The Modulation of the Bioactivity of Bovine Luteinizing Hormone by Somatotropin and Nutritional Manipulation

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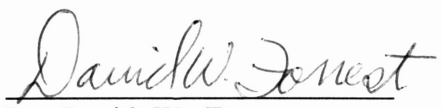

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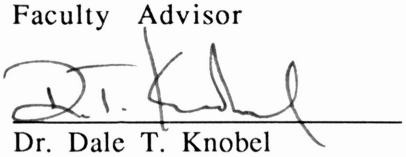

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ABSTRACT

THE MODULATION OF THE BIOACTIVITY OF BOVINE LUTEINIZING HORMONE BY SOMATOTROPIN AND NUTRITIONAL MANIPULATION

(APRIL 1991)

Eric Wynne McIntush

Twenty Angus X Holstein heifers were randomly assigned to one of four treatment groups (n=5) in a 2 X 2 factorial arrangement to evaluate the effects of recombinant bovine somatotropin (bST) and(or) nutritional manipulation on bioactive LH. The four treatment groups were arranged as follows: (I) continuous growth (CG) + excipient (EX), (II) CG + ST, (III) discontinuous growth (IG) + EX and (IV) IG + ST. CG heifers were fed a 68% concentrate diet formulated to provide for a growth rate of .8 kg/d throughout treatment. Two successive periods of intermittent growth were imposed on IG heifers by alternately feeding a 68% concentrate diet formulated for growth at .25 kg/d (restricted growth) for 3 months and then realimentation on a 90% concentrate diet fed ad libitum (compensatory growth) for 2 months. EX groups received excipient injections, while ST groups received injections (sc) of 500 mg bST every 14-d. Blood samples were collected at 20-min intervals for 6 h at ages 9, 11 and 14 mo (sample periods 1, 2 and 3, respectively). Bioactive LH was quantified by a validated *in vitro* rat Leydig cell testosterone assay. There was a sample period x treatment interaction ($P<.05$) for mean bioactive LH concentration. Mean bioactive LH concentration across sample period was greater ($P<.05$) for CG + EX heifers (.20 ng/ml) than for IG + ST heifers (.07 ng/ml). There was an effect of sample period ($P<.05$) on number of LH pulses and mean amplitude of LH pulses. Treatment affected ($P<.05$) mean amplitude of LH pulses but not number of LH pulses. Mean amplitude was greater for

group CG + EX than for group IG + ST (.36 and .09 ng/ml, respectively). These results suggest that nutritional manipulation designed to achieve intermittent growth patterns in combination with somatotropin administration reduces mean concentration and pulse amplitude of bioactive LH in heifers.

ACKNOWLEDGEMENTS

I am deeply appreciative for the guidance of Dr. D. W. Forrest as my advisor. His endless patience and insight has made my introduction into the world of research an enlightening and motivating experience.

Thank you to Sandra Scarborough, Scott Jacques and Christine Rumuly for making the laboratory work all the more enjoyable. All the long hours put into this project were made into a valuable learning endeavor. The laughs we shared will be remembered always.

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INTRODUCTION

One of the primary limitations to significant gains in efficiency of beef cattle production is the interval from the birth of a heifer to delivery of her first calf. Various means have been studied to analyze potential means of decreasing the length of this interval, but new and innovative approaches are still needed.

Recombinant bovine somatotropin has been shown to increase persistency in dairy cattle, but only a small number of studies have been conducted to determine the influence of somatotropin administration on beef heifers. To date studies on somatotropin's influence on luteinizing hormone (LH) in beef cattle have been limited to analysis of immunologically active LH plasma concentration. The effects of somatotropin on plasma concentration of biologically active LH have not been recorded.

In the context of this thesis, nutritional manipulation involves regulating the nutritional status of an animal to achieve intermittent growth patterns. By restricting dietary intake for a period of time, a heifer responds to increases in nutritional level by increasing the percentage of lean tissue deposition as compared to a heifer which has been on a diet that provides the nutrients required for a continuous growth rate. This principle has been employed in a variety of instances, but the influence of intermittent growth patterns on the bioactivity of LH has not been determined.

The objectives of this study are to determine the influence of exogenous somatotropin administration and nutritional manipulation on the bioactivity of LH. The mean concentration and the number and amplitude of pulses of biologically active LH are analyzed to determine the effects of these treatments on the reproductive development of beef heifers from weaning to the expected age at puberty.

*The following pages follow the style of the Journal of Animal Science.

LITERATURE REVIEW

Introduction to Luteinizing Hormone

The gonadotropin luteinizing hormone (LH) is a glycoprotein hormone that is synthesized by the anterior pituitary and secreted in a pulsatile fashion into the blood where LH acts on the testis and ovary. Secretions of luteinizing hormone-releasing hormone (LHRH) by the hypothalamus elicit the secretion of LH where the known functions performed by LH on the the testis and ovary include the regulation of (1) steroidogenesis (Payne et al., 1985); (2) gamete release, through the production of plasminogen activators and prostaglandins; and (3) gonadal peptides such as inhibin, growth factors and enzymes which, in turn, exhibit feedback control of gonadotropin synthesis and secretion (Wilson et al., 1990). It has been known for some time that discrepancies exist between the biological and immunological potencies of several polypeptide hormones. For this reason there have been many research thrusts to elucidate the molecular basis by which biological activity is conferred to LH and other polypeptide hormones.

LH is a member of a family of glycoprotein hormones that consists of the noncovalent association of a common α-subunit with a unique β-subunit which confers biological specificity to the hormone (Pierce and Parsons, 1981). The α-subunit of LH is the same α-subunit (i.e. contains the same amino acid sequence) which is found in pituitary hormones, follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), as well as the placental hormone, chorionic gonadotropin (CG). It is the β-subunit, which has a distinct amino acid sequence for each respective hormone, that confers hormonal specificity (Keel and Grotjan, 1989). The subunits have no known biological activity when they exist independently: the formation of a

heterodimer by the association of the two subunits is essential for biological activity (Gharib et al., 1990).

Each subunit possesses N-linked carbohydrate chains attached to specific asparagine residues of its polypeptide structure. There is one N-linked oligosaccharide on the α-subunit and two on the β-subunit (Gharib, 1990). Due to the variation in the structure of the carbohydrate chains, LH exhibits microheterogeneity and exists as a series of isohormones which differ in molecular weight, isoelectric point, circulatory half-life, receptor binding activity and biological activity (Keel and Grotjan, 1989). Wilson (1990) has stated that the biopotency of LH appears to be strongly influenced by the charge conferred by the acid radicals attached to the terminal groups on the oligosaccharide structures. Basic isoforms have a greater biopotency *in vitro* but a shorter half-life *in vivo*. Acidic isoforms have a lesser biopotency *in vitro* but a longer circulatory time and are, thus, suspected to be more active *in vivo*.

Measurement of LH Bioactivity:

Numerous reports from this lab (Hines et al., 1985; Weesner et al., 1987) and others (Dufau et al., 1977; Neill et al., 1977; Marut et al., 1981) have shown marked disparity in the bioactivity of LH. The most common method for the expression of LH bioactivity is to relate the concentration of bioactive LH isoforms (BLH) to the immunologically active LH isoforms (ILH) in a B:I ratio.

BLH can by detected by the implementation of a variety of bioassay techniques. Parlow (1958) described a cytochemical bioassay method based on the ability of LH to reduce the concentration of ascorbic acid in the ovary. A modification of Parlow's cytochemical bioassay in which ovarian sections are utilized has been described by Buckingham and Hodges (1981). The ability of

LH to elicit a testosterone response by the interstitial cells from the testis of mice (Van Damme et al., 1974; Baraghini et al., 1983; Celina et al., 1985; Ding and Huhtaniemi, 1989) or rats (Dufau et al., 1974; Burstein et al., 1985; Weesner et al., 1987) has also been used as an *in vitro* method for the determination of BLH. Measurement of ILH has been made using a variety of radioimmunoassay procedures (Niswender et al., 1969; Golter et al., 1973; Forrest et al. 1980).

Significance of Changes in LH Bioactivity:

Changes in B:I ratios at different stages during sexual development as well as at different stages of the female reproductive cycle have been reported by numerous investigators in a variety of species. Mathison et al. (1986) reported that in the cow, the biological activity of LH, as measured by B:I ratios, was greatest during the luteal phase with a decrease during the follicular phase and lowest during the LH surges. The biochemical basis for this disparity has not been fully elucidated. Some reports have attempted to link the changes in B:I ratios to changes in the charge microheterogeneity. Although Burnstein et al. (1985) suggested that the B:I disparity is a function of the standard employed in the two assay systems, there are numerous indications that this is not the case. Buckingham and Wilson (1985) suggest that there are abrupt changes in the nature of LH at puberty and that ovulatory cycles commence only when an adult form of LH with a full spectrum of biological activity is released from the pituitary gland. Hutchinson (1988) has suggested that there is a preferential secretion of less bioactive forms with a more prolonged action for chronic gonadal effects such as follicle maturation, while more bioactive forms with shorter half-lives are secreted for more dynamic events such as ovulation.

Schenken et al. (1985) have found that in Rhesus monkeys the mid-cycle BLH surge is qualitatively and quantitatively distinct from that of ILH. In man it has been found that BLH secretion occurs in discrete burstlike episodes (Dufau et al., 1983; Veldhuis et al., 1989). Torressani et al. (1983) have seen an increase in circulating BLH and a marked dissociation of BLH and ILH during early infancy, analogous to observations during puberty. Pituitary BLH reserve is greater during midpuberty than before or after puberty in man (Rich et al., 1982). Data from Mukhopadhyay et al. (1979) indicate that the B:I ratio of LH released *in vitro* in response to LHRH can be influenced by gonadal steroids. Norris et al. (1989) found that in the ewe, BLH and ILH are suppressed during late gestation and that ILH escapes from the suppressive effects of late gestation more rapidly than BLH. Furthermore, Norris et al. (1989) found that the B:I ratio varies during the estrous cycle and differences in biopotency are associated with the preovulatory LH surge.

The Influence of Pulsatile LH Secretion

"Frequency of LH pulses appears to increase gradually from birth to puberty" (Day et al., 1984). It is believed that the pulses increase in frequency until a point is reached at which the frequency of pulses provides more LH than is being metabolized. The frequent pulses build up LH in the system and the surge of LH necessary to stimulate the maturation of the follicle results, inducing ovulation (D. W. Forrest, personal communication). Anderson et al. (1986) concluded that in Holstein heifers the ovarian inhibition of pulsatile LH secretion is established by 6 weeks of age. The gradual increase in the frequency of LH pulses is due to the changing role of estradiol in LH regulation.

It has been suggested that a decrease in the sensitivity of the hypothalamo-pituitary centers controlling LH secretion to estradiol (E_2) negative feedback is necessary for the onset of puberty (Ramirez and McCann, 1963). This classical "gonadostat" theory has been challenged by Andrews et al. (1981) who suggest that decreased E_2 negative feedback was a consequence rather than a controlling mechanism of the onset of puberty in the female rat. LH secretion in prepubertal heifers is responsive to E_2 negative feedback, but the negative feedback decreases during the prepubertal period in beef heifers (Day et al., 1984). The negative feedback of E_2 on LH secretion is followed by a period of positive feedback after pubertal age is surpassed (Kinder et al., 1983; Day et al., 1986).

Following the initiation of puberty, frequency of LH pulses varies during different stages of the estrous cycle. Pulsatile secretion of biological and immunological LH occurs concurrently in the bovine female (Mathison et al., 1986), with lowest pulse frequency during the midluteal stage of the cycle (Rahe et al., 1980; Mathison et al., 1986).

Nutritional Influences on LH

Few studies to date have been published which analyze the effect of intermittent growth patterns on B:I ratios of prepubertal beef heifers approaching the expected age for the onset of puberty. Published data in related areas include: (1) the correlation between condition score and B:I ratios in multiparous cows; (2) the effect of limited energy intake on puberty in heifers; and (3) the effect of nutritional restriction on ILH during gestation in crossbred heifers.

The nutritional status of bovine females is influential in regulating the quantity and quality of LH. Bastidas et al. (1989) reported that the nutritional

status of postpartum multiparous cows altered the B:I ratio of LH. Cows losing energy reserves also showed a decrease in metabolic rate and longer interval to first estrus following parturition. Day et al. (1986) speculated that delay of puberty in heifers on diets which restricted intake resulted from the failure of ILH secretion to increase. These reports indicate that nutritional state influences LH synthesis and(or) secretion in the bovine female.

Data on the influence of nutritional restriction on LH in the female bovine which has attained puberty have been accumulated. Low circulating concentrations of LH and lack of pulsatile secretion of LH due to anestrus as a result of restricted dietary intake have been suggested as the reason that estrus is not initiated after treatment with a norgestomet-estradiol valerate combination (Imakawa et al., 1983). Reports indicate that LH secretion is inhibited by E_2 prior to (Imakawa et al., 1987) and following the onset of nutritional anestrus in the heifer but that the responsiveness to E_2 did not subside with the re-initiation of estrous cycles (Imakawa et al., 1986). Nutritional restriction of heifers during gestation impacts pre- and post-partum pituitary response to LHRH as measured by radioimmunoassay for ILH (Killen et al., 1989a). Results reported by Killen et al. (1989b) also suggest that intake and retention of energy during gestation appear to be inversely related to LHRH-induced LH secretion before parturition. These reports indicate that nutrition influences physiological function after the onset of puberty.

Exogenous Somatotropin Administration and the Female

Bovine somatotropin (bST), or bovine growth hormone, is a natural substance produced by the pituitary. Somatotropin (ST) is produced by all species of animals and is important in such physiological functions as growth, development, and other body functions (Hartwig, 1991). With the recent

advances in biotechnology, production of synthetic ST has been made possible by recombinant technology. The feasibility of administration of exogenous recombinant somatotropin (rST) has been investigated in attempts to discover possible applications to dairy cattle, swine, and beef cattle production. Although there is limited data on the bioactivity of LH in response to recombinant bST (rbST) administration, several studies have been conducted which analyzed the effect of bST administration on bovine ILH and other reproductive parameters.

A significant improvement in persistency, the ability of cows to maintain relatively high levels of production throughout lactation, is a primary response of dairy cattle to rbST treatment (Bauman, 1989). Gallo et al. (1989) have suggested that LHRH-induced LH response is enhanced in rbST-treated dairy cows, and that the delay in the resumption of estrus following parturition is due to the negative effects of lowered energy balance on endogenous LHRH due to the increase in milk production. Moseley (1989) showed no significant effect of rbST injections on the magnitude of LHRH-induced LH response in pre- and post-pubertal dairy heifers. Morbeck et al. (1989) have indicated that the administration of high doses (20 mg/d) of rbST affected estrous cycle length and may have suppressed expression of estrus, but that lower doses of rbST did not adversely affect reproduction.

The injection of porcine rST (rpST) has been investigated to determine the effect of rpST injection on growth patterns and reproductive traits. The growth pattern of finishing pigs has been shifted from fat deposition to lean tissue deposition in pigs injected with rpST (Busby and Stender, 1991). Although rpST administration increases rate of gain in prepubertal gilts and average age at puberty is correlated with body weight, Busby and Stender (1991) have stated that "gilts injected with rpST during the growing-finishing

phase reached puberty at similar ages as untreated gilts and were equally fertile to the control group at breeding." Terlouw et al. (1989) have reported that treatment of gilts with rpST from day 57 to day 108 of age did not significantly affect ovarian weight, follicular size or number of follicles.

MATERIALS AND METHODS

Animals

Twenty Angus X Holstein heifers from the Texas Agricultural Experiment Station Research Center at McGregor were used in this experiment. At 5 months of age, heifers were randomly assigned to one of four treatment groups (n=5) in a 2 X 2 factorial arrangement for evaluation over the following 9-month period. The four treatment groups were arranged as follows: (I) continuous growth (CG) + excipient (EX), (II) CG + bovine somatotropin (ST), (III) discontinuous growth (IG) + EX and (IV) IG + ST. Heifers were housed in pens equipped with Calan electronic gate feeders to monitor individual feed intake. Throughout treatment CG heifers were fed a 68% concentrate diet ad libitum, formulated to provide for a growth rate of .8 kg/d. Two successive periods of intermittent growth were imposed on IG heifers by alternately feeding a 68% concentrate diet formulated for growth at .25 kg/d (restricted growth) for 3 months and then realimentation on a 90% concentrate diet fed ad libitum (compensatory growth) for 2 months. EX heifers received excipient injections (sc), and ST heifers received injections (sc) of 500 mg of recombinant bST every 14 days.

Blood Sample Collection

Blood samples were collected over 6-hour periods at 20-minute intervals via indwelling jugular vein catheters which had been fitted to each heifer the day prior to sampling. Samples were taken at 9, 11 and 14 months of age (sample periods 1, 2 and 3, respectively). CG heifers were in stages of

continuous growth at each of the three sample periods. IG heifers were in a stage of restricted growth at sample period 2 and stages of compensatory growth at sample periods 1 and 3.

Each blood sample was placed in a labeled, heparinized, 12 mm X 75 mm glass culture tube, inverted several times and placed on ice until centrifugation (20 min, 100 X G, 4° C). All samples were centrifuged within 16 hours of collection. The plasma was aspirated with a Pastuer pipette and stored at -20° C.

Bioassay of LH

Bioactive LH concentration in plasma was quantified by a validated *in vitro* rat interstitial cell testosterone (RICT) assay. The procedure is based on that of Dufau et al. (1976) with modifications for bovine LH in our laboratory by Weesner et al. (1987). Four- to 7-week old Sprague-Dawley rats were used in these experiments.

Rats were maintained on a standard laboratory diet (Teklad Laboratory Rodent Food) with water ad libitum. For each assay, three rats were euthanized with CO_2; and the testes were removed, decapsulated, and placed in a 50 ml conical polypropylene centrifuge tube containing digestive solution (2 mg collagenase, Sigma, Type I, C-0130; .1 mg DNAse, Sigma, Type III, D-4638; 8 ml Medium 199 (M199) with Earle's Salts, pH 7.2). The centrifuge tube was placed in a 37° C oscillating water bath (1.67 Hz) for 10 to 15 minutes to allow seminiferous tubules to separate into individual units.

Digestion was stopped by adding 40 ml M199 to the dispersion tube. The tube was gently inverted several times, and then the seminiferous tubules were allowed to settle to the bottom of the tube. The supernatant was aspirated

using a 35 ml plastic syringe with a 10 cm tygon tubing extension, and the supernatant was equally divided and placed in two 50 ml polypropylene centrifuge tubes. The tubes were centrifuged at 100 X G for 5 minutes. The supernatant was discarded and the cells of one tube were resuspended in 20 ml of M199 and added to the other tube. The cells were washed by centrifugation and resuspension in 20 ml M199 three times.

Following the third wash, the cells were suspended in 20 ml of assay buffer. The assay buffer consisted of 300 mg defatted BSA (Sigma, Fraction V, powder), 66 mg 3-isobutyl-1-methylxanthine (Sigma), and 9 mg soybean tripsin inhibitor (Sigma), dissolved in 150 ml M199. Interstitial cells were counted (with a hemacytometer) and diluted in sufficient assay buffer to obtain a concentration of 800,000 cells/ml. The cell suspension (500 µl) was added to 100 µl of LH standard or 100 µl of the sample dilution to yield a final assay volume of 600 µl.

Fresh bovine LH standards (NIH-LH-B10 from NIADDK) were prepared for each assay. The concentration of the LH stock solution was 100 µg/200 ml. Serial dilutions of the LH stock solution were made with M199 to obtain concentrations of 100 pg/100 µl, 75 pg/100 µl, 50 pg/100 µl, 25 pg/100 µl, 12.5 pg/100 µl, 6.75 pg/100 µl, 3.125 pg/100 µl, 1.5626 pg/100 µl and .78125 pg/100 µl. Each of these LH standard concentrations (100 µl) and M199 (100 µl) were pipetted in triplicate into 12 X 75 mm polypropylene tubes. Unknown and reference samples were prepared for assay by diluting (using a Micromedic Automatic Dilutor) plasma at 30 µl with M199 to yield a final volume of 100 µl. All assay tubes were incubated in an oscillating water bath at 37° C (.67 Hz) for 2 hours under an atmosphere of 95% O_2 + 5% CO_2. Incubation was halted by placing tubes in an ice water bath for 15 min. Tubes were stored at -20° C until conducting a testosterone RIA.

The resulting medium was assayed for testosterone using the RIA procedures of Harrison et al. (1985) with modifications. In a 12 X 75 mm glass tube, 100 µl of incubation media from each bioassay tube was added to 300 µl PBS-Gel buffer. The antisera was diluted to 1:80,0000 with 1:400 normal sheep serum in PBS-Gel buffer. LH values were calculated using a 4 parameter logistic curve fitting model for data transformation (Rodbard and Hutt, 1974). Pulses of LH were defined as values that exceeded the preceding value by three times the preceding value's standard deviation.

Radioimmunoassay of LH

Immunoactive LH concentration for each pulse was quantified by a double antibody RIA described by Niswender et al. (1969) with modifications by Forrest et al. (1980). The radioimmunoassay is outlined in Appendix A. The LH standard used was NIH-LH-B10 from NIADDK, with a sensitivity of .2 ng/ml. The first antibody (TEA#35) was from J. J. Reeves of Washington State University used at a final dilution of 1:30,000. The second antibody (sheep anti-rabbit gamma globulin; #1256, P4) was from Antibodies, Inc., Davis, California used at a final dilution of 1:400.

Statistics

A split plot ANOVA (SAS, 1985) was used to analyze the effects of treatment, individual(treatment), sample period and sample period X treatment on bioactive LH concentration, number of bioactive LH pulses and amplitude of bioactive LH pulses. Differences between means were defined by Duncan's Multiple Range Test (SAS, 1985).

RESULTS

Bioactive LH

There was a sample period X treatment interaction ($P<.05$) for mean bioactive LH (Table 1). Mean bioactive LH concentration increased between sample periods 1 and 3 for all groups, except IG + ST treated heifers as seen in Figure 1. At sample period 1, all treatment groups were similar. At sample period 2, the maximum mean concentration of bioactive LH was seen in the CG + ST group; while, IG + EX and IG + ST groups showed minimum mean concentrations of bioactive LH. At sample period three, similar mean concentrations of mean bioactive LH were seen for CG + EX, CG + ST and IG + EX heifers, but IG + ST heifers showed no increase. There was an effect of sampling period ($P<.05$) on mean number of LH pulses (Figure 2) and mean amplitude of LH pulses (Figure 3). An increase in the number of LH pulses occurred between sampling period 1 and period 2 for CG + EX heifers, but not for CG + ST, IG + EX or IG + ST heifers. Mean number of LH pulses increased from period 2 to period 3 in all heifers but IG + ST treated heifers (Figure 4). Mean amplitude of bioactive LH pulse increased between sample periods 1 and 2 for CG + EX heifers, but not CG + ST, IG + EX and IG + ST. Mean amplitude increased between sample periods 2 and 3 for CG + ST and IG + EX groups, but not for CG + EX and IG + ST groups (Figure 5). Treatment effected mean amplitude of LH pulses (Figure 6) but not number of LH pulses (Figure 7). CG + ST and IG + EX did not differ from CG + EX in mean amplitude of bioactive LH pulse, but mean amplitude was greater ($P<.05$) for CG + EX heifers than IG + ST heifers (Figure 8).

Table 1: Mean bioactive LH plasma concentration for each treatment group by sample periods 1, 2 and 3.

	TREATMENT			
SAMPLE PERIOD	CG+EX	CG+ST	IG+EX	IG+ST
1	.08 ng/ml	.06 ng/ml	.07 ng/ml	.08 ng/ml
2	.29 ng/ml	.13 ng/ml	.07 ng/ml	.06 ng/ml
3	.24 ng/ml	.27 ng/ml	.24 ng/ml	.08 ng/ml

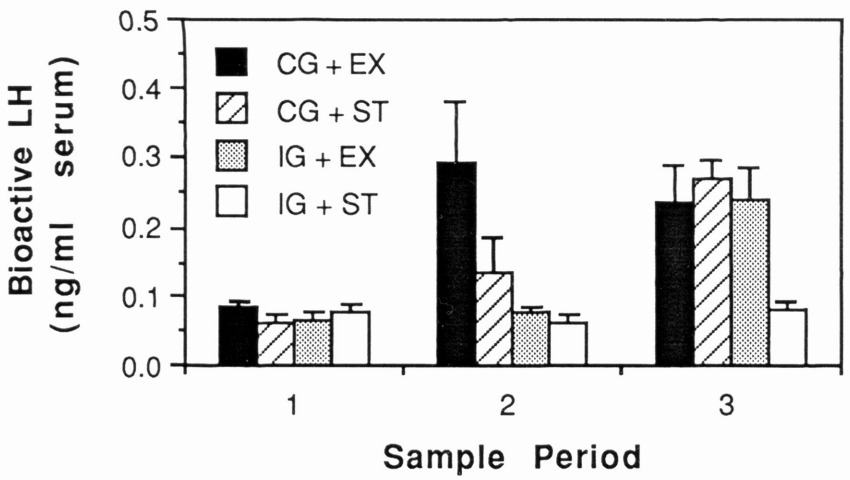

Figure 1: Mean plasma concentration of bioactive LH for each of four treatment groups by sample period 1, 2 and 3.

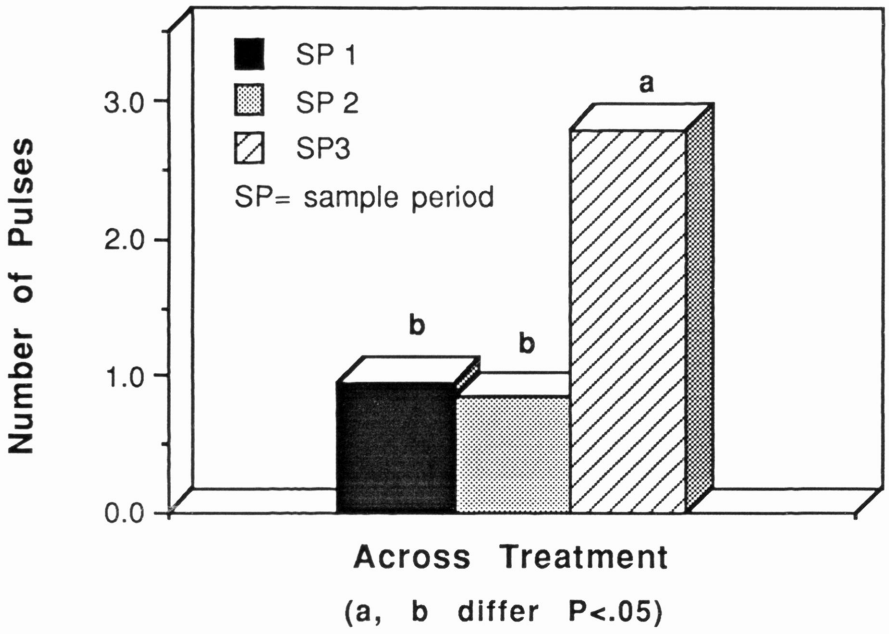

Figure 2: Mean number of bioactive LH pulses for each sample period across treatments.

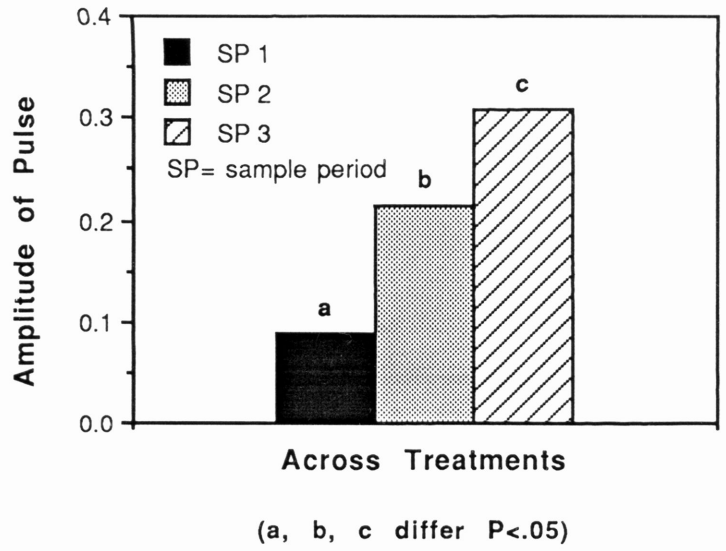

Figure 3: Mean amplitude of bioactive LH pulses for each sample period across treatments.

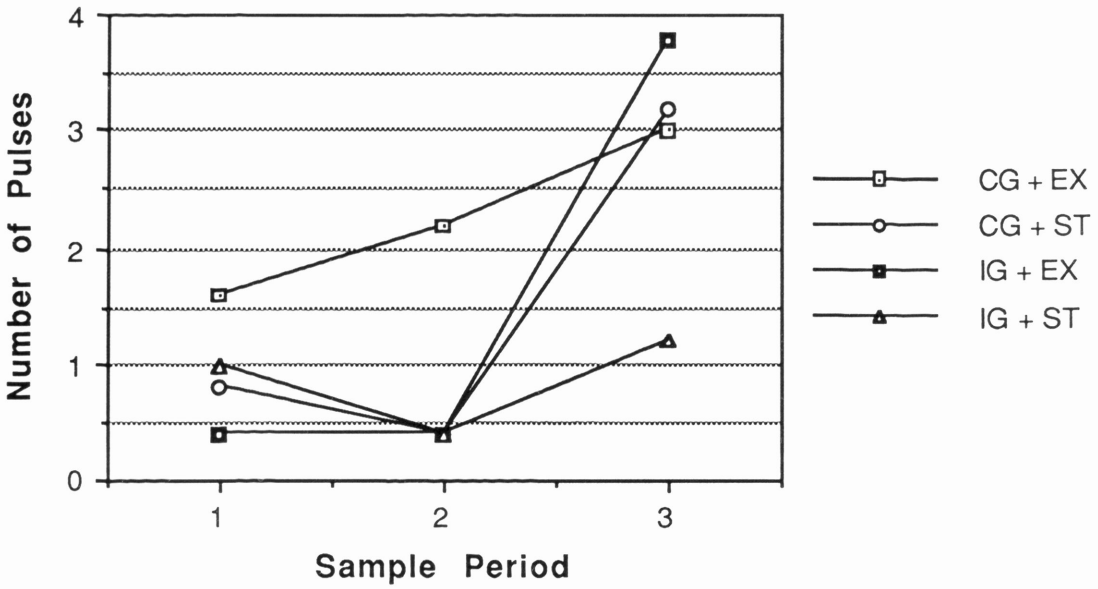

Figure 4: Mean number of bioactive LH pulses for each of four treatment groups by sample period 1, 2 and 3.

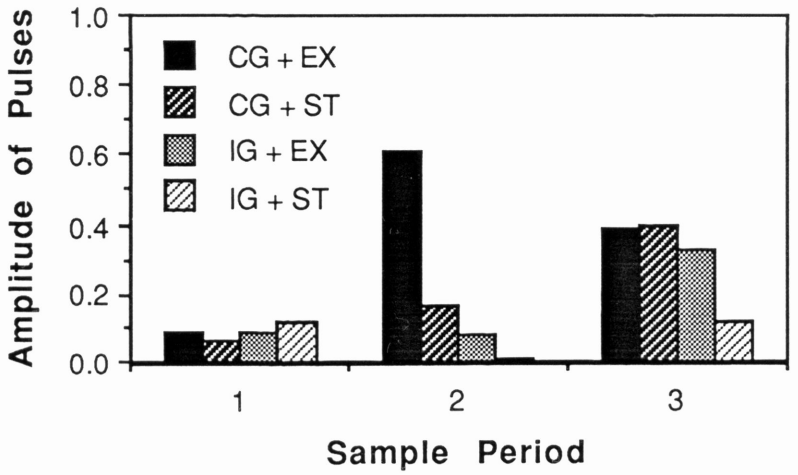

Figure 5: Mean amplitude of bioactive LH pulses for each of four treatment groups by sample period.

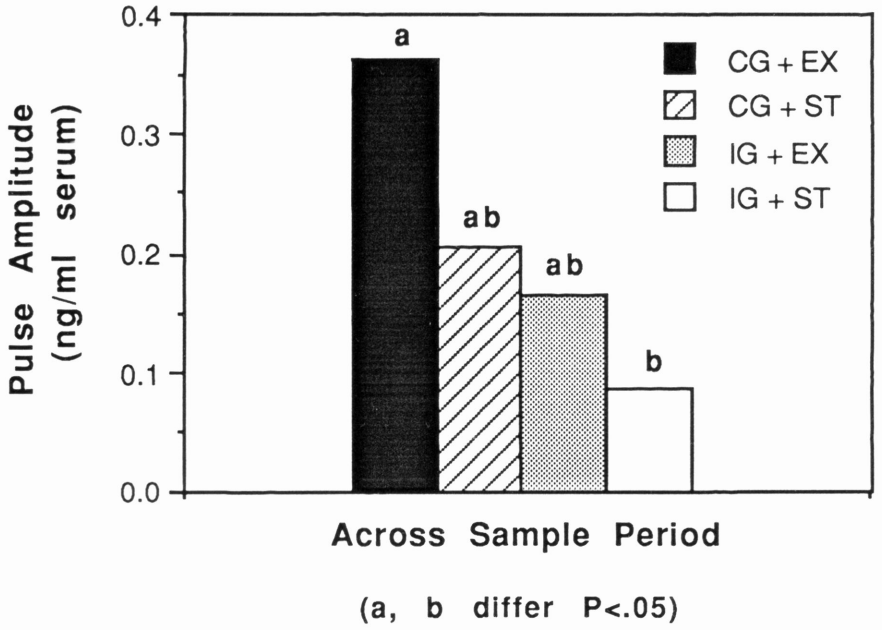

Figure 6: Mean amplitude of bioactive LH pulses for each treatment group across sample period.

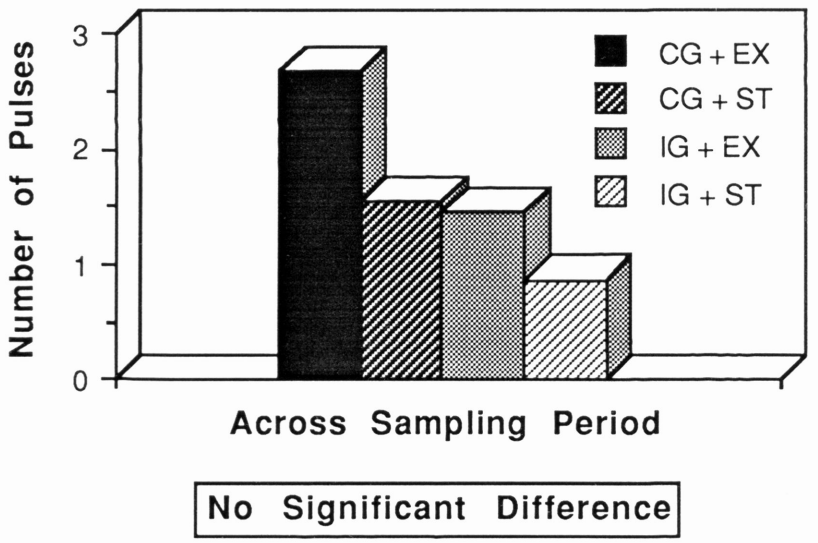

Figure 7: Mean number of bioactive LH pulses for each treatment group across sample period.

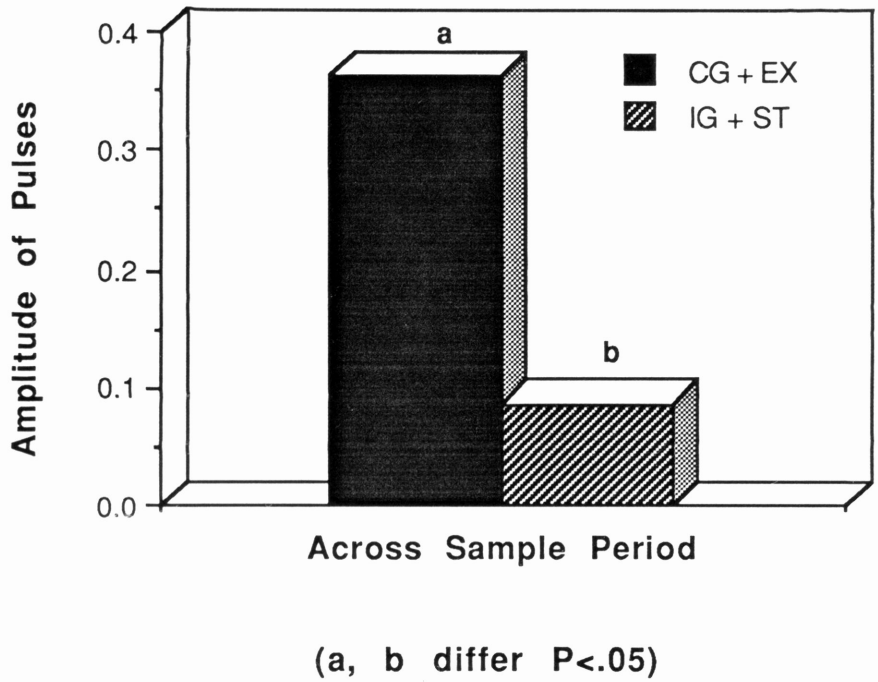

Figure 8: Mean amplitude of LH pulses for CG + EX and IG + ST groups across sample period.

Discussion

The attainment of puberty is a dynamic process the analysis of which requires that a number of aspects be considered. The quantity and quality of circulatory LH is influential in determining the age at which puberty occurs, and analysis of LH requires that the biological potency, number of pulses and amplitude of pulses be taken into consideration. The bioassay employed in collecting the data contained within this thesis allows for an accurate analysis of both the quantity and quality of LH.

Significant increases in the concentration of biologically active LH during pre-pubertal development were reported by Luckey et al. (1980) in humans. They reported that concentration of biologically active forms of LH increased 23.1-fold. It was concluded that this increase is highly influential in determining the initiation of the physiological changes associated with puberty. During the same time, the concentration of immunologically active forms increased only 4.9-fold, and therefore, the relatively large increase in biologically active LH is a more reliable indicator of pubertal development.

Biologically active LH is required for normal pubertal development. According to our data, continuous growth patterns alone and in combination with somatotropin, as well as intermittent growth patterns in the absence of exogenous somatotropin, showed no significant difference in the mean biologically active LH concentration. Therefore treatment with somatotropin or intermittent growth alone does not appear to inhibit normal reproductive development through depressed concentrations of biologically active forms of LH in the circulation. Our data indicate that intermittent growth patterns in combination with somatotropin administration as compared to continuous

growth without somatotropin may inhibit pre-pubertal development through delaying increases in the concentration of biologically active forms of LH.

Our results indicate that the mean number of bioactive LH pulses across sample period increased for all groups. This suggests that the number of bioactive LH pulses across sample period may not be influenced by treatment with intermittent growth and(or) exogenous somatotropin. Visual analysis of the number of pulses by sample period indicates that continuous growth heifers showed a gradual increase in number of bioactive LH pulses at each successive sample period when somatotropin treatment was not applied, indicating that reproductive development was occurring throughout treatment. Continuous growth heifers that did receive somatotropin, as well as intermittent growth heifers with and without somatotropin appear to have experienced a delay in the time at which number of LH pulses increased. These data indicate that somatotropin treatment may delay the time at which increases in the number of bioactive LH pulses occur.

At the first sample period, all animals were on diets which did not restrict the nutritional level to sub-normal. All treatment groups had a comparable number of bioactive LH pulses. At the second sampling period, both intermittent growth groups were undergoing their second growth restriction phase. Therefore, our data would appear to coincide with the findings of Day et al (1986) in which it was speculated that puberty was delayed in heifers on restricted diets through inhibiting increases in LH secretion. At the third sample period, intermittent growth heifers were in the second compensatory gain phase of treatment. At this time, the number of pulses was comparable for all groups, suggesting that intermittent growth heifers had recovered from the influence of restricted diet to the extent that the number of pulses had returned to levels comparable with continuous

growth heifers. Therefore, these data indicate that nutritional manipulation designed to achieve intermittent growth patterns may not inhibit normal pubertal development through restricting the number of bioactive LH pulses.

Our data suggest that mean amplitude of bioactive LH pulses appears to be inhibited by treatment with intermittent growth in conjunction with exogenous somatotropin but not by somatotropin or intermittent growth treatment alone. Visual appraisal of the mean amplitude of LH pulses by sample period for each treatment indicates that heifers subjected to continuous growth in the absence of somatotropin experience increases in mean amplitude of bioactive LH pulses at an earlier age than heifers treated with somatotropin, intermittent growth or a combination of the two. These results indicate that treatment with intermittent growth patterns without somatotropin or somatotropin alone may delay the age at which increases in mean amplitude of bioactive LH pulses occur.

Analysis of the changes in LH microheterogeneity as a result of treatment with intermittent growth treatment and administration of somatotropin should give an indication of the possible influences that these two treatments have on the LH molecule. The biochemical basis for differences in biopotency of circulating LH as a result of somatotropin and intermittent growth treatment may be changes in the charge of the carbohydrate moieties. Procedures such as chromatofocusing, isoelectric focusing and high performance liquid chromatography could be employed to determine the charges that reside on the oligosaccharide chains of the LH molecule from animals receiving treatments similar to those described here. It is plausible that these treatments are acting on LH through influencing LHRH secretion by the hypothalamus. Additional studies that analyze the

effects of nutritional manipulation and somatotropin on LHRH should be conducted.

These data indicate that treatment of prepubertal heifers with somatotropin or intermittent growth patterns does not inhibit puberty through decreasing the mean concentration of biologically active LH, number or amplitude of pulses of biologically active LH. Treatment with both somatotropin and intermittent growth appears to hinder normal reproductive development by decreasing the mean plasma concentration of biologically active LH and depressing the amplitude of bioactive LH pulses.

REFERENCES

Anderson, W. J., D. W. Forrest, B. A. Goff, A. A. Shaikh and P. G. Harms. 1986. Ontogeny of ovarian inhibition of pulsatile luteinizing hormone secretion in postnatal Holstein heifers. Domest. Anim. Endocrinol. 3:107.

Andrews, W. W., J. P. Advis and S. R. Ojeda. 1981. The maturation of estradiol negative feedback in female rats: evidence that the resetting of the hypothalamic "gonadostat" does not precede the first pre-ovulatory surge of gonadotropins. Endocrinol. 109:2022.

Baraghini, C. F., A. A. Zaidi, V. Montanini, M. F. Celani, K. Cioni, C. Carani, D. Cavani, L. F. Agnati, F. Morabito, M. Resentini, F. Cogliati, B. Bonati and P. Marrama. 1983. Changes in Biologically Active Serum Luteinizing Hormone at Different Ages: Methodological Problems, in *Recent Advances in Male Reproduction: Molecular Basis and Clinical Implications*. D'Agata, R., M. B. Lipsett, P. Polosa and H. J. van der Molen, ed. Ravin Press, New York. 7:279-285.

Bastidas, P., D. W. Forrest, R. P. Del Vecchio and R. D. Randel. 1990. Biological and immunological luteinizing hormone activity and blood metabolites in postpartum Brahman cows. J. Anim. Sci. 68:2771.

Bauman, D. E. 1989. Biology of bovine somatotropin. Advanced technologies facing the dairy industry: BST. Cornell University, Nov 10-11. pp 1-8.

Buckingham, J. C. and J. R. Hodges. 1981. A cytochemical bioassay method for the determination of luteinizing hormone in biological fluids and tissues. J. Pharmac. 73:111.

Burstein, S., E. Schaff-Blass, J. Blass and R. L. Rosenfield. 1985. The changing ratio of bioactive to immunoreactive luteinizing hormone (LH) through puberty principally reflects changing LH radioimmunoassay dose-response characteristics. J. Clin. Endocrinol. Metab. 61:508.

Busby, D. and D. Stender. 1991. Porcine somatotropin: a review. Dairy, Food and Environ. Sanitat. 11:144.

Celani, M. F., V. Montanini and P. Marrama. 1985. Effects of luteinizing hormone-releasing hormone (LHRH) upon the bioactive and immunoreactive serum LH in patients with Turner's syndrome before and after oestrogen treatment. Acta Endocrinol. 109:304.

Day, M. L., K. Imakawa, M. Garcia-Winder, D. D. Zalesky, B. D. Schanbacher, R. J. Kittok and J. E. Kinder. 1984. Endocrine mechanisms of puberty in heifers: estradiol negative feedback regulation of luteinizing hormone secretion. Biol. Reprod. 31:332.

Day, M. L., K. Imakawa, M. Garcia-Winder, R. J. Kittok, B. D. Schanbacher and J. E. Kinder. 1986. Influence of prepubertal ovariectomy and estradiol replacement therapy on secretion of luteinizing hormone before and after pubertal age in heifers. Domest. Anim. Endocrinol. 3:17.

Ding, Y. and I. Huhtaniemi. 1989. Human serum LH inhibitor(s): behaviour and contribution to in vitro bioassay of LH using dispersed mouse Leydig cells. Acta Endocrinol. 121:46.

Dufau, M. L., R. Pock, A. Nuebauer and K. J. Catt. 1976. In vitro bioassay of LH in human serum: the rat interstitial cell testosterone (RICT) assay. J. Clinic. Endocrinol. Metab. 42:958.

Dufua, M. L., K. Nozu, A. Dehejia, A. Garcia Vela, R. A. Solano, F. Fraioli, K. J. Catt. 1982. Biological activity and target cell actions of luteinizing hormone, in *Pituitary Hormones and Related Peptides*. Motta, M., M. Zanisi, F. Piva eds. Academic Press. New York. pp 117-138.

Dufau, M. L., J. D. Velduis, F. Fraioli, M. L. Johnson and I. Z. Beitins. 1983. Mode of secretion of bioactive luteinizing hormone in man. J. Clin. Endocrinol. Metab. 57:993.

Forrest, D. W., J. L. Fleeger, C. R. Long, A. M. Sorensen,Jr. and P. G. Harms. 1980. Effects of exogenous prolactin on peripheral luteinizing hormone levels in ovariectomized cows. Biol. Reprod. 22:197.

Gallo, G. F. and E. Block. 1989. Effects of recombinant bovine somatotropin (rbST) on circulating concentrations of progesterone in plasma during estrous cycles and pregnancy in lactating dairy cows. J. Anim. Sci. (Suppl. 1):343.

Gharib, S. D., M. E. Wierman, M. A. Shupnik and W. W. Chin. 1990. Molecular biology of the pituitary gonadotropins. Endocrinol. Reviews. 11:177.

Golter, T. D., J. J. Reeves, C. C. O'Mary, A. Arimura and A. V. Schalley. 1973. Serum LH levels in bulls treated with synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH). J. Anim. Sci. 37:123.

Harrison, L. M., R. D. Randel, D. W. Forrest, J. G. Betts, W. D. Humphrey and D. R. Hardin. 1985. Cloprostenol induced luteal regression in the beef cow. I. Ovarian response and endocrine changes. Theriogenology 23:11.

Hartwig, N. R. 1991. Bovine somatotropin: an overview. Dairy, Food and Environ. Sanitat. 11:140.

Hines, K. K., J. L. Kreider, G. D. Potter, S. L. Hodge, D. W. Forrest and P. G. Harms. 1985. Biological vs. immunological levels of LH in mares at different levels of body condition. Biol. Reprod. 32 (Suppl. 1):165.

Hutchinson, J. S. M. 1988. The interpretation of pituitary gonadotrophin assays-a continuing challenge. J. Endocrinol. 118:169.

Imakawa, K., R. J. Kittok and J. E. Kinder. 1984. Luteinizing hormone secretion after withdrawal of exogenous progesterone in heifers fed three levels of dietary intake. J. Anim. Sci. 58:151.

Imakawa, K., M. L. Day, M. Garcia-Winder, D. D. Zalesky, R. J. Kittok, B. D. Schanbacher and J. E. Kinder. 1986. Endocrine changes during restoration of estrous cycles following induction of anestrus by restricted nutrient intake in beef heifers. J. Anim. Sci. 63:565.

Imakawa, K., M. L. Day, D. D. Zalesky, A. Cutter, R. J. Kittok and J. E. Kinder. 1987. Effects of 17β-estradiol and diets varying in energy on secretion of luteinizing hormone in beef heifers. J. Anim. Sci. 64:805.

Keel, B. A. and H. E. Grotjan. 1989. Luteinizing Hormone Microheterogeneity, in *Microheterogeneity of Glycoprotein Hormones*. CRC Press, Boca Raton, Florida. pp149-184.

Killen, J. H., D. W. Forrest, F. M. Byers, G. T. Schelling and C. R. Long. 1989a. Gonadotropin-releasing hormone-induced luteinizing hormone release in heifers:effect of nutrition during gestation. J. Anim. Sci. 67:496.

Killen, J. H., D. W. Forrest, F. M. Byers, G. T. Schelling and C. R. Long. 1989b. Effects of nutritional level and biological type on gonadotropin-releasing hormone-induced luteinizing hormone release and plasma progesterone, estrone and estradiol concentrations in pre- and post-partum beef heifers. J. Anim. Sci. 67:3379.

Kinder, J. E., M. Garcia-Winder, K. Imakawa, M. L. Day, D. D. Zalesky, M. L. D'Occhio, R. J. Kittok and B. D. Schanbacher. 1983. Influence of different estrogen doses on concentration of serum LH in acute and chronic ovariectomized cows. J. Anim. Sci. (Suppl. 1):350.

Luckey, A. W., B. H. Rich, R. L. Rosenfeld, V. S. Fong, N. Roche-Bender. 1980. LH bioactivity increases more than immunoactivity during puberty. J. Pediatr. 97:205.

Marut, E. L., R. F. Williams, B. D. Cowan, A. Lynch, S. P. Lerner and G. D. Hogden. 1981. Pulsatile pituitary gonadotropin secretion during maturation of the dominant follicle in monkeys: estrogen positive feedback enhances the biological activity of luteinizing hormone. Endocrinol. 109:2270.

Mathison, B. D., H. M. Schoenemann and J. J. Reeves. 1986. Differences in immunological and biological activity of serum luteinizing hormone during the bovine estrous cycle. Domest. Anim. Endocrinol. 3:1.

Morbeck, D. E., B. T. McDaniel and J. H. Britt. 1989. Reproductive and metabolic performance of primiparous Holstein cows treated with recombinant bovine somatotropin (rbST). J. Anim. Sci. (Suppl. 1):345.

Moseley, W. M. 1989. Bovine somatotropin (bSt) treatment does not enhance the GnRH-induced LH response in pre- and postpubertal Holstein Heifers. J. Anim. Sci. (Suppl. 1):344.

Mukhopadhyay, A. K., F. A. Leidenberger and V. Lichtenberg. 1979. A comparison of bioactivity and immunoactivity of luteinizing hormone stored in and released in vitro from pituitary glands of rats under various gonadal states. Endocrinol. 104:925.

Neill, J. D., R. A. Dailey, R. C. Tsou and L. E. Reichert, Jr. 1977. Immunoreactive LH-like substances in serum of hypophysectomized and prepubertal monkeys: inactive in an in vitro LH bioassay. Endocrinol. 100:856.

Niswender, G. D., L. E. Reichert, A. G. Migdely and A. V. Nalbandov. 1969. Radioimmunoassay of bovine and ovine luteinizing hormone. Endocrinol. 84:1166.

Norris, T. A., G. D. Weesner, J. M. Shelton, P. G. Harms and D. W. Forrest. 1989. Biological activity of LH during the peripartum period and the estrous cycle of the ewe. Domest. Anim. Endocrinol. 6:25.

Parlow, A. F. 1958. A rapid bioassay method for LH and factors stimulating LH secretion. Fedn. Proc. 17:402.

Payne, A. H., P. G. Quinn and J. R. D. Stalvey. 1985. The Stimulation of Steroid Biosynthesis by Luteinizing Hormone, in *Luteinizing Hormone Action and Receptors.* Ascoli, M., Ed., CRC Press, Boca Raton, Fla., chap. 4.

Pierce, J. G. and T. F. Parsons. 1981. Glycoprotein hormones: structure and function. *Annual Reviews of Biochemistry.* 50:465-495.

Rahe, C. H., R. E. Owens, J. L. Fleeger, H. J. Newton and P. G. Harms. 1980. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. Endocrinol. 107:498.

Ramirez, D. V. and S. M. McCann. 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinol. 72:452.

Rich, B. H., R. L. Rosenfield, G. W. Moll, Jr., A. W. Lucky, N. Roche-Bender and V. Fang. 1982. Bioactive luteinizing hormone pituitary reserves during normal and abnormal male puberty. J. Clin. Endocrinol. Metab. 55:140.

Rodbard, D., D. M. Hutt. 1974. Statistical analysis of radioimmunoassays and immunoradiometric (labelled antibody) assays. A generalized weighted, iterative, least-squares method for logistic curve fitting, in *Proceedings, Symposium of Radioimmunoassay and Related Procedures in Medicine.* Unipub, New York. pp. 165-178.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, N. C.

Schenken, R. S., L. B. Werlin, R. F. Williams, T. J. Prihoda and G. D. Hodgen. 1985. Periovulatory hormonal dynamics: relationship of immunoassayable gonadotropins and ovarian steroids to the bioassayable luteinizing hormone surge in Rhesus monkeys. J. Clin. Endocrinol. Metab. 60:886.

Terlouw, S. L., A. Rieke, T. Cantley and B. N. Day. 1989. Recombinant porcine somatotropin (rpST) effects on prepubertal reproductive tract development in the pig. J. Anim. Sci. (Suppl. 1):344.

Torresani, T., E. Schuster and R. Illig. 1983. Bioactivity of plasma luteinizing hormone in infants and young children. Acta Endocrinol. 103:326.

Van Damme, M. P., D. M. Robertson and E. Diczfalusy. 1974. An improved in vitro bioassay method for the measuring luteinizing hormone (LH) activity using mouse Leydig cell preparations. Acta Endocrinol. 77:655.

Veldhuis, J. D., M. L. Johnson and M. L. Dufau. 1989. Physiological attributes of endogenous bioactive luteinizing hormone secretory bursts in man. Amer. J. Phys. 256:199.

Weesner, G. D., T. A. Norris, D. W. Forrest and P. G. Harms. 1987. Biological activity of luteinizing hormone in the peripartum cow: least activity at parturition with an increase throughout the postpartum interval. Biol. Reprod. 37:851.

Wilson, C. A., A. J. Leigh and A. J. Chapman. 1990. Gonadotrophin glycosylation and function. J. Endocrinol. 125:3.

Appendix A: Radioimmunoassay for Bovine Luteinizing Hormone

The bovine LH radioimmunoassay is a five-day, double antibody assay. The assay procedure is outlined by day.

DAY 1

1. The diluent [.1% egg white-phosphate buffer solution (PBS), pH 7.0; .05 M disodium ethylenediamine tetraacetate (EDTA)-PBS, pH 7.0] is added to all assay tubes.

2. The LH standard curve, total count (TC) and total binding (TB) tubes are prepared in triplicate. Six nonspecific binding (NSB) tubes are added to the standard. The standard consists of nine concentration/dilutions ranging from .05 ng to 20 ng made in the diluent.

3. Duplicates of unknown samples are added to the assay at volumes of 50 µl to 400 µl.

4. Anti-bovine LH (TEA #35), diluted in normal rabbit serum-EDTA-PBS (1:50,000) is added to all assay tubes except TC and NSB.

5. All assay tubes are vortexed and stored at 4° C for 24 hours. Assay volume equals 700 µl.

Day 2

1. I^{125}-bovine LH, approximately 40,000 counts per minute/100 µl, is added to all assay tubes.

2. All assay tubes are vortexed and stored at 4° C for 24 hours.

Day 3

1. 200 µl of 2nd antibody, sheep anti-rabbit gamma-globulin, diluted to 1:80 in diluent is added to all assay tubes except TC.

2. Tubes are vortexed and stored at 4° C for 48 hours.

Day 5

1. Ice cold PBS (1.5 ml) is added to all tubes except TC.

2. Tubes are centrifuged at 2000 X G for 40 minutes (4° C).

3. Supernatant is aspirated and discarded from all tubes except TC. Tubes are allowed to dry inverted for 30 minutes.

4. Cotton-tipped applicators are used to remove any excess moisture from the sides of the tubes.

5. Tubes are placed into a Packard gamma counter and counted for one minute.

Luteinizing hormone concentrations are calculated using a logit-log data transformation with final values expressed in ng/ml.