, h	5.0	6.8	6.6	5.1	1.9	NS	NS
Mounts, no.	6.7	15.8	11.4	10.8	3.6	NS	NS

[a]Low = 1.2 kg/d and high = 2.5 kg/d of a 42% CP supplement.

[b]Thin, BCS < 5; Moderate, BCS ≥ 5.

[c]Nutrition x BCS, $P > 0.1$.

Table 3. Effects of body condition score (BCS) at calving and postpartum protein supplementation on size of the ovulatory follicle, plasma concentrations of estradiol at the first postpartum estrus, and percentage of cows that became pregnant to artificial insemination

	Low[a]		High[a]			P-Value[c]	
Item	Thin[b]	Moderate[b]	Thin[b]	Moderate[b]	SEM	BCS	Supplementation
Cows, no	9	6	9	10			
Follicle size, mm	13.2	14.2	13.6	16.3	0.6	0.01	0.07
Estradiol, pg/mL	2.7	2.9	2.7	2.2	0.7	NS	NS
Pregnancy rate, %	44	83	67	90	-	0.05	NS

[a]Low = 1.2 kg/d and high = 2.5 kg/d of a 42% CP supplement.

[b]Thin, BCS < 5; Moderate, BCS ≥ 5.

[c]Nutrition x BCS, $P > 0.1$.

Figure 1. Least squares means for concentrations of IGF-I in plasma during 3 wk before and 3 wk after treatment. There was a BCS effect ($P < 0.08$).

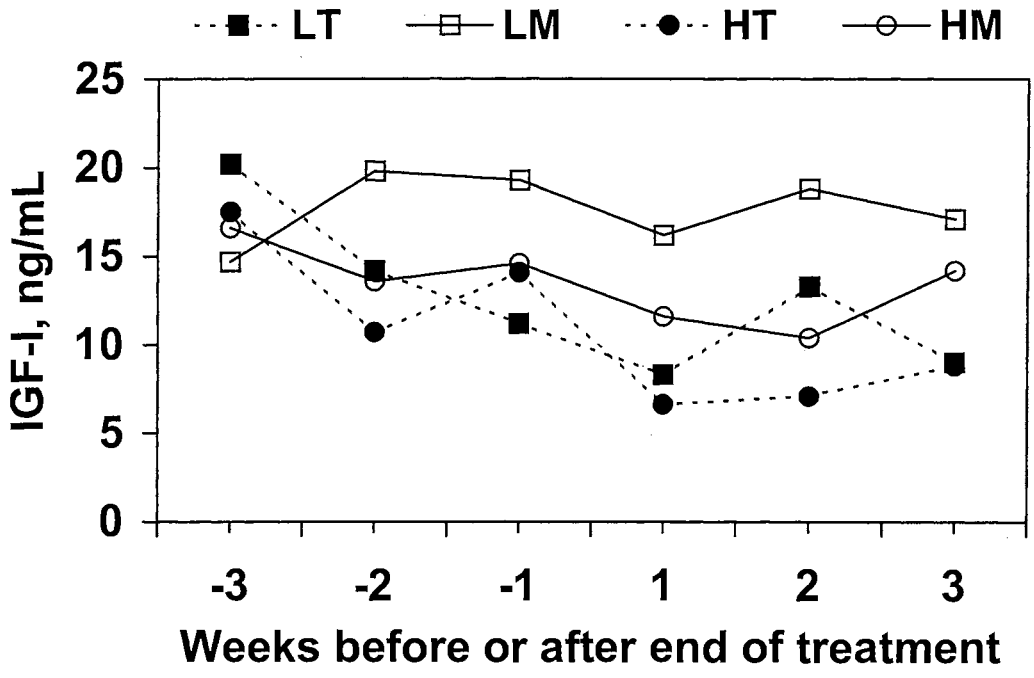
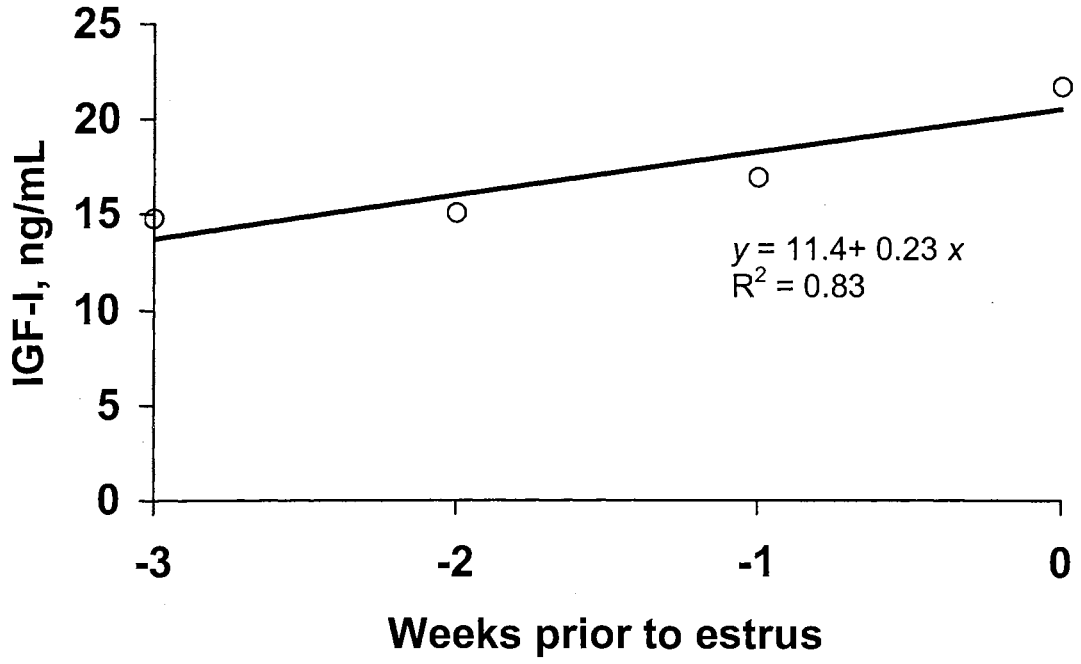

LT
LM
HT
HM
25
20
15
10
5
0
IGF-I, ng/mL
-3
-2
-1
1
2
3
Weeks before or after end of treatment

Figure 2. Least squares means (symbols) and least squares regressions (line) for plasma concentrations of insulin-like growth factor-I (IGF-I) prior to estrus. Concentrations of IGF-I increased ($P < 0.03$) linearly from three weeks (-3) prior to estrus through the week of estrus (0).


25
20
15
10
5
0
IGF-I, ng/mL
-3
-2
-1
0
Weeks prior to estrus
y = 11.4+ 0.23 x
R2 = 0.83

CHAPTER IV

EFFECTS OF ACUTE NUTRITIONAL RESTRICTION ON METABOLIC AND ENDOCRINE CHANGES ASSOCIATED WITH ALTERED GONADOTROPIN SECRETION AND ANOVULATION IN BEEF HEIFERS

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ABSTRACT: Angus x Hereford heifers (BCS = 5.5 ± 0.1; 387 ± 7 kg BW) exhibiting normal estrous cycles at 15 mo of age were used to determine the effects of acute nutritional restriction on concentrations of LH in serum, leptin receptor in the brain, and the incidence of anovulation. Heifers were maintained in individual pens and adapted to a 1.2 x maintenance (M) diet for 10 d. On d 0, heifers were randomly assigned to 0.4 M (n = 15) or 1.2 M (n = 8). Heifers were treated with PGF2α on d –10, 0, and 10 of treatment. Daily blood samples were obtained by tail venipuncture and progesterone in plasma was quantified. Jugular catheters were inserted on d 8. Blood samples were

collected every 10 min for 8 h on d 9, 10 and 11, and LH in serum was quantified. On d 12, 10 heifers (0.4 M, n = 6; 1.2 M, n = 4) were exsanguinated. Hypothalamic and anterior pituitary tissues were removed and frozen. Beginning on d 12, blood samples were collected from the remaining 0.4 M (n = 9) and 1.2 M (n = 4) heifers every 4 h for 48 h to quantify estradiol in plasma and the ovulatory surge of LH. Heifers with plasma progesterone less than 0.5 ng/mL on d 14 to 21 were classified as anovulatory. On d 12, 0.4 M heifers weighed less ($P < 0.01$) than 1.2 M heifers (353 ± 9 kg vs 391 ± 7 kg, respectively), but BCS was not influenced by treatment (5.4 ± 0.1). Concentrations of NEFA were greater ($P < 0.05$) in 0.4 M heifers, but IGF-I was not influenced by treatment ($P < 0.10$). Between d 14 and 21, 44% (4 of 9) of 0.4 M heifers were anovulatory and all 1.2 M heifers ovulated. Concentrations of LH and amplitude of LH pulses in serum on d 9, 10, or 11 were not influenced by treatment, but 0.4 M heifers had fewer ($P < 0.05$) LH pulses on d 9 than 1.2 M heifers. Anovulatory heifers lacked an ovulatory surge of LH and concentrations of estradiol were minimal on days 2, 3, and 4 after PGF2α (1.1 ± 0.1 pg/mL). Amount of leptin receptor mRNA in the median eminence, arcuate nucleus, and anterior pituitary were not influenced by treatment. In conclusion, acute nutritional restriction for 14 d induced anovulation in 44% of heifers , did not influence mean concentrations of LH in serum during follicular growth, or leptin receptor mRNA in the brain, but inhibited the proestrous increase in estradiol and the ovulatory surge of LH.

Key Words: Heifers, Luteinizing hormone, Nutrition, Ovulation, Leptin, Receptor

Introduction

Nutrient intake and body condition are important regulators of postpartum anestrous (Dunn and Kaltenbach, 1980; Rutter and Randel, 1984; Short et al., 1990), which is caused by insufficient secretion of LH (Short et al., 1972; Ingalls et al., 1973). Prolonged nutrient restriction of beef cattle causes loss of body weight and body condition, reduces secretion of LH and decreases follicular growth, and causes cessation of estrous cycles (Richards et al., 1989a; Rhodes et al., 1995; Bossis et al., 1999).

Leptin, a hormone secreted primarily from fat cells, has been implicated as a regulator of reproduction. Exogenous leptin hastened the onset of puberty in rodents (Ahima et al., 1997; Chehab et al., 1997; Cheung et al., 1997), but decreases estradiol production by rat (Duggal et al., 2002) and bovine (Spicer and Francisco et al., 1997). granulosa cells. When ob/ob mice are treated with leptin, concentrations of LH are increased and fertility is restored (Mounzih et al., 1997; Yu et al., 1997). Chronic nutritional deprivation reduces pulsatile secretion of LH in cattle (Rhodes et al., 1996; Bossis et al., 1999) and plasma concentrations of leptin are reduced (Tsuchiya et al., 1998; Amstalden et al., 2000). Insulin-like growth factor-I may be a regulator of reproduction in cattle (Granger et all, 1989; Nugent et al., 1993; Wettemann and Bossis, 2000).

Mechanisms regulating anovulation are difficult to study with many animal models because of the inability to predict when ovulation will cease. Short-term nutritional restriction induces predictable anovulation in beef heifers (Mackey et al., 1999; 2000; White et al., 2000). The objective of this study was to evaluate the effects of acute

nutritional restriction on metabolic and endocrine function associated with anovulation in beef heifers.

Materials and Methods

Animals and Experimental Design

Angus x Hereford heifers exhibiting normal estrous cycles at 15 mo of age and with moderate to good body condition scores (n = 23; **BCS** = 5.5 ± 0.1; 1 = emaciated and 9 = obese, Wagner et al., 1988; BW = 387 ± 7 kg) were used in three replicates during September and October. Heifers were housed at 21 ± 4°C in individual pens and adapted to a diet supplying 1.2 x maintenance (**1.2 M**) energy requirements for 10 d. On d 0, heifers were randomly assigned to diets of **0.4 M** (n = 15) or 1.2 M (n = 8). Net energy required for maintenance was calculated from the NRC (1984). The energy density of the diet (Table 1) was then used to determine the amount of feed for 0.4 M and 1.2 M (2.0 kg/d and 6.4 kg/d, respectively). Approximately twice as many heifers were assigned to 0.4 M as to 1.2 M to produce a sufficient number of anovulatory heifers to evaluate metabolic and endocrine functions. Body weight and BCS were recorded on d –10, 0, and 12. Heifers were treated with PGF2α (25 mg, Lutalyse®; Pharmacia & Upjohn, Kalamazoo, MI) on d –10 and d 0 to synchronize estrus and follicular development. On d 10, heifers were treated with PGF2α to cause luteal regression and ovulation of the dominant follicle that emerged from the first wave after initiation of nutritional restriction (d 0). Heifers were determined to be ovulatory if they had concentrations of progesterone of ≥ 0.5 ng/mL in plasma on d 14 to 21. The experimental design is summarized in Figure 1.

Blood sampling

Daily blood samples. Prior to feeding on d 0 to d 21, a blood sample was collected from each heifer via tail venipuncture into a 10 mL tube containing EDTA (0.1 mL of a 15% solution) and cooled to 4°C. Plasma was obtained by centrifugation (2000 x *g* at 4°C for 15 min) and stored at –20°C until analyzed.

Acute blood samples. Concentrations of LH were determined in serum from blood samples (10 mL) collected every 10 min for 8 h on d 9, 10, and 11 (0.4 M, n = 15; 1.2 M, n = 8), and FSH was quantified in samples on d 11. The day before sampling, a polyvinyl cannula (Bolab Inc., BB 317-V/11; i.d. 1.68 mm, o.d. 2.39 mm; Lake Havasu City, AZ) was inserted into a jugular vein of each heifer, and animals were confined to stalls. Blood samples were allowed to clot for 24 h at 4°C, then centrifuged (2000 x *g* at 4°C for 30 min), and serum was decanted and stored at –20°C until analyzed. Beginning at 1200 on d 12, serum and plasma samples were collected from 0.4 M (n = 9) and 1.2 M (n = 4) heifers every 4 h for 48 h for quantification of estradiol-17β in plasma and LH in serum.

Tissue collection

Ten heifers (0.4 M, n = 6; 1.2 M, n = 4) were randomly selected for exsanguination, in three replications (0.4 M, n = 2 and 1.2 M, n = 1 or 2 heifers in each replication). At 0600 on d 12, heifers were transported to the abattoir and hypothalami and pituitaries were collected. Within 10 min after exsanguination, the brain was carefully removed intact and an area of tissue encompassing the medial basal hypothalamus was dissected as one block similar to Sesti and Britt (1993). Briefly, the medial basal hypothalamus was

defined as a block of tissue that was bound rostrally by the optic chiasma, caudally by the mammillary body, dorsally by the third ventricle. Lateral dissection was made by transverse cuts 20 mm from the stalk median eminence. The arcuate nucleus (**ARC**) was defined as tissue within 5 mm of the base of the third ventricle and the median eminence (**ME**) was defined as tissue 5mm dorsal to the stalk median eminence. Hypothalami sections were snap frozen in liquid nitrogen, and stored at –80°C until analyzed. Pituitary glands were then removed, placed on ice, trimmed, midsagitally sectioned, and the posterior lobe discarded. Anterior pituitary glands were weighed, snap frozen in liquid nitrogen, and stored at –80°C until analyzed. Ovaries were collected, the type of corpus luteum present was recorded, and the surface diameter of the largest follicle present was measured with calipers.

Hormone and metabolite assays

Insulin, glucose, NEFA, and IGF-I were quantified in plasma from heifers from d 0 to d 12, and only nonexsaguinated heifers on d 14. All samples were blocked by treatment within assay. Plasma concentrations of progesterone were quantified with a solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA; Vizcarra et al., 1997). Interassay coefficient of variation (n = 4 assays) was 6%. Concentrations of estradiol-17β in plasma were determined by RIA (Serono Estradiol MAIA assay kit, Biodata SpA, Montecelio, Italy) with modifications (Vizcarra et al., 1997). Intra- and interassay coefficients of variation (n = 2 assays) were 24 and 18%, respectively. Serum concentrations of LH were quantified by RIA (Bishop and Wettemann, 1993) with NIH LH-B9 for standards. Intra- and interassay coefficients of variation (n = 19 assays) were 26 and 30%, respectively. Concentrations of FSH in

serum were determined by RIA (Vizcarra et al., 1997) with USDA-bFSH-I-2 for standards. Intra- and interassay coefficients of variation (n = 7 assays) were 14 and 23%, respectively. Concentrations of IGF-I in plasma were determined by RIA with acid-ethanol extraction (Echternkamp et al., 1990). Recombinant human IGF-I (R&D Systems, Minneapolis, MN) was used for standards. Intra- and interassay coefficients of variation (n = 2 assays) were 6 and 35%, respectively. Concentrations of insulin in plasma were determined by solid phase RIA for human insulin (Coat-A-Count Insulin kit, Diagnostic Products Corp., Los Angeles, CA) using bovine pancreatic insulin (Sigma Chem. Co., St Louis, MO) for standards (Bossis et al., 1999). Intra- and interassay coefficients of variation (n = 3 assays) were 7 and 9%, respectively. Concentrations of glucose in plasma were quantified by an enzymatic colorimetric procedure (Sigma, No. 510, Sigma Chemical Co., St. Louis, MO). Intra- and interassay coefficients of variation were 2 and 5%, respectively (n = 3 assays). Concentrations of NEFA in plasma were determined by an enzymatic colorimetric procedure (Wako-NEFA C, Wako Chemicals Inc., Dallas, TX) with modifications (McCutcheon and Bauman, 1986).

Analyses of mRNA

RNA isolation. Total RNA was isolated from pituitary and hypothalamic tissue using TRIzol reagent (Invitrogen Corp., Carlsbad, CA). Briefly, approximately 0.5 g of tissue was homogenized in 5 mL of TRIzol reagent using a VirTishear® homogenizer (Virtis Co., Inc., Gardiner, NY). After samples were incubated for 5 min at room temperature, 1 mL of chloroform (Fisher Scientific BP 1145-1, Fair Lawn, NY) was added, the samples mixed for 1 min, then incubated at room temperature for 3 min. Samples were then centrifuged at 3500 x *g* for 30 min at 4°C to remove cellular debris and the aqueous phase

was transferred to a new tube. Then 2.5 mL of isopropanol (Fisher Scientific) was added to precipitate the RNA. Following centrifugation (3500 x *g* for 15 min at 4°C), the supernatant was removed, the RNA pellet was washed with 1 mL of 80% ethanol and centrifuged (3500 x *g* for 5 min at 4°C). The supernatant was removed, the pellet air dried for 5 min, resuspended in 100 μL of Tris-EDTA buffer, and stored at –80°C until analyzed. Total RNA was quantified by spectrophotometery at an absorbence of 260 nm, and purity was determined from calculations of 260/280 nm ratios.

Quantitative RT-PCR probes and primers. The partial sequence of bovine leptin receptor (456 bp; GenBank Accession U62385), homologous to bp 130 to 582 of the long form of the leptin receptor in sheep (Dyer et al., 1997), was analyzed for primer and probe locations. A BLAST (NCBI) search was conducted to assure that probes and primers were not designed in a homologous region that would code for other proteins. The primers (Forward primer, 5'-CAATGCAGCAGTGCTCAATTC-3', bp 73 to 93; Reverse primer, 5'-GGGCTGTCTCCTGCTCTCAT-3', bp 128 to 147) were used to amplify a RT-PCR product which was electrophoresed on a 2% agarose gel. This resulted in a single DNA product which was cut out, extracted (Qiagen, Valencia, CA), and sequenced at the biochemistry core facility. Once the sequence was verified by cycle sequencing, the primers were used with the fluorescent labeled probe (5'-6FAM-CTCACAGGTTATGTCTGTGCTCTCAGCCTCA-TAMRA-3', bp 96 to 126) in a RT-PCR 5'-exonuclease assay to measure relative differences in amount of mRNA for leptin receptor.

Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Quantification of mRNA was evaluated using one-step RT-PCR and a 5' exonuclease

assay (PE Biosystems, Foster City, CA). Specific sequence detection primers and a fluorescent labeled probe contained a 5' reporter dye (6-FAM) and a 3' quencher dye (TAMRA) were used to measure expression of leptin receptor in hypothalamic and pituitary tissue. The total reaction volume (25 μL) used with each reaction contained 12.5 μL of 2x TaqMan Master Mix without uracil-N-glycosylase (P/N 4309169; PE Biosystems), 0.625 μL of 40x MultiScribe and Rnase Inhibitor mix, 1.5 μL of 200 nM forward primer, 1.5 μL of 200 nM reverse primer, 0.25 μL of 250 nM fluorescent-labeled probe, 2.5 μL of 5 μM RNA (25 ng of total RNA), and 6.125 μL of RNase free water. The PCR amplification was carried out in a ABI PRISM® 7700 Sequence Detection System (PE Biosystems). Thermal cycling conditions were 48°C for 30 min, 95°C for 10 min, followed by 40 repetitive cycles of 95°C for 15 sec and 60°C for 1 min. As a normalization control for variation in RNA loading, the ribosomal 18S RNA control kit (43108993E, PE Biosystems) was used according to the manufacturers specifications. Specific mRNA detection and the corresponding 18S normalization reactions were carried out in the same multi-well plate during a single RT-PCR run, and the amount of mRNA for leptin receptor in the ME, ARC and anterior pituitary were done in individual assays. The same reaction conditions were used for amplification of 18S as previously described for mRNA except for the use of 50 nM forward and reverse primer, 200 nM fluorescent-labeled probe, and 25 pg of total RNA. Amplification of 18S rRNA and target mRNA was determined to be parallel and linear over a range of known amounts of total RNA loading (100, 10, 1, 0.1, and 0.01 ng of total RNA for target mRNA; 500, 50, 10, 5, and 1 pg of total RNA for 18S ribosomal RNA).

The comparative C_T method (PE Biosystems) was used to evaluate relative gene expression as previously described by Hettinger et al. (2001). Briefly, after examining the semi-log view of the amplification plot, a threshold (C_T) was set in the geometric region of the plot. Samples with greater amounts of target mRNA initiate increases of fluorescence at earlier PCR cycles than samples with less target mRNA. Therefore, the cycle at which fluorescence is measured at an arbitrary threshold is proportional to the number of mRNA transcripts. The ΔC_T value was determined by subtracting the C_T of each sample from its corresponding ribosomal 18S C_T value. Higher ΔC_T values indicate less target mRNA and lower ΔC_T values indicate more target mRNA.

Pulse analysis

Frequency and amplitude of LH and FSH pulses were determined using the pulsar program (Merriam and Wachter, 1982). To determine if variation in hormone concentrations in serial samples are pulses of hormone secretion or just random variation in concentrations, appropriate G values are chosen for each experiment. The value for G1 is usually set at 99 to prevent identifying a one sample increase in concentration as a pulse, which by our definition of a pulse could not occur due to the frequency at which samples were collected. The G5 value is also set at 99 to avoid false positive determination of small increases, followed by a return to baseline concentrations, as a pulse. The G values used for LH were G1=99, G2=3.25, G3=2.75, G4=2.25, G5=99. The G values used for FSH were G1=99, G2=3.25, G3=3, G4=2.5, G5=99.

Statistical analyses

Data from one 1.2 M heifer that was exsanguinated on d 12 were not included in statistical analyses because she became anovulatory and did not have plasma concentrations of progesterone ≥ 0.5 ng/mL before and during treatment. Chi-square analyses were used to evaluate the effect of treatment on the percentage of heifers that became anovulatory, percentage of heifers that exhibited a proestrous increase in estradiol, and percentage of heifers with an ovulatory surge of LH. A mixed model analyses of variance for repeated measures was used to determine effects of acute nutritional restriction on daily concentrations of hormones. Replication and assay were included as blocks with day being a repeated measure. Mixed model analyses of variance for repeated measures were used to determine nutritional effects on mean concentration, amplitude and frequency of LH pulses. The model included treatment and the treatment x day interaction. Assay and replication were used as blocks and day was used as a repeated measure. Least squares analyses of variance was used to determine the effects of nutrition on mean concentration, amplitude and frequency of FSH pulses on d 11. The model included treatment with assay as a block. Fishers-LSD were used to compare means when treatment or day effects were significant ($P < 0.05$). Least squares analyses of variance were used to determine the effects of treatment on amount of mRNA for leptin receptor in pituitary and hypothalamic tissue, as well as size of the largest follicle for exsanguinated heifers. The model included treatment as the source of variation.

Results

Body weight on d 0 was similar ($P = 0.93$) for heifers on both treatments, however on d 12, 1.2 M heifers had greater ($P < 0.01$) BW than 0.4 M heifers (Table 2). Heifers

fed 0.4 M lost 35 ± 4 kg during treatment and 1.2 M heifers maintained BW. Treatment did not influence BCS on d 0 ($P = 0.57$) and 12 ($P = 0.19$), or change in BCS ($P = 0.75$) during treatment (Table 2).

There was a treatment x day effect for plasma concentrations of NEFA ($P < 0.001$). Concentrations of NEFA were similar on d 0, but 0.4 M heifers had greater ($P < 0.001$) concentrations of NEFA on d 2 to 14 than 1.2 M heifers (Figure 2). Neither treatment ($P = 0.62$) nor treatment x day ($P = 0.17$) influenced concentrations of IGF-I in plasma, but there was a day effect ($P < 0.001$). Concentrations of IGF-I in plasma were less ($P < 0.01$) on d 10 and 12 of treatment compared with d 0 to 8 (Figure 2).

There was not a treatment x day effect ($P = 0.53$) on concentrations of glucose in plasma. Plasma concentrations of glucose were influenced ($P < 0.001$) by day of sample, and there was a tendency ($P = 0.10$) for 1.2 M heifers to have greater plasma glucose than 0.4 M heifers (Figure 3). There was a treatment x day effect ($P < 0.05$) on plasma concentrations of insulin. Heifers fed 1.2 M had greater ($P < 0.05$) concentrations of insulin in plasma on d 10 to 14 than 0.4 M heifers (Figure 3).

Treatment ($P = 0.88$) and treatment x day effect ($P = 0.80$) did not influence concentrations of LH in serum on d 9, 10, and 11 (Table 3). Mean concentrations of LH were greater ($P < 0.01$) for 0.4 M and 1.2 M heifers on d 10 and 11 (6.2 ± 0.4 and 6.4 ± 0.5 ng/mL, respectively) than on d 9 (4.7 ± 0.4 ng/mL). Treatment and day did not influence amplitude ($P = 0.50$) of LH pulses on d 9, 10, and 11, however, 1.2 M heifers tended ($P < 0.07$) to have a greater frequency of LH pulses on d 9 than 0.4 M heifers (Table 3). Heifers fed 0.4 M had fewer ($P < 0.05$) LH pulses on d 9 than on d 10 and 11. Frequency of LH pulses was greater ($P < 0.05$) for 1.2 M than 0.4 M heifers on d 9, but

number of LH pulses was similar ($P = 0.58$) between treatments on d 10 and 11. Frequency of LH pulses was not influenced ($P = 0.61$) by day of treatment, but amplitude of LH pulses was greater ($P < 0.01$) on d 10 and 11 than on d 9.

Treatment did not influence concentrations of FSH ($P = 0.66$) in serum on d 11. Amplitude ($P = 0.96$) and frequency ($P = 0.65$) of FSH pulses on d 11 were similar for 1.2 M and 0.4 M heifers (Table 4). Treatment did not influence ($P = 0.65$) concentrations of estradiol in plasma on d 11 (Table 4).

On d 14, 44% (4 of 9) of 0.4 M heifers were anovulatory and all (4 of 4) 1.2 M heifers ovulated ($P < 0.1$). A proestrous increase in plasma concentrations of estradiol was detected in 50% (2 of 4) of 1.2 M heifers and 80% (4 of 5) of 0.4 M heifers that ovulated. A proestrous increase in estradiol was not detected for heifers (0 of 4) fed 0.4 M that were anovulatory (1.1 ± 0.4 pg/mL; Figure 4), however, mean plasma concentrations of estradiol during 48 to 72 h after PGF2α were similar ($P = 0.40$) for heifers fed 1.2 M and 0.4 M that ovulated (1.8 ± 0.4 pg/mL).. A preovulatory surge of LH was detected in 25% (1 of 4) of 1.2 M heifers and 80% (4 of 5) of 0.4 M heifers that ovulated. An ovulatory surge of LH was not detected in any of the heifers that were fed 0.4 M that did not ovulate (0 of 4; figure 4). All heifers that were exsanguinated on d 12 had a regressing corpus luteum. Treatment did not influence ($P = 0.16$) size of the largest follicle on the ovary at exsanguination (13.3 ± 0.6 mm vs 11.6 ± 0.9 mm for 1.2 M and 0.4 M heifers, respectively).

The amounts of mRNA for leptin receptor in the median eminence ($P = 0.19$), arcuate nucleus ($P = 0.61$) and anterior pituitary ($P = 0.32$) were similar for 1.2 M and 0.4 M heifers (Table 5).

Discussion

Body Weight and Anovulation

Restricting heifers to 0.4 M for 14 d caused a 9% decrease in BW, and induced anovulation in 44% of heifers. Previously we determined that heifers fed 0.4 M for 14 d lost 4.7% of their BW and 70% failed to ovulate (White et al., 2000). However, heifers in that study were 30 d younger and 70 kg lighter than heifers in the current experiment. Mackey et al. (1999) found that feeding heifers 0.4 M caused a decrease of approximately 7.5% in BW and anovulation occurred in 60% of heifers. Chronic nutritional restriction causes loss of BCS and BW, and causes cessation of ovarian activity (Richards et al., 1989). When beef heifers were fed approximately 70% of maintenance energy requirements, they lost 23% of their BW before they failed to ovulate after 4 to 7 mo of restriction (Rhodes et al., 1995; Bossis et al., 1999). Although treatment significantly decreased BW, BCS was not altered by acute nutritional restriction for 14 d. This is in agreement with others (Mackey et al., 1999; 2000; White et al., 2000).

Metabolic Hormones

Feeding heifers 0.4 M for 14 d increased plasma concentrations of NEFA compared with 1.2 M heifers. This is consistent with previous reports (Mackey et al., 2000; White et al., 2000). Acute nutritional restriction for 3 to 6 d increased NEFA in plasma of cows (Looper et al., 1997) and steers (Ojeda et al., 1996). Plasma concentrations of NEFA are increased in nutrient restricted cows (Richards et al., 1989b) and heifers (McShane et al., 1989; Yelich et al., 1995; Bossis et al., 1999). In the current experiment, acute nutritional restriction caused an increase in plasma NEFA that was similar to the response to chronic

nutritional restriction of heifers (Bossis et al., 1999). With both acute and chronic nutritional restriction, the increase in plasma NEFA occurs much earlier than the onset of anovulation. Concentrations of NEFA were not indicative of the onset of postpartum luteal activity in primiparous beef cows (Vizcarra et al., 1998). Changes in NEFA indicate that fat cells are capable of responding to nutritional restriction very quickly. Because fat cells respond to changes in nutrition and alter metabolic activity rapidly, other hormones secreted from fat cells may be acutely altered in response to nutritional deprivation.

Heifers fed 0.4 M tended to have decreased plasma concentrations of glucose compared with 1.2 M heifers. Fasting decreases plasma concentrations of glucose in cattle (Harrison and Randel, 1986; McCann and Hansel, 1986). Concentrations of glucose are decreased before anovulation in chronically nutritionally restricted cows (Richards et al., 1989b) and heifers (Bossis et al., 1999). Intravenous infusion of 2-deoxy-D-glucose (**2DG**), an antagonist of glucose metabolism, decreased plasma LH in sheep (Crump et al., 1982; Funston et al., 1995) and delayed the onset of the estradiol induced LH surge (Crump and Rodway, 1986; Medina et al., 1998). Decreased glucose in plasma may alter LH secretion via a central mechanism at the hypothalamus. Central administration of glucose to ovariectomized ewes increased plasma concentrations of LH (Daniel et al., 2000). Intracerebroventricular (Bucholtz et al., 1996; Ohkura et al., 2000) administration of 2DG to sheep decreased plasma LH, but did not alter LH release in response to exogenous GnRH (Crump et al., 1982). Reduced concentrations of glucose in 0.4 M heifers may have compromised hypothalamic function so that GnRH secretion

was inadequate for some heifers to have adequate pulsatile secretion of LH on d 9 and to initiate an ovulatory surge of LH.

Concentrations of insulin in plasma were less in heifers fed 0.4 M than 1.2 M heifers after 10 d of nutritional restriction. Mackey et al.(2000) also found that heifers fed 0.4 M had reduced insulin in plasma. These results indicate that reduced plasma glucose in 0.4 M heifers results in less insulin secretion. Feed restriction causes plasma concentrations of insulin to decrease (Trenkle, 1978; McCann and Hansel 1986; Bossis et al., 1999). Insulin in serum is decreased before the onset of nutritionally induced anestrus in mature cows (Richards et al., 1989b) and heifers (Bossis et al., 1999). When nutritionally restricted cows were initiating anestrus, they had decreased disappearance of glucose and prolonged secretion of insulin in response to glucose treatment (Richards et al., 1989b). Intracerebroventricular infusion of insulin to ovariectomized ewes increased serum concentrations of LH (Daniel et al., 2000) and increased frequency of LH pulses (Tanaka et al., 2000), or had no effects (Hileman et al., 1993). Intravenous infusion of insulin did not alter LH in feed restricted heifers (Harrison and Randel, 1986). Based on our research and others, it is unclear whether insulin has a direct effect in altering LH secretion.

Plasma concentrations of IGF-I were not significantly altered by treatments. Mackey et al. (2000) and White et al. (2001) found that feeding heifers 0.4 M for 14 d caused a decrease in plasma IGF-I. Reduced nutrient intake decreases peripheral concentrations of IGF-I in cattle (Granger et al., 1989; Rutter et al., 1989; Spicer et al., 1992) due to decreased binding of GH to liver membranes (Brier et al., 1988). Insulin-like growth factor-I is a known regulator of reproduction (Spicer and Echternkamp, 1995). Decreased IGF-I is associated with increased postpartum anestrous intervals (Rutter et

al., 1989; Nugent et al., 1993; Roberts et al., 1997) and concentrations of IGF-I are reduced prior to anovulation in cows (Richards et al., 1991) and heifers (Bossis et al., 1999). Circulating concentrations of IGF-I increased from 2 to 10 wks after calving in cows that resumed estrous cycles, but were unchanged in cows that remained anestrous (Roberts et al., 1997). Plasma concentrations of IGF-I were increased during 2 wk of realimentation of acutely restricted heifers (White et al., 2001) and increased during the follicular wave before resumption of ovulation in chronic nutritionally induced anovulatory heifers undergoing realimentation (Bossis et al., 2000). Changes in plasma IGF-I may effect hypothalamic function. Insulin-like growth factor-I stimulates *in vitro* gonadotropin secretion from rat pituitaries (Kanematsu et al., 1991; Soldani et al., 1995). Additionally, reduced concentrations of IGF-I may have a direct effect on pituitary function. There is mRNA for IGF-I receptors in the pituitaries of rats, and IGF-I binding is altered by stage of the estrous cycle (Michels, et al., 1993). Insulin-like growth factor-I increased basal LH secretion *in vitro* from pituitary cells of rats (Kanematsu et al., 1991; Soldani et al., 1994) and pigs (Whitley et al., 1995).

Actions of IGF-I are modulated by high affinity insulin-like growth factor binding proteins (IGFBP), which are responsive to nutritional status. In cattle, plasma IGFBP-2 is increased with chronic (Vandeharr et al., 1995) and acute (McGuire et al., 1995) feed restriction. Plasma IGFBP-3 is reduced in anestrous cows (Roberts et al., 1997) and in ewes that are in thin body condition (Snyder et al. 1999). Insulin-like growth factor binding proteins are present in the pituitary of sheep (Snyder et al., 1999) and cattle (Funston et al, 1995) and are sensitive to changes in nutrition (Snyder et al., 1999). Thus potential changes in IGFBP caused by acute nutritional restriction may have altered bio-

availability of IGF-I at the pituitary and(or) higher brain centers to inhibit the release of an ovulatory surge of LH.

Gonadotropins and Estradiol

Decreased nutrient intake in cows is associated with reduced concentrations of LH in serum (Richards et al., 1989; Perry et al., 1991). We found that 12 d of nutritional restriction did not effect concentrations of LH in serum or amplitude of LH pulses. However, the frequency of LH pulses were less on d 9 in 0.4 M than 1.2 M heifers. Mackey et al. (1999; 2000) found that feeding heifers 0.4 M for 14 d did not alter mean concentrations of LH in blood or frequency and amplitude of LH pulses. Failure of chronic nutritionally restricted heifers to ovulate was associated with decreased concentrations of LH in serum (Rhodes et al., 1996; Bossis et al., 1999) and reduced frequency and amplitude of LH pulses (Bossis et al., 1999). Treatment did not influence mean concentrations of FSH in serum or frequency and amplitude of FSH pulses 24 h after PGF2α treatment. We previously reported that chronically restricted heifers had greater concentrations of FSH in serum and frequency and amplitude of FSH pulses 48 h after PGF2α treatment during the cycle that they became anovulatory (Bossis et al., 1999). Mackey et al. (2000) found that concentrations of FSH were greater during pre-emergence phase of follicular development of 0.4 M heifers, but that nadir concentrations of FSH during the post-emergence phase were not different compared with full fed controls.

Size of the dominant follicle that became anovulatory and concentrations of estradiol in plasma after luteolysis were reduced with chronic nutritional restriction (Rhodes et al.,

1995; 1996; Bossis et al., 1999). Presumably, reduced concentrations of estradiol prevented sufficient secretion of LH for final maturation of follicles, preventing heifers from ovulating. In the current study, plasma concentrations of estradiol at 24 h after PGF2α treatment were similar and size of the largest follicle present on the ovary was not different for 0.4 M and 1.2 M heifers, . Mackey et al. (2000) also found that acute nutritional restriction did not alter concentrations of estradiol in plasma 3 to 4 d prior to estrus, although follicle size was reduced 20%.

Proestrus increases in plasma concentrations of estradiol cause a surge of LH to be released from the pituitary which results in ovulation of the follicle. Because heifers were confined to stalls, we were unable to determine when heifers began estrus. Heifers were determined to be ovulatory if they had concentrations of progesterone of ≥ 0.5 ng/mL in plasma on d 14 to 21. We failed to detect a proestrus increase of estradiol in plasma or an ovulatory surge of LH in serum of one 0.4 M heifer that was classified as ovulatory. Based on plasma concentrations of progesterone, it was determined that she ovulated between d 11 and 12. Similarly, we did not detect a proestrus increase in estradiol in 2 of 4 1.2 M heifers, and no ovulatory surge was detected in 3 of 4 1.2 M heifers. Based on plasma concentrations of progesterone, it was determined that these heifers did not ovulate until after d 14. We sampled heifers every 4 h beginning at 1200h on d 12 until 1200 h on d 14 to measure changes in estradiol and LH associated with ovulation. However, we failed to detect changes in estradiol and(or) LH in these 4 heifers because they did not ovulate during this time. Mackey et al. (1999; 2000) did not detect a proestrus increase in plasma concentrations of estradiol in heifers fed 0.4 M that failed to ovulate. Similarly, the preovulatory increase in estradiol did not occur during the

cycle that chronic nutritionally restricted heifers became anovulatory (Rhodes et al, 1996; Bossis et al., 1999).

Leptin, a hormone synthesized and secreted primarily by adipose tissue, is an important regulator of energy balance (for reviews see Friedmann and Halaas, 1998; Houseknecht et al., 1998) and reproduction (for reviews see Barb, 1999; Clarke and Henry, 1999; Keisler et al., 1999; Spicer, 2001). Plasma concentrations of leptin are correlated with body fat in humans (Rosenbaum et al., 1996) and rodents (Frederich et al., 1995). Body condition accounted for 30% of the variation in leptin in plasma of sheep (Blache et al., 2000; Delavaud et al., 2000; Thomas et al., 2001). Feed intake decreased and body weight loss increased when rodents were treated with leptin (Camplfield et al., 1995; Weigle et al., 1995). Concentrations of leptin in plasma are decreased with reduced nutrient intake in rodents (Maffei et al., 1995; Trayhurn et al., 1995; Ahima et al., 1996), swine (Barb et al., 2001), and cattle (Ciccioli et al., 2001). The ob/ob mouse, which does not produce leptin, is obese and infertile. Treating ob/ob mice with exogenous leptin reduces body weight (Yu et al., 1997), renews pulsatile secretion of LH (Barash et al., 1996; Yu et al., 1997), and restores fertility (Mounzih et al., 1997; Yu et al., 1997). The time to onset of puberty in rodents is reduced when they are treated with leptin (Ahima et al., 1997; Chehab et al., 1997; Cheung et al., 1997).

Leptin may regulate reproduction by affecting gonadotropin secretion. Leptin caused the release of GnRH from rat hypothalamic cultures (Yu et al., 1997; Parent et al., 2000) and treating rat hypothalamic explants with leptin reduced the interpulse interval of GnRH (Lebrethon et al., 2000). Treating pituitary cultures of rats (Yu et al., 1997) and pigs (Barb, 1999) with leptin increased LH release. Intraperitoneal treatment of fasted

rats with leptin prevented decreased pulse frequency of LH (Nagatani et al., 1998). Leptin in plasma of sheep was decreased with fasting (Adam et al., 2002) or reduced nutrient intake (Blache et al., 2000; Morrison et al., 2001; Thomas et al., 2001), which also reduce concentrations of LH (Foster et al., 1989). Subcutaneous injections of leptin to sheep prevented concentrations of LH from decreasing during fasting (Nagatani et al., 2000). Fasting beef heifers (Amstalden et al., 2000) or cows (Amstalden et al., 2002b) for 24 to 60 h decreased plasma concentrations of leptin and expression of leptin mRNA in subcutaneous adipose tissue by as much as 47% (Tsuchiya et al., 1998; Amstalden et al., 2000; Amstalden et al., 2002). However, plasma concentrations of LH were unchanged (Amstalden et al., 2000; Amstalden et al., 2002b). Luteinizing hormone secretion from anterior pituitary explants of fasted cows was increased when treated with leptin (Amstalden et al., 2002a). In the current experiment, acute nutritional restriction abolished the ovulatory surge of LH in 44% of the heifers. Potential changes in leptin concentrations may have compromised the ability of 0.4 M heifers to generate an ovulatory surge of LH. Previously we determined that plasma concentrations of leptin were not different for heifers fed 1.2 M or 0.4 M (White et al., 2000). The lack of difference in leptin for nutrient restricted and full fed heifers may be due to the time that plasma samples were taken. Heifers in that experiment consumed all their feed within an hour and samples were collected immediately prior to feeding. As a result, plasma samples reflected concentrations of leptin in heifers after 23 h without feed intake. Fasting reduced plasma concentrations of leptin within 24 h in sheep (Thomas et al., 2001). Amstalden et al. (2000) found that leptin in plasma of prepuberal heifers was

reduced with a 48 h fast. The greatest reduction in leptin was observed in the first 24 h of the fast.

Leptin receptor mRNA has been found in the pituitary (Zamorano et al., 1997; Jin et al., 2000) and hypothalamus (Magni et al., 1999; Matsuda et al., 1999; Garcia et al., 2000) of rodents and monkeys (Fin et al., 2000). Leptin receptors have also been found in the hypothalamus and pituitary of sheep (Dyer et al., 1997; Daniel et al., 2000), and were localized on gonadotropes (Iqbal et al., 2000).

We found mRNA transcripts for leptin receptor in the ARC and ME of the hypothalamus as well as the anterior pituitary of beef heifers. The amount mRNA for leptin receptor in these tissues was not influenced by nutrient intake. In sheep, leptin receptor has been localized in the paraventricular, ventromedial, and ARC nucleus of the hypothalamus (Iqbal et al., 2001; Adam et al., 2002; Sorensen et al., 2002) as well as the anterior and lateral hypothalami (Iqbal et al., 2001). When sheep were fasted for 4 d (Adam et al., 2002), or feed restricted for 3 wk (Dyer et al., 1997), expression of leptin receptor was increased in the ARC, which corresponded with reduced concentrations of leptin in plasma. Lack of difference in amount of mRNA for leptin receptor in this experiment as compared with reports with sheep may be attributed to species differences. In addition, heifers in our experiment received reduced nutrient intake rather than a complete fast. More aggressive nutrient restriction or fasting may induce greater changes in the expression of leptin receptor in the hypothalamus. Differential effects of nutrition on expression of leptin receptor in the hypothalamus of sheep have been observed. Although expression of leptin receptor in the ARC was increased with a 4 d fast, leptin receptor was unchanged in the ventromedial hypothalamus (Adam et al., 2002), but was

increased in the ventromedial hypothalamus with 3 wk of feed restriction (Dyer et al., 1997).

Acute nutritional restriction of beef heifers impairs follicular growth (Mackey et al., 1999; 2000). Metabolic and endocrine changes can alter ovarian function. Diabetic pigs have decreased follicular growth (Cox et al., 1994; Edwards et al., 1996) and reduced intrafollicular concentrations of IGF-I (White et al., 1993; Cox et al., 1994; Edwards et al., 1996). Insulin-like growth factor-I potentiates ovarian responsiveness to gonadotropins (Gong et al., 1993; Spicer et al., 1993) and increases ovarian steroidogenesis (Stewart et al., 1995; Spicer et al., 1996) in cattle. Leptin can potentially affect ovarian function in cattle as well (Spicer et al., 1997; Spicer and Francisco, 1998; Spicer et al., 2000), but whether expression of leptin receptor was altered in the ovary in heifers in the current study is unknown. Although direct acute nutritional effects on ovarian function can not be ruled out, the primary cause of anovulation in acutely restricted heifers was impaired hypothalamic-pituitary function and the inability of heifers to generate an ovulatory surge of LH.

Implications

Heifers should be managed to avoid short-term nutrient restriction to maintain normal estrous cycles during the breeding season. Acute nutritional restriction of beef heifers can prevent ovulation by abolishing the ovulatory surge of LH. Since mRNA for leptin receptor is present within the hypothalamus, leptin may mediate nutritional effects on gonadotropin secretion and reproduction in beef cattle.

Table 1. Diet composition

Ingredient	% DM
Rolled Corn	40
Alfalfa Pellets	35
Cottonseed Hulls	22
Molasses	3
Trace mineralized salt	0.25
NEm, Mcal/kg	1.65
NEg, Mcal/kg	0.92
CP, %	10.3

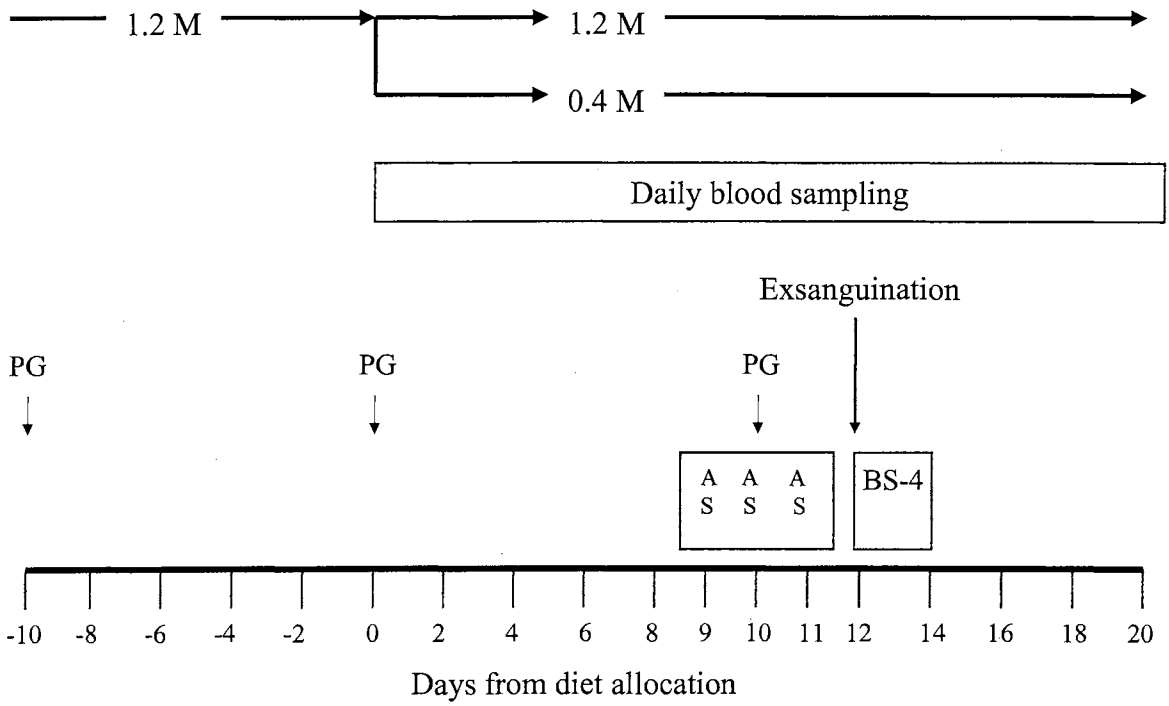

Figure 1. Experimental design of estrous synchronization, dietary treatments, acute blood sampling, and exsanguination of heifers fed 0.4 M or 1.2 M. Heifers were treated with PGF2α (**PG**) on d –10, 0, and 10 to cause luteal regression and ovulation. During acute sampling (**AS**; d 9, 10, and 11), blood samples were collected every 10 min for 8 h. Starting on d 12, blood samples were collected every 4 h (**BS-4**) for 48 h. Ten of 23 heifers were exsanguinated on d 12.

Table 2. Least squares means for initial and final BW and body condition score[a] (BCS), and change in BW during treatment[b]

	Treatment		
Item	1.2M	0.4M	SEM
No.	7	15	
Initial BW, kg	388	388	7
Final BW, kg	392[c]	353[d]	8
Change in BW, kg	4[c]	-35[d]	4
Initial BCS	5.5	5.4	0.1
Final BCS	5.5	5.3	0.1

[a]1 = emaciated, 9 = obese.
[b]Initial BW and BCS determined on d 0; final BW and BCS determined on d 12.
[c,d]Means without a common superscript letter differ ($P < 0.01$).

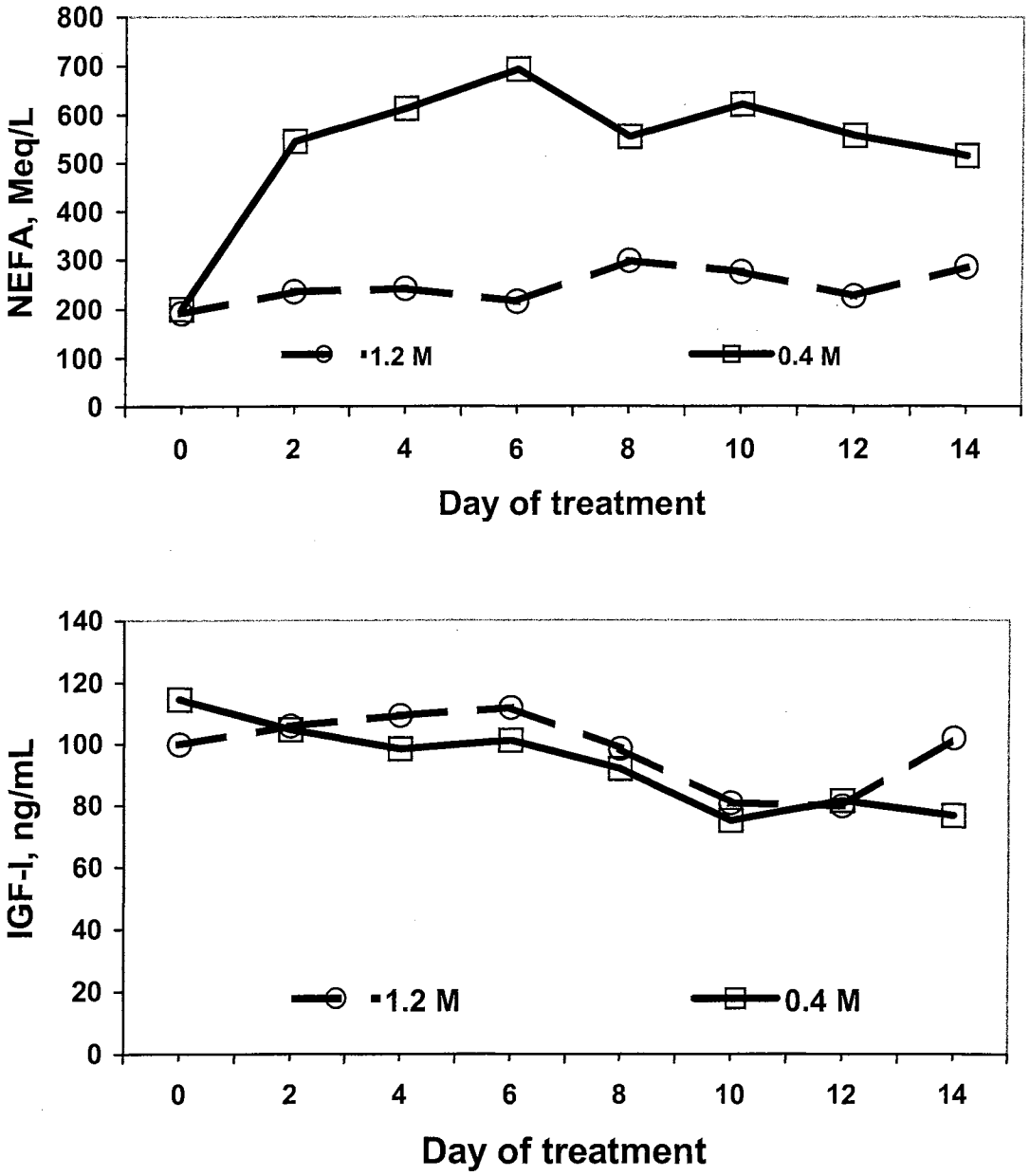

Figure 2. Least squares means for plasma concentrations of NEFA (SEM ± 47) and insulin-like growth factor-I (IGF-I; SEM ± 10) from d 0 to d 14 of treatment for heifers fed 1.2 M or 0.4 M. There was a treatment x day effect for NEFA ($P < 0.001$). Plasma IGF-I was influenced by day of sample ($P < 0.01$) but not by treatment ($P = 0.62$) or treatment x day ($P = 0.17$).

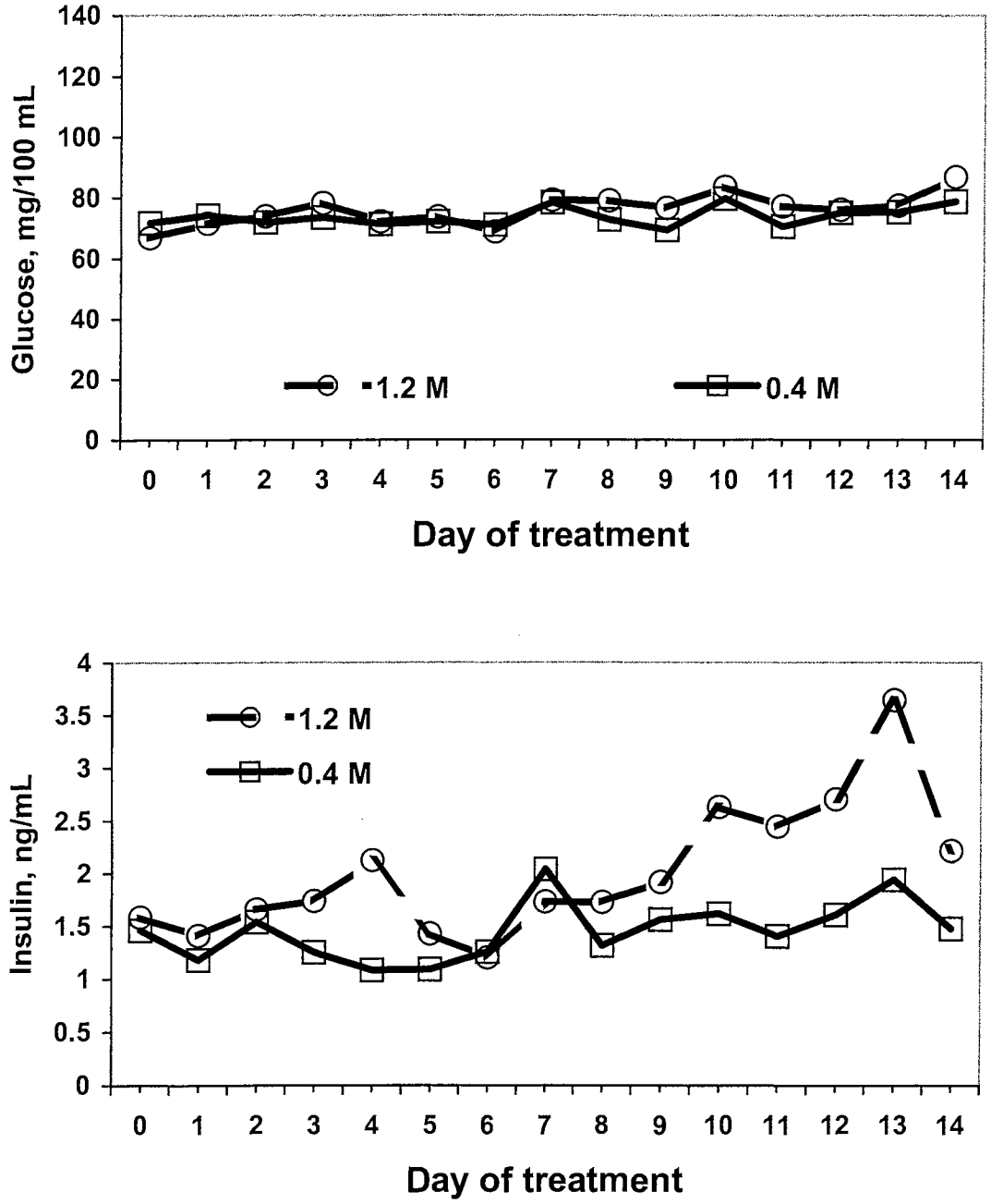

Figure 3. Least squares means for plasma concentrations of glucose and insulin from d 0 to d 14 of treatment for heifers fed 1.2 M or 0.4 M. There was no treatment x day effect ($P = 0.53$; SEM ± 2.7) for glucose. There was a day effect ($P < 0.0001$), and a tendency ($P = 0.10$) for 1.2 M heifers to have greater plasma glucose than 0.4 M heifers. There was a treatment x day effect for insulin ($P < 0.001$; SEM ± 0.2).

Figure 4. Concentrations of estradiol in plasma and concentrations of LH in serum during d 10 to 14 of a heifer fed 0.4 M that ovulated and a heifer fed 0.4 M that was anovulatory on d 14 of treatment. Heifers were treated with $PGF_{2\alpha}$ on d 10.

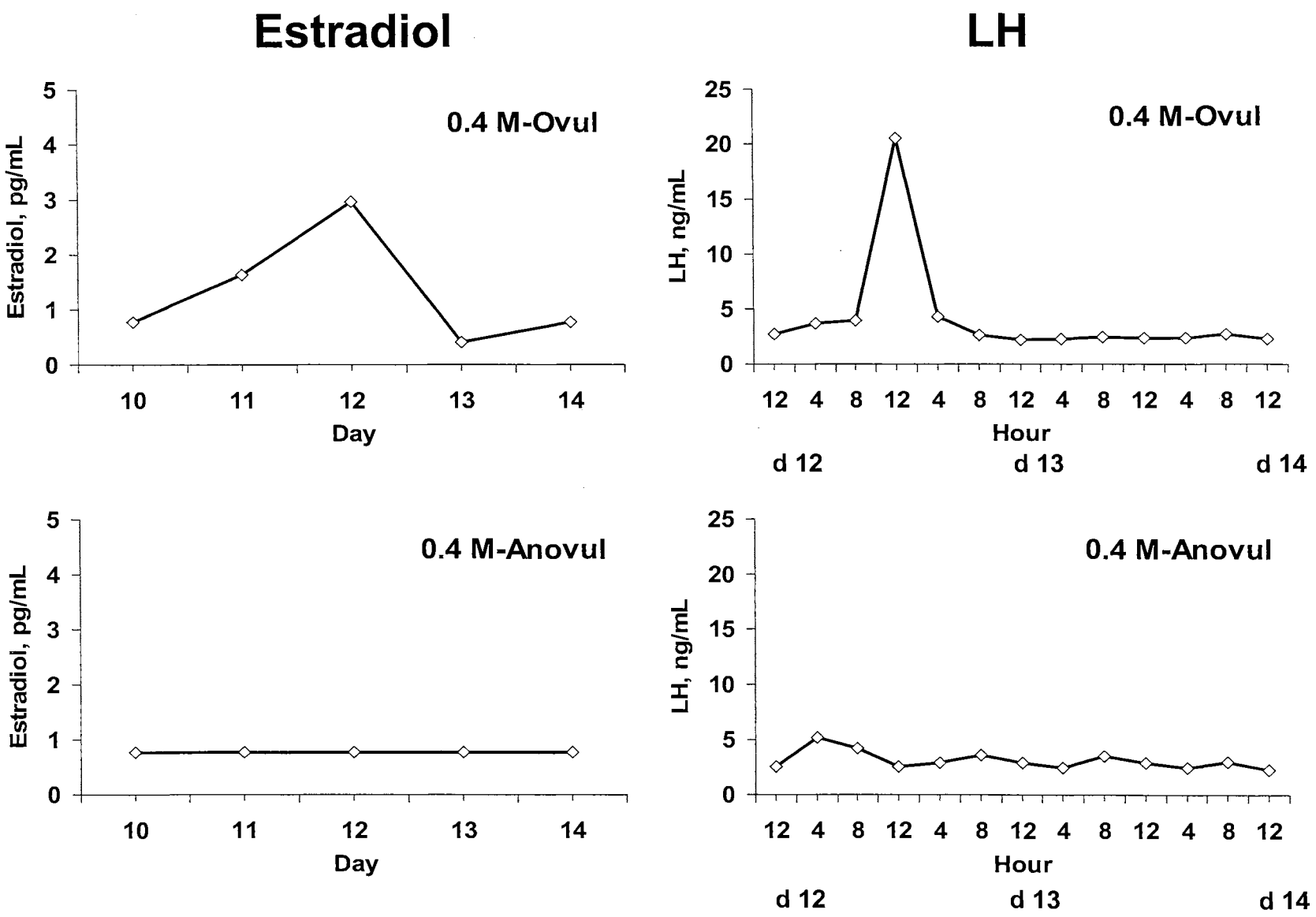
Estradiol
LH
0.4 M-Ovul
0.4 M-Anovul
Estradiol, pg/mL
LH, ng/mL
Day
Hour
10
11
12
13
14
12 4 8 12 4 8 12 4 8 12 4 8 12
d 12
d 13
d 14

Table 3. Effects of treatment and day on mean concentration, frequency and amplitude of LH pulses in serum on d 9, 10, and 11 of treatment for heifers fed 1.2 M or 0.4 M

	1.2 M			0.4 M			
Item	d 9	d 10	d 11	d 9	d 10	d 11	SEM
Concentration, ng/mL[a]	4.8[a]	6.0[b]	6.5[b]	4.5[a]	6.3[b]	6.3[b]	0.6
Pulse frequency, pulses/8 h[b]	5.0[b]	4.8[b]	4.7[b]	3.7[a]	4.5[b]	4.6[b]	0.2
Pulse amplitude, ng/mL	2.3[a]	3.6[b]	3.6[b]	2.3[a]	3.2[b]	3.3[b]	0.5

[a,b]Means without a common superscript letter differ ($P < 0.05$).

Table 4. Effects of treatment on mean concentration of FSH, and frequency and amplitude of FSH pulses in serum, and estradiol in plasma on d 11 of treatment for heifers fed 1.2 M or 0.4 M

Item	1.2 M	0.4 M	SEM
FSH			
Concentration, ng/mL	0.22	0.27	0.07
Pulse frequency, pulses/8 hr	5.3	5.8	0.6
Pulse amplitude, ng/mL	0.10	0.10	0.02
Estradiol, pg/mL	1.9	1.6	0.4

Table 5. Effects of treatment on amounts of mRNA for leptin receptor in various tissues using the comparative C_T method.

Tissue	Treatment	mRNA target average C_T[a]	18s average C_T[a]	ΔC_T[b]
Median eminence	1.2 M	29.75 ± 0.63	21.83 ± 0.70	7.92 ± 0.76
	0.4 M	28.81 ± 0.44	22.14 ± 0.50	6.68 ± 0.54
Arcuate nucleus	1.2 M	25.56 ± 1.95	17.96 ± 0.32	7.61 ± 2.10
	0.4 M	27.23 ± 1.51	18.12 ± 0.25	9.11 ± 1.63
Anterior pituitary	1.2 M	25.98 ± 0.27	19.07 ± 0.21	6.91 ± 0.44
	0.4 M	26.36 ± 0.19	19.11 ± 0.15	7.25 ± 0.31

[a]C_T = Cycle threshold; cycle number at which detection of fluorescence crosses the threshold set in the geometric region of the amplification plot. C_T is directly related to the number of RNA transcripts in a sample.
[b]ΔC_T = mRNA C_T – 18s C_T; This is the C_T value normalized for amount of RNA used.

CHAPTER V

SUMMARY AND CONCLUSIONS

Nutrient intake and body condition score are major regulators of reproduction in beef cattle (Wettemann, 1980; Short et al., 1990; Wettemann and Bossis, 2000). Endocrine and metabolic signals are important in mediating the effects of nutrition and body energy reserves on reproduction. The objective of these experiments was to determine mechanisms by which nutrition and BCS influence reproductive function in beef cattle.

In the first experiment, multiparous Angus x Hereford cows were used to determine the effects of BCS at parturition and the amount of protein supplement after calving on estrous behavior and follicle development. Cows calved were fed supplemental protein during gestation so that they had either thin (< 5) or moderate (≥5) BCS at calving. The thin and moderate cows were randomly allotted at calving to either low (1.5 kg/d) or high (2.9 kg/d) amounts of a 42% CP supplement for an average of 49 d after parturition (2 x 2 factorial). The interval from parturition to first estrus was longer for cows calving in thin condition compared with cows calving in moderate condition. The amount of protein supplementation after calving did not influence the interval to first estrus. Duration of estrus and number of mounts received at the first postpartum estrus was not influenced by BCS or nutrition, but were less compared with reports for nonlactating cyclic cows. Less

intense behavior at the first postpartum estrus may result in fewer inseminations or matings during the breeding season and(or) reduced pregnancy rates .

Protein supplementation and BCS altered size of the ovulatory follicle at the first postpartum estrus. Cows that had greater BCS at calving or those that were fed greater amounts of protein after calving had larger ovulatory follicles at the first postpartum estrus. Conception rates are greater for cows that receive a greater level of nutrition after calving. This may be due to a larger follicle at the first estrus, with an ovum with greater potential fertility, and(or) formation of a corpus luteum with enhanced ability to maintain pregnancy.

In the second experiment, Angus x Hereford heifers were fed either 1.2 or 0.4 times their maintenance requirements for 14 d. The objective was to determine the relationship between metabolic and endocrine function with ovulation. Restricted heifers lost more BW during treatment, but BCS was similar for both groups throughout the treatment period. On d 14, 44% of 0.4 M heifers were anovulatory and all of 1.2 M heifers ovulated. Plasma concentrations of insulin were greater for 1.2 M heifers. Concentrations of glucose in plasma tended to be greater for 1.2 M heifers compared with heifers fed 0.4 M. Concentrations of NEFA were greater for 0.4 M heifers compared with 1.2 M heifers but plasma IGF-I was not different between treatments. Treatment did not alter serum concentrations of LH or amplitude of LH pulses during maturation of the dominant follicle, but frequency of LH pulses was greater on d 9 for 1.2 M than 0.4 M heifers. Treatment did not influence serum concentrations of FSH or frequency and amplitude of FSH pulses. A proestrus increase in plasma concentrations of estradiol and an ovulatory surge of LH was not detected in heifers that failed to ovulate. Acute

nutritional restriction causes changes in metabolic and endocrine functions. Changes in these hormonal and metabolic signals relay information to the central nervous system about environmental and metabolic changes. Severe perturbations to energy balance compromised hypothalamic-pituitary function of some heifers and resulted in the abolition of the ovulatory surge of LH. The fact that some heifers continued to maintain normal reproductive function in the face of severe metabolic and endocrine insults may indicate genetic variation in efficiency of energy utilization. Further identification and characterization of how nutrition regulates reproductive function will lead to identifying potential genetic differences in cattle that have increased efficiency of reproduction and growth.

Hypothalamic-pituitary function was altered in nutritionally induced anovulatory heifers since they failed to have an ovulatory surge of LH. There are several potential metabolic signals by which nutrition may influence reproduction. Leptin, a hormone synthesized and secreted from fat cells, is thought to relay information about energy balance to the brain. We found that restricting nutrient intake of heifers to 40% of maintenance for 12 d did not influence the amount of mRNA for leptin receptor in the median eminence, arcuate nucleus, or anterior pituitary. In sheep, nutritional restriction abolishes pulsatile secretion of LH (Foster et al., 1989), reduces plasma concentrations of leptin (Blache et al., 2000; Morrison et al., 2001; Thomas et al., 2001), and increases leptin receptor in the hypothalamus (Adam et al., 2002). Our results indicate that the leptin signaling system is present in the hypothalamic and pituitary tissue of beef cattle. Potential changes in leptin may regulate reproductive efficiency by altering hypothalamic-pituitary function.

Nutrition and body energy stores can modulate the timing of reproductive events. Chronic nutritional restriction causes decreased gonadotropin secretion and quiescent ovaries. Acute nutritional restriction of beef heifers can also cause anovulation, but the mechanism is not known. These data indicate that both hypothalamic-pituitary and ovarian functions are responsive to changes in nutrient availability, body energy stores, and endocrine status. Almost certainly, different reproductively important tissues could be affected via different mechanisms, although some similarities may exist. These experiments indicate that changes in leptin receptor in the hypothalamus may be an important component in the regulation of GnRH secretion. A more complete understanding of mechanisms by which nutrition regulates reproductive processes will allow development of management strategies to improve reproductive efficiency of beef cattle.

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VITA 2

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