MONTANA STATE UNIVERSITY

The relationship between androgen levels, LH levels and puberty in bull calves
by Gary Eugene Moss

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE In Animal Science
Montana State University
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Abstract:
Eight crossbred bulls, 7-13 months of age, were bled weekly by jugular venipuncture for 6 months. The plasma was removed and frozen until assayed following centrifugation. Plasma LH levels were determined by double antibody RIA and plasma testosterone levels were determined by RIA without chromatography. The plasma testosterone binding ability was determined by saturation of the plasma with testosterone and removal of the unbound testosterone with florisil. Results were analyzed collectively through the 6 month period and with puberty defined as week 0. The differences between the 4 Beef x Beef crossbred bulls and 4 Dairy x Beef crossbred bulls were also examined. Individual LH levels varied erratically and ranged from nondetectable levels to a peak of 7.45 ng/ml of plasma in Bull 130 seven weeks after puberty. LH levels for the 8 bulls collectively over the 6-month period had a mean of 1.9+l.33 ng/ml of plasma and increased linearly (P<.05) . LH levels after puberty increased quadratically (P<.05) in all 8 bulls and linearly in the beef bulls (P<.01), Testosterone levels ranged from nondetectable levels to .2351 pg/ml of plasma in Bull 107. Testosterone levels collectively over the 6-month period had a mean of 438.271+339.27 pg/ml of plasma and changed quadratically (P<.05). No regression was significant before or after puberty collectively or in the beef bull and dairy bull groups. Levels of testosterone bound/ml of plasma ranged from 1.3-4.31 ng/ml of plasma. Plasma testosterone binding ability increased linearly in puberty (P<.05) but followed no regression significantly after puberty. In addition, the plasma testosterone binding ability increased linearly both before and after puberty in the dairy bull group (P<.05). Collective LH levels over the 6-month period and before puberty were positively correlated (P<.01) with the plasma testosterone binding ability. LH and plasma testosterone binding were not correlated after puberty. In the dairy and beef bull groups, LH was positively correlated (P <.01 and P<.05, respectively), with testosterone binding before puberty. LH and testosterone levels were negatively correlated (P<.Ol) over the entire 6-month period and before puberty (P<.Ol). Beef bull prepubertal LH levels were positively correlated (P<.05) before puberty and dairy bull postpubertal LH and testosterone levels were negatively correlated (P<.01). Testosterone and plasma testosterone binding ability were not significantly correlated at any time.

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Signature Gary E. Moss

Date December 29, 1972

THE RELATIONSHIP BETWEEN ANDROGEN LEVELS, LH LEVELS AND PUBERTY IN BULL CALVES

by

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

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in

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ABSTRACT

Eight crossbred bulls, 7-13 months of age, were bled weekly by jugular venipuncture for 6 months. The plasma was removed and frozen until assayed following centrifugation. Plasma LH levels were determined by double antibody RIA and plasma testosterone levels were determined by RIA without chromatography. The plasma testosterone binding ability was determined by saturation of the plasma with testosterone and removal of the unbound testosterone with florisil. Results were analyzed collectively through the 6 month period and with puberty defined as week 0. The differences between the 4 Beef x Beef crossbred bulls and 4 Dairy x Beef crossbred bulls were also examined. Individual LH levels varied erratically and ranged from nondetectable levels to a peak of 7.46 ng/ml of plasma in Bull 130 seven weeks after puberty. LH levels for the 8 bulls collectively over the 6-month period had a mean of 1.9 ± 1.33 ng/ml of plasma and increased linearly ($P<.05$). LH levels after puberty increased quadratically ($P<.05$) in all 8 bulls and linearly in the beef bulls ($P<.01$). Testosterone levels ranged from nondetectable levels to 2351 pg/ml of plasma in Bull 107. Testosterone levels collectively over the 6-month period had a mean of 438.27 ± 339.27 pg/ml of plasma and changed quadratically ($P<.05$). No regression was significant before or after puberty collectively or in the beef bull and dairy bull groups. Levels of testosterone bound/ml of plasma ranged from 1.3-4.31 ng/ml of plasma. Plasma testosterone binding ability increased linearly in puberty ($P<.05$) but followed no regression significantly after puberty. In addition, the plasma testosterone binding ability increased linearly both before and after puberty in the dairy bull group ($P<.05$). Collective LH levels over the 6-month period and before puberty were positively correlated ($P<.01$) with the plasma testosterone binding ability. LH and plasma testosterone binding were not correlated after puberty. In the dairy and beef bull groups, LH was positively correlated ($P<.01$ and $P<.05$, respectively), with testosterone binding before puberty. LH and testosterone levels were negatively correlated ($P<.01$) over the entire 6-month period and before puberty ($P<.01$). Beef bull prepubertal LH levels were positively correlated ($P<.05$) before puberty and dairy bull postpubertal LH and testosterone levels were negatively correlated ($P<.01$). Testosterone and plasma testosterone binding ability were not significantly correlated at any time.

INTRODUCTION

The many hormonal and neural interrelationships which lead to the initiation of puberty are poorly understood. An understanding of these relationships has been greatly enhanced by the recent development of RIA techniques which are sensitive enough to detect very minute changes in hormone levels. With the advent of precise and sensitive techniques, the mechanism that initiates puberty has been receiving considerable attention.

The events leading to the initiation of puberty in males and females, as well as the events which stimulate the preovulatory LH surge, appear to be similar. An understanding of these events might lead one to conject practical applications such as; by controlling the preovulatory LH surge fertility could be affected, puberty could be hastened in heifers so they could be bred at a younger age, and bulls could be progeny tested earlier. However, a basic understanding of the mechanisms involved must be obtained before final applications are planned.

This experiment was initiated to determine the levels and relationships of plasma LH, plasma testosterone, and the plasma testosterone binding ability in bulls through puberty. Eight bulls, 7-13 months of age, were bled weekly by jugular venipuncture and the plasma was removed and frozen after centrifugation. The plasma LH and testosterone levels were determined by RIA and the plasma testosterone binding ability was determined by a simple saturation technique.

REVIEW OF LITERATURE

Mechanism of Puberty

Kragt and Masken (1972) reviewed the events associated with puberty. They stated that "a neural signal, which is mediated by hypothalmic amines, initiates the events leading to puberty by increasing the release of FSH-RH to induce the release and synthesis of FSH". They further postulated that FSH sensitizes the gonads to LH by activating the enzyme responsible for the conversion of androstenedione to testosterone, and in males LH "potentiates" the testosterone production by providing additional progesterone precursor, possibly pregnenolone.

The preovulatory LH surge observed in females appears to be stimulated by a mechanism similar to that which initiates puberty in males and females (Kragt and Masken, 1972). Hopkins and Pincus (1963) demonstrated that reserpine, a drug which has a tranquilizing effect on the hypothalmus, could decrease the ability of exogenous gonadotropins (PMS and HCG) to stimulate ovulation in rats. A single injection of reserpine given one minute prior to HCG administration will completely block ovulation. Hopkins and Clayton-Hopkins (1970) demonstrated that factors which enhance the action of gonadotropins can be extracted from cerebral cortical tissues in rats.

The Pineal has long been suspected of producing substances which delay reproductive development (Kitay and Altschule, 1954). Pinealectomy in male rats does not change pituitary weight, but testicular, prostate, and seminal vesicular weights are increased 12 days after the

operation. This observation suggests that the Pineal retards the release of FSH and LH (Martini, Fraschine, and Motta, 1968). Systemic administration of melatonin, which is produced by the Pineal, causes the prostate and seminal vesicles to atrophy slightly. In immature female rats, melatonin administration retards vaginal opening and causes a decrease in ovarian weight (Wurtman, Axelrod, and Kelly, 1968). Melatonin implants in the median eminance supressed LH release (Martini, Fraschine, and Motta, 1968). Peat and Kenson (1971) demonstrated that melatonin can act directly on steroidogenesis, apparently by suppressing the production of testosterone and androstenedione from cholesterol and pregnenolone. Serotonin, a melatonin precursor, has effects similar to melatonin on rats and mice. A biphasic response is seen to both melatonin and serotonin, suggesting multiple sites of action (Kragt and Masken, 1972). Martini, Fraschine, and Motta (1968) suggest that these factors exert a central nervous system control as well as a direct effect.

Turner (1967) stated that testosterone begins to differentiate the hypothalmus of a male rat within 5 days after birth; then the male hypothalmus is characterized by a steady secretion of LH. Siperstein et al. (1954) observed, morphologically, an increased granulation in the male pituitary as the animal matures, whereas there is a decreased granulation and glycoprotein content as puberty approaches in the female rat. Kragt and Masken (1972) stated that the cells of the anterior pituitary of female rats possess all the necessary components to store and synthesize

FSH and LH as early as 5-10 days of age, however, this ability decreases at about 20 days of age. The male rat develops this storage and synthesizing ability about 10 days later than the female, but this ability is retained throughout his lifetime.

Kragt and Masken (1972) observed slightly elevated levels of both FSH and LH in the immature male rat at 10-20 days of age. However, these levels never reached those of the 15-day-old female. At 20-40 days of age, FSH and LH are both low, but near the onset of spermatogenesis at 40 days, FSH increases three fold and LH less than two times.

The changes in pituitary FSH, in female gilts, which occur at an earlier age than LH, led Hollandbeck (1956) to speculate that "the greater potencies of prepubertal gilt pituitaries were due to relatively greater levels of FSH than LH and that the initiation of cyclic activity at puberty was the result of increased LH secretion which gave a more functionally balanced FSH/LH ratio". Hoogstra and Paesi (1955) also suggested a shift in the FSH/LH ratio at puberty and at the ovulatory surge in the adult female rat.

Means and Hall (1969) demonstrated that exogenous FSH stimulated testicular protein biosynthesis in rats. Ramirez (1970) observed a decrease in pituitary FSH at puberty and a positive relationship between plasma FSH and testicular weight in rats. Swanson et al. (1971) reported a negative correlation between pituitary concentration of FSH and gonadal sperm numbers in Hereford bulls one year of age. Pituitary FSH was the

greatest at 5-6 months of age. They hypothesized that the "retarded release of FSH may limit puberty, therefore, FSH may play a more important role in determining the onset of puberty in the male". Yuhara, Cohen and Frieden (1963) demonstrated that the in vitro addition of FSH to slices of rat ovaries prompts the release of estrogen and androstenedione; thus FSH may also promote testosterone formation. Turner (1967) stated that "LH functions to actuate the interstitial cells of the testes to cause androgen production". In both male and female rats FSH release is elevated just prior to LH release, and in the male rat this LH release coincides with the secretion of testosterone (Knorr, Vanka-Perttula, and Lipsett, 1970). At this time, a shift occurs in the androstenedione:testosterone secretion ratio in rats and bulls (Inano, Hori and Tamaski, 1967, Lunder and Mann, 1960, Rawlings, Hafs and Swanson, 1972). Swanson et al. (1971) found no difference in pituitary LH with increasing age, however, serum LH increased with age until 10-12 months then dropped at 12.5 months. McMillan and Hafs (1968) reported LH levels in bulls to be biphasic, there was no change from 0-2 months, an increase at 4 months and another at 6-10 months of age.

Kragt and Masken (1972) feel that LH release immediately after FSH secretion may increase testosterone production by increasing cholesterol conversion to progesterone. Oshima (1967) suggested that LH acts to stimulate the hydroxylation of the 17th carbon of androstenedione through

action on 17-hydrosteroid dehydrogenase. Swanson et al. (1971) reported that serum testosterone in bulls decreases slightly from 10-12 months of age, but androstenedione decreases proportionally greater. Near the onset of spermatogenesis, at about 5 months of age, the testosterone: androstenedione ratio changes. Testosterone:androstenedione at 4 months of age was 1:1 and changed to 10:1 at 9 months of age. Strickland (1970) states "the high levels of androstenedione in the testes and plasma of bulls less than 6 months of age provide enough substrate for testosterone synthesis; but testosterone becomes the prominent androgen only after approximately 5 months of age". This suggests that the elevated release of LH caused elevated testosterone secretion. Rawlings, Hafs, and Swanson (1972) and Swanson et al. (1971) demonstrated that little relationship can be shown between plasma LH and testicular testosterone concentration until the bull reaches about 9 months of age. They interpret this to suggest that LH maintains, rather than initiates androgen secretion.

Rawlings, Hafs, and Swanson (1972) reported a negative correlation between plasma testosterone and testicular testosterone from 4-6 months of age in the bull; and a positive correlation thereafter. Plasma testosterone was positively correlated with vesicular fructose concentration and testicular sperm concentration, but not with sperm numbers.

Prolactin secretion has been shown to affect steroidogenesis, and the onset of puberty, by inhibiting the conversion of progesterone to

20α-hydroxyprogesterone, thus promoting sex steroid formation. Swanson et al. (1971) found a negative correlation between pituitary prolactin and vesicular fructose and no significant correlation between serum prolactin and serum androgen. This was interpreted to demonstrate that prolactin also stimulates the seminal vesicles independently of the testes.

McMillan and Hafs (1968), upon examination of puberty versus increase in body weight with increasing age, concluded that puberty is not associated with any marked changes in rate of body growth. Anterior pituitary weight increased linearly from birth to 6 months of age, then it decreased from .96 g at 6 months to .8 g at 7 months. An increase to 1.24 g occurred at 9 months of age, then little change occurred from 9-12 months of age. This decline in weight of the anterior pituitary suggests that the anterior pituitary may become more sensitive to increasing levels of androgen at this time, when testosterone becomes the dominant androgen (Swanson et al. 1971).

Testosterone--Mode of Action

Testosterone and androgens in general, are steroids produced primarily by the testes of the male (Hafez, 1968). Testosterone is also synthesized peripherally from such steroids as epiandrosterone sulfate and androstenedione (Lipsett et al. 1966).

Testosterone is not stored to an appreciable amount, but is rapidly degraded and excreted through the urine and feces. The

principle : urinary products being androsterone, epiandosterone and eticholanolone (Hafez, 1968).

Testosterone synthesized peripheally may be conjugated before it mixes with the plasma testosterone. In this case some of the urinary testosterone glucoronide will be derived from an area not in equilibrium with the plasma testosterone; thereby yielding erroneous high values of plasma testosterone when it is attempted to calculate plasma testosterone levels from urinary products (Lipsett et al., 1966). Siiteri, Vande Wiele, and Lieberman (1963) demonstrated that dehydoisoandrosterone gluronoside is derived not only from dehydroisoandrosterone but is also secreted by the adrenals.

The general modes of action of testosterone are: To stimulate spermatogenesis in hypophysectiomized animals and hastened the onset of spermatogenesis in seasonal breeders, prolong the life span of epididymal sperm; promote growth, development and secretory activity of the secondary sex organs, stimulate sexual behavior, and induce nitrogen retention (protein anabolic activity) (Hafez, 1968).

Baulieu, Raynaud and Milgrom (1970) suggest that testosterone is metabolized to dihydroxytestosterone (DHT) in rat prostate tissue. Both DHT and testosterone are active regarding increased height, secretory activity, hyperplasia of the epithelial cells. Bashrelahi and Villee (1970) demonstrated that DHT incubated for 30 minutes with isolated nuclei of castrated rat prostates caused an increased incorporation of uridine

in the nucleus. Testosterone had no effect on prostatic nuclei. This was presented as evidence for DHT being the active form of testosterone in the rat prostate nuclei.

Lang (1971), in a review of the cellular action of steroids in general, suggests that steroid hormones bring about their expression of affecting protein synthesis at the transcriptional or translational level to induce enzyme synthesis.

Cortisol, a steroid hormone which has been used as a model for the action of steroid hormones in general, causes an increase in the appearance of the liver enzyme, tryptophan pyrrolase. Feigelson (1962) demonstrated that the increase in enzyme activity was due to an increase in the net amount of enzyme protein molecules. Radioactive cortisol has been shown to be present in the nucleus within a few minutes after interperitionial administration, suggesting that cortisol acts at the nuclear level (as reviewed by Lang, 1971).

Ecdysone, a developmental steroid hormone in the blowfly, Calliphora erythrocephalia, is the best known example of hormone action on enzyme induction at the nuclear level (Lang, 1971). Briefly, ecdysone brings about the synthesis of a new enzyme, dopadecarboxylase, which opens the pathway for n-acetyldopamine synthesis from dopamine. N-acetyldopamine is the precursor of the melon pigment, or tanning agent, which causes the formation of the hard, colored cuticles of the pupae. Injections of ecdysone into the larvae of the midge, Cheronomus tentans, causes

puffs on certain gene loci of giant chromosomes. These puffs indicate an active gene structure where RNA and protein synthesis takes place. This leads to the synthesis of a specific mRNA which is released into the cytoplasm to serve as a template for specific protein synthesis (reviewed by Lang, 1971).

Lang (1971) further hypothesized that steroid hormones may act at a post-transcriptional level by antagonizing a cytoplasmic receptor which is assumed to be a protein. Evidence for this was shown with cortisol, which induces the production of tyrosine transaminase. When RNA synthesis was inhibited with actinomycin D after full induction has occurred, enzyme formation continues at the induced rate. Lang (1971) explained this by dividing the enzyme formation into two phases, inducible and non-inducible phase. During the inducible phase the structural gene produces a repressor which inhibits the translation of mRNA into the enzyme and promotes its degradation. In the presence of inducer, cortisol in this case, the repressor is inactivated thus allowing an increase in mRNA which leads to enzyme formation. The non-inducible phase is characterized by basal enzyme synthesis enabled by a stable mRNA without control by the genes which are repressed by a process insensitive to the inducer. Means and O'Malley (1971) state the "testosterone has a primary nuclear site of action after the steroid complexes with a cytoplasmic "receptor" protein. Interaction of the hormone-receptor complex with the gene causes an increase in RNA synthesis which codes

for the translation of a new specific protein".

Farnsworth (1967) noted that testosterone stimulated the biosynthesis of citrate from radio-labeled sodium acetate in minces of rat ventral prostates, enhanced the production of free fatty acids, lactate, and accelerated the incorporation of amino acids into proteins. Lang (1971) interpreted this to mean that the increased synthetic activity in response to testosterone was a result of decreased citric acid content, which would mobilize acetyl coenzyme A precursors to return citrate concentration to normal. The energy released from the increased rate of glycolysis would then be available for the synthesis of lipids, proteins and nucleic acids. Lostroh and Johnson (1966) demonstrated an increased production of citrate in response to testosterone in rat ventral prostates, however, they observed that insulin could maintain the level of citrate secretion for several days and is necessary for a net increase in the synthesis of protein by prostatic tissue. This suggests a passive role for insulin in the action of androgens on prostatic tissue (Lang, 1971).

Farnsworth (1971) demonstrated that testosterone also affects tissue uptake of potassium into minces of rat ventral prostate, suggesting a steroid specific increased activation of sodium and potassium dependent ATPase of the isolated rat ventral prostate microsomes occurs. This reaction is a membrane ATPase reaction that occurs in two steps. The first, a sodium dependent phosphorylation of the membrane by ATP

to form a phosphoprotein, is unaffected by testosterone. Secondly, a potassium dependent cleavage of the externalized phosphoprotein occurs and is specifically stimulated by testosterone. Thus, Farnsworth (1971) postulated that prostate membranes may be a primary receptor site for the action of androgenic steroids.

Breuer and Florine (1966) demonstrated that testosterone propionate affects the template activity of DNA. Single injections of testosterone propionate were given to femoral muscles of hormone deficient rats with a subsequent stimulation of the activity of RNA polymerase. There was a considerable lag period prior to the effects observed on RNA synthesis indication that the primary response of muscle to testosterone propionate may involve other sites in the cell. Potter (1964) suggested that several steroid hormones act by converting inactive DNA to active DNA. The inactive form of DNA is bound by histones which act as a repressor (Bonner, 1965). He said that the hormones may remove the histones from the DNA by combining with them and thus rendering the DNA active. He postulated that the hormone combines with an unidentified protein (X) to form protein (Y), protein Y then becomes the stripping agent.

Plasma Testosterone Binding Proteins

Mercier, Alfsen, and Baulieu presented evidence for a sex steroid binding protein in 1966 (Mercier et al., 1970). Pearlman and Crepy (1967) demonstrated the existence of a plasma testosterone binding protein by illustrating a greater affinity for testosterone than albumin or

Sephadex dialysis, the latter technique being used to separate unbound and bound steroids.

Baulieu, Raynaud, and Milgrom (1970) discussed the binding proteins found in plasma. They are: corticosteroid binding globulin (CBG), which tightly binds cortisol, corticosterone, progesterone, 17α-hydroxy-progesterone and testosterone and aldosterone to a lesser extent; sex steroid binding plasma protein (SBP) which has high affinity for estradiol and testosterone but also binds 17-β-hydroxy-5α androstane steroids; and albumin which has non-specific low affinity bindings. Baulieu, Raynaud and Milgrom (1970) suggested that plasma binding proteins are important as a reservoir or buffer system for the active unbound steroids. A role in the transport of steroids was also suggested for the passage of hormones from their tissues of origin into the blood or the transport of steroids into their target issues.

Murphy (1968) stated that SBP or testosterone Binding Globulin (TeBG) is a β-globulin which increases during pregnancy and cirrhosis. She suggested that the binding of estradiol and testosterone occurs on a common site on the globulin and that it could be used for a radioassay for both hormones. Florisil was used to separate the protein-bound and unbound steroids and by means of a competitive protein binding assay approach she achieved results similar to equilibrium dialysis and Sephadex filtration. Murphy (1968) stated that estradiol was 68 percent as effective as testosterone in binding TeBG. Other estrogens

and androgens had smaller effects while the competition of cholesterol for binding was only 0.14 percent that of testosterone.

Rosenfield (1971) tested the relationship of plasma-free testosterone levels and TeBG levels in human hirsute patients. He demonstrated that hirsutism was associated with elevated levels of either plasma-free testosterone, low TeBG levels, or elevated 17 -hydroxysteroid levels which would compete with testosterone for TeBG binding sites.

Ramaley (1971) studied the binding of estradiol-6,7-^{3}H, 17 -progesterone-4-C^{14}, testosterone-4-C^{14}, and androsterone 1-2-^{3}H to serum proteins in prepubertal male and female rats by equilibrium dialysis. The onset of puberty occurred at 38±1.4 days and at 61±3.4 days in females and males, respectively. More estrogen was bound in male plasma than testosterone at any time except at day 60, when they were about equal. Testosterone increased very slightly from days 52-60, then declined to its previous plateau level. Estradiol increased and remained high. Progesterone binding until day 60 was intermediate to estradiol and testosterone, at day 60 progesterone binding levels increased and remained elevated. Androsterone binding did not change with increasing age. Castration at weaning did not change the pattern of estradiol or testosterone binding, but an increase was seen in testosterone binding as the animal matured until day 68, when testosterone binding was higher in intact male rats. Progesterone and androsterone binding were both increased by castration.

Testosterone Assay

Testosterone production has been determined in many ways, biologically and chemically. The most common biological assays utilize observations of the effects of systemic or direct administration of testosterone on the combs of cockerals or capons. An increase in comb weight is used as an index for the quantity of testosterone present. Other biological assays include observation of the effects of testosterone administration on prostate or vesicular glands in immature or castrated rats and mice (Hafez, 1968).

The first attempt to determine plasma testosterone levels utilized the urinary isotope dilution technique (Korenman, Wilson, and Lipsett, 1963). This method, which was previously used for aldosterone and estradiol, depends upon the presence of a unique urinary metabolite from the unique plasma pool in question. It was assumed that testosterone glucuronide was a unique metabolite of testosterone, an assumption which was proven incorrect and resulted in an overestimation of plasma testosterone (Korenman and Lipsett, 1964). This led to the development of various methods to determine plasma testosterone levels directly.

Finkelstein, Farchielli and Dorfman (1961) developed one of the earliest assays for plasma testosterone based on the enzymatic conversion of testosterone to estradiol-17β and estrone. The latter, estrone and estradiol-17β, are then measured by fluorometric techniques. The

sensitivity of this method is approximately .1 ug/100 ml plasma. Riondel et al. (1963) developed a double isotope derivative method to determine plasma testosterone levels in 10 ml plasma. This method used S^{35} thiosemicarbazide as the derivative forming agent and testosterone-1,2^{3}-H as an indicator. Overall recovery for this method was 5 percent with a sensitivity of 0.02 ug/100 ml of plasma (30% error). Lipsett et al. (1966), with a double isotope derivative method, determined that plasma levels of testosterone in men between 30-45 years of age averaged .7 ug/100 ml of plasma and .1 ug/100 ml of plasma in men close to 21 years of age. This method involved chromatographic steps with either glass fiber paper or thin layer; acetylation and methoxime formation and gas chromatography to reduce non-specific tritium contamination. Saroff et al. (1967) further developed the double isotope derivative method for measuring testosterone, androstenedione, and dehydroepiandrosterone in one 5-10 ml plasma sample. Bromine-82 was used as a derivative forming agent following chromatographic separation of the three steroids and aromitization to estradiol with a microsomal fraction from human term placenta. Swanson et al. (1971) determined testosterone levels in prepubertal bull blood plasma and testicular homogenates using a double isotope derivative method. The method briefly consisted of isolation by thin-layer and partition chromatography and quantitation by gas-liquid chromatography in the form of heptafluorobutyric anaydride esters. Using bulls from

4 to 12.5 months of age, testicular testosterone concentration was high at 5 months, low at 6 months, increased from 6 to 11 months, then fell to the lowest level at 12 months of age. Plasma testosterone concentration was highly variable among bulls within age groups, however, it increased erratically from birth (.03 ng/ml) to 11 months (3.73±1.80 ng/ml) followed by a decrease at 12 months (.5±0.16 ng/ml).

Because the previous methods are complex, costly, and not suited for routine analysis of large numbers of samples, new procedures for testosterone measurement were developed.

Murphy and Pattee (1964) and Nugent and Mayes (1966) developed competitive protein binding assays for cortisol. Murphy and Pattee (1964) identified three components required for application of protein binding assays, they are: (1) a specific binding protein for the substance being determined, (2) a labeled form of the substance, (3) some method for separating the protein-bound material from unbound material. Pearlman and Crepy (1967) demonstrated the existence of a testosterone binding globulin and suggested a competitive protein-binding assay. Horton, Kato, and Sherins (1967) were the first to describe a competitive protein-binding assay (CPBA) for testosterone. Basically the assay utilizes displacement of labeled testosterone from a specific binding protein by testosterone in the sample, known amounts of testosterone are added to the labeled testosterone-protein complex to establish a standard curve. Following this development, numerous workers developed

CPBA for testosterone which differed in method of extraction and separation of free and bound testosterone (Frick and Kincl, 1969, Rosenfield, Eberlein, and Bongiovanni, 1969, Winter and Grant, 1971, Amando de la Pina and Goldzieher, 1971, and Katongole, 1971). Katongole (1971) developed a CPBA to measure testosterone in bull and ram plasma using paper chromatography for purification and Sephadex G-25 for separation of free and bound hormone. He reported a range of 2-24 ng/ml testosterone concentration in bull and ram plasma.

Wright and Taylor (1967) discussed radioimmunoassays (RIA) of hormones, this method depends upon highly-specific reactions between a protein hormone and its antibody. Competition between the radioactive labeled hormone and unlabeled hormone for the specific sites on the antibody compared against known amounts of hormone added to identical tubes to establish a standard curve is the basic principle. Berson and Yalow (1964) felt the advantages of the RIA were their high sensitivity, simplicity, and applicability to assaying large numbers of samples at a time. RIA were first developed for insulin (Wright and Taylor, 1967). Several methods were utilized to separate free and antibody-bound hormone, including electrophoresis, double antibody precipitation, salt precipitation, gel filtration, charcoal and silica, ion exchange resins, and solid phase antibody methods. The solid phase principle is based on the ability of an insoluble copolymer of styrene and polytetraflouroethylene to bind antibodies. Midgley (1966) using anti-HCG developed a RIA for

human LH. This assay was sensitive enough to allow quantitation of LH in .1 ml serum. Antisera was obtained by injecting, rabbits with repeated subcutaneous injections of HCG in Freud's adjuvant. HCG was iodinated with I^{131} by the method of Greenwood, Hunter and Glover (1963). This method uses chloramine-T to oxidize iodide to iodine, the iodine then reacts with the tyrosine residues in HCG. Sodium metabisulfate was then added to stop the reaction by reducing the excess iodine to iodide. Anti-rabbit gamma globulin was added as a secondary antibody to bind the anti-HCG, then the assay tubes were centrifuged to form a precipitate which was counted on a gamma counter.

Midgley et al. (1969) proposed a general procedure for the estimation of steroids by RIA. This proposal was based on the principle that steroids, acting as haptens, become immunogenic when conjugated to a protein. They suggested conjugating estradiol-17β to bovine serum albumin to form an antigenic substance. Abraham (1969) developed the first hapten RIA for estradiol-17β. He obtained antisera against estradiol-17β by immunizing a ewe with estradiol-17β-succinyl-bovine serum albumin. To precipitate the antibodies against the haptenic group, the antisera was absorbed with bovine serum albumin, then the albumin was removed by treatment with .4 percent Rivanol. The excess Rivanol was removed by absorption with charcoal, which was subsequently centrifuged down. The solid phase method of Catt (1967) was used for separation of the free and bound steroid. The sensitivity of this assay

was about 6-10 pg/ml plasma and recovery ranged from 30-36 percent.

Furuyama et al. (1970) developed a RIA for plasma testosterone utilizing a solvent partitioning step for purification and absorption chromatography on micro-columns of AI_2O_3 for separation of free and bound testosterone. Ismael, Niswender, and Midgley (1972) developed a RIA for testosterone which had the advantage of not requiring chromatography for purification. This method achieved partial purification by separating the fraction containing sex hormone binding globulin from the unbound steroids and those bound to other proteins. Antisera was obtained by injecting rabbits with testosterone-3-oxime BSA. Radiolabeled hormone was testosterone-3-oxime-tyrosine methyl ester-1^{125}.

Midgley et al. (1971) described the preparation of radio-labeled steroids. Steroids were conjugated to the methyl ester of tyrosine making the phenolic tyrosine ring available for iodination. I^{125} was selected as a label over C^{14} and H^3 because of the higher specific activity of I^{125}. The free and bound hormone was separated by precipitation with sheep anti-rabbit gamma globulin and centrifugation. The precipitate was counted on a gamma counter and counts per minute were compared with known amounts added to the system. The sensitivity of this assay was 10±3 pg/.2 ml serum. This procedure has the advantage of being sensitive, not complex and fast.

Hafs (1972) described a simple and rapid RIA for testosterone. Extraction for this method involved mixing a .5 ml serum sample with

benzene:hexane (1:2 v/v) followed by storage at -20°C for one hour. The supernant was poured off and dried to 2.5-3 ml. This extract was then assayed. Radio-labeled steroid used was testosterone-1,2-H^3 and separation of free and bound testosterone was achieved by means of dextran coated charcoal and centrifugation. Sensitivity for this method is about 50 pg/.5 ml serum.

Mode of Action of Lutinizing Hormone (LH)

Dorfman (1971) outlined the probable action of LH as; "in association with Follicle Stimulating Hormone (FSH) LH induces ovulation, forms the corpus luteum, partially controls the biosynthesis of estrogens, androgens, and progesterone by the ovary, and stimulates the interstitial cells of the testes to biosynthesize and secrete androgens and estrogens".

LH is a glycoprotein hormone with characteristics that vary between species. Its molecular weight ranges from 30,000 in sheep to 100,000 in hogs (Hafez, 1968).

Sutherland (1970) presented a general cellular mode of action for protein hormones. This postulates that the hormone, or first messenger, travels to its target tissue and binds to specific receptor sites on the plasma membrane where it activates the mebrane bound adenyl cyclase. The activated adenyl cyclase then stimulates the formation of cyclic AMP, or second messenger, from ATP. Cyclic AMP mediates the action of the specific hormone. Sutherland (1970) based his hypothesis on the

observation that, in fat ghost cells in rats a single adenyl cyclase enzyme activation occurs in response to glucagon, secretin, ACTH, epinephrine, thyrotropin, and LH through sites specific for each hormone. Thus, he postulated that animal adenyl cyclase systems contain distinct molecular components called "discriminators" which react with specific hormones to activate adenyl cyclase.

Marsh and Savard (1966) demonstrated, in bovine luteal tissue, an increase in cyclic AMP production by LH stimulation. The addition of puromycin, which inhibits protein synthesis, inhibited steroidogenesis but had no effect on cyclic AMP production. This suggested that cyclic AMP acts as an intercellular mediator of LH action.

Goswami, Skipper, and Williams (1968) found that FSH and LH, individually or combined to a greater extent, stimulated the synthesis of polyunsaturated fatty acids in rat testicular tissue. They suggested that the hormonal stimulation of spermatogenesis is associated with an increase in the synthesis of polyunsaturated fatty acids and that some stimulatory factor is transferred from the nucleus to the mitochrondria. This latter assumption was based on the obervation that only nuclear preparations from hormone treated rats caused an increase in fatty acid synthesis in isolated mitchrondria.

Behrman and Armstrong (1969) suggested that LH stimulates the availability of cholesterol for steroid hormone synthesis. The interveneous administration of LH was followed as early as 15 minutes, by

an increase in cholesterol esterase which converts cholesterol ester to free mitochrondrial cholesterol. They reported that the action of LH in this case did not appear to be mediated by cyclic AMP, however, stimulation did not occur in the absence of intact testicular cells.

Dorfman (1972) summarized the effects of LH on the testes as being two-fold. The first, the stimulation of cyclic AMP caused an increase in phosphorylase activity which increased glucose-6-phosphate formation from glycogen, resulting in an increase in NADPH. NADPH, as a cofactor, is required in the conversion of cholesterol to pregnenolone. The second action is that the LH on cholesterol esterase activity which is independent of cyclic AMP.

LH Assay

LH has been measured by numerous biological assays, classically, total gonadtrophin content has been determined by observing the increase in uterine weight in hypophysectomized rats and mice following treatment with pituitary or urinary extracts (Hafez, 1968).

Apostolakes and Loraine (1967) discussed various LH assays. Biological assays have included observation of the effects of LH on the repair of interstitial tissue in hypophsectomized female rats, enlargement of the ventral lobe of the prostate in hypophysectomized immature male rats, ovulation in intact immature female rats and mice, ovarian ascorbic acid depletion (OAAD) in intact immature rats, and ovarian

cholesterol depletion (OCD) in intact immature rats.

The OAAD test was described by Parlow in 1958 (Apostolakes and Loraine, 1967). This assay is based on the ability of LH to decrease the ascorbic acid content in immature rat ovaries pretreated with Pregnant Mare Serum (PMS) and Human Choronic Gonadotropin (HCG). The advantages of this assay over previous methods were a greater sensitivity and specificity. The disadvantages included the inability to measure LH in crude extracts and low precision in some animal strains.

The OCD assay, first described by Bell in 1964 (Apostolakis and Loraine, 1967), is very similar to the OAAD assay differing in that depletion in ovarian cholesterol is determined. This assay is much more sensitive than the OAAD assay, but poor precision and extensive control of the test animals environment hampered its general use.

Berson and Yalow (1964) discussed the complement fixation method of Brody and Carlstrom for determining HCG and LH concentration. This method is based on the phenomena that in the presence of a fixed amount of HCG antibody and an excess of complement, the addition of increasing amounts of HCG of LH caused an increasing fixation of complement. The sensitivity for sheep LH was .01 ug/ml of plasma. A disadvantage of this system is that some antigens and antiserums inactivate complement, and rigid control is necessary with respect to all the reactants and conditions.

The development of RIA was discussed in the testosterone

literature review section. Ely and Chen (1966) developed a rabbit antisera which was specific for sheep LH. This was accomplished by absorbing the antisera with FSH, PMS, HCG, and TSH. Niswender et al. (1969) developed double antibody RIA which were specific for bovine and ovine LH. The sensitivity was .0003 ug/200 ul of serum; LH levels in steers were reported to be 3-5 ng/ml of plasma. Goding et al. (1969) developed a solid-phase RIA for sheep LH using antibody coated polystyrene tubes for the assay. Snook, Saatman, and Hansel (1971) subsequently measured LH by solid-phase RIA in cycling cows, basal LH levels of 2-4 ng/ml were reported. Bogdanove et al. (1971) examined the agreement between OAAD bioassay and RIA estimates of LH concentration. The OAAD bioassay gave higher levels of serum LH than the RIA in castrated rats. They attributed this on an overestimation on the part of the OAAD assay, since serum levels were near the limit of sensitivity for the OAAD assay.

Swanson et al. (1971) and Rawlings, Hafs and Swanson (1972) determined LH levels in prepubertal bulls by the double antibody RIA method developed by Niswender et al. (1969). They reported two periods of marked LH elevation, from 2-5 months and 11-12 months. Total plasma LH increased between 1 and 2 months, plateaued to 10 months (1.93 ng/ml), increased at 11 and 12 months (3.22 ng/ml), then declined at 12.5 months (2.22 ng/ml). LH levels in Hereford bulls were reported to be twice as high as Holstein bulls at 12 months. Seminal vesicular fructose

and citrate were correspondingly elevated in Hereford bulls. Rawlings, Hafs, and Swanson (1972) concluded that "the release of LH is related to the onset of spermatogenesis and testosterone secretion".

MATERIALS AND METHODS

Eight bull calves approximately 7 months old at the onset of the experiment (for specific data see Table 1), were bled weekly for six months by jugular venipuncture. The bulls were housed at the Montana State University beef barns and were kept on an adequate level of nutrition throughout the test.

Approximately 90 ml of blood was collected weekly into heparinized centrifuge tubes, which were covered with parafilm and refrigerated until the following day. The samples were centrifuged[1/] for 30 minutes at 2500 RPM and the plasma was placed in whirl-paks[2/], labeled, and stored at -10°C until assayed.

LH Assay

The LH was assayed by the double antibody RIA described by Niswender et al. (1969, and personal communications). This assay is based on the principle of a competition system between labeled and unlabeled hormone for antibody binding sites. A sheep anti-rabbit gamma globulin is used to separate free and unbound hormone.

Rabbit antisera[3/] against bovine LH was obtained from Dr. G. Niswender. The antibody received was diluted 1:400 with .05 M EDTA-PBS pH 7, containing 1:10,000 merthiolate, and frozen. Subsequent dilutions were made to a working concentration of 1:50,000 with Normal Rabbit Serum

1/ HNS-centrifuge, manufactured by International Equipment Company, Needham Heights, Mass.
2/ Nasco Whirl-Pak, Van Waters and Rogers Catalog No. 11216
3/ Lot No. 225

TABLE 1. PUBERTY, BIRTH DATES AND BREED OF THE INDIVIDUAL BULLS

Bull No.	Experiment week number	Date	Total sperm concentration	Bull birth date	(Dam x Sire) Breed [a]
107	11	2/29	153×10^6	7/8/71	H x Hol
108	12	3/7	160×10^6	5/8/71	A x A
111	11	2/29	55×10^6	5/10/71	H x H
112	15	3/31	468×10^6	5/10/71	A x Hol
120	10	2/23	164×10^6	5/13/71	A x Jer
127	10	2/23	120×10^6	5/15/71	A x H
130	11	2/29	115×10^6	5/15/71	H x A
134	19	4/28	70×10^6	5/17/71	A x Jer

[a] H = Hereford; Hol = Holstein; A = Angus; Jer = Jersey

(NRS), diluted 1:400 in the previous buffer.

The second antibody, sheep anti-rabbit gamma globulin used to precipitate the LH-antibody complex, was prepared by injecting sheep subcutaneously with rabbit gamma globulin (Pentix Fraction II) in Freud's adjuvant. The anti-RGG was diluted 1:3 with the .05 M EDTA-PBS, with 1:10,000 merthiolate, and frozen in amounts required for one assay.

Standard[4/] solutions, corresponding to .05, .1, .2, .4, .8, 1.6, 3.2, 6.4, 12.8, and 25.6 ng, were weighed and diluted in .5 ml PBS-1% egg white (0.14M NaCl, .01 M $NaPO_4$, pH 7, with 1:10,000 merthiolate). Standards were frozen in aliquots necessary for one assay.

Purified LH for iodination was obtained from Dr. L. Reichert[5/]. The iodination procedure was modified from that of Greenwood, Hunter, and Glover (1963) for Growth Hormone and consisted of adding 1 mc I^{131}[6/], in 10 ul NaPhosphate buffer, pH 7.5, to 2.5 ug LH. Chloramine-T, 15 ug in 15 µl .05 M $NaPO_4$, pH 7.5, was added and mixed to initiate the reaction. The reaction was allowed to procede for 2 minutes before 12 ug sodium metabisulfate, 50 ul in .05 M $NaPO_4$, pH 7.6, was mixed in to stop the reaction. To separate the labeled LH, free I^{131}, and unlabeled LH the mixture was transferred with 100 ul of 16 percent sucrose

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4/ NIH-LH-B7
5/ LER-1072-2
6/ I^{131}, NaI in 0.1 N NaOH, New England Nuclear, Boston, Mass.

(containing 1 mg KI and .01 mg bromophenol blue) to the surface of a Biogel P-60 column[7]. The reaction vial was rinsed once with 70 ul of 8 percent sucrose (containing 1 mg KI and .01 mg bromophenol blue) and twice with the buffer, .05 M $NaPO_4$ pH 7.5. Each rinse was added to the surface of the gel. Fractions were collected in 1 ml aliquots in tubes containing 1 ml PBS-5% E.W. by not allowing the elutate to run down the sides of the tubes. A .1 ml aliquot was removed from each tube and counted on an automatic gamma counter[8] to determine the peak tube. The contents of the peak tube were subsequently diluted, dropwise, in 100 ml PBS-1% E.W. to approximately 50-60,000 cpm in .1 ml. This diluted solution ("IX" solution) and the extra stock solution were frozen in small vials by immersion in dry ice and isopentane.

The assay was conducted in 112 12x75 mm glass disposable culture tubes of which 2 served as total count tubes, 2 served as a check on the binding of the NRS, 20 served as duplicates of the 10 standard points (0-25.6 ng) and the remaining 88 served as duplicates for 44 samples. The assay proper was conducted in a stepwise fashion; first, 600 ul PBS-1% E.W. was added to the NRS control tubes and 0 point on the standard curve and 300 ul PBS-1% E.W. was added to the sample tubes; secondly, the standards (500 ul each) and samples (200 ul)

7/ 1 x 22 cm Biogel P-60, 50-100 mesh, Bio-Rad Laboratories, Richmond, CA
8/ Automatic Gamma Counting System, Model 4233, Manufactured by Nuclear, Chicago Co., Denver, Colorado.

were added. Each addition was followed by a brief mixing and the individual tube racks (20 tubes each) were placed in the refrigerator between additions. The final step for the first day was the addition of 20 ul NRS (diluted 1:400) to the 2 NRS control tubes and the addition of rabbit anti-bovine LH (200 ul diluted 1:50,000) to all except the total count tubes. Following this latter step the tubes were briefly mixed[9/] and incubated at 4°C for 24 hours. The sole addition for the second day consisted of adding 100 ul LH-I^{131}, with a microliter syringe[10/], to all the assay tubes, a brief mixing and another 24 hour incubation of 4°C followed. Sheep anti-rabbit gamma globulin, 200 ul diluted 1:3, was added on the third day to all except the total count tubes preceding a 72 hour 4°C incubation period. The total assay was centrifuged at 2500 RPM for 30 minutes in a refrigerated centrifuge equipped with swinging bucket heads[11/], following the addition of 3 ml cold (0°C) PBS to each tube. The supernatant was discarded and the remaining precipitate was counted 2 hours later on the automated gamma counter. Counting was delayed 2 hours to allow the supernatant remaining in the tubes time to settle to the bottom of the tube.

9/ Catalog No. 58810, distributed by Van Waters and Rogers Scientific, Seattle, WA.

10/ Manufactured by Hamilton Co., Inc., Whittier, CA

11/ Model PR-2 centrifuge, International Equipment Co., Boston, Mass.

Percent bound (100%=Opt on the standard curve) versus the logarithm of the standard points was the basis on which the standard curve was constructed. Values for the unknowns were then calculated directly off this curve and multiplied by 5 to put the values on a ng/ml basis.

Testosterone Assay

Testosterone was assayed by RIA as described by Hafs (1972). Basically this assay is very similar to the LH assay with the exception that dextran coated charcoal was used for separation on the bound and free hormone rather than a second antibody.

Lypholized rabbit antisera to bovine testosterone-3-BSA was obtained from Dr. G. Niswinder[12]. The antisera was restored to full strength with distilled water, diluted 1:400 with 0.1% gelatin-PBS, pH 7, and stored in small aliquots for the assay.

Standards[13] were prepared in double distilled ethanol with concentrations equal to 0, 15, 25, 50, 100, 200, 400, 800, and 1600 pg/ml ethanol. Standards were stored at 5°C and were added to the assay at room temperature.

Testosterone-1,2-^{3}H[14] was initially diluted with absolute ethanol to form a stock solution of 160 ng/ml. Aliquots of the stock solution

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12/ Pool #667.
13/ Control No. 9509, purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.
14/ 45 curies/mM, Lot No. 531-259, New England Nuclear, Boston, Mass.

were subsequently diluted with 0.1% gelatin-PBS, pH 7, to form an assay solution containing approximately 56,000 dpm/200 ul of solution. A recovery tracer solution was made by dilution of the stock solution with absolute ethanol to a final concentration of approximately 3000 dpm/50 ul of solution.

Dextran[15] and "Norit A" charcoal[16] were mixed as 0.025% dextran and 0.25% charcoal in distilled water. One ml was used for each tube to separate the free and bound hormone.

The extraction procedure consisted of adding 3000 dpm testosterone-1,2-^{3}H (for recovery determination) and .5 ml of the plasma samples to 15 ml glass stoppered centrifuge tubes. The plasma was mixed with ml benzene:hexane (1:2 v/v) by repeated inversion for 30 minutes then stored at -20°C for one hour. The supernatant was then poured off into 12 ml graduated conical tubes (precoated with 5% trimethylchorosilane in toluene and air dried) and dried under nitrogen to a volume of 2.5-3 mls. For recovery determination, .5 ml from each extract was added to 15 ml Bray's solution[17] and counted on a Beckman liquid scintillation counter[18] for 10 minutes.

The assay was initiated by the addition of standards (250 ul) and extracts (.5 ml), in duplicate, to 12x75 mm disposable glass culture

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15/ No. 24751, Sigma Chem. Co., St. Louis, MO
16/ Matheson Coleman and Bell, Los Angeles, CA
17/ 100 gm napthaline, 7 gm PPO, .3 gm POPOP, and 1 liter 1,4 dioxane
18/ Model LS-100 Liquid Scintillation System

tubes, which were dried under nitrogen. Antisera (200 ul) diluted 1:400 in 0.1% gelatin-PBS was added to room temperature and allowed to incubate for 10 minutes. The addition of 200 ul testosterone-1, 2-^{3}H (56,000 dpm) in 0.1% gelatin-PBS was followed by a brief mixing and a 12-18 hour incubation period at 5°C. Separation of the bound and free testosterone, at the end of the incubation period, was achieved by the addition of 1 ml cold 0.025% dextran and 0.25% "Norit A" in distilled water. The tubes were briefly mixed on a vortex mixer, incubated at 4°C for 10 minutes, then centrifuged at 2500 x g for 10 minutes in a refrigerated centrifuge. A .5 ml aliquot was removed[19/], added to 15 ml Bray's solution and counted for 10 minutes on an automatic liquid scintillation counter.

Values for the individual sample extracts were calculated directly from a composite standard curve constructed from the average values of the standard point values established throughout the experiment (figure 1). Percent bound versus logarithm testosterone concentration were plotted on the Y and X axis, respectively. All extract values had to be corrected for their separate dilution values, depending on the level to which the extract was dried, and multiplied by 2 to place them on a pg/ml basis.

19/ Automatic Dilutor, Labindustries, Berkley, CA

Testosterone Binding Assay

An estimation of the change in total testosterone binding capacity in beef plasma was accomplished with a saturation technique. The principles developed by Murphy (1968) and Neil et al. (1967) for testosterone, estradiol and progesterone competitive protein binding assays were used as guide lines.

Testosterone-1,2-^{3}H, diluted to 160 ng/ml in absolute ethanol, was added in a quanity sufficient to saturate the plasma testosterone binding sites. The addition of testosterone-1,2-^{3}H in amounts greater than 8 ng testosterone/ml plasma did not result in an increased testosterone-1,2-^{3}H binding in bull plasma.

Incubation of the steroid and plasma was in two phases; a short 5 minute 45°C incubation in a metabolic shaker[20/] was performed to establish equilibrium, and a 1 hour 4°C incubation was performed to allow binding to occur. The 1 hour cold incubation time was determined by noting, at 10 minute intervals, the time at which no further increase in binding occurred.

Separation of the bound and free testosterone was accomplished by mixing 80 mg of Florisil[21/] with the diluted plasma for 30 seconds on a Vortex mixer. Murphy (1968) illustrated that Florisil absorption of

20/ Dubnoff Precision Scientific Co., Sub. of GCA Corp. Chicago, Ill.
21/ 60-100 mesh, F-100, Fisher Scientific Co.

steroids is a reliable method of separation and the time interval following mixing is not critical.

The details of the method in stepwise fashion consisted of:

1. Adding 1 ml serum to 9 ml distilled water in 16x75 mm disposable glass tubes and mix briefly.
2. Remove 2 ml, 3 times, from each tube and place in separate 12x75 mm disposable glass culture tubes.
3. Add 10 ul (1.6 ng) testosterone-1,2-^{3}H to each tube with a microliter syringe.
4. Incubate the assay for 5 minutes with gentle shaking in a 45°C water bath for 1 hour at 4°C.
5. Remove .5 ml from each tube and mix with 15 ml Bray's solution to determine total counts (C tubes).
6. Add 80 mg Florisil to each tube and mix on a Vortex mixer for 30 seconds.
7. Remove .5 ml from each tube and mix with 15 ml Bray's solution (A tubes).
8. Count the entire assay for 10 minutes/vial on a liquid scintillation counter.

The assay tubes and reagents were continously kept in ice water except for the brief 45°C incubation and 30 second mixing.

The percent of testosterone-1,2-^{3}H bound by the plasma was calculated by subtracting the average cpm bound by the six water blanks from the cpm bound by the average of the 3 plasma (A) tubes and dividing by the average total cpm added (A tubes-water blanks/C tubes cpm). The ng of testosterone bound by the plasma was derived by multiplying percent bound x ng of testosterone added (1.6 ng) divided by the dilution

factor (.2).

Each assay consisted of the entire samples for one bull to avoid the possibility of a shift in binding levels due to assay variation. It was not attempted to determine assay differences because the individual changes in binding levels in each bull through time was felt to be most important.

Data Analysis

Birth date and breed are indicated in Table 1. The age difference between bulls was ignored during data analysis since all were born within 9 days of each other. Breed difference between bulls was examined on the basis of a comparison between 4 bulls with dairy breeding and 4 bulls with beef breeding. Individual breed differences could not be looked at because of the small number of any specific breed.

Data was analyzed, both as a complete group and in groups of beef and dairy by least-squares analysis for breed difference, weekly differences, and orthogonal polynomial regression over the entire 24 week sampling period. In addition, the regression of each hormone was determined before and after puberty. The correlation coefficients between hormones were determined for both methods of analysis.

Individual samples with non-detectable levels of hormone present were assigned a value one-half that of the minimum detectable level for analysis. Puberty was defined as the first occurrence of a minimum 50×10^6 total sperm count in an ejaculate and was noted by weekly

electroejaculation in a separate experiment.

RESULTS

LH Assay

The minimum detectable level of LH was approximately 1 ng/ml of plasma. In a few cases the sensitivity was lowered to approximately .5 ng/ml if the standard curve retained its linearity.

Individual LH levels, represented in figures 13-20 and appendix table 2, followed an erratic pattern throughout the entire 24 week period.

Bull 111 (figure 2) had two substantial prepubertal LH peaks of 2.95 and 3.6 ng/ml plasma. A two week period of nondetectable LH levels immediately followed puberty, prior to an erratic but general increase to 3.55 ng/ml of plasma 12 weeks postpuberty.

Bull 112 (figure 3) had substantial LH peaks before and after puberty, 4.3 ng/ml plasma, however, while LH levels were lower at puberty 3.3 ng/ml of plasma, they were readily detectable. A low level of LH occurred (1.8 ng/ml plasma) 3 weeks before puberty but was followed by an increase to 3.55 ng/ml of plasma 1 week before puberty.

Bull 127 (figure 4) with a LH peak of 3.65 ng/ml 9 weeks before puberty and low LH levels following this (1.55 ng/ml of plasma to non-detectable), increased to 2.2 ng LH/ml of plasma at puberty and 3.3 ng 1 week after puberty. A high level of LH appeared 7 weeks after puberty (6 ng/ml).

Bull 130 (figure 5), with very erratic and dramatic changes in LH levels between bleedings reached 4.5 ng/ml 1 week before puberty then

dropped to nondetectable levels by 3 weeks after puberty. A high peak of 7.45 ng/ml occurred 8 weeks after puberty.

Bull 108 (figure 6) was characterized by very low LH levels throughout the entire period. A two week increase from nondetectable levels to 0.7 ng/ml occurred 5 and 6 weeks before puberty then returned to nondetectable levels. Except for a brief increase to .95 ng/ml which occurred 1 week after puberty, LH levels were nearly nondetectable until an increase began at 11-12 weeks after puberty, 1.75 and 2.4 ng/ml, respectively.

Bull 107 (figure 7) followed the pattern of high LH levels one week before and at puberty, 2.25 and 2.2 ng/ml, respectively. LH levels thereafter remained low for 10 weeks then increased to 2.5 ng/ml 11 weeks after puberty.

Bull 120 (figure 8) had very erratic prepubertal LH levels. Five peaks of 2.5, 3.65, 4.7, 5.5, and 5.75 ng/ml occurred and increased pregressively as puberty neared. At puberty LH levels began to decrease from approximately 3.5 ng/ml to less than 1 ng/ml five weeks after puberty. At the sixth postpubertal week an abrupt LH surge occurred (5.25 ng/ml) then declined again.

Bull 134 (figure 9) with two prepubertal LH peaks of 4.4 and 7 ng/ml at the fifth and eighth weeks before puberty, respectively, followed with nondetectable levels for four weeks before puberty. At puberty, there was an increase in LH levels to 5 ng/ml, a drop to nondetectable

for one week after puberty, and another increase to 3.95 ng/ml two weeks later.

LH levels for the eight bulls collectively over the entire 24-week period had a mean of 1.9±1.33 ng/ml of plasma and increased linearally (figure 10) ($P < .05$). LH levels in the prepubertal period on all 8 bulls increased as puberty neared, however, they did not follow any regression significantly. After puberty, LH levels in all 8 bulls increased quadratically ($P < .05$) (figure 11).

Beef and dairy bull plasma LH levels throughout the 24-week period are illustrated in appendix figure 1. Dairy bull LH levels averaged 1.97±1.4 ng/ml of plasma with no significant regression while beef bull LH levels, with a mean of 1.8±1.3 ng/ml of plasma, followed a linear regression ($P < .05$) over the entire period. No significant regression in LH levels was seen in either the beef or dairy bull groups before puberty. After puberty no significant regression existed for LH levels in the dairy bull group, however, in the beef bull group LH levels increased linearally ($P < .01$) (figure 12).

Testosterone Assay

Recovery estimates from the benzene:hexane extracts ranged from 65-100 percent with a mean 84 percent recovery. The level to which the extract was, prior to the removal of aliquots for recovery determinations and assay additions varied by 0.6 ml. This variation was corrected by determining the percent of the total extract that was

assayed.

The sensitivity of the testosterone assay was limited by the blank benzene:hexane 0.5 ml aliquots which were added to the assay as unknowns. Blank values ranged from 0-19 pg, therefore, the lower detectable limit was set at 20 pg/.5 ml benzene:hexane extract or approximately 300 pg/ml of plasma.

Testosterone levels for the 8 bulls for the entire 24-week period are illustrated in figures 13-20 and appendix table 2. In all the bulls, the testosterone peaks increased in amplitude near puberty. Individually, Bull 107 (figure 13) had the most dramatic changes in testosterone levels. Peak levels before puberty reached 1906 pg/ml of plasma and 2351 pg/ml of plasma after puberty. A sharp decrease in testosterone levels (296 pg/ml of plasma) one week before puberty is evident, after which the testosterone levels begin to increase and reach their peak levels 2 weeks after puberty.

Bull 108 (figure 14) had testosterone levels similar in pattern to Bull 107 but on a much smaller scale. Prepubertal testosterone levels increased to 973 pg/ml of plasma 4 weeks before puberty then declined to nondetectable levels 1 week before puberty. At puberty, the testosterone levels increased to 355 pg/ml of plasma and by 2 weeks after puberty, reached their highest level of 1031 pg/ml of plasma.

Bull 111 (figure 15) differed from the previous two bulls by having a larger prepubertal testosterone peak (1114 pg/ml of plasma) than after

puberty (1023 pg/ml of plasma). Testosterone levels were nondetectable at puberty and one week following, after which an abrupt increase to 1023 pg/ml of plasma occurred 3 weeks after puberty.

Bull 112 (figure 16) had the lowest overall testosterone levels of any bull in the group. The highest testosterone level recorded was 651 pg/ml of plasma 5 weeks before puberty, this declined to 469 pg/ml of plasma 2 weeks before puberty and became nondetectable at puberty. Post pubertal testosterone levels remained near the non-detectable level.

Bull 120 (figure 17) with 678 pg/ml of plasma two weeks before puberty, declined to nondetectable levels one week before puberty then increased to 466 pg/ml of plasma 2 weeks after puberty. A peak level of 990 pg/ml of plasma was seen 9 weeks after puberty which was followed by a return to nondetectable levels 14 weeks after puberty.

Bull 127 (figure 18) was characterized by very low prepubertal testosterone levels. A solitary prepubertal testosterone peak (727 pg/ml of plasma) occurred at puberty and was followed 2 weeks later by a peak of 623 pg/ml of plasma.

Testosterone levels in Bull 130 (figure 19) were characterized by nondetectable levels for 4 weeks after puberty. Two small testosterone peaks of 503 and 488 pg/ml of plasma occurred at 6 and 8 weeks after puberty, respectively.

Bull 134 (figure 20) reached 601 pg/ml of plasma at puberty then

declined to nondetectable levels for three weeks. A small increase to 370 pg/ml of plasma occurred 4 weeks after puberty.

Testosterone levels over the 24-week period for the 8 bulls collectively had a mean of 438.27±339.27 pg/ml of plasma. Regression analysis revealed that testosterone levels changed quadratically over this period ($P < .05$) (figure 21). Testosterone levels on all 8 bulls before puberty had a mean of 430.93±326.03 pg/ml of plasma and a mean of 445.26±352.26 pg/ml of plasma after puberty. Testosterone levels did not follow any significant regression when examined before and after puberty. The testosterone levels of the beef and dairy groups are illustrated in appendix figure 2.

Testosterone Binding Assay

The levels of testosterone bound by one ml of plasma are individually illustrated in figures 13-20 and appendix table 3. Levels of testosterone bound by the plasma differed between bulls and ranged from 1.3 ng/ml of plasma to 4.31 ng/ml of plasma. Testosterone binding levels do not appear to differ in binding ability present at the start and end of the experiment. Analysis did not reveal any significant regression for all eight bulls combined over the 24-week period.

Plasma testosterone binding ability on all eight bulls before puberty increased linearally to puberty ($P < .05$) (figure 11), however, no regression was significant after puberty. Testosterone binding did not follow any regression significantly before or after puberty in the

beef group. In contrast, testosterone binding in the dairy group increased linearally both before and after puberty ($P < .05$) (figure 12).

Intrahormonal and Plasma Testosterone Binding Ability Relationships

Figure 22 illustrates the relationships between LH, testosterone, and the plasma testosterone binding ability before and after puberty from the average of all 8 bulls (week 1 = time of puberty).

Collective LH levels were positively correlated ($r=.2302$, $P < .01$) with the plasma testosterone binding ability over the entire 24-week period. Analysis before puberty revealed a positive correlation ($r=.3683$, $P < .01$) between LH and testosterone binding. LH and testosterone binding were not significantly correlated after puberty.

In both the dairy and beef groups, LH was positively correlated with testosterone binding before puberty ($r=-.3959$, $P<.01$, $r=-.3158$, $P<.05$, respectively). LH and testosterone levels were positively correlated before puberty ($r=-.3336$, $P<.05$) in the beef bulls but not significantly correlated thereafter. In the dairy group, postpubertal LH and testosterone levels were negatively correlated ($r=-.4707$, $P < .01$), but not significantly correlated before puberty. Testosterone and plasma testosterone binding were not significantly correlated at any time.

LH and testosterone levels were negatively correlated ($r=-.2302$, $P < .01$) throughout the entire 24-week period. Analysis before and after puberty revealed a negative correlation between LH and testosterone ($r=-.3446$, $P < .01$) after puberty, but no correlation before puberty.

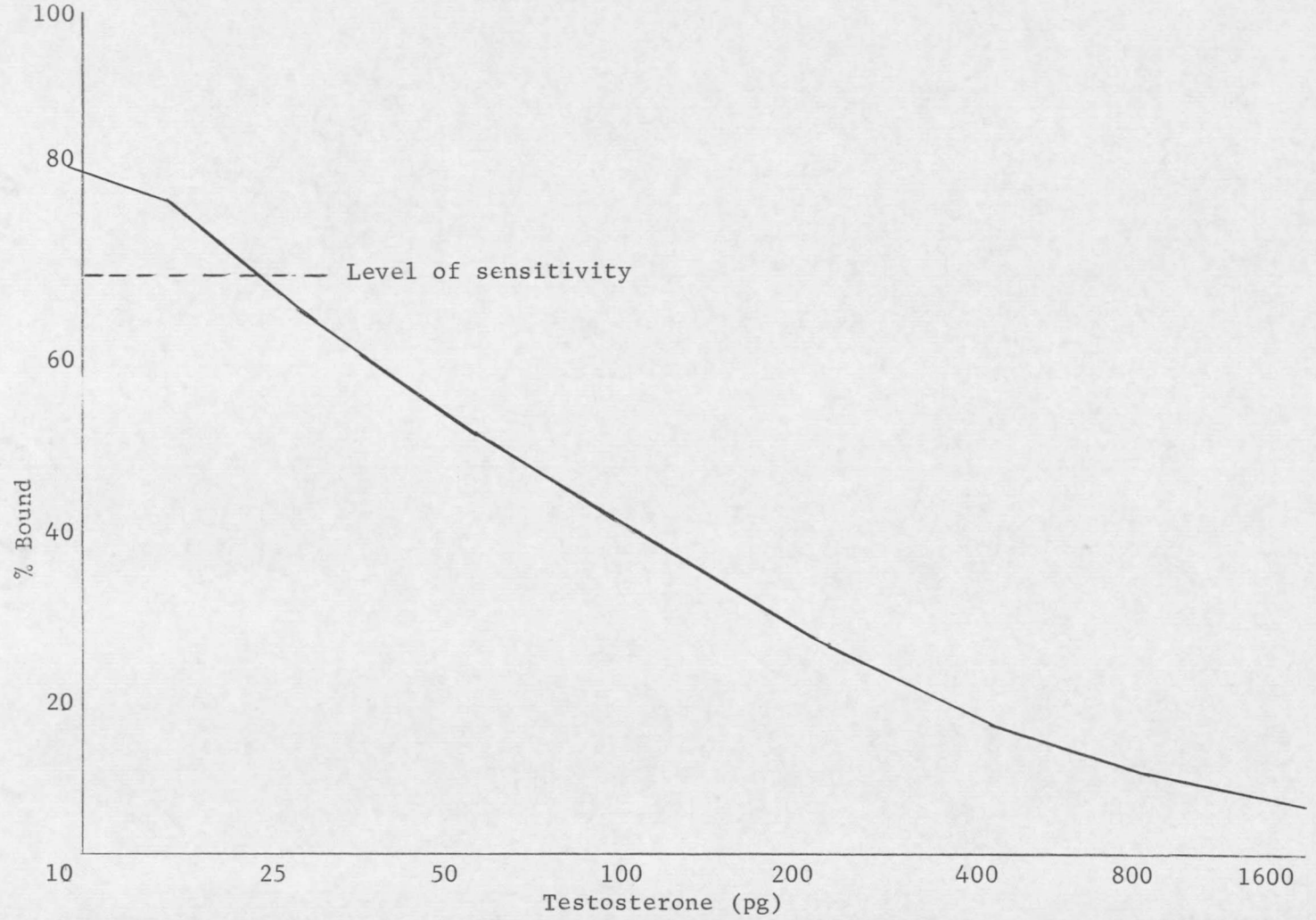


Figure 1. Composite testosterone standard curve established from the average of the five individual standard curves ran with each assay.

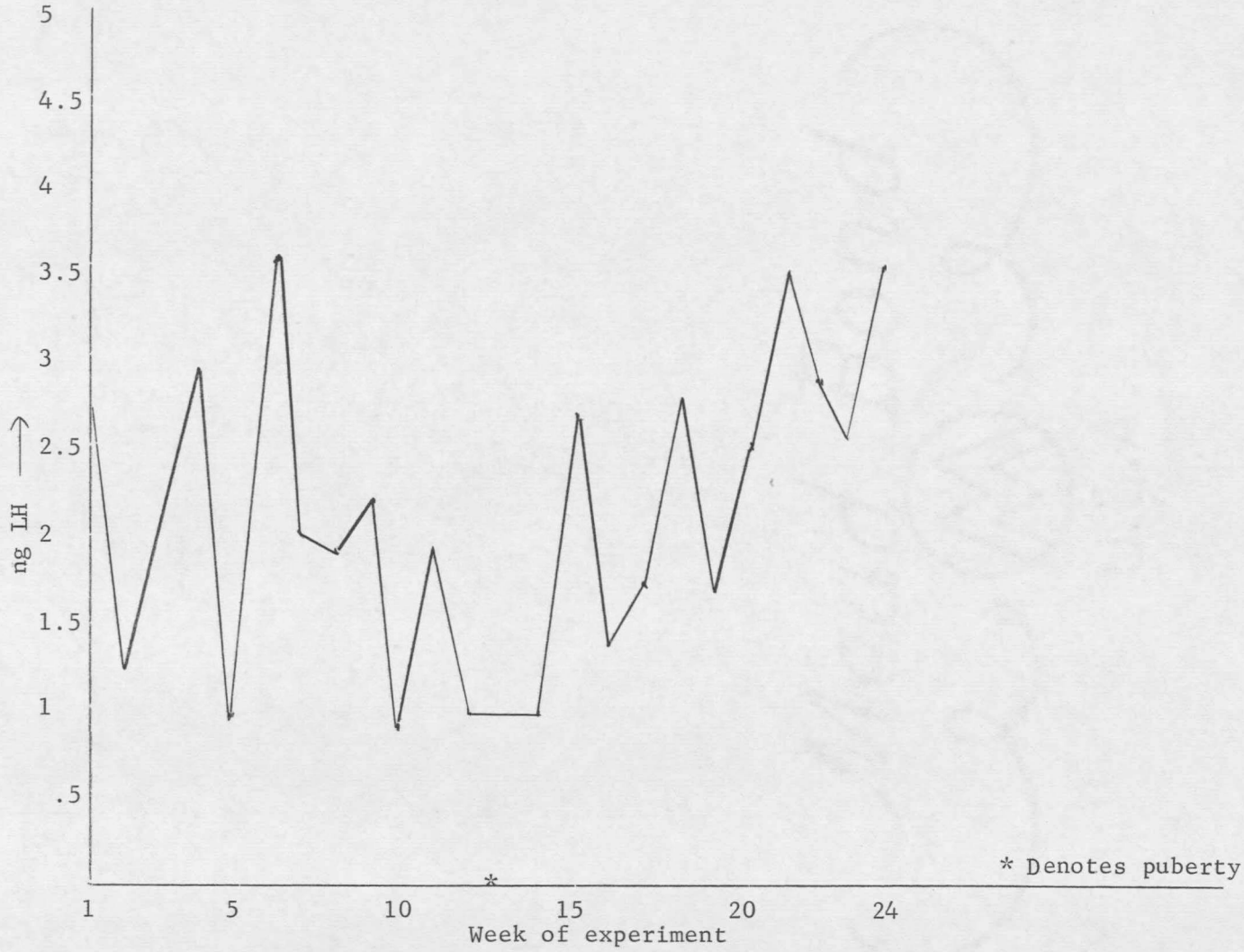


Figure 2. Plasma LH levels in bull 111 from 7-13 months of age.

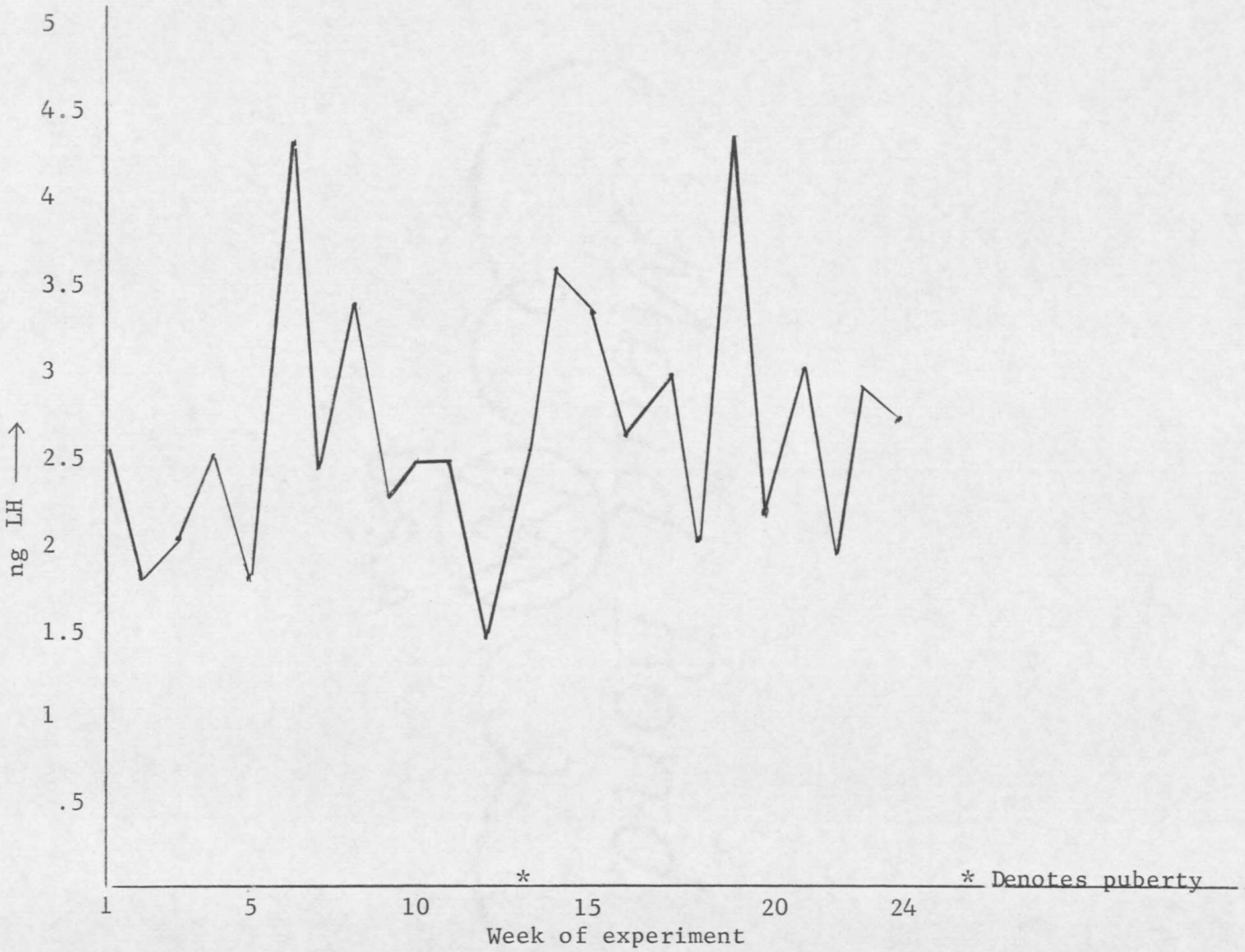


Figure 3. Plasma LH levels in bull 112 from 7-13 months of age.

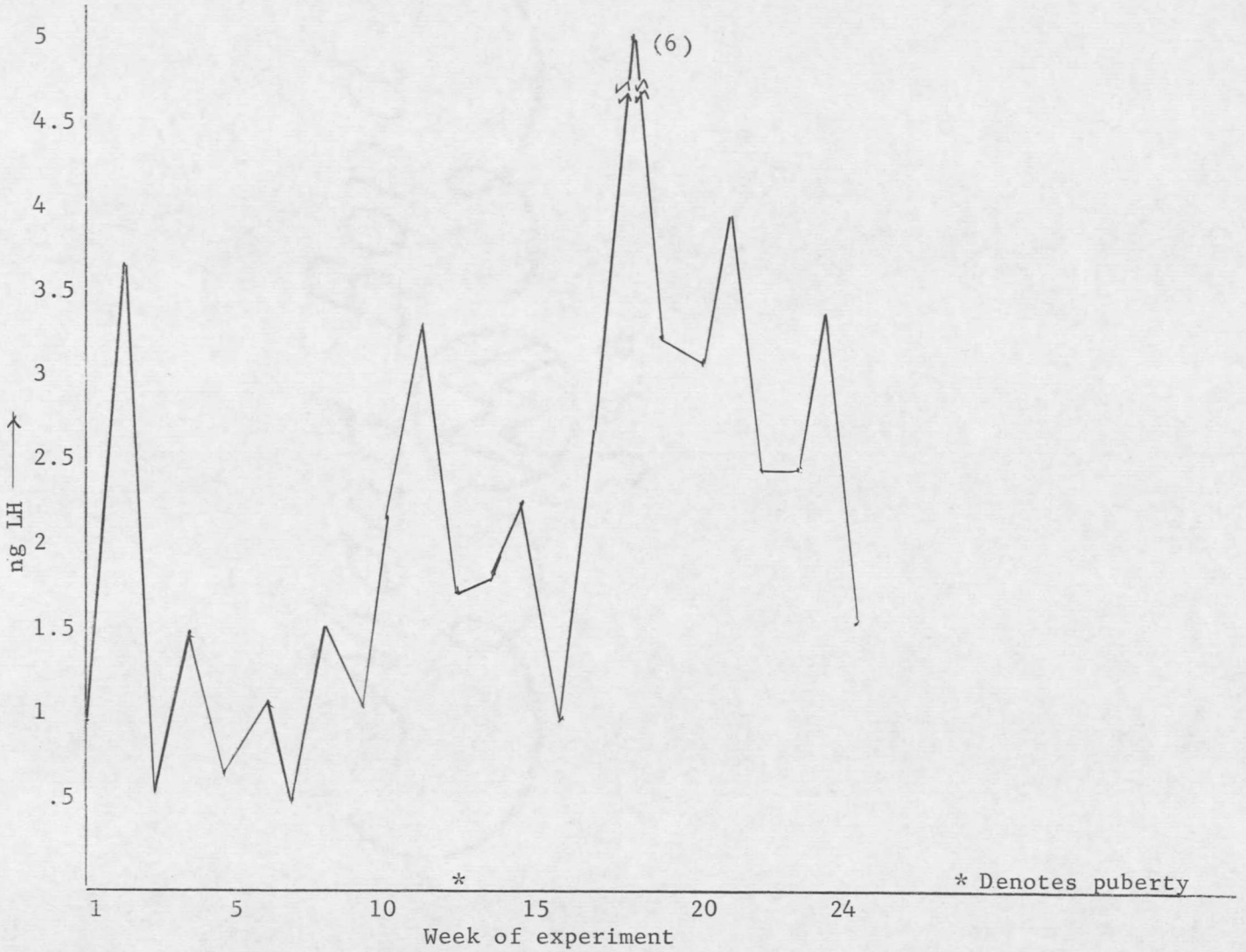


Figure 4. Plasma LH levels in bull 127 from 7-13 months of age.

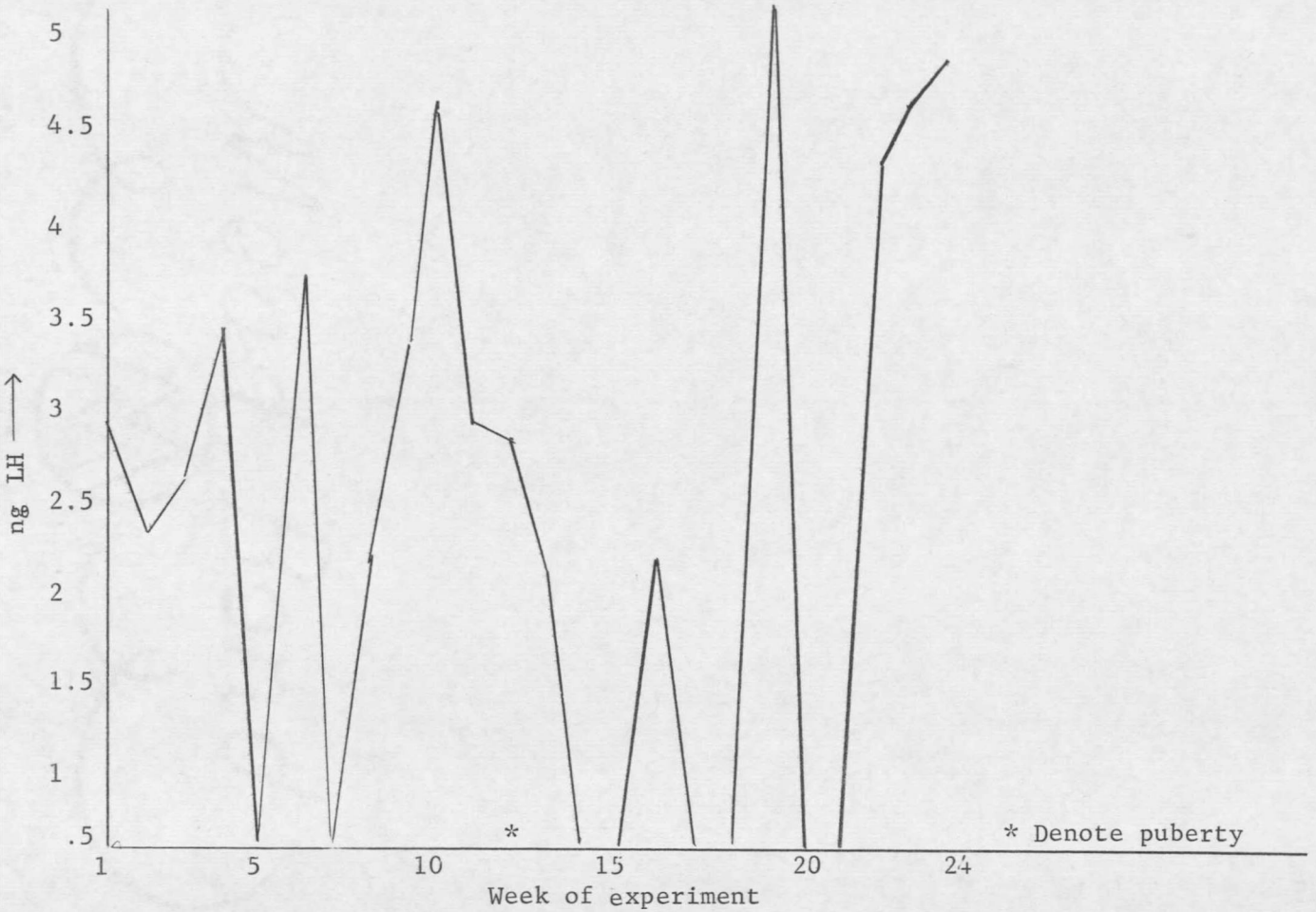


Figure 5. Plasma LH levels in bull 130 from 7-13 months of age.

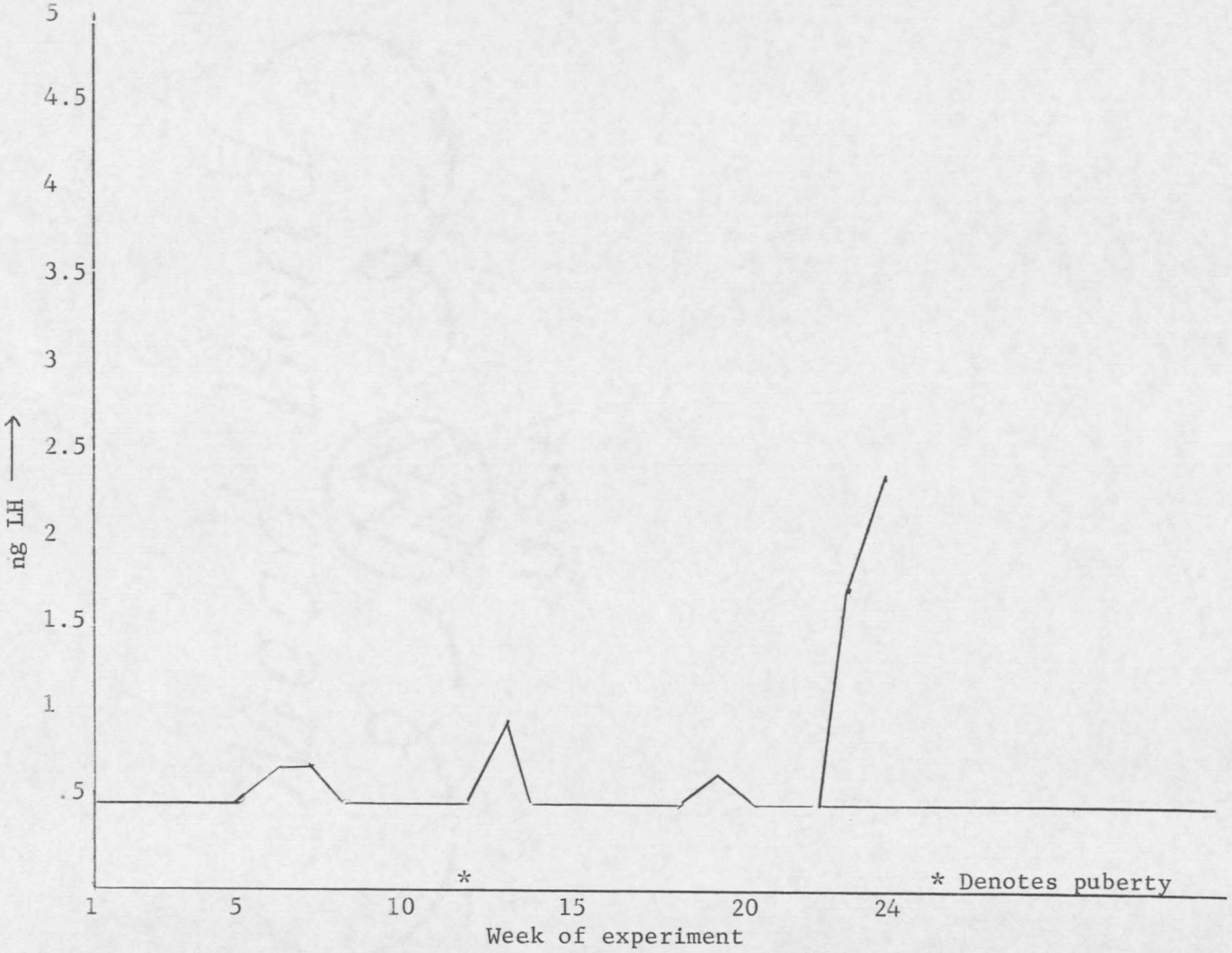


Figure 6. Plasma LH levels in bull 108 from 7-13 months of age.

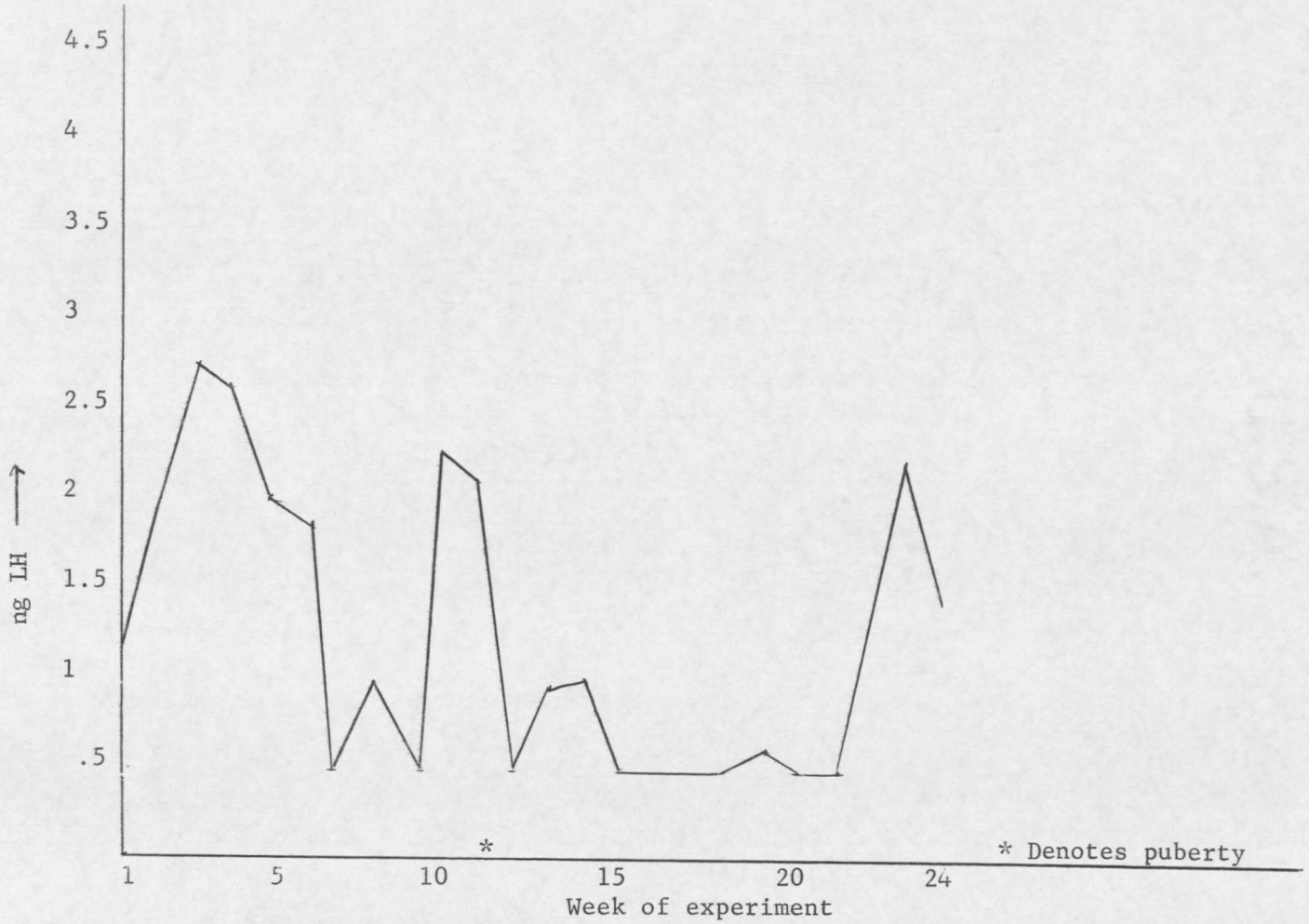


Figure 7. Plasma LH levels in bull 107 from 7-13 months of age.

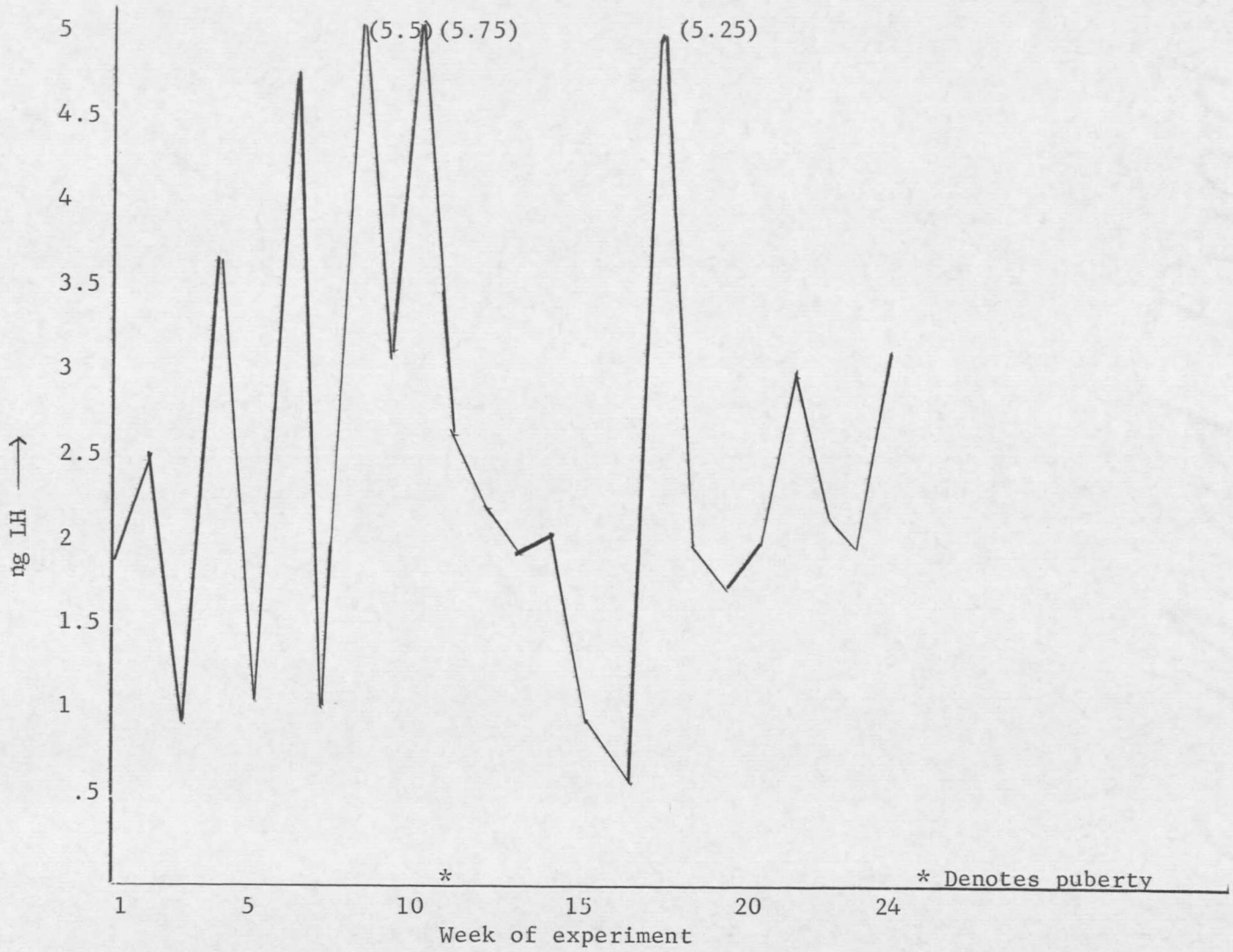


Figure 8. Plasma LH levels in bull 120 from 7-13 months of age.

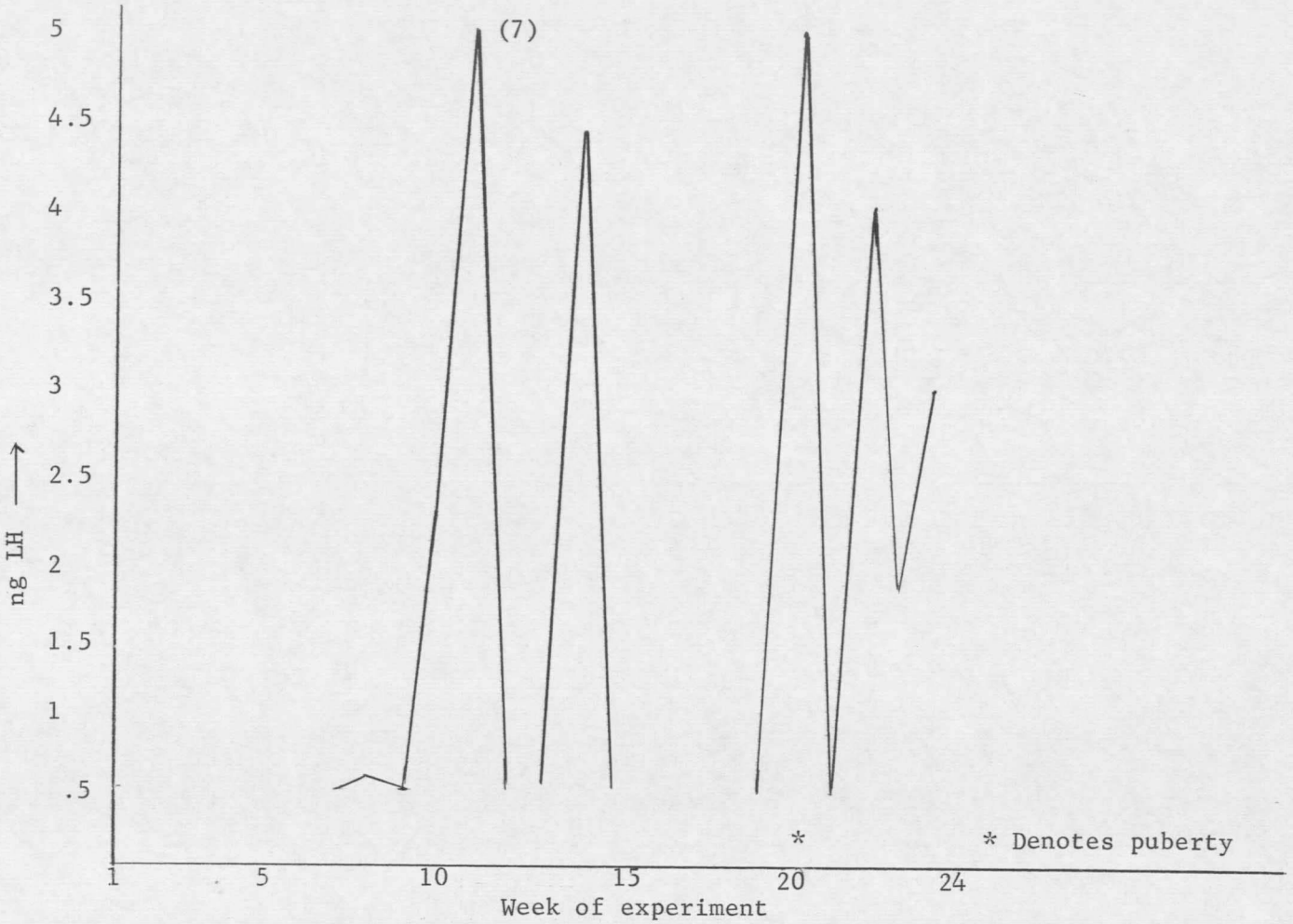


Figure 9. Plasma LH levels in bull 134 from 7-13 months of age.

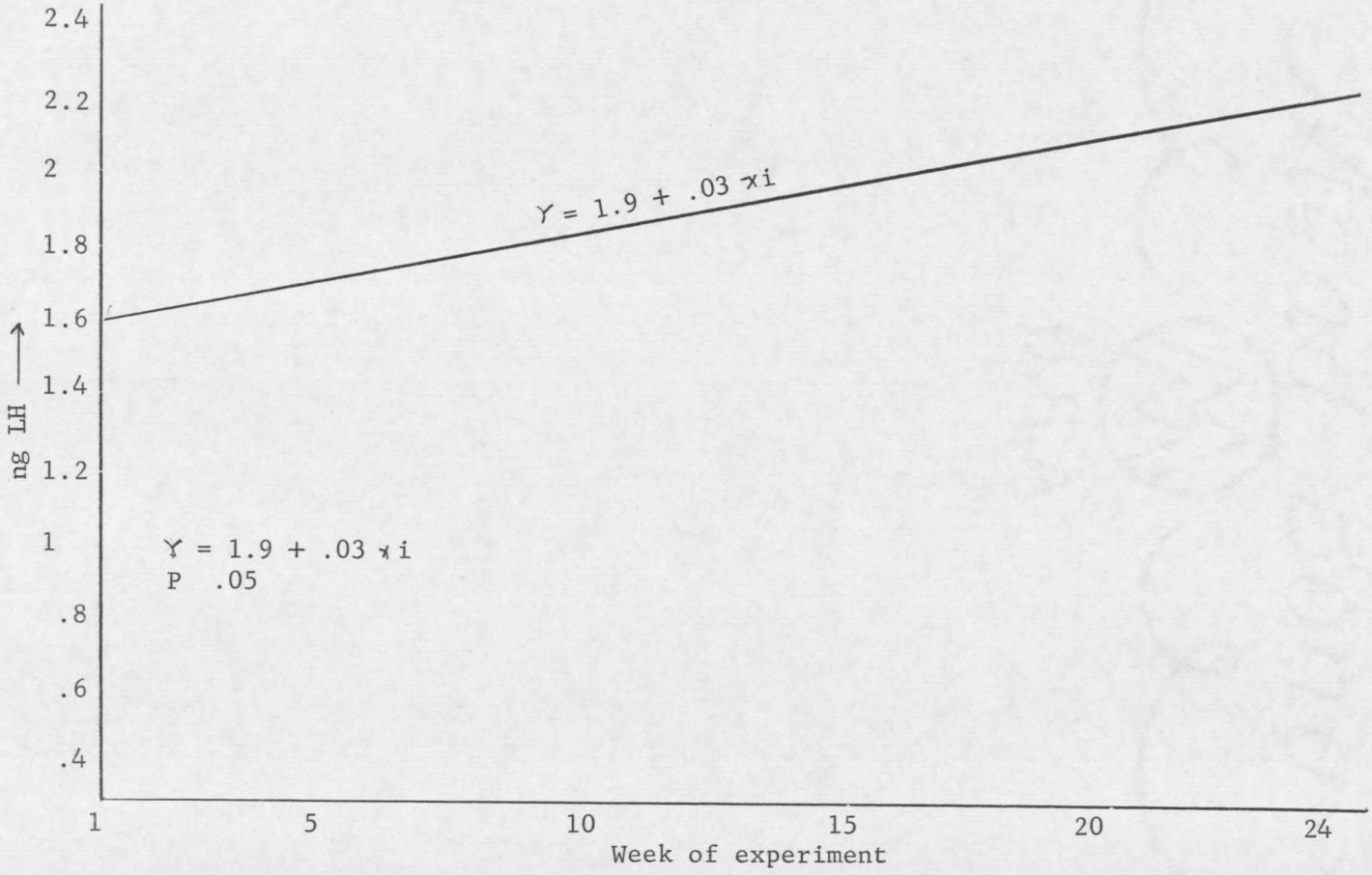


Figure 10. Linear regression of LH over the 24-week sampling period for 8 bulls 7-13 months of age.

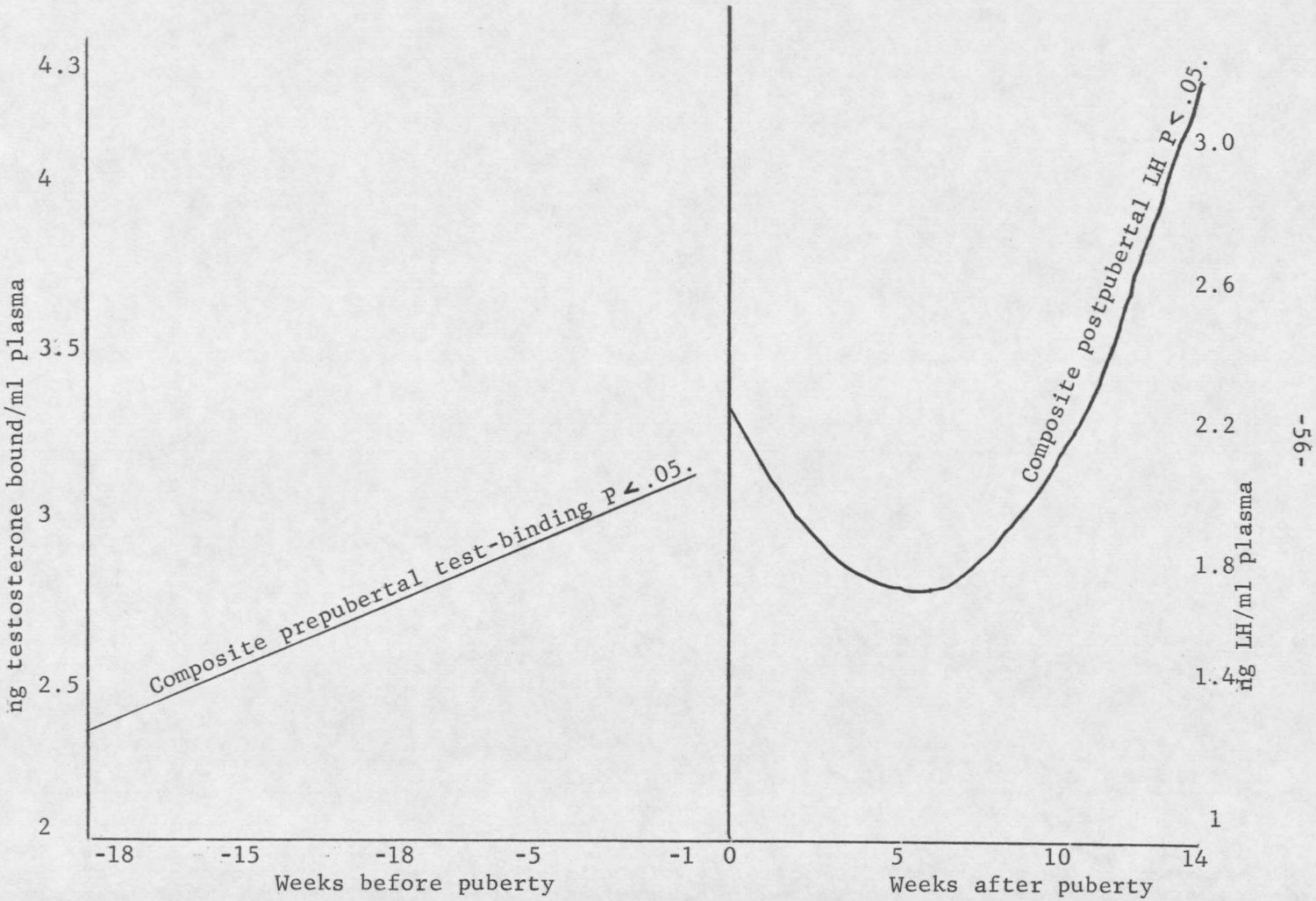


Figure 11. Linear regression of plasma testosterone binding before puberty for all 8 bulls ($P < .05$), left scale and quadratic regression of LH levels on all 8 bulls after puberty ($P < .05$).

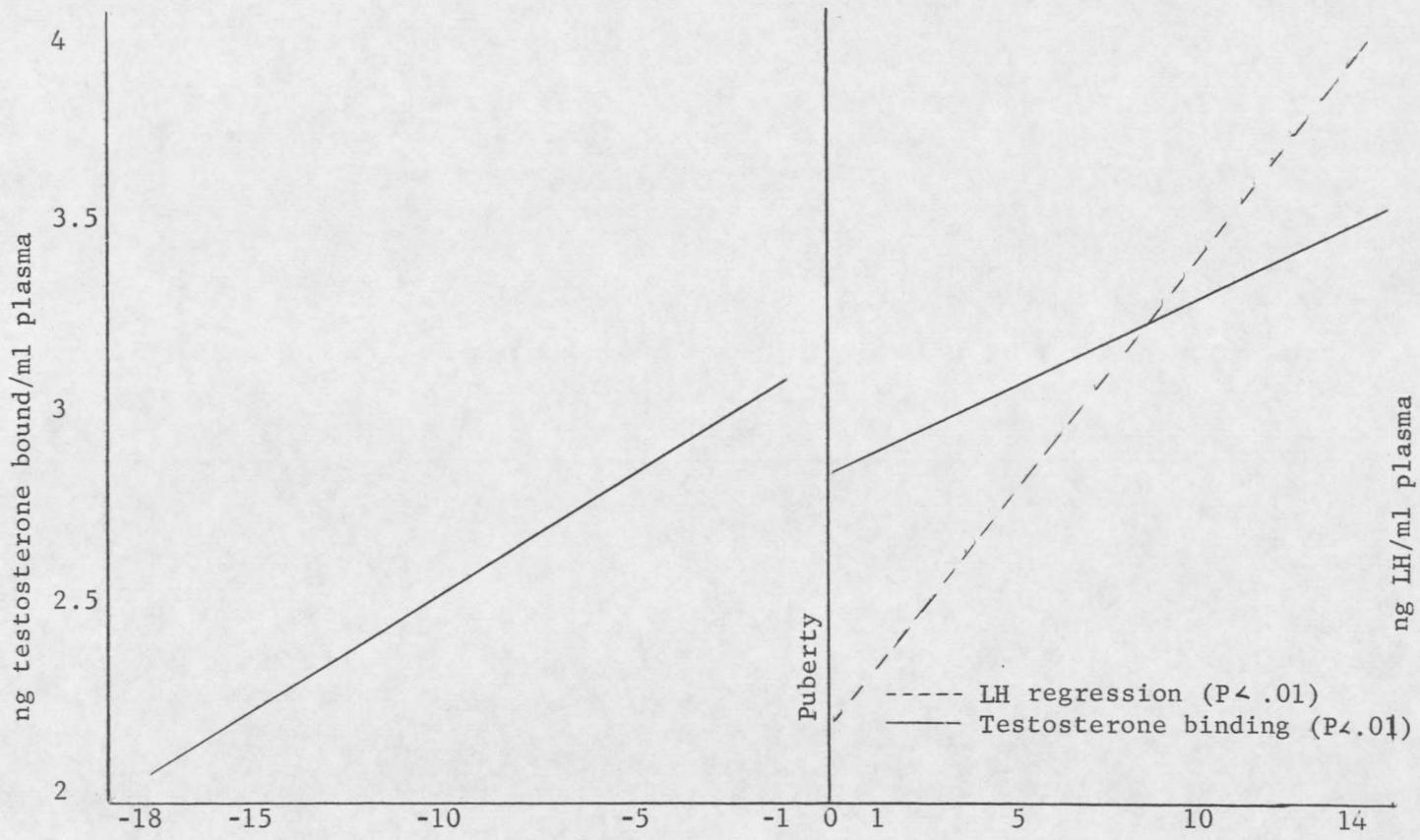


Figure 12. Left. Linear regressions of testosterone bound by plasma before puberty in 4 dairy cross bulls (P< .05). Right. Linear regression of testosterone bound by plasma after puberty in 4 dairy cross bulls and the linear regression of LH levels after puberty in 4 beef bulls.

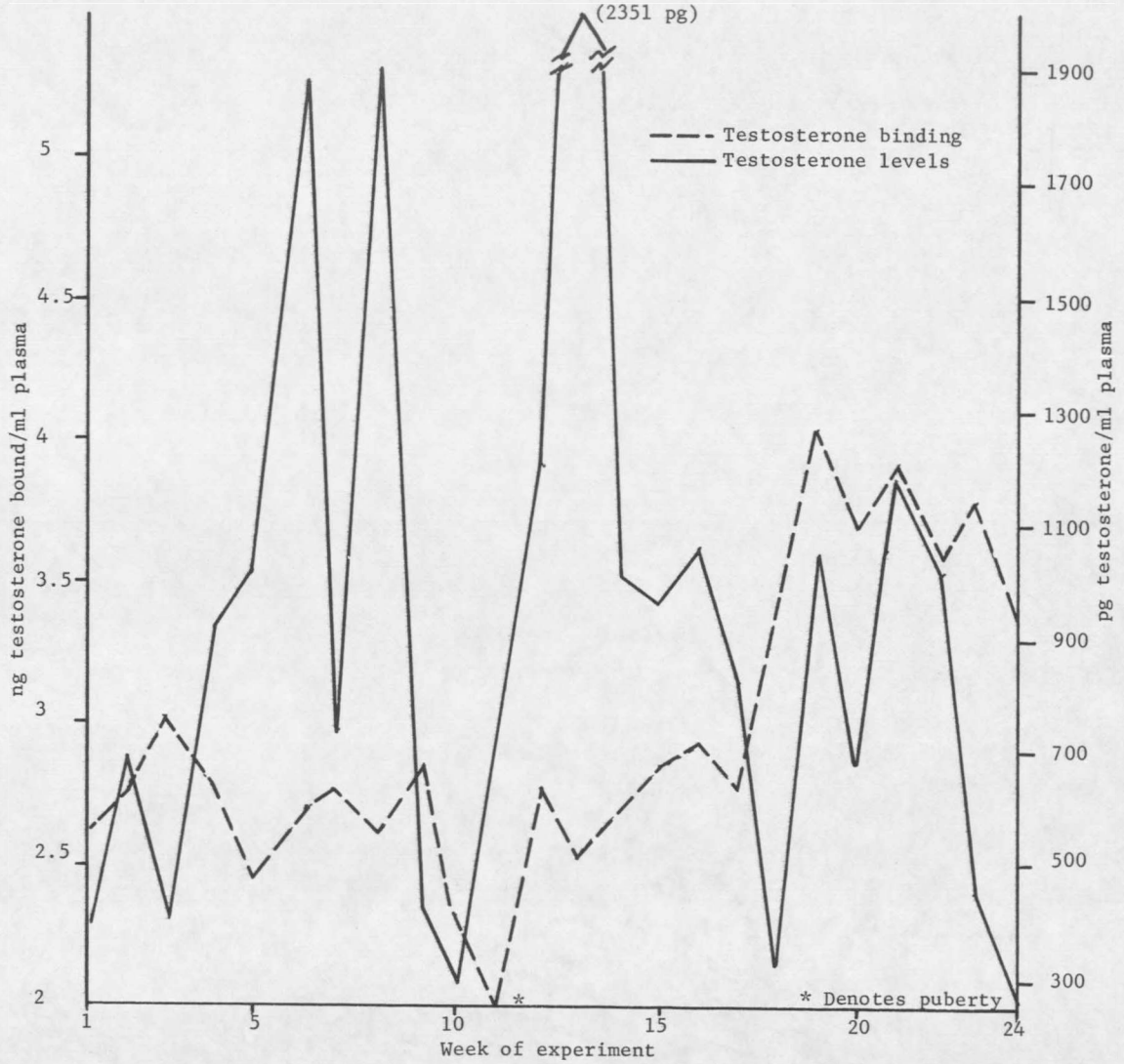


Figure 13. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 107 from 7-13 months of age.

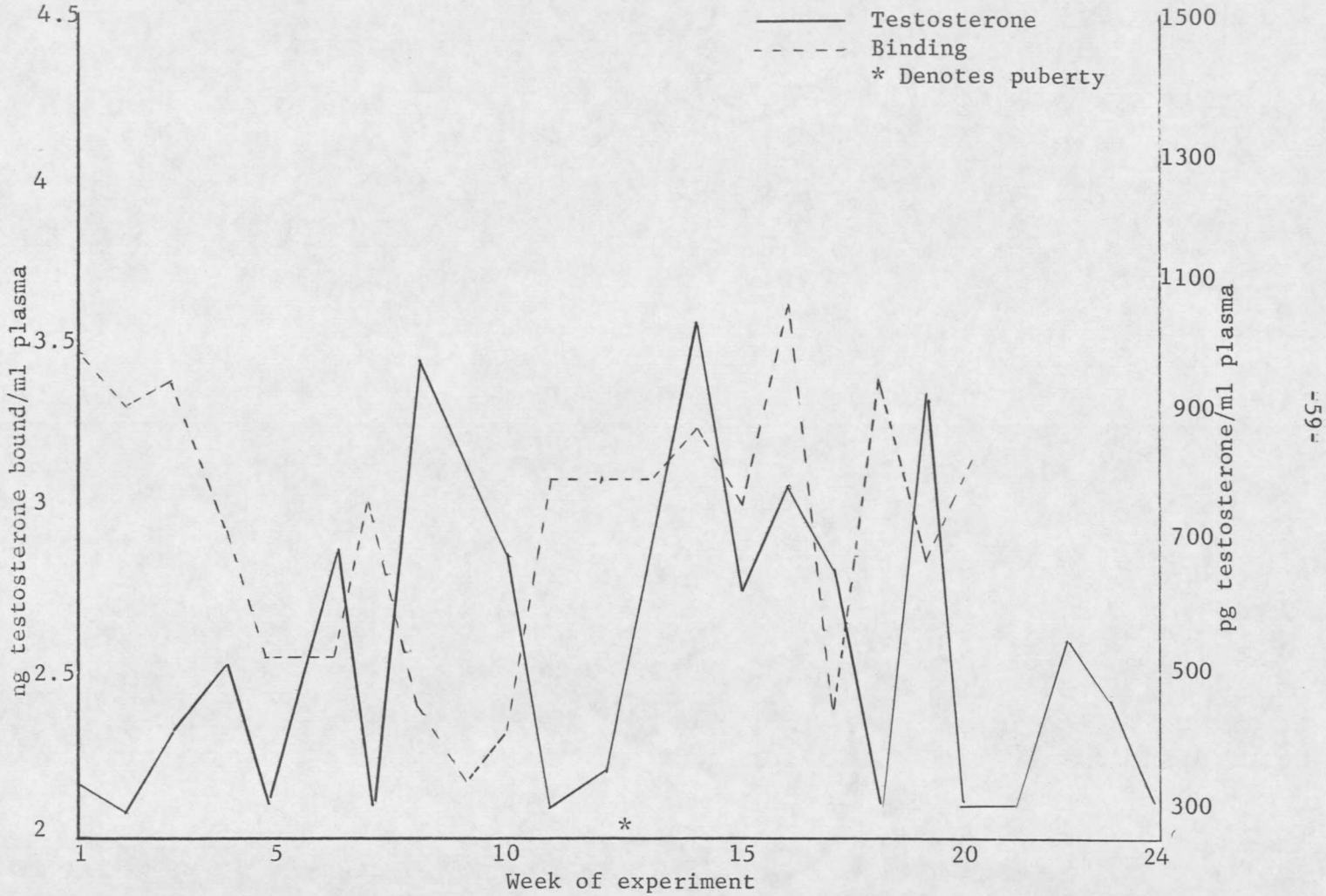


Figure 14. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 108 from 7-13 months of age.

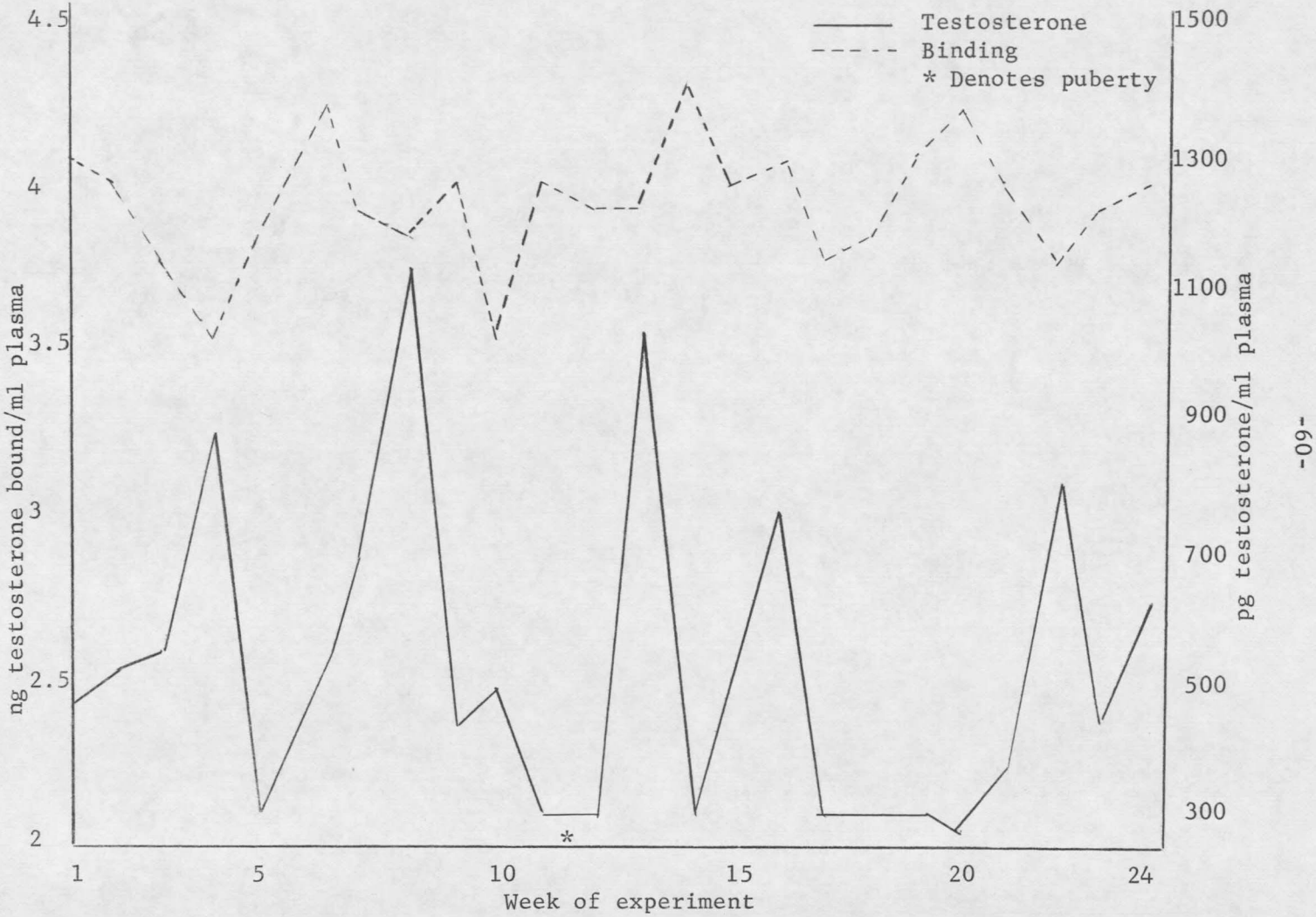


Figure 15. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 111 from 7-13 months of age.

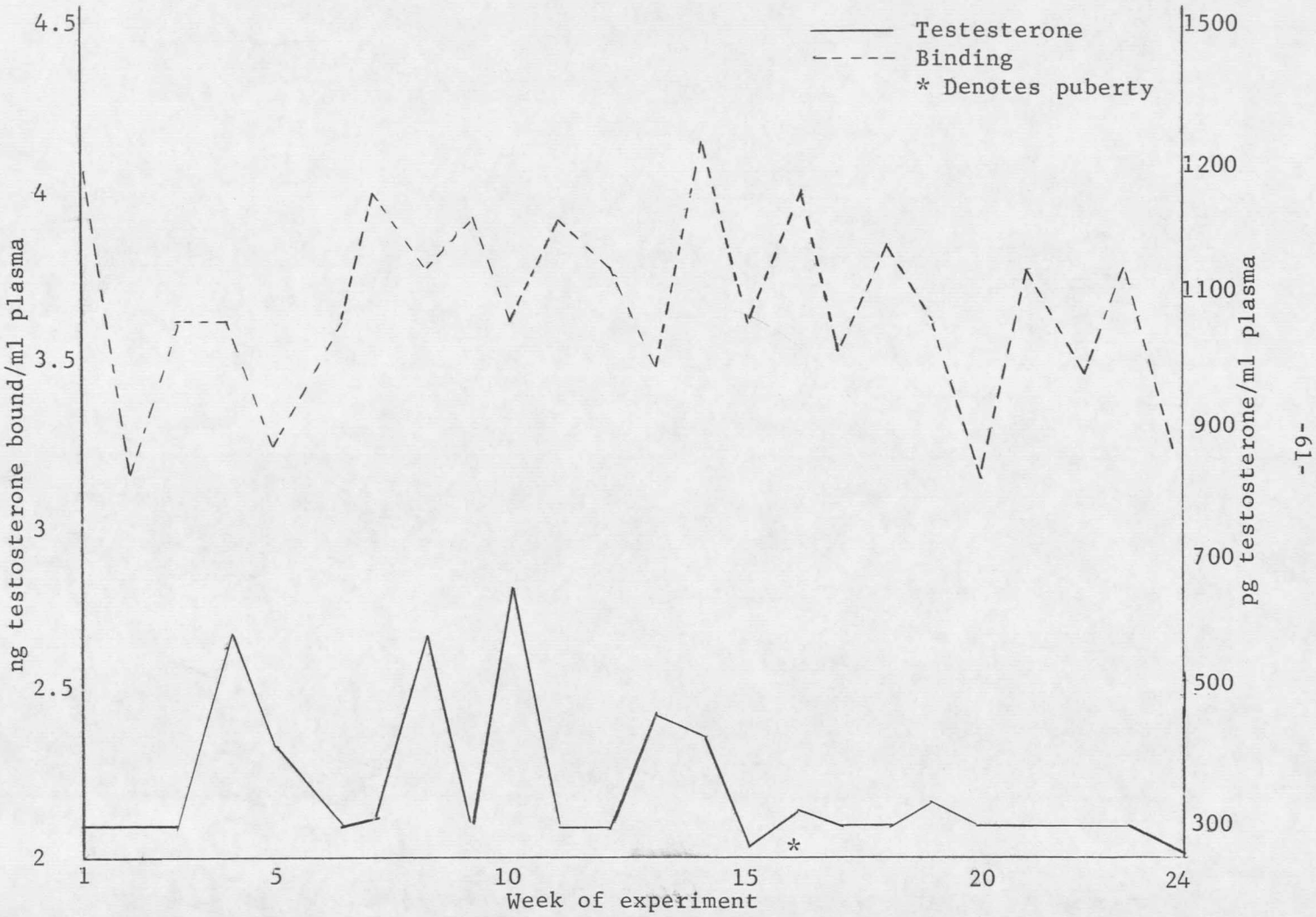


Figure 16. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 112 from 7-13 months of age.

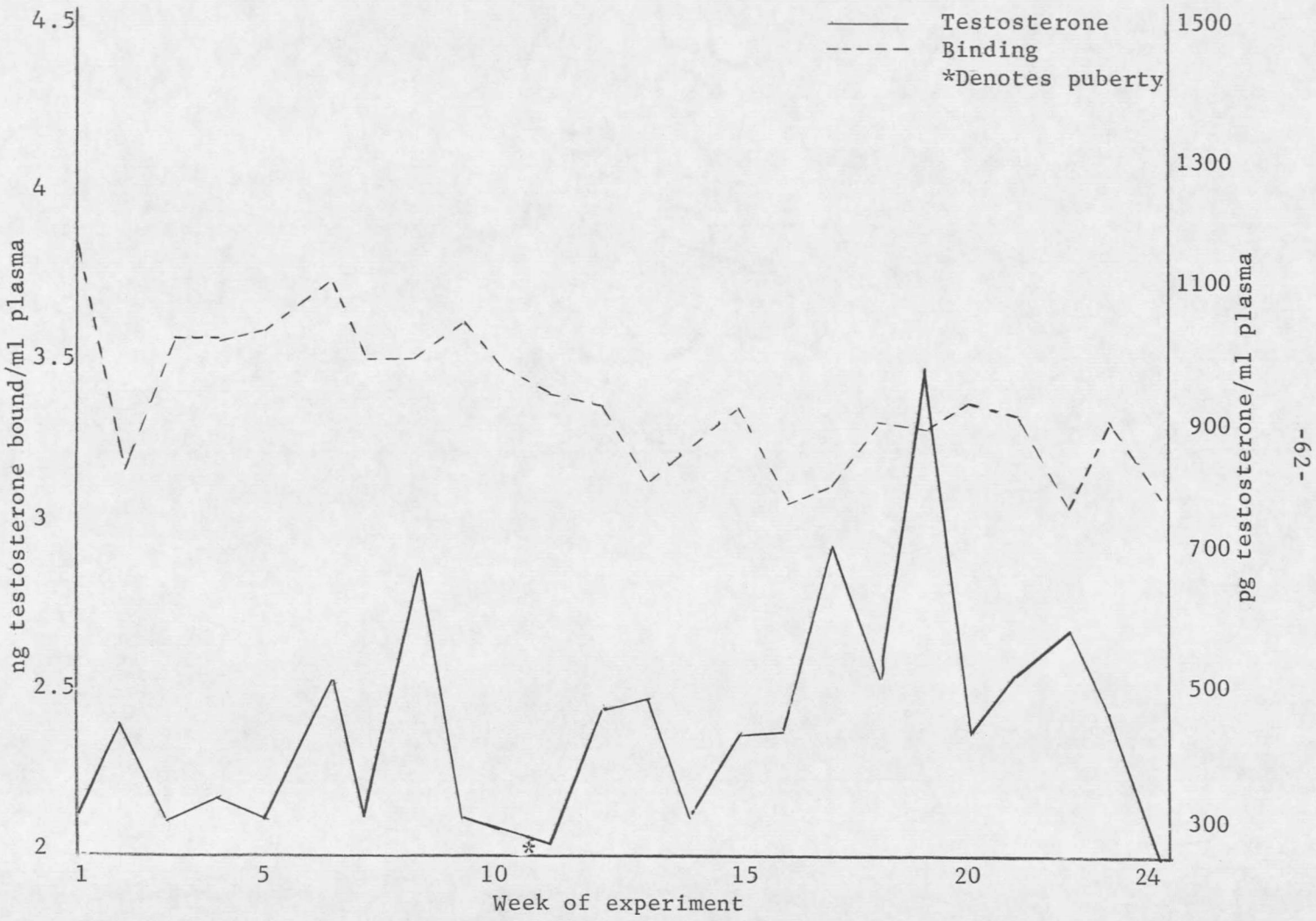


Figure 17. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 120 from 7-13 months of age.

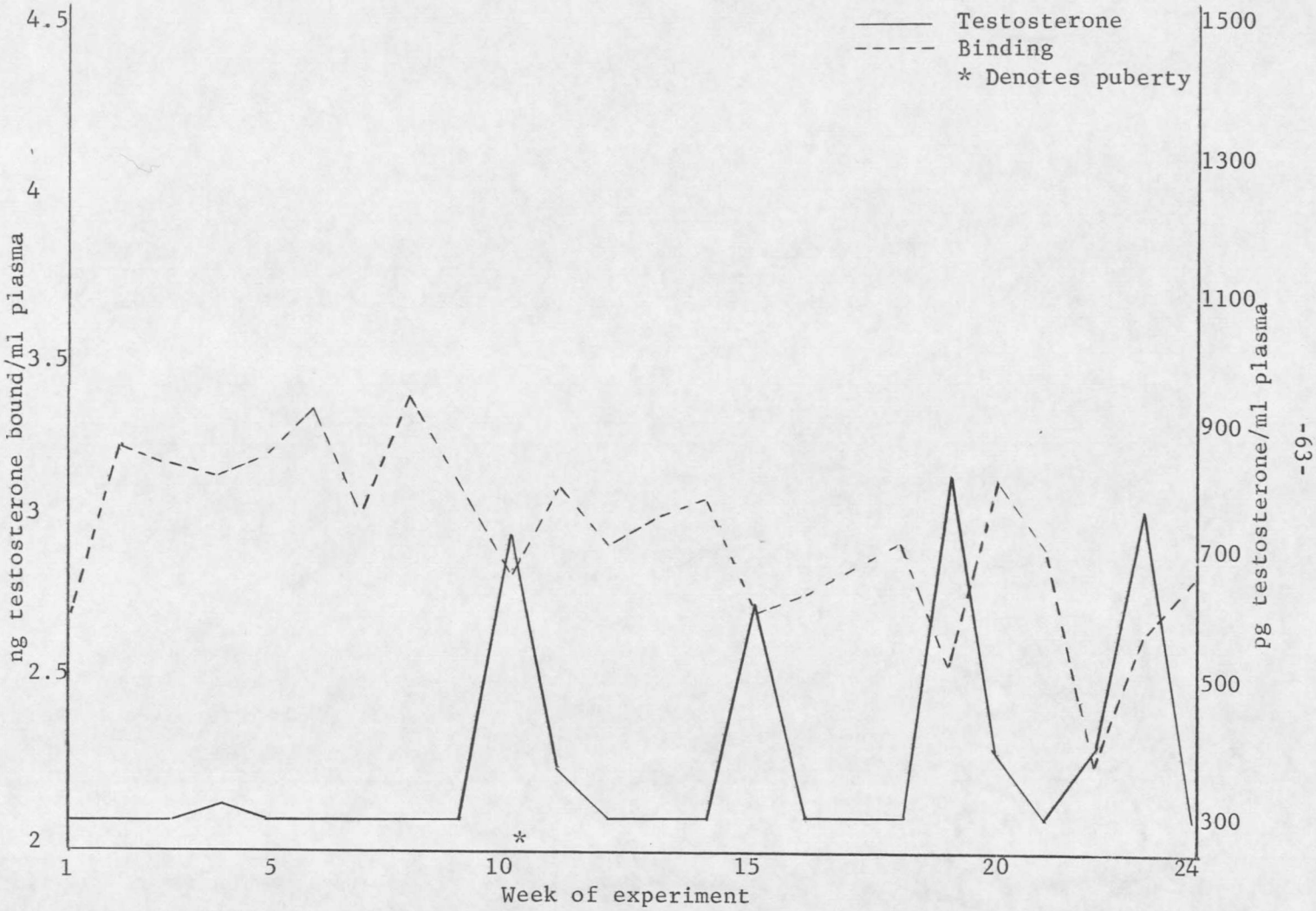


Figure 18. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 127 from 7-13 months of age.

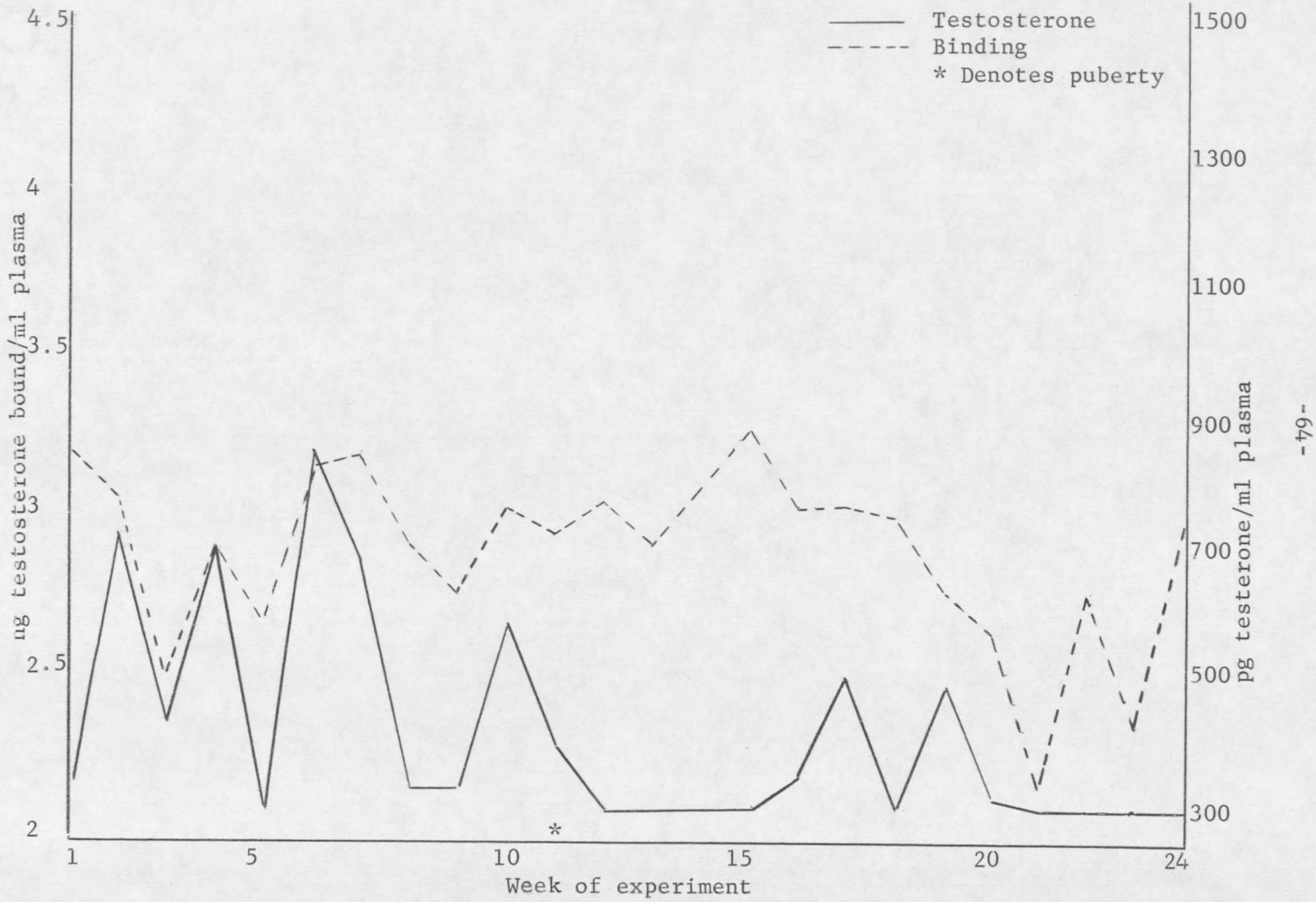


Figure 19. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 130 from 7-13 months of age.

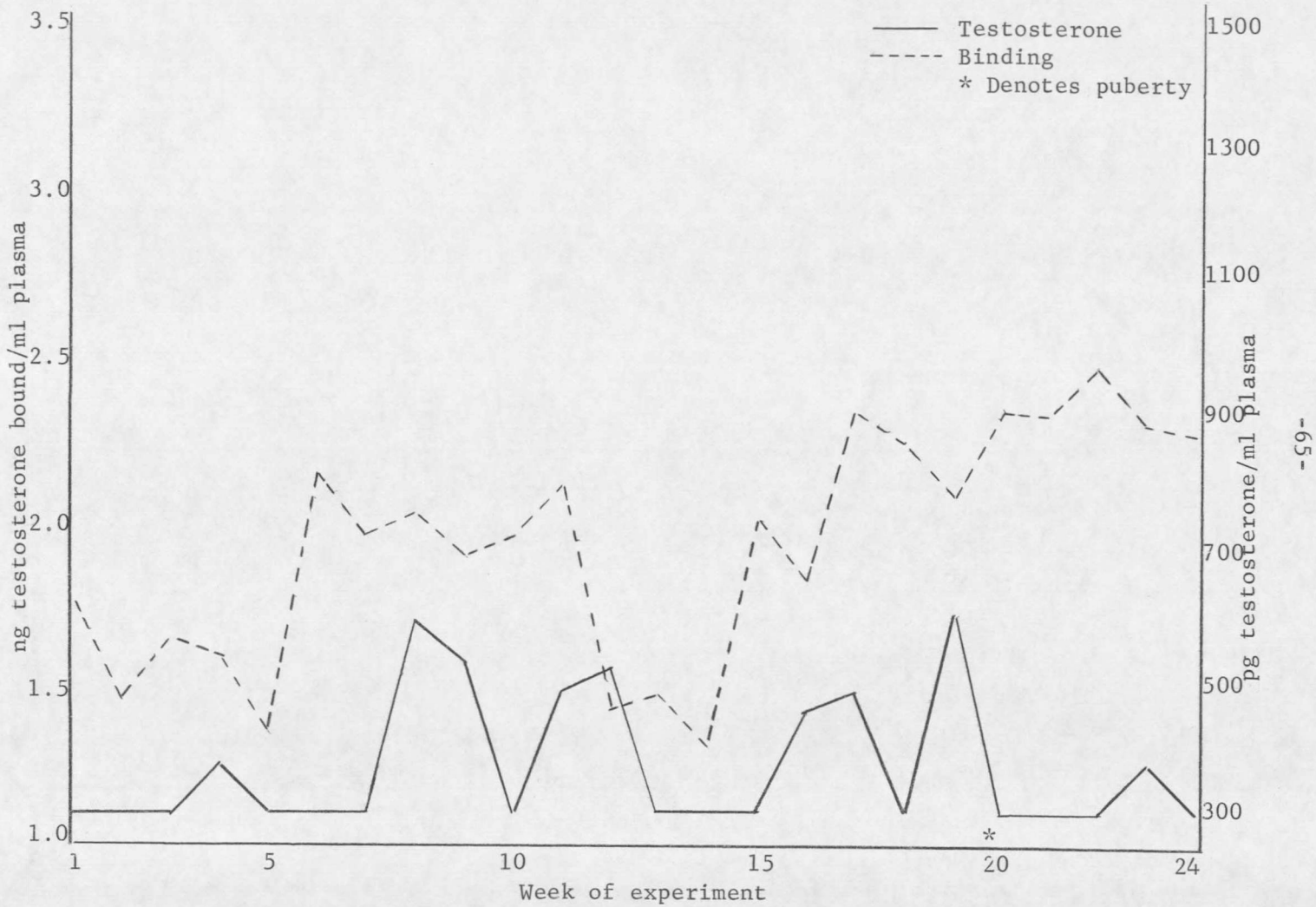


Figure 20. Plasma testosterone levels and levels of testosterone bound/ml of plasma in bull 134 from 7-13 months of age.

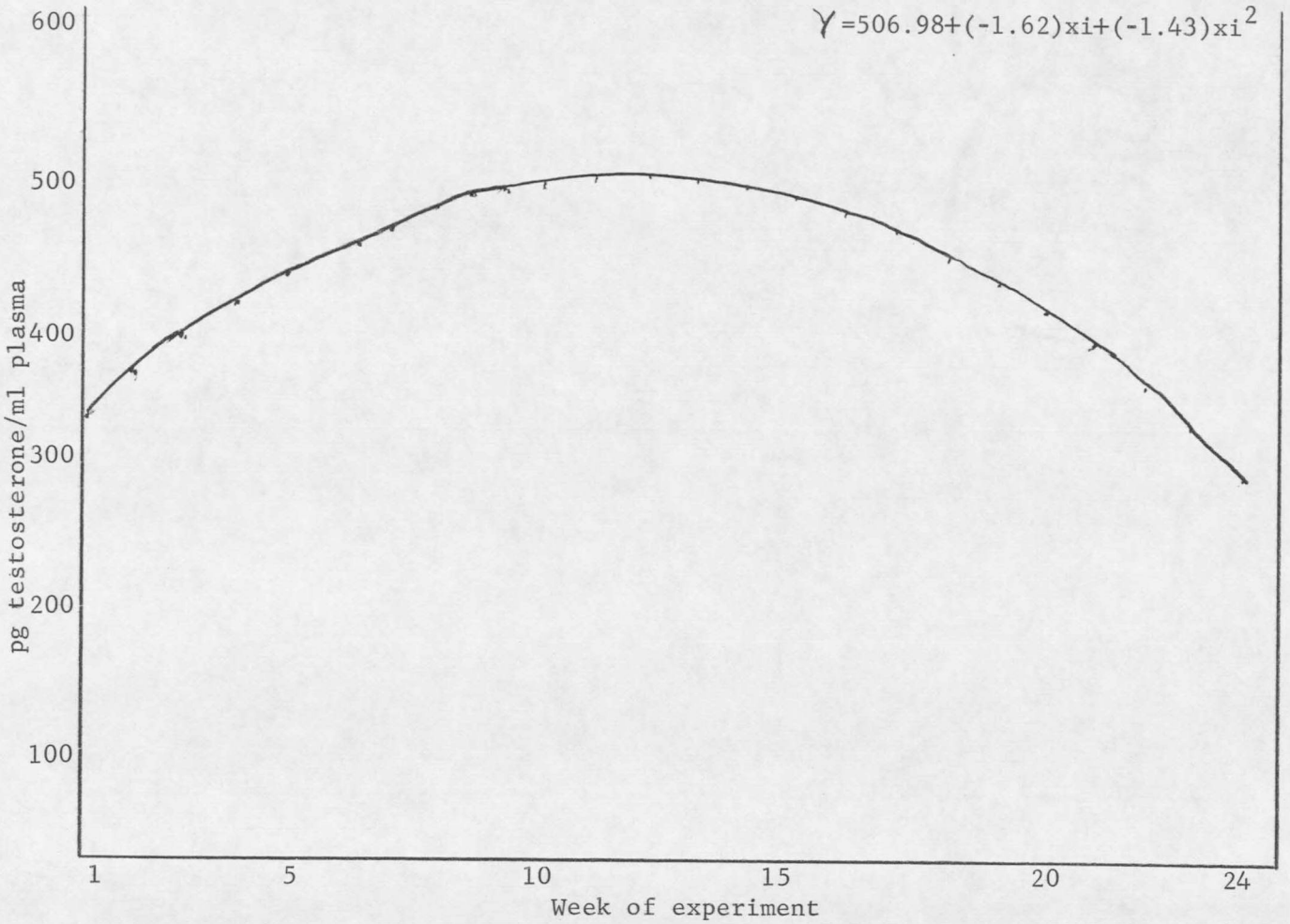


Figure 21. Quadratic regression of all 8 bulls testosterone levels over the 24-week period from 7-13 months of age.

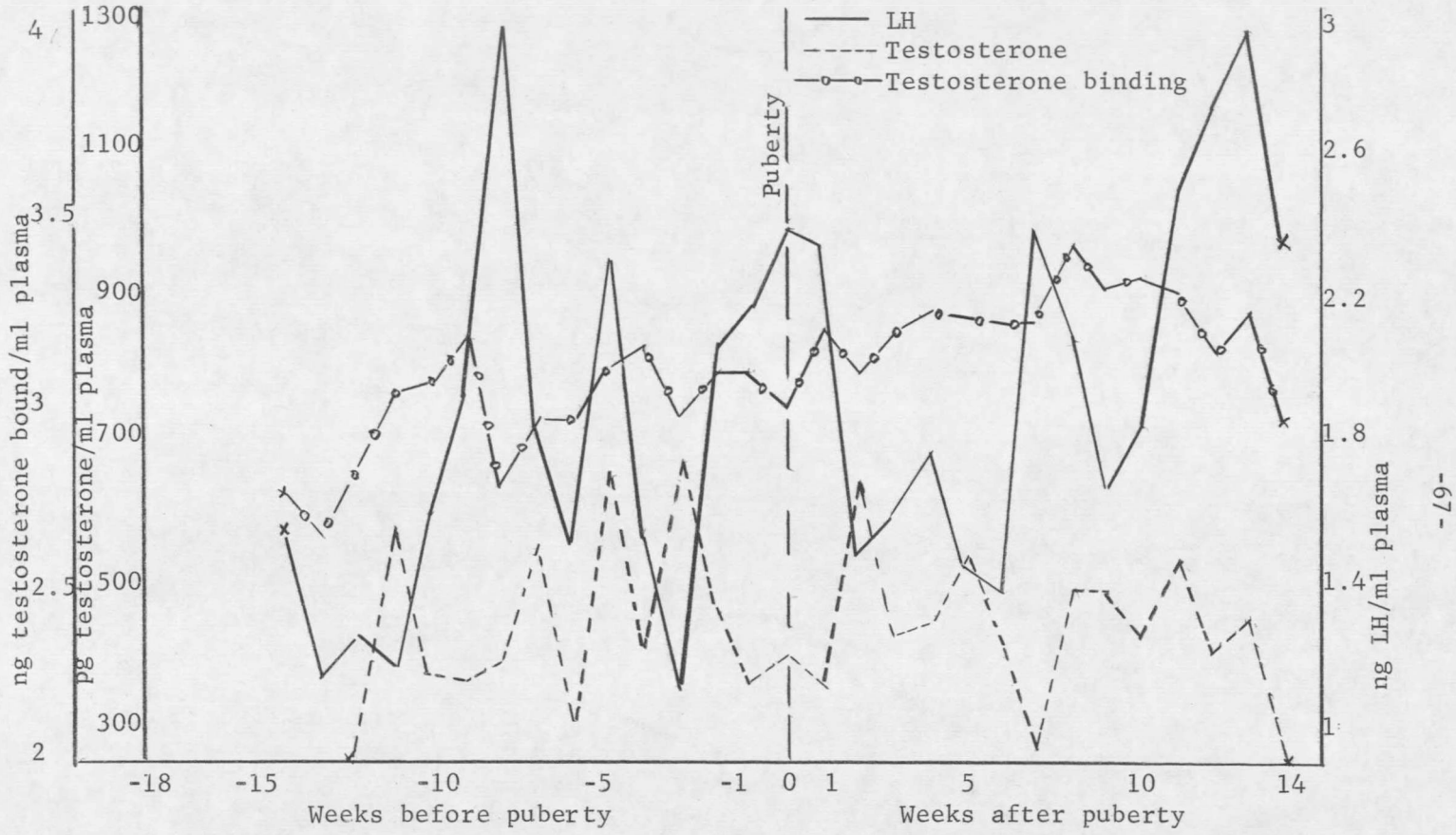


Figure 22. Relationship between LH, testosterone, and plasma testosterone binding for the average of all 8 bulls before and after puberty.

DISCUSSION

The term "puberty" in the male, is a rather abstract period of time during which sexual maturation is achieved. Since sexual maturation occurs over a prolonged period of time, puberty was defined as the first occurrence of a minimum $50x10^6$ total sperm count in an ejaculate (Wolf, Almquist, and Hale, 1965). This level of sperm production seemed to occur during a sharp increase which followed a low base line level of sperm production. Since total sperm numbers increased so rapidly at this time, it was not possible to specify the exact date at which puberty was attained.

Plasma LH

Plasma LH levels were determined by the double antibody radioimmunoassay technique developed by Niswender et al. (1969). The sensitivity of this assay was approximately 0.2 ng in 0.5 ml of plasma. In future studies with bull plasma it would be beneficial to try to increase the sensitivity of this assay because several samples were in the nondetectable range.

The bulls in this experiment were approximately 7 months old at the onset of the experiment which continued for 6 months. Weekly blood collections revealed, collectively, that an erratic but general linear (figure 10) increase in plasma LH levels occurred as the bulls advanced in age. This increase is apparent in Bulls 108, 111, 112, 127, and 130 (figures 2, 3, 4, 5, respectively). Bulls 107, 120, and 134 (figures 7, 8, 9) do not readily reveal an increase in plasma LH with advancing age,

however, high LH levels are apparent.

Rawlings, Hafs, and Swanson (1972) reported a biphasic increase in LH levels occurs in bulls from 2-5 months of age and at 11-12 months of age. This observation was based on monthly blood collection data.

Analysis of LH levels before and after puberty, separately, revealed that LH levels before puberty did not change with any regression significantly, and LH levels after puberty increased quadratically (figure 11). Thus it appears that LH levels until puberty are rather sporatic in nature then increase quite rapidly after puberty. This rapid increase after puberty seems to coincide with the increase in LH in bulls at 11-12 months reported by Rawlings, Hafs, and Swanson (1972). Kragt and Masken (1972) suggest that at puberty a decrease in the sensitivity to the negative feedback mechanism occurs. This would allow LH levels to increase at this time. Since puberty could not be defined exactly it is difficult to describe the relationship of LH levels to the exact time of puberty. However, it appears from the average LH levels at puberty (figure 22) that LH levels increase just prior to puberty and remain high until about 1 week after puberty. Kragt and Masken (1972) reported that in male rats at 40 days of age FSH increases three fold and LH two times. It was suggested that this increase corresponds to the onset of spermatogenesis. Swanson et al. (1971) observed an inverse relationship between pituitary FSH and puberty and suggested that a change in the FSH/LH ratio occurs at puberty. The measurement of

plasma FSH levels is needed to clarify what actually happens at this point.

The linear increase observed in the beef bulls (figure 13) over the entire 24-week period was not significant for the dairy bulls. In addition, when dairy and beef groups were analyzed separately, before and after puberty, the only significant regression found was a post-pubertal linear increase in the LH levels in the beef group (figure 12). These differences between beef and dairy bulls need more study to clarify whether the differences are due to the individual variation which would be seen with such small groups or are due to actual physiological differences between dairy and beef cattle. On the basis of this study it appears that beef bulls reach a steady level of LH secretion at an earlier age than dairy bulls. This difference could be subtly manifest in behavioral characteristics such as aggressiveness frequently associated with dairy bulls.

Testosterone RIA and Plasma Testosterone Levels

Plasma testosterone levels were determined by the radioimmunoassay technique developed by Hafs (1972). This method is rapid and simple, requiring three days (8 hours total working time) to process approximately 40 samples with the standard curve. The sensitivity, of about 20 pg/0.5 ml of extract, could probably be improved by using more refined solvents or by labeling the testosterone with an isotope with a greater specific activity and diluting the antibody to a greater degree.

The great advantage this assay has, in comparison to other methods, is that only a simple solvent extraction with no additional purification is required prior to the assay proper.

The high testosterone levels observed in Bull 107 (figure 13) seemed to be due to individual variation, since reruns on various samples gave similar results and Bull 108, whose samples were assayed in part with Bull 107, had much lower testosterone levels. Bull 112 (figure 16), with the lowest testosterone levels, had normal sperm concentrations. Further studies need to be initiated on these bulls to see if the differences in testosterone levels are reflected in other indicators of semen quality. Rawlings, Hafs and Swanson (1972) report a positive correlation exists between plasma testosterone concentration and seminal fructose concentration.

Plasma levels of testosterone (figures 13-20) tended to be erratic throughout the sampling period during which the bulls progressed from 7 to 13 months of age. There appears to be at least 2 prepubertal testosterone peaks which decline just prior to puberty. Postpubertal testosterone levels, in general, appear to increase soon after puberty then slowly decline (figure 22). Regression analysis of testosterone levels over the entire 24-week period defines testosterone levels as following a quadratic expression (figure 21). This is in agreement with the findings of Swanson et al. (1971) in which they reported an increase in testosterone levels until 10-12 months at which time they started to

decrease. This decrease in testosterone levels after puberty would appear to suggest that the need for testosterone decreases as the animal matures. However, Swanson et al. (1971) reported that although testosterone decreased from 10-12 months of age androstenedione decreased proportionally greater. Strickland (1970) stated that the ratio of testosterone:androstenedione, which changes from 1:1 at 4 months of age to 10:1 at 9 months of age, is what determines which androgen is dominant. It appears that testosterone dominance is more essential than actual plasma levels at puberty. Ramarez and McConn (1963) suggested that the onset of puberty in the female is related to a decrease in the sensitivity of the estrogen negative feedback mechanism. If such a mechanism exists for testosterone it would explain the reduced postpubertal testosterone levels in conjunction with increasing LH levels.

Regression analysis of testosterone levels obviously masks weekly changes in hormone levels. Visual observation of testosterone levels at puberty (figure 22), based on the average of the 8 bulls, shows that testosterone levels are low from 1 week before puberty until 1 week after puberty. There was no significant regression when testosterone levels were analyzed collectively or in groups of beef and dairy bulls before and after puberty. This lack of significant regressions before a and after puberty is probably due to the erratic testosterone levels observed.

Testosterone Binding In Plasma

The amount of testosterone that could be bound by bull plasma was determined by a simple saturation technique. The excess unbound testosterone was removed by absorption to Florisil after a specified incubation period.

The amount of testosterone that was bound by 1 ml of plasma is illustrated in figures 13-20. Levels changed erratically from week to week but did not follow any regression significantly over the 24-week period. Plasma testosterone binding ability did not seem to change in regard to an overall increase or decrease in binding ability at the two endpoints.

Ramaley (1971) reported that an increase in testosterone binding ability was seen at puberty in rats, this increase soon returned to the previous plateau level after puberty. This bull data, when examined before and after puberty, revealed that testosterone binding ability increased linearally ($P < .05$) (figure 11) until puberty then did not follow any regression significantly. Visual exmaination of the average testosterone binding levels (figure 22) does not reveal any marked increase in testosterone binding levels near puberty, although a general increase in testosterone binding does seem to be apparent.

A confusing factor in these observations is that the beef testosterone binding levels did not follow any significant regression before or after puberty while the dairy bulls binding levels increased

linearally both before and after puberty (figure 12) ($P < .05$). Perhaps this is indicative of the difference in aggressiveness between beef and dairy bulls. Whalen (1971) related testosterone levels and aggressiveness in rats.

Intrahormonal and Plasma Testosterone Binding Ability Relationships

LH levels and testosterone levels were negatively correlated ($r=-.2302$, $P<.01$) throughout the 24-week period. Analysis before and after puberty revealed a negative LH:testosterone correlation ($r=-.3446$, $P<.01$) after puberty but not before puberty. The beef and dairy groups had a positive LH:testosterone correlation ($r=.3336$, $P<.05$) before puberty for the beef bulls and a negative LH:testosterone correlation ($r=-.4707$, $P<.01$) after puberty for the dairy bulls. Katongole, Naftolin and Short (1971) demonstrated that elevated plasma testosterone follows elevated plasma LH by about 30 minutes in bulls. Thus a negative correlation between LH and testosterone after puberty could be indicative of a testosterone negative feedback mechanism, whereby LH caused the production of testosterone precursor (Dorfman, 1972). Swanson et al. (1971) reported a positive relationship ($r=.18$ to $.29$) between LH and testosterone in bulls 10-12 months of age, however, it was not significant. Skinner, Mann, and Rowson (1968) suggested that androstenedione may be a more potent inhibitor of LH release than testosterone. The shift in the androstenedione:testosterone ratio, which occurs near puberty, could

explain the shift from a positive LH:testosterone correlation before puberty to a negative LH:testosterone correlation after puberty. It would appear that androstenedione, or an earlier precursor, acts to decrease LH release avoiding the detection of a lag period. It is apparent that the overall 24-week LH:testosterone correlation would be negative because there were more postpubertal observations present in this study than prepubertal observations.

LH was positively correlated ($r=.2302$, $P<.01$) with plasma testosterone binding ability over the 24-week period and before puberty ($r=.3683$, $P<.01$) but not after puberty. Dorfman (1972) summarized the effects of LH as causing an increase in testosterone precursors, notably pregnenolone, which result in an increased androgen and estrogen production. This, coupled with the observation that testosterone is not significantly correlated with plasma testosterone binding ability at any time, suggests that testosterone precursors produced by LH stimulation affect the plasma testosterone binding ability.

SUMMARY

Eight bulls, 7 months of age at the onset of the experiment, were bled weekly for six months by jugular venipuncture. Blood samples were heparinized, centrifuged, and the plasma was frozen until assayed.

Puberty, defined as the first occurrence of a minimum $50x10^6$ total sperm, was determined by weekly electroejaculation as a portion of a separate experiment.

LH and testosterone levels were determined by RIA. Plasma testosterone binding ability was determined by a saturation technique which utilized florisil absorption to separate bound and unbound testosterone.

The results were analyzed by least-squares analysis for correlations and polyorthogonal regressions. The correlations between hormones and regression for each hormone was examined over the entire 24-week period and separately, before and after puberty. In addition the differences between the 4 beef bulls and 4 dairy bulls were examined.

LH levels tended to increase erratically until one week before puberty when there appears to be high LH levels until one week after puberty. It was suggested that this LH increase at puberty is associated with a change in the FSH/LH ratio which occurs at this time. Postpubertal LH levels, which increased quadratically ($P<.05$), seem to increase more rapidly than prepubertal LH levels. This increased rate of LH release could be due to a decrease in the sensitivity of the

testosterone negative feedback mechanism.

The quadratic regression ($P<.05$), which defined plasma testosterone levels over the 6-month period, supports the concept of an erratic but general increase in testosterone levels as puberty approaches decline in plasma testosterone levels. Low or nondetectable plasma testosterone levels appear to occur one week prior to and after puberty.

The plasma testosterone binding ability, although erratic, did not appear to increase or decrease significantly with regards to plasma testosterone binding ability present at the start and termination of the experiment. A linear increase in plasma testosterone binding ability was observed before puberty ($P<.05$).

LH and testosterone levels were negatively correlated ($P<.01$) throughout the 24-week period but were positively correlated ($P<.05$) before puberty and negatively correlated ($P<.01$) after puberty. This shift from a prepubertal positive correlation to a postpubertal negative correlation could be due to the shift in androstenedione: testosterone ratio which occurs at puberty. The change in the testosterone:androstenedione ratio at puberty appears to be associated with a more efficient conversion of testosterone precursors to testosterone after puberty.

LH was positively correlated ($P<.01$) with plasma testosterone binding ability before puberty while testosterone and plasma testosterone binding ability were not correlated at any time. This suggests that other androgenic compounds present affect the plasma testosterone binding ability.

APPENDIX

APPENDIX TABLE 1. PLASMA LH LEVELS (ng/ml plasma)

Date	Week No.	Bull 107	Bull 108	Bull 111	Bull 112	Bull 120	Bull 127	Bull 130	Bull 134
		ng	ng	ng	ng	ng	ng	ng	ng
12/16/71	1	1.15	*	2.7	2.6	1.9	.95	2.8	*
12/23/71	2	1.95	*	1.25	1.8	2.5	3.65	2.2	*
12/30/71	3	2.75	*	2.1	2.0	.95	.55	2.5	*
1/7/72	4	2.35	*	2.95	2.5	3.65	1.5	3.3	*
1/14/72	5	2.00	*	.95	1.8	1.05	.65	*	*
1/24/72	6	1.85	.7	3.6	4.3	4.7	1.1	3.6	*
1/28/72	7	*	.7	2.0	2.4	1.0	*	*	*
2/6/72	8	1.0	*	1.9	3.35	5.5	1.55	2.5	.55
2/13/72	9	*	*	2.2	2.25	3.1	1.05	3.2	*
2/19/72	10	2.25	*	1.9	2.45	5.75	2.2	4.5	2.3
2/26/72	11	2.2	*	1.9	2.45	2.65	3.3	2.8	7.0
3/5/72	12	*	*	1.0	1.45	2.2	1.75	2.7	*
3/12/72	13	.95	.95	1.0	2.5	1.95	1.85	2.0	*
3/19/72	14	1.0	*	1.0	3.55	2.05	2.3	*	4.4
3/26/72	15	*	*	2.0	3.3	.95	1.0	*	*
4/2/72	16	*	*	1.4	2.6	.60	2.7	2.05	*
4/9/72	17	*	*	1.75	2.95	5.25	6.0	*	*
4/16/72	18	*	*	2.8	2.0	2.0	3.25	*	*
4/23/72	19	.6	.65	1.7	4.3	1.75	3.1	7.45	*
4/30/72	20	*	*	2.5	2.15	2.0	3.95	*	5.0
4/7/72	21	*	*	3.5	3.0	3.0	2.5	*	*
5/15/72	22	*	*	2.9	1.95	2.15	2.5	4.2	3.95
5/21/72	23	2.2	1.75	2.6	2.9	2.0	3.4	4.5	1.9
5/29/72	24	1.45	2.4	3.55	2.7	3.1	1.6	4.75	3.0

* Denotes nondetectable levels. Values of .5 ng for analysis were used for these points.

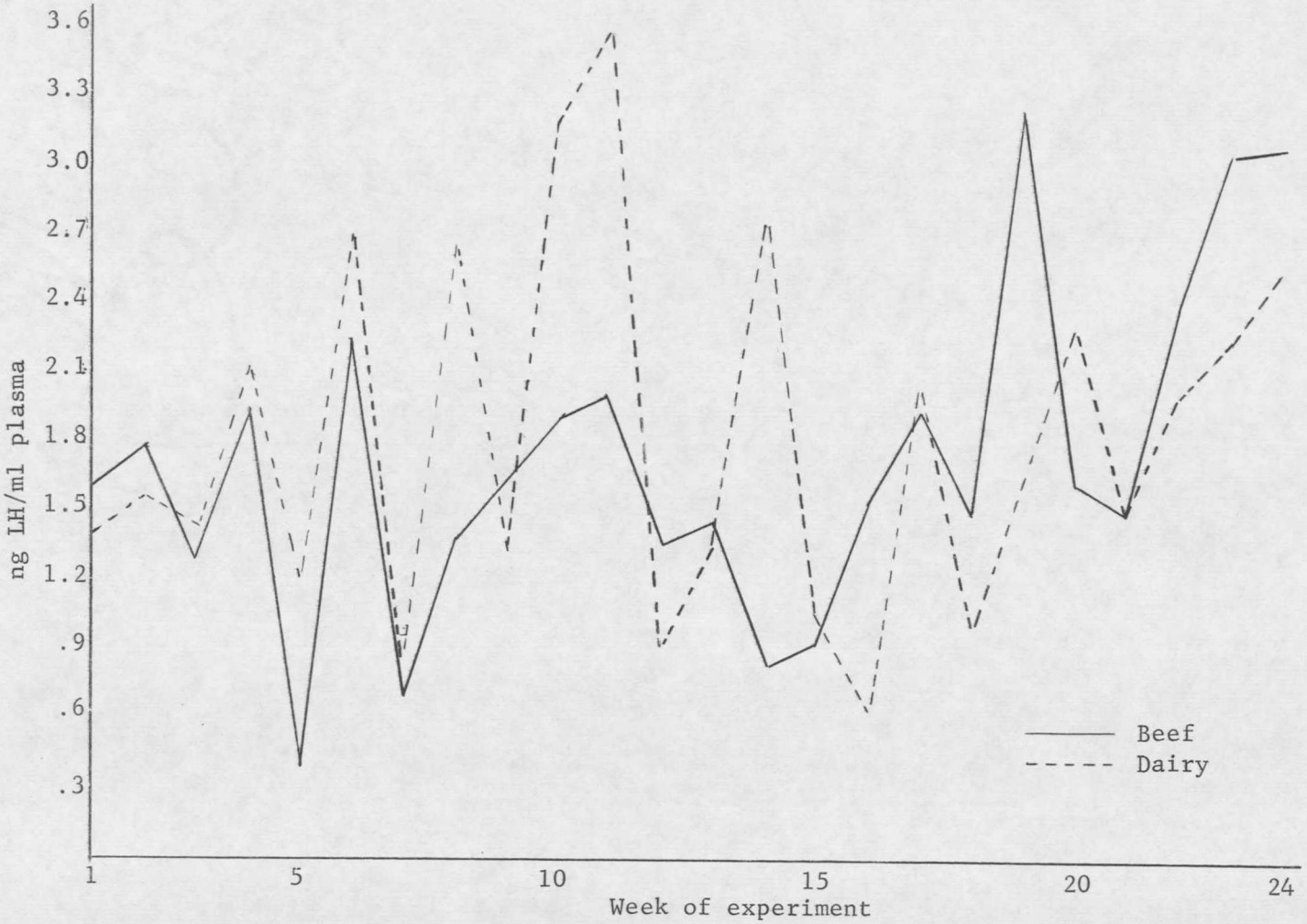
APPENDIX TABLE 2. PLASMA TESTOSTERONE LEVELS (pg/ml)

Date	Week No.	Bull 107	Bull 108	Bull 111	Bull 112	Bull 120	Bull 127	Bull 130	Bull 134
		pg	pg	pg	pg	pg	pg	pg	pg
12/16/71	1	389	329	463	*	*	296	*	*
12/23/71	2	689	292	510	*	448	*	716	250
12/30/71	3	397	408	540	*	*	*	426	*
1/7/72	4	915	559	877	583	336	322	691	372
1/14/72	5	1013	*	*	415	*	284	*	*
1/24/72	6	1876	674	526	*	510	*	849	*
1/29/72	7	740	*	687	303	306	*	670	265
2/6/72	8	1906	973	1114	580	678	*	331	590
2/13/72	9	427	802	430	*	*	*	331	524
2/19/72	10	296	685	484	651	295	727	582	*
2/26/72	11	671	*	*	*	261	372	399	481
3/5/72	12	1208	355	*	*	466	*	*	512
3/12/72	13	2351	722	1023	469	483	*	*	*
3/19/72	14	1012	1031	*	433	306	*	270	252
3/26/72	15	962	622	509	222	427	623	278	251
4/2/72	16	1066	779	752	319	434	*	348	451
4/9/72	17	826	654	*	*	717	*	503	478
4/16/72	18	328	*	*	*	518	*	273	*
4/23/72	19	1043	926	*	335	990	818	488	601
4/30/72	20	680	*	273	*	430	393	311	*
5/7/72	21	1179	*	373	*	520	*	*	*
5/15/72	22	1015	457	803	*	598	413	*	*
5/21/72	23	457	360	437	*	468	763	*	370
5/29/72	24	205	*	622	*	*	*	*	*

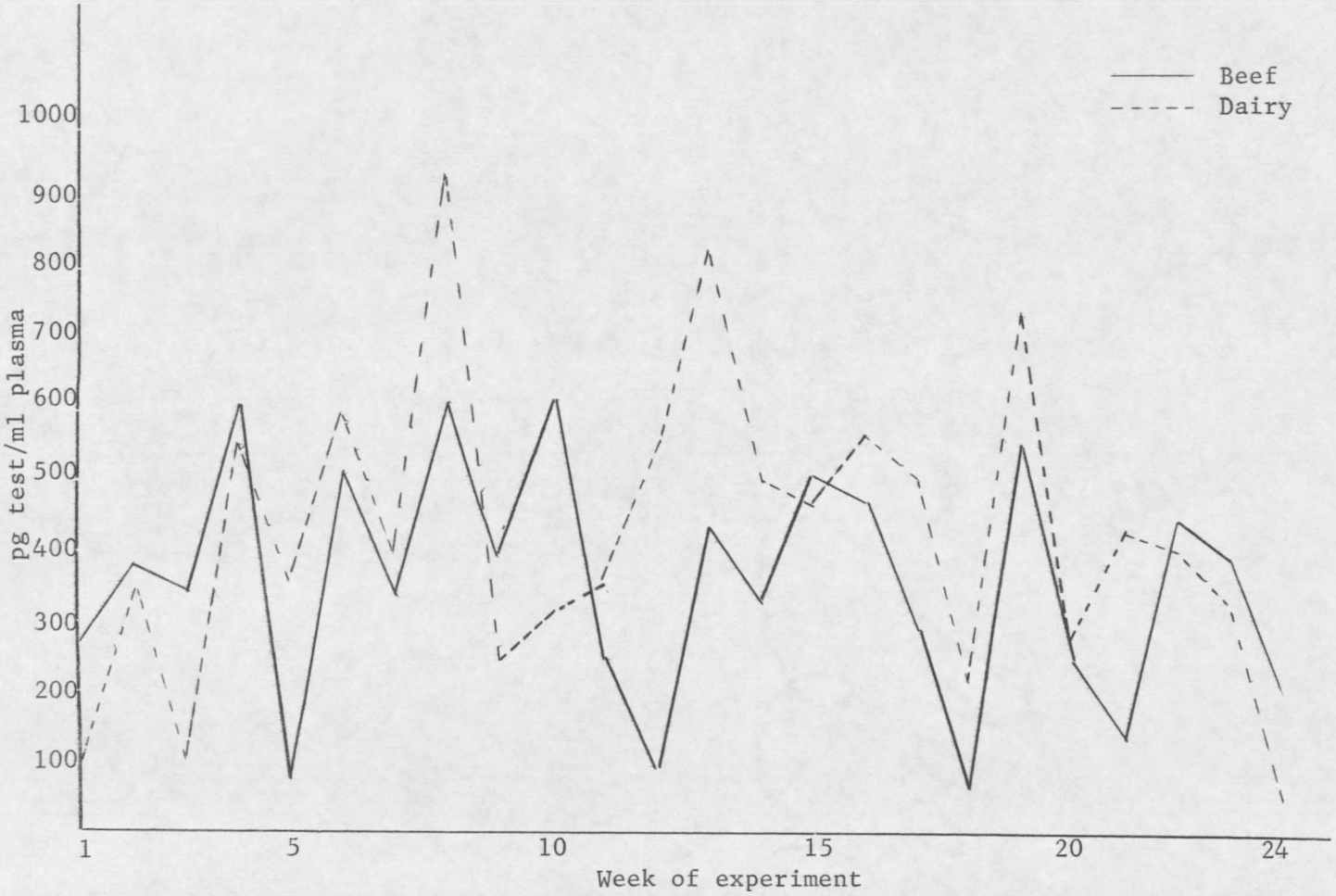
* Denotes nondetectable levels. Values of 150 pg, for analysis, were used for these points.

APPENDIX TABLE 3. PLASMA TESTOSTERONE BINDING LEVELS (ng/ml).

Date	Week No.	Bull 107	Bull 108	Bull 111	Bull 112	Bull 120	Bull 127	Bull 130	Bull 134
		ng	ng	ng	ng	ng	ng	ng	ng
12/16/71	1	2.62	3.47	4.08	4.16	3.86	2.71	3.20	1.76
12/23/71	2	2.77	3.31	4.01	3.16	3.17	3.23	3.05	1.43
12/30/71	3	3.01	3.39	3.78	3.62	3.57	3.18	2.50	1.62
1/7/72	4	2.77	2.93	3.54	3.62	3.56	3.14	2.90	1.57
1/14/72	5	2.47	2.54	3.85	3.24	3.58	3.20	2.68	1.34
1/24/72	6	2.70	2.54	4.24	3.62	3.73	3.35	3.14	2.13
1/29/72	7	2.77	3.01	3.93	4.01	3.50	3.02	3.18	1.94
2/6/72	8	2.62	2.39	3.85	3.78	3.51	3.39	2.91	2.01
2/13/72	9	2.85	2.16	4.01	3.93	3.61	3.11	2.75	1.88
2/19/72	10	2.31	2.31	3.54	3.62	3.46	2.85	3.02	1.94
2/26/72	11	2.00	3.08	4.01	3.93	3.39	3.12	2.94	2.10
3/5/72	12	2.77	3.08	3.93	3.78	3.36	2.93	3.04	1.41
3/12/72	13	2.54	3.08	3.93	3.47	3.12	3.02	2.91	1.46
3/19/72	14	2.70	3.24	4.31	4.16	3.23	3.07	3.08	1.30
3/26/72	15	2.85	3.01	4.01	3.62	3.36	2.73	3.26	2.00
4/2/72	16	2.93	3.62	4.08	4.01	3.07	2.78	3.02	1.81
4/9/72	17	2.77	2.39	3.78	3.54	3.11	2.86	3.03	2.33
4/16/72	18	3.47	3.39	3.85	3.85	3.31	2.94	2.99	2.23
4/23/72	19	4.02	2.85	4.08	3.62	3.29	2.55	2.77	2.07
4/30/72	20	3.59	3.16	4.24	3.16	3.36	3.13	2.54	2.33
5/7/72	21	3.9	3.68	4.01	3.78	3.33	2.92	2.16	2.32
5/15/72	22	3.59	3.47	3.78	3.47	3.05	2.25	2.76	2.47
5/21.72	23	3.78	3.47	3.93	3.78	3.32	2.65	2.35	2.29
5/29/72	24	3.36	3.47	4.01	3.24	3.08	2.82	2.97	2.26



Appendix Figure 1. Plasma levels of LH (ng/ml plasma) in beef and dairy bulls from 7-13 months of age.



Appendix Figure 2. Plasma testosterone levels (pg/ml of plasma) in beef and dairy bulls from 7-13 months of age.

LITERATURE CITED

Abraham, G. E. 1969. Solid phase RIA of estradiol-17β. J. Clin. Endo. 29:866.

Almquist, J. O. and D. C. Cunningham. 1965. Reproductive capacity of beef bulls. I. Postpubertal changes in semen production at different ejaculation frequencies. J. Anim. Sci. 26:174.

Apostolakes, Michael and J. A. Loraine. 1967. Pituitary Gondotrophins In, Hormones in Blood. Gary and Bacharach, Academic Press, N.Y. 1:273.

Baranas, S. 1969. Effect of nutrition and androgens on composition of bovine blood plasma and seminal plasma at puberty. Brit. J. Nutr. 23:191.

Bartke, A. and C. W. Lloyd. 1970. Influence of prolactin and pituitary isografts on spermatogenesis in dwarf mice and hypophysectomized rats. J. Endo. 46:321.

Baulieu, E. E., J. P. Raynaud and E. Milgrom. 1970. Measurement of steroid binding proteins. In. Steroid Assay by Protein Binding. Karolinska Symposia on Res. Methods. in Repro. Endo. 2:104. Stockholm.

Berson, S. A., and R. S. Yalow. 1964. The Hormones. Pincus, Thermann, and Astwood, Academic Press, N.Y. 4:557.

Birnbaumer, L., Stephen L. Pohl, and Martin Rodbell. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. J. Biol Chem. 246:1867.

Bloch, G. J., C. L. Kragt, and J. F. Masken. 1971. Plasma LH levels in male rats of various ages: Effects of castration and treatment with testosterone proprionate (T.P.). Fed. Proc. 30:475 (Abstr.).

Bogdonove, E. M., Neena B. Schwartz, L. E. Riechert, Jr. and A. R. Midgley, Jr. 1971. Comparisons of pituitary: Serum LH ratios in the castrated rat by radioimmunoassay and OAAD bioassay. Endo. 88:644.

Bonner, J. 1965. The template activity of chromatin. I. Cell. and Comp. Physiol., 66:77.

Breuer, C. B. and J. R. Florine. 1966. Effects of ammonium sulfate, growth hormone and testosterone propionate on ribonucleic acid polymerase and chromatin activities in rat skeletal muscles. Biochem. 5:3857.

Byrnes, W. W., R. K. Meyer. L951. The inhibition of gonadotropic hormone secretion by physiological doses of estrogen. Endo. 48:133. (Kargt, 1972).

Catt, K. J. 1967. Solid-phase RIA in Ab-coated tubes. Science 158:1570.

DeLa, Penna, Amando and J. W. Goldzieher. 1971. Separation of free and bound steroid in the competitive protein binding assay for testosterone: Accuracy and Reproductability. Steroids. 18:195.

Dorfman, Ralph I. 1972. Mechanism of action of gonadotropins and prolactin. In: Biochemical Actions of Hormones. Gerald Litwack. Academic Press, N. Y. Vol. II:296.

Ely, Charles A. and Beli-Loo Chen. 1966. Immunological studies of the antigenic components of sheep lutenizing hormone and the development of a serologically specific antiserum. Endo. 79:362.

Farnsworth, Wells E. 1971. Androgen stimulation of prostatic cation-dependent ATP-ASE. In: The Biochemistry of Steroid Hormone Action. Biochem. Soc. Symp. #32. Smellie, Academic Press, N. Y.

Finkelstein, M., Enrico Forchielli and R. I. Dorfman. 1961. Estimation of testosterone in human plasma. J. Clin. Endo. and Met. 21:98.

Frick, J. and F. A. Kincl. 1969. The measurement of plasma testosterone by CPBA. Steroids. 134.

Furuyama, Shunsuke, D. M. Mayes, and C. A. Nugent. 1970. A radio-immunoassay for plasma testosterone. Steroids. 16:415.

Goding, J. R., K. J. Catt, J. M. Brown, C. C. Kaltenbach, I. A. Cumming, B. J. Mole. 1969. Radioimmunoassay for ovine lutenizing hormone. Secretion of LH during estrus and following estrogen administration in sheep. Endo. 85:133.

Goswami, A., J. K. Skipper and W. L. Williams. 1968. Stimulation of fatty acid synthesis in vitro by gonadotropin induced testicular RNA. Biochem. J. 108:147.

Greenwood, F. C., and W. M. Hunter. 1963. The reparation of ^{131}I labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114.

Gudzenowicz, Benjamin J. 1967. Gas chromatographic analysis of drugs and pesticides. Marcel Dekker, Inc. N. Y.

Hafez, E. S. E. 1968. Reproduction in Farm Animals. (2nd Ed.), Lea and Ferbinger. Philadelphia.

Hafs, H. D. 1972. Personal communications.

Hollandbeck, R., B. Baker, Jr., H. W. Norton, and A. V. Nalbandov. 1956. Gonadotrophic hormone content of swine pituitary glands in relation to age. J. Anim. Sci. 15:418.

Hoogstra, M. J. and F. J. A. Paesi. 1966. A comparison between the FSH and ICSH contents of the hypophysis of adult and immature rats. Acta Physiol. Pharm. Neerl. 4:395. (Kragt and Maskew, 1972).

Hoogstra, M. J. and S. E. Dejongh. 1954. The effect of oestradiol-benzoate on the release of gonadotropens by the anterior pituitary following hemicastration. Acta Physiol. Pharm. Neerl. 3;465.

Hopkins, T. F. and G. Pincus. 1963. Effects of reserpine on gonadotropin-induced ovulation in immature rats. Endo. 73:775.

Hopkins, T. F. and J. A. Clayton-Hopkins. 1970. Gonadotropin-enhancing factors in rat cerebral tissue fractions. II. Continuation of studies of their biological activity. Endo. 86:843.

Horton, R., T. Kato, and R. Sherins. 1967. A rapid method for the estimation of testosterone in male plasma. Steroids. 10;245.

Inano, H., Y. Hori and B. Tamaoki. 1967. Effection of age on testicular enzymes related to steroid bioconversion. Ciba Foundation Colloquial on Endo. 16:105.

Inskeep, E. K., C. E. Johnson, J. E. McNary and P. F. Hall. 1967. Variations in Biosynthesis of progesterone and response to ICSH in vitro in bovine corpora lutea. J. Anima. Sci. 26:540.

Ismail, Adel A., Gordon D. Niswender and A. Rees Midgley, Jr. 1972. RIA of testosterone without chromatography. J. Clin. Endo. 34:177.

Kalvert, M. and E. Bloch. 1968. Conversion of 4 ^{14}C-dehydroepiandrosterone to estrone and 17β-estradiol by the rat ovary with observations on variations during the estrous cycle. Endo. 82:1021.

Kar, A. B. 1966. Effect of nonprimate gonadotropins on testes of prepubertal Rhesus monkeys. Acta Biol. Med. Gen. 16:450.

Katongole, C. B. 1971. A competitive protein-binding assay for testosterone in the plasma of the bull and ram. J. Endo. 51:303.

Kitay, J. and M. D. Altschule. 1954. The Pineal Gland. Harvard Univ. Press, Cambridge.

Knorr, D. W. T., Vanha-Perttula and M. B. Lipsett. 1970. Structure and function of rat testis through pubescence. Endo. 86:1298.

Korenman, S. G., H. Wilson and M. B. Lipsett. 1963. Testosterone production rates in normal adults. J. Clin. Invest. 42:1753.

Kragt, C. L., and J. F. Masken. 1972. Puberty-physiological mechanisms of control. X. Biennial symposium on animal reproduction. J. Anim. Sci. 34: Suppl. I.

Kragt, C. L. and W. F. Ganong. 1968. Pituitary FSH content in male rats at various ages. Proc. Soc. Exp. Biol. Med. 128:965.

Lang, N. 1971. Steroid hormones and enzyme induction. In, The Biochemistry of Steroid Hormone Action. Biochem. Soc. Symp. #32 Smellie, Academic Press, N. Y.

Lerner, A. B., J. D. Case and H. Takahashi, 1960. Isolation of melatonin and S-methoxindole-3 acetic acid from bovine pineal glands. J. Biol. Chem. 235:192.

Lindner, H. R. and T. Mann. 1960. Relationship between the content of androgenic steroids in the testes and the secretory activity of the seminal vesicles in the bull. J. Endo. 21:341.

Lipsett, Mortimer B., Hildegard Wilson, Marvin A. Kirshner, Stanley G. Korenman, Lawrence M. Fishman, Gordon A. Sarfaty and C. Wayne Barden. 1966. III, Steroid Hormones. Rec. Prog. Hor. Res. 22:245.

Lostroh, A. J. and R. E. Johnson. 1966. Amounts of interstitial cell-stimulating hormone and follicle-stimulating hormone required for follicular development, uterine growth and ovulation in the hypophysectomized rat. Endo. 79:991.

McMillan, K. L., H. D. Hafs. 1968. Pituitary and hypothalmic endocrine changes associated with reproductive development of Holstein bulls. J. Anim. Sci. 27:1614.

McMillan, K. L. and H. D. Hafs. 1969. The reproductive tract of Holstein bulls through puberty. J. Anim. Sci. 28:233.

Martini, L. F., F. Fraschini and M. Motta. 1968. Neural control of anterior pituitary functions. Rec. Prog. Hor. Res. 24:439.

Means, A. R. and B. W. O'Malley. 1971. Assessment of sex steroid action. In. In vitro Methods in Reproductive Cell Biology. Karolinska Symposia on Res. Methods in Repro. Endo. 3:318, Stockholm.

Means, A. R. and P. F. Hall. 1969. Protein biosynthesis in the testes. V. Concerning the mechanism of stimulation of FSH. Biochem. 8:4293.

Mercier-Bodard, C., A. Alfsen and E. E. Baulieu. 1970. Sex steroid binding plasma protein. In. Steroid Assay by Protein Binding. Karolinska Symposia on Res. Methods in Repro. Endo. 2:204, Stockholm.

Midgley, A. Rees, Jr. 1966. Radioimmunoassay: A method for human chorionic gonadotropin and human lutenizing hormone. Endo. 79:10.

Midgley, A. Rees, Jr., G. D. Niswender and J. Sri Ram. 1968. Hapten radioimmunoassay: A general procedure for estimation of steroidal and other haptenic substances. Steroids. 134:731.

Midgley, A. Rees, Jr., G. D. Niswender, Vernon L. Gay and L. E. Reichert, Jr. 1971. Use of antibodies for characterization of gonadotropins and steroids. Rec. Prog. Hor. Res. 27:235.

Murphy, Beverly E. P. 1967. Some studies of the protein-binding of steroids and their application to the routine micro and ultra micro measurement of various steroids in body fluids by competitive protein-binding radioassay. J. Clin. Endo. 27:973.

Murphy, Beverly E. P. 1968. Binding of testosterone and estradiol in plasma. Can. J. Biochem. 46:299.

Murphy, B. E. P. and C. J. Pattee. 1964. Determination of plasma corticoids by competitive protein-binding analysis using gel filtration. J. Clin. Endo. 24:919.

Neill, J. D., E. D. B. Johansson, J. K. Datta, J. F. Knobil. 1967. Relationship between the plasma levels of lutenizing hormone and progesterone during the normal menstral cycle. J. Clin. Endo. 27:1167.

Niswender, Gordon D., Leo E. Reichert, Jr., A. R. Midgley, Jr., and A. V. Nalbondov. 1968. Radioimmunoassay for bovine and ovine lutenizing hormone. Endo. 84:1166.

Nugent, C. A. 1970. Reliability of plasma testosterone assays by C.A.B. methods. In. Steroid Assay by Protein Binding. Karolinska Symposia on Res. Methods in Repro. Endo. 2:257.

Nugent, C. A., and D. A. Mayes. 1966. Plasma corticosteroids determined by use of corticosteroid-binding globulin and dextran coated charcoal. J. Clin. Endo. 26:1116.

Oshima, H. 1967. Influence of administration of testosterone upon testicular enzymes of rats related to androgen formation. Endo. Japon 14:75.

O'Steen, W. K. 1965. Supression of ovarian activity in immature rats by serotonin. Endo. 77:937.

Pearlman, W. H. and Odette Crepy. 1967. Steroid-protein interaction with particular reference to testosterone binding by human serum. J. Biol. Chem. 242:182.

Peat, Frances and G. A. Kenson. 1971. Testicular steroidogenesis in vitro in the rat in response to binding, pinealectomy and to the addition of melatonin. Steroids. 17:2.

Potter, V. R. 1965. Summary of 1965 Biology Research Conference. J. Cell. and Comp. Physiol., 66:175-182.

Ramaley, Judith A. 1971. Steroid binding to serum proteins in maturing male and female rats, Endo. 89:545.

Ramirez, D. 1970. Second conference on steroid hormones and brain functions, Pacific Palisades, Calif.

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

Rawlings, N. C., H. D. Hafs and L. V. Swanson. 1972. Testicular and blood plasma androgens in Holstein bulls from birth through puberty. J. Anim. Sci. 34:435.

Riondel, A., J. F. Tait, M. Gut, S. A. S. Tait, E. Joachim and B. Little. 1963. Estimation of testosterone in human peripheral blood using S^{35} thiosemicarbazide, J. Clin. Endo. and Met. 23;620.

Robson, J. M. and M. Botros. 1961. The effect of 5-hydroxy-tryptamine and of monoamine oxidase inhibitors on sexual maturity. J. Endo. 22:165.

Robison, G. A., R. W. Butcher and E. W. Sutherland. 1968. Cyclic AMP, Annual Review of Biochem. 37:149.

Rosenfield, Robert L. 1971. Plasma testosterone binding globulin and indexes of the concentration of unbound plasma androgens in normal and hersute subjects. J. Clin, Endo. 32:717.

Rosenfield, R. L., W. R. Eberlein and A. M. Bongiovanni. 1969. Measurment of plasma testosterone by means of CPB analysis. J. Clin. Endo. 29:854.

Saroff, J., R. E. Keenan, A. A. Sandberg and W. R. Slaunwhite, Jr. 1967. Determination of testosterone, androstenedione and dehydroepindrosterone in human plasma using bromine-82. Steroids. 10:15.

Siitteri, Penti k., R. L. Vande Wiele and Seymour Lieberman. 1963. Occurrence of dehydroisoandrosterone glucuronoside in normal human urine. J. Clin. Endo, and Met. 23:588.

Siperstein, E., C. W. Nichols, Jr., W. E. Griesbach and I. L. Chaikoff. 1954. Cytological changes in the rat anterior pituitary from birth to maturity. Anat. Rec. 118:593.

Skinner, J. D., T. Mann and L. E. A. Rowson. 1968. Androstendione in relation to puberty and growth of the male calf. J. Endo. 40:261.

Snook, R. B., R. R. Saatman, W. Hansel. 1971. Serum progesterone and lutenizing hormone levels during the bovine estrous cycle. Endo. 88:678.

Strickland, A. L., S. N. Nayfeh, F. and S. F. French. 1970. Conversion of cholesterol to testosterone and androstenedione in testicular homogenates of immature and mature rats. Steroids. 15:373.

Sutherland, E. W. 1970. On the biological role of cyclic A.M.P. Amer. Med. Assoc. 214:1281.

Swanson, L. V., R. P. Willemann, N. C. Rawlings, H. D. Hafs, and W. T. Magee. 1971. Pubertal relationships of some endocrine and reproductive criteria in Hereford bulls. J. Anim. Sci. 33:823.

Turner, C. Donnell. 1967. General Endocrinology. 4th Ed. W. B. Saunders Co. Philadelphia.

Vermeulen, A. 1970. Testosterone assays by competitive protein binding. In. Steroid Assay by Protein Binding. Karolinska Symposia on Res. Methods in Repro. Endo. 2:239. Stockholm.

Winter, J. S. D., and D. R. Grant. 1971. A rapid and sensitive assay for plasma testosterone in adults and children. Anal. Biochem. 40:440.

Wolf, F. R., J. O. Almquist and E. B. Hale. 1965. Prepubertal behavior and prepubertal characteristics of beef bulls on high nutrient allowance. J. Anim. Sci. 24:761.

Wright, A. D. and K. W. Taylor. 1967. Hormones in Blood. Gray and Bacharach, Academic Press, N.Y. 1:24.

Wurtman, R. J. 1970. The role of brain and pineal indoles in neuro-endocrine mechanisms. In. The Hypothalmus. Martini, Motta and Franschini (ed). Academic Press, N.Y.

Wurtman, R. J., J. Axelrod and E. W. Chu. 1963. Melatonin, a pineal substance: Effect on the rat ovary. Science. 141:277.

Yamamoto, Makato, N. B. Diebel and E. M. Bagdonove. 1970. Radio-immunoassay of serum and pituitary LH and FSH levels in intact male rats and of serum and pituitary LH in castrated rats in both sexes-apparent absence of diurnal rhythms. Endo. 87:798.

Yuhara, M., A. I. Cohen and E. H. Frieden. 1963. Stimulation by pituitary gonadotropin of the synthesis of estrogen by rat ovary slices in vitro. Proc. Soc. Exp. Biol. Med. 113:907.

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