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Body Condition, Leptin, and Reproductive Characteristics in Horses.

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BODY CONDITION, LEPTIN, AND REPRODUCTIVE CHARACTERISTICS IN HORSES

A Dissertation

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and Dairy Sciences

by
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DEDICATION

This dissertation is dedicated with love to the three most important men in my life: my father, my husband and my son. First, I dedicate this dissertation to the memory of my father, Harry L. Roland, Jr. He once told me that one of the proudest moments of his life was when I graduated with honors from my high school. I hope that in his death I have made him proud of me once again by receiving my PhD. Second, I dedicate this dissertation to my husband, Glen T. Gentry, Jr. The most truthful statement I could ever make is that this dissertation would not have happened without him. I respect and admire him for his strength, intelligence and hard work and I am very proud to have him not only as my husband, but also as a fellow colleague. Last but by far, not least, I dedicate this dissertation to my son, Cameron. I requested a great deal of patience and understanding and help from such a young man, and he never faltered. This dissertation belongs to the three of you as much, if not more, than it does to myself.

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ABSTRACT

A series of experiments was conducted to gain a better understanding of the effects of nutrition and hormonal manipulation on the reproductive processes of the horse. In Experiment 1, body condition score (BCS) and ultrasonic backfat measurements were highly correlated in mares, especially over the tailhead area. In Experiment 2, high BCS (HBCS) mares continued to ovulate during the winter anestrous period, whereas low BCS (LBCS) mares went deeply anestrous. Daily hormone concentrations were not affected by BCS, however, responses to hormonal challenges indicated cyclicity in HBCS mares and reproductive quiescence in LBCS mares. In Experiment 3, treatment of average BCS mares with gonadotropin releasing hormone analog (GnRHa) and thyrotropin releasing hormone (TRH) tended to increase ($P < 0.09$) the number of medium-sized follicles, and GnRHa increased ($P < 0.0002$) synthesis and storage of luteinizing hormone. In HBCS and LBCS mares (Experiment 4), equine growth hormone (eGH) increased ($P < 0.0001$) insulin-like growth factor-1 (IGF-1), and IGF-1 was higher ($P < 0.02$) in HBCS mares. Treatment with GnRHa after eGH increased ($P < 0.002$) the number of follicles and two LBCS mares ovulated; however, these mares soon reverted back to an anestrous state. In Experiment 5, leptin concentrations were higher ($P < 0.009$) in HBCS mares and decreased from September to January; however, eGH treatment did not affect leptin concentrations. Dexamethasone treatment increased ($P < 0.001$) leptin concentrations in HBCS mares. Also, pregnant mares had higher ($P < 0.0001$) leptin concentrations prior to parturition than after, indicating that leptin may be produced in part by the placenta. Lastly, leptin

concentrations increased ($P < 0.004$) in foals not treated with eGH and remained unchanged in foals treated with eGH for 12 months. These results indicate that although hormones, such as eGH and GnRHa, may play a role in alleviating some of the deleterious affects of nutritional anestrous, the amount of body condition may be the most important factor affecting reproductive activity in the horse. Also, plasma concentrations of leptin are directly related to the amount of body condition indicating that leptin may also influence reproductive activity to a large degree.

CHAPTER I

INTRODUCTION

It is well known that nutrition plays a crucial role in the reproductive performance of various species, including humans. Several researchers have reported problems such as prolonged puberty, decreased cyclicity and a longer than normal postpartum anestrous period in animals (particularly ruminants) that are malnourished, as well as calving difficulties in excessively fat beef cows and temporary sterility in overly fat bulls. More indepth studies have shown that reproductive hormones in these animals are affected by level of nutrition. For instance, the episodic release of luteinizing hormone (LH) has been shown to be suppressed by dietary energy restriction in both cattle (Imakawa et al., 1987) and sheep (Thomas et al., 1990).

To date, little research has been conducted in the horse to determine the effects of nutrition and body condition score (BCS) on hormone concentrations and reproductive performance. The data that are available appear to be conflicting. Some believe mares should be thin upon entering the breeding season and then placed on an increasing plane of nutrition, whereas others have found that mares that were moderate to fat in body condition (BC) at the beginning of the breeding season cycled earlier, required fewer cycles per conception and had higher conception rates (Ginther, 1974; Henneke et al., 1984). No direct studies have been reported characterizing the reproductive and hormonal profiles of mares of either very high BCS (HBCS) or very low BCS (LBCS) during the traditional seasonal anestrus period and through transition. Therefore, the objectives of Experiments 1 and 2 were to: 1) monitor BCS in mares and correlate that

with actual ultrasonic backfat measurements during periods of HBCS or LBCS and 2) characterize hormonal profiles and ovarian follicular activity in mares of either very HBCS vs mares of very LBCS during the seasonal anovulatory period.

The anovulatory season for horses is during the winter months when the amount of daylight is minimum. In nonpregnant mares, seasonal anestrus and anovulation have been shown to be due in part to reduced hypothalamic production and secretion of gonadotropin releasing hormone (GnRH), which ultimately results in reduced production and secretion of LH and, to a lesser degree, follicle stimulating hormone (FSH; Mumford et al., 1994a). Administration of GnRH or one of its analogs twice daily (Fitzgerald et al., 1987) or continuously (Allen et al., 1987; Hyland et al., 1987) has been shown to initiate follicular growth and induce ovulation in acyclic mares. This may be important for those who wish to breed their mares earlier to coincide with foals being born as close to the January 1 birthdate as possible. Two other hormones, growth hormone (GH) and thyroid hormone have also received a great deal of interest lately for their role in the reproductive process. Fitzgerald and Davison (1997) reported that mares that continued to cycle throughout the winter in Kentucky had higher thyroxine (T_4) concentrations in plasma than mares that went anestrous. Cochran et al. (1999a) showed that treatment with equine GH (eGH) caused an increase in circulating insulin like growth factor-1 (IGF-1) concentrations along with an increase in the number of small follicles in mares. Cochran et al. (1999b) also reported that eGH in combination with daily injections of a GnRH agonist (GnRHa) enhanced the ovulatory response of mares of average BC. Therefore, Experiments 2 and 3 were conducted to determine 1) what effect daily injections of

GnRHa, thyrotropin releasing hormone (TRH) or a combination of both would have on follicular and hormonal patterns of mares of average BC during the traditional seasonal anestrus period, and 2) what effect eGH and GnRHa would have on follicular and hormonal patterns of mares of either HBCS or LBCS during the traditional seasonal anestrus period.

Another hormone which appears to play a major role in the reproductive process is leptin. Many believe leptin, the product of the obese gene, is the long sought signal for nutritional status that allows reproductive processes to proceed (Houseknecht et al., 1998). It has been postulated that low concentrations of leptin inhibit the activity of the neuroendocrine reproductive axis by acting as a "metabolic gate" (Cunningham et al., 1999). In both rats and mice, exogenous leptin increased basal hormone concentrations and increased reproductive organ weights in both males and females; it also corrected the sterility defect in female ob/ob (leptin deficient) mice (Barash et al., 1996; Carro et al., 1997; Kohsaka et al., 1999). Most of the effects of leptin on reproduction have been studied in rodents, and little work has been done to determine its role in the horse. Therefore, the last series of experiments was conducted to determine the role of leptin in anestrous mares of HBCS vs LBCS, mares of HBCS given eGH vs mares of LBCS given eGH, dexamethasone (DEX) effects on mares of HBCS vs LBCS, pregnant vs foaling mares (of average BC), and foals given eGH.

CHAPTER II

REVIEW OF LITERATURE

Assessment of BCS and backfat

Nonequine species. Body condition is defined as the amount of stored fat in the animal's body. Several BCS systems are in use in the world today, however, in the United States, the scoring system developed in Virginia is the most widely used for dairy cows (Reneau and Linn, 1989). The scale ranges from 1 to 5 using visual appraisal and palpation of the loin, rump and tailhead regions. A score of 1 indicates the animal is severely underconditioned and 5 indicates the animal is extremely obese (Perkins et al., 1985; Reneau and Linn, 1989). In beef cattle, the scale is much broader, ranging from 1 to 9 with a score of 1 being extremely thin and emaciated and a score of 9 being extremely obese (Whitman, 1975). Convincingly, it has been shown that body reserves are better reflected by BCS than by live weight change (Ducker et al., 1985; Reneau and Linn, 1989).

Although using the BCS system is an effective method to monitor the energy intake of cows and gives a surprisingly accurate assessment of a live animal's energy reserves (Reneau and Linn, 1989), livestock producers have been faced with a dilemma because of the lack of accurate methods for measuring carcass value prior to slaughter (Houghton and Turlington, 1992). Therefore, many have become interested in using ultrasound to determine fat thickness and muscle development in the live animal (Houghton and Turlington, 1992; Perkins et al., 1992; Herring et al., 1994; Brethour, 2000). Since the mid 1950's, researchers have been using ultrasound technology to

predict fat and muscle composition in domestic livestock (Temple et al., 1956; Price et al., 1958; Stouffer et al., 1959). According to Perkins et al. (1992), the ability of ultrasound to accurately estimate live carcass composition could be important for a value-based marketing system, which would encourage production of carcasses that yield as much lean tissue as possible with little external and seam fat and would respond to increased consumer demands for a leaner product. The research conducted by Perkins et al. (1992) indicated that ultrasonic measurements of backfat and longissimus muscle area taken before slaughter may be relatively accurate predictors of final carcass fat thickness and longissimus muscle area in beef cattle. Likewise, Brethour (2000) showed that ultrasound estimates of backfat and marbling made during the feeding period could be used to predict carcass merit at slaughter. However, Houghton and Turlington (1992) reported that although a single ultrasonic measurement of fat can be helpful in predicting days on feed in yearling cattle, when used alone, it does not provide adequate accuracy.

Horses. To date, little research has been conducted to specifically assess BCS and(or) backfat in the horse. As a matter of fact, before the early 1980's, there was no consistent basis for the evaluation of BC in the horse. Henneke et al. (1983) developed a system for the accurate comparison of stored body fat in the horse similar to that used in beef cattle (Whitman, 1975). The scoring system is based on visual appraisal and palpable fat cover at six areas of the horse's body. Those areas are the ribs, the area behind the shoulder, the area along the neck, the area along the withers, the crease down the back, and the tailhead. The score ranges from 1 to 9 with a score of 1 being extremely thin, to the point of emaciation, and a score of 9 being extremely fat (Table 2.1).

Table 2.1. Description (abbreviated) of individual condition scores (Henneke et al., 1983).

Score	Description
1 Poor	Animal extremely emaciated. No fatty tissue can be felt.
2 Very Thin	Animal emaciated. Slight fat covering over base of spinous processes, transverse processes of lumbar vertebrae feel rounded.
3 Thin	Fat build up about halfway on spinous processes, transverse processes cannot be felt. Slight fat cover over ribs. Tailhead prominent.
4 Moderately Thin	Negative crease along back. Faint outline of ribs discernable. Fat can be felt around tailhead.
5 Moderate	Back level. Ribs cannot be visually distinguished but can be easily felt. Fat around tailhead beginning to feel spongy.
6 Moderately Fleshy	May have slight crease down back. Fat over ribs feels spongy. Fat around tailhead feels soft. Fat beginning to be deposited along side of withers, behind shoulders and along sides of neck.
7 Fleshy	May have crease down back. Individual ribs can be felt, but noticeable filling between ribs with fat. Fat around tailhead is soft. Fat deposited along side of withers, behind shoulders and along sides of neck.
8 Fat	Crease down back. Difficult to feel ribs. Fat around tailhead very soft. Wither area and area behind shoulder filled with fat. Noticeable thickening of neck.
9 Extremely Fat	Obvious crease down back. Patchy fat over ribs. Bulging fat around tailhead, along withers, behind shoulders and along neck. Flank filled with fat.

In the study by Henneke et al. (1983), simple correlations were used to determine the relationship between condition score, physical measurements and percent body fat in mares. Results showed that condition score was positively correlated to body fat percentage ($r^2 = 0.65$), but no significant correlations were found between body fat percentage and weight, height or heart girth.

Another study was conducted by Kane et al. (1987) in horses using real-time ultrasonography to estimate fat thickness at five sites over the croup and then measuring actual fat thickness at each site upon slaughter. Those researchers determined that actual and ultrasonic measurements of fat thickness correlated well ($r^2 = 0.88$ to 0.98) with each other at four of the five sites. However, prediction equations for estimating empty body fat from actual and ultrasonic measurement of fat thickness varied greatly according to measurement site.

Nutritional manipulation of reproduction

Malnutrition (nonequine species). A great deal of information is available on the effects of malnutrition in the ruminant animal. Several studies have shown that the onset of puberty can be delayed when the prepubertal animal is inadequately fed or is malnourished. According to Kinder et al. (1987), the prepubertal increase in LH pulse frequency of cattle and sheep, which results from a decrease in responsiveness of the hypothalamic-pituitary axis to estrogen negative feedback, is the critical event initiating the onset of puberty. In prepubertal heifers maintained on a low-energy diet, no increase in LH pulse frequency was seen; however, heifers fed an adequate diet for growth did exhibit increased LH pulse frequencies and therefore attained puberty (Day et al., 1986).

Also, Kurz et al. (1990) reported that the prepubertal increase in LH pulse frequency in intact and ovariectomized heifers (with or without estradiol treatment) was prevented by restriction of dietary energy. Foster and Olster (1985) showed that undernutrition in ovariectomized ewe lambs reduced pulsatile LH secretion both in the presence or absence of estradiol negative feedback. These researchers demonstrated that ewe lambs allowed ad libitum feed became pubertal while ewe lambs fed to maintain their weaning weight remained anovulatory.

In addition to problems with delayed puberty, malnutrition has been shown to induce anestrus in mature, cycling cattle (Shillo, 1992). Richards et al. (1989) reported that Hereford cows on a restricted diet for several weeks did not have luteal activity. In another study by Imakawa et al. (1986), anestrus occurred in heifers that had been fed 50% of the energy required for maintenance for 185 d. Also, in Hereford-cross cows fed a restricted diet, follicular development was arrested at the medium-sized follicle stage without going on to a preovulatory stage. Likewise, Bossis et al. (1999) reported that growth rate and diameter of the ovulatory follicle, as well as concentrations of LH, estradiol, and IGF-1, are reduced before the onset of nutritionally induced anovulation in beef heifers.

Reduced ovulation rates were seen in adult ewes maintained on low planes of nutrition (Haresign, 1981) and estrous cycles ceased in gilts after 46 d of feed restriction (Armstrong and Britt, 1987). Richards et al. (1989) reported that weekly concentrations of serum LH, as well as LH pulse frequency, were reduced in the restricted cows. According to Schillo (1992), the negative effects of inadequate nutrition on LH release

probably involve reduced GnRH release; however, direct effects on the ovary are also possible.

Problems associated with nutritional deficiencies in the dairy female are of particular importance due to the economics associated with having a healthy, high-producing herd. Beam and Butler (1999) reported that pregnancy rates in lactating dairy cows over the past four decades have declined as the demand for greater milk production has increased. Domecq et al. (1997) reported that decreased BC during the first 4 weeks of lactation was associated with a decreased likelihood of conception. Dairy cows with a BCS of 3 had a significantly better first service conception rate than cows with a BCS of < 3 in seven North German cattle herds (Klindworth et al., 2001). In a study by Prandi et al. (1999), dairy cows that lost more than 20% in BCS due to negative energy balance at 30 d postpartum had the lowest reproductive efficiency of all groups studied. According to Roche et al. (2000), negative energy balance in early postpartum decreases LH pulse frequency, as well as growth rate and diameter of the dominant follicle, which can have damaging effects on the follicle or the corpus luteum (CL). Blood metabolites and hormones, such as glucose, insulin and IGF-1 are decreased while GH is increased, which results in loss of BCS and a higher percent of cows in the herd that are anestrus (Roche et al., 2000). According to Whitley et al. (1998), the mechanism of action of insulin and porcine GH on reproductive function in the sow may be direct or could involve modulation of IGF-1, which has positive effects on follicular development.

In contrast to the studies by Domecq et al. (1997) and Klindworth et al. (2001), Markusfeld et al. (1997) reported that although high BCS cows were less prone to go

anestrus after calving, they did not conceive more successfully to first service than cows that lost more BC. However, multiparous cows from that same study that lost more BC during the dry period did have inactive ovaries and were more likely to be open 150 d after calving in the next lactation.

Other problems associated with below average BC in the dairy animal include longer intervals to first ovulation (Beam and Butler, 1999) and increased days open (Suriyasathaporn et al., 1998), as well as endometritis occurring between calving and 20 d of lactation (Heuer et al., 1999). Also, Markusfeld et al. (1997) reported that the risk of retained placenta was greater for cows that were underconditioned at drying off, and cows that lost more BC during the dry period suffered more from both retained placenta and metritis.

Similar problems that have been reported for dairy cattle have been shown to occur in beef animals as well. DeRouen et al. (1994) reported that cows with BCS 6.0 and 7.0 at calving had higher postpartum pregnancy rates than cows of BCS 4.0 and 5.0. Also, interval to pregnancy for cows with a BCS 4.0 at calving was 10 to 18 d longer than for cows with a BCS $\geq$ 5.0. Lalman et al. (1997) investigated the relationship between change in weight (and BC) on postpartum interval of thin first-calf heifers and found that as dietary energy density increased, postpartum interval decreased. In addition to the 1997 study, Lalman et al. (2000) showed BCS was positively associated with IGF-1 and insulin concentrations but was negatively associated with GH concentrations. In a study with spring-calving beef cows, Morrison et al. (1999) determined that large changes in

body energy reserves during mid- to late gestation of cows calving in moderate BC did not influence subsequent reproductive performance of those cows.

Several events, such as recovery of the pituitary gland and uterus after pregnancy, escape from suckling-induced inhibition of pulsatile LH secretion, initiation of follicular development, estrus and subsequent ovulation, are all required for postpartum cows to successfully be rebred (Randel, 1990; Schillo, 1992). In his review, Randel (1990) states that the nutritional mechanism controlling ovarian activity may be having its effect on the hypothalamus, pituitary gland or ovary. Because ovarian function is controlled by gonadotropin secretion from the pituitary gland, the hypothalamic-pituitary axis is most likely where nutritional influence on the ovary occurs (Randel, 1990).

Malnutrition (horses). Unlike other species, data on malnutrition in the equine are limited. The research that is available on the relationship between BC and reproduction in the mare is vague and often misinterpreted (Henneke et al., 1983, 1984). Day (1939) reported that mares of high BCS had higher fertility rates at mating than mares of low BCS. In addition to those findings, Ginther (1974) reported that the onset of the breeding season was significantly earlier in mares which gained weight prior to the breeding season than in mares that lost weight. Research in the early 1970's, however, showed that mares should be relatively thin upon entering the breeding season and then placed on an increasing plane of nutrition (Voss et al., 1974; Witherspoon et al., 1977). Like Ginther (1974), Henneke et al. (1984) reported that mares moderate in weight or fat prior to the breeding season cycled earlier, required fewer cycles per conception and had higher conception rates than did thin mares. It was concluded that improving the BC of thin

mares prior to the breeding season does not improve reproductive performance over mares already in good BC. Kubiak et al. (1987) reported similar results and concluded that nonlactating mares should be brought into the breeding season with a body fat content $\geq$ 15% and a BCS $\geq$ 5.0. They also stated that mares should then be maintained in a positive energy balance in order to attain an earlier ovulation and a shorter initial estrus. For example, nonfoaling mares with BCS < 5.0 had average intervals from February 1 to first estrus and first ovulation of 39 and 63 d, respectively, whereas corresponding intervals for mares with BCS > 5.0 were 26 and 37 d (Henneke et al., 1984).

In the lactating mare, a fertile postpartum estrus (foal heat), characterized by normal follicular development and ovulation, occurs within 20 d of foaling (McKinnon and Voss, 1993). According to Fitzgerald et al. (1985), concentrations of LH during the first postpartum estrus are comparable to those noted during subsequent estrous cycles. This appears to be different than in the nonpregnant (dry) mare, in which LH concentrations are lower during the first estrous cycle of the breeding season (Freedman et al., 1979). Much less work has been done to study the relationships of the reproductive hormones in the mare than in other species; therefore, Hines et al. (1987) conducted a study to examine LH concentrations in mares of high vs low BCS during late pregnancy and into the first and second estrous cycles. These researchers reported that their mares differed in the regularity of estrous cycles following parturition. In the high BCS mares, the total amount of LH released during the estrous surge and the peak amount of LH released during estrus were the same for the first and second cycles. In contrast, low BCS

mares released more LH and had higher peak LH concentrations during the second cycle. According to Hines et al. (1987), this pattern is similar to that observed in mares during transition into and out of the breeding season.

Obesity (nonequine species). Many potential health risks in cattle exist from obesity caused by overfeeding. Although most reproductive failures are mainly due to improper nutrition and low BC, obesity is a particular problem in heifers (Mangione, 1992). Fat beef heifers in the growing phase normally have lower than average reproductive rates (Ferrell, 1982; Mangione, 1992), and studies show that dairy heifers fed for maximum gains have limited lifetime potentials for milk production (Walker et al., 1994). According to Reneau and Linn (1989), dairy cows with BCS > 4 have lower milk yields, more metabolic disease and lower reproductive performance. Excess BC of cows at calving increases losses in body weight and BC during lactation and decreases dry matter intake and milk production (Treacher et al., 1986; Smith et al., 1997). Arnett et al. (1971) reported that obese cows required more services per conception, more assistance at calving, lost more calves, produced less milk and produced lighter calves compared with normal cows. Wallace et al. (1996) reported that overfeeding the adolescent pregnant ewe causes rapid maternal growth at the expense of the fetus. This overfeeding leads to growth restriction in both the placenta and fetus compared with pregnant ewes of similar age that were moderately fed (Thomas et al., 2001).

Obesity (horses). For many years, it was commonly believed that obesity was a major cause of infertility in the mare (McKinnon and Voss, 1993). Simmerman and Greene (1978) reported smaller foals and reduced rebreeding efficiency in obese mares,

however, Kubiak et al. (1987) reported no adverse effects of obesity on these characteristics. In fact, Kubiak et al. (1987) reported no differences between treatment groups for all times associated with the process of parturition, the degree of cervical and vaginal bruising incurred during foaling, or the presence of angular limb deformities to suggest increase foaling difficulties in excessively fat mares. As stated earlier, Henneke et al. (1984) showed that breeding efficiency was enhanced in mares entering the breeding season or foaling at a BCS of 5.0 or greater. These researchers also reported that initial excess stores of body fat did in fact enhance fertility, and there were no detrimental effects of excess body fat stored in late gestation.

Hormonal manipulation of reproduction

GnRH. The mare is a seasonal breeder having normal estrous cycles from May to October (Ginther, 1979; Harrison et al., 1990). Most nonpregnant mares go through a winter anestrous period in which they are reproductively inactive (quiescent). This seasonal anestrous and anovulation period in nonpregnant mares is known to be due in part to reduced hypothalamic production and secretion of GnRH (Hart et al., 1984), resulting in reduced production and secretion of LH with lesser effects on FSH (Mumford et al., 1994a). Then, in early spring, a period of transition occurs in which there is renewed follicular growth and large ovarian follicles begin to develop, some more than 30 mm in diameter that fail to ovulate and then regress. The transition from winter anestrus into the breeding season likely occurs due to the restoration of hypothalamic GnRH and therefore increased synthesis and secretion of LH (Silvia et al., 1987).

The horse is unique in that most breed registries in the United States have a standardized birth date of January 1 for all foals born in a calendar year. Many breeders attempt to manipulate the mare's cycle so that foals will be born as close to that January 1st date as possible. For many years, scientists have been researching methods to hasten the onset of normal cyclicity so that this can be achieved. The use of GnRH or its analogs to induce ovulation in seasonally anestrous mares, hasten the onset of regular cycles in transitional mares, and shorten the follicular phase in cyclic mares has been attempted (Irvine et al., 1975; Silvia et al., 1987; Becker and Johnson, 1992). Several investigators have shown that both LH and FSH (Evans and Irvine, 1976; Alexander and Irvine, 1986; Johnson, 1987) are released from the pituitary when anestrous mares are given exogenous GnRH.

Several GnRH products and treatment protocols have been studied for the induction of ovulation in the anestrous mare. Evans and Irvine (1977) attempted to reproduce the gonadotropin surges responsible for causing follicular growth and ovulation during the normal estrous cycle by giving acyclic mares three courses of GnRH treatment. Although they reported that follicular development, maturation and ovulation resulted from GnRH-induced gonadotropin surges, 5 of the 10 mares originally used in the experiment were excluded from the results because their data suggested that those mares may have had spontaneous FSH release. Therefore, the ovulation that occurred may not have been induced solely by the GnRH treatment. More promising and definitive results occurred when high doses of GnRH were administered to anestrous mares in a pulsatile pattern (Johnson, 1986). In that study, a preovulatory-like LH surge

occurred and ovulation was induced in an average of 9 d (Johnson, 1986). A follow-up study by Johnson (1987) revealed that follicular development and ovulation in seasonally anovulatory mares can be induced with pulsatile administration of substantially lower doses of GnRH. In agreement with those studies, Hyland et al. (1986), Sanderson et al. (1986), and Allen et al. (1987) showed that continuous administration of GnRH or one of its analogs can initiate follicular growth and induce ovulation in acyclic mares as well. Harrison et al. (1990) gave subcutaneous implants of the GnRH agonist, buserelin, to anestrous mares and found that ovulation could be induced, although the response time and reliability of results were more variable compared to intravenous pulses of GnRH (Becker and Johnson, 1992). Studies using twice daily injections of GnRH or a GnRH analog (Fitzgerald et al., 1987; Harrison et al., 1990) produced results similar to pulsatile administration.

TRH. To date, very little research has been conducted on the role of thyroid hormones in reproductive processes of the mare. In several species, TRH has been shown to increase both PRL and TSH (Davis and Borger, 1972; Convey et al., 1973; McMahon et al., 1979; Thompson et al., 1984). Prolactin is a pituitary hormone, which, in the mare, varies relative to season (Johnson, 1986; Thompson et al., 1986). It is highest in June through August (time of reproductive activity) and lowest from November through February (Johnson, 1987); therefore, it may play a role in reproduction, although the exact mechanism has yet to be determined. Roser et al. (1987) reported that the frequency and amplitude of PRL surges tended to change depending on the time of the estrous cycle in the mare, which suggested that PRL is involved in ovarian function.

According to Johnson (1987), the stimulatory effect of TRH on PRL secretion is influenced by season. In several mammalian species, PRL as well as T_4 and triiodothyronine (T_3) have been shown to vary when photoperiod, temperature and(or) reproductive state changes (Bourne and Tucker, 1975; Johnson, 1986). In the horse, Fitzgerald and Davison (1997) reported that mares that did not become anestrus and continued to cycle throughout the winter in Kentucky had higher T_4 concentrations in plasma than mares that went anestrous. It is possible that a decrease in thyroid function, which may be due to reduced nutrition or energy stores in winter, could result in mares becoming anestrous.

In addition, TRH stimulates the secretion of GH in both cattle and sheep (McMahon et al., 2001), and GH has been shown to play an important role in reproductive processes (see following section on GH). More research is warranted to determine the role of TRH in reproductive processes of the horse.

GH. In contrast to TRH, much work has been conducted over the past 15 years to understand the actions of GH in domestic animals (Etherton and Bauman, 1998). Growth hormone, also called somatotropic hormone or somatotropin, is secreted by the adenohypophysis and exerts its effects on all or almost all tissues of the body (Guyton, 1991). According to McMahon et al. (2001), GH is essential for postnatal somatic growth and maintenance of lean tissue at maturity in livestock. Increased concentrations of GH are of economic importance because they are associated with faster growth, less fat stores, and more efficient milk production in dairy cows.

Although its main function is to cause growth of all tissues of the body that are capable of growing, GH has been shown to have a very important reproductive role as well. For instance, administration of GH has been shown to have beneficial effects on ovarian activity and in particular, increased follicle numbers in many species including cattle (Buratini et al., 2000; Lucy, 2000; Tripp et al., 2000), sheep (Joyce et al., 1998; Joyce et al., 2000), swine (Spicer et al., 1992; Echternkamp et al., 1994; Whitley et al., 1998), and horses (Cochran et al., 1999).

In the study by Tripp et al. (2000), yearling beef heifers were treated with bovine GH (bGH) for 142 d and follicles were measured ultrasonically and aspirated. The heifers treated with bGH had significantly more follicles than control heifers. In the same study, however, heifers that were restricted to 75% of ad libitum intake and treated with bGH did not have more follicles than heifers fed ad libitum. Buratini et al. (2000) reported that not only did treatment with recombinant bGH result in a 36% increase in the number of small follicles in Nelore heifers, plasma IGF-1 concentrations were increased two-fold. According to Lucy (2000), in vivo administration of recombinant bGH does cause greater ovarian follicular development, and this may be due to either direct effects of GH (given that GH receptors are found within granulosa cells as well as oocytes), or indirectly by increased IGF-1 and(or) nutrient partitioning that occurs after recombinant bGH treatment.

Cochran et al. (1999a) determined that treatment with eGH significantly increased plasma IGF-1 concentrations and increased the number of small follicles on the ovaries of mares treated early in the estrous cycle and at the onset of standing estrus. In contrast to

these results, Scaramuzzi et al (1999) reported that short-term treatment of sheep with ovine GH had no effect on ovulation rate or follicular development. Similarly, Eckery et al. (1994) reported no increase in ovulation rate or number of small antral follicles when ewes were treated with recombinant bGH.

Leptin. It has been known for many years that the amount of body fat (or BC) influences reproductive performance of several species. Many have sought to determine the "signal" that tells the brain that the body is nutritionally fit for reproduction. According to Houseknecht et al. (1998), many believe that leptin, the product of the obese gene, is the long sought indicator of nutritional status that allows reproductive processes to proceed. Leptin is an adipocyte-derived 16 kDa polypeptide that has been found to regulate food intake and thermogenesis in rodents by modulating stimulatory and inhibitory pathways in the feeding circuitry of the hypothalamus (Brunetti et al., 1999). Concentrations of plasma leptin vary directly with body mass index and percentage body fat, and it contributes to the regulation of body weight and intake (Prolo et al., 1998; Chilliard et al., 2000). In fact, in two independent studies in which leptin (recombinant human leptin, Henry et al., 1998; recombinant ovine leptin, Morrison et al., 1998) was infused into the third ventricles of well fed ewes, food intake was reduced. In the study by Morrison et al. (1998), food intake was decreased by d 4 and ceased by d 7 of infusion in well fed ewes. Feed-restricted ewes in the same study, however, limited their intake only while receiving maximum amounts of leptin. Barb et al. (1998) reported similar results in pigs in which intracerebral ventricular injections of leptin caused food intake to decrease substantially in a dose-dependent manner.

Recent studies with cattle and sheep have shown that plasma leptin concentrations are directly related to the plane of nutrition. Ehrhardt et al. (2000) reported that not only is there a direct relationship between leptin and level of nutrition in growing calves and lambs, but plasma leptin is linearly related to fat content of the empty carcass in growing cattle and to BCS in lactating dairy cows. Delavaud et al. (2000) reported similar results for nonlactating, nonpregnant ovariectomized ewes, in which there was a significant positive correlation between body fatness or BCS and plasma leptin concentrations. Like Delavaud et al. (2000), Bocquier et al. (1998) showed that plasma leptin is reduced in sheep fed below liveweight-maintenance requirements, and Amstalden et al. (2000) reported decreased plasma leptin in cattle subjected to a 48 h fast. In feed restricted gilts, leptin as well as plasma IGF-1 and insulin concentrations were lower than in gilts fed 240% of energy requirements for maintenance (Louveau et al., 2000). In obese humans, small reductions (10%) in body weight result in approximately 50% reductions in serum leptin concentrations, and a 10% increase in body weight causes a 300% increase in serum leptin (Houseknecht et al., 1998). Therefore, leptin functions not only as an "adipostat" to signal the status of body energy stores to the brain but also as a sensor of energy balance (Houseknecht et al., 1998).

Plasma concentrations of leptin are also influenced by sex, metabolic hormones, and body energy requirements. In women, circulating plasma leptin concentrations are higher than in men, even after correction for body fat (Castracane et al., 1998; Pineiro et al., 1999). Aging, however, has no apparent effect on serum leptin concentrations in women or men (Castracane et al., 1998; Koistinen et al., 1998). In sheep, Blache et al.

(2000) reported that plasma concentrations of leptin were higher in female sheep than in castrated or intact male sheep. Also, Clarke et al. (2001) showed that in spring, leptin had a profound inhibitory effect on voluntary feed intake in female sheep, but only a slight effect in male sheep. Centrally administered leptin had relatively no effect on feed intake in either sex during autumn (Clarke et al., 2001). In their review of leptin, Fruhbeck et al. (1998) stated that some researchers attribute gender differences to the stimulating role of estrogens and(or) the suppressive effect of circulating androgens, but other investigators have not been able to ascribe the sexual dimorphism to sex hormones.

Insulin, a metabolic protein hormone, may play an important role in the regulation of leptin. Hyperinsulinemia has been shown to increase leptin concentrations within 3 to 5 h in both rats and humans (Cusin et al., 1995; Saladin et al., 1995). More recently, it has been shown that improvements in insulin-stimulated skeletal muscle glucose uptake and transport following chronic leptin treatment result from increased responsiveness to insulin (Yaspelkis et al., 1999). Ceddia et al. (1999) showed that in soleus muscle of rats, leptin exerted a direct and acute insulin-like effect, stimulating glucose uptake, glycogen synthesis, lactate formation and glucose oxidation. Although Marie et al. (2001) reported a positive relationship between plasma leptin and plasma insulin concentrations in sheep exposed to short-term changes in energy balance, Kauter et al. (2000) reported that plasma leptin concentrations were not responsive to short-term changes in blood glucose or insulin in adult merino wethers. Also, Barb et al. (1998) showed that insulin, IGF-1, T_4, glucose and non-esterified fatty acids were not affected by leptin treatment in swine.

Glucocorticoids, in combination with insulin, increase the expression of genes important in regulating lipid deposition in the adipocyte (Papaspyrou-Rao et al., 1997), and glucocorticoids have been shown to be potent regulators of leptin expression (Fruhbeck et al., 1998; Houseknecht et al., 1998). Dexamethasone (DEX) not only increased the relative abundance of leptin mRNA in abdominal and gluteal adipose tissues by about 70%, it also increased serum leptin by 80% and insulin by 83% without affecting serum glucose in humans (Papaspyrou-Rao et al., 1997).

In another study with obese subjects, DEX increased plasma leptin concentrations by 64 to 111% in 2 to 4 d (Dagogo-Jack et al., 1997). It was suggested that increased leptin secretion might be a counter-regulatory attempt to limit glucocorticoid-induced hyperphagia and weight gain (Dagogo-Jack et al., 1997). Russell et al. (1998) reported that DEX increased leptin mRNA almost 2-fold in subcutaneous and omental adipose tissue after 1 d of culture, but leptin secretion was only increased in omental adipose tissue. The same researchers reported that DEX and insulin together increased leptin mRNA and leptin secretion approximately 2 to 3-fold in both omental and subcutaneous adipose tissue at d 1 and maintained leptin expression for 7 more days of culture.

Growth hormone is another hormone that is known to interact with adipose tissue and to induce lipolysis. Adult pituitary GH is affected by metabolic status and body mass. Human GH secretion is suppressed in obesity and is restored after weight loss (Shimon et al., 1998). Miyakawa et al. (1998) suggested that excess GH/IGF-1 reduced serum leptin concentrations by reducing body fat mass (or by unknown mechanisms). Shimon et al. (1998) reported that GH secretion from human fetal pituitary cultures was

stimulated with recombinant human leptin without altering fetal adrenocorticotropic hormone, PRL or gonadotropin secretion. In pigs, Barb et al. (1998) showed that leptin increased GH when it was given at supraphysiological levels, but it reduced GH response to a GH releasing factor. In estrogen-treated yearling wethers, recombinant human leptin prevented the fall in LH pulse frequency that was seen during feed restriction in the vehicle-treated animals (Nagatani et al., 2000). Also, there was a much greater increase in plasma GH concentrations during leptin treatment than occurred with feed restriction alone. Henry et al. (2001) reported similar results in that leptin increased the secretion of LH in feed-restricted sheep and increased plasma GH concentrations irrespective of body weight. Morrison et al. (2001) showed that in diet-restricted lambs treated with leptin, mean GH did not differ on day 0, but increased in response to leptin treatment whereas treatment of fed lambs with leptin did not affect serum GH. According to Nagatani et al. (2000), by regulating both LH and GH secretion in sheep, leptin conveys information about nutrition to mechanisms controlling neuroendocrine function in ruminants. Cunningham et al. (1999), in their review of leptin concur, and state that under conditions of inadequate energy reserves, low leptin concentrations act as a metabolic "gate" to inhibit the activity of the neuroendocrine reproductive axis.

Leptin has been proposed to have numerous effects on reproduction, particularly in rodents. Recent data indicate that leptin may play an important regulatory role in the hypothalamic-pituitary-gonadal axis. A 3-d starvation in rats completely abolished both LH and PRL surges, but leptin resumed these hormonal surges to the levels of normally fed rats (Kohsaka et al., 1999). Also, leptin treatment increased basal LH concentrations

and ovarian and uterine weights, and corrected the sterility defect in female ob/ob mice, while it increased FSH concentrations, testicular and seminal vesical weights, as well as sperm counts in male ob/ob mice (Barash et al., 1996). Rats treated with leptin antiserum showed an impairment of reproductive function with all rats remaining anestrous (Carro et al., 1997).

In human females, leptin increases before appearance of reproductive hormones related to puberty (Garcia-Mayor et al., 1997), and leptin concentrations are higher in the luteal phase than in the follicular phase (Ramsey, 1999). According to Foster and Nagatani (1999), leptin (perhaps in a permissive role) permits pubertal maturation to occur with attainment of energy stores adequate for reproduction. In his review of leptin, Ramsey (1999) states that the effects of leptin on reproduction may be through an alteration of GnRH secretion and hence, LH and FSH secretion. He goes on to state that not only may leptin alter gonadal steroid secretion by indirect mechanisms mediated via the hypothalamic-pituitary axis, but it may also have direct effects on the ovary and ovarian function.

During pregnancy, plasma leptin concentrations have been shown to be high in women (Schubring et al., 1997; Helland et al., 1998; Tamura et al., 1998) and rodents (Kawai et al., 1997; Herrera et al., 2000). However, according to Gonzalez et al. (2000), leptin does not seem to be a critical molecule for implantation, gestation, and parturition in rodents, but in humans, leptin may be considered a novel placental hormone.

Although most of the research with leptin has been conducted in rodents and humans, little work has been reported concerning the role of leptin on reproductive

performance of the horse. To date, the only published research is a study by McManus and Fitzgerald (2000), in which mares were deprived of feed for 24 h and plasma leptin concentrations determined. Although short-term feed restriction was associated with a decrease in serum leptin concentrations, no changes in gonadotropin or PRL secretion were reported to occur. Therefore, many questions still remain about the role of leptin in signaling nutritional status to the brain.

CHAPTER III

THE RELATIONSHIP BETWEEN BODY CONDITION SCORE AND ULTRASONIC BACKFAT MEASUREMENTS IN MARES

Introduction

Research has shown that nutrition plays an important role in the reproductive performance of various species. According to Frisch (1980), a minimum level of BC (the amount of stored fat in an animal's body) is needed to ensure adequate reproductive activity in mammals. The amount of BC can also have an influence on the animal's production and health, as well as reproduction (Perkins, 1985). An accurate assessment of BC would, therefore, be beneficial to producers, by allowing them a way to monitor their animal's health and potentially maximize performance.

Henneke et al. (1983) conducted a study to develop a system for accurate comparison of stored body fat in horses and came up with a scoring system comparable to that used in beef cattle. This system was based on visual appraisal and palpable fat cover at certain areas of the horse's body. In the same study, simple correlations showed BC to be positively related to body fat percentage. The objective of the current study was to correlate BCS in the horse with ultrasonic backfat measurements taken at four locations along the back and to determine which site is the most reliable area of measurement.

Experimental procedures

Animals and treatments. Twenty-four light horse mares (mean BW = 496 kg; mean BCS = 7.0 [range of 6.5 to 8.0]) were grouped by breed and age and randomly allotted to either the HBCS group (8.0 to 8.5) or LBCS group (3.0 to 3.5). Mares were

housed at the Louisiana State University Idlewild Research Station in Clinton, Louisiana, from September to May. The mean ambient temperature from September to May is depicted in Table 3.1.

Mares in the HBCS group were given free choice access to Alicia bermudagrass pasture plus ryegrass pasture supplemented with a good quality bermudagrass hay. Mares in the LBCS group were limit-grazed on Alicia bermudagrass pasture and then ryegrass pasture for decreasing periods of time until the mares reached the desired BCS of 3.0 to 3.5. A good quality bermudagrass hay was supplemented as necessary. All mares had ad libitum access to water and a trace-mineralized salt block (Champions Choice; Cargill, Inc., Minneapolis, MN). Also, all mares were on a normal herd health schedule.

During the study, mares in both groups were dewormed at least once with Quest Paste wormer (Fort Dodge Animal Health, Overland Park, KS). Due to high fecal egg counts, mares in the LBCS group had to be dewormed more frequently over the course of the experiment. Once mares reached their desired BCS (Figure 3.1), they were managed in a manner allowing them to maintain their respective BCS until the end of the project. This experiment was approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

Determination of condition and backfat. Body weights, BCS and backfat data were recorded every 14 d from September to May. Body condition score was determined throughout the experiment by the same technician by visual appraisal and palpation of the six areas suggested by Henneke et al. (1983) as being most indicative of changes in stored body fat (Figure 3.2). Backfat thickness data were obtained using an Aloka SSD 500V

Table 3.1. Mean temperature and average high and low from September to May.

	Temperature (°C)		
Month	Mean	Average High	Average Low
September	24	31	17
October	19	26	22
November	15	22	8
December	11	17	4
January	11	17	5
February	14	21	7
March	17	24	11
April	18	24	11
May	24	31	19

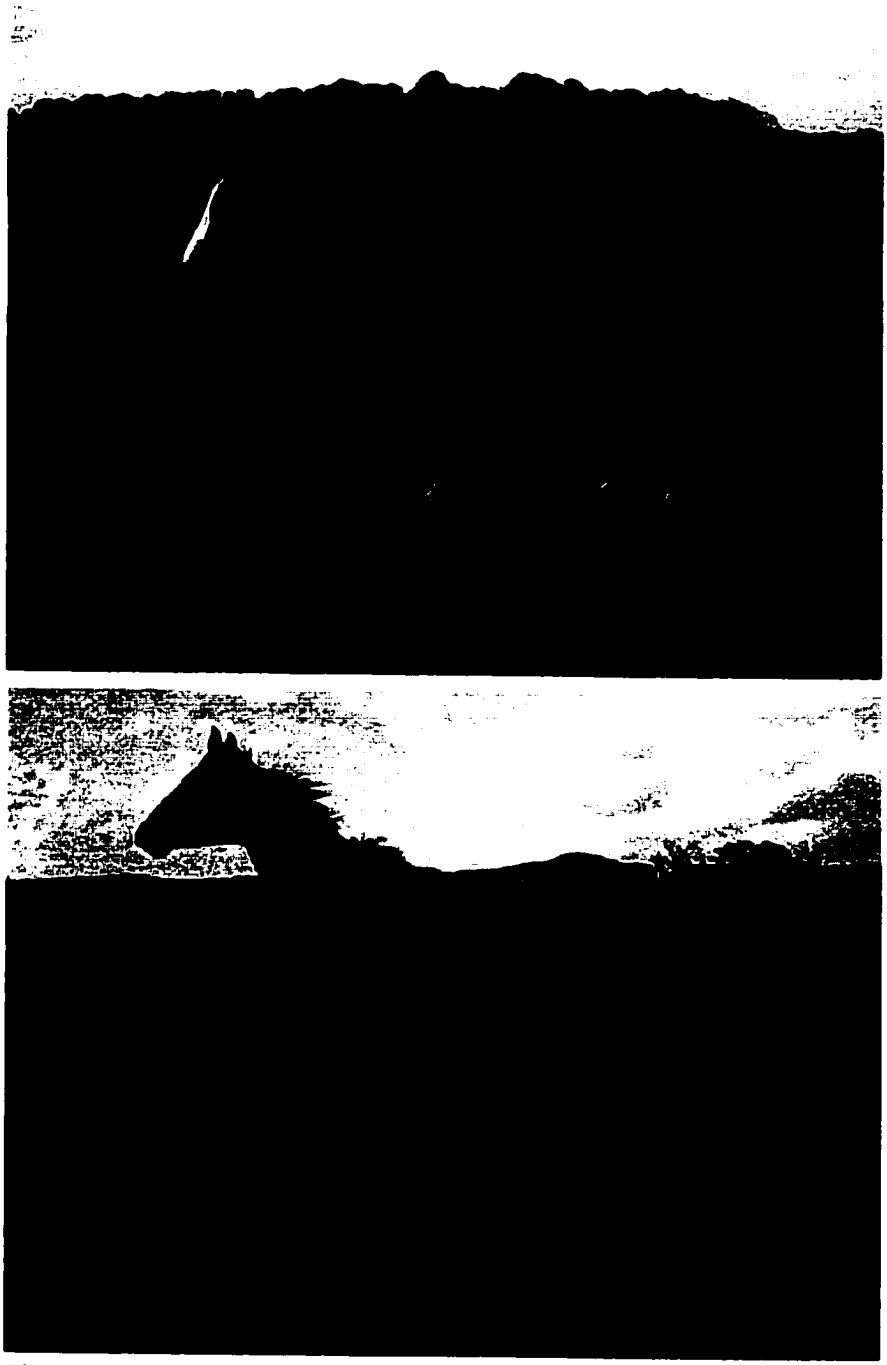

Figure 3.1. Mare of HBCS (BCS of 8.0; top panel) and mare of LBCS (BCS of 3.0; bottom panel).

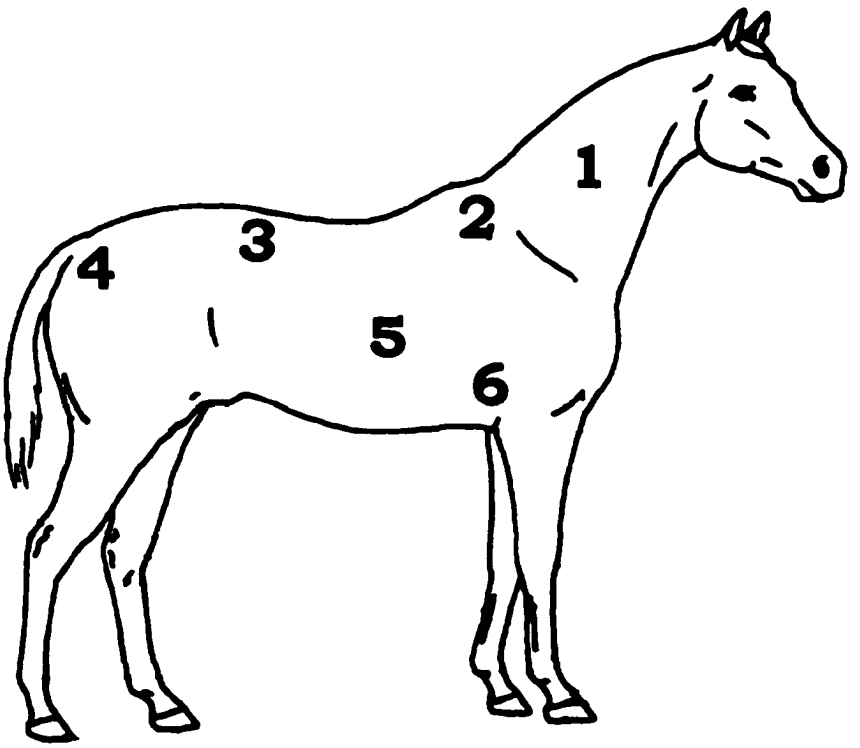

Figure 3.2. Areas used to visually appraise or palpate to estimate body fat and condition score in the horse. 1 = along the neck; 2 = along the withers; 3 = crease down the back; 4 = tailhead; 5 = area of the ribs; 6 = behind the shoulder. Figure taken from Henneke et al. (1983).

ultrasound equipped with a 3.5 megahertz linear-array transducer placed in a transducer guide (Aloka Science and Humanity, Wallingford, CT). All ultrasonic images were presented in B-mode. Four sites on each mare were selected for a series of successive measurements. Each image in the series was recorded on VHS video tape. The images were played back, and fat thickness was measured using the AniMorph computer program Version 1.41, on calibration setting 5044 (Woods Hole Educational Associates, Woods Hole, MA).

All examinations were conducted on the right side of each mare. Site one was on the flat area on the rear of the rump approximately 7.62 cm anterior from the tailhead, the second site was along the mid portion (crown) of the rump, the third site was along the mid-flank (13^{th} rib) and the fourth site was positioned just behind the withers (Figure 3.3). It should be noted that in the original protocol, only the tailhead, rump and 13^{th} rib areas were to be used for determination of backfat measurements. After d 1, it was decided that the withers area should be included in order to get a more complete picture of exactly where fat is deposited and how it is correlated to BCS. Therefore, measurements at the withers did not begin until d 2 of data collection.

Statistical analyses. To determine the correlation between BCS and backfat, data were analyzed using Proc Reg Corr (SAS Inst. Inc., Cary, NC) with both stepwise and backward regressions run on the data. This analysis was done to determine which variables should be included in the model to maximize fit and which variables should be removed from the model because they contributed least to the fit. Once the appropriate model was determined, backfat measurements taken over time were analyzed using

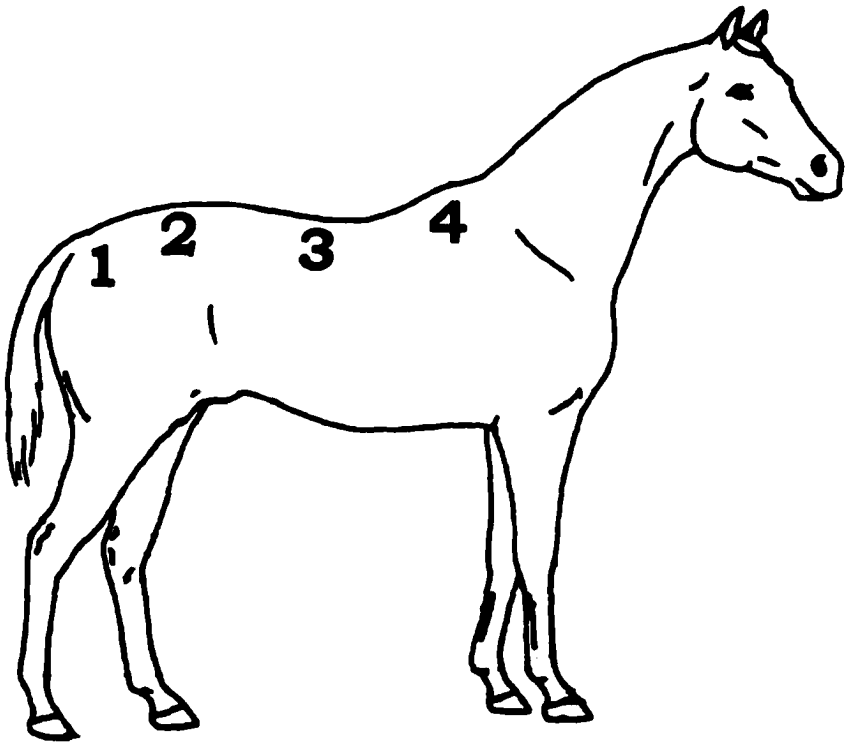

Figure 3.3. The four sites (locations) used to measure backfat thickness in the horse. Backfat thickness data were obtained via ultrasonography. Site 1 = tailhead; Site 2 = rump; Site 3 = 13th rib; Site 4 = withers.

univariate analysis of variance, which took into account the repetitive nature of the sampling (split plot; Gill and Hafs, 1971) and tested treatment, day and the treatment x day interaction.

Results

Average BCS for each of the two groups over time is presented in Figure 3.4. At the beginning of the experiment, all mares had average BCS of 7.0. Over the course of several months, mares in both groups reached their respective desired BCS (3.0 to 3.5 for the LBCS group and 8.0 to 8.5 for the HBCS group). In order to correlate BCS with the amount of backfat, backfat (in cm) at each of the four sites (tailhead, rump, 13th rib and withers) was measured over time, and the average for each group is shown in Figure 3.5. Overall, mares in the HBCS group had more ($P < 0.0001$) backfat than mares in the LBCS group. Also, over time, mares in the LBCS group lost backfat at all four sites and mares in the HBCS group deposited backfat at all four sites ($P < 0.02$; Figures 3.6 to 3.9). In both the HBCS group and the LBCS group, backfat at the rump changed much less over the course of the experiment than did the amount of backfat at the tailhead, 13th rib and withers (Figure 3.10).

Correlation coefficients between BCS and backfat thickness were: 0.87, 0.84, 0.82 and 0.86 for the tailhead, rump, 13th rib and withers, respectively. Stepwise regression analysis showed that the tailhead area accounted for the majority of the variation in BCS with an $r^2 = 0.75$. Overall, 78% of the variation could be accounted for when the 13th rib area and withers area were combined with the tailhead area; however, the rump area did not significantly contribute or account for variation.

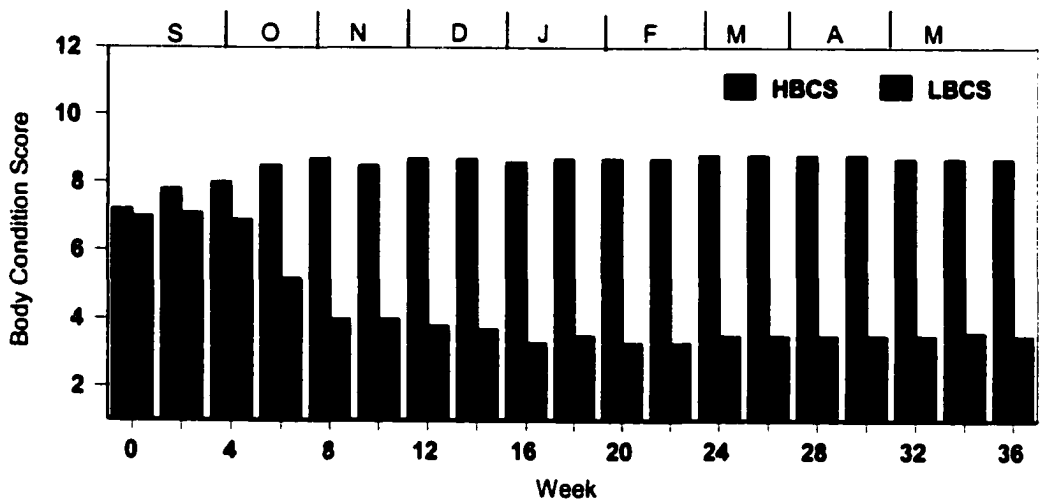

Figure 3.4. Average BCS of mares of HBCS vs mares of LBCS from September to May. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment.

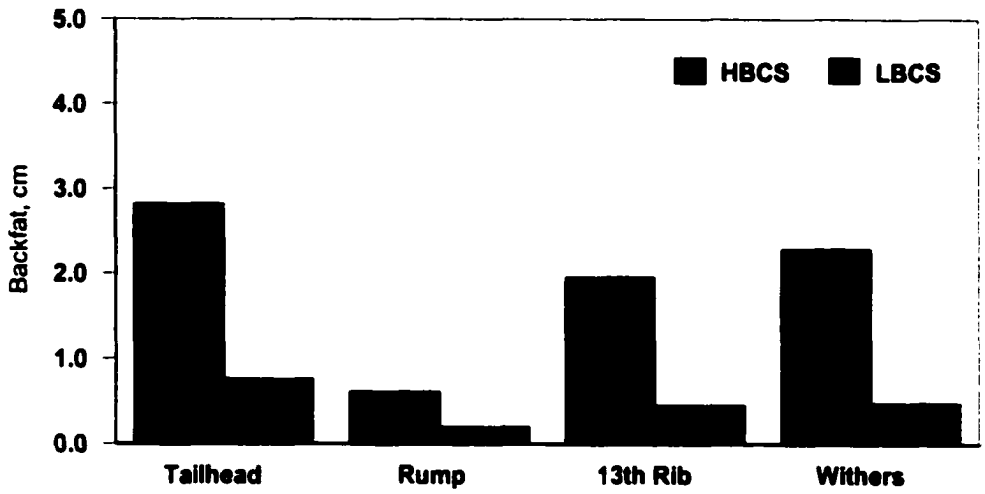

Figure 3.5. Average backfat (in cm) at the tailhead, rump, 13th rib, and withers in mares of HBCS vs mares of LBCS. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Experimental period was from September to May. The pooled SEM was 0.05 cm.

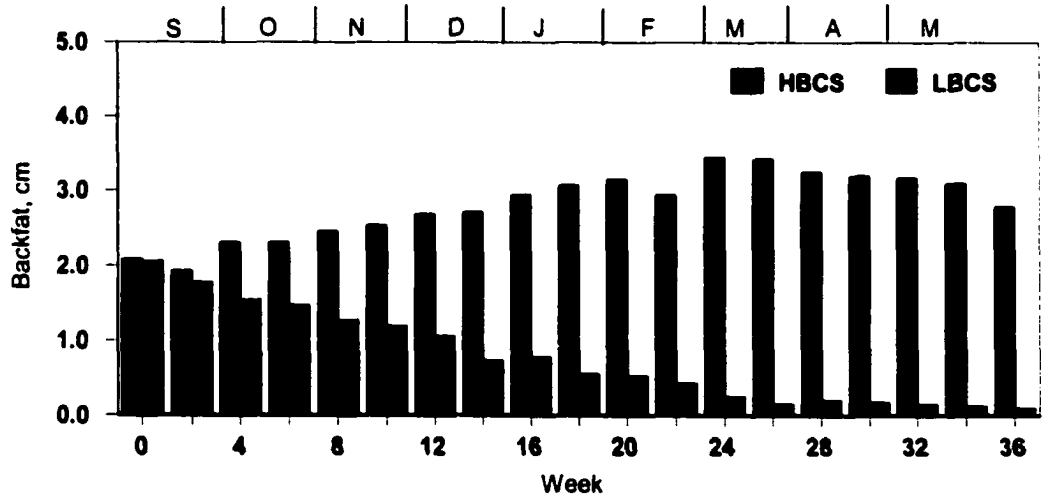

Figure 3.6. Average backfat (in cm) at the tailhead area in mares of HBCS vs mares of LBCS. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Experimental period was from September to May. Months are designated at the top of the graph and correspond to weeks of the experiment. The pooled SEM was 0.12 cm.

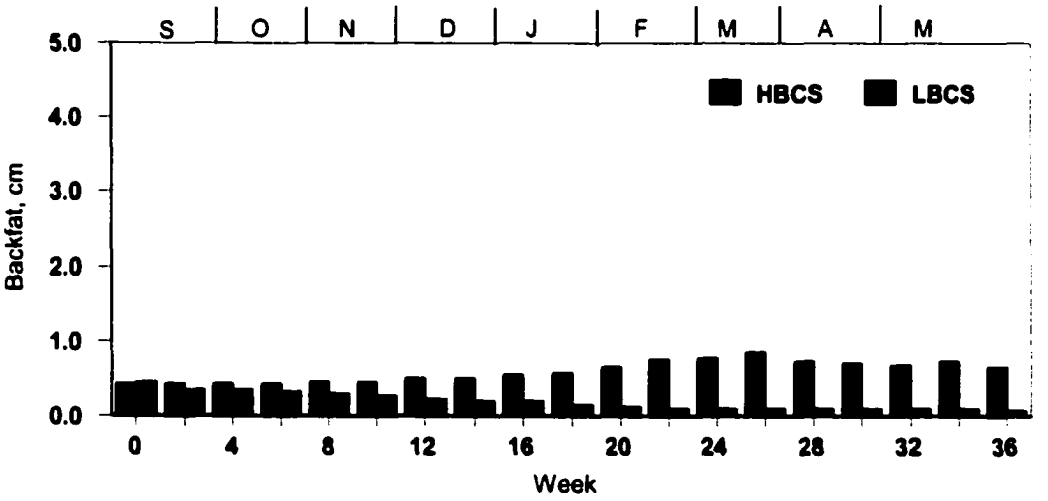

Figure 3.7. Average backfat (in cm) at the rump area in mares of HBCS vs mares of LBCS. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Experimental period was from September to May. Months are designated at the top of the graph and correspond to weeks of the experiment. The pooled SEM was 0.012 cm.

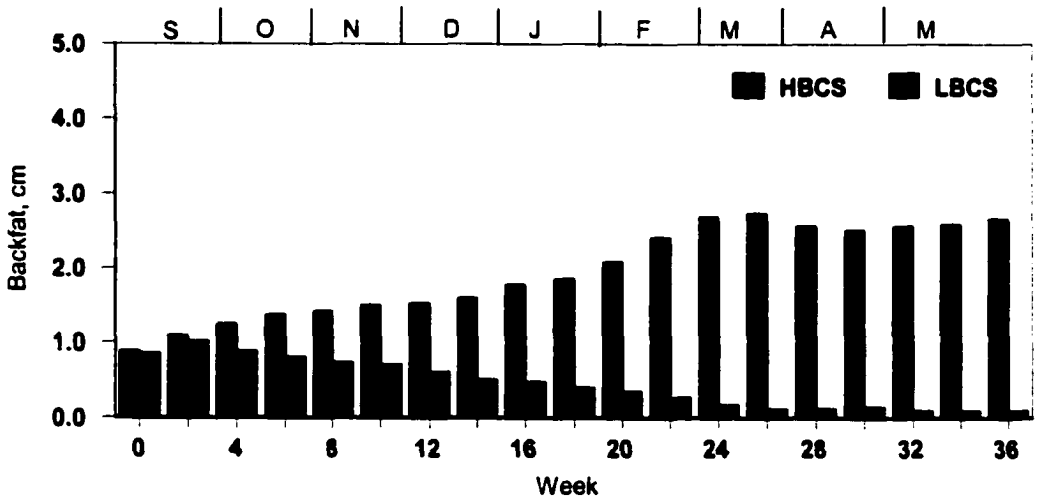

Figure 3.8. Average backfat (in cm) at the 13th rib area in mares of HBCS vs mares of LBCS. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Experimental period was from September to May. Months are designated at the top of the graph and correspond to weeks of the experiment. The pooled SEM was 0.05 cm.

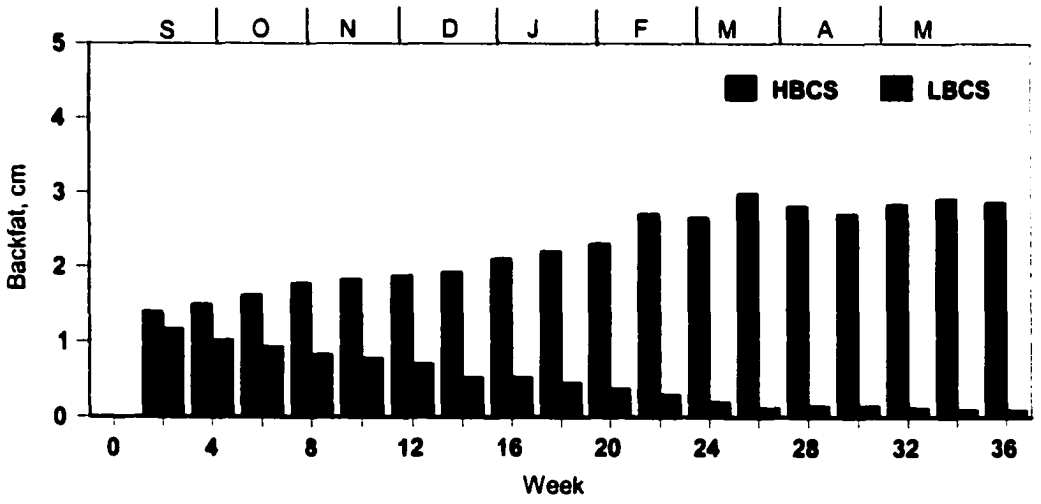

Figure 3.9. Average backfat (in cm) at the withers area in mares of HBCS vs mares of LBCS. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Experimental period was from September to May. Months are designated at the top of the graph and correspond to weeks of the experiment. The pooled SEM was 0.05 cm.

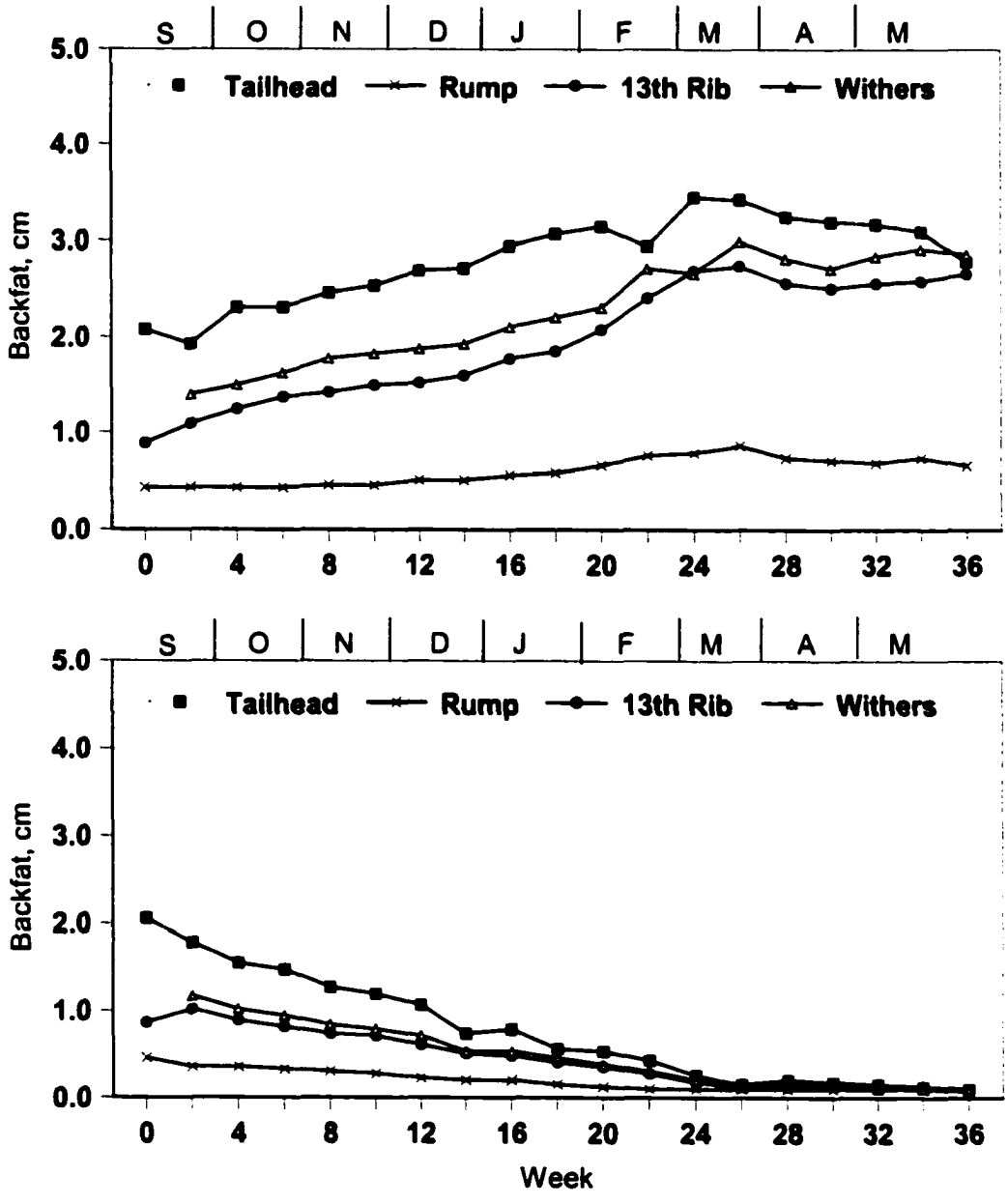

Figure 3.10. Average backfat (in cm) at the tailhead, rump, 13th rib, and withers in mares of HBCS vs mares of LBCS. Mares in the HBCS group (top panel) had average BCS of 8.0 to 8.5. Mares in the LBCS group (bottom panel) had average BCS of 3.0 to 3.5. Experimental period was from September to May. Months are designated at the top of the graph and correspond to weeks of the experiment.

Discussion

In the cattle industry, BCS has emerged as an effective tool for monitoring the energy intake of cows (Ducker et al., 1985; Treacher et al., 1986; Reneau and Linn, 1989). Research and field experience have shown that BC, or the degree of fattening, can influence the animal's production, reproduction and health (Perkins et al., 1985). In all animals, body fat reserves are important because fat represents the amount of energy reserves the animal has for periods of stress. If fat reserves are low and energy is needed, protein will be broken down from muscle to meet the energy requirements. Using the BCS system developed by Henneke et al. (1983), mares in this study started the project at an average BCS of 7.0. The mares were fleshy with a noticeable filling between the ribs with fat. Mares in the LBCS group were limit grazed until they reached a BCS of 3.0 to 3.5. These mares became thin with slight fat cover over the ribs, the ribs easily discernable and the tailhead prominent. Mares in the HBCS group became very fat with areas such as the tailhead, withers and neck filled with fat (BCS of 8.0 to 8.5). The differences seen in BCS of these mares is reflected in the amounts of backfat measured in each group. Since the BCS system developed by Henneke et al. (1983) is based on visual appraisal as well as palpable fat cover, it makes sense that mares in the HBCS group would have more backfat overall than LBCS mares. Several studies in cattle have shown that the use of ultrasonic backfat measurements (as well as marbling and longissimus muscle area) are very useful in predicting carcass merit at slaughter (Perkins, 1992; Herring et al., 1994; Brethour 2000).

It is interesting that over time, the amount of backfat changed only a very small amount over the rump area, whereas the amount of change at the other three locations was much greater. This indicates that the rump would not be a good indicator of BCS in the horse. This is in contrast to the study conducted by Westervelt et al. (1976) in which the rump area of the horse was indicative of total body fat. In a study by Kane et al. (1987), actual backfat thickness and ultrasonic fat thickness were measured at five sites over the croup, with site 1 being closest to the tailhead area and site 5 being closest to the top of the rump area. In their study, fat thickness was greatest at site 1 and progressively decreased toward site 5 in mares of good BC. They found that ultrasound determination of fat thickness correlated well at sites 1 through 4, however, the poorer correlation of ultrasound measurement to empty body fat at site 5 was attributable to the decrease in fat thickness.

In our study, the rump area (which would correspond closely to their site 5) was also the site with the least amount of backfat (especially when mares were average BCS). Regression analysis showed that backfat measurements at the tailhead were most representative of BCS and least representative at the rump. These data are in agreement with Kane et al. (1987). In most species, fat is laid down from front to back and top to bottom. In our study, the tailhead area represented the most change in backfat as BCS changed. Therefore, as the animal gets progressively fatter, the tailhead area acts as a repository for the fat. Also, as the animal gets progressively thinner, more fat is removed from the tailhead area. Backfat at the 13th rib and withers also changed with changes in BCS, however, the magnitude of the change was less than at the tailhead.

Implications

Due to the importance of body condition to the general overall health of the animal, as well as the reproductive correlations to body condition that exist for several species, it is essential that producers be able to accurately assess the body condition of their animals. Data obtained in this experiment show that body condition score and backfat measurements are correlated, especially at the tailhead area, and that by using the system developed by Henneke et al. (1983), as well as putting added emphasis on the amount of fat in areas such as the tailhead, 13th rib and withers, producers should have an accurate indication of the condition and nutritional health of their horses.

CHAPTER IV

THE RELATIONSHIP BETWEEN BODY CONDITION AND REPRODUCTIVE AND HORMONAL CHARACTERISTICS OF MARES DURING THE SEASONAL ANOVULATORY PERIOD

Introduction

Inadequate nutrition impairs reproductive function in many farm animals (Rutter and Randel, 1984; Schillo, 1992), including delayed onset of puberty of ruminants (Short and Bellows, 1971; Day et al., 1986), induction of anestrus in cyclic females (Richards et al., 1989; Rhodes et al., 1996) and prolonged postpartum anestrus (Wiltbank et al., 1964; Selk et al., 1988). A similar relationship between inadequate nutrition and reproductive inefficiency has been observed in the horse (Henneke et. al, 1983, 1984; Hines et al., 1987). In both cattle and sheep, dietary energy restriction suppresses episodic release of LH (Schillo, 1992); however, interrelationships among the reproductive hormones, such as LH and FSH, are much less understood in the mare (Hines et al., 1987). Research concerning mares that are overly fat is not only limited, but conflicting, particularly for mares at the onset of the breeding season or during gestation (Henneke et al., 1984). Thus, the objective of the present experiment was to contrast the reproductive and hormonal profiles of mares of LBCS vs HBCS during the seasonal anovulatory period.

Experimental procedures

Animals and treatments. Twenty-four light horse mares (mean BW = 496 kg; mean BCS = 7.0 [range of 6.5 to 8.0]) were grouped by breed and age and randomly allotted to either the HBCS group (8.0 to 8.5) or LBCS group (3.0 to 3.5). Mares were

housed at the Louisiana State University Idlewild Research Station in Clinton, Louisiana, from September to January (period of increasing or decreasing weight and BCS). The mean ambient temperature from September to January is depicted in Table 4.1.

Mares in the HBCS group were given free choice access to Alicia bermudagrass pasture plus ryegrass pasture supplemented with a good quality bermudagrass hay. Mares in the LBCS group were limit-grazed on Alicia bermudagrass pasture and then ryegrass pasture for decreasing periods of time until the mares reached the desired BCS of 3.0 to 3.5. A good quality bermudagrass hay was supplemented as necessary. All mares had ad libitum access to water and a trace-mineralized salt block (Champions Choice; Cargill, Inc., Minneapolis, MN). Also, all mares were on a normal herd health schedule.

During the study, mares in both groups were dewormed at least once with Quest Paste wormer (Fort Dodge Animal Health, Overland Park, KS). Due to high fecal egg counts, mares in the LBCS group had to be dewormed more frequently over the course of the experiment. This experiment was approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

Determination of condition and stage of cyclicity. Body weights and BCS were recorded weekly throughout the study. Body condition score was determined each time by the same technician by visual appraisal and palpation of the six areas suggested by Henneke et al. (1983) as being most indicative of changes in stored body fat. Once the mares approached their target BCS (December), ultrasound scans and teasing with a stallion began and continued throughout the experiment. Mares were teased every 72 h (after blood sampling) by one of two stallions. Immediately after teasing, all mares'

Table 4.1. Mean temperature and average high and low from September to Janurary.

	Temperature (°C)		
Month	Mean	Average High	Average Low
September	24	31	17
October	19	26	22
November	15	22	8
December	11	17	4
January	11	17	5

ovaries were assessed for ovary size, follicle number and size, number of ovulations and number of CL. Transrectal ultrasonagraphy was performed with the use of an Aloka 550V ultrasound machine with a 5 megahertz linear-array transducer (Aloka Science and Humanity, Wallingford, CT). Follicles were assigned to categories based on their size (small $\leq$ 10 mm, medium 11 to 19 mm, large $\geq$ 20 mm). Once a follicle reached 30 mm, that mare's ovaries were examined daily until the follicle ovulated or regressed to below 20 mm.

Blood sampling and challenges. Beginning in September, weekly blood samples were collected at 0730 h by jugular venipuncture into two 7-mL evacuated tubes (one containing potassium oxalate and sodium fluoride and one containing sodium heparin (Vacutainer; Becton and Dickinson, Franklin Lakes, NJ). These samples were centrifuged and the plasma harvested and stored at -15°C in 12 mm x 75 mm polystyrene tubes until analyzed for progesterone (P_4), LH, FSH, GH, IGF-1, PRL, thyroid stimulating hormone (TSH), insulin and glucose concentrations. Luteinizing hormone, FSH, GH, IGF-1, PRL, TSH and insulin were analyzed by RIA as previously described for horses (Thompson et al., 1983a,b; Colborn et al., 1991; Thompson et al., 1992; DePew et al., 1994; Sticker et al, 1995; Sticker et al., 2001). Glucose concentrations were determined colorimetrically using commercial kits (Sigma Tech. Bull. No. 315; Sigma Chemical, St. Louis, MO).

Also, during the month of January, several hormonal challenges were conducted to determine how BC affects hormonal profiles of these mares. On January 7, mares were catheterized, allowed to rest 60 min, and an i.v. sulpiride challenge conducted. Blood

samples were collected at -15, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min relative to 0.1 mg/kg sulpiride administered at 0 min. Samples were centrifuged and the plasma stored at -15°C until analyzed for PRL concentrations.

On January 12, mares were again catheterized, allowed to rest for 60 min and blood samples collected every 15 min for 12-h. This was done to determine the patterns of basal reproductive hormone concentrations during the day. Samples were centrifuged and the plasma frozen and later analyzed for LH, FSH, PRL and GH concentrations as described earlier. Immediately following the last blood sample of the 12-h window of frequent blood sampling, mares were given 10 μg/kg BW of EP51389 i.v. (Deghenghi, 1997), a potent GH secretagogue. Blood samples were then collected at 10, 20, 30, 40, 50, 60, 75 and 90 min after secretagogue injection. Blood samples were centrifuged and the plasma stored at -15°C and later analyzed for GH concentrations.

On January 19, mares were once again catheterized, allowed to rest 60 min and a GnRH/TRH challenge conducted. Blood samples were collected at -10, 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min with mares receiving 1 μg/kg BW GnRH and 4 μg/kg BW TRH (in a single injection) at 0 min. Blood samples were centrifuged and the plasma stored at -15°C and later analyzed for LH, FSH, PRL and TSH as previously described.

Statistical analyses. Data were analyzed by using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Follicle data (number of follicles, number of follicles within a size category and number of CL/treatment group) were analyzed as a completely randomized design by analysis of variance (Steele and Torrie, 1980). Hormone data as

well as glucose data taken over many days throughout the experiment were analyzed by univariate analysis of variance that took into account the repetitive nature of the sampling (split plot; Gill and Hafs, 1971) and tested treatment, day and the treatment x day interaction. Hormonal data from the various bleeding challenges was also analyzed as a split-plot design which tested treatment, time and the treatment x time interaction.

Results

From September to the end of December, body weights increased in mares in the HBCS group and decreased in mares in the LBCS group ($P < 0.005$, Figure 4.1). Once weight and BCS stabilized within groups, mares were maintained throughout the experiment at their respective body weights.

Average P_4 concentrations from September to January are shown in Figure 4.2. Follicle data (during January) are shown in Table 4.2. Mares in the HBCS group continued to ovulate, as evidenced by higher P_4 concentrations ($P < 0.034$), a greater number of CL ($P < 0.006$; HBCS = 5, LBCS = 0) and during January, a greater number of large follicles ($P < 0.008$). Although data for this experiment were collected and analyzed from September to January, mares were subsequently followed with ultrasonography and blood sampling (for P_4 analysis) until late spring when mares would normally have begun to cycle. One mare in the HBCS did go anestrus (mid November until late spring) and four mares did go through a very brief period with low P_4 concentrations (late December to mid February); however, they continued to have several large follicles.

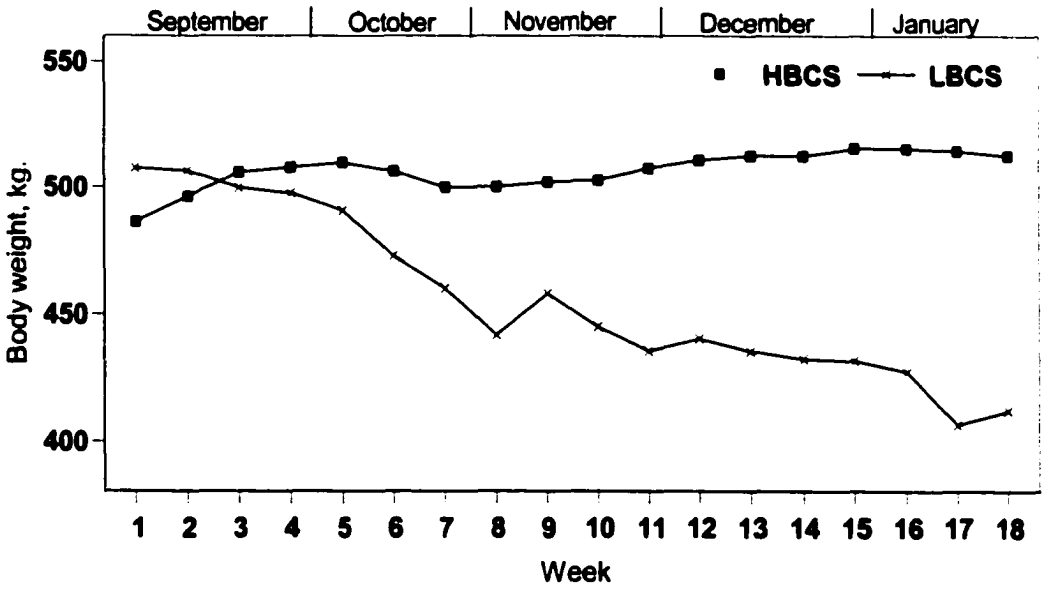

Figure 4.1. Body weight of mares of HBCS vs mares of LBCS from September to January. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment. There was an effect of BCS ($P < 0.005$) in the analysis of variance. The pooled SEM was 3.1 kg.

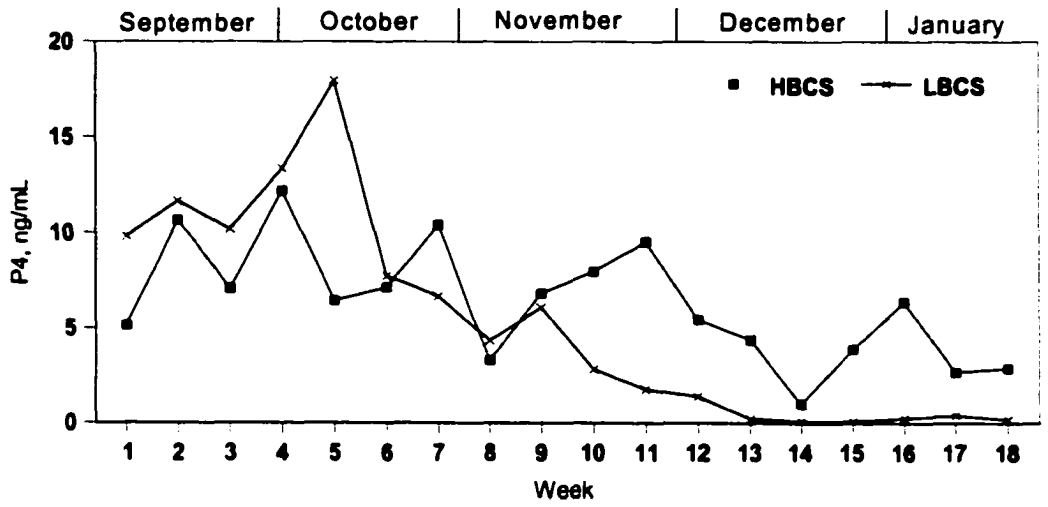

Figure 4.2. Weekly P_4 concentrations in mares of HBCS vs mares of LBCS from September to January. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment. There was an effect of BCS ($P < 0.034$) in the analysis of variance. The pooled SEM was 0.03 ng/mL.

Table 4.2. Mean follicle diameter of HBCS vs LBCS mares during January.

Follicle Size[a]	Treatment Group[b]	Mean Follicle Diameter	± SEM[c]
Large Follicles[d]	HBCS	0.86	0.20
	LBCS	0.03	0.20
Medium Follicles[e]	HBCS	4.75	0.69
	LBCS	2.90	0.68
Small Follicles	HBCS	9.19	0.59
	LBCS	7.86	0.59

[a] Follicle Size: large = follicle > 20 mm; medium = follicle >10 mm and ≤ 19 mm; small = follicle ≤ 10 mm.
[b] HBCS = high body condition score treatment group; LBCS = low body condition score treatment group.
[c] Standard error of the mean.
[d] BCS effect, ($P < 0.008$).
[e] BCS effect, ($P = 0.07$).

None of the mares in the LBCS group cycled (P_4 values < 1 ng/mL with ovaries very small and inactive) from October until April and into May. However, some of the mares did show a behavioral type estrus when exposed to the stallion. This behavior was characterized by the mares squatting, urinating and winking as soon as they were aware of the stallion's presence.

Blood samples collected weekly were analyzed to characterize baseline concentrations of hormones in LBCS mares compared to HBCS mares. Figure 4.3 shows data for LH and FSH, Figure 4.4 shows data for PRL and TSH and Figure 4.5 shows data for GH and IGF-1. No differences between treatments were detected for LH, FSH, TSH or GH. Plasma PRL was higher ($P < 0.02$) in the HBCS group and decreased over time ($P < 0.008$). Insulin-like growth factor-1 tended ($P = 0.1$) to be higher in the HBCS group than the LBCS group.

Plasma glucose and insulin concentrations are shown in Figure 4.6. No differences ($P > 0.1$) were detected between treatments for either glucose or insulin over the course of the experiment. However, both HBCS mares and LBCS mares had a spike in insulin concentrations on January 5.

Plasma PRL concentrations increased ($P < 0.0001$) within 15 min after sulpiride injections in both groups (Figure 4.7, top panel), but no differences ($P > 0.1$) were detected due to BCS. Mares in the HBCS group exhibited a greater ($P < 0.0001$) release of PRL in response to the TRH challenge than did mares in the LBCS group (Figure 4.7, bottom panel). Thyroid stimulating hormone response to TRH was not affected by BC (data not shown). Luteinizing hormone and FSH were both affected by BC in response to

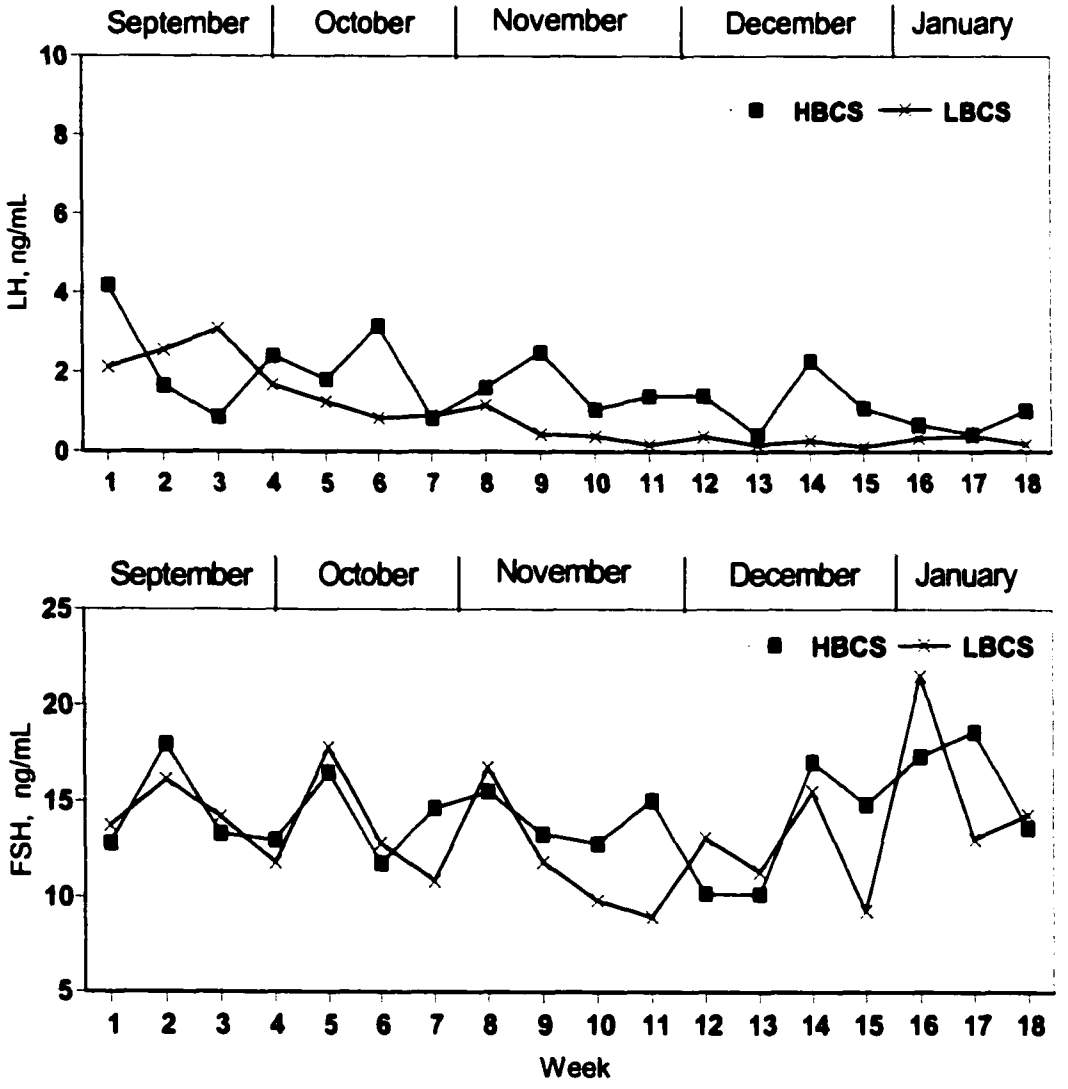

Figure 4.3. Weekly LH (top panel) and FSH (bottom panel) concentrations in mares of HBCS vs mares of LBCS from September to January. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment. There were no differences ($P > 0.1$) between groups for LH or FSH. The pooled SEM were 0.35 and 1.80 ng/mL for LH and FSH concentrations, respectively.

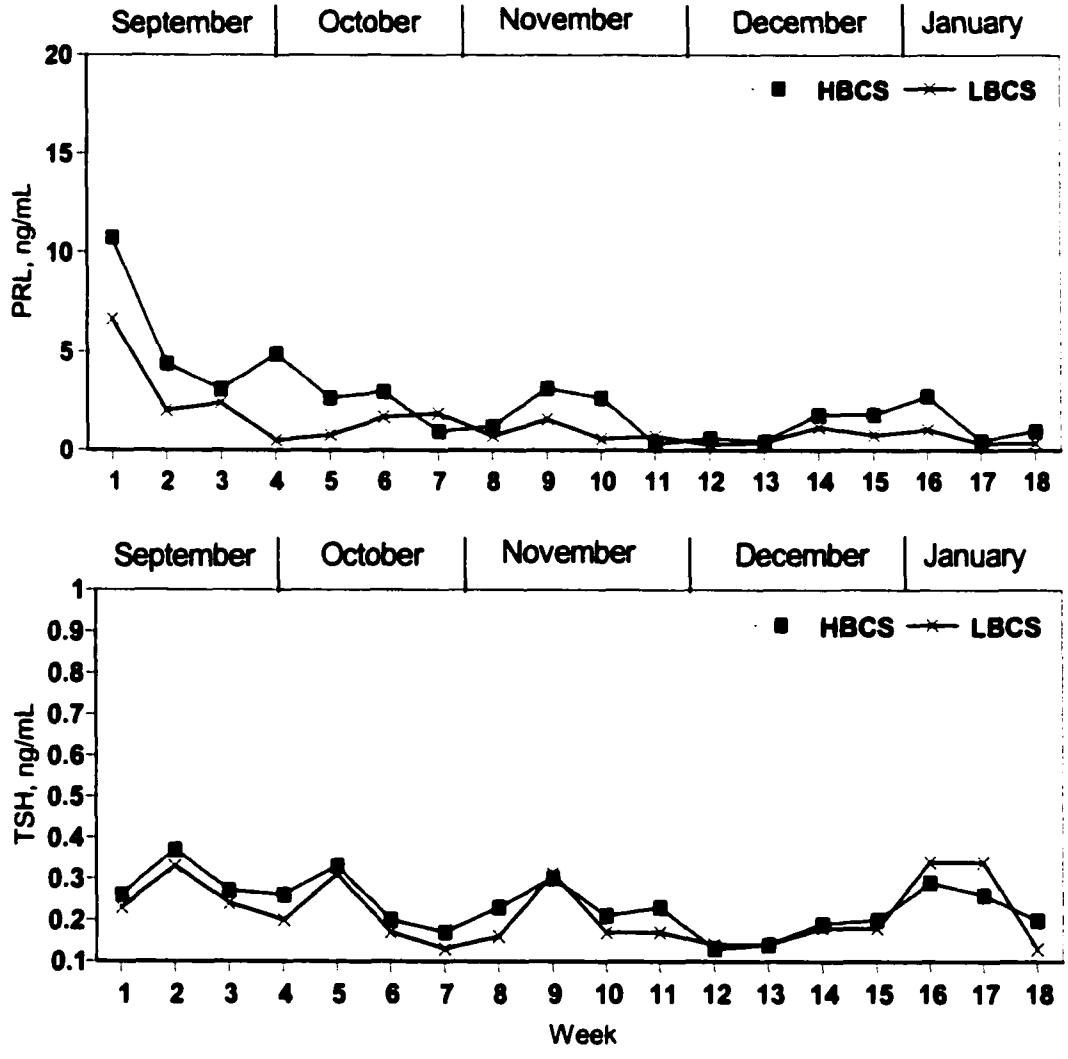

Figure 4.4. Weekly PRL (top panel) and TSH (bottom panel) concentrations in mares of HBCS vs mares of LBCS from September to January. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment. There was an effect of BCS ($P < 0.02$) and day ($P < 0.008$) for PRL concentrations, but there was no difference ($P > 0.1$) between groups for TSH concentrations in the analysis of variance. The pooled SEM were 0.31 and 0.03 ng/mL for PRL and TSH concentrations, respectively.

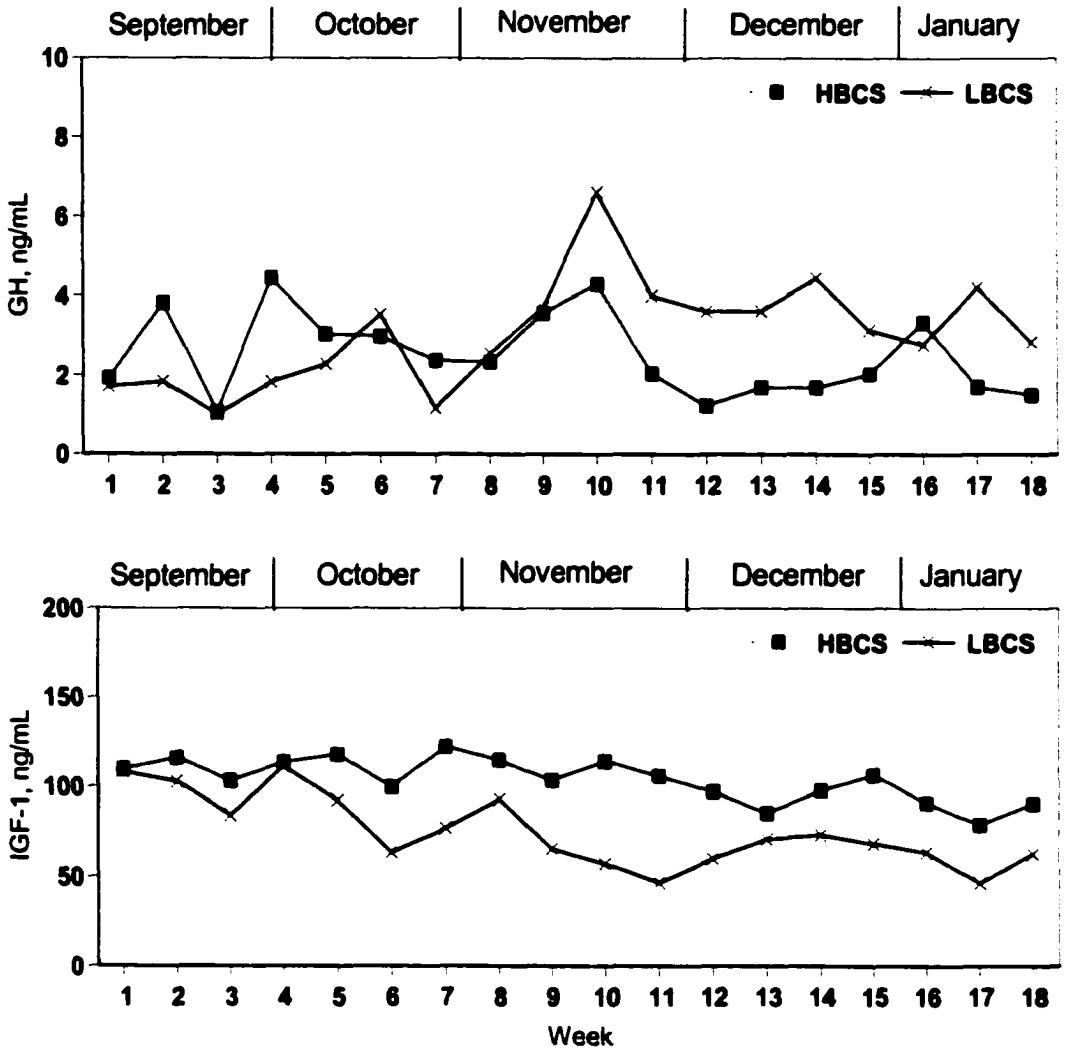

Figure 4.5. Weekly GH (top panel) and IGF-1(bottom panel) concentrations in mares of HBCS vs mares of LBCS from September to January. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment. There was no difference ($P > 0.1$) between groups for GH concentrations in the analysis of variance. Insulin-like growth factor 1 tended ($P = 0.1$) to be higher in the HBCS mares. The pooled SEM were 3.40 and 13.10 ng/mL for GH and IGF-1 concentrations, respectively.

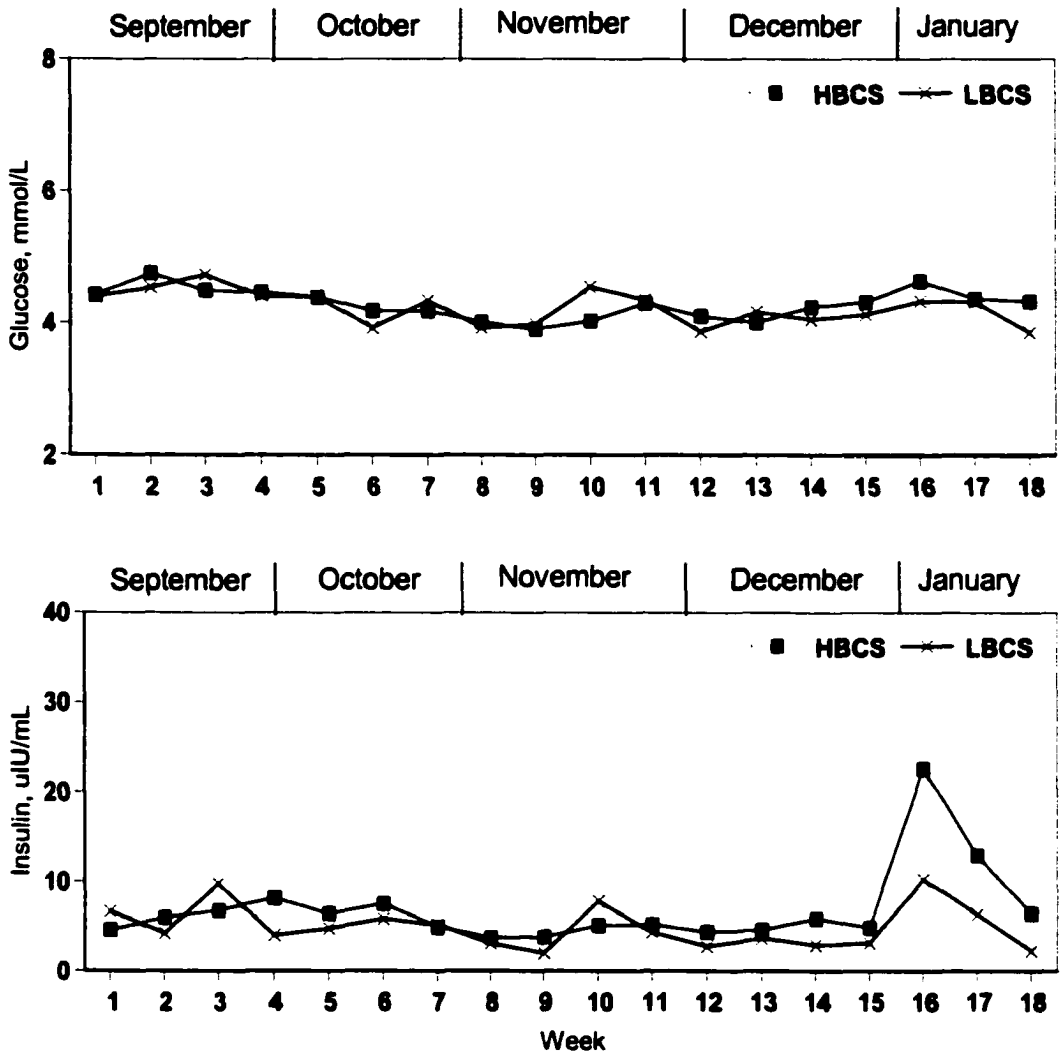

Figure 4.6. Weekly glucose (top panel) and insulin (bottom panel) concentrations in mares of HBCS vs mares of LBCS from September to January. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. Months are designated at the top of the graph and correspond to weeks of the experiment. There was no difference ($P > 0.1$) between groups for glucose or insulin concentrations in the analysis of variance. The pooled SEM were 0.06 mmol/L and 0.82 uIU/mL for glucose and insulin concentrations, respectively.

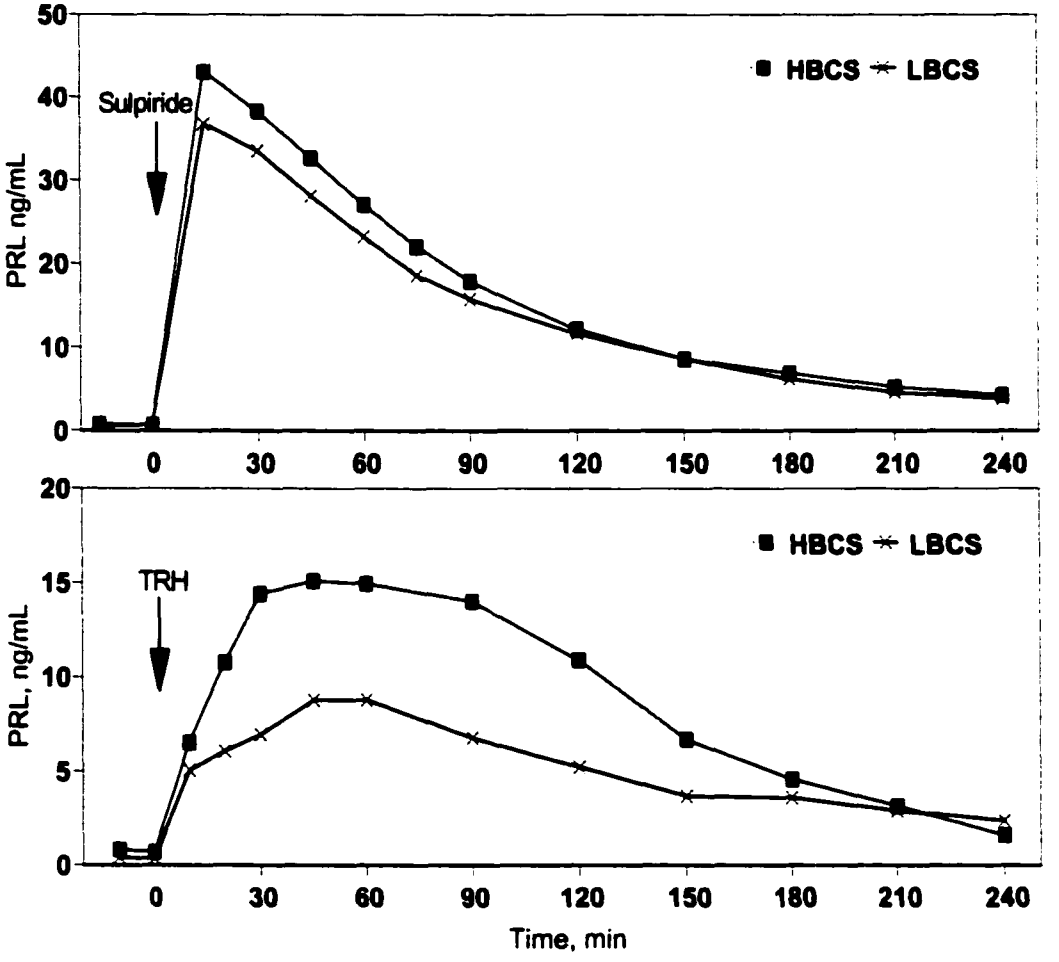

Figure 4.7. Plasma PRL concentrations (top panel) in response to a sulpiride challenge and plasma PRL concentrations (bottom panel) in response to a TRH challenge in mares of HBCS vs mares of LBCS in January. Sulpiride (0.1mg/kg BW) was administered on January 7, 2000; TRH (1 ug/kg BW) was administered on January 19, 2000. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. There was no difference ($P > 0.1$) between groups, but there was a time effect ($P < 0.0001$) for PRL concentrations in response to sulpiride. There was an effect of BCS ($P < 0.01$) for PRL concentrations in response to TRH in the analysis of variance. The pooled SEM were 1.20 and 0.85 ng/mL for PRL concentrations during sulpiride and PRL concentrations during TRH, respectively.

the GnRH challenge, with LH rising to 4 ng/mL post-injection in the HBCS group ($P < 0.0001$) vs < 1.0 ng/mL in the LBCS group (Figure 4.8, top panel). The FSH response to GnRH was reversed, being lower ($P < 0.002$) in the HBCS group compared to the LBCS group (Figure 4.8, bottom panel). During the 12-h period of frequent blood sampling, LH and FSH concentrations were higher ($P < 0.0001$), PRL concentrations were not different ($P > .1$), and GH concentrations were lower ($P < 0.0001$) in the HBCS vs LBCS group (Figure 4.9 and 4.10). The GH response to EP51389 was also lower ($P < 0.0001$) in the HBCS vs LBCS group (Figure 4.11).

Discussion

Mares in the LBCS group had low P_4 concentrations, lacked significant follicular activity and were anestrous for six to seven months, whereas, most of the mares in the HBCS group continued to cycle throughout and did not appear to be affected adversely by being overly conditioned. Henneke et al. (1984) showed that breeding efficiency was enhanced in mares entering the breeding season or foaling at a BCS of 5.0 or greater. These researchers also reported that initial excess stores of body fat did in fact enhance fertility. Also, in the study by Henneke et al. (1984), nonfoaling mares with BCS > 5.0 had average intervals from February 1 to first estrus and first ovulation of 26 and 37 d, respectively, whereas, corresponding intervals for mares with BCS < 5.0 were much longer (39 and 63 d, respectively). The mares in this study were followed with ultrasound, teasing and blood sample collection for P_4 analysis until all mares went through transition and ovulated or until the end of May. Generally, the HBCS mares continued to cycle with only one of the HBCS mares going into a classical anestrous

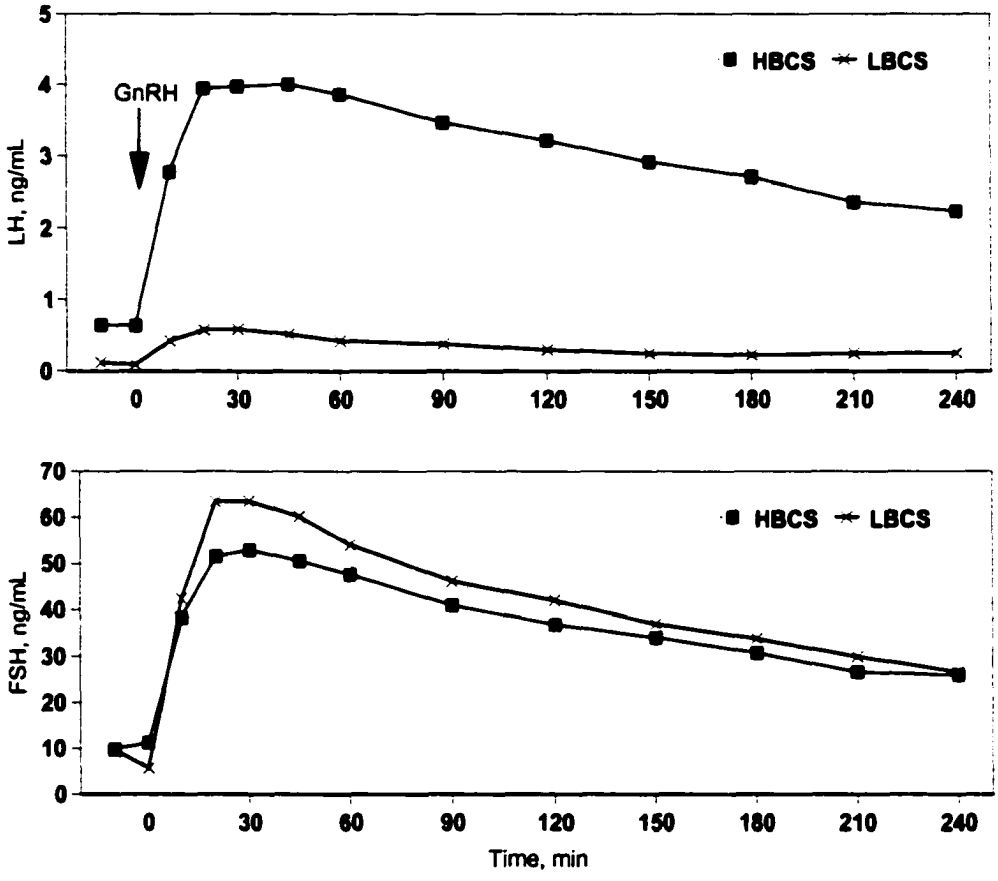

Figure 4.8. Plasma LH (top panel) and FSH (bottom panel) concentrations in response to a GnRH challenge in mares of HBCS vs mares of LBCS in January. The GnRH (1 ug/kg BW) was administered on January 19, 2000. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. There was an effect of BCS ($P < 0.02$) for LH concentrations in the analysis of variance. There was also an effect of BCS ($P < 0.002$) for FSH concentrations in the analysis of variance. The pooled SEM were 0.72 and 1.30 ng/mL for LH and FSH concentrations, respectively.

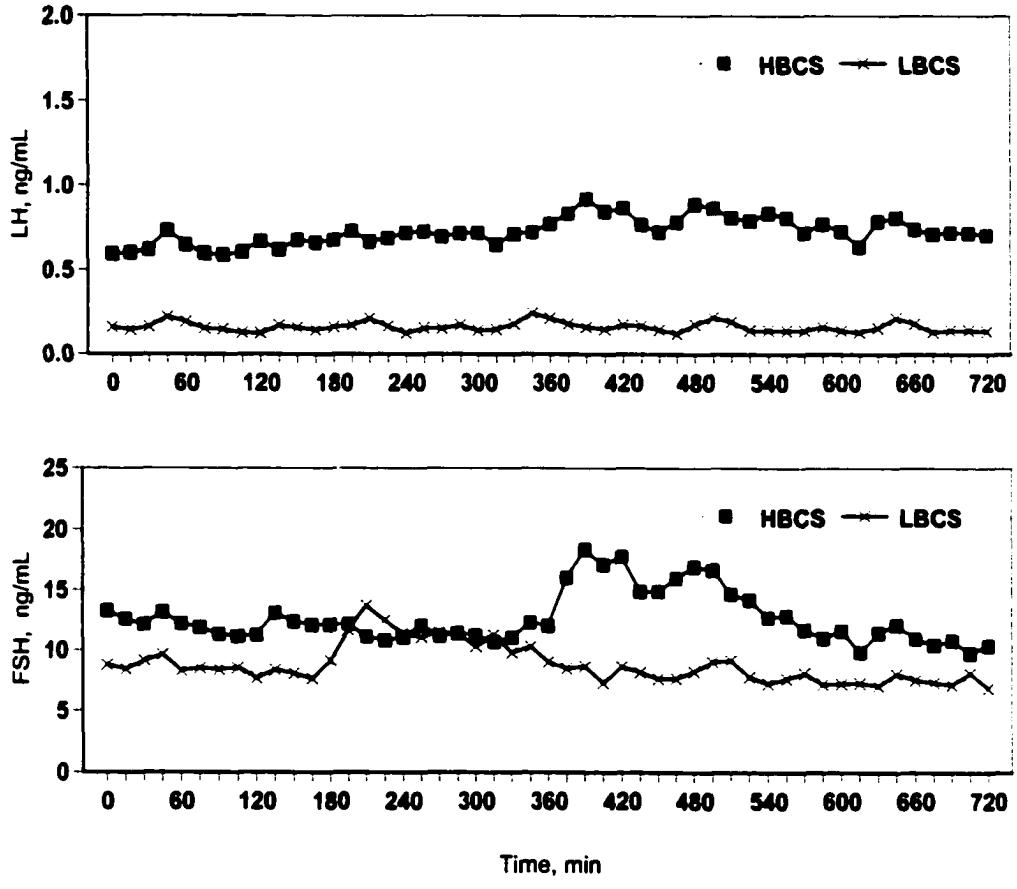

Figure 4.9. Plasma LH (top panel) and FSH (bottom panel) concentrations over 12 h in mares of HBCS vs mares of LBCS in January. The 12 h period of blood sampling was conducted on January 12, 2000. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. There was an effect of BCS ($P < 0.0001$) for both LH and FSH concentrations in the analysis of variance. The pooled SEM were 0.24 and 2.13 ng/mL for LH and FSH concentrations, respectively

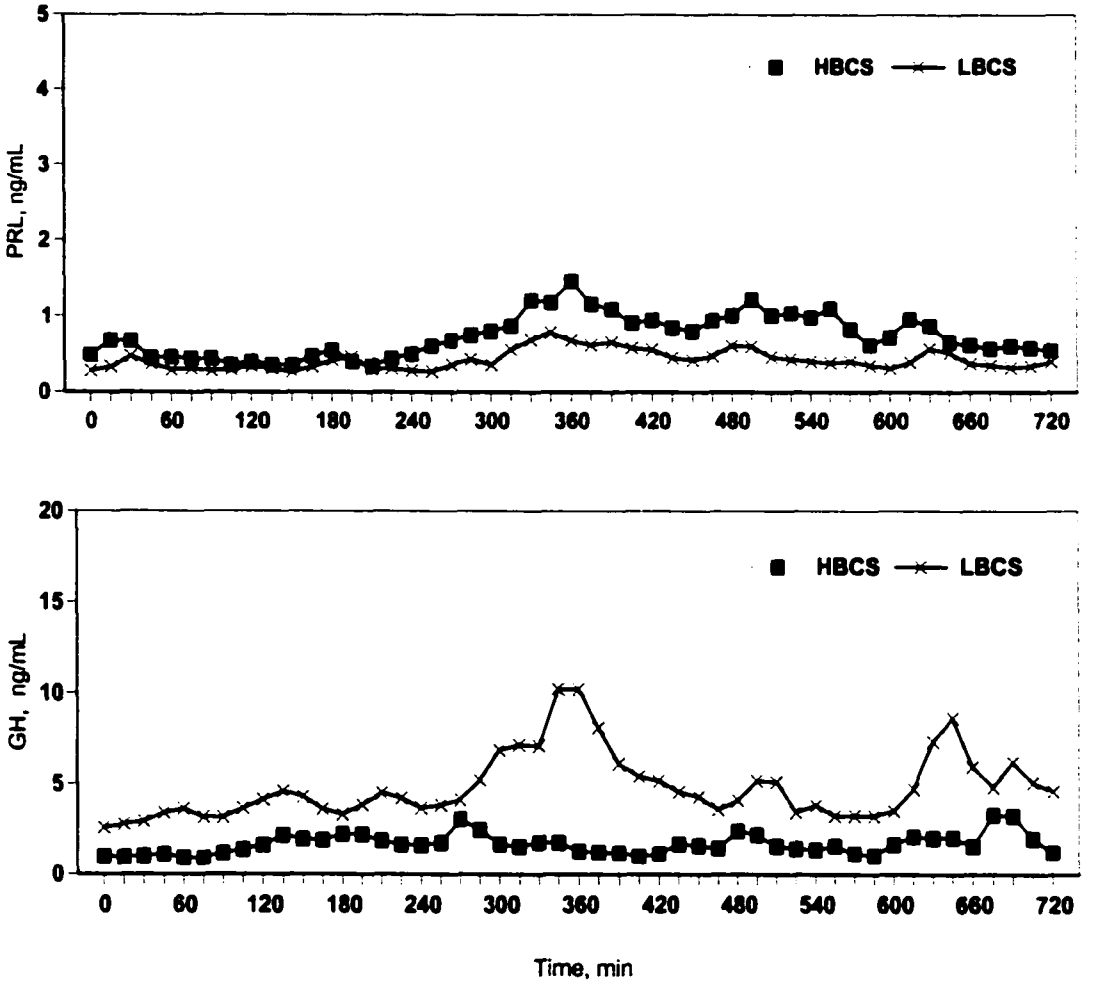

Figure 4.10. Plasma PRL (top panel) and GH (bottom panel) concentrations over 12 h in mares of HBCS vs mares of LBCS in January. The 12 h period of blood sampling was conducted on January 12, 2000. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. There was no difference ($P > 0.1$) between groups for PRL concentrations, but there was an effect of BCS ($P < 0.0001$) for GH concentrations in the analysis of variance. The pooled SEM were 0.92 and 0.93 ng/mL for PRL and GH concentrations, respectively.

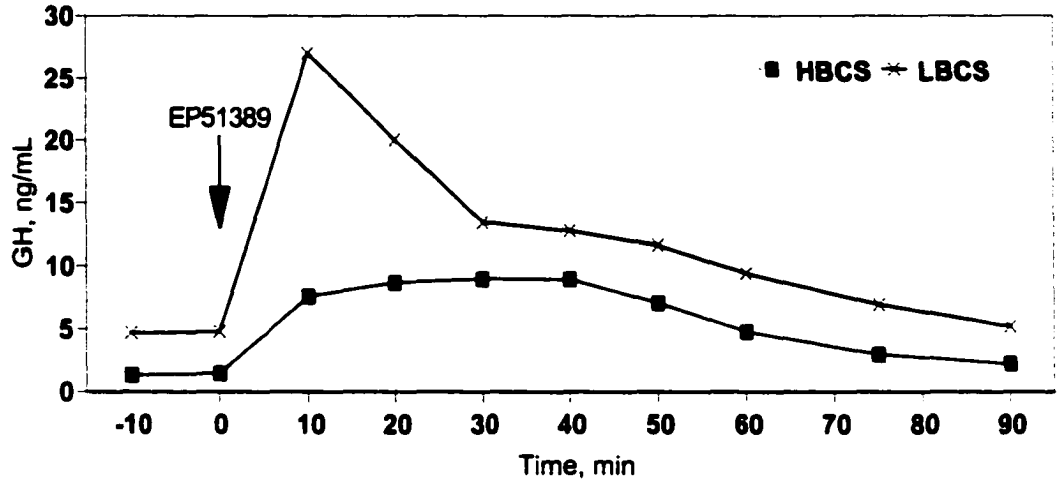

Figure 4.11. Plasma GH concentrations in response to EP51389 in mares of HBCS vs mares of LBCS in January. The EP51389, a potent GH secretagogue, was administered (10 ug/kg BW) on January 12, 2000 immediately following the 12 h period of blood sampling. Mares in the HBCS group had average BCS of 8.0 to 8.5. Mares in the LBCS group had average BCS of 3.0 to 3.5. There was an effect of BCS ($P < 0.02$) in the analysis of variance. The pooled SEM was 0.39 ng/mL.

state. The only difference between this mare and the others in her treatment group was that she was younger than the rest of the mares in her group. Also, although four of the HBCS mares did have low P_4 values (< 1 ng/mL) for a brief period from late December to mid February, it must be noted that these mares did have several follicles in the large category (> 20 mm). In addition, it should be emphasized that the anestrus period in the LBCS mares extended from around mid October to April and into May, a period of six to seven months. In Henneke's study, whether mares were a BCS > 5.0 or < 5.0, ovulation still occurred by about April 1. The time frame differences between the two studies may be due to the fact that mares in this study were at the two extremes of BCS for the entire experimental period. Although some mares in the study by Henneke et al. (1984) started the experimental period at a BCS of 3.0 to 3.5, mares were fed to gain weight.

In contrast to results seen in our study, Morris et al. (1987) reported no differences in the number of mares ovulating in fat vs lean transitional mares in Colorado. Similar to the study by Henneke et al. (1984), mares in the study by Morris et al. (1987) were considered fat if they were BCS of 5.75 or greater and lean if they were BCS below 5.75. So, in essence, there were no extreme differences in BCS between the groups as in our study.

Although the LBCS mares were in deep anestrus, three of the mares did show a behavioral (paradoxical) type estrus when exposed to the stallion. These mares showed all the outward signs of estrus (squatting, urinating, winking), however, upon examination of the ovaries and analysis of blood for P_4, it was determined that they were reproductively inactive. Therefore, although teasing with a stallion is helpful for

determining when mares are in estrus, behavioral displays of estrus are possible in the absence of significant follicular activity.

In the present study, no differences were detected in weekly plasma samples for LH, FSH, GH, TSH, glucose or insulin. Plasma PRL though was higher in HBCS mares and decreased over time from September to January. This is in agreement with studies that have shown PRL to be lower during the winter months (Thompson et al., 1986; Johnson, 1987). DePew et al. (1994) reported that plasma PRL increased relative to feeding in both mares and stallions, which may explain why concentrations were higher in mares in the HBCS group. Also, Bryant et al. (1970) reported that plasma concentrations of PRL were increased after feeding and were low during feed restriction in goats; McAtee and Trenkle (1971) reported similar results in cattle. In contrast, McManus and Fitzgerald (2000) reported that circulating concentrations of PRL were similar in feed-restricted mares compared with controls, however, the restricted period was short-term (24 h). Also, Sticker et al. (1995) reported that plasma PRL concentrations were not influenced by 72 h of feed restriction in mares.

Sejrsen et al. (1983) showed that heifers fed ad libitum had higher PRL and insulin concentrations than heifers fed a restricted diet. Likewise, McFadden et al. (1990) showed that insulin was greater and GH lower in lambs fed a high energy ration ad libitum than in restricted lambs limited to an ADG of only about 120 g. Although plasma insulin concentrations were not different between groups over time, both HBCS mares and LBCS mares had a large spike of plasma insulin release on January 5. This may have been due to stress placed on the mares from extremely bad weather conditions the day

(and night) before, which may have caused the release of large amounts of stress hormones, such as cortisol or epinephrine. For instance, ambient temperature decreased for 40 h at a rate of 0.7°C per h. Temperature dropped from 24°C at 1200 h on January 3 to -3°C at 0600 h on January 5.

The fact that LH and FSH concentrations in twice weekly blood samples were not different is in contrast to results reported by Foster et al. (1989) in which nutritionally growth restricted lambs (chronically maintained at a body weight comparable with that at weaning) became hypogonadotropic, with a low frequency of episodic LH discharges and decreased FSH concentrations. These researchers did find, however, that although GH concentrations changed inversely with level of nutrition, PRL concentrations were not altered by changes in nutrition. In mares, McManus and Fitzgerald (2000) reported that (in addition to PRL) circulating concentrations of FSH and LH were not different from controls during a 24 h feed restriction. These same researchers also reported no differences in serum glucose concentrations during the same period.

Although no differences were detected in GH concentrations, plasma IGF-1 concentrations tended to be higher in mares of HBCS vs LBCS. Sticker et al. (1995) showed that plasma IGF-1 concentrations were significantly lower in mares fed restricted protein and(or) energy than in mares fed adequate protein and energy. Similar results were reported by Granger et al. (1989) in heifers that had lost weight due to nutritional deficiencies. In contrast, McGuire et al. (1992) reported no differences in plasma IGF-1 concentrations in lactating cows on a restricted diet.

The fact that PRL concentrations were not different between groups in response to sulpiride is in agreement with Nadal et al. (1997) in which the PRL response to daily sulpiride administration only tended to differ between feed-deprived mares compared with control mares. In the present experiment, PRL was secreted in response to TRH in both groups, however, the response was greater in the HBCS mares. The exact mechanism is unknown, however, it seems that PRL does play an important role in reproductive function (Thompson et al., 1997). Based on daily PRL concentrations, mares in the HBCS group had greater pituitary release of PRL than LBCS mares. Although plasma PRL is at a minimum during winter (Thompson et al., 1987), both sulpiride and TRH are potent secretagogues of PRL and the fact that PRL concentrations rose in response to both secretagogues indicates that PRL was stored in the pituitary gland (of both groups) and available to be released.

When the TRH challenge was administered 12 d following the sulpiride challenge, not only was the PRL response diminished in both groups, but mares in the LBCS group released significantly less PRL, possibly indicating less pituitary storage of PRL overall. Both basal and TRH-induced prolactin secretion is affected by season, being greater in June compared with January, suggesting that photoperiod plays an important role in controlling circulating PRL concentrations (Thompson et al., 1986; Johnson, 1987). Evans et al. (1991) reported similar results with horses both in vivo and in vitro. It is possible, that factors such as nutritional status of the animal, as occurred in this study, are also important in modulating the seasonal rhythym of plasma PRL. However, more research needs to be conducted in anestrous, nutritionally stressed mares.

More LH, but less FSH, was secreted from the pituitary of mares in the HBCS group in response to GnRH. The reduced FSH response was indicative of mares in the follicular phase, when ovarian products inhibit normal FSH secretion, which is consistent with the HBCS mares being cyclic and not going anestrous (Ginther, 1990; Hines et al., 1991). Several investigators have shown that both LH and FSH (Evans and Irvine, 1976; Alexander and Irvine, 1986; Johnson, 1987) are released from the pituitary when anestrous mares are given exogenous GnRH; however, those studies used mares of average BC and the GnRH was given over several weeks time. These mares did have higher FSH concentrations (as well as LH) over the 12-h sampling period when compared with the LBCS mares, which is consistent with higher follicular activity and cyclicity.

Also, GH concentrations during the 12-h sampling period as well as during the secretagogue challenge (EP51389) were elevated for mares in the LBCS group. In mares, protein restriction (50% of maintenance) slightly increased the occurrence of GH episodes during a 14 h feeding period (Sticker et al., 1995). Ogawa et al. (1996) evaluated the effects of recombinant bGH on the IGF-1 axis and protein metabolism during fasting induced metabolic stress in young lambs. They reported that the rate of net protein catabolism was reduced and there was a smaller fall in IGF-1 concentrations in feed restricted lambs treated with recombinant bGH vs those treated with saline. In a report by Breier (1999), decreased nutrition led to elevated GH secretion but reduced hepatic GH receptor number and plasma concentrations of IGF-1. The higher GH concentrations seen in this study could be indicative of nutritional stress in the LBCS group.

Implications

It is clear from this study that there is a relationship between the level of nutrition and the reproductive status of the mare, particularly for mares that are malnourished. A great deal of research has been done with hormone therapy and hormone deficiency studies in other species that show that the endocrine system regulates metabolism. Although an attempt was made here to characterize hormonal and reproductive patterns in mares on either extreme of body condition score, more in depth studies are needed to understand the interrelationships of hormones, nutrition and reproductive status of the horse.

CHAPTER V

THYROTROPIN-RELEASING HORMONE (TRH) AND GONADOTROPIN-RELEASING HORMONE ANALOG (GnRHa) INTERACTIONS IN SEASONALLY ANOVULATORY MARES OF AVERAGE BODY CONDITION

Introduction

Seasonal anestrus and anovulation in nonpregnant mares (of average body condition) is known to be due in part to reduced hypothalamic production and secretion of GnRH, which results in reduced production and secretion of LH with lesser effects on FSH (Mumford et al., 1994a). Luteinizing hormone has been shown to be seasonally regulated in the mare but FSH does not appear to be regulated by season (Garcia et al., 1979). Several investigators have reported that GnRH administered to anestrous mares induces the release of both LH and FSH (Evans and Irvine, 1976; Alexander and Irvine, 1986; Johnson, 1987; Mumford et al., 1994a). Also, twice-daily injections of GnRH or a GnRHa (Fitzgerald et al., 1987) or the continuous administration of either GnRH (Hyland et al., 1987) or a GnRHa (Allen et al., 1987) has been shown to initiate follicular growth and to induce ovulation in acyclic mares.

Much less research has been conducted to determine the role of thyroid hormones in reproductive processes in the horse. Fitzgerald and Davison (1997) reported that mares that continued to display estrous cycles throughout the winter in Kentucky had higher T_4 concentrations in plasma than mares that went anestrous. It is not known whether this association is cause-and-effect or simply a coincident occurrence. The present experiment was conducted to determine the effects of daily TRH injections given to

anovulatory mares of average BC in winter on thyroid hormone and PRL secretion and the possible interaction with the reproductive effects of daily GnRHa injections.

Experimental procedures

Animals and treatments. Starting two months before initiation of the study (November 1), open mares at the Louisiana State University Agricultural Center Central Research Station in Baton Rouge, Louisiana, had jugular blood samples drawn twice weekly for P_4 analysis and had their ovaries assessed for follicular activity once weekly via rectal palpation and ultrasonic scanning. Mares with low P_4 (< 1 ng/mL) for at least 28 d were randomly allotted to one of four treatments in a 2 x 2 factorial arrangement. The four mares assigned to Treatment 1 received 5 μg TRH (pGlu-His-Pro-amide, Sigma Chem. Co.; P2161)/kg BW daily. Mares (n = 4) in Treatment 2 received 50 ng GnRHa (des-Gly^{10},[D-His(Bzl)^6]-LHRH-ethylamide; Sigma Chem. Co.; L2761)/kg BW daily, Treatment 3 mares (n = 5) received both 5 μg TRH/kg BW and 50 ng GnRHa/kg BW daily and Treatment 4 mares (n = 4) received 0.0033 mL saline/kg BW daily from January 5 to February 1. All injections were given i.m. in the neck at the same time each morning. In order to keep from biasing any results obtained from ultrasound scans and rectal palpation, treatments were color-coded and otherwise unknown to the personnel involved with data collection. Ovaries of each mare were scanned at 72 h intervals using an Aloka 550V ultrasound machine with a 5 megahertz linear-array transducer (Aloka Science and Humanity, Wallingford, CT), and size and number of follicles were recorded for each ovary. Once a follicle ≥ 30 mm in diameter was detected, the ovaries of that mare were scanned daily until ovulation or regression of the follicle to < 20 mm.

Blood sampling and challenges. Blood samples were collected every 3 d throughout the experiment in the morning before treatment injections. Jugular blood was collected into 7-mL heparinized, evacuated tubes (Vacutainer; Becton and Dickinson, Franklin Lakes, NJ), centrifuged at 1,500 x g at 4°C for 15 min, and the plasma harvested and stored in 12 mm x 75 mm polystyrene tubes at -15°C. Samples were analyzed for LH, FSH, PRL, P_4 and T_4.

To determine the responses to the releasing hormones over the course of the experiment, seven periods of frequent blood sampling (d 1, 2, 4, 7, 11, 16 and 22) were conducted over the course of the experiment. On these days, mares were fitted with indwelling jugular vein catheters (Quik-Cath, Baxter Healthcare Corp., Deerfield, IL) and allowed to rest for 60 min. Blood samples were then collected at -10, 0 10, 20, 40, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min relative to treatment injections. Samples were processed in a similar manner as the daily blood samples and stored until analyzed for LH, FSH, PRL and TSH concentrations. Also on these days of frequent sampling, samples of hair were collected from each mare in three regions of the rib cage area, pooled and weighed for determination of hair shedding. Rectal temperatures were also taken at the beginning of the sample collection period and again at the end of the sample collection period on each of the 7 d of frequent bleeding and recorded.

At the end of the experiment (d 29), all mares received jugular catheters and were allowed to rest for 60 min. Blood samples were collected at -10, 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 relative to each mare receiving 1 μg/kg GnRH, 4 μg/kg TRH, 1 mg/kg sulpiride and 10 μg/kg EP51389 (a GH-releasing tripeptide) in a cocktail,

followed by 5 mL saline and 1.5 mL sodium citrate. Samples were processed as above and stored until analyzed for LH, FSH, PRL, TSH and GH. Although the actual experimental period ended on d 29, blood samples were continually collected every 3 d through d 89 to monitor changes in P_4 concentrations indicative of ovulation and(or) CL formation.

Statistical analyses. Data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The design was a split-plot in time with a 2 x 2 factorial arrangement of treatments testing GnRH, TRH and their interaction. Data from one mare in the saline group were not included in the analysis due to unusually high hormone values, which was not representative of the group.

Results

No consistent differences ($P > 0.1$) due to treatment were observed for LH, FSH, PRL or T_4 concentrations in samples collected every 3 d. Over the course of the experiment, no differences ($P > 0.1$) were detected in follicle size or number of follicles. However, mares not receiving TRH tended to have more follicles between 11 mm and 19 mm diameter over time, except at the very beginning and on the last day of ultrasonography (TRH x day, $P < 0.09$; Figure 5.1). Also, there was a GnRH x TRH interaction ($P < 0.029$) in that mares receiving both GnRH and TRH had a higher number of 11 mm to 19 mm follicles than those receiving TRH, but a similar number as those receiving just GnRH (Figure 5.2). None of the mares ovulated as assessed by both ultrasonography and low P_4 values throughout the treatment period (d 29) and by low P_4 values monitored out to d 89.

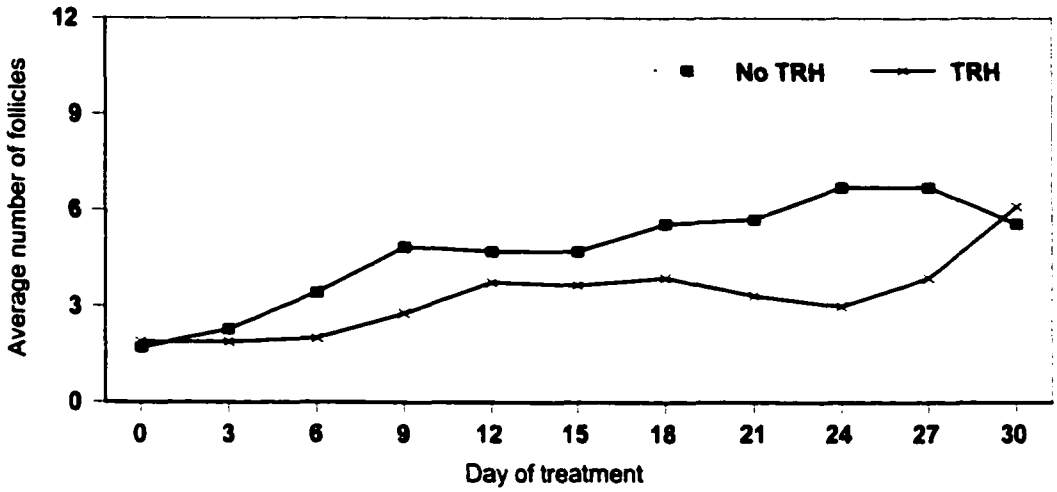

Figure 5.1. The effect of TRH on number of 11 mm to 19 mm diameter follicles in anovulatory mares of average BCS. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d during January. There tended to be a treatment x day interaction ($P < 0.09$) in the analysis of variance. The pooled SEM was 0.99.

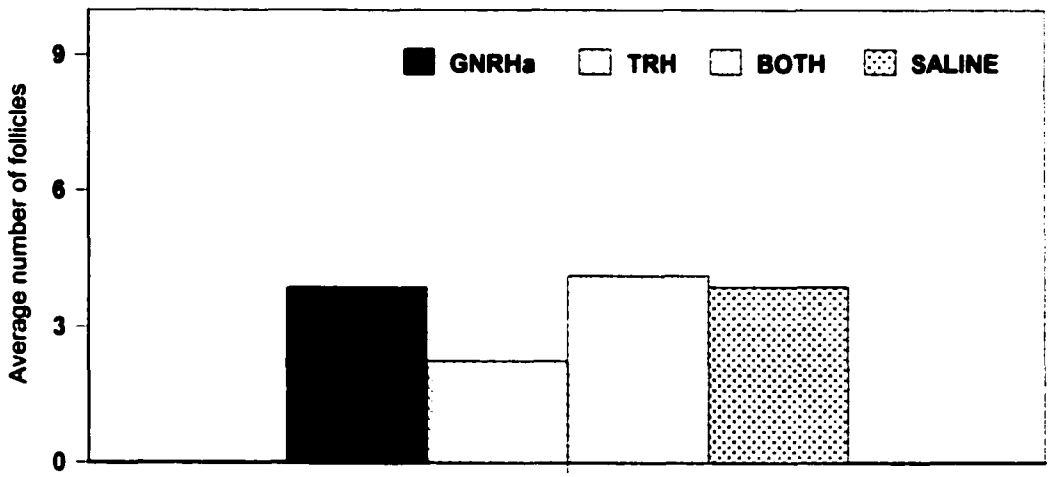

Figure 5.2. The effect of treatment with GnRHa, TRH, both GnRHa and TRH, or saline on number of 11 mm to 19 mm diameter follicles in anovulatory mares of average BCS. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d during January. There was an effect of treatment ($P < 0.029$) for follicle number in the analysis of variance. The pooled SEM was 0.99.

During the seven periods of frequent blood sampling, LH concentrations in all mares receiving GnRHa were higher ($P < 0.0001$) than mares not receiving the GnRHa, and the magnitude of the response increased from d 1 to d 27 ($P < 0.003$; Figure 5.3, top panel). Follicle stimulating hormone followed a similar pattern as LH in that FSH concentrations were higher in mares receiving GnRHa than mares not receiving GnRHa ($P < 0.001$) and also increased over days of treatment ($P < 0.004$; Figure 5.3, bottom panel). Administration of TRH caused concentrations of both PRL ($P < 0.001$) and TSH ($P < 0.0001$) to increase, however, the greatest responses were during the first 2 d of treatment; subsequent responses were minimal (Figure 5.4). No consistent differences ($P > 0.1$) were found for hair weights collected from three areas of the ribcage or for rectal temperatures taken twice during each period of blood sampling (data not shown).

When mares were administered the secretagogue cocktail on d 29, the LH response to the cocktail was greater (GnRH x time; $P < 0.0002$) in mares that had previously received the GnRHa, with peak concentrations of 1.72 ng/mL occurring at 20 min after injection (Figure 5.5). The FSH response, however, was not affected ($P > 0.1$) by previous treatment (Figure 5.6). In mares that had previously received TRH, the PRL response was greater (TRH x time; $P < 0.014$) than in mares that had not received TRH, with peak concentrations of 40.9 ng/mL occurring at 30 min post injection (Figure 5.7). Concentrations of TSH, however, were lower (TRH x time; $P < 0.0005$) in mares that had previously received TRH (Figure 5.8). Growth hormone concentrations were not affected ($P > 0.1$) by previous treatment (data not shown).

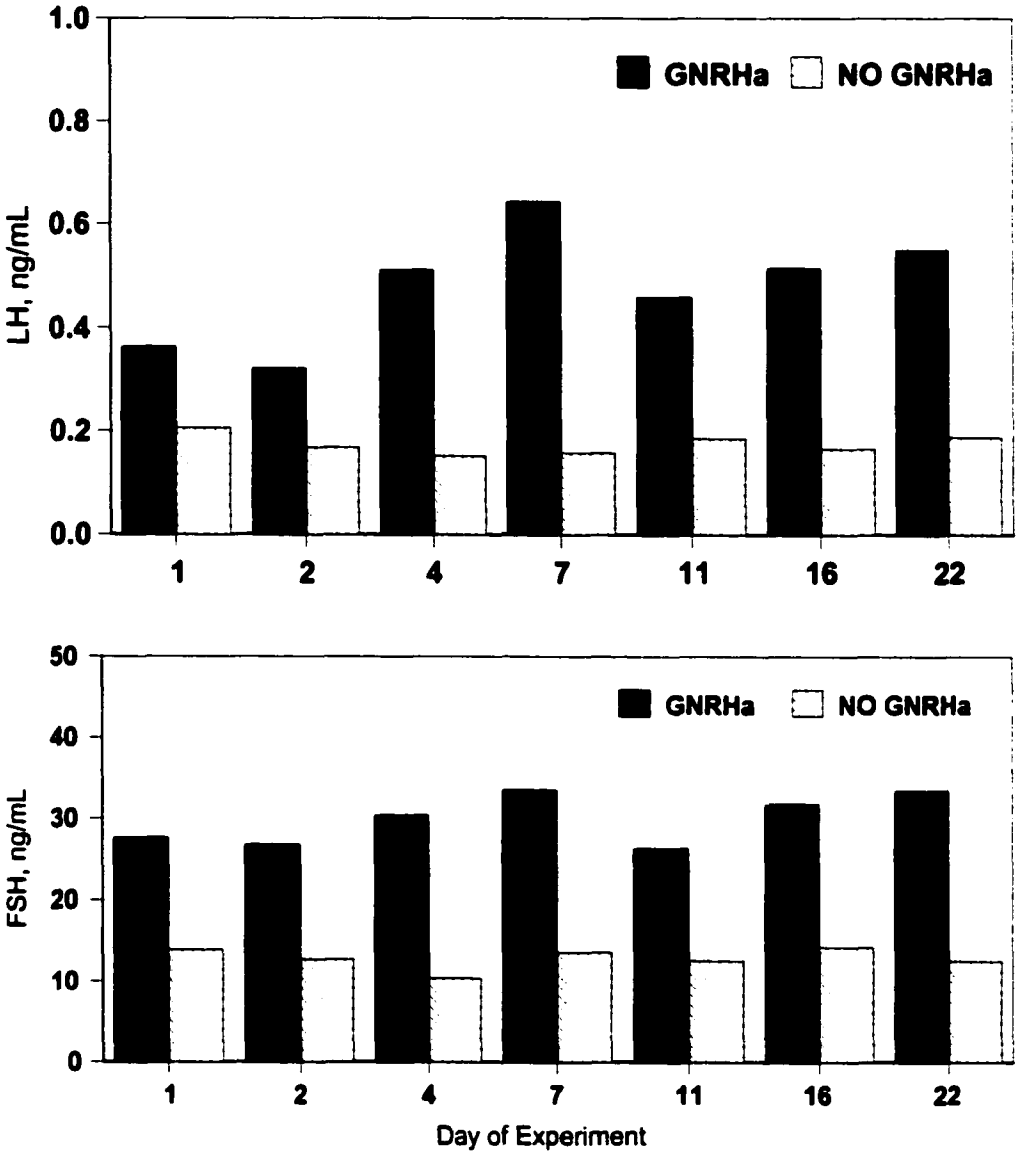

Figure 5.3. The effect of GnRHa on LH (top panel) and FSH (bottom panel) concentrations in anovulatory mares of average BCS during seven periods of frequent blood sampling. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d in January. Means represent the average concentrations over each 8 h frequent blood sampling period. There was an effect of GnRHa treatment ($P < 0.0001$) and a GnRHa x day interaction ($P < 0.003$) for LH concentrations in the analysis of variance. There was an effect of GnRHa treatment ($P < 0.001$) and a GnRHa x day interaction ($P < 0.004$) for FSH concentrations in the analysis of variance. The pooled SEM were 0.06 and 3.08 ng/mL for LH and FSH concentrations, respectively.

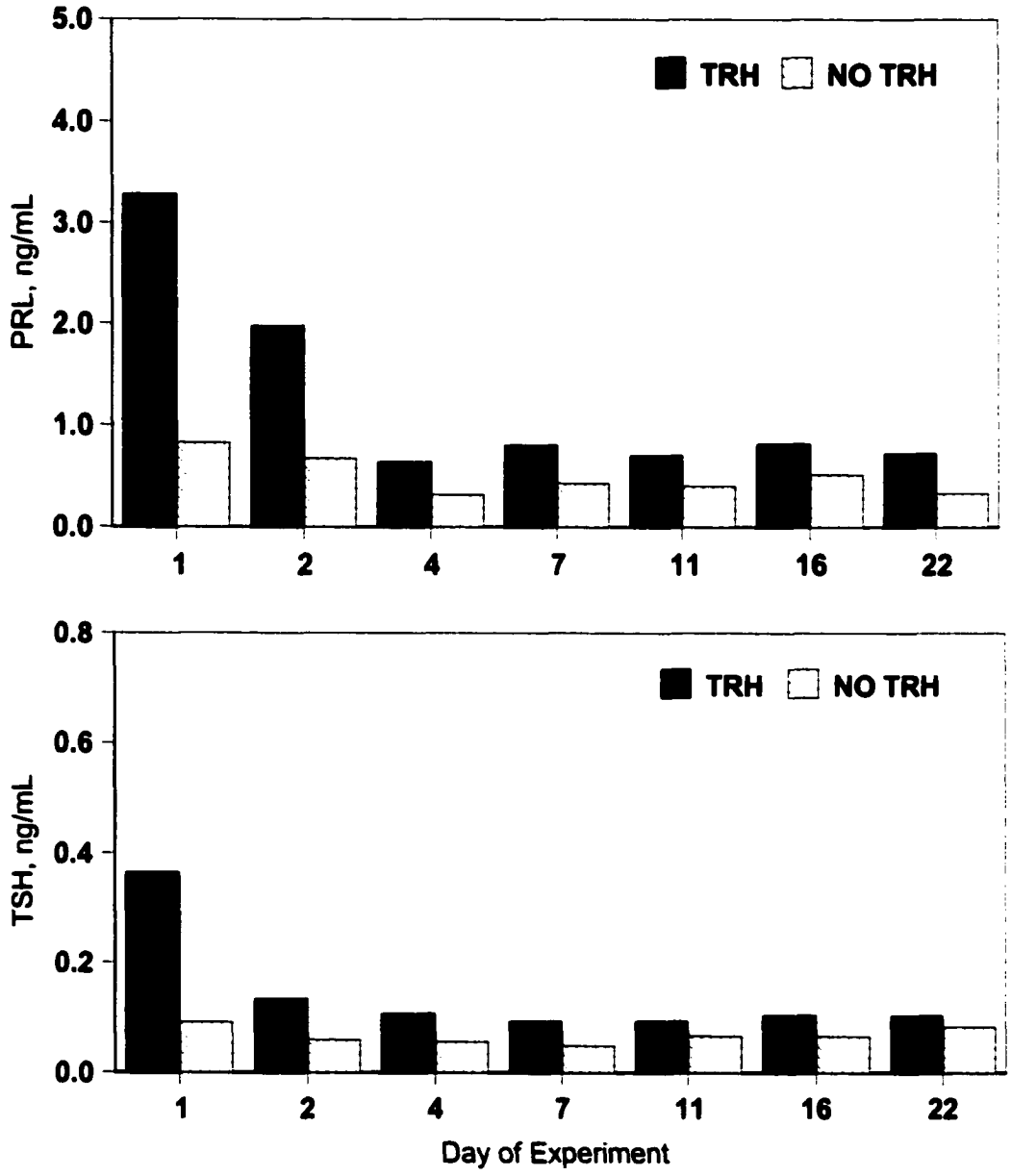

Figure 5.4. The effect of TRH on PRL (top panel) and TSH (bottom panel) concentrations in anovulatory mares of average BCS during seven periods of frequent blood sampling. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d in January. Means represent the average concentrations over each 8 h frequent blood sampling period. There was a TRH x day interaction ($P < 0.001$) for PRL concentrations and a TRH x day interaction ($P < 0.0001$) for TSH concentrations in the analysis of variance. The pooled SEM were 0.46 and 0.03 ng/mL for PRL and TSH concentrations, respectively.

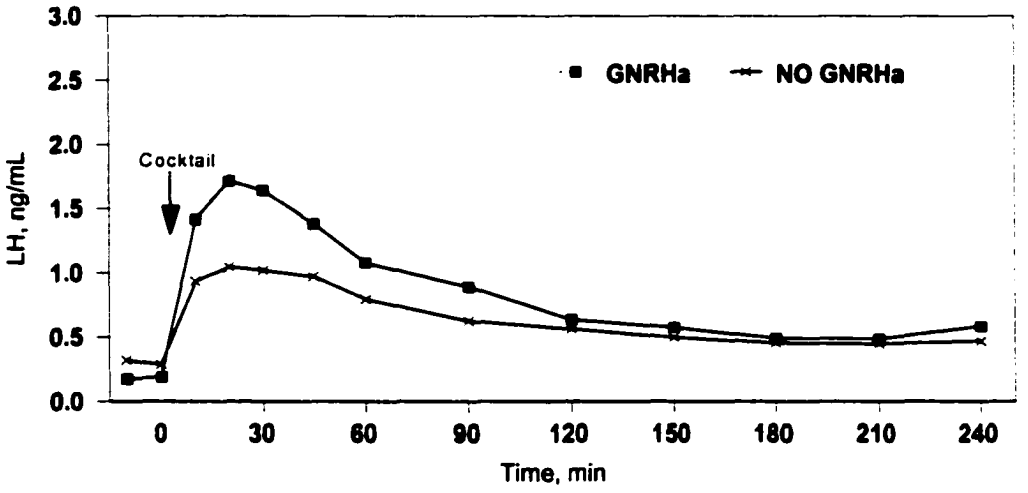

Figure 5.5. The effect of GnRHa on LH concentrations during a challenge with a cocktail consisting of GnRH, TRH, sulpiride and EP51389 in anovulatory mares of average BCS. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d in January. The cocktail was administered on d 29. There was a treatment x time interaction ($P < 0.0002$) for LH concentrations in the analysis of variance. The pooled SEM was 0.13 ng/mL.

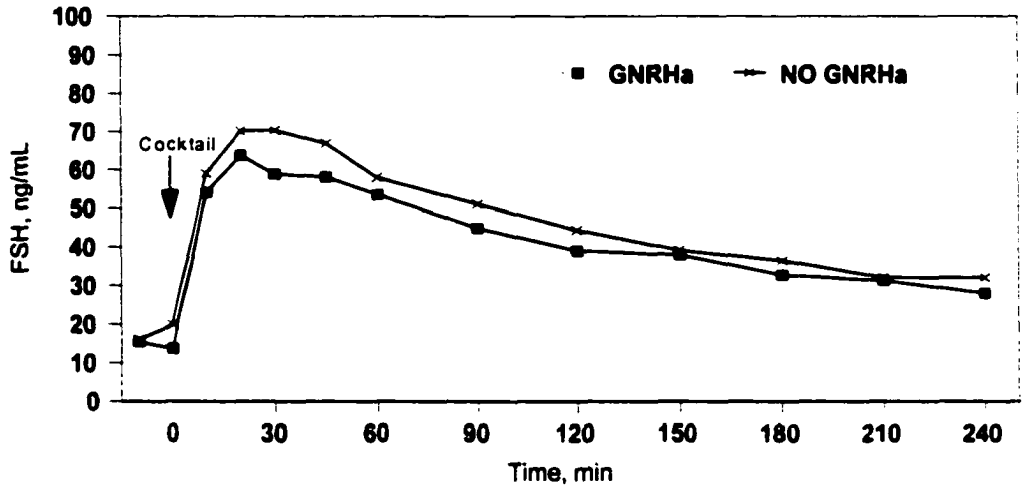

Figure 5.6. The effect of GnRHa on FSH concentrations during a challenge with a cocktail consisting of GnRH, TRH, sulpiride and EP51389 in anovulatory mares of average BCS. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d in January. The cocktail was administered on d 29. There was no effect ($P > 0.1$) in the analysis of variance. The pooled SEM was 3.6 ng/mL.

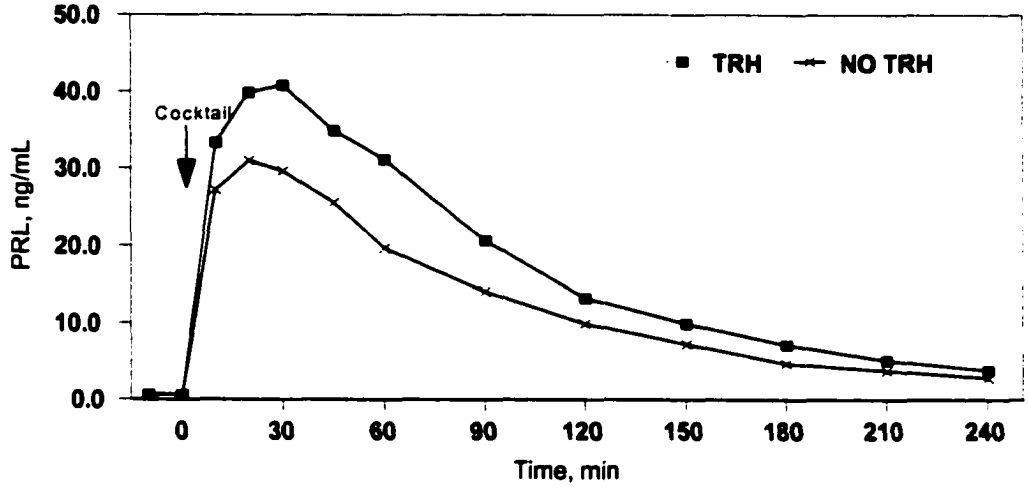

Figure 5.7. The effect of TRH on PRL concentrations during a challenge with a cocktail consisting of GnRH, TRH, sulpiride and EP51389 in anovulatory mares of average BCS. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d in January. The cocktail was administered on d 29. There was a treatment x time interaction ($P < 0.014$) for PRL concentrations in the analysis of variance. The pooled SEM was 2.5 ng/mL.

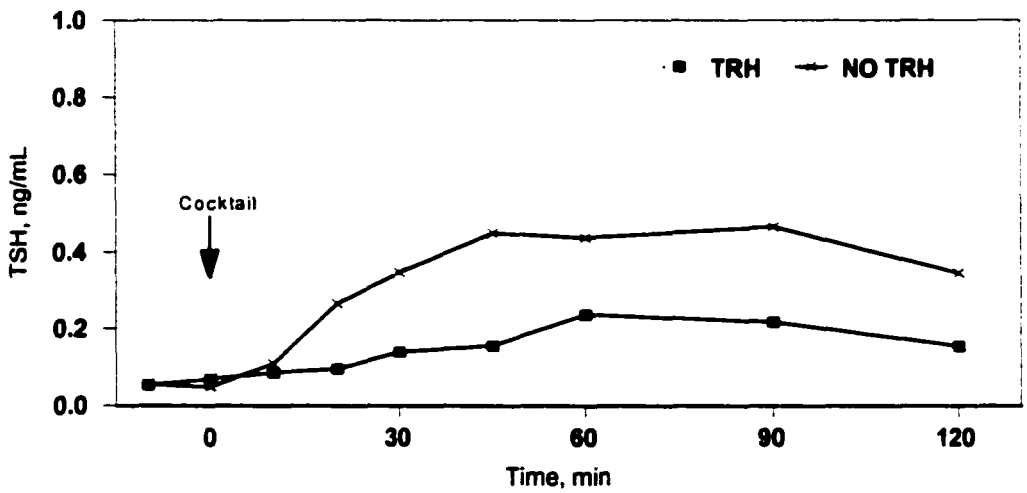

Figure 5.8. The effect of TRH on TSH concentrations during a challenge with a cocktail consisting of GnRH, TRH, sulpiride and EP51389 in anovulatory mares of average BCS. Mares were treated with 50 ng/kg BW GnRHa, 5 μg/kg BW TRH, both 50 ng/kg BW GnRHa and 5 μg/kg BW TRH, or 0.0033 mL/kg BW saline for 29 d in January. The cocktail was administered on d 29. There was a treatment x time interaction ($P < 0.0005$) for TSH concentrations in the analysis of variance. The pooled SEM was 0.05 ng/mL.

Discussion

In the present study, concentrations of LH, FSH, PRL and T_4 in twice-weekly blood samples showed no consistent changes due to treatment with either GnRHa, TRH, or both, over controls. Given that these samples were collected in the morning immediately before that day's treatment, it appears that any changes occurring in response to injection decayed before the next treatment injection. Harrison et al. (1990) showed an increased pituitary response of LH to a GnRHa by d 7 in mares given an implant subcutaneously and by d 21 for mares injected with GnRHa twice daily. Other researchers have shown that treatment with GnRH causes the release of both LH and FSH from the pituitary (Ginther and Wentworth, 1974; Silva et al., 1987). In the mare, photoperiod plays an important role in controlling circulating concentrations of PRL, with highest concentrations occurring from June to August and lowest concentrations occurring from November to February (Johnson, 1986, 1987). Johnson (1987) reported that TRH induced PRL secretion was greater in June than January.

Overall in this experiment, no increases in follicular development occurred, although mares that received both GnRHa and TRH tended to have a higher number of medium-sized (11 mm to 19 mm) follicles than those mares receiving just TRH. Apparently, TRH treatment alone had no effect on follicles, but GnRHa treatment with TRH did. Mumford et al. (1994b) reported no increase in follicular development for GnRH-implanted mares over 28 d. In contrast, Turner and Irvine (1991) showed that frequent GnRH injections increased follicular development in mares as did pulsatile doses of GnRH (Johnson, 1986). The fact that none of these mares ovulated and

follicular activity was not greatly affected, along with the inconsistent concentrations of daily hormone concentrations, indicates that these mares were still anestrous and had not gone through vernal transition. It is possible that once a day injections may not have been frequent enough to elicit a consistent response.

Although daily responses of hormones were variable, GnRHa injections did cause concentrations of both LH and FSH to rise during each of the seven frequent blood sampling events. Also, concentrations of each of these hormones increased as time went on (d 1 to d 27), indicating that GnRHa administration not only induced the immediate secretion of releasable gonadotropin stores from the pituitary but also stimulated the cellular production machinery such that even more hormone was made over time than was secreted.

Mumford and colleagues (1994b) showed no difference in either peak LH or area under the response curve (AUC) among treatment groups in response to a GnRH challenge. However, in a companion study, Mumford et al. (1994a) reported that peak LH and LH AUC were greater for mares administered GnRH and GnRH plus estradiol than for mares given estradiol alone. According to Thompson et al. (1991), E_2 (in the absence of androgen or progestogens) is a potent stimulator of LH secretion and storage in horses. In their study, LH secretion, storage and response to exogenous GnRH were all highly correlated ($r \geq 0.77$) and that storage of FSH and response to exogenous GnRH were also related ($r = 0.62$). Similarly, Silvia et al. (1987) determined that a correlation of $r = 0.72$ exists between LH released and pituitary tissue content of LH after a GnRH

challenge, thereby allowing the GnRH challenge to be a reliable method of determining relative pituitary LH stores.

During the seven periods of frequent blood sampling, TRH caused concentrations of both PRL and TSH to increase, particularly during the first 2 d of treatment. In several species, TRH has been shown to increase both PRL and TSH (Davis and Borger, 1972; Convey et al., 1973; McMahon et al., 1979; Thompson et al., 1984). Johnson (1987) challenged mares with TRH during January (short photoperiod) and again in June (long photoperiod) and reported that although TRH induced the release of PRL during both periods, the response was much greater in mares exposed to the long photoperiod. Thompson et al. (1985) reported similar results in anestrous mares in winter and estrous mares in summer.

In our study, the periods of frequent blood sampling occurred during a time of short photoperiod in anovulatory mares. Although the initial rise in PRL and TSH concentrations confirm that the TRH was reaching the pituitary gland and causing release, the fact that subsequent responses became minimal may indicate a desensitization of PRL and TSH receptors to the TRH treatment over time. Also, the secretagogue cocktail was administered on d 29 to assess pituitary responsiveness at the end of the experiment. The cocktail contained sulpiride (in addition to TRH), which is a dopamine antagonist that causes immediate increases in PRL concentrations in horses (Colborn et al., 1991). The PRL response to the cocktail was greater in mares that had previously received TRH, thereby indicating that pituitary stores of PRL were elevated in these mares and the mares were responding to the sulpiride. The TSH response to the cocktail, however, was less in

mares that had previously received TRH, lending further credence to the possibility of a desensitization of TSH receptors to TRH treatment.

Unlike PRL, which is mainly under constant negative restraint by dopamine from the hypothalamus, LH is highly dependent on GnRH from the hypothalamus. Gonadotropin releasing hormone had a greater effect on LH response in mares that had previously received the GnRHa, indicating that the releasable stores in the pituitary had not been depleted, but in fact were apparently stimulated (i.e., an increase in LH production). Follicle stimulating hormone, which is also highly dependent on GnRH from the hypothalamus was not affected by previous treatment. It must be noted, however, that FSH was released in response to the cocktail, indicating that pituitary stores were not reduced due to GnRHa treatment for 29 d. This is especially evident given the fact that FSH concentrations were higher during each of the seven periods of frequent blood sampling in mares treated with GnRHa.

Implications

Results from the current experiment show that in agreement with other studies, treatment of anovulatory mares in winter with daily injections of gonadotropin releasing hormone analog was effective in increasing concentrations of both luteinizing hormone and follicle stimulating hormone released from the pituitary. Also, mares that received treatment of thyrotropin releasing hormone did have slightly higher pituitary stores of prolactin. However, no overall increase in follicular development occurred, except for an increase in the number of medium-sized follicles in mares treated with both gonadotropin releasing hormone analog and thyrotropin releasing hormone. Therefore, it appears that

treatment with both gonadotropin releasing hormone analog and thyrotropin releasing hormone would not be of benefit for inducing ovulation in seasonally anovulatory mares.

CHAPTER VI

GROWTH HORMONE (GH) AND GONADOTROPIN-RELEASING HORMONE ANALOG (GnRHa) INTERACTIONS IN MARES OF LOW BODY CONDITION SCORE OR HIGH BODY CONDITION SCORE

Introduction

Growth hormone, also called somatotropic hormone or somatotropin, is secreted by the anterior pituitary and exerts its effects on all or almost all tissues of the body (Guyton, 1991). Although its main function is to cause growth of all tissues of the body that are capable of growing, GH has been shown to have a very important reproductive role as well. For instance, administration of GH has been shown to have beneficial effects on ovarian activity and in particular, increased follicle numbers, in cattle (Buratini et al., 2000; Lucy, 2000; Tripp et al., 2000), sheep (Joyce et al., 1998; Joyce et al., 2000), swine (Spicer et al., 1992; Echternkamp et al., 1994; Whitley et al., 1998) and horses (Cochran et al., 1999a,b). Growth hormone, along with IGF-1, appears to work synergistically with gonadotropins, thereby allowing an increased response of follicular cells to available gonadotropins (Cochran, 2000).

In the nonpregnant mare, the seasonal anestrus and anovulation period is known to be due in part to reduced hypothalamic production and secretion of GnRH (Hart et al., 1984). The use of GnRH or its analogs to induce ovulation in seasonally anestrous mares, to hasten the onset of regular cycles in transitional mares and to shorten the follicular phase in cyclic mares has been attempted by several (Irvine et al., 1975; Silvia et al., 1987; Becker and Johnson, 1992). Johnson (1987) has shown that follicular development

and ovulation in seasonally anovulatory mares can be induced with pulsatile administration of low doses of GnRH. Research by Hyland et al. (1987), Sanderson et al. (1986) and Allen et al. (1987) indicated that continuous administration of GnRH or one of its analogs can initiate follicular growth and induce ovulation in acyclic mares as well.

In a study by Cochran et al. (1999b), it was shown that treatment with eGH increases circulating IGF-1 concentrations along with an increase in the number of small follicles in mares of average BCS. A follow-up study to determine if eGH would enhance the ovulatory response of seasonally anovulatory mares (of average BCS) treated with a GnRHa showed that eGH in combination with once daily injections of GnRHa did indeed enhance the ovulatory response of those average BCS mares (Cochran et al., 1999a). The objective of this study was to determine the effects of eGH and a GnRHa on follicular growth and ovulation of mares of either very LBCS or very HBCS.

Experimental procedures

Animals and treatments. Beginning January 20, 12 light horse mares of LBCS (3.0 to 3.5) were randomly allotted to receive either daily injections of 25 μg/kg BW equine GH (eGH; BresaGen, Ltd., Adelaide, Australia; n = 6) or daily injections of vehicle (n = 6) for a period of 14 d. Likewise, 12 light horse mares of HBCS (8.0 to 8.5) were randomly divided into two groups and treated in a similar manner (Phase 1). At the end of the 2 week treatment period, all mares in both groups received daily injections of a GnRHa (50 ng/kg BW; des-Gly^{10}, [D-His(Bzl)^{6}]-LHRH ethylamide; Sigma; L-2761) for 21 d or until ovulation occurred (Phase 2). This experiment was approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

Stage of cyclicity and blood sampling. Every 72 h throughout the experiment mares were teased with a stallion and had their ovaries palpated for determination of ovary size. A scale of 1 to 5 was used, with a score of 1 being smallest and 5 being largest. Also, follicular development was monitored via transrectal ultrasonagraphy using an Aloka 550V ultrasound machine with a 5 megahertz linear-array transducer (Aloka Science and Humanity, Wallingford, CT) until a follicle 30 mm or greater was detected. That mare's ovaries were then examined daily thereafter until ovulation or luteinization occurred. Once it was determined that a mare had ovulated, GnRHa injections for that particular mare ceased. Blood samples were collected daily throughout the treatment injection period by jugular venipuncture into 7-mL evacuated tubes, one containing potassium oxalate and sodium fluoride and 1 containing sodium heparin (Vacutainer; Becton and Dickinson, Franklin Lakes, NJ). Samples were centrifuged and the plasma harvested and stored in 12 mm x 75 mm polystyrene tubes at -15°C until analyzed for P_4, GH, IGF-1, LH and FSH. Progesterone was analyzed by RIA using a commercially available kit (DSL-3400; Diagnostic Systems Laboratories, Inc., Webster, TX) and LH, FSH, GH and IGF-1, were analyzed by RIA as previously described for horses (Thompson et al., 1983a,b; Thompson et al., 1992; Sticker et al, 1995, respectively).

Statistical analyses. Data were analyzed using the PROC mixed procedure of SAS (SAS Inst. Inc., Cary, NC) as a completely randomized design with a 2 x 2 factorial arrangement of treatments testing BCS, GH, phase and their interactions using the residual error. Phase was included in the model since GnRHa was administered to all horses for 21 d (or until ovulation) immediately following the last day of eGH treatment.

Data collected throughout the experiment were analyzed by univariate analysis of variance, which took into account the repetitive nature of the sampling (split plot; Gill and Hafs, 1971) and tested treatment, day and the treatment x day interaction.

Results

Mares in the HBCS group had larger ovaries ($P < 0.002$; Figure 6.1, top panel) and more CL (BCS, $P < 0.05$; BCS x phase x day, $P < 0.0003$; Figure 6.1, bottom panel) than mares in the LBCS group, especially during eGH treatment. Also, analysis of P_4 data showed that mares in the HBCS group had higher P_4 concentrations during eGH treatment, but were not different by d 6 of GnRHa treatment (BCS x day, $P < 0.04$; phase, $P < 0.015$; Figure 6.2). On average, mares in the HBCS group had more large follicles (1.85 vs 0.76) than mares in the LBCS group ($P < 0.02$; pooled SEM = 0.34), and the number of large follicles was greater during phase 2 (BCS x phase, $P < 0.002$; Figure 6.3; top panel). Similar to results obtained for the number of large follicles, more medium sized follicles were observed toward the end of the experiment than at the beginning, and mares in the HBCS group had more medium follicles than the LBCS group or a similar amount during the course of the study (BCS x phase x day, $P < 0.05$; Figure 6.3; middle panel). The number of small follicles, however, did not differ between groups, although there tended to be a GH x phase x day interaction ($P = 0.06$; Figure 6.3; bottom panel).

Data collected from the mares prior to the experiment showed that although it was the normal winter anestrous period, mares in the HBCS group had continued to cycle throughout the winter months without going anestrus. Mares in the LBCS group on the other hand, had been in a classic anestrous state. Toward the end of GnRHa treatment,

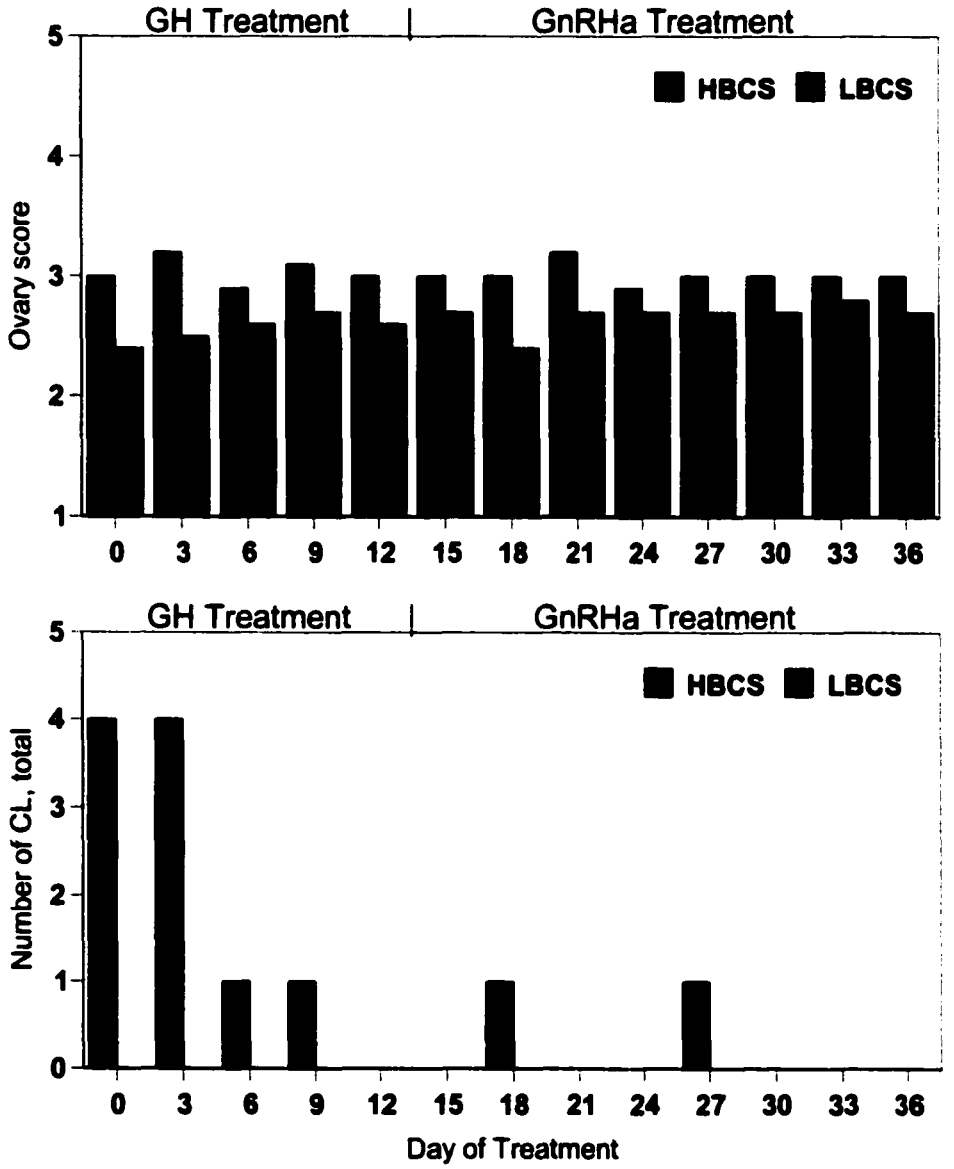

Figure 6.1. Ovary score (top panel) and number of CL (bottom panel) in mares of HBCS vs mares of LBCS. Mares in each BCS group were treated with (n = 6) or without (n = 6) 25 ug/kg BW eGH for 14 d. All mares received GnRHa (50 ng/kg BW) injections for 21 d thereafter, or until ovulation occurred. There was a treatment effect ($P < 0.002$) for ovary score, a treatment effect ($P < 0.05$) for number of CL, and a BCS x phase x day interaction ($P < 0.0003$) for number of CL in the analysis of variance. The pooled SEM were 0.46 and 0.04 for ovary score and CL, respectively.

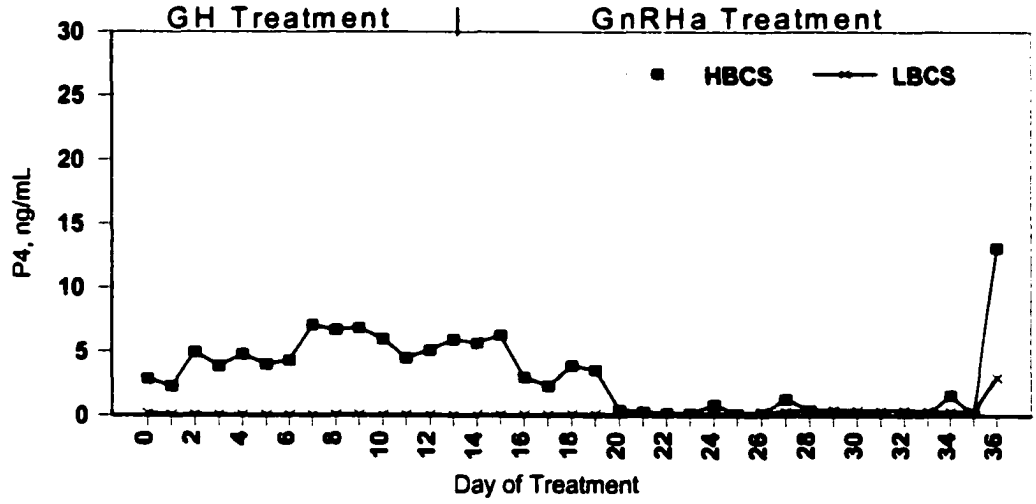

Figure 6.2. Plasma P_4 concentrations in mares of HBCS vs mares of LBCS. Mares in each BCS group were treated with (n = 6) or without (n = 6) 25 ug eGH/kg BW for 14 d. All mares received GnRHa (50 ng/kg BW) injections for 21 d thereafter, or until ovulation occurred. There was an effect of phase ($P < 0.015$), and a BCS x day interaction ($P < 0.04$) for P_4 concentrations in the analysis of variance. The pooled SEM was 0.43 ng/mL.

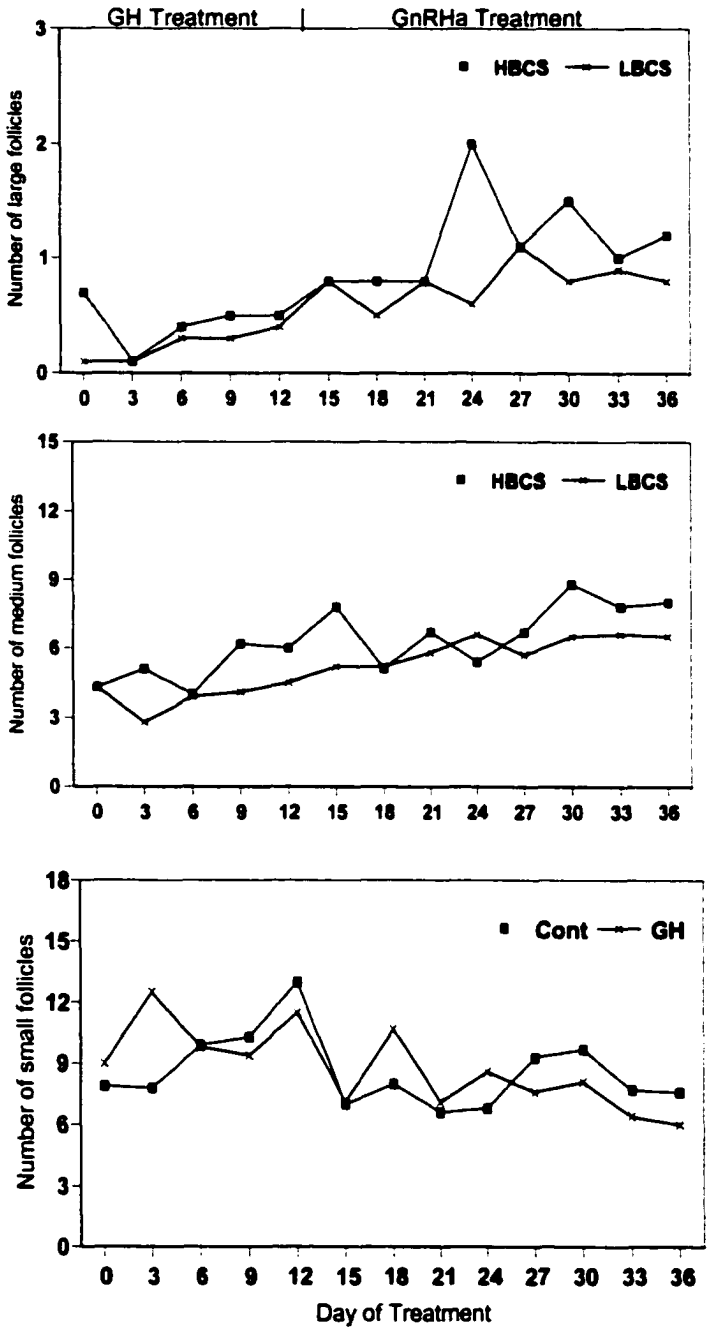

Figure 6.3. Average number of large follicles (top panel), medium follicles (middle panel) and small follicles (bottom panel) in mares of HBCS vs mares of LBCS. Mares in each BCS group were treated with (n = 6) or without (n = 6) eGH for 14 d. All mares received GnRHa injections for 21 d thereafter, or until ovulation occurred. There was a BCS x phase interaction ($P < 0.002$) for large follicles, a BCS x phase x day interaction ($P < 0.05$) for medium follicles, and a GH x phase x day interaction ($P = 0.06$) for small follicles in the analysis of variance. The pooled SEM were 0.40, 0.55, and 0.66 for large, medium, and small follicles, respectively.

two mares in the LBCS group did ovulate, one which had received the eGH treatment and one which had not.

No differences ($P > 0.1$) in GH concentrations were detected in mares of HBCS vs LBCS, but there was a GH x phase x day interaction ($P < 0.05$). On d 10 of eGH treatment and on d 10 of GnRHa treatment, plasma GH concentrations of mares not treated with eGH spiked to a high of 9.21 ng/mL and 9.78 ng/mL, respectively (Figure 6.4; top panel). These spikes, however, were due to only 4 of the 12 mares in that treatment group.

Plasma IGF-1 concentrations were higher in mares treated with eGH compared to mares given vehicle ($P < 0.0001$). Mares of HBCS had higher IGF-1 concentrations than LBCS mares ($P < 0.02$). A GH x BCS x phase x day interaction ($P < 0.0001$) was observed in which IGF-1 concentrations approximately tripled by d 14 in eGH treated mares but remained relatively constant in mares receiving the vehicle, with all mares having similar IGF-1 values by the end of the experiment (Figure 6.4; bottom panel).

Concentrations of LH and FSH are presented in Figure 6.5. Mares of HBCS had higher LH concentrations than mares of LBCS, and the difference was greater during phase 2 (BCS, $P < 0.02$; BCS x phase x day, $P < 0.0001$; top panel). Mares not treated with eGH did have higher FSH concentrations toward the end of GnRHa treatment than mares treated with eGH (GH x Phase, $P = .05$; bottom panel).

Discussion

In previous studies in other species, administration of GH has been shown to have beneficial effects on ovarian activity, particularly increased follicle numbers. For

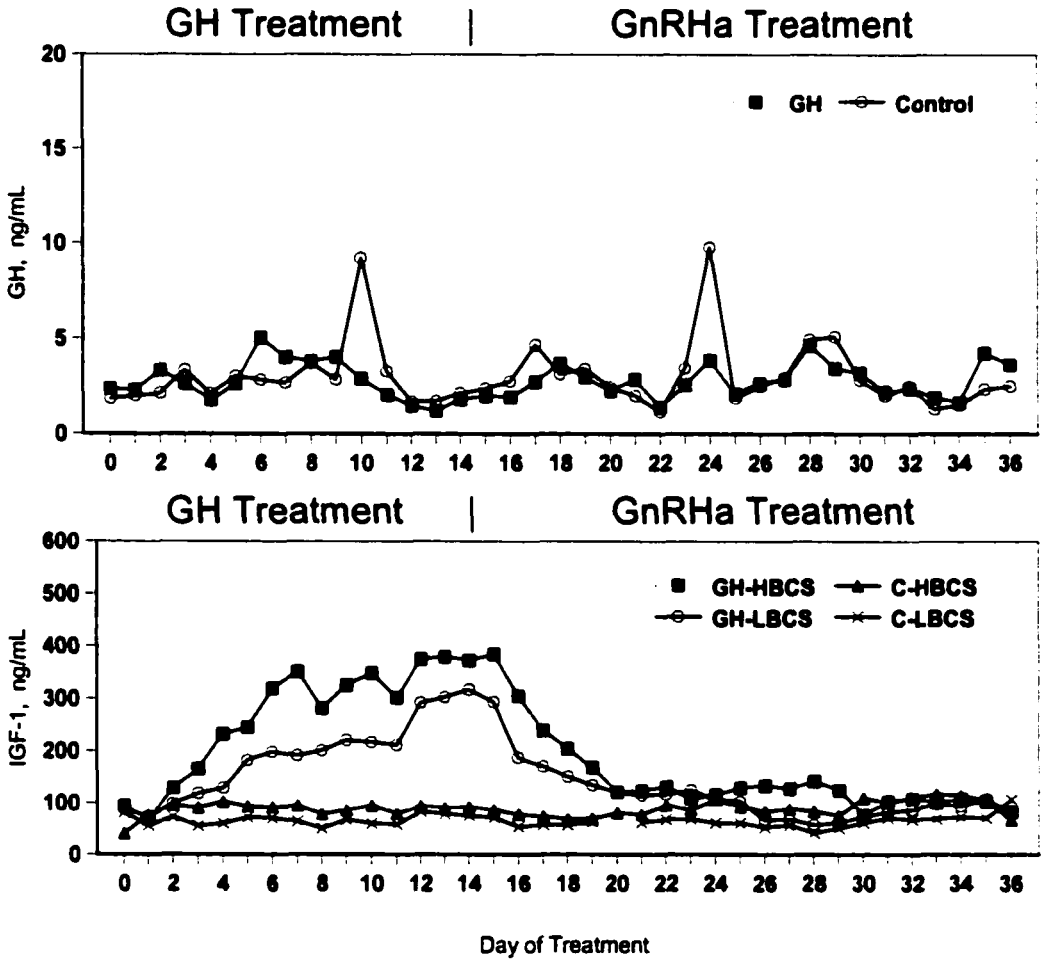

Figure 6.4. Plasma GH (top panel) and IGF-1 (bottom panel) concentrations in mares of HBCS vs mares of LBCS. Mares in each BCS group were treated with (n = 6) or without (n = 6) eGH for 14 d. All mares received GnRHa injections for 21 d thereafter, or until ovulation occurred. There was a GH x phase x day interaction ($P < 0.05$) for GH concentrations and a BCS effect ($P < 0.02$), a GH effect ($P < 0.0001$), and a GH x BCS x phase x day interaction ($P < 0.0001$) for IGF-1 concentrations in the analysis of variance. The pooled SEM were 0.10 and 1.86 ng/mL for GH and IGF-1 concentrations, respectively.

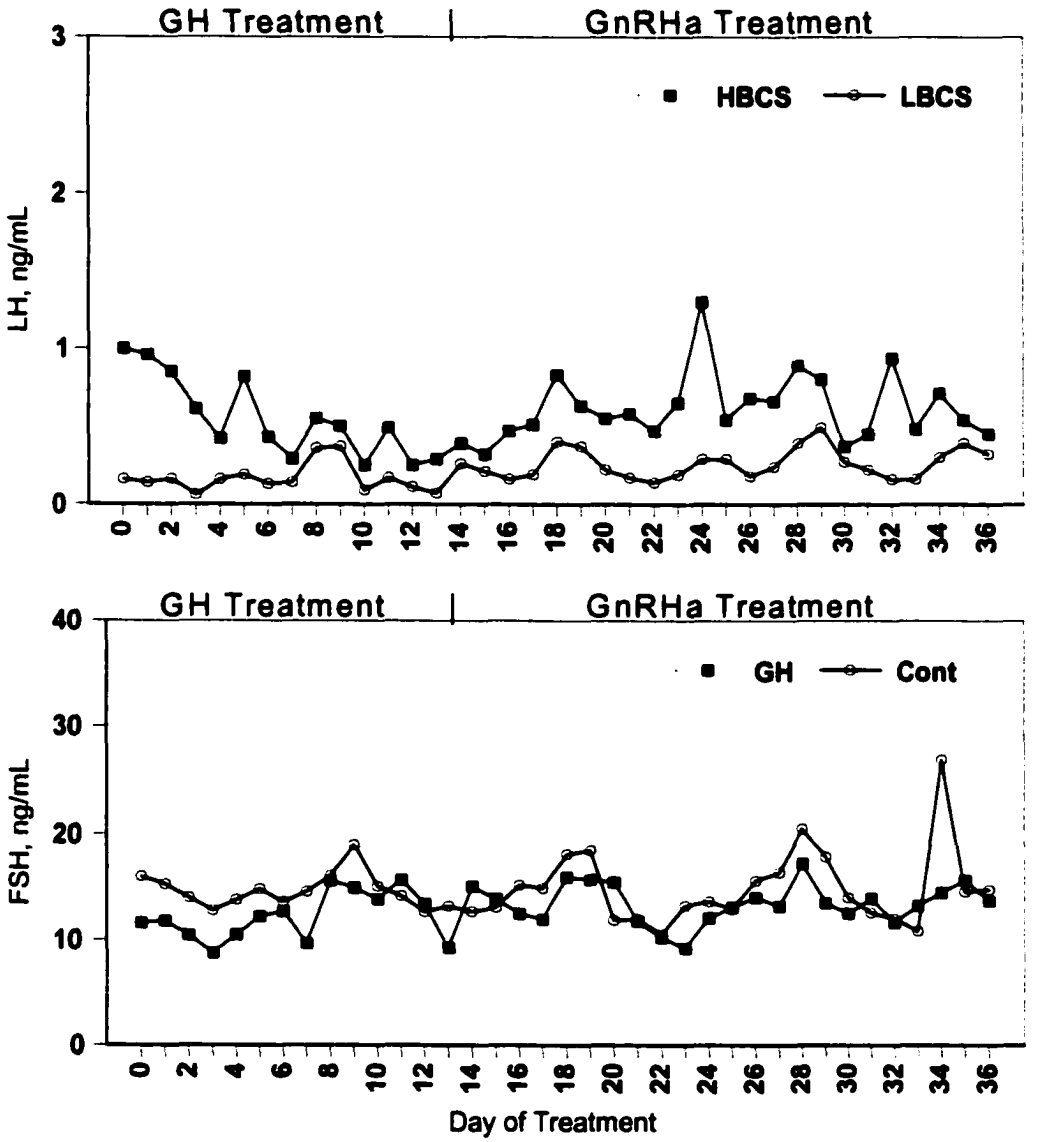

Figure 6.5. Plasma LH (top panel) and FSH (bottom panel) concentrations in mares of HBCS vs LBCS. Mares in each BCS group were treated with (n = 6) or without (n = 6) eGH for 14 d. All mares received GnRHa injections for 21 d thereafter, or until ovulation occurred. There was a BCS effect ($P < 0.02$) and a BCS x phase x day interaction ($P < 0.0001$) for LH concentrations and a GH x phase interaction ($P < 0.05$) for FSH concentrations in the analysis of variance. The pooled SEM were 0.02 and 0.26 ng/mL for LH and FSH concentrations, respectively.

instance, yearling beef heifers treated with bGH for 142 d had more total follicles than control heifers (Tripp et al., 2000). Lucy (2000) reported that administration of recombinant bGH increased ovarian follicular development, and it was suggested that this is due to either direct effects of GH (since GH receptors are found within granulosa cells as well as oocytes) or to indirect effects of increased IGF-1 and(or) nutrient partitioning that occurs after recombinant bGH treatment. Buratini et al. (2000) substantiated that finding by showing that not only did treatment with recombinant bGH result in a 36% increase in small follicle numbers in Nelore heifers, but plasma IGF-1 concentrations increased 2-fold.

In the mare, Cochran et al. (1999a) reported that treatment with eGH significantly increased the number of small follicles by the seventh day of treatment, and plasma IGF-1 concentrations were also greater by d 3 of treatment. The mares in that particular study were of average BCS, and all mares were anovulatory. In the present study, there was only a tendency for eGH to increase the number of small follicles, even though plasma IGF-1 concentrations increased almost 4-fold during eGH treatment. The mares in this study were either very HBCS or very LBCS. The HBCS mares already had numerous follicles of all sizes (large, medium and small) because they had continued to cycle and did not go deeply anestrus, as did the LBCS mares, which is why they had larger ovaries, more CL, and more large follicles. It is possible that a major increase in follicles in the LBCS mares treated with eGH did not occur because all efforts were being put into survival and there were not enough nutrients available for repartitioning. Also, in contrast to Tripp et al. (2000), these mares were only on eGH treatment for 14 d, a

relatively short time compared with the 142 d that the heifers were on treatment. In sheep, short-term treatment with ovine GH had no effect on ovulation rate or follicular development (Scaramuzzi et al., 1999). Moreover, in agreement with our findings, Tripp et al. (2000) reported that heifers that were restricted to 75% of ad libitum intake and treated with bGH did not have more follicles than heifers fed ad libitum.

In this study, treatment of mares with a GnRHa increased the number of large and medium-sized follicles. Cochran (2000) also reported that once daily injections of GnRHa caused an increase in the number of large follicles; however, treatment was given in conjunction with eGH. Twice daily injections (Harrison et al., 1990) as well as pulsatile administration (Johnson, 1987) of GnRHa to anovulatory mares has been shown to induce follicular development and ovulation in seasonally anovulatory mares. In this study, two mares in the LBCS group did ovulate toward the end of GnRHa treatment. Although Cochran et al. (1999a) suggested that eGH in conjunction with daily GnRHa injections can enhance the ovulatory response of mares, these two ovulations do not follow that pattern since one mare was in the eGH treatment group and the other received vehicle. Apparently the mares were responding to the GnRHa injections only. It is interesting to note though, that although these mares did ovulate, analysis of P_4 samples taken every 3 d from the beginning of treatment until the end of May showed that these mares reverted back to an anestrous state and did not actually go through transition and begin to cycle regularly until late April and early May, similar to the other mares in the LBCS group.

Unexpectedly, plasma GH concentrations were not different between mares in the HBCS group vs mares in the LBCS group. Higher plasma GH concentrations in the LBCS group would be indicative of nutritional stress. Plasma GH concentrations were higher on d 10 of both eGH treatment and GnRHa treatment in mares not treated with eGH, however, this was due to only 4 of the 12 mares in that treatment group (3 from the HBCS group and 1 from the LBCS group). The reason for such high GH concentrations in these mares on those particular days is unknown.

Breier (1999) stated that, in sheep, decreased nutrition results in elevated GH secretion but reduced plasma IGF-1 concentrations. In this case, treatment with eGH increased plasma IGF-1 concentrations in both HBCS mares and LBCS mares, and concentrations were higher in mares of HBCS. Smith et al. (1999) reported that daily plasma GH and IGF-1 concentrations were higher in geldings receiving recombinant GH relative to controls. In mares fed control protein and energy, plasma IGF-1 concentrations were higher than in mares fed restricted protein and(or) energy (Sticker et al., 1995). In a review, McGuire et al. (1992) reported that humans, rats and ruminants with severe nutritional deficiencies have decreased plasma IGF-1 concentrations and that plasma GH concentrations do not stimulate the release of IGF-1 under these conditions. The reduction in IGF-1 concentrations during feed deprivation (as detected in our study) supports the uncoupling of the stimulatory effect of GH on IGF-1 concentrations as is seen in reduced intake (McGuire et al., 1992).

Luteinizing hormone concentrations were higher in HBCS vs LBCS mares, especially during treatment with GnRHa. Several investigators have shown that both LH

and FSH (Evans and Irvine, 1976; Alexander and Irvine, 1986; Johnson, 1987) are released from the pituitary when anestrous mares are given exogenous GnRH. In this study, the HBCS mares had continued to cycle, whereas, the LBCS mares were anestrous. It has been well documented that during winter when mares are normally anestrous, hypothalamic GnRH content is severely reduced, causing pituitary LH output to be almost non-existent (Reeves et al., 1972; Hart et al., 1984; Mumford et al., 1984; Silva et al., 1987). Although LH concentrations increased slightly over time, it may be possible that the LBCS mares were so malnourished and deeply anestrous that the GnRHa given to them was not able to increase LH and FSH. Follicle stimulating hormone did increase in mares not treated with eGH toward the end of GnRHa treatment. This is in contrast to results by others in both cattle (Gong et al., 1991) and horses (Cochran, 2000) in which no differences were seen in either LH or FSH in animals treated with or without GH.

Implications

Treatment of mares with equine growth hormone did not significantly increase ovarian or follicular activity. The mares in the high body condition score group were very fat, and they did not go anovulatory as would be expected for mares during the winter. The added equine growth hormone was therefore of no benefit to these mares because they were continually cycling. The low body condition score mares were indeed anovulatory and in a deep anestrous state. Due to the low plane of nutrition of these mares, the addition of equine growth hormone (as well as gonadotropin releasing hormone analog) was not enough to overcome this state of extremely low energy reserves and cause them to ovulate and begin cycling. Others have shown that administration of

growth hormone and gonadotropin releasing hormone analog can have beneficial effects on reproduction of animals of average body condition. These data confirm the importance of proper nutrition to the animal, particularly from a reproductive standpoint.

CHAPTER VII

THE EFFECTS OF BC, PREGNANCY STATUS, GH AND DEXAMETHASONE ON LEPTIN CONCENTRATIONS IN THE HORSE

Introduction

Leptin is an adipocyte-derived 16 kDa polypeptide that has received a great deal of attention since 1994, when the leptin gene was cloned by a group at Rockefeller University (Zhang et al., 1994). At that time, it was suggested that leptin may be the long sought "adipostat" that would help maintain whole-body energy balance and therefore be the answer to body weight regulation. Since that time, research has shown leptin to be involved in various systems of the body. The most consistent role of leptin is as a regulator in food intake and energy expenditure (Fruhbeck et al., 1998; Houseknecht et al., 1998; Ramsey, 1999), and it has been shown that plasma leptin concentrations vary directly with body mass index and percentage of body fat (Prolo et al., 1998; Chilliard et al., 2000). Research with humans and rodents has shown that concentrations of leptin are influenced by gender (Castracane et al., 1998; Koistinen et al., 1998; Pineiro et al., 1999), metabolic hormones (Cusin et al., 1995; Miyakawa et al., 1998; Shimon et al., 1998) and reproductive status (Barash et al., 1996; Kohsaka et al., 1999). In normal female mice, leptin induced early puberty (Chehab et al., 1997). In ob/ob mice, leptin increased LH as well as ovary and uterine weight in females and increased FSH as well as testes and seminal vessicle weight in males (Barash et al., 1996). To date, although a considerable amount of knowledge on leptin's actions has been accumulated and research is expanding to include areas such as animal agriculture, there is little information regarding the role of

leptin in the horse. Therefore, this experiment was conducted to characterize plasma leptin concentrations under various conditions in the horse.

Experimental procedures

Experiment 7.1: LBCS vs HBCS mares. Data for this experiment were obtained from the mares used in the experiment cited in chapter IV on the relationship between body condition and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. A detailed description of the experimental procedures can be found in chapter IV. For this experiment, weekly blood samples (from September to January) were collected at 0730 h by jugular venipuncture into 7-mL evacuated tubes containing sodium heparin. These samples were centrifuged and the plasma harvested and stored at -15°C in 12 mm x 75 mm polystyrene tubes until analyzed for plasma leptin concentrations. For all experiments (7.1 to 7.5), plasma leptin was analyzed by RIA using a commercially available kit (Linco Research, St. Charles, MO) previously validated for horse tissues by McManus and Fitzgerald (2000), with values based on human equivalents of leptin.

Experiment 7.2: GH/GnRHa in mares. Data for this experiment were obtained from the mares used in the experiment cited in Chapter VI on GH and GnRHa interactions in mares of very LBCS or very HBCS. A detailed description of the experimental procedures can be found in Chapter VI. For this experiment, daily blood samples were collected at 0730 h by jugular venipuncture into 7-mL evacuated tubes containing sodium heparin. These samples were centrifuged and the plasma harvested

and stored at -15°C in 12 mm x 75 mm polystyrene tubes until analyzed for plasma leptin concentrations.

Experiment 7.3: Dexamethasone treatment of mares. Twelve mares of LBCS (3.0 to 3.5), which had been anestrus during the winter period and 12 mares of HBCS (8.0 to 8.5) which continued to ovulate throughout the winter period were used in this study. Cyclicity in mares was determined in three ways: blood sampling over time for P_4 analysis, teasing with a stallion on a regular basis and transrectal ultrasonography using an Aloka 550V ultrasound machine with a 5 megahertz linear-array transducer (Aloka Science and Humanity, Wallingford, CT). Mares had been monitored closely over the winter period and into the spring months. Mares in the HBCS group were obese and continued to cycle throughout the traditional seasonal anestrus period. Mares in the LBCS group went into a deep anestrous state until late spring. Once it was determined that a LBCS mare had ovulated for the first time (in the spring), she was then paired with a mare from the HBCS group, which had just ovulated, and both mares were treated with 125 μg/kg BW DEX i.m. for 4 d. Jugular blood samples were collected at 0800 h and 2000 h during treatment and then once daily at 0800 h for 4 more days. Samples were centrifuged and the plasma harvested and stored at -15°C in 12 mm x 75 mm polystyrene tubes until analyzed for plasma leptin concentrations as well as plasma glucose and insulin.

Experiment 7.4: Pregnant vs foaling mares. Beginning in late February and continuing weekly until late May, blood samples were collected from mares at a local breeding and training farm (Clear Creek Stud, Folsom, LA) within one week of foaling

and again within one week after foaling. All mares were of average BCS (5.0 to 6.0) and under normal herd health and nutrition regimes. Blood samples were drawn by venipuncture into 7-mL evacuated tubes containing sodium heparin. Immediately after withdrawal, blood samples were put on ice until centrifuged for 15 min at 1,500 x g at 4°C. Plasma was harvested and stored at -15°C in 12 mm x 75 mm polystyrene tubes. These samples were used for measurement of plasma leptin concentrations.

Experiment 7.5: GH in foals. Blood samples were previously collected from twelve light horse foals (predominantly Quarter Horse breeding) and two pony-Arabian cross foals, which were part of an ongoing experiment (Capshaw et al., 2001). Briefly, foals were housed at the Louisiana State University Agricultural Center Central Research Station in Baton Rouge, Louisiana. Males and females were equally distributed to treatment and control groups by first pairing the foals based on similar gender, size, parentage and type. One foal from each pair was then randomly assigned at weaning to receive either a daily i.m. injection of eGH (EquiGen) at 20 μg/kg of BW in the neck or an equivalent daily injection of vehicle for 12 months. Blood samples were drawn weekly by venipuncture into 7-mL evacuated tubes containing sodium heparin. Blood samples were centrifuged and the plasma harvested and stored at -15°C in 12 mm x 75 mm polystyrene tubes. These samples were used for measurement of plasma leptin concentrations.

Statistical analyses. Data from Experiments 7.1, 7.2, 7.3 and 7.5 were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). For Experiment 7.4, data were analyzed using the proc t-test procedure of SAS (SAS Inst. Inc., Cary, NC) to

determine differences in leptin concentrations pre- and post-foaling. Also, the proc univariate procedure of SAS (SAS Inst. Inc., Cary, NC) and Shipero Wilkes normality test was used to determine outliers. In Experiments 7.1 and 7.3, a completely randomized design tested BCS using the residual error. In Exp. 7.2, a completely randomized design with a 2 x 2 factorial arrangement of treatments tested BCS, GH treatment and their interaction using the residual error. In Exp. 7.5, the data were analyzed as a randomized complete block design. Foal pairs were the blocking factor for this experiment. Leptin (and glucose and insulin, Exp. 7.3) data taken over days throughout each of the experiments were analyzed by univariate analysis of variance which took into account the repetitive nature of the sampling (split plot; Gill and Hafs, 1971).

Results

Experiment 7.1. Plasma leptin concentrations from September to January are shown in Figure 7.1. Plasma leptin concentrations were higher ($P < 0.009$) in the HBCS group than the LBCS group throughout the experiment and decreased over time (trt x week, $P < 0.0001$). Due to pre-treatment (d 0) differences in plasma leptin concentrations, leptin data were sorted by mares with leptin concentrations < 10 ng/mL and mares with leptin concentrations ≥ 10 ng/mL on d 0 and that data is shown in Figure 7.2.

Experiment 7.2. Plasma leptin concentrations were higher ($P < 0.002$) in HBCS mares than LBCS mares throughout the experiment with no effect ($P > 0.1$) of eGH treatment. Also, an interaction ($P < 0.003$) between BCS and phase was detected in that leptin concentrations increased in the HBCS group throughout the period of eGH

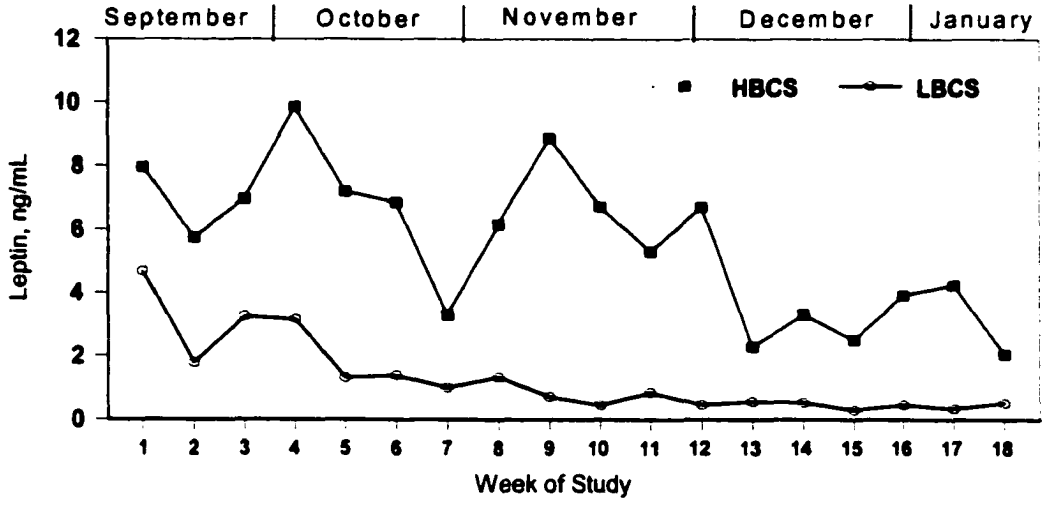

Figure 7.1. Weekly plasma leptin concentrations in mares of HBCS vs mares of LBCS from September to January. There was a BCS effect ($P < 0.009$) and a BCS x week interaction ($P < 0.0001$) in the analysis of variance. The pooled SEM was 0.05 ng/mL.

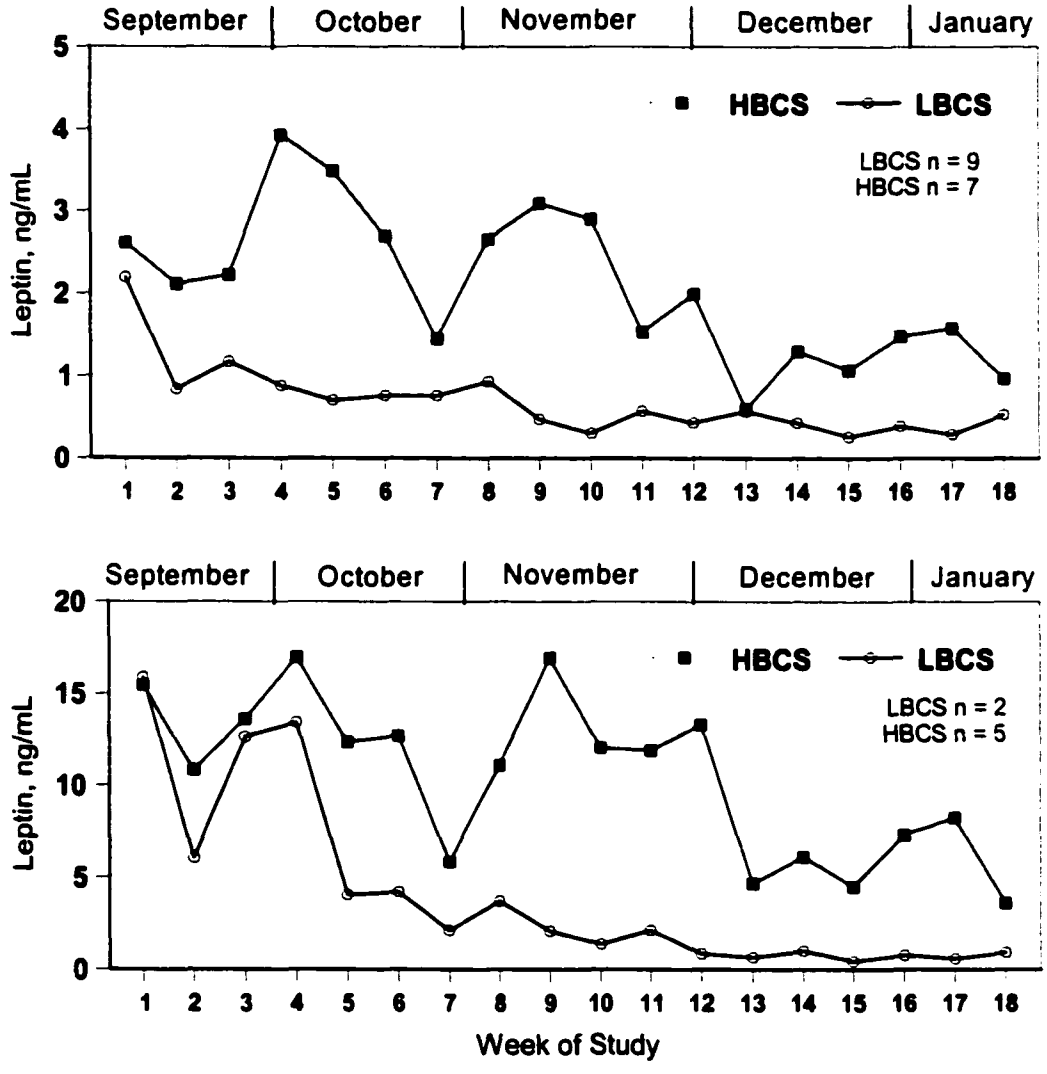

Figure 7.2. Weekly plasma leptin concentrations in mares of HBCS vs mares of LBCS from September to January analyzed based on d 0 leptin concentrations. Mares with leptin concentrations < 10 ng/mL at d 0 are shown in the top panel. There was a BCS effect ($P < 0.02$) and a BCS x week interaction ($P < 0.001$). Mares with leptin concentrations ≥ 10 ng/mL at d 0 are shown in the bottom panel. There was a BCS effect ($P < 0.072$) and a BCS x week interaction ($P < 0.02$). The pooled SEM were 0.05 ng/mL.

treatment (phase 1), peaked by d 7 of GnRHa treatment (phase 2, d 21 of experimental period) and declined over time after that (Figure 7.3).

Experiment 7.3. Dexamethasone treatment increased ($P < 0.001$) leptin concentrations in HBCS mares within 12 h of treatment, and leptin concentrations continued to rise until d 3 before the morning injection. Leptin concentrations in the LBCS group were not affected by DEX, and remained low and unchanged (Figure 7.4).

Both glucose and insulin responded to DEX treatment in both the HBCS and LBCS groups, however, the magnitude of the response was greater ($P < 0.0001$) in the HBCS mares (Figure 7.5). Plasma glucose concentrations increased ($P < 0.0001$) throughout the treatment injection period and peaked at 8.8 and 6.5 mmol/L for HBCS and LBCS groups, respectively, within 12 h after the last treatment injection (Figure 7.5). Plasma insulin, on the other hand, peaked at approximately 81 μIU/mL by the morning of d 3 before treatment injections in the HBCS group whereas plasma insulin concentrations reached approximately 24 μIU/mL by the evening of d 2 in the LBCS and plateaued after that (day, $P < 0.04$; Figure 7.5).

Experiment 7.4. In mares of average BCS, leptin concentrations were higher ($P < 0.0001$) immediately prior to foaling than immediately after foaling (3.4 vs 2.0 ng/mL leptin; Figure 7.6).

Experiment 7.5. Over the course of the experiment, leptin concentrations increased ($P < 0.004$) in foals not treated with eGH and remained relatively unchanged in those foals that were treated with eGH for 12 months (Figure 7.7, top panel). Also, leptin

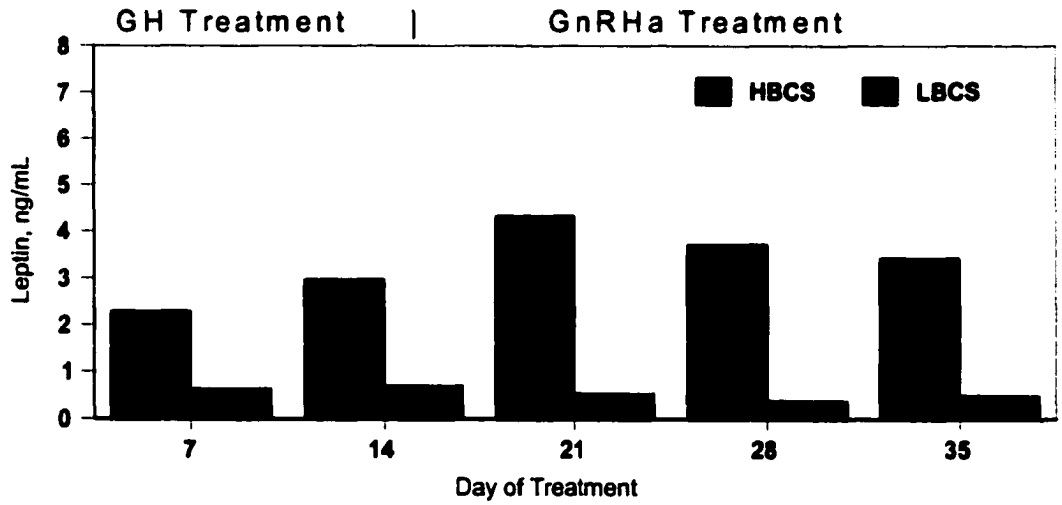

Figure 7.3. Plasma leptin concentrations in mares of HBCS vs mares of LBCS. Mares in each BCS group were treated with (n = 6) or without (n = 6) 25 ug/kg BW eGH for 14 d. All mares received GnRHa (50 ng/kg BW) injections for 21 d thereafter, or until ovulation occurred. There was a BCS effect ($P < 0.002$) and a BCS x phase interaction ($P < 0.003$) for leptin concentrations in the analysis of variance. The pooled SEM was 0.87 ng/mL.

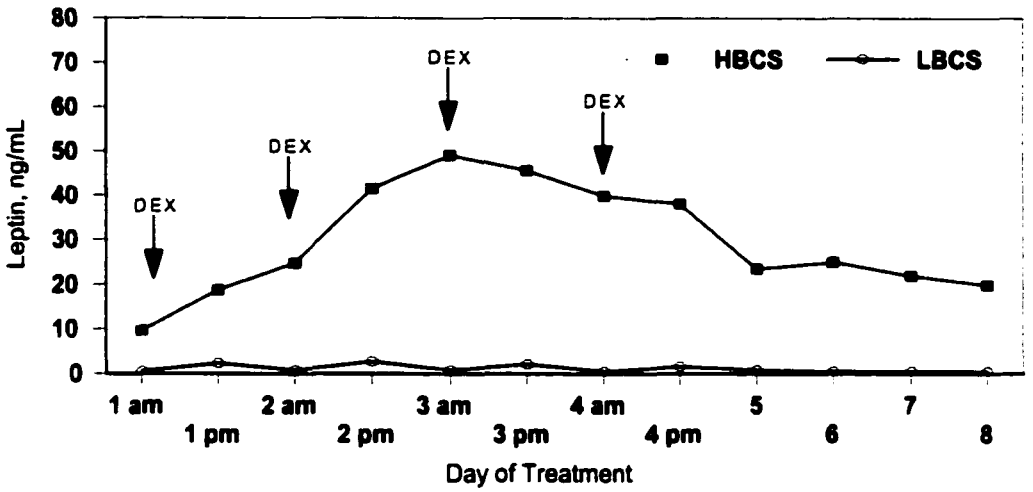

Figure 7.4. Plasma leptin concentrations in mares of HBCS vs mares of LBCS during treatment with dexamethasone. Blood samples were collected every 12 h for 4 d and then for 4 d following treatment. There was a BCS effect ($P < 0.001$) for leptin concentrations in the analysis of variance. The pooled SEM was 5.4 ng/mL.

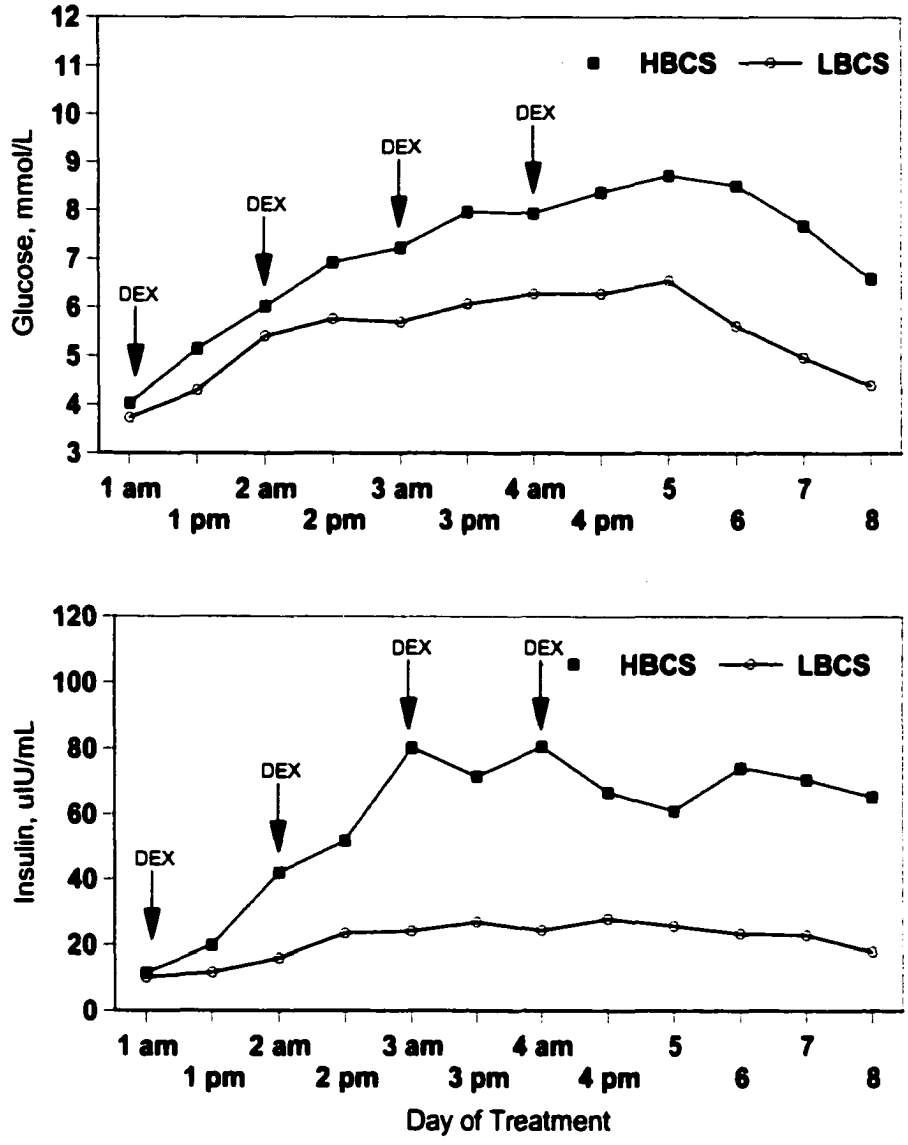

Figure 7.5. Plasma glucose (top panel) and insulin (bottom panel) concentrations in mares of HBCS vs mares of LBCS during treatment with dexamethasone. Mares were treated every 12 h for 4 d and for then 4 d following treatment. There was a BCS effect ($P < 0.0001$) and a day effect ($P < 0.0001$) for glucose concentrations and a BCS effect ($P < 0.0001$) and a day effect ($P < 0.04$) for insulin concentrations in the analysis of variance. The pooled SEM were 0.18 mmol/L and 3.7 uIU/mL for glucose and insulin concentrations, respectively.

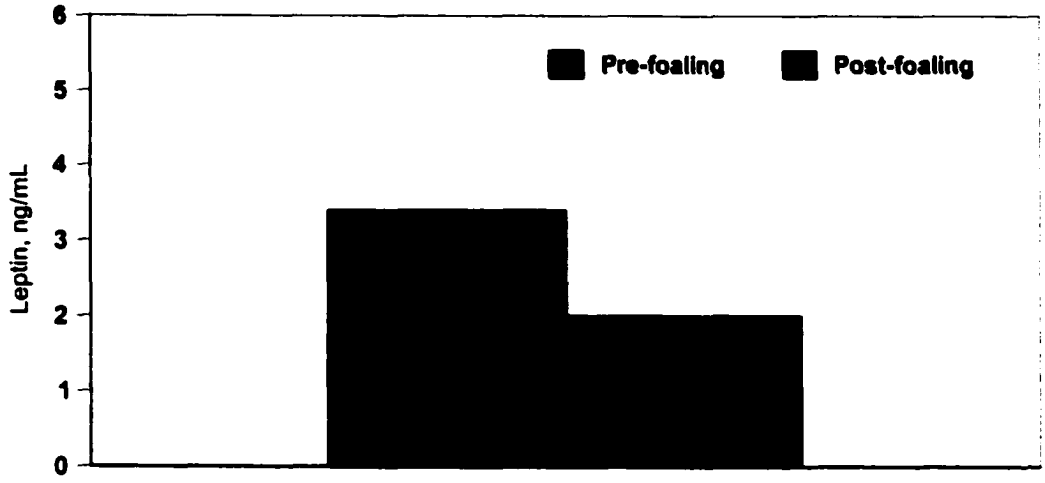

Figure 7.6. Plasma leptin concentrations prior to foaling vs after foaling in average BCS mares. Jugular blood samples were collected from mares within one week of foaling and again within one week after foaling. There was an effect ($P < 0.0001$) for leptin concentrations in the analysis of variance.

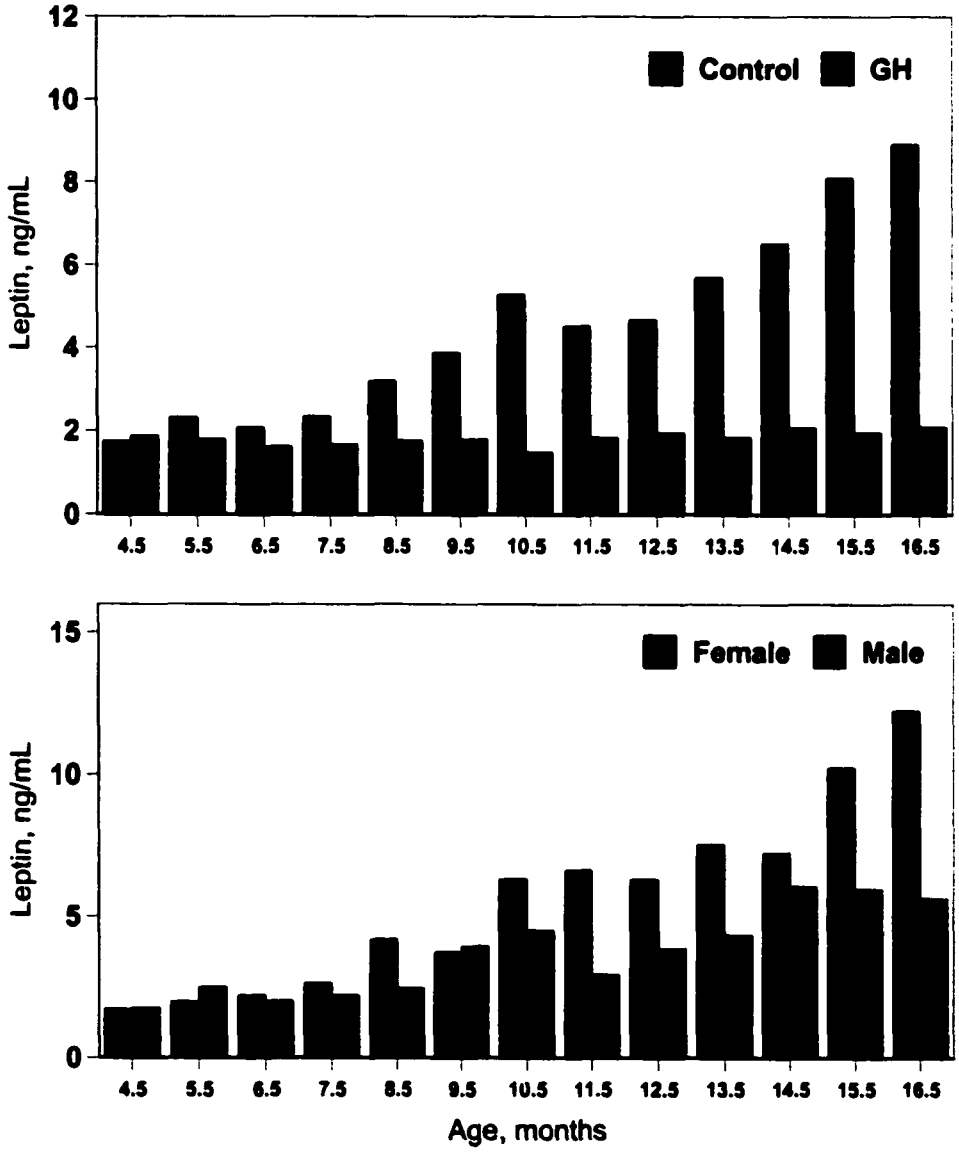

Figure 7.7. Plasma leptin concentrations in foals treated with or without eGH for 12 months. There was a treatment effect ($P < 0.004$; top panel) and there tended to be a gender effect ($P = 0.1$; bottom panel) in leptin concentrations in the analysis of variance. The pooled SEM were 0.84 ng/mL.

concentrations tended to be higher (P = 0.1) in females than males over the 12-month period (Figure 7.7; bottom panel).

Discussion

Experiment 7.1. In the analysis of the raw data in Experiment 1, plasma leptin concentrations were higher in the HBCS mares than the LBCS mares, even on d 0, before they were placed on their respective planes of nutrition. Examination of the individual mares indicated that they fell into two distinct catagories on d 0 - those with leptin concentrations $\geq$ 10 ng/mL, and those with leptin concentrations of 5 ng/mL or less. Thus, a second analysis was performed with d 0 leptin concentrations as a blocking factor in the analysis of variance. Due to chance, there were 5 of 12 mares with leptin concentrations $\geq$ 10 ng/mL in the group that was destined to be the HBCS group, and only two in the group that was destined to be the LBCS group. The results from this analysis (Figure 7.2) showed that reducing BC did indeed reduce leptin concentrations in mares with high d 0 leptin concentrations.

In addition to the effect of BC on leptin concentrations, leptin concentrations also decreased throughout the experiment in both groups. In the LBCS mares, leptin concentrations decreased more rapidly as BC decreased. The decreased leptin concentrations in both groups (from September to January) seen in this study are in agreement with McManus and Fitzgerald (2000), in which leptin concentrations were shown to be higher in the summer than in the winter in both young and mature mares. Therefore, it appears that there is an overriding factor (such as photoperiod, temperature or season) causing leptin concentrations to decrease in both groups.

Leptin is an adipocyte-derived protein hormone and it has been shown to vary directly with body mass index and percentage body fat in humans (Prolo et al., 1998; Chilliard et al., 2000). Therefore, it would be expected that the HBCS mares (with a lot of fat cover) would have higher leptin concentrations than the LBCS mares (with very little fat cover).

Houseknecht et al. (1998) proposed that leptin may be the long sought indicator of nutritional status that allows reproductive processes to proceed. In this study, although LBCS mares went deeply anestrous, only one mare in the HBCS group went truly anestrous during the normal winter anestrus period. Seven of the HBCS mares cycled regularly from September to January and four others only went through a brief period (December to mid February) with low P_4 concentrations. Although there appears to be a link between leptin concentrations, fat content and reproductive activity, more research is needed to determine exactly what role leptin plays in the reproductive processes of the horse.

Experiment 7.2. Plasma leptin concentrations were higher in mares of HBCS vs mares of LBCS throughout the experimental period. This was expected given that leptin is secreted mostly by the adipocytes, and mares in the HBCS were obese and mares in the LBCS group had no visible fat cover. No differences were detected, though, due to treatment with eGH. Excess GH (and/or IGF-1) have been shown to reduce serum leptin concentrations by reducing body fat mass (Miyakawa et al., 1998). However, this effect may have been masked since the HBCS mares were so obese and the LBCS mares did not have any fat left to be reduced. An interaction between BCS and phase was observed in

that leptin concentrations increased in the HBCS group throughout eGH treatment and peaked by d 7 of GnRHa treatment. Leptin concentrations gradually decreased from d 7 to d 21 of treatment. As was stated above, this may be due to the fact that the HBCS mares were still cycling and therefore some mares were in the luteal phase while more may have been in the follicular phase.

Experiment 7.3. Several studies have shown that glucocorticoids upregulate leptin secretion and serum leptin concentrations (Wabitsch et al., 1996; Elimam et al., 1998; Wauters et al., 2000). In this study, DEX increased leptin concentrations approximately 4-fold in mares of HBCS by d 2, but leptin concentrations were virtually unchanged in the LBCS group. Continual injections of DEX, however, did not stimulate leptin to rise further in the HBCS mares. In fact, leptin concentrations began a steady decline throughout the remainder of the injection schedule and then slowly declined thereafter. Elimam et al. (1998) found an increase in plasma leptin during low dose DEX treatment. Also, Dagago-Jack et al. (1997) found that DEX increased plasma leptin concentrations by 64 to 111% above baseline within 2 to 4 d, particularly in obese subjects.

Plasma glucose and insulin were also both increased after DEX administration. In vitro, insulin has been shown to stimulate mRNA expression and secretion of leptin in cultured rat and human adipocytes (Hardie et al., 1996; Wabitsch et al., 1996). The increase in leptin secretion could be due to the elevated insulin concentrations induced by DEX administration (Zakrzewska et al., 1997).

Experiment 7.4. The fact that plasma leptin concentrations were higher in mares before foaling than after foaling is in agreement with reports in both rodents and humans in which leptin rises in the maternal circulation towards the end of gestation with a fall to below pregestational concentrations at around birth (Hardie et al., 1997; Masuzaki et al., 1997; Fruhbeck et al., 1998; Lewandowski, et al., 1999; Herrera et al., 2000; Wauters et al., 2000; Thomas et al., 2001). Thomas et al. (2001) indicated there are a few possible explanations for the increase in leptin during late pregnancy. One is that leptin is synthesized by the placenta (Gonzales et al., 2000; Thomas et al., 2001) while another theory states that leptin is secreted by specific adipose tissue depots (Tomimatsu et al., 1997).

Wauters et al. (1999) suggested that even changes in other hormones which take place during pregnancy may influence leptin concentrations. In sheep, leptin receptor gene expression was detected in the placenta, suggesting that the placenta is a target organ for leptin; however, between species, the relative level of placental leptin expression is different (Thomas et al., 2001). These theories may apply to the mare as well since leptin concentrations are high before foaling and are lower after foaling, however, more research is needed to determine the exact source of the leptin in late gestation.

Experiment 7.5. In this study, administration of eGH to foals did not stimulate the secretion of leptin. As a matter of fact, plasma leptin remained unchanged in foals treated with eGH for 12 months. In contrast, leptin concentrations steadily increased in foals not treated with eGH. Growth hormone exerts its effects on all or almost all tissues of the

body (Guyton, 1991). In addition to growth and development, GH has a very important impact on body composition and fat distribution through its influence on energy metabolism (Wauters et al., 2000). According to De Boer et al. (1995), exogenous GH stimulates energy expenditure and decreases the amount of body fat. In growing animals in positive energy balance, exogenous GH treatment reduces food intake which suggests that GH may act on central feeding centers in addition to regulating peripheral tissue metabolism (Houseknecht et al., 2000). Because leptin concentrations vary directly with body mass and percentage body fat, it would make sense that the foals treated with eGH in this study had lower leptin concentrations. Although most studies indicate an inhibitory effect of the GH axis on leptin secretion, it is not clear if it is a direct effect or and indirect effect through stimulation of lipolysis in the adipocytes (Wauters et al., 2000).

Also, in the present study, fillies tended to have higher leptin concentrations than young geldings. In sheep, plasma leptin was higher in female sheep than in castrated or intact male sheep (Blache et al., 2000). In studies with humans it has been reported that leptin concentrations are much higher (as much as 2 to 3 times higher) in females than in males (Hamilton et al., 1995). Females generally have a higher percentage of body fat and a higher ratio of subcutaneous to visceral fat, and plasma leptin is strongly related to fat mass (Houseknecht, et al., 1998; Chilliard et al., 2000) as well as subcutaneous fat (Wauters et al., 1998). It is interesting to note that other factors, such as sex steroids (of females vs males), may play a role in the differences in leptin concentrations. In vitro studies have shown that estrogens stimulate leptin secretion by adipocytes from women

and not men (Lahlou et al., 1997), and androgens were shown to have an inhibitory effect on leptin secretion (Wabitsch et al., 1997).

Implications

In these experiments, leptin concentrations were affected by body condition, pregnancy status and treatment with equine growth hormone or dexamethasone. Therefore, it appears that leptin may be involved in the various systems of the horses body including reproduction. However, more research is needed to learn as much as possible about the role of leptin in the horse. Although obesity is not as much a problem in the horse industry, the information gained here is an important first step in becoming more familiar with the systems involved in leptin production, secretion and activity in the body.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

The fact that nutrition plays a crucial role in the reproductive performance of many species, including humans, is well known; however, information that is available in the horse is limited and conflicting. Although it has been shown in the horse that inadequate nutrition and reproductive inefficiency are related, it is not known how reproductive hormones and ovarian activity are affected. Also, research with overly fat mares is inconclusive. Therefore, the first objective was to contrast the reproductive and hormonal profiles of HBCS compared with LBCS mares during the traditional seasonal anestrous period. It was also important to correlate BCS with actual backfat measurements. Body condition scoring, which is a method used to assess the live animal's energy reserves, can be very effective if used properly. Data from the first experiment showed that BCS is highly correlated to ultrasonic backfat measurements. Results showed that backfat measurements taken at the tailhead area are most highly correlated to BCS, and backfat measurements taken over the rump area are least correlated. The areas of the 13th rib and the withers are also good indicators of the amount of condition. By utilizing the BCS system developed by Henneke et al. (1983), as well as looking closely at the areas of the tailhead, 13th rib and withers, the condition and nutritional status of the horse can be more accurately assessed.

The data obtained in Experiment 2 demonstrate the importance of nutrition for reproduction of the horse. Mares that were in low body condition went into a deep anestrous state for a much longer period than normal. The HBCS mares continued to

cycle throughout the anestrous period and therefore had larger ovaries, more large follicles, more ovulations and more CL. Hormonal patterns were different also, as would be expected. The release of PRL was greater in response to TRH in the HBCS mares. Also, LH was higher and FSH lower, in response to GnRH in the HBCS group. The reduced FSH response was indicative of mares in the follicular phase, when ovarian products inhibit normal FSH secretion. Over 12 h, LH and FSH increased, and GH decreased, in the HBCS mares. The lower GH concentrations over 12 h, as well as lower GH in response to a potent GH secretagogue, is a clear indication that the LBCS mares were stressed.

The second set of objectives were to determine how different hormonal manipulations affect reproduction of seasonally anovulatory mares. Experiment 3 tested the effects of two releasing hormones, GnRHa and TRH, either together or alone, and Experiment 4 tested the effects of eGH with GnRHa. Gonadoptropin releasing hormone is a hypothalamic hormone, which is known to be low during the seasonal anestrous and anovulation period. This results in reduced production and secretion of gonadotropins. Several experiments have shown that exogenous GnRH does induce the release of both LH and FSH (Evans and Irvine, 1976; Alexander and Irvine, 1986; Johnson, 1987). The effect of TRH is not really known, except that plasma T_4 concentrations were higher in mares that continued to cycle during winter in Kentucky (Fitzgerald and Davison, 1997). Gonadotropin releasing hormone analog treatment with TRH did increase the number of medium-sized follicles over TRH treatment alone. Also, both LH and FSH increased over days indicating that GnRHa administration not only induced secretion of releasable

gonadotropins from the pituitary, but that more hormones were made. Pituitary content of PRL was slightly higher after TRH treatment, which is important because PRL appears to play a role in reproduction, although the exact mechanism still needs to be determined.

Growth hormone is another hormone that may have a very important reproductive role. Administration of GH has been shown to have beneficial effects on ovarian activity such as increased follicle numbers in animals of average BCS (Spicer et al., 1992; Cochran, 1999; Buratini et al., 2000; Joyce et al., 2000). Growth hormone, in conjunction with GnRHa, was shown to significantly increase the number of anovulatory mares of average BCS ovulating over mares just treated with GnRHa (Spicer et al., 1992; Cochran, 1999; Buratini et al., 2000; Joyce et al., 2000). Experiment 4 was conducted to determine if eGH treatment immediately followed with GnRHa treatment would benefit mares of very LBCS and initiate larger follicle growth and ovulations. Two mares in the LBCS group did ovulate, however, these mares went back into a deep anestrous state and did not go through transition until late spring (similar to the other LBCS mares).

The last experiment was conducted to evaluate the role of leptin in the horse and how leptin and reproduction are related. Leptin has gained a lot of attention lately, and it is thought that leptin may act as a metabolic "gate" to inhibit the activity of the neuroendocrine reproductive axis. As expected, leptin was higher in mares of HBCS over time and decreased to almost non-existent levels in mares of LBCS. Although data is now emerging which suggests that leptin is secreted from other cells and locations in the body, it is mostly an adipocyte secreted protein. Based on that fact that leptin is synthesized by the adipocytes, it would make sense that differences were seen in these

mares. Also, DEX caused leptin to increase significantly in mares of HBCS and had virtually no affect on leptin concentrations in LBCS mares. Several studies have shown that glucocorticoids upregulate leptin secretion and serum leptin concentrations (Wabitsch et al., 1996; Elimam et al., 1998; Wauters et al., 2000). In agreement with other studies in humans (Schubring et al., 1997) and rodents (Herrera et al., 2000), leptin concentrations were higher immediately prior to foaling and decreased significantly right after foaling. Thus it appears that leptin is made in the placenta also. Also, in agreement with other studies, eGH treatment suppressed leptin concentrations in foals and leptin concentrations tended to be higher in fillies than young geldings.

In conclusion, these results show how important nutrition is to the reproductive processes of the horse. However, the complexity of the nutritional-neuroendocrine-reproductive axis requires that much more research be conducted to determine exactly how each is related to the other and the exact roles that the various hormones have in this process.

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VITA

Laura Roland Gentry, daughter of Harry Lewman Roland, Sr. and Ethel Melancon Roland, was born in Baton Rouge, Louisiana. She graduated Valedictorian from Tara High School, Baton Rouge, Louisiana, in 1982 and received her bachelor of science degree in 1986 in animal science and her master of science degree in 1989 in ruminant nutrition at Louisiana State University. Laura was married to Glen T. Gentry, Jr. on August 14, 1989, and they moved to Lowell, Arkansas. While in Arkansas, Laura worked for the Poultry Science Department at the University of Arkansas in Fayetteville and rode racehorses on the side. In 1991, Laura and Glen moved to Clinton, Louisiana, and Laura went to work at Exxon in the Catalyst Preparation Laboratory. In 1993, Laura accepted a position as Research Associate for the Poultry Science Department at Louisiana State University working specifically with ratites. Then, in 1994, Laura became Research Associate with a joint appointment in the departments of Animal and Dairy Science. In 1997, Laura became Research Associate under the supervisor of Dr. Donald L. Thompson, Jr. in the area of equine physiology in the Department of Animal Science, during which she pursued the doctor of philosophy degree with an emphasis in equine nutritional and reproductive physiology. Laura and Glen have one son, Jacob Cameron Gentry.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Animal and Dairy Sciences

Title of Dissertation: Body Condition, Leptin, and Reproductive Characteristics in Horses

Approved:

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November 6, 2001