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Prepuberal levels of gonadotrophins and their relationship to the hypothalamo-hypophoseal response to a 17B-Estradiol challenge and performance criteria in the beef heifer

Robert L. Cato

To the Graduate Council:

I am submitting herewith a thesis written by Robert L. Cato entitled "Prepuberal levels of gonadotrophins and their relationship to the hypothalamo-hypophoseal response to a 17B-Estradiol challenge and performance criteria in the beef heifer." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Bert H. Erickson, Major Professor

We have read this thesis and recommend its acceptance:

H. G. Kattesh, T. T. Chen

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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and recommend its acceptance:

H. G. Kattesh

Accepted for the Council:

Lew Minkel

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Date May 23, 1986

PREPUBERAL LEVELS OF GONADOTROPHINS AND THEIR RELATIONSHIP TO THE HYPOTHALAMO-HYPOPHOSEAL RESPONSE TO A 17β-ESTRADIOL CHALLENGE AND PERFORMANCE CRITERIA IN THE BEEF HEIFER

A Thesis

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Degree

The University of Tennessee, Knoxville

Robert L. Cato, Jr.

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ABSTRACT

Reproductive efficiency is a major concern in the production of livestock. In an effort to determine if predictive criteria for reproductive performance could be developed in the prepuberal female bovine, heifers were used to establish and compare prepuberal LH and FSH secretion profiles and evaluate the hypothalamic response to an E_2 (17β-estradiol) challenge. This information was then related to reproductive performance through the first calving.

Blood samples were collected at various prepuberal ages to establish profiles of LH and FSH secretion. The E_2 challenge was effected by injecting 1 mg of E_2-benzoate intramuscularly and collecting blood samples at 8, 11 and 14 hours after injection. Radioimmunoassays (RIAs) were developed to measure plasma levels of LH and FSH.

Ages studied ranged from 6 to 424 days. A significant difference ($P>0.01$) in levels could be seen between the pre- (31±14 days) and post- (387±22 days) weaning periods. Mean FSH level for the age-range studied was 12±5.6 ng/ml with a range of 3.8 to 39.7 ng/ml. LH averaged 388±271 pg/ml with a range of 135 to 3148 pg/ml and was significantly higher ($P>0.01$) during the post-weaning period (387±22 days). A comparison of the hormonal profiles of the heifers with the highest and lowest levels of FSH and LH secretion during the first 100 days after birth revealed that the FSH levels of the respective groups were significantly different ($P<0.01$) but the LH levels were not ($P>0.10$).

The effect of E_2 on LH secretion was significant ($P<0.01$) but it was not for FSH secretion. Ages tested ranged from 117 to 193 days. Of the 38 animals tested, 20 did not respond, 16 responded positively (any level 2 or more times greater than the 8 hr sample but less than 10 ng/ml) and 2 responded with a preovulatory-like LH surge (LH peaks $\geq$10 ng/ml, Schillo et al., 1983). The number of responders was evenly distributed throughout all of the ages tested. Responses of higher magnitude occurred between the ages of 162 to 193 days. Although the FSH response was not significant, 4 of the 38 animals responded positively to the E_2 challenge. The responses occurred between 162 and 193 days of age.

Of the 41 heifers used to relate prepuberal LH and FSH secretion to reproductive performance, 19 calved within one estrous cycle of one another, 2 were open and 20 were exposed to an infertile bull which made them ineligible for further study. Due to the low number of animals studied, no significant differences ($P>0.10$) between the heifers that calved and those open were observed in either the profile of LH or FSH secretion. Although not statistically significant, FSH concentration was higher in the heifers that calved. Comparing the prepuberal E_2 challenge to reproductive performance showed that the heifers that calved had a higher LH response. This difference was not significant compared to the open heifers, which was probably due to an insufficient number of animals.

The results suggest that prepuberal FSH concentrations are higher in heifers that calve. Fertile heifers also respond to the

E_2 challenge with higher levels of LH. Further study is needed to validate the results, but at this point there seems to be potential for developing predictive criteria, based on prepuberal gonadotrophin levels, for reproductive performance.

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CHAPTER I

INTRODUCTION

Economic pressure has caused efficiency to be the major concern of beef producers today. Efficiency results in low costs and high profitability. Efficiency also means that more is obtained from what is presently available and this is currently a need of the beef industry. Recent changes in the beef industry have been due to a relatively new division of management, reproductive management. Technological advances such as artificial insemination, estrous synchronization and embryo transfer form the foundation for this managerial program. Instead of being mainly concerned with feeding the animal, the efficient production of beef requires that more attention be paid to reproduction.

Reproductive management involves planning production in a way that creates conditions that allow maximum expression of the animals' reproductive ability. To improve reproductive efficiency the manager can provide adequate nutrition, good breeding and an external evaluation of an animal's merit, but after that he/she can only hope the heifer or cow will be capable of an efficient and viable reproductive life. If the manager selects an animal only to find marginal reproductive capabilities, the business suffers a significant economic loss. Being able to evaluate reproductive capacity during prepuberal life would greatly enhance the effectiveness of reproductive management.

For the female, puberty is defined as a stage in development when an individual is initially capable of conceiving and carrying a conceptus to term. The definition indicates that puberty is a culmination of changes associated with adequate growth and development. Recent research has revealed that puberty is the end of a series of physiological events that begin at fertilization and parallels the growth and development of an individual. It seems that sexual development functions as a system which is dependent upon, yet separate from, growth. The onset of puberty occurs within days in the rat and takes several years in humans. Each system of development is designed to assure the survival of its own kind. Researchers have shown in the bovine that many exogenous as well as endogenous factors can enhance or deter the physiological steps necessary for puberty. With this in mind, the prepuberal attributes of an individual could well indicate its reproductive potential and, within a given environment, age at which puberty would occur.

Although there are still many questions pertaining to the "how" of reproductive development and function, we are now at a point that allows study of whether criteria associated with prepuberal development can be used to predict reproductive capacity. Therefore, the following study was conducted to identify possible hormonal relationships linking prepuberal status to postpuberal performance. It is hoped that results from a study of prepuberal events would have practical as well as technical applications.

CHAPTER II

REVIEW OF THE LITERATURE

Function and Control of Gonadotrophins in the Adult Mammalian Female

The concern for prepuberal study of gonadotrophins arises from the intricate role the hormones play in the mature cyclic adult. Follicle stimulating hormore (FSH) and luteinizing hormore (LH), glycoproteins with a molecular weight of approximately 30,000 (Baird, 1972) and commonly referred to as gonadotrophins because of their stimulatory effect on the gonads (Bearden and Fuquay, 1984a), are responsible for follicle development and ovulation in the female. More specifically, FSH is responsible for follicular growth and estrogen production through the granulosa cells in the ovary (Bearden and Fuquay, 1984a). Important functions of LH are to cause ovulation and in turn cause the ovulated follicle to form a corpus luteum (Bearden and Fuquay, 1984a). LH also stimulates the production of estrogen in conjunction with FSH. Through the stimulatory effect of LH the theca interna cells produce androgen which moves to the granulosa cells and with the assistance of FSH is converted to estrogen, principally 17β-estradiol (Bearden and Fuquay, 1984a). Both LH and FSH, though having different effects, are similar in molecular composition, differing only in their subunits. Each molecule of FSH and LH consists of two polypeptide

chains. Both have a common α subunit but each has a specific β subunit which distinguishes one from the other (Sherwood and McShan, 1977). The anterior pituitary is the source of these hormones (Bearden and Fuquay, 1984a). Secretion of gonadotrophins is dependent upon the hypothalamus which manufactures a peptide hormone called gonadotrophin releasing hormone (GnRH) that causes the pituitary to release the gonadotrophins (Bearden and Fuquay, 1984a).

GnRH synthesis and release, among many other factors, is controlled by estrogen. Estrogen is a steroid produced by the ovary. As mentioned earlier, estrogen synthesis is governed directly by the gonadotrophins and indirectly by GnRH. The site of its production is the Graafian follicle (Bearden and Fuquay, 1984a). Having many responsibilities in the reproductive cycle, estrogen has been deemed the "female sex hormone" (Bearden and Fuquay, 1984a). After ovulation, estrogen levels are low due to the feedback effects of the progesterone produced by the corpus luteum on the hypothalamus (Bearden and Fuquay, 1984a). Gonadotrophin secretion is thus reduced to a low level. Preovulatory levels of gonadotrophins are also influenced by 17β-estradiol. As the corpus luteum regresses and consequent progesterone levels decline at proestrus, gonadotrophin levels rise, follicles mature and their estrogen-output elicits a "positive feedback" stimulation of the hypothalamus and pituitary. This stimulation results in the gonadotrophin surge (Bearden and Fuquay, 1984b). The pattern of gonadotrophin secretion and its

control in mammals are in general the same. Events in the ovarian cycle differ such as menstruation in humans and estrus in domestic animals (Short, 1972).

Prepuberal Development

The first evidence of luteinizing hormone (LH) and follicle stimulating hormore (FSH) occurs in the fetus. Sklar et al. (1981) detected varying levels of gonadotrophins in ovine fetuses as young as 59d of age. Levels subsided as the fetuses went to term. In a similar study, Clark et al. (1984) observed a pulsatile mode of LH secretion by the ovine fetal pituitary as early as 81d. An FSH pulse was revealed in fetuses between the ages of 106d and 115d. The evidence suggests that a hypothalamic GnRH pulse generator is developed to a considerable extent by midgestation (Clark et al., 1984). A study in the bovine by Oxender et al. (1972) revealed that levels of LH were greatest in 90d fetuses, the youngest age studied, and that gonadotrophin levels decreased toward term. LH secretion continued to decline until 1 week after birth. Levels of LH were greater in fetal than in the maternal circulation which suggests that a placental barrier exists for LH and indicates that the fetal pituitary was the source of the LH secretion (Oxender et al., 1972).

Postnatal gonadotrophin secretion has been most thoroughly defined in rats. In the female rat, sexual maturation has been separated into four phases; neonatal (first week of life), early

juvenile period (second week of life), the late juvenile phase (weaning age, approximately 20-22 days), and the peripubertal phase (ovulation) (Ramaley, 1979). Gonadotrophins are secreted at high levels during the neonatal phase but levels decrease as the female approaches the early juvenile stage (Döhler and Wuttke, 1975). Tonic levels of FSH begin to rise during the early juvenile phase (Döhler and Wuttke, 1975). At the beginning of the late juvenile phase, which is just prior to puberty, tonic FSH secretion begins to decrease and continues to do so for the remainder of this phase (Döhler and Wuttke, 1975). LH pulse frequency remains low throughout prepuberty with the exception that between the ages of 13 to 18 days LH pulse frequency varies in a circadian manner with LH peaks occurring in the early morning (3.00hr) and mid-afternoon (15.00hr) (Döhler and Wuttke, 1975, 1976).

In the bovine, Dodson et al. (1985) have shown that gonadotrophin concentrations decline during the first half of prepuberal development and increases during the second half. Using Hereford x Friesian crossbred heifers they found that mean levels of FSH were approximately 33.3±4.2 ng/ml between 3 and 7 weeks of age, falling to 23.1±3.5 ng/ml at 15 weeks and increasing to 37.8±3.5 ng/ml at 35 weeks. LH secretion was somewhat different with mean levels decreasing at 3 to 7 weeks from 0.96±0.9 ng/ml to 0.76±0.9 ng/ml at 15 weeks. At 35 weeks LH concentrations increased to 1.21±0.6 ng/ml. LH pulse frequency increased with age. Mean pulse rates at 3, 7 and 35 weeks of age were 0.6±0.4, 2.9±0.6 and 5.1±0.3 per 24 hr

respectively. Age at puberty for these animals was 314±12 days.

The pattern of gonadotrophin secretion in the prepuberal ovine is unlike that of the bovine with mean levels of 0.2±.01 ng/ml LH and 46±3 ng/ml FSH at 3 to 8 weeks of age, 2.8±0.8 ng/ml and 88±21 ng/ml FSH at 11 weeks. Little change occurred in LH levels for the remainder of prepuberal development, but FSH declined somewhat to 79±3 ng/ml between 11 and 37 weeks of age (Foster et al., 1975). LH pulse frequency ranged from 1 to 7 pulses per 6-hour period from 11 weeks of age to puberty.

Porcine gonadotrophin secretion has been described by Camous et al. (1985). Mean concentrations of FSH ranged from 8.3±0.5 ng/ml to 11.1±1.0 ng/ml between 15 and 54 days, then decreased between 125 and 168 days from 7.8±0.5 ng/ml to 3.1±0.4 ng/ml, and remained at approximately 3.0±0.4 ng/ml for the remainder of the sampling period (192d). LH concentrations began at approximately 1.2±0.3 ng/ml at 15 days and decreased to 0.8±0.2 ng/ml by 68 days. Between 83 and 125 days, LH ranged from 1.0±0.3 ng/ml and 1.5±0.5 ng/ml. After 125 days tonic LH concentrations decreased steadily from 1.5±0.5 at 125 days to 0.6±0.1 ng/ml at 192 days. LH pulse frequency changed with age. Pulses per 6-hour period averaged 1.1±0.1 between 15 and 68 days, increased to 2.2±0.3 between 83 and 125 days, and decreased to 1.2±0.2 between 137 and 192 days.

A comparison of prepuberal gonadotrophin secretion shows that tonic LH and FSH concentrations are high during early life

in the rat, bovine and porcine, but are relatively low in the ovine species. Main interspecies differences occur during the first and second half of prepuberal development. In the rat, tonic FSH and LH levels decline during early juvenile life, but FSH begins to increase later in the juvenile period. Unlike the rat, tonic gonadotrophin secretion levels in the ovine are increasing while the bovine concentrations are decreasing during this same time. The porcine is also unique in the fact that tonic FSH secretion decreases and LH concentrations increase throughout the first half of prepuberal development. As seen in the rat and ovine during the second half of prepuberal development, tonic FSH secretion decreases while LH concentrations stabilize. At the same time in the bovine, basal concentrations of LH and FSH steadily increase, while gonadotrophin concentrations in the porcine gradually decrease.

Prepuberal Control of Gonadotrophin Secretion

In the female, tonic gonadotrophin secretion is primarily controlled by ovarian derived estrogen in the form of 17β-estradiol (E_2). Studies in the rat show that estrogen has a negative-feedback effect on the hypothalamus which, through its secretion of gonadotrophin-releasing hormone (GnRH), is responsible for stimulating gonadotrophin secretion by the pituitary gland (Andrews and Ojeda, 1981). This is also the case in sheep (Bolt, 1981) and cattle (Butler et al., 1983). In addition to E_2, the release of FSH is also controlled by the negative-feedback effect of inhibin, a proteinaceous

hormore produced by the granulosa cells of vessicular follicles (Erickson and Hsueh, 1978) that causes its effect through the pituitary gland. Ovulation is dependent upon a preovulatory surge of LH and this occurs as a result of the positive feedback effect of E_2 on the hypothalamus and pituitary. The LH surge has been described in sheep (Webb et al., 1981) and cattle (Rzepkowski et al., 1982), as well as other mammals (Karsch, 1984). Development of the positive-feedback mechanism in the hypothalamus is dependent upon ovarian maturation and the subsequent production of E_2 (Foxcroft et al., 1984). The positive-feedback effect of estrogen on the hypothalamus is evident prior to puberty. For example, an exogenous dose of E_2 will cause a preovulatory LH surge in rats of approximately 16 days of age (Andrews et al., 1981). As for sheep, a preovulatory LH surge can be induced by E_2 in lambs as young as 19 weeks of age (Squires et al., 1972). Control of gonadotrophin secretion by estrogen in the bovine is much the same as that in the rat and sheep except for a difference in the age at which estrogen can induce an LH surge. The ability of the bovine hypothalamus to produce a preovulatory LH surge in response to exogenous E_2 occurs between the ages of 3 and 8 months (Schillo et al., 1983; Staigmiller et al., 1979).

Onset of Puberty

Increased tonic gonadotrophin secretion, primarily LH, is a key element in the induction of puberty. LH is important

because it stimulates the theca cells to produce androgen which is used by the granulosa cells to produce estrogen. Estrogen is essential for the tonic and surge modes of gonadotrophin secretion. The first preovulatory surge and subsequent ovulation marks the initiation of puberty. For the female rat, Ramaley (1979) suggests two possible theories for the onset of puberty: 1) a change in the hypothalamic neurons controlling GnRH secretion and 2) a shift in the sensitivity of the gonads to gonadotrophin secretion. Other work with the rat suggests that ovarian maturation may be the most important factor in determining the onset of puberty (Andrews et al., 1981). Recent studies suggest that a change in the molecular composition of LH may also be a contributing factor in the initiation of puberty. Differences in the character of LH were found between peripubertal female rats classified as large ($\geq$ 60 g) that were capable of responding to induced ovulation and small rats (<60 g) that were not capable of response to induced ovulation (Buckingham and Wilson, 1985). Plasma LH concentration from the larger rats could be detected by a radioimmunoassay and a cytochemical bioassay. In the smaller rats, plasma LH was active in a radioimmunoassay and not by cytochemical bioassay.

Elevated tonic concentrations of LH are associated with the oncet of puberty in heifers. Increased tonic LH concentrations are evident just prior to the onset of puberty (Gonzalez-Padilla et al., 1975; Morrow et al., 1976). Gonzalez-Padilla and associates (1975) found no change in GnRH secretion relative to the increase

in tonic LH concentration. Tonic LH secretion also decreased just before the initial preovulatory LH surge marking the onset of puberty. FSH concentration did not change relative to the onset of puberty. Pubertal onset in the heifer has also been related to hypothalamic activity. Anderson et al. (1981) confirmed the importance of the hypothalamus by showing that puberal estrus and ovarian growth were inhibited in hypophysial-stalk transectioned heifers. An increase in LH pulse frequency presumably due to hypothalamic modulation by GnRH has been determined as a major factor contributing to the onset of puberty in sheep (Foster et al., 1984; Huffman and Goodman, 1985). Camous et al. (1985) showed in the female pig that both FSH and LH tonic concentrations decline and remain low before and during the onset of puberty, which may suggest that, in this species, high levels of LH and FSH are not required for the initiation of puberty.

Factors such as temperature, photoperiod, genetic background and nutrition have been found to enhance or deter puberty. These factors are known to affect gonadotrophin secretion (Karsch, 1984). For example in rats, noise, crowding and extreme temperatures can alter the onset of puberty (Moltz, 1975).

The effect of growth on age at puberty is very important. A correlation between weight gain and onset of puberty has been established in rats but there is not a specific weight associated with the attainment of puberty (Glass and Swerdoff, 1977). Restricted diets result in retarded growth, lowered LH secretion and delayed

puberty in the female sheep (Foster and Olster, 1985). It has also been found that photoperiod, which controls seasonal breeding in sheep, can affect age at puberty in female lambs (Foster, 1983). Premature exposure of the lambs to short days disrupted the negative feedback effect of estrogen on tonic LH secretion causing abnormal ovarian cycles which delayed the onset of puberty in this case.

Attainment of puberty has been shown to differ among various breeds and types of cattle (Dow, Jr., et al., 1982). The interaction of diet and genotype in heifers has also been shown to affect pubertal onset (Grass et al., 1982). Day et al. (1984) showed that restricted diet delayed the increase in tonic LH secretion needed for puberty in the heifer. Exposing heifers to increased light also affects attainment of puberty. In a study where heifers were exposed to either a natural or an increased photoperiod, the age at puberty was reduced in the heifers exposed to increased light (Hansen et al., 1983). No changes were observed in tonic LH secretion, however there was a considerable difference in ovarian size. Heifers exposed to increased light had significantly larger ovaries.

In conclusion, Foster et al. (1985) have proposed a detailed hypothesis explaining the onset of puberty in sheep. The signals that control growth and melatonin govern the GnRH pulse generator which controls the tonic and surge modes of LH secretion. Upon the occurrence of a favorable photoperiod and the attainment of a body of sufficient size, the hypothalamus becomes capable of

inducing a preovulatory LH surge which causes the first ovulation. Subsequent ovulations can lead to pregnancy and, by definition, a female capable of being impregnated has attained puberty.

Advancements in Predicting Reproductive Performance

An early study by Findlay and Bindon (1976) using Merino lambs selected on the basis of litter size (high, medium, low) showed that the level of tonic gonadotrophin secretion was greater in lambs selected for high lambing rate. Also in 1976, Eckternkamp and Laster, using ovulation rate as a measure of fecundity, found no relationship between prepuberal tonic LH secretion and fecundity in various breeds of sheep. Chiquette et al. (1984) also showed that prepuberal tonic LH secretion and fecundity could not be related across breeds of sheep but they did find that higher levels of LH secretion occurred in the larger lambs within a breed. In other studies, the level of FSH secretion has been found to be an important factor for identifying breeds of sheep with higher reproductive protential (Lahlou-Kassi et al., 1984). Gonadotrophin secretion was measured and compared during the estrous cycle in two breeds of sheep with high and low ovulation rates. Timahdite sheep which had a low ovulation rate (1 CL/cycle) and D'man sheep which had a high ovulation rate (3 CL/cycle) were used. When comparing gonadotrophin secretion overall, LH concentration was highest in the Timahdite sheep, whereas FSH concentration during pro-estrus and estrus was highest in the D'man sheep, showing

that higher levels of FSH secretion are related to higher fecundity in sheep. Recent evidence has revealed genetic differences relative to tonic gonadotrophin secretion within a breed of sheep. Bindon et al. (1985) comparing tonic gonadotrophin secretion in female Booroola lambs of high, medium and low prolificacy showed that the more prolific lambs had a higher level of tonic FSH secretion at 30 and 45 days of age. However, tonic LH secretion was not significantly different between the groups tested. The lambs were not kept for study of mature reproductive performance. Though much work has been completed in sheep, studies on predicting reproductive performance in cattle have not been attempted.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

The study was separated into three parts: 1) an evaluation of prepuberal gonadotrophin secretion, 2) a test of hypothalamic response to a 17β-estradiol (E_2) challenge and 3) a study of the relationship between hormonal profiles and reproductive performance. In the first study the entire 1984 heifer calf crop (39) from the Hereford brood cow herd at the Tobacco Experiment Station was used to develop a prepuberal profile of basal levels of LH and FSH. The herd was maintained on pasture with supplemental feeding of silage and hay during the winter. Ten milliliters of heparinized blood were collected from each heifer at varying times during pre- and post-weaning periods. Plasma was obtained from calves ranging in age from 6d to 424d. Intervals between age groups ranged from 10 to 20 days with 5 to 20 animals evaluated per group. The blood samples were centrifuged at 3000 rpm for 20 minutes and the plasma was stored at -20°C until assayed for gonadotrophins. From this data a prepuberal pattern for the secretion of LH and FSH was established.

In the second study, the same animals were used to measure the hypothalamic and pituitary response to an E_2 challenge. Calves ranging in age from 117 to 193 days were injected intramuscularly

with 1 mg of E_2-benzoate and blood samples were taken at 8, 11 and 14hr post injection. The objective of this study was to determine whether the hypothalamic-pituitary axis of the calf was capable of producing a surge of LH in response to a high level of E_2. A preovulatory LH surge is defined at any level $\geq$ 10 ng/ml (Schillo et al., 1983). The animals were placed into age groups ranging from 125 to 183d of age with intervals of 10 to 20d. Numbers of animals per age group ranged from 5 to 15. The existence and magnitude of the LH surge was then compared between age groups and the pretest gonadotrophin level of each calf.

A third study considered the relationship between reproductive performance and the LH and FSH profiles of 41 heifers. These heifers were part of the herds at the Knoxville and Tobacco stations. Twenty heifers from Blount Farm assigned to this study were eliminated because they were subjected to an infertile bull. Hormonal data from these animals were, therefore, not included in this study. Indexes of reproductive performance were whether or not the animal conceived and age at conception. These criteria were compared to LH and FSH profiles. The same indexes of reproductive performance were also compared to whether or not the animals responded to an E_2 challenge.

Development of Radioiodination Techniques

Inexperience in the practice of radioiodination and the testing of the iodinated hormone resulted in many unsuccessful attempts. Without detailing an entire radioiodination procedure

the following points were found to be essential to a success. The hormone should be stored in plastic vials--hormones will adhere to glass and cannot be quantitatively recovered. A glass reaction vessel should not be used for iodination unless the "iodogen" method is employed. A plastic reaction vessel resulted in a greater quantity of protein with higher specific activity. "Mixing" should be "vigorous" rather than "gentle," but splashing should be avoided.

FSH

Purified FSH, USDA BP3, was graciously supplied for radioiodination by Dr. D. J. bolt, USDA, Beltsville, MD. Radioiodination of FSH was accomplished with the iodogen technique developed by D. J. Bolt (1983, unpublished) with modification. In brief, a 1.5 ml glass serum vial (Wheaton Co.) is plated with 2 ug of iodogen (Pierce Chemical Co.). The iodogen is dissolved in chloroform at a rate of 1 ug/25 ul. Fifty ul of the iodogen/chloroform mixture is added to the 1.5 ml serum vial and dried gently with a light stream of nitrogen. The iodogen is hardly visible in the vial. To the iodogen coated vial 5 ug of USDA BP3 FSH in 35 ul of 0.5 M sodium phosphate buffer, pH 7.4, is added, followed by 400 uCi of I^{125} (Amersham Co.). The mixture is allowed to react 10 minutes under constant vortexing. Vortex at a rate capable of sustaining visible agitation of the solution but avoid a speed that may splash the mixture beyond contact of the iodogen. Termination

of the reaction is accomplished by the successive addition of 200 ul of sodium metabisulfite (25 ug/ml) in 0.25 M sodium phosphate buffer, pH 7.4, and 200 ul of potassium iodide (1 mg/ml) in 0.5 M dibasic sodium phosphate buffer. After addition, mix these reducing agents for approximately 10 seconds to insure dispersion. The reaction volume is then loaded onto a sephadex G25M gel column for separation of the radiolabelled protein from the free I^{125}.

LH

Of the many methods tried, Chloramine T was the preferred technique for iodinating LH. The method was a modification of the technique of Brown et al. (1983). Purified bLH, LER 1072-2, for iodination was supplied by Dr. Leo Reichert of the Albany Medical School, Albany, New York. In brief, using a 1.5 ml plastic microcentrifuge tube containing 2.5 ug of LH, LER 1072-2, in 25 ul of 0.5 M sodium phosphate buffer, pH 7.5, 500 uCi of I^{125} was added. The reaction was initiated by the addition of 15 ug (1 ug/ul) of chloramine T in 0.05 M sodium phosphate buffer, pH 7.5. The mixture was allowed to react for 1 minute with periodic agitation by finger tapping. Termination of the reaction was accomplished by adding 60 ug (2 ug/ul) of sodium metabisulfite in 0.05 M sodium phosphate buffer, pH 7.5, and again finger tapping the mixture for about 10 seconds. One hundred ul of eluent buffer (0.05 M - sodium phosphate buffer - 0.1% bovine serum albumin) was added to

the reaction mixture and the entire contents were then transferred to a sephadex G25M gel column for isolation of the radiolabelled LH.

Column Preparation

Gel filtration was used to separate both FSH and LH radiolabelled compounds. A 1 x 20 cm glass column containing Sephadex G25M gel (Pharmacia Co.) suspended in 0.05 M sodium phosphate buffer was prepared using the technique recommended by the Sephadex gel chromatography manual. Buffer pH for FSH and LH was 7.4 and 7.5, respectively. The column was rinsed with about 10 mls of 0.05 M - sodium phosphate buffer - 0.1% bovine serum albumin (BSA), which is also the elution buffer, just prior to use.

Isolation Technique

Upon loading the iodinated material on the G25M column, isolation of the radiolabelled protein was accomplished by fractionating the eluded material in 1 ml aliquots. Radioactivity was monitored with a portable geiger counter. The first peak of radioactivity that comes off of the column is the radiolabelled protein and the second peak is that of the free iodide. The radiolabelled product is eluted after approximately 15 fractions. Elution of the free iodide is complete at about 30 fractions.

Characterization and Validation of Tracer

The quality of radiolabelled hormone was determined by taking 10 ul aliquots from each column fraction and measuring

the radioactivity (CPM) on a gamma counter. Average I^{125} incorporation for FSH and LH was 57% and 59% respectively. Specific activity was approximately 82 uCi/ug for FSH and 83 uCi/ug for LH. After characterization, the tracer was then diluted to a concentration of approximately 30,000 cpm/ul in RIA buffer (0.01 M phosphate buffered saline - 0.01% BSA - 0.01% thimerosal) and stored at 4° C. A trichloroacetic acid (TCA) test (Tower et al., 1978) was employed to establish the amount of hormone actually bound to I^{125} in the tracer batch. Twenty-five ul of diluted tracer from the peak fractions was placed in each of four 1.5 ml plastic microcentrifuge tubes. Two tubes are controls and to the remaining tubes 100 ul of cold (4° C) 0.05 M - 1% sodium phosphate buffer - BSA and 200 ul of cold (4° C) 0.05 M - 18% sodium phosphate buffer - TCA were added in sequence. The treated tubes were vortexed approximately 10 seconds and then centrifuged at 3000 rpm for 5 minutes. The supernatant was poured off and all tubes were counted using a gamma counter. Hormone incorporation of 80% or greater qualified the radiolabelled material for use. Tracer addition and antibody titer were tested to quantify the amount of each needed to produce a total binding capacity of 25% for FSH and 30% for LH. For LH approximately 18,000 cpm and a 1:40,000 final dilution of antibody were required for 30% total binding. Rates for FSH were approximately 20,000 cpm and a 1:10,000 antibody dilution for 25% total binding. The diluted tracer was stored at 4° C and had a viable life of 4 to 6 weeks.

Radioimmunoassay of FSH

FSH levels were determined by radioimmunoassay using the method developed by Bolt and Rollins (1983) with modification. Table 1 contains an outline of the FSH RIA protocol used. All buffers and reagents used along with the basic RIA protocol were previously described by Bolt (1981). The RIA buffer used was different since phenol red was not used and the preservative (thimerosal) was used at a 0.01% concentration. Also bovine serum albumin (BSA) was used instead of gelatin. FSH reference standard (USDA-bFSH-B1) and first antibody (USDA-5-0122) were supplied by Dr. D. J. Bolt, USDA, Beltsville, MD. Bolt and Rollins (1983) showed that the FSH β-subunit antiserum (USDA-5-0122) cross reacted with bovine luteinizing hormone, bovine growth hormone and bovine prolactin at < 0.09%. Bovine thyroid-stimulating hormone had a 0.5% cross reactivity. The RIA was sensitive to 1 ng. Samples, in triplicate, containing concentrations from 1 to 128 ng of the standard (USDA-bFSH-B1) were used to construct a reference curve. Unknowns were compared thereto to quantify the plasma levels of FSH. The second antibody (goat-antirabbit IgG) was purchased from Miles Scientific Ltd. The main differences in the RIA procedure were antibody dilution, amount of tracer added and total binding capacity. FSH antibody concentration was 1:10,000 and about 20,000 cpm of FSH-I^{125} were added to each sample tube with an average total binding capacity of 25.7%. Nonspecific binding was less than 2.0%. Control of variation

Table 1. Protocol for FSH and LH RIA.

Sequence → Tubes	1 RIA Buffer	2 Sample	3 Stock Ab	4 1st Ab	5 Tracer
TC	------	------	------	------	100 ul
NSB	700 ul	------	------	------	100 ul
Bo	500 ul	------	------	200 ul	100 ul
Standards	300 ul	200 ul	------	200 ul	100 ul
Poolled Sample	100 ul	400 ul	------	200 ul	100 ul
Max Bo	500 ul	-----	200 ul	------	100 ul
Sample	100 ul	400 ul	------	200 ul	100 ul

Legend:

TC - Total count (total amount of tracer added to each tube.

NSB - Non-specific binding.

Bo - Total binding (%) capacity of the working dilution of tracer and antibody to be used as the basis for determining hormone concentrations.

Standards - Known amounts of hormone used to construct the standard reference curve.

Poolled sample - A standard unknown sample used in each assay to control inter- and intra-assay variation.

Max Bo - Maximum binding (%) capacity of the tracer and antibody to control the quality of the tracer.

Sample - The unknown amount of hormone that is to be measured.

between assays was monitored by measuring a standard batch of pooled plasma from heifers in each assay. Tracer quality was also monitored by using a maximum binding test (D. J. Bolt, personal communication) combining stock FSH-Ab (1:300) with the recommended tracer addition (∿20,000 cpm). Interassay variation between poolled samples was 11% and the intrassay variation between the poolled sample replicates was <10%. The average maximum binding capacity was 65.9% with an interassay variation of 8.9%. The variation within the sample replications was <3.0%.

Radioimmunoassay of LH

LH was measured using the basic RIA developed by Niswender et al. (1969) as modified by D. J. Bolt (unpublished). The protocol is the same as previously described for FSH. Table 1 also applies to the basic RIA protocol used for LH. LH reference standard (LER 1056) was supplied by Dr. Leo Riechert, of the Albany Medical School, Albany, NY. The antibody (#15 anti-ovine LH) was supplied by Dr. Gordon Niswender, Colorado State University. The LH RIA was sensitive to 31 pg. Performance of the LH antibody was not significantly affected by FSH, thyroid-stimulating-hormone and growth hormone (Niswender et al., 1969). Eight concentrations, in triplicate, of the standard (LER 1056) ranging from 31 to 4000 pg were used to construct a reference curve. Plasma levels of LH were quantified by comparison to the reference curve. The antibody was used at a dilution of 1:40,000 with a tracer addition of about

18,000 cpm LH-I^{125} to obtain an average total binding capacity of 34.3%. Nonspecific binding was <5.0%. Variation between assays was controlled using the same method as used for FSH. Variation of the poolled samples was 11% and intrassay variation between the poolled sample replicates was <5.0%. The average maximum binding capacity was 60% with an interassay variation of 11%. The stock antibody dilution was 1:400. Variation between sample replicates was <5.0%.

Statistical Methods

Differences in prepuberal levels of FSH and LH were tested for significance using one-way analysis of variance, least squares means (LSM) and student-newman-keuls (SNK). The effect of age, weight and pulse rate (of LH and FSH secretion) were also tested separately against levels of FSH and LH in a one-way analysis of variance. The equation that resulted in a "best fitting" line describing the regression of FSH level on prepuberal age was $\hat{Y} = a + bx$ and that for LH was $\hat{Y} = a - b'x' + b^2 x^2$. An analysis of variance was also performed to test for significant differences in levels of FSH and LH between individual heifers.

In the study on the hypothalamo-hypophoseal response to an E_2 challenge the effect of E_2 on FSH and LH secretion was tested using a one-way analysis of variance.

The third study was conducted to determine whether differences in prepuberal hormone levels and the response to an E_2 challenge

were related to reproductive capacity. Analysis of variance was used to identify potentially meaningful asociations. Finally correlations (Pearson's product-moment correlation coefficient) between basal LH levels and the LH response to an E_2 challenge were calculated.

All means, standard deviations, coefficients of variation and correlations were computed with programs provided by the statistical analysis system (SAS, 1982a). Analyses of variance and regression, LSM and SNK were completed using SAS (1982b). The standard deviation and the coefficient of variation (standard deviation divided by the mean) were employed as indexes of variation.

CHAPTER IV

RESULTS

Study 1--FSH and LH Levels in the Prepuberal Heifer

Figures 1 and 2 show the means and standard deviations of FSH and LH at various prepuberal ages in the beef heifer. Each group is identified by its age when sampled. Ages studied varied from 6 to 424 days. The pre-weaning period is represented by ages of 11 to 116 days and the post-weaning period is represented by ages of 258 to 416 days. The unusually high mean LH level in group 22 is due to one outlying sample of 3148 pg/ml of plasma. That particular animal had only this one extraordinary value. All other samples were within the general range of the other animals tested (it is suspected that this high level of LH was due to the sample being taken during the peak of an LH pulse). Both FSH and LH levels were dependent on age ($P<0.01$). Significant differences between ages were evident for levels of FSH ($P<0.01$) and LH ($P<0.05$). Overall, the coefficient of variation for FSH was 43% and that for LH was 67%. FSH levels were significantly higher ($P<0.01$) in the younger heifers (31±14 days) and LH levels were higher ($P<0.10$) in the older heifers (387±22 days).

During days 9 through 124, two blood samples were taken at a 15 minute interval. Even though pulses of LH are released very infrequently in the heifer calf, minor pulses of FSH occur at

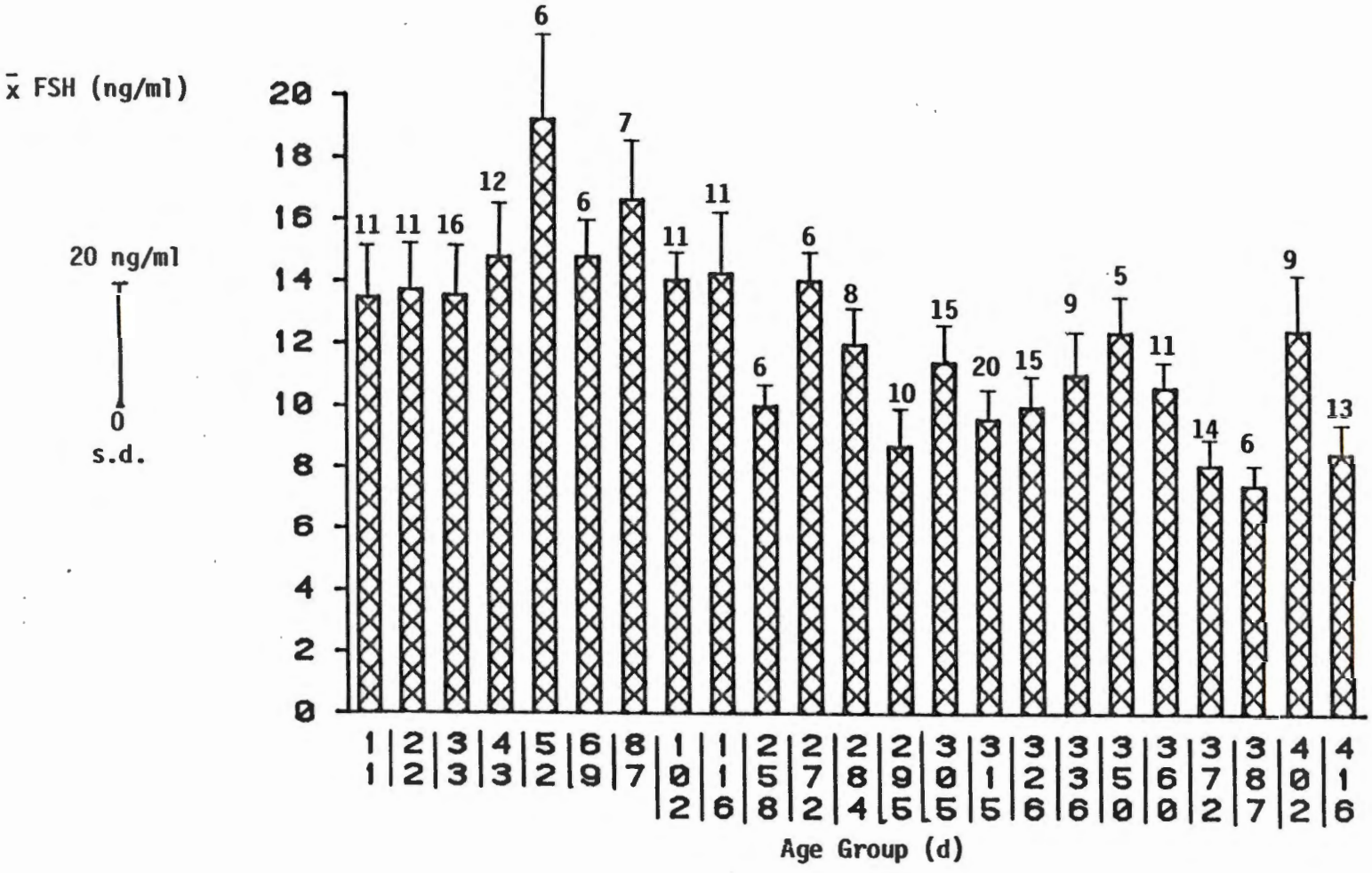

Figure 1. Concentration of FSH in the plasma of Hereford heifers of varying ages. (Note: The number at the top of the bar is the number of animals studied in that particular age grouping.)

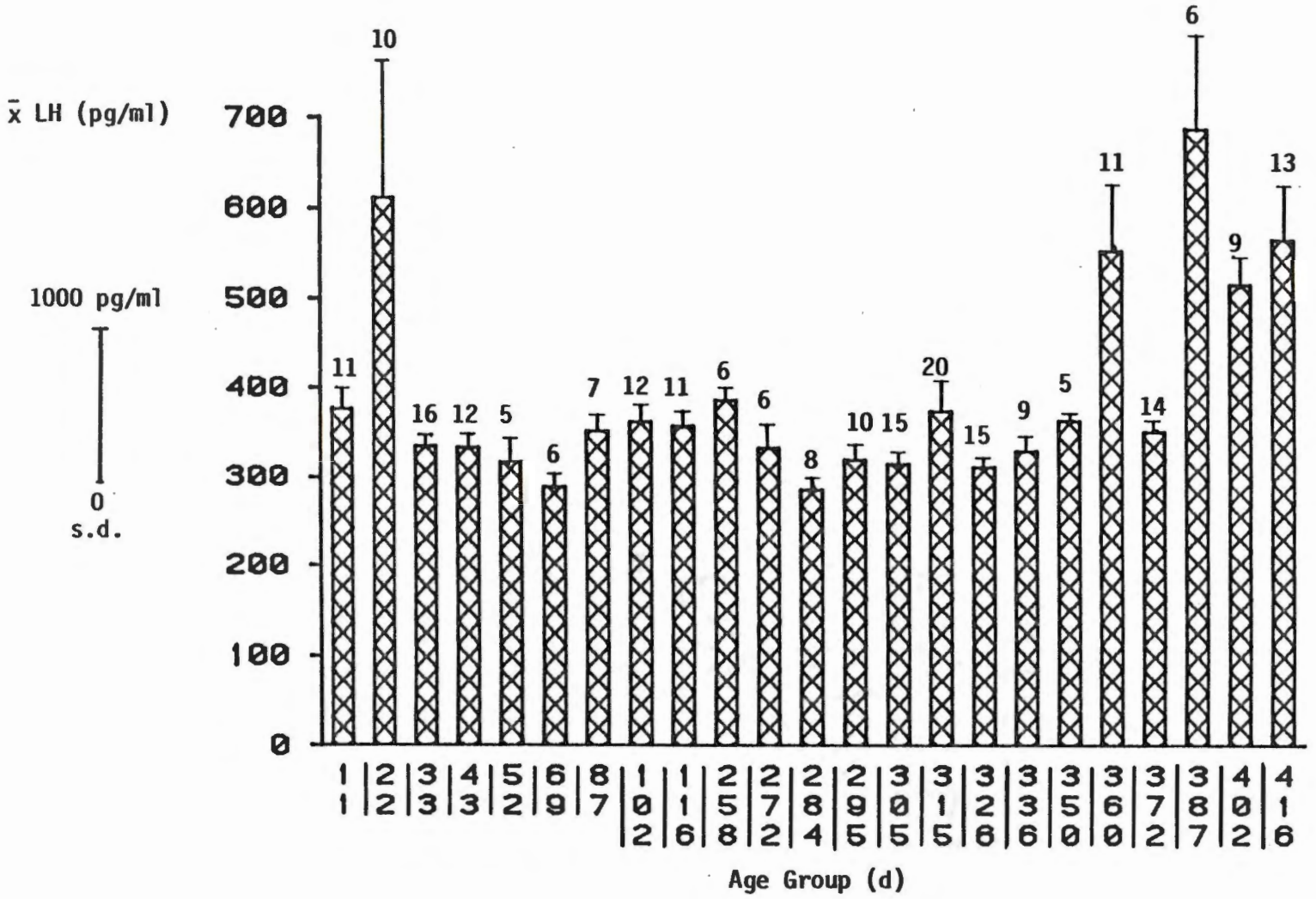

Figure 2. Concentration of LH in the plasma of Hereford heifers of varying ages. (Note: the number at the top of the bar is the number of animals studied in that particular age grouping.)

approximately a 15-minute interval (McLeod et al., 1984). There was no significant difference in either FSH or LH levels between the two sampling periods.

A comparison of growth rate to levels of FSH and LH secretion was also made. Table 2 shows weights, FSH, LH and standard deviations at various ages. Although there was no significant ($P>0.10$) relation between weight and hormone level; trends were observed for both FSH and LH. LH levels increased as weight and age increased (Table 2). FSH decreased slightly as weight and age increased (Table 2).

Table 2. Body weights, FSH and LH levels and their standard deviations in Hereford heifers at various post-weaning ages (days 303 to 424).

n	Age±s.d. (d)	Weight±s.d. (kg)	FSH±s.d. (ng/ml)	LH±s.d. (pg/ml)
9	322±10	397±51	11±4.3	325±43
27	361±9	458±83	10±4.3	425±293
18	390±11	529±72	10±5.8	529±72
14	416±5	563±77	8±3.2	563±77

Figures 3 and 4 show the regression of FSH and LH values on age in the prepuberal heifer. This analysis provides a "best estimate" of the pattern of change in gonadotrophin level with age. The measured values are indicated by (*) and the estimated values by (—). The outlying LH value (3148pg/ml) was not included in this analysis.

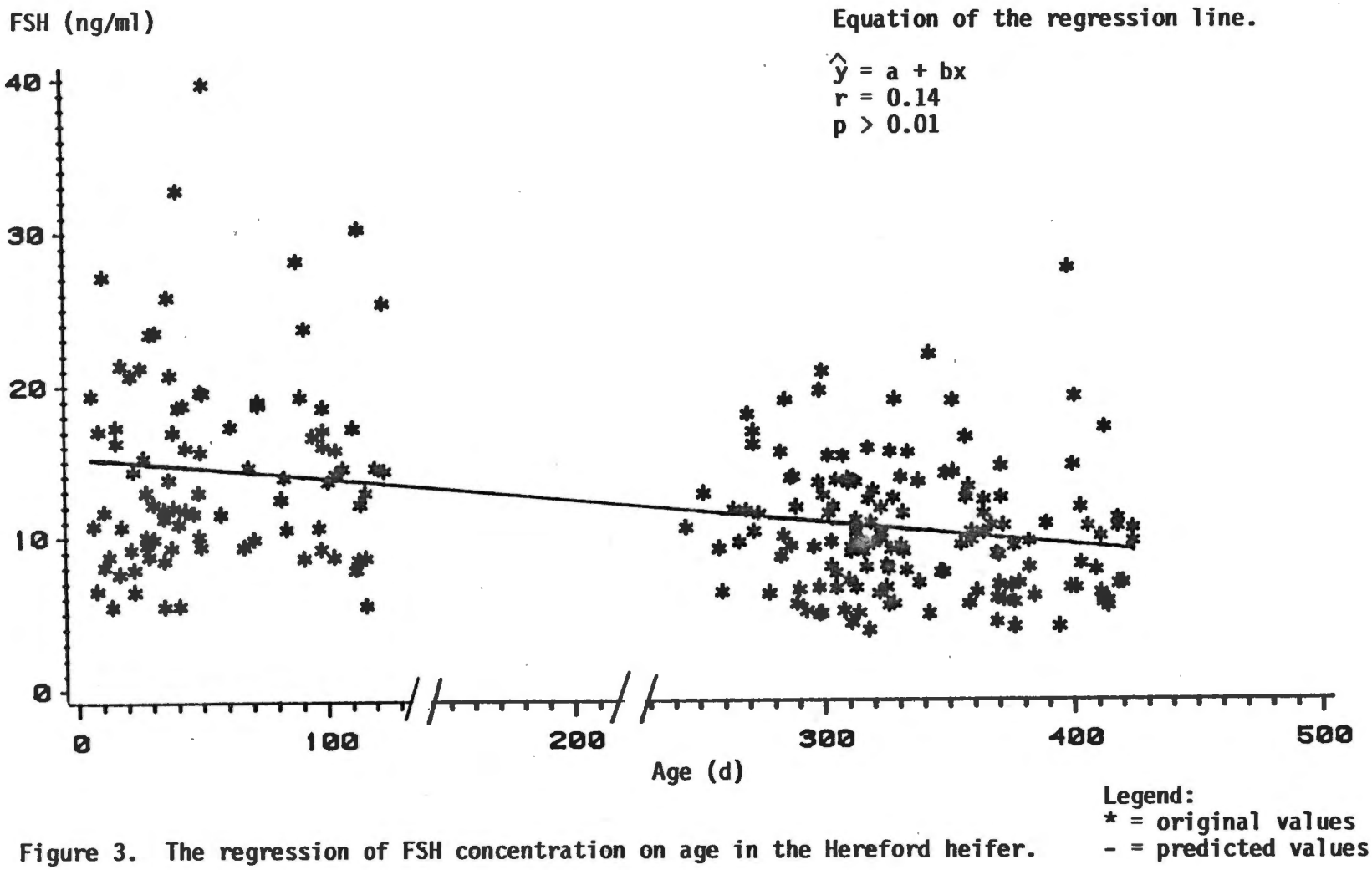

Figure 3. The regression of FSH concentration on age in the Hereford heifer.

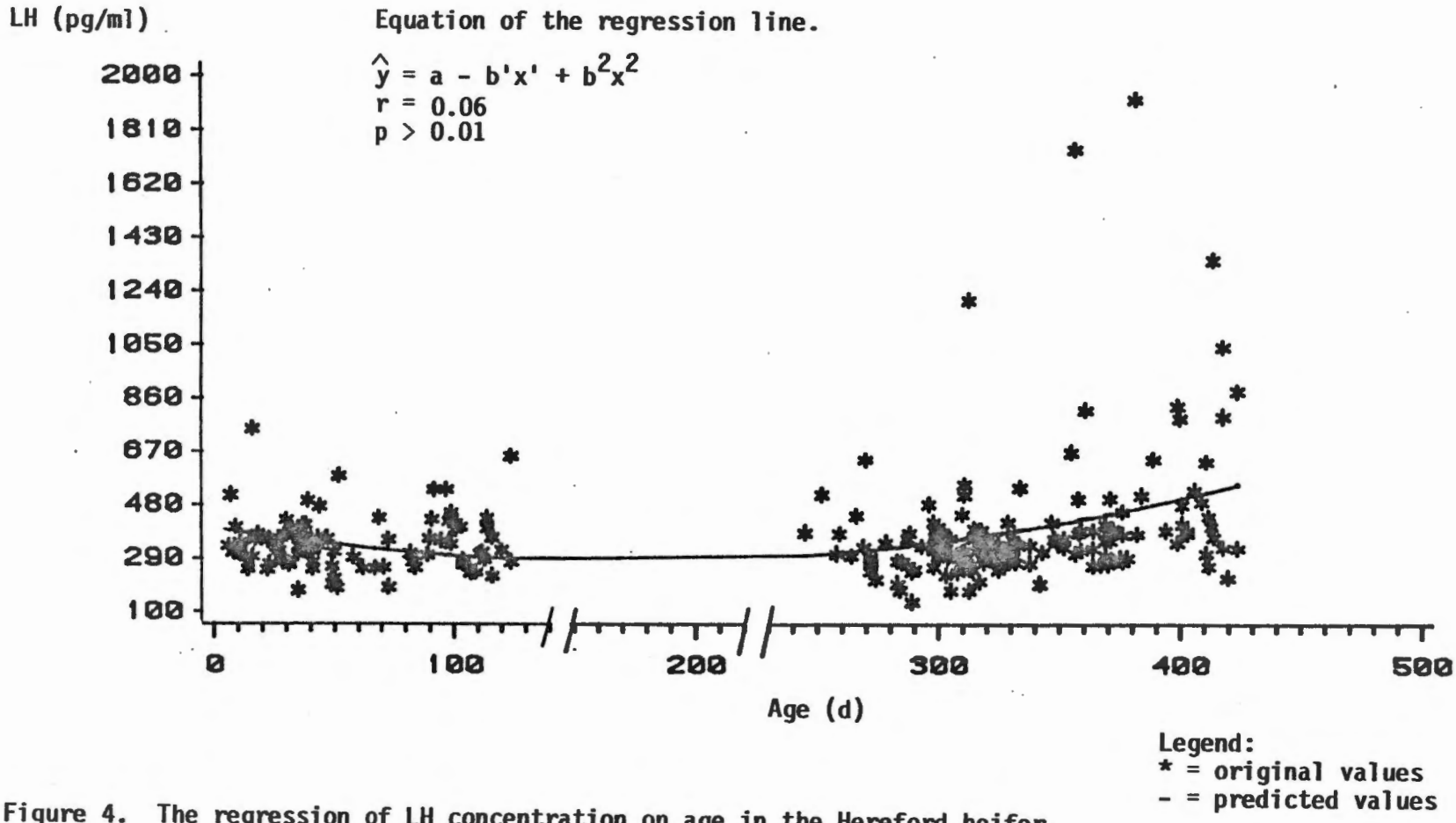

Figure 4. The regression of LH concentration on age in the Hereford heifer.

The overall mean for FSH representing 238 samples, pre- and post-weaning combined, was 12±5.6 ng/ml. FSH values for all ages ranged from 3.8 to 39.7 ng/ml of plasma. Coefficients of variation ranged from 22.5 to 56.5% for all ages tested. For FSH the degree of variation did not change significantly with age (Figure 1). The mean for LH from all samples at all ages (237) was 388±271 pg/ml. Levels of LH ranged from 135 to 3148 pg/ml. Coefficients of variation ranged from 9 to 146% for all ages tested. The degree of variability for LH did not change significantly, with the exception of age group 22, until about 360 days of age (Figure 2). FSH levels were more stable than LH, presumably because of the difference in the pulse frequency of LH and FSH. As a percent of the basal level, LH pulses are large and infrequent, while FSH pulses are small and frequent (McLeod et al., 1984).

The pattern established for FSH secretion during the prepuberal period (Figure 3) shows that levels of FSH begin high and decrease with age. LH secretion also starts slightly higher but decreases during the pre-weaning period (day 6 through 124, Figure 4) and increases during the post-weaning period (days 245 through 424, Figure 4).

In an attempt to determine whether a given level of gonadotrophin secretion characterized a heifer throughout prepuberal life, calves in the 21 to 24 day age group were designated as either high or low secretors and their status was observed at varying ages thereafter. For FSH, eight heifers with the highest (H) level of initial secretion were compared to seven with the lowest (L) initial secretion. For LH, five of the highest were compared to

five of the lowest. The objective was to determine how the difference between H and L would change with age. There was a significant ($P<0.01$) difference between the overall H and L secretion profiles for FSH but not for LH. FSH levels in the plasma of H and L heifers differed significantly to about 100 days. The groups converged at 311 days and diverged again around puberty (382 days, Figure 5). Although different at 24 days, high and low secretors of LH were not distinguishable at any age thereafter (Figure 6), suggesting that LH secretion may not provide a useful means of identifying reproductive capacity.

The relative stability of FSH secretion suggests, however, that plasma levels of this hormone could have potential for indicating reproductive capacity.

Study 2--Response to an E_2 Challenge

The response of the hypothalamus and pituitary of the prepuberal heifer as reflected by plasma levels of FSH and LH to an E_2 challenge is shown in Figures 7 and 8. The values shown are the means at each postinjection interval for each age group. The effect of E_2 on LH secretion was significant ($P<0.01$) but it was not for FSH secretion. The animals' response to the E_2 challenge was categorized in three ways: (1) no response to the E_2 injection; 2) a detectable response which is defined as any LH or FSH level at the 14hr sampling time which was 2 or more times greater than the 8hr level but less than 10 ng/ml in the case of LH;

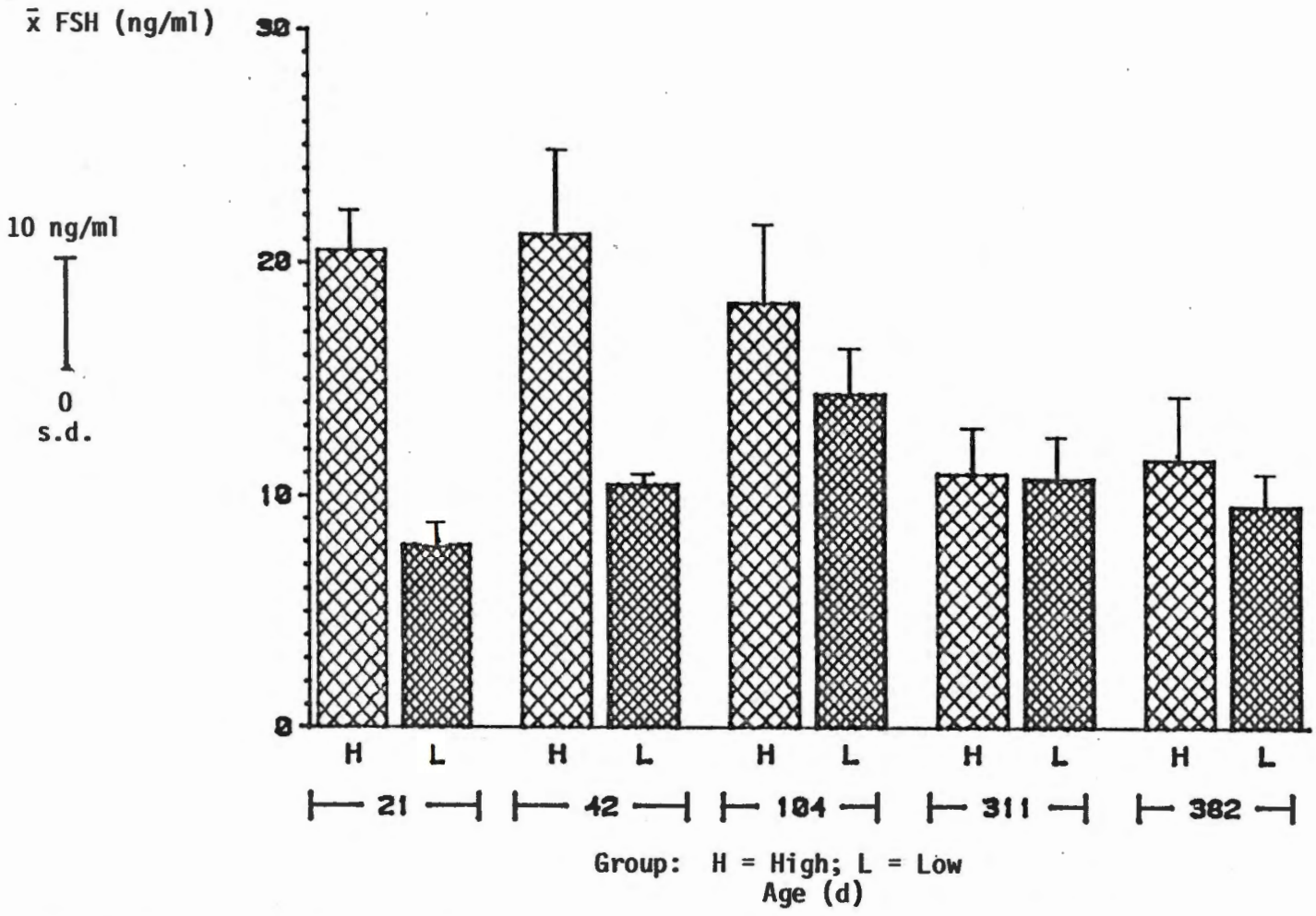

Figure 5. Effect of age on the magnitude of the difference between high and low secretors of FSH. (Note: High and low secretors of FSH were identified in the 21 day age group [N = 8 for high group and N = 7 for low group] and the effect of age on their status at succeeding ages was noted.)

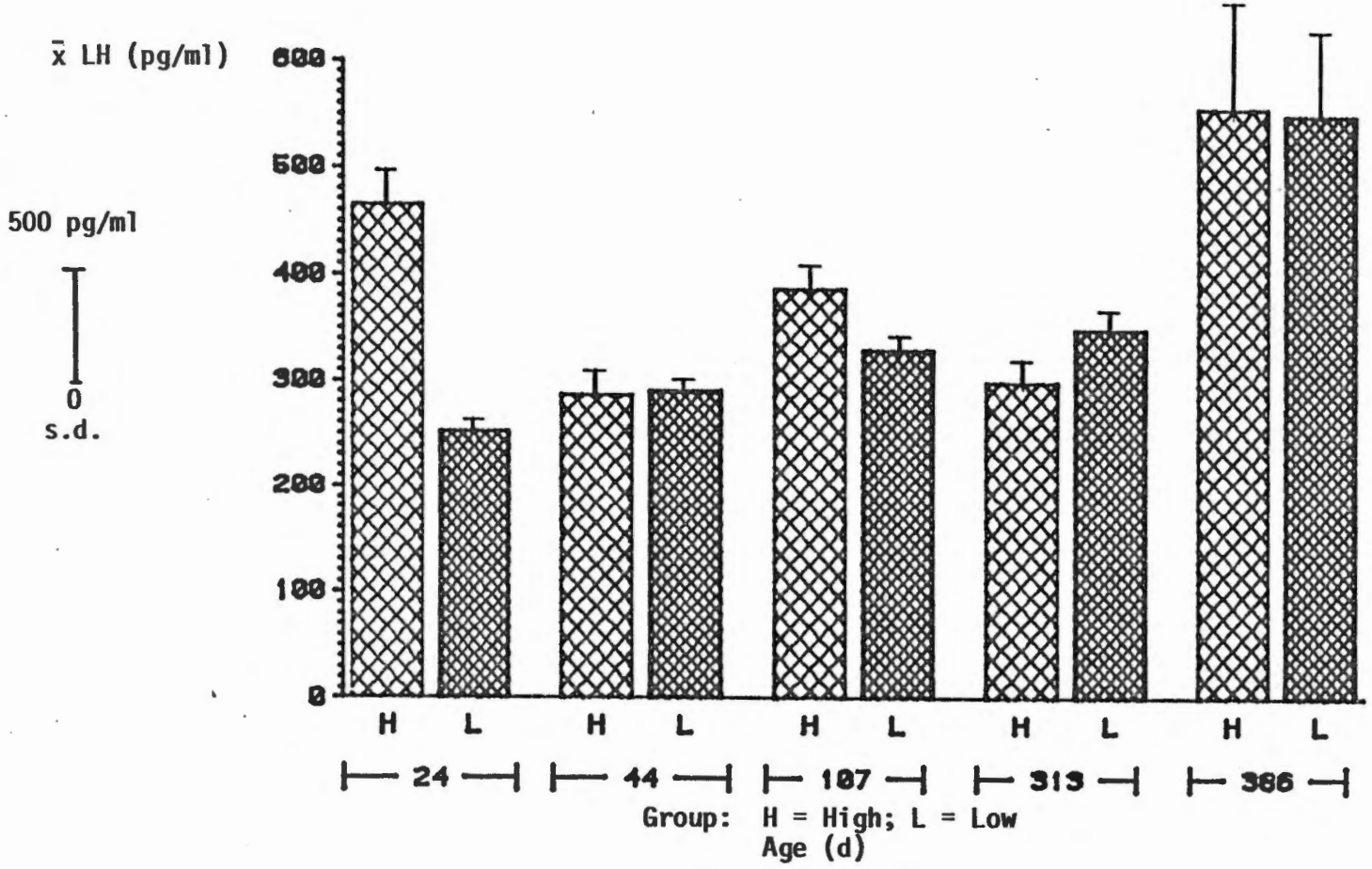

Figure 6. Effect on age on the magnitude of the difference between high and low secretors of LH. (Note: High and low secretors of LH were identified in the 24 day age group [N = 5 for high group and N = 5 for low group] and the effect of age on their status at succeeding ages was noted.)

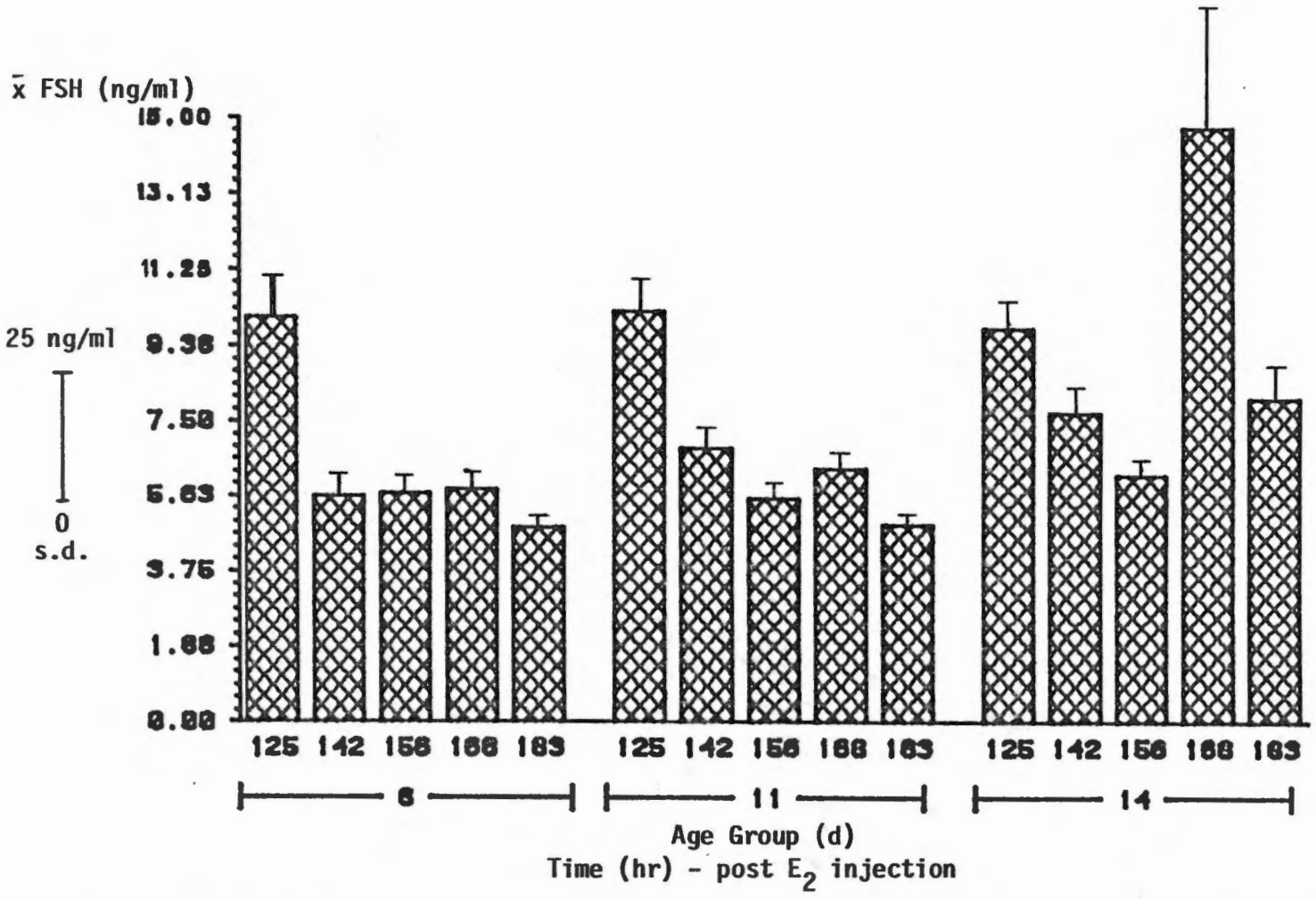

Figure 7. Response of the prepuberal heifer of varying ages to an E_2 challenge as reflected by FSH secretion.

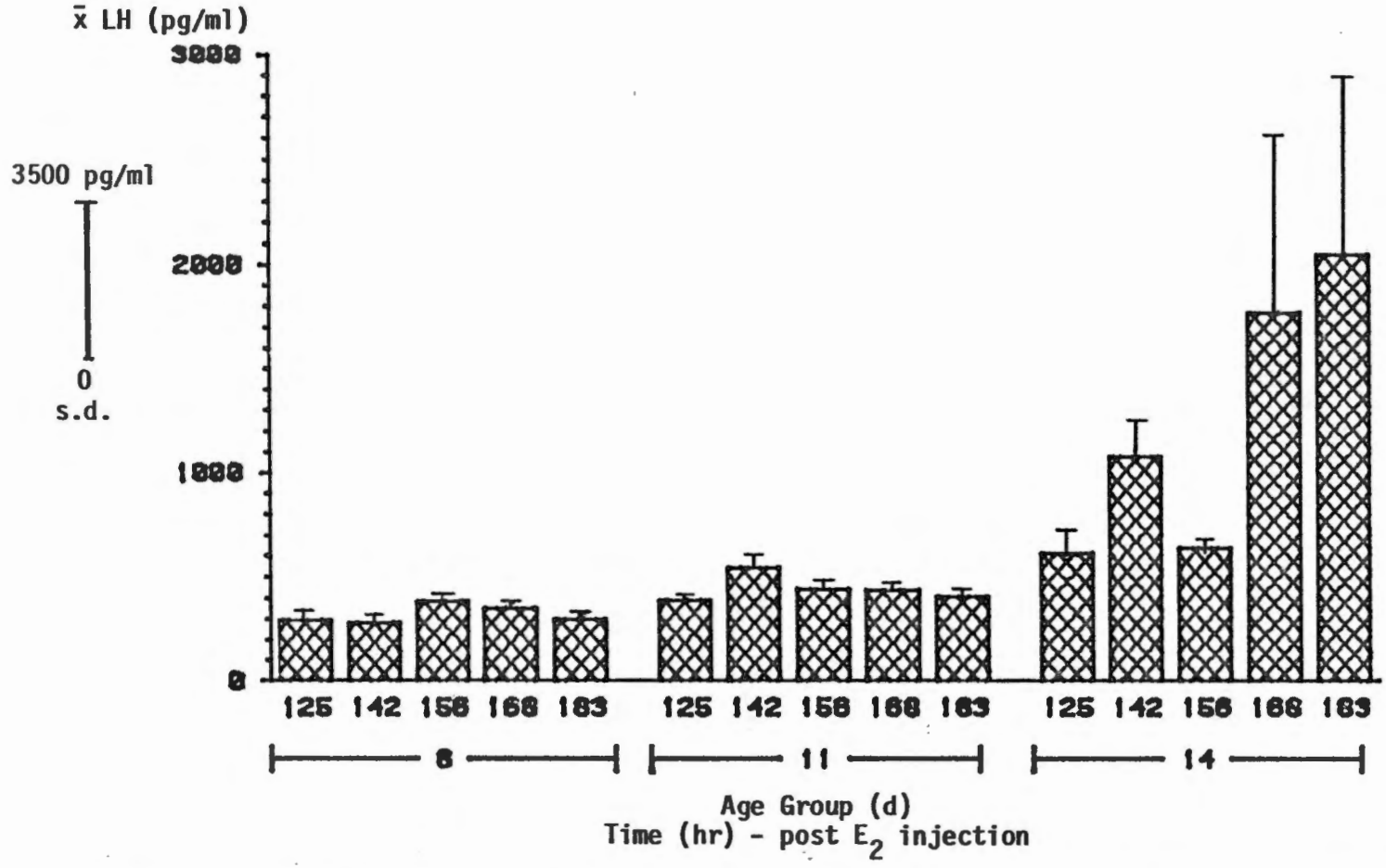

Figure 8. Response of the prepuberal heifer of varying ages to an E_2 challenge as reflected by LH secretion.

Table 3. Means and standard deviations of FSH and LH secretion in the prepuberal Hereford heifer at varying ages in response to an E_2 challenge (1 mg/intramuscular).

Group (x age/group)	FSH±s.d. (ng/ml) 8hr	11hr	14hr	LH±s.d. (pg/ml) 8hr	11hr	14hr	Responders/no. challenged FSH	LH
125±6	10±8	10±6	10±5	293±141	338±36	611±422	0/5	2/5
142±2	6±4	7±4	8±5	281±83	544±226	1079±1333	0/5	3/5
156±3	6±3	6±3	6±3	386±8	441±161	575±148	0/4	1/4
168±3	6±3	6±3	14±23	352±98	437±110	1673±3270	1*/8	3*/8
183±6	5±2	5±2	8±6	298±117	406±91	2049±3249	3*/16	9*/16

*One animal responded with a preovulatory-like FSH or LH surge.

responded within each group. Of the 38 heifers challenged with 1 mg of E_2, 20 exhibited no response, 16 responded positively and 2 responded with a preovulatory-like LH surge. The ages tested varied from 117 to 193 days. LH responses of greater magnitude occurred in heifers of 162 to 193 days of age. Four of the 38 heifers exhibited an FSH response. Ages of the FSH responders ranged from 162 to 193 days. Both heifers that exhibited an LH surge also exhibited a surge of FSH.

Study 3--The Relationship between Reproductive Performance, Prepuberal FSH and LH Levels and the Prepuberal E_2 Challenge

Of the 41 heifers used in the experiment, 19 conceived and calved within one estrous cycle of one another. Two failed to conceive and 20 were exposed to an infertile bull which made them ineligible for further study. An attempt was made to establish age at puberty by measuring the level of progesterone. A level of 1 ng/ml of plasma progesterone was used as the criteria for pubertal attainment (Glencross, 1984). Progesterone assays were completed courtesy of Dr. H. G. Kattesh, University of Tennessee. Only three of the heifers had attained puberty by the final post-weaning sample period. Ages of the heifers varied from 352 to 424 days ($\bar{x}$ = 399±20 days) at this time.

Heifers that calved (fertile) and heifers that were open (infertile) were compared as to their FSH and LH profiles. No significant differences ($P>0.10$) between the groups were observed in either the profile for FSH or LH. Table 4 shows the mean and standard deviation for each group

Table 4. Means and standard deviations for FSH and LH levels of fertile (1) and infertile (2) heifers at the various ages tested.

GRP	N	Age (d)	FSH±s.d. (ng/ml)	c.v. (%)	LH±s.d. (pg/ml)	c.v. (%)
1	19	31±8	13±5	39.2	338±129	38.0
2	2	32±13	9±2	17.7	339±81	23.8
1	19	109±10	14±3	21.5	385±49	12.6
2	2	111±18	12±4	30.6	347±102	29.3
1	19	352±41	11±4	35.9	440±190	43.2
2	2	355±44	10±4	36.5	474±238	50.2

of animals at each age tested. As revealed from the data in study 2, a comparison of the groups' response to an E_2 challenge also exhibited a notable difference. Table 5 shows the mean hormone response and the standard deviation of the response to an E_2 challenge. As can be seen in Table 5, the group 1 heifers (fertile) had a higher mean LH response to the E_2 challenge than the group 2 heifers (infertile). Eight of the 12 heifers in group 1 responded to the E_2 challenge whereas none of the heifers in group 2 responded. Although this difference was not significant ($P>0.10$), which was probably due to an insufficient number of animals, the data does suggest a meaningful difference might result from a more extensive study. There was no significant correlation between the basal levels of LH and the LH response to an E_2 challenge. There was neither a statistical or observable difference in the FSH response to an E_2 challenge between the groups (Table 5). None of the heifers responded with a preovulatory-like surge of LH or FSH.

There was no significant intergroup difference in the body weights ($P>0.10$). Weights were taken just prior to entry into the breeding herd. Group 1 heifers (fertile) at a mean age of 327±7 days weighed 214±5 kgs. Heifers in group 2 (infertile) at a mean age of 391±14 days weighed 214±13 kgs.

Table 5. Means and standard deviations of FSH and LH in the fertile (1) and infertile (2) heifer in response to an E_2 challenge (1 mg, intramuscular).

GRP	Responders/No. Challenged		$\bar{x}$ Prepuberal Profile		$\bar{x}$ All E_2 Samples		$\bar{x}$ Peak E_2 Response	
	FSH	LH	FSH (ng/ml)	LH (pg/ml)	FSH	LH	FSH	LH
1	0/12	8/12	12.6±1.2	388±42	6.2±2.8	569±630	7.3±3.3	983±974
2	0/2	0/2	10.3±1.2	387±62	5.8±3.3	387±57	7.7±5.9	420±104

NOTE: Ages of the heifers in group 1 at the time of the E_2 challenge ranged from 123 to 189 days and for group 2 168 and 193 days respectively.

NOTE: E_2 data was only available for 14 heifers.

CHAPTER V

DISCUSSION

Study 1--FSH and LH Levels in the Prepuberal Heifer

The pattern of prepuberal LH secretion followed that established by Dodson et al. (1985). Levels of FSH secretion, however, conflicted with the pattern described by these same workers. The level of FSH secretion in the present study was high at birth and decreased as the animal aged. Dodson et al. (1985) also showed that FSH secretion was at a high level at birth, which decreased through the succeeding 15 weeks, but increased during the remainder of the prepuberal period. The difference in the respective patterns of FSH secretion may be due to a difference in the ovarian endowment of antral follicles. Heifers in this study could have had more antral follicles which would result in higher inhibin production, thereby suppressing FSH release. This, however, is not a likely reason for the difference; since the heifers in this study were not well developed for their age (Table 2, p. 29). Retarded development is very likely to be associated with suppressed folliculogenesis since Foster and Olster (1985) found in sheep that undernutrition impaired hypothalamic function which restricted the LH pulses necessary for follicular development.

LH and FSH levels were lower (Figure 1, p. 27; Figure 2, p. 28) than those established by Dodson et al. (1985). At 7, 15 and 35 weeks of age the levels of FSH observed by Dodson and

associates were 33.3±4.2 ng/ml, 23.1±3.5 ng/ml and 37.8±3.5 ng/ml compared to 15.1±4.6 ng/ml, 14±3.3 ng/ml and 9.8±2.2 ng/ml found in this study. Their LH values at 3, 15 and 35 weeks were 960±90 pg/ml, 760±90 pg/ml and 1210±60 pg/ml compared to 329±45 pg/ml, 361±93 pg/ml and 386±82 pg/ml in this study. Several possible reasons exist for such a difference in gonadotrophin levels. The heifers of this study were underdeveloped, weighing only an average of 250±32 kgs at 399±20 days. As reported by Kirkpatrick (1980), a well developed heifer should weigh 318 kgs by this age. Breed differences in the level of gonadotrophin secretion are also likely. It has been shown in sheep that breeds differ widely in levels of LH secretion (Chiquette et al., 1984).

Differences in age at pubertal attainment were also seen, but this is not unusual. In cattle, age at puberty can vary from 11.5 to 19.5 months in various breed of Bos taurus and Bos indicus x Bos taurus heifers (Dow, Jr., et al., 1982). The animals used by Dodson et al. (1985) attained puberty at 314±12 days, whereas in the present study only 3 animals had attained puberty by 399±20 days. Average age at puberty for the Hereford heifer is 415 days (U.S.D.A., 1976). Puberty in this herd was likely delayed due to undernutrition which is also a possible cause for lower gonadotrophin secretion. As shown by Grass et al. (1982), heifers fed low energy diets attained puberty later than those fed a high energy diet. The stress of undernutrition is, therefore, probably the main reason for the low level of gonadotrophin secretion and the delay of puberty in the heifers in this study.

Stress induced by shock caused an increased release of β-endorphin in rats (Akil et al., 1984). Opiates are known to suppress gonadotrophin secretion by blocking the release of GnRH (Sirinathsinghji et al., 1985; Piva et al., 1985). Undernutrition may also cause an increase in the release of β-endorphin which would suppress gonadotrophin secretion. Delayed puberty would result from the reduced levels of gonadotrophin secretion.

Results from the comparison of high (H) and low (L) secretions of FSH and LH indicate that FSH may be useful for predicting reproductive performance. Significant differences ($P<0.01$) in FSH concentration can be seen between H and L groups out to about 100 days (Figure 5, p. 34). After 100 days FSH begins to decrease and the H and L patterns become indistinguishable but diverge again around puberty (Figure 5). This decrease in FSH is probably due to the increase in follicular growth which occurs at about 60-80 days in the heifer (Erickson, 1966). Increased follicular growth would result in increased release of inhibin (Erickson and Hsueh, 1978) which suppresses FSH secretion (Ireland et al., 1983). Two of the heifers which had high initial levels of FSH also had high initial levels of LH. Three of the heifers exhibited both low FSH and LH levels of initial secretion. None of the high FSH secretors were in the low LH group but one high LH secretor was in the low FSH group.

If FSH is to be useful for predicting reproductive performance, samples must be taken at 100 days or less and possibly around puberty. It may also be beneficial to take a sample at 100 to 120 days to see

if levels are decreasing in order to establish the occurrence of follicular growth.

Differences between H and L secretors of LH were not consistent and LH secretion may, therefore, not be useful for predicting reproductive performance (Figure 6, p. 35).

Study 2--Response to an E_2 Challenge

In mammals the preovulatory gonadotrophin surge is caused by the "positive feedback" of E_2 on the hypothalamus and pituitary. The development of the positive feedback mechanism within the hypothalamus is dependent upon ovarian maturation and associated E_2 production (Foxcroft et al., 1984). Since undernutrition delays puberty (Foster and Olster, 1985; Grass et al., 1982), maturation of the "positive feedback" response is also likely to be delayed.

Overall, the responsiveness of the heifers in this study to an E_2 challenge was low. All of the animals, 3 out of 3, 5 to 6 months of age used by Schillo et al. (1983) responded with a preovulatory-like LH surge following a dose of 500 ug of E_2. This compares to only 2 out of 30 animals of the same age given 1 mg of E_2 per animal in the present study. The magnitude of the preovulatory LH surge was also much lower in these heifers when compared to those used by Schillo et al. (1983) (10.9±0.55 ng/ml vs. 108±29.6 ng/ml). None of the animals below 5 months of age responded with an LH surge. This coincides with data provided by Schillo et al. (1983) showing that only 1 in 5 heifers 3 to 4 months of age responded with a preovulatory LH surge. Sixteen of the 38 animals tested at ages ranging from 117 to 193 days

responded positively to the E_2 challenge, thereby providing evidence of positive-feedback response to the exogenous dose of E_2. Ages of the positive responders were evenly distributed across the range of ages tested (Table 3, p. 38). However, the magnitude of the LH response was greater in animals 162 to 193 days of age.

There was also evidence of a positive FSH response to the E_2 challenge. Regarding previous studies on the prepuberal gonadotrophin surge, FSH has been neglected; however, it is known that FSH release is also influenced by the positive feedback effect of E_2 (Karsch, 1984). The neglect to study the FSH surge is most likely because of the passive role of FSH during the preovulatory surge. LH is the hormone of major concern because it is responsible for ovulation. The primary role of FSH is the promotion of follicular growth. As observed in this study, FSH release in response to E_2 is not as great as is LH in the prepuberal animal. Of the 38 heifers challenged with E_2, 4 responded positively at ages ranging from 162 to 193 days (Table 3). Two of the responders were the same ones that exhibited a preovulatory-like LH surge. Although it is not known what level constitutes a preovulatory FSH surge in the prepuberal bovine, it is possible that 2 of the 4 heifers responded with a preovulatory-like surge. The level of FSH increased 21x and 6x the baseline sample respectively in each of these 2 heifers. In the mature bovine, Rzepkowski et al. (1982) show that the FSH surge increases to about 3.5x the basal level. There was no FSH response to the E_2 challenge below 162 days (Table 3). Before puberty, FSH

secretion seems not as sensitive as LH secretion to the positive-feedback of E_2.

One possible reason for the low response of FSH is that inhibin which is produced by the growing follicles may be blocking the release of FSH. The hypothalamus may not be releasing enough GnRH to override the effects of inhibin. Another possibility is that the gonadotrophs of the pituitary may not have enough FSH stored before puberty to allow a surge.

As was the case with tonic levels of gonadotrophin secretion, the lack of a larger number of animals responding to the E_2 challenge was probably caused from underdevelopment. As discussed earlier, the stress caused by underdevelopment may have caused an increased release of β-endorphin which blocks the release of GnRH from the hypothalamus (Sirnathsinghji et al., 1985; Piva et al., 1985). Another possible reason for the limited surge response is that the hypothalamus could have been at a lesser stage of maturity (Foxcroft et al., 1984). Furthermore, there may have been a difference in the time at which the peak response occurred after the injection of E_2. As shown by Schillo et al. (1983), the peak of the preovulatory-like surge during prepuberty occurs at 18 hrs in heifers 3 to 4 months old and 14 hrs in heifers 5 to 6 months old. Duration of the peak lasted from 8 to 10 hrs. In future studies, an 18 and 20 hr post injection sample should be taken to confirm the presence or absence of a surge.

In conclusion, the overall low number of responders to the E_2 challenge suggests that the ovaries and hypothalami were underdeveloped.

Study 3--The Relationship between Reproductive Performance, Prepuberal FSH and LH Levels and the E_2 Challenge

A potentially important outcome of this study is the positive correlation between levels of FSH and fertility in the heifer. Although there was no statistical differences in either LH or FSH between the fertile and infertile heifers, as a probable result of the low number of animals studied, a notable difference can be seen for FSH. The heifers that calved had a higher level of FSH at the three ages tested than those that were open. This result is similar to that found in sheep. FSH levels were highest in sheep with higher fecundity (Findlay and Bindon, 1976; Lahlou-Kassi et al., 1984; Bindon et al., 1985). This is further supported by McNatty et al. (1985), showing that FSH is influential to follicle viability and ovulation rate in sheep. The fact that LH was not different between the fertile and infertile heifers coincided with evidence provided by Eckternkamp and Laster (1976) in sheep. They found that LH concentration was not a good indicator of high fecundity due to its high variability. LH secretion was also more variable than FSH in the heifer (Table 4, p. 40). Again, due both to a low number of responders and a low number of animals studied, differences between the levels of the FSH and LH response to an E_2 challenge in the fertile and infertile heifers were not statistically different. The results, however, do suggest that the heifers that calved had a higher level of LH output following the E_2 challenge than the heifers that were infertile (Table 5, p. 42).

Following the injection of E_2, there was no observable difference in FSH response between the fertile and infertile heifers (Table 5, p. 42).

Results of this study suggest that it should be followed by a more thorough investigation that will allow the study of the attributes of a greater number of fertile and infertile heifers. And, as to the use of gonadotrophin levels as an index to be used in selecting replacement heifers, it was encouraging to note that the average FSH levels of fertile heifers was higher than that of the infertile heifers at all ages and that a consistent level of FSH secretion characterizes a heifer for the first 100 days of her prepuberal life.

It is also worthy of note that the FSH output (9.2±1.6 ng/ml) of the two infertile heifers was very low at birth, but since the FSH secretion of one of the fertile heifers was also low (8.45 ng/ml), the relationship of fecundity to FSH level in the bovine requires further study.

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