STUDIES OF PUBERTAL DEVELOPMENT IN BOARS: RELATIONSHIPS BETWEEN REPRODUCTIVE ORGAN DEVELOPMENT AND PERIPHERAL PLASMA LEVELS OF LUTEINIZING HORMONE AND TESTOSTERONE

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by Simplicia Valenzuela FlorCruz

Two studies were conducted to obtain fundamental data on the process of puberty in Large White x Landrace boars. The first was a longitudinal study designed to evaluate the course of gonadal and epididymal development and the concurrent endocrine changes during the period of sexual maturation.

Twenty-four animals were castrated sequentially at ages ranging from 43 to 241 days. Increases in testicular weight were more highly correlated with body weight ($r = 0.953$, $P < 0.001$) and age ($r = 0.919$, $P < 0.001$) than were similar increases in epididymal weight ($r = 0.558$ and 0.593, $P < 0.01$, respectively). The age at onset of spermatogenesis varied between boars in the range 90 to 127 days. First observations of spermatozoa in seminiferous and epididymal tubules were made at 127 and 146 days of age, respectively.

Longitudinal profiles of LH and testosterone secretion were investigated by assaying plasma samples collected at fortnightly intervals from each of ten boars aged 41 to 236 days. Until 82 days of age mean LH levels were low (0.20 - 1.25 ng/ml), then rose to a peak of 2.19 ng/ml at 110 days. Later LH levels declined gradually and after 166 days fluctuated between 0.5 and

1.0 ng/ml. Mean testosterone levels also were low in the prepubertal period (0.10 - 0.27 ng/ml), then between 110 and 138 days increased from 0.60 to 8.00 ng/ml. Subsequently testosterone concentrations fell slowly, then except for an isolated peak of 7.73 ng/ml at 194 days, fluctuated between 1.40 and 3.80 ng/ml. These results indicated that the major changes in LH and testosterone secretion during puberty in boars were similar to those which have been reported to occur in males of other species.

Two short term studies of LH and testosterone secretory profiles were carried out to evaluate the effects of stage of sexual maturity on the patterns of secretion of these hormones. Four pubertal and three post-pubertal boars were subjected to plasma sampling every twenty minutes for 24 hours. During puberty, plasma profiles of LH varied in a manner indicative of a highly pulsatile mode of secretion. Likewise, large fluctuations in plasma testosterone levels were noted at this age but they were not as frequent as those of LH. In contrast, plasma LH and testosterone profiles of post-pubertal boars showed fewer and smaller fluctuations in hormone concentrations. The respective overall mean levels of LH and testosterone were 0.82 and 1.04 ng/ml in pubertal boars, and 0.39 and 0.81 ng/ml in post-pubertal boars. These values were consistent with those obtained in the longitudinal study and confirmed the greater output of both hormones during puberty and their decline thereafter. At both pubertal and post-pubertal ages, associations between LH and testosterone pulses were not always consistent, nor was there any evidence of diurnal variations in plasma hormone concentrations.

The results reported in this thesis indicated that puberty in boars occurred between the ages of 90 and 146 days, at which ages, mean body weights were 35.3 and 58.6 kg, respectively. This period was characterized by a rapid growth of the testes and epididymides and was accompanied by concurrent elevations of plasma LH and testosterone concentrations. The less pulsatile nature of LH and testosterone secretion recorded from the post-pubertal boars probably reflected the maturation (increased sensitivity) of hypothalamic negative feedback mechanisms which normally regulate hormone secretion. The existence of such a mechanism was confirmed by an elevation of plasma LH levels following castration of 5 boars at 215 days of age.

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CHAPTER I

INTRODUCTION

A. PHYSIOLOGY OF PUBERTY IN MALES

1. General Considerations

The term "puberty" is derived from the Latin word pubes which referred to the first appearance of pubic hair, a specific characteristic of the process in humans (Tanner, 1962). Today, puberty is taken to refer to that phase of bodily development leading to the attainment of the ability to reproduce. In the male, it has been related to the development of the spermatogenic and androgenic functions of the testis. Often, puberty has been equated to sexual maturity but some authors draw distinction between the two terms (Asdell, 1964; Emmens, 1969; Courot, 1970). Although spermatogenesis is established and sexual behaviour manifested during puberty, a full cellular yield of the spermatogenic process is not achieved for a considerable time after puberty, until the attainment of sexual maturity.

Puberty in male animals is not characterized by such obvious manifestations as the occurrence of the first behavioural estrus in females, which makes the assessment of the process in males less precise. Moreover, different authors have utilized different criteria, thus considerable variations in the age of attainment of puberty have been reported for most species. Asdell (1964) and Courot (1970) identified the completion of puberty with the first appearance of spermatozoa in the testes. Donovan and van der Werff ten Bosch (1965), Skinner and Rowson (1968), and Lincoln (1971) related the attainment of puberty with the establishment of the androgenic and spermatogenic functions of the testes. The development of libido or testing the ability to mate also have been utilized as criteria of puberty in boars (Wiggins *et al*., 1951). Likewise, parameters such as rates of bodily and testicular growth, and chronologic age have been considered in the assessment of pubertal onset (Donovan and van der Werff ten Bosch, 1965; Ramirez, 1973).

2. Physiological Changes Associated with Puberty

a. Testicular Development

In most species, testicular growth between post-natal life and adulthood follows a sigmoid curve, being slow and fairly constant neonatally, accelerating during puberty, then slowing and plateauing again when sexual maturity is reached. This pattern of testicular growth has been observed in humans (Scammon, 1931), rams (Skinner and Rowson, 1968), bulls (Abdel-Raouf, 1960), horses (Nishikawa and Horei, 1955), and boars (Niwa and Mizuho, 1954; Egbunike and Steinbach, 1972). Growth in testicular mass is considered to be due mainly to increases in the diameter and length of the seminiferous tubules, coincident with the establishment of spermatogenesis and Leydig cell function.

Histologically, the post-natal development of mammalian testes follows a fairly definite pattern (Albert, 1961). During the neonatal period, the seminiferous tubules are solid, cord-like structures, in which only two types of cells are distinguishable. These are the numerous supporting cells, which form a pallisade-like layer along the basement membrane and a smaller number of gonocytes which are located more centrally within the tubules. The supporting cells are considered to be the precursors of the Sertoli cells, and the gonocytes the spermatogenic primordia

(Clermont and Perey, 1957; Courot, 1962 a). Both types of cells divide by mitosis but do not differentiate until the onset of puberty. As puberty commences, the seminiferous tubules undergo rapid growth in diameter and length, the center of tubules becomes vacuolated and later lumina appear. At the same time, supporting cells start to assume Sertoli cell morphology (with irregularly shaped nuclei, dust-like nuclear chromatin, and two or more nucleoli), spermatogonia appear and undergo mitoses. The cells originating from the last spermatogonial division are the primary spermatocytes which in turn undergo meiotic divisions to form secondary spermatocytes. These cells divide to form spermatids which finally metamorphose into spermatozoa (Clermont and Perey, 1957; Courot, 1962 a; Ortavant, 1969). Once established, the spermatogenic cycle continues with an increasing spermatogenic yield until sexual maturity is attained (Rowson, 1969).

With respect to the development of testicular androgenic function, it is accepted that androgen production occurs primarily in the interstitial cells of Leydig (Hall, 1970). The genesis of these cells has been described in humans (Sniffen, 1950; Vilar et al., 1970) and rats (Roosen-Runge and Anderson, 1959; Nieme and Ikonen, 1963). Two generations of Leydig cells were noted: a foetal generation which is active during foetal life but which regresses post-natally, and a second generation whose appearance coincides with puberty. Both generations of Leydig cells originate from fibroblasts whose differentiation involves progressive changes in size and shape of cells, modification in their histochemical reactions, loss of basophilia, increased activity of oxidative enzymes (lipases, esterases and hydroxysteroid dehydrogenases) and the appearance of lipids (Vilar, 1970).

b. Development of Other Organs of Reproduction

(1) The Epididymides

Studies in bulls (Abdel-Raouf, 1960), rams (Watson *et al.*, 1956), boars (Niwa and Mizuho, 1954) and stallions (Horei and Nishikawa, 1955) have demonstrated that epididymides increase in weight during puberty in parallel with the increase in testicular weight. Epididymal tubule and epididymal tubule lumen diameters in turn increase in parallel with the growth in epididymal weight. After production of spermatozoa commences, increasing numbers of spermatozoa may be noted in epididymal sections.

The histological development of epididymides during sexual maturation follows a similar pattern in most species (Asdell,1964). As the epididymal tubule diameter increases, differentiation of the epithelium from simple columnar to a pseudostratified type, as well as increases in epithelial height occur simultaneously. Also part of the differentiation of the epithelial cells is the acquisition of stereocilia on their luminal borders.

(2) The Accessory Sex Glands

The accessory sex glands comprise the seminal vesicles, bulbo-urethrals and prostate glands. The growth of these organs was found to be more highly correlated with testicular weight than with body weight (Asdell,1964). Hooker (1942) reported that the seminal vesicles of rats exhibited greatly increased responsiveness to androgens at puberty. In rams, fructose and citric acid contents of the seminal vesicles, which are highly correlated with testicular androgen content, increase markedly during puberty (Mann, 1964; Skinner and Rowson, 1968).

(3) The External Genitalia

Both the penis and scrotum exhibit rapid growth during puberty and penile adhesions become detached allowing the penis to move freely within the prepuce. This separation process between the glans penis and the penile prepuce has been described in some species and occurs under the influence of increased testosterone secretion during puberty (Watson et al., 1956; Abdel-Raouf, 1960; Skinner and Rowson, 1968).

c. Development of Secondary Sex Characters

Growth and development of the secondary sex characters are very obvious changes which take place during puberty in most species. These generally involve changes in the skin and other structures, i.e. growth of the mane of the lion, antlers of the stag, plumage and head pieces of the rooster, and activation of pheromonal glands in boars and goats (Donovan and van der Werff ten Bosch, 1965). Differential development of general body structure between male and female animals also is characteristic of puberty. For instance, the mature body weight of bulls frequently is twice that of cows of the same breed, because of the more massive development of the skeleton and musculature in the males (McDonald, 1971).

Signs of sexual behaviour involving attempts to mount companion animals and the ability to detect estrous females are characteristic indications of libido in males. However, mounting behaviour cannot always be considered as indicative of the emergence of puberty since it can be observed in very young lambs, pigs, calves, and kids (Cheng et al., 1964; Fraser, 1968).

d. Hormonal Changes Associated with Pubertal Development

(1) Gonadotrophins

The pattern of gonadotrophin secretion during sexual maturation has been described for the males of several species. Levels of FSH and LH rise towards puberty in humans (Yen et al., 1969), rams (Courot et al., 1975; Lee et al., 1976 a), and rats (Payne et al., 1977), but there are considerable variations in the longitudinal hormone secretion profiles between species. An interesting phenomenon in human adolescents is the occurrence of sleep-related spurts of LH secretion, an event considered to be one of the biological indices of puberty in humans (Boyar et al., 1972).

Some workers have suggested that a shift in the FSH/LH ratio also takes place during puberty in males. In rats FSH levels were reported to decline following the onset of spermatogenesis while LH levels continued to rise to adult levels (Payne et al., 1977). A similar decline in plasma FSH levels was reported in developing rams following the first appearance of spermatids (Lee et al., 1976 a).

(2) Gonadal Steroids

Most of the phenotypic changes characteristic of puberty in mammals are produced by steroids secreted by the testes, although Tanner (1962) observed that in human males adrenal steroids could also function in the induction of morphological changes.

Several criteria have been used for measuring the gonadal hormone secretion of the testes, including the effects of androgens on responsive tissues, androgen levels in peripheral blood and in testicular extracts, the presence of enzymes for steroid synthesis, and the histological structure of the interstitial cells of Leydig. Data accumulated from such research, based mostly on data from humans and rodents (Hall, 1970; Hooker, 1970), has revealed a diphasic pattern of testicular androgen secretion. This profile is characterized by rising levels in early pregnancy, falling towards birth, remaining low during neonatal and prepubertal life, then at puberty levels rise again until adult values are reached. Levels of testosterone in bulls (Secchiari et al., 1976), rams (Cotta et al., 1975; Lee et al., 1976 a; Wilson, 1977), and boars (Carlson et al., 1971; Gray et al., 1971; Elsaesser et al., 1976 b) also have been reported to increase during puberty. These increases in androgen levels were found to be correlated significantly with testicular weight, as well as with the concentrations of fructose and citric acid in seminal vesicles of rams (Skinner and Rowson, 1968).

Qualitative changes in androgen secretion during the maturation process have been shown to occur in some species. Changes in the ratio of the two major androgens, testosterone and androstenedione were demonstrated in bulls (Lindner and Mann, 1960). These authors found that androstenedione predominates over testosterone prepubertally, but as puberty proceeds, testosterone increases so that it becomes the predominant androgen at 9 months of age. In calves younger than 4 months of age, injection of Human Chorionic Gonadotrophin (HCG) did not promote the conversion of androstenedione to testosterone, but merely an increase in spermatic venous plasma androstenedione concentrations (Lindner and Mann, 1960). Thus it appears that the increased capacity of Leydig cells to convert androstenedione to testosterone develops only during puberty, presumably as a result of increased testicular 17 β -hydroxy steroid dehydrogenase activity.

(3) Other Hormones

(a) Adrenal Sex Steroids

In humans, some of the changes observed during puberty, particularly the growth of axillary and pubic hairs, are attributed to increased production of androgens by the adrenal cortex (Tanner, 1962). Clinical studies have shown that men with testicular hypofunction, or women with ovarian agenesis, grow pubic and axillary hair at about the usual age of puberty (Donovan and van der Werff ten Bosch, 1965). Significant elevations in levels of dehydroepiandrosterone, androstenedione, and progesterone were reported to occur during the second half of puberty in boys (Lee and Midgeon, 1975). Evidence derived from gonadectomy and adrenalectomy experiments indicated that both the testes and adrenal cortex synthesize androgenic precursors in increased amounts during puberty, indicating a simultaneous onset of adrenarche (Parkes, 1945).

(b) Prolactin

In male rats, prolactin exerts a synergistic effect with LH in promoting testosterone synthesis (Bartke, 1973). The concentrations of prolactin in plasma of male rats were found to increase during puberty (Negro-Vilar, 1973 a; Dohler and Wuttke, 1974), while in lambs, a rapid increase in prolactin secretion occurred simultaneously with the beginning of the rapid increase in testicular weight and spermatogenic activity (Ravault and Courot, 1975).

(c) Growth Hormone (GH)

Short term studies of GH secretion in humans indicated that GH is secreted episodically and that the total amount of GH secreted and the timing of secretory episodes change with age (Finkelstein *et al*., 1972). These investigators found that prepubertal boys secreted GH mainly during sleep, while adolescents secreted GH both during sleep and waking periods and had total secretory rates greater than prepubertal boys. As adulthood is reached the pattern of GH secretion reverts to the prepubertal pattern.

3. Neuroendocrine Regulation of Puberty in Males

a. General

Detailed studies of the structural relationships between the central nervous system (CNS) and the anterior pituitary, together with the development of sensitive and specific radioimmunoassay techniques for pituitary, steroid, and releasing hormones, all have contributed to a dramatic increase in the current understanding of the functional relationships between the hypothalamus, pituitary, and gonads in the control of mammalian reproduction. Although most of the experimental evidence utilized for drawing conclusions about these neuroendocrine mechanisms comes from the rat, it is possible to glean from the literature some data which indicates that similar control mechanisms operate in other species.

b. The Hypothalamo-Anterior Pituitary-Testicular Axis

(1) The Hypothalamus

The hypothalamus occupies a unique position in neuroendocrinology, functioning as an integrator of central and peripheral endocrine functions. This vital coordination is accomplished by neurons whose secretions are concerned with regulating pituitary functions and by reciprocal hypothalamic neural connections with higher brain centers (Motta *et al.*, 1973). Local hormone implants and microhormone injection techniques have established the presence of discrete hormone receptor elements in the medial basal hypothalamus which appear to be sensitive not only to gonadal steroids, but also to gonadotrophins (Sawyer, 1969; Motta *et al.*, 1973). The hypothalamus must therefore be regarded as a target organ in itself, as well as being an endocrine organ which releases hormones to regulate adenohypophyseal and neurohypophyseal secretion.

The hypothalamus has widespread neural connections with adjacent brain areas. Neuronal circuits from the limbic system and midbrain reticular formation interact with the hypothalamus and thus exert profound influences on both pituitary and gonadal functions and on reproductive behaviour (Motta et al., 1973).

The existence of a hypothalamic decapeptide, referred to as Gonadotrophin Releasing Hormone (GnRH), which is capable of stimulating the release of both FSH and LH in a variety of mammalian species, is now generally accepted (Schally, 1972). However, the precise anatomical site(s) of its production in the hypothalamus has/have not been identified.

Recently, RIA measurements of GnRH content of the male rat hypothalamus demonstrated an increasing concentration of the decapeptide during sexual maturation (Payne et al., 1977). However, measurement of GnRH levels in the peripheral circulation failed to detect increased releasing hormone concentrations during development (McCann, 1976).

(2) The Anterior Pituitary Gland

The anterior pituitary gland is linked functionally with the hypothalamus by way of the releasing hormones delivered by the hypothalamo-hypophyseal portal vessels (Green and Harris, 1949; Guillemin, 1967; McCann et al., 1968). This functional relationship between the hypothalamus and anterior pituitary gland forms the basis for current concepts of hypothalamic regulation of secretion of the anterior pituitary hormones.

The vital role of the anterior pituitary gland in the control of reproduction is based on its ability to elaborate the gonadotrophins, FSH and LH, which link the gonads with the hypothalamo-pituitary axis. Based on staining affinity, immunohistochemical localization, and electron-microscopic studies, the gonadotrophin producing cells are classified as basophils and are localized in the dorso-cephalic zone of the *pars distalis* (Baker, 1974). However , it is still not clear whether different gonadotrophin secreting cells are responsible for the synthesis of FSH and LH. The similar α-subunits but dissimilar β-subunits, and the discovery of only one releasing hormone for both gonadotrophins, may be interpreted as indicative of the existence of only one type of gonadotroph.

The induction of gonadotrophin release by GnRH appears to involve the mediation of cyclic AMP (Zor *et al*., 1969). McCann (1970) postulated that the cyclic nucleotide probably alters the cell membrane permeability and causes Ca^{++} uptake by the cell, the Ca^{++} in turn is required for the extrusion of the hormone secretory granules.

It is important to note that the stimulatory action of GnRH on the pituitary gonadotropic cells can be modified by circulating steroid hormones. *In vitro* perfusion techniques have been utilized to demonstrate that testosterone and dihydrotestosterone can inhibit GnRH stimulation of LH and FSH discharge from the pituitary(Wei Liu and Weiz, 1975), indicating the presence of androgen negative feedback at the pituitary level.

(3) The Testis

The morphological and histological development of postnatal testes has been described in section 2.a of this chapter. Experimental investigations in some species have demonstrated that the structure and functions of the testes at any stage of development are governed primarily by the gonadotrophins (Ortavant *et al.*, 1969; Courot, 1970). However, the specific roles of FSH and LH in the establishment and maintenance of testicular functions are not clearly defined. It is accepted that the germinal epithelium is maintained and stimulated directly by FSH, and the interstitial cells by LH, but synergistic effects between the two gonadotrophins in initiating and maintaining spermatogenesis following hypophysectomy also have been documented (Lostroh, 1963 and 1969; Steinberger, 1971; Courot, 1970).

Most studies implicate FSH and androgens as the main hormones which regulate spermatogenesis but there is still no universal agreement on the specific stages of the process controlled by each hormone. While androgen or LH alone was shown to be capable of maintaining and initiating spermatogenesis when given immediately following hypophysectomy, the minimum dose required for the maintenance of the process was much less when either of these hormones was given together with FSH (Lostroh, 1969; Kalra and Prasad, 1967).

The concept that LH influences spermatogenesis through its stimulation of Leydig cell androgen production has been supported by studies which showed that LH receptors were localized in the interstitial cells rather than in the seminiferous tubules (Dufau *et al.*, 1971; Cooke *et al.*, 1972). On the other hand, it is evident that receptors for both FSH and androgens are localized in the Sertoli cells, and that these cells are the targets for both hormones (Hannsson *et al.*, 1976; Facunding *et al.*, 1976).

The dependence of the testes on pituitary gonadotrophins starts very early in life. Hypophysectomy of neonatal and prepubertal rats induced testicular regression while administration of gonadotrophins reversed the effects of the operation (Courot, 1970). In this respect, FSH was found to have a greater stimulating effect on testis weight than LH, but the latter was found to be the more effective hormone in preventing testicular regression in adult hypophysectomized rats and rams, provided the therapy was instituted immediately after the operation. Once post-hypophysectomy atrophy of the testes took place, both FSH and LH were required to reinitiate spermatogenesis (Lostroh, 1969; Ortavant et al., 1969). It was suggested that the differentiation of spermatogonial cells primarily requires FSH, but LH exerts a synergistic effect on this process.

While the structural and functional integrity of the testes are dependent on gonadotrophin stimulation, the testes by way of their secretions in turn regulate gonadotrophin release. Testicular androgens are known to exert a major inhibitory feedback on LH secretion both at the hypothalamic and pituitary levels (Davidson, 1969; Wei Liu and Weiz, 1975). On the other hand, the control of FSH appears to involve the participation of a testicular substance other than androgens, since testosterone replacement therapy effectively suppressed the post-castration rise of LH, but not of FSH levels (Bogdanove, 1975). Increasing evidence indicates that a testicular factor termed "inhibin" isolated from rete-testis fluid and identified as a non-steroidal, water-soluble, and proteinaceous substance, is capable of selectively suppressing the elevated levels of FSH which can be induced either by castration or by testicular irradiation (Franchimont et al., 1972; Setchell, 1973; Lee et al., 1976 c). The cellular source of inhibin is unknown but in view of several studies of the relationship between Sertoli cells and FSH levels, and the capacity of

Sertoli cells to synthesize proteins (e.g. androgen-binding-protein), it was suggested that Sertoli cells are the most likely site of production of the FSH inhibitory substance (Castro et al., 1970; Hansson et al., 1976; Lee et al., 1976 c).

Besides their regulatory role on gonadotrophin secretion, gonadal androgens also are considered to induce the hypothalamic male sex differentiation (development of the "tonic" type of gonadotrophin secretion), and to activate growth of the male sex structures during the foetal (primates and sheep) or early neonatal (rats) periods (Jost, 1970; Barraclough, 1971; Short, 1974; Resko, 1975). Androgens are responsible for the rapid growth of the gonads and accessory sex organs and the development of secondary sex characters during puberty (Donovan and van der Werff ten Bosch, 1965) and the maintenance of the function of these reproductive structures post-pubertally.

c. Neuroendocrine Mechanisms Involved in the Initiation of Puberty

Some investigations undertaken to elucidate the neuroendocrine mechanisms regulating the time of onset of puberty have focussed on the age-related changes in the functional interrelationships between the hypothalamus, pituitary and testes (Ramirez, 1973; Swerdloff et al., 1971; Payne et al., 1977). Interactions within this system during early reproductive development have been indicated by alterations observed in the levels of gonadotrophins following hemicastration in rats (Ojeda and Ramirez, 1972), by testicular atrophy following hypophysectomy in immature rats and lambs (Ortavant et al., 1969; Courot, 1970), and by the demonstration of measurable levels of gonadotrophins during the early post-natal life of males in many species (Courot et al., 1975; Swerdloff et al., 1971; Payne et al., 1977; Lee et al., 1976 a; Ford and Schanbacher, 1977).

The factors which initiate rapid maturation of the testes during puberty are not clearly defined. The elevation of gonadotrophin levels during sexual maturation (see section A-2.d.) which causes increasing stimulation of the gametogenic and androgenic components of the testes is considered to be the major event in puberty, and two hypotheses have been proposed to explain this increase of FSH and LH secretion: (a) a change in the sensitivity of the hypothalamo-pituitary axis to inhibition by gonadal steroids (Ramirez, 1973; Ramirez and McCann, 1965; Smith and Davidson, 1969), and (b) an increase in the sensitivity of the anterior pituitary to GnRH stimulation (Debeljuk et al., 1972; Lee et al., 1976 b). According to the first hypothesis, the hypothalamo-hypophyseal system of the immature animal is highly sensitive to testicular steroid, but as sexual maturation proceeds, the sensitivity to inhibition decreases, resulting in increased FSH and LH secretion. This theory is based on the finding that smaller doses of testossterone were capable of inhibiting LH release in prepubertal or immature males, than in adult male rats (Smith and Davidson, 1969; Ojeda and Ramirez, 1972; Negro-Vilar et al., 1973 b).

Increasing pituitary sensitivity to GnRH stimulation could be another cause of the pubertal elevation of gonadotrophin levels. This has been reported in rams (Lee et al., 1976 b) and rats (Debeljuk et al., 1972) in which a greater release of gonadotrophins occurs in response to GnRH administration to pubertal males as compared to immature or adult animals. However, data published by Miyachi et al. (1973) for rats, Pomerantz et al. (1974) for boars, and Mongkopunya et al. (1975) for bulls, showed that pituitary responses to GnRH do not vary during sexual maturation. Discrepancies between the results of this type of investigation may have been caused by variations in the methods calculating the GnRH dosage.

A change in gonadal responsiveness to gonadotrophins also was suggested to be involved in the initiation of puberty (Swerdloff *et al*., 1971; Odell *et al*., 1973). Investigations by these authors indicated that testicular responsiveness to LH increases with age and that this increased responsiveness is dependent on previous exposure of the immature testes to FSH. Chen *et al*. (1976) also showed that FSH treatment of immature hypophysectomized rats resulted in a marked increase in the capacity of Leydig cells to respond *in vitro* to LH stimulation. Whether FSH acts to stimulate the synthesis of 17 β-hydroxysteroid dehydrogenase (which catalyzes the final step in testosterone synthesis), or whether it increases the number of LH receptors, has not been investigated, but the above evidence points to an important role for FSH in the induction of testicular capacity to secrete androgens.

It thus appears that the whole hypothalamo-pituitary-testicular system undergoes progressive maturational changes during puberty, but the underlying neuroendocrine mechanisms which trigger such changes are still poorly inderstood. Experiments with immature female rats produced convincing evidence that extrahypothalamic influences from the amygdala and pineal glands are important in preventing the onset of puberty. Complete deafferentation of the medial basal hypothalamus by surgical isolation resulted in precocious puberty (Ramaley and Gorski, 1967). Destruction of the amygdala or sectioning of the tract which connects it with the hypothalamus (*stria terminalis*) similarly caused premature vaginal opening, whereas electrical stimulation of the amygdaloid area delayed puberty (Critchlow and Bar-Sela, 1967). The involvement of the pineal gland in the timing of pubertal onset is suggested by the occurrence of precocious puberty in boys and girls with pineal tumours, although some authors regarded that result as being secondary to the pressure exerted by such tumours on adjacent neural structures (Critchlow and Bar-Sela, 1967). However, the finding that pinealectomy of immature female rats accelerated puberty (Relkin, 1971) and the characterization of 2 pineal substances with antigonadotrophic effects (melatonin, Wurtman _et al_., 1964, and pineal peptides, Pavel and Petrescu, 1966) indicate that it is possible that this organ may have some role in mediating the onset of puberty. Moreover, in buffalo calves, the number of pinealocytes and the vascularity of the gland was found to be higher than in adults (Rao and Saigal, 1971), and the activity of the pineal enzyme HIOMT (hydroxyindole-O-methyl transferase) which catalyzes the formation of melatonin, declines at the time of puberty (Hoerman, 1971). This evidence indicates that the pineal gland is more active prepubertally and could exert antigonadotrophic influences during the period of sexual immaturity.

The exact mechanisms by which these extrahypothalamic structures affect the functional maturation of the hypothalamo-pituitary-gonadal system during puberty are unknown. Likewise, the means by which gonadal steroids could modulate hypothalamic sensitivity remains speculative. Current experimental observations are rather difficult to integrate and no unified theory has yet been formulated to explain the control of onset of puberty, particularly for the male gender.

d. Factors Affecting Puberty in Males

The age of occurrence of puberty varies considerably within any one species. Although the ontogeny of the process is largely determined by genetics, the normal rate of maturation can be modified by the environment (Donovan and van der Werff ten Bosch, 1965; Critchlow and Bar-Sela, 1967; Ramirez, 1973). Factors which influence pubertal onset are: genetic constitution, season, light, temperature, stress, nutrition, and social and sexual stimuli.

(1) Genetics

The existence of genetic factors which influence the age of puberty is indicated clearly by the earlier occurrence of sexual maturity in smaller rather than larger breeds of a particular species e.g. miniature pigs and dogs attain puberty earlier than larger breeds of these species (Mount *et al.*, 1971; McDonald, 1969). Also triplet boys were reported to attain puberty at identical ages while significant variations in ages at puberty occurred between non-twin brothers (Donovan and van der Werff ten Bosch, 1965).

(2) Season, Light, Temperature and Stress

The delaying effects of shortened day-length, extreme temperatures and stressful manipulations on the occurrence of puberty have been reported in female rodents but have not been investigated in males (Donovan and van der Werff ten Bosch, 1965). In ram lambs, Skinner and Rowson (1968) suggested that seasonal factors such as environmental daylight might influence sexual development; they recorded delayed reproductive development in lambs born during late summer compared to those born in spring.

(3) Nutrition

Markedly inadequate caloric intake during the prepubertal period of life was suggested to delay the onset of sexual maturity (Moustgaard, 1959); this hypothesis has been confirmed for bulls (Flipse *et al.*, 1953; Mann *et al.*, 1967), rams (Watson *et al.*, 1956; Courot, 1962 *a*), and boars (Niwa, 1954; Dutt and Barnhart, 1959). Further, it has been reported that in rams fed inadequately, the androgenic function of the testes is retarded more markedly than was the onset of spermatogenesis (Mann _et al_., 1967; Skinner and Rowson, 1968). Mann _et al_. (1967) claimed that the delay which underfeeding caused in the onset of androgenic function was due to lack of gonadotrophins from the pituitary and not to an inability of the testes themselves to produce testosterone. However, the investigations of Gombe and Hansel (1973) in heifers showed that gonadal hypofunction under conditions of restricted energy intake was not due to reduced circulating levels of LH, but to a reduced ability of the ovarian tissue to respond to LH.

(4) Social and Sexual Stimuli

While the effects of social environment and sexual stimuli on the age of puberty have been studied extensively in females (Parkes, 1961; Whitten, 1966), only limited studies have been conducted in males. Vandenberg (1971) reported that rearing of immature male mice in the presence of adult females had a stimulatory effect on testicular and accessory sex gland development.

The establishment of social hierarchies amongst boars (Fraser, 1974) has been suggested as a possible factor which influences the androgenic activity of the testes. Subordinate boars in the group were found to have lower plasma levels of pheromonal steroids and a delayed onset of testicular steroidogenesis (Andresen, 1976).

B. PHYSIOLOGY AND ENDOCRINOLOGY OF REPRODUCTIVE DEVELOPMENT IN BOARS

The domestic pig (_Sus scrofa domestica_) belongs to the class of monogastric ungulates with even-toed hooves (Hafez, 1974). Generally, boars are reported to reach sexual maturity by 8 months of age, but reports on the age of puberty vary considerably, ranging from as early as 110 days (Andrews and Warnick, 1949) to as late as 200 days (Wiggins et al., 1951). This variation may be attributed primarily to the different criteria utilized by those investigators, the former being based on the first appearance of sperm in the seminiferous tubules, and the latter on the ability to mate.

1. Reproductive Development

The development of the reproductive organs of boars has been described by Phillips and Andrews (1936), Andrews and Warnick (1949), Hausser et al. (1949), Niwa and Mizuho (1954), Asdell (1964) and Egbunike and Steinbach (1972) for the domestic breeds and by McFee et al. (1967) for the miniature breeds. Descent of the testes into the scrotum normally occurs during the last quarter of foetal life (Backhouse and Butler, 1960), while the growth curve of the testes from birth to adulthood is sigmoid in shape (Niwa and Mizuho, 1954), with the most rapid growth occurring between 4 to 10 months of age. Increases in the diameter of seminiferous tubules approximately parallel testicular weight changes until a maximum diameter of 200 μm is reached (Niwa and Mizuho, 1954; Egbunike and Steinbach, 1972).

Histologically, the testes are of an immature type similar to that described for other mammalian species from birth until 1½ months of age. By 3 months, the seminiferous tubules have acquired lumina and primary spermatocytes appear, while spermatozoa are detectable between 4 to 6 months; at 6 months 85 % of the tubules contained sperm (Rowson, 1969).

The development of the epididymis also was reported to parallel the growth of the testis, while the accelerated growth of the accessory sex glands occurred later, between 5 to 10 months of age (Niwa and Mizuho, 1954). Completion of separation between the penis and the penile part of the prepuce occurs at 5 months (Hafez, 1974), with penile growth being most rapid at 7 - 8 months of age (Niwa and Mizuho, 1954). Although boars could ejaculate at 6 - 7 months of age, the first ejaculates contained many immature gametes (Rowson, 1969). The number of sperm and the volume of ejaculates both increased until boars reached 18 months of age, at which time the ejaculate contains 20 to 100 x 10^9 sperm in 200 to 400 ml of semen (Leman and Rodeffer, 1976). Swierstra (1968) estimated the duration of spermatogenesis in boars to be 34.4 days.

The principal factors which determine the time of onset of puberty in boars are breed and nutrition. The influence of breed is indicated clearly by the early attainment of reproductive capability in miniature boars of Gottingen strain. Spermatozoa are detectable as early as 4 weeks of age in these animals and sexual maturity is attained between 8 and 12 weeks of age (Haring et al., 1966). However, miniature boars of the Pittman-Moore strain only reach sexual maturity at an age (23 weeks) comparable to boars of domestic breeds (McFee et al., 1967). Among domestic breeds some degree of variation also has been noted. Berkshires and Middle Whites were shown to attain sexual maturity at 8 months while Poland China and Large White boars reach sexual maturity at 11 months of age (Niwa and Mizuho, 1954). Cross-breeds were found to have an earlier onset of spermatogenesis than pure-bred boars (Hausser, 1949).

Individual variations within breeds have been observed and appear to be related to nutrition and body growth. Niwa (1954) investigated the relationship between body growth and spermatogenic function and found that boars with a normal body growth rate had greater testicular weights and an earlier onset of spermatogenesis than animals with retarded body growth. Dutt and Barnhart (1959) also reported a delayed onset of puberty in boars under restricted feeding, but found no deleterious effects of reduced nutrition on semen volume, sperm density, sperm motility, or the fertility of sexually mature boars. Seasonal variations in the time of birth were reported to have no significant effect on the attainment of puberty in boars (Wiggins et al., 1951).

2. Endocrinology of Reproductive Development in Boars

Knowledge of the endocrine control of reproduction in boars, particularly of hypothalamo-pituitary-testicular relationships, is still very limited. A growing interest in the subject has followed the advent of radioimmunoassays for hormone measurment, and the use of miniature swine as experimental animals. However, in view of the much earlier age of onset of sexual maturity in miniature breeds, caution must be taken in interpreting results derived from these animals in relation to domestic breeds.

a. Gonadotrophins

Very limited data on plasma levels of gonadotrophins are available at present. A little information has been published for LH, but data for FSH and prolactin for the porcine species are completely lacking. Plasma LH levels in miniature boars were first reported by Pomerantz et al. (1974) who found that pubertal boars had resting plasma LH levels of 0.6 ng/ml, intact adults - 0.7 ng/ml and castrated adults - 3.0 ng/ml. The same group of workers further characterized the patterns of plasma LH levels from birth to 12 weeks of age , and noted no significant change in LH levels throughout this period (Elsaesser et al., 1976 b). Recently, Ford and Schanbacher (1977) measured serum LH levels in domestic boars from birth to 9 weeks of age and reported an elevated concentration from birth to 21 days of age (mean value = 1.46 ng/ml), this then declined between days 21 and 28 to levels comparable to those observed in adult boars (0.58 ng/ml). To date, no complete profile of LH secretion from post-natal life to adulthood has been published.

b. Testicular Steroids

In contrast to the meager LH data available for boars, considerable information on the patterns of testicular steroid secretion has been reported.

Testosterone is the principal steroid secreted by the boar testes (Lindner, 1961 b; Elsaesser et al., 1972). Plasma levels of this steroid in spermatic vein blood of domestic boars, aged 3 to 9 months, were measured by Carlson et al. (1971) and Gray et al., (1971) using thin layer chromotography technique. Both groups of authors recorded increased concentrations of testosterone towards puberty. Gray et al. (1971) also recorded the highest testosterone concentration in 5 - 7 month old animals, while lower levels were registered in boars aged 8 - 9 months. Andresen (1976) measured peripheral plasma concentrations of testosterone and recorded maximal levels between 115 and 157 days of age and lower levels thereafter. Moussy-Dessole (1976) reported a profile of testosterone secretion in boars analogous to that recorded in humans, and showing a large rise after birth, between 5 and 17 days, followed by a prepubertal decline, then a progressive increase during puberty until adult values were attained. On the other hand, Elsaesser et al. (1976 b) and Ford and Schanbacher (1977) found low and constant levels of testosterone from birth up to 5 weeks of age. Elsaesser et al. (1972) and Booth (1975) found that the concentration of testosterone in boar testes was higher than that of androstenedione throughout all stages of reproductive development, in contrast to the findings of Lindner and Mann (1960) in bulls. During foetal life, Raeside and Sigman (1975) confirmed that testosterone was the principal androgen in foetal boar testes, being 100 times more concentrated than any other androgen. During postnatal development, a higher concentration of testosterone rather than androstenedione in the testes also was reported, irrespective of age (Elsaesser et al., 1972; Booth, 1975).

Besides testosterone and androstenedione, a number of androgens were identified as being elaborated by boar testes, including various musk smelling 16 unsaturated C_{19} steroids (Patterson, 1968 a; Claus et al., 1971), dehydroepiandrosterone (Huis in't Veld et al., 1964; Booth et al., 1973), and estrogens (Velle, 1958 a; Raeside, 1965).

The 16-androstene C_{19} steroids possess little or no androgenic activity (Saat et al., 1972) but are considered to serve as pheromones (Sink, 1967). Patterson (1968 b) identified one of these steroids, 5α-androstenone, in boar fat; this steroid is the major component of the sex odour complex of the boar and causes "taint" in boar's meat. Further studies by Claus et al. (1971) revealed that fatty tissues and salivary glands (parotid and submaxillary) serve as stores for 16-androstenes. The presence of these steroids in saliva (Gower, 1972) and sweat glands (Stinson and Patterson, 1972) of boars is of considerable interest since they have been shown to have pheromonal activity which facilitates the detection of estrus in sows (Reed et al., 1972) and advances puberty in gilts (Brooks et al., 1970). Concentrations of 5α-androstenone in plasma and fat tissue were reported to increase between puberty and adulthood (Andresen, 1976).

Significant amounts of dehydroepiandrosterone (DHA) also have been identified in boar testes and peripheral plasma. That the main source of origin of this steroid is the testes and not the adrenal cortex was confirmed by marked alterations in its levels following castration and HCG injection (Huis in't Veld et al., 1964). It was suggested that DHA might act as a precursors for testosterone synthesis (Booth et al., 1973).

Besides androgens, boars also secrete large quantities of estrogens. The testes are considered to be the source of these estrogens since castration greatly reduced the urinary levels of these steroids (Velle, 1958 b; Raeside, 1965). The physiological significance of high levels of estrogen in boars is not clear. Investigations by Joshie and Raeside (1973) demonstrated that estrogens could exert synergistic effects with testosterone in stimulating the secretory activity of the accessory glands, and also on sexual behaviour of boars, when administered as steroid replacement therapy following castration. Also, estrogen may participate in the negative feedback control of gonadotrophin secretion (Ford and Schanbacher, 1977).

It has been suggested that testicular steroidogenesis in boars can be affected by external factors. For example, stressful stimuli such as confinement in metabolism crates was shown to lower plasma LH activity, estimated by the rat ovarian cholesterol depletion method (Liptrap and Raeside, 1968). Social factors i.e. establishment of social hierarchies (see section A-3.d. of this chapter) may also affect the endocrine status of individual animals. Sexual stimuli such as copulation also have been reported by Andresen (1976) to induce an increase in the peripheral plasma levels of testosterone and 5α-androstenone within a few minutes.

In contrast, Ellendorff *et al*. (1975) found no significant elevation of plasma testosterone concentrations following copulation although an elevation of plasma LH levels was noted. Further investigations are needed to clarify the effect of mating or sexual stimuli on the reproductive hormone secretion of boars.

c. Hypothalamo-Anterior Pituitary-Testicular Relationships during Puberty

Only preliminary investigations of the physiology of the hypothalamo-pituitary-testicular axis in boars have been reported. The response to GnRH infusion in pubertal, intact adult, and castrated miniature boars were investigated by Pomerantz *et al*., (1974). Their experiments revealed that in all animals the LH responses were dependent on the dose of GnRH, however the decay constant of LH was significantly longer in intact adult boars (76 min) compared to pubertal (25 min) or castrate animals (29 min). They hypothesized that there was no difference in pituitary sensitivity to GnRH stimulation between pubertal and adult boars. Also, it has been demonstrated that the steroid negative feedback mechanism is relatively insensitive during the neonatal period (Elsaesser *et al*., 1976 *a*; Ford and Schanbacher, 1977); while neonatal castration reduces the levels of testosterone, no elevation of LH levels was noted. The lack of a negative feedback system shortly after birth was further indicated by findings that neither norethindrone acetate nor estradiol benzoate administered on day 2 had any effect on plasma LH levels, but both were effective in decreasing the plasma LH concentrations in 5 month old domestic boars (Ford and Schanbacher, 1977). In miniature breeds, Elsaesser *et al*. (1976 *a*) reported that testosterone propionate administered to orchidectomized boars significantly suppressed LH in prepubertal but not in pubertal animals. They interpreted this finding as indicative of a declining sensitivity of the hypothalamus to testosterone negative feedback.

C. THE PURPOSE OF THE PRESENT STUDY

The studies described in this thesis were carried out to obtain fundamental data on the process of puberty in boars and to evaluate the neuroendocrine mechanism regulating the process.

The experiments in chapter III were designed to study the course of gonadal and epididymal development during sexual maturation and the concomitant changes in plasma levels of LH and testosterone. The experiments described in chapter IV were conducted to determine the 24 hr LH and testosterone secretory profiles of pubertal and post-pubertal boars.

CHAPTER II

MATERIALS AND METHODS

A. ANIMALS

Thirty-two Large White x Landrace boars derived from seven litters born in April, 1976 were utilized in the experiments described in this thesis. All animals were weaned at five weeks of age, at body weights of 10 - 12 kg.

B. ANIMAL MANAGEMENT

The boars were reared at the Pig Research Center, Massey University. Littermates were grouped together in concrete pens provided with wooden platforms. A standard weaner meal (composed of barley - 41.5 %, maize - 30.0 %, skimmed milk powder - 7.5 %, fish meal - 3.0 %, lupin seed meal - 6.0 %, lucerne - 2.0 %, bone flour - 3.0 % and salt-premix - 1.0 %) was fed *ad libitum* to the animals from weaning until 3 months of age. Thereafter, the feed was changed to grower meal (consisting of barley - 75.0 %, dried blood - 10.0 %, bone flour - 6.0 %, fish meal - 8.0 % and salt-premix - 1.0 %) given as a restricted ration of approximately 2 kg/animal/day. Water was provided *ad libitum* through automatic waterers.

As required by the experimental design for Experiment 2, some of the boars in that experiment were transferred to the Animal Physiology Unit for two weeks for the 24 hr samplings. During this period each boar was held in an individual steel crate inside a controlled climate room maintained at 15°C. Lighting was provided by two flourescent lights (80 W) controlled by automatic time switches and a 10:14 h light : dark lighting regime was instituted.

All animals remained in good health throughout the course of both experiments.

C. SURGICAL TECHNIQUES

1. Anaesthesia

Prior to induction of anaesthesia, azaperone ('Stresnil', Ethnor Limited, N.Z.) at a dose rate of 2 mg/kg body weight was administered as a tranquilizer. Subsequently, anaesthesia was induced and maintained with halothane ('Flouthane', I.C.I., U.K.) administered via a face mask. Halothane vapour concentrations of approximately 8 % and 3 % (v/v), respectively, in oxygen were utilized for induction and maintenance of anaesthesia.

2. Jugular Vein Catheterization

a. Insertion of the cannula into the jugular veins

The animals were placed on an operating table in dorsal recumbency then the neck, shoulders, and back were clipped and sterilized with 0.5 % (w/v) chlorhexidine ('Hibitane', I.C.I.) in 70 % v/v ethanol prior to surgery. On each side of the neck an 8 cm incision was made extending cranially and slightly laterally from a point midway between the points of the cariniform cartilage and the anterior tuberosity of the humerus. This site of incision parallels the jugular furrow lateral to the trachea. Subcutaneous adipose tissue and muscle layers were separated by blunt dissection to expose the jugular vein lateral to the trachea and medial to sternocephalic muscle. The vein was freed from the surrounding connective tissue by blunt dissection, then bull dog clamps placed on either side of the proposed point of cannulation, to prevent blood flow through the vessel. A 2 - 0 plain gut purse string suture was inserted in the vessel wall then a small V-shaped incision made in the center of the purse string with fine pointed scissors. At this stage, the centrally placed bull dog clip was released, then the cannula (Silastic, Dow Corning, Michigan, USA; 1.57 mm I.D. and 2.41 mm O.D.) was inserted so that two silastic retention collars, 10-12 cm from the end of the cannula, came to lie within the lumen of the vessel. The ends of the purse string suture were pulled tight and tied, then the peripherally placed bull dog clip was released to re-establish the flow of blood.

b. Exteriorization of the cannula

Once patency of the cannula had been checked, its peripheral end was exteriorized through a subcutaneous tunnel passing towards the dorsum of the animal. The tunnel was made by pushing a 25 cm trochar (6 mm O.D. and 4 mm I.D.) through the subcutaneous tissues until it protruded dorsally. The skin over the end of the trochar was incised, the head of the trochar removed, and the cannula passed through the trochar lumen. A retention ligature was made at the point of entry of the cannula to the subcutaneous tunnel, then the patency of the cannula checked again. Finally, the neck incision was closed by interrupted nylon sutures.

To allow for the possible failure of cannula patency both left and right jugular veins were catheterized.

3. Castration

The scrotal area was clipped and sterilized with hibitane prior to surgery. A primary skin incision was made along the median raphe of the scrotum. One testicle was exteriorized through the incision by incising and blunt dissecting the underlying fascia and parietal tunica vaginalis. After cutting the reflected tunica vaginalis propria the testis was withdrawn to expose the spermatic cord. Two Kelly hemostats were placed on the cord, which also was ligated with chromic gut at a point between clamps. Then the testicle was severed distal to the point of ligation. After removing the second testis by a similar procedure, the skin incision was closed with interrupted non-absorbable sutures.

4. Post-Surgical Care

After all surgical procedures, antibiotic therapy (procaine penicillin-streptromycin suspension, 'streptopen', 5 ml/animal) was instituted for five days as prophylaxis against infection. Skin sutures were removed approximately seven days post-operatively.

D. BLOOD COLLECTION

1. Fortnightly Sampling

For Experiment 1 blood samples (10 mls) were collected once every two weeks by puncture of the anterior vena cava, following the method of Carle and Dewhirst (1942). Animals less than 30 kg were bled while restrained on their back by an assistant. An appropriate sized needle was inserted into the depression formed by the superficial neck muscles just anterior and lateral to the sternal cartilage and the anterior border of the first rib. The needle was directed dorsally and caudally. Large animals were bled standing while restrained by a snubbing rope around the upper jaw. In this position, the needle was directed into the depression then medially, dorsally and caudally. For pigs below 10 weeks of age, 3.8 cm - 18 gauge needles were used, while 9 - 11.5 cm - 17 gauge needles were used for older boars. Blood samples were collected into heparinized vacutainers then kept in an ice bucket for up to 1 hr until conveyed to the laboratory where plasma was separated by centrifugation at 3,000 rpm for 30 minutes. Plasma was stored at -18°C until required for hormone assay procedures.

2. Twenty-four Hour Sampling

To facilitate the frequent withdrawal of blood for Experiment 2 without stressing the animals, indwelling catheters were inserted into the jugular veins as described in section C-2 of this chapter. Catheter patency was maintained by connecting them to infusion pumps which infused heparinized saline (containing 25 I.U./ml of sodium heparin) at a rate of 1 ml/hr. Blood sampling and saline infusion were undertaken from a remote sampling station outside the room housing the pigs so that the animals were unaware of these procedures.

Sampling commenced a week after the catheterization; 8 ml of blood was withdrawn into heparinized tubes once every twenty min for 24 hr. Again plasma was separated by centrifugation, then frozen until assayed for LH and testosterone concentrations.

E. HORMONE RADIOIMMUNOASSAY PROCEDURES

1. LH Assay

a. Materials and Procedure

Plasma LH concentrations were estimated by the double antibody porcine LH radioimmunoassay described by Niswender *et al.* (1970).

Purified porcine LH (LER 786-3, courtesy of Dr. L.E. Reichert, Emory University, Atlanta, Georgia) was labelled with ^{125}I using a chloramine T method based on that of Greenwood, Hunter, and Glover (1963). Standard solutions of purified porcine LH (LER 778-4, also from Dr. Reichert) were prepared to provide a standard curve corresponding to a range of plasma concentrations from 0 to 16 ng/ml.

Plasma samples and standards were assayed in triplicate in polystyrene tubes (11 x 75 mm). To each tube was added 200 µl of porcine LH antiserum (courtesy of Dr. G.D. Niswender, Colorado State University, USA) diluted 1 in 40,000 with 0.05 M EDTA (ethylene diamine tetra acetic acid di-sodium salt) - PBS [phosphate buffered saline - 0.01 % (w/v) sodium merthiolate as a preservative, pH = 7.3] containing non-immune rabbit serum (1:400); 200 µl of standard porcine LH solution or plasma sample; and 300 µl of PBS containing 1 % egg white. The mixture was incubated for 24 hr at 4°C after which 100 µl of radioiodinated porcine LH (50,000 cpm) was added. After a further incubation at 4°C for 24 hr 200 µl of precipitating antiserum (sheep anti-rabbit gamma globulin serum diluted 1:25 in 0.05 M EDTA-PBS) was added. Then after a final incubation for 72 hr at 4°C, the separation of bound and unbound hormone was completed by centrifugation at 3,000 rpm for 30 min at 4°C. The supernatant was aspirated, then the radioactivity of the precipitate was counted in a Packard Auto-Gamma Scintillation Spectrometer (Model 5285).

An IBM 1620 computer was used to determine plasma LH concentrations by the method of Burger, Lee and Rennie (1972). With this technique a best-fit expression for the standard curve was calculated, then values for the samples (means ± standard deviations) were computed. A composite standard curve representing the mean values from four consecutive assays is shown in Figure 2.1.

b. Assay Validation

The specificity of the porcine LH antiserum was determined by Niswender *et al*. (1970). These authors found that porcine

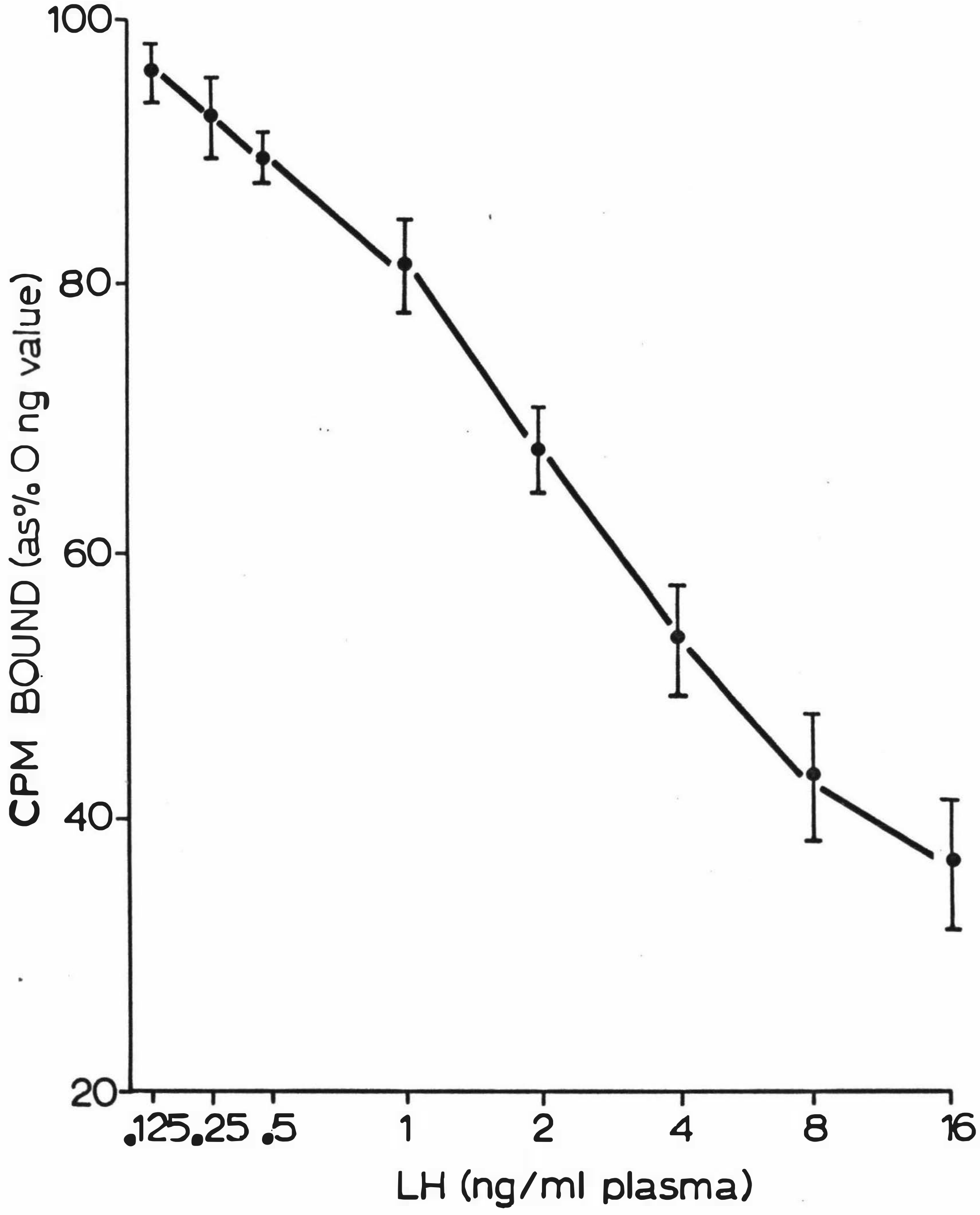


Figure 2.1 : Composite standard curve (mean ± S.E. of 4 consecutive assays) for porcine LH radioimmunoassay.

Follicle Stimulating Hormone (FSH) and porcine Thyroid Stimulating Hormone (TSH) did not affect radioimmunoassay estimates of LH.

Precision of the assay was checked by assaying 3 reference plasma samples repeatedly in each of the four assays. The between- and within-assay coefficients of variation (C.V.) for each reference plasma sample were:

Pool	Plasma number	Mean LH Concentration (ng/ml)	Within-Assay C.V.(%)	Between-Assay C.V.(%)
Z	1	2.01	14.00	32.1
X	2	3.23	16.00	22.9
Y	3	8.87	13.25	19.1

Assay sensitivity, defined as the minimal detectable concentration of the hormone significantly different from zero ranged between 0.10 and 0.19 ng/ml. This range of values corresponded to the lowest point on the standard curve with a coefficient of variation equal to 50 % and with 95 % fiducial limits which did not overlap zero (Burger, Lee and Rennie, 1972).

Based on these validation tests it was considered that this assay provided reliable estimates of porcine LH levels in 200 μl plasma.

2. Testosterone Assay

a. Materials and Procedure

Testosterone was analyzed by radioimmunoassay without chromatography following the procedure (RIA II) of Barrell, (1976). This was a modification of the assay of Smith and Hafs (1973) and incorporated some methods described by Terqui and Thimonier (1974). Tritiated testosterone (1,2,6,7 Tritiated testosterone) with specific activity of 86.4 Ci/m mol was obtained from the

Radiochemical Centre, Amersham, UK, while anti-testosterone-3-BSA serum (#S-250) raised in rabbits was supplied by Dr. G.D. Niswender.

Extraction of steroid was achieved by mechanically shaking 9 mls of Toluene : Hexane (1:2) with 500 µl of plasma in screw-capped glass culture tubes for 10 min. After freezing the plasma, the solvent phase was decanted into glass test tubes and dried under air in a water bath at 35°C. Tube walls were rinsed with 1 ml of dichloromethane which then was evaporated under air. Then 500 µl of absolute ethanol was added to dissolve the testosterone, and three 100 µl aliquots of the plasma extract in ethanol were dispensed into polystyrene tubes for assay, as also were triplicate 100 µl aliquots of testosterone in ethanol (standard testosterone solutions corresponded to a range of plasma concentrations from zero to 50 ng/ml). After drying the ethanol under air, 200 µl anti-testosterone (diluted 1 in 25,000 with 1 in 200 non-immune rabbit serum in PBS 0.1 % gelatin and 200 µl ^{3}H-testosterone also in PBS 0.1 % gelatin (containing approximately 25,000 cpm), were added to each tube. Tubes were incubated in a 35°C water bath for 30 min, then at 4°C for 2 hr. Free and antibody bound steroid were separated by adding 300 µl of a 0.5 % charcoal suspension in 1 % Dextran T 70. Tubes were incubated for 10 min at 4°C then centrifuged at 3,000 rpm for 10 min. 500 µl aliquots of supernatant were added to scintillation vials with 5 ml scintillation fluid [9 g PPO (2,5-Diphenyl oxazole); 300 mg POPOP (1,4 (2-5-phenyloxazolyl)-Benzene); and 1,000 ml Triton X 100 / 2 L Toluene] and counted in a Beckman Liquid Scintillation Counter (Model LS 350) for 2 min per vial.

Assay results were estimated by an IBM computer as described for the LH assay. A composite standard curve from 11 consecutive assays is shown in Figure 2.2.

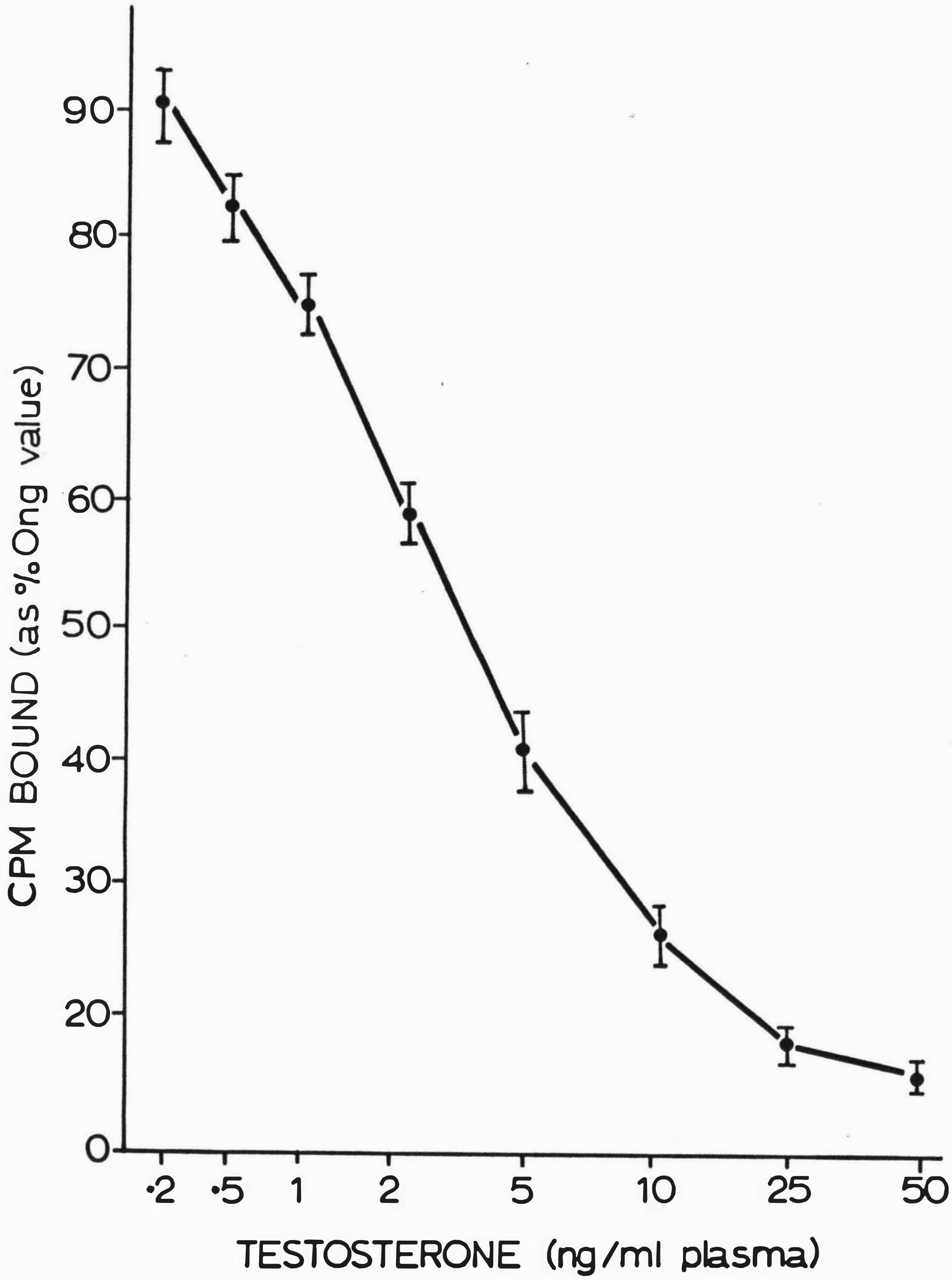


Figure 2.2 : Composite standard curve (means ± S.E. of 11 consecutive assays) for testosterone radioimmunoassay.

b. Assay Validation

The antiserum utilized in the present assay was characterized by Dr. V.C. Gay, University of Pittsburg and was shown to cross-react with other androgens:- Dihydrotesterone (69 %), 3α-androstanediol (14 %), and 3β-androstanediol (22 %). Previous studies (Lindner, 1961 b and Elsaesser et al., 1976) indicated that these androgens were present in very low concentration in boar plasma so that their possible interference in the estimation of testosterone concentrations may be considered negligible.

Parallelism of the standard curve and a curve obtained by assaying varying quantities of testosterone, extracted from a plasma sample, was checked by assaying the sample undiluted (11.45 ng/ml), diluted 1:1 (6.59 ng/ml) and diluted 1:3 (3.69 ng/ml) in barrow plasma (n = 6).

Assay sensitivity, defined as the lowest testosterone concentration significantly different from zero, varied between 0.09 and 0.58 ng/ml.

Estimates of the within- and between-assay C.V., previously measured by Mr. P.R. Wilson, Massey University, were as follows:

Sample	Mean testosterone Concentration (ng/ml)	No. of Assays	Within Assay C.V.(%)	Between Assay C.V.(%)
1	0.31	25	27.60	22.40
2	5.07	27	8.41	13.36
3	9.29	12	7.04	5.54

Further evidence for the validity of the assay was indicated by the drop in testosterone levels to below the limit of sensitivity of the assay following castration of 5 boars in Experiment 1.2. Also, testosterone was not detectable in plasma collected from barrows or gilts.

F. EXPERIMENTAL DESIGNS AND ANALYSES

Details of the experimental designs and methods of statistical analyses for each experiment are described in the appropriate chapters.

CHAPTER III

A LONGITUDINAL STUDY OF PUBERTAL DEVELOPMENT IN BOARS: INVESTIGATION OF THE RELATIONSHIPS BETWEEN GONADAL AND EPIDIDYMAL DEVELOPMENT AND PLASMA LH AND TESTOSTERONE PROFILES

A. INTRODUCTION

Relatively little work has been conducted on the course pubertal development in boars. Earlier workers established the age of pubertal onset by testing the ability of boars to mate (Wiggins et al., 1951) and by macroscopic and microscopic studies of testicles and epididymides (Phillips and Andrews, 1936; Hausser et al., 1949; Niwa and Mizuho, 1954; McFee et al., 1967).

Studies of the endocrine changes occurring during sexual maturation also have been limited. Linder (1961 b) used paper chromatography to identify testosterone and androstenedione in the spermatic venous effluent of boars; similar studies by Gray et al. (1971) and Carlson et al. (1971) comfirmed Lindner's results. More recently German workers (Elsaesser et al., 1975; Elsaesser et al., 1976; Ellendorff et al., 1975; Pomerantz et al., 1974) have used radioimmunoassay methods to measure plasma LH and testosterone levels in foetal, neonatal, pubertal and adult male and female miniature pigs. However, as yet no reports have been published describing systematic studies of hormone profiles during pubertal development in boars. Neither have there been adequate studies of the interrelationships between hormone profiles and the stages of gonadal and bodily growth in this species.

The experiments described in this chapter were designed as a longitudinal study of the course gonadal and epididymal development during sexual maturation in boars, and of the concomitant changes in plasma levels of LH and testosterone. Post-pubertally, the existence of a steroid negative feedback mechanism was investigated by examining the effects of castration on plasma hormone levels.

B. MATERIALS AND METHODS

1. Animals and Experimental Procedure

In Experiment 1.1 twenty four boars from six litters born at approximately the same time were selected at random for castration at the following ages: 1½, 3, 4, 4½, 5, 5½, 6, 7, and 8 months. Three boars were castrated at each age except at 4, 5, and 8 months when the number was two. Body weights were recorded at two weekly intervals.

Immediately following castration, testes and epididymides were trimmed free of any adhering tissue and the combined weights of left and right testes and epididymides recorded. Next, approximately 5 mm thick tissue wedges were removed from the center of the testes and the body of epididymides and fixed in Bouin's fluid for 24 hr before transfer to 70 % ethanol to await histological processing. Fixed tissues were embedded in paraffin wax after automatic processing, then 5 µm sections were stained with Hematoxylin-Eosin. Seminiferous tubule diameters were measured on a microscope fitted with an eyepiece micrometer and the mean diameter for each testis was calculated as the mean recorded from 20 round tubules; duplicate measurements at right angles were made for each tubule (modified from the method of Skinner and Van Heerden, 1971). Mean epididymal diameters and mean epididymal tubule lumen diameters were estimated similarly from duplicate measurements made on each of five round epididymal tubules. Epididymal spermatozoal reserves at each age were determined by making quadruplicate direct counts of diluted epididymal homogenates prepared by a technique similar to that described by Lino (1972).

In Experiment 1.2 boars selected from two litters born on April 22nd, 1976 were bled between 09.00 and 10.00 h at fortnightly intervals, from 41 to 236 days of age. At 215 days of age five boars were chosen randomly and castrated while the remaining five were kept as entires. All plasma samples were assayed for concentrations of LH and testosterone.

2. Statistical Analysis

The associations of the rates of increase in testicular and epididymal weights with age and with body weight were estimated by calculating their correlation coeffecients and drawing linear regression lines. The slope of the linear regressions was estimated by the method of least squares with the equation:

$$y_i = a + b_{x_i}$$

where y_i represents the paired testicular/epididymal ewight

and x_i is the age/body weight

Differences in the plasma levels of LH and testosterone between castrates and entires, and between the two litter groups, were tested for significance using Student's 't' test.

C. RESULTS

1. Testicular, Epididymal and Body weights

See Tables 3.1 and 3.2 and Figures 3.1 to 3.5.

Mean body weights and weights of testes and epididymides increased throughout the period of sampling from 43 to 241 days

Table 3.1

Body Weights, Testicular Weights, Epididymal Weights and Diameters of Seminiferous Tubules, Epididymal Tubules and Epididymal Tubule Lumina in Individual Boars.

Boar Number	Age (Days)	Body Weight (kg)	Testis Weight (g)	Epididymal Weight (g)	Seminiferous Tubule Diameter (µm)	Epididymal Tubule Diameter (µm)	Epididymal Tubule Lumen Diameter (µm)
24/59	43	18	7.79	2.34	49.25	103	75
25/6	43	22	9.01	2.14	53.76	112	75
23/59	44	12	6.21	3.30	53.36	106	77
23/9	89	38	33.48	8.73	77.52	225	73
24/9	88	30	24.29	13.02	58.06	202	89
21/7	94	38	49.79	13.09	95.28	241	100
21/567	125	48	126.18	30.84	121.49	309	131
24/56	129	50	66.76	19.93	105.62	233	107
23/58	145	60	87.60	30.90	115.99	266	134
24/57	144	51	119.70	34.00	113.37	307	142
21/8	150	59	268.00	59.40	164.62	337	143
24/567	161	55	160.93	34.10	149.43	320	155
23/68	162	70	177.34	44.41	159.62	320	150
25/7	175	82	301.60	66.37	186.43	410	233
24/67	175	80	262.28	58.77	170.92	361	199
24/6	175	80	226.02	56.42	186.73	292	134
25/57	187	90	363.33	70.82	195.68	449	296
23/6	188	90	311.91	73.73	207.68	412	248
24/69	187	93	289.60	69.71	197.68	494	264
19/67	216	114	413.62	100.02	218.49	480	282
19/8	216	116	443.28	100.26	212.99	480	323
20/68	216	93	466.33	104.77	209.43	504	346
19/5	241	130	634.45	142.93	197.99	524	406
19/57	241	123	489.68	119.93	212.67	456	284

Table 3.2

Mean Body Weights, Testicular Weights, Epididymal Weights and Diameters of Seminiferous Tubules, Epididymal Tubules, and Epididymal Tubule Lumina in Boars Between 43 and 241 Days of Age.

Age (Days)	Body Weight (kg)	Testis Weight (g)	Epididymal weight (g)	Seminiferous tubule Diameter (μm)	Epididymal tubule Diameter (μm)	Epididymal tubule Diameter (μm)
43	17.50	7.67	2.26	52.12	107.5	75.8
90	35.33	32.22	11.61	76.95	214.8	87.8
127	49.00	96.47	25.38	113.55	266.9	117.00
146	58.66	158.26	40.43	131.32	303.4	139.9
161	62.50	169.13	40.25	154.52	320.0	152.0
175	80.66	263.30	60.52	181.36	354.0	188.0
187	91.00	321.63	71.42	200.34	427.9	272.0
216	107.66	441.07	101.69	213.63	487.5	348.6
241	126.50	562.06	131.43	205.33	492.0	345.0

Note: n=3 except at ages 146, 161 and 241 days where only 2 animals were sampled.

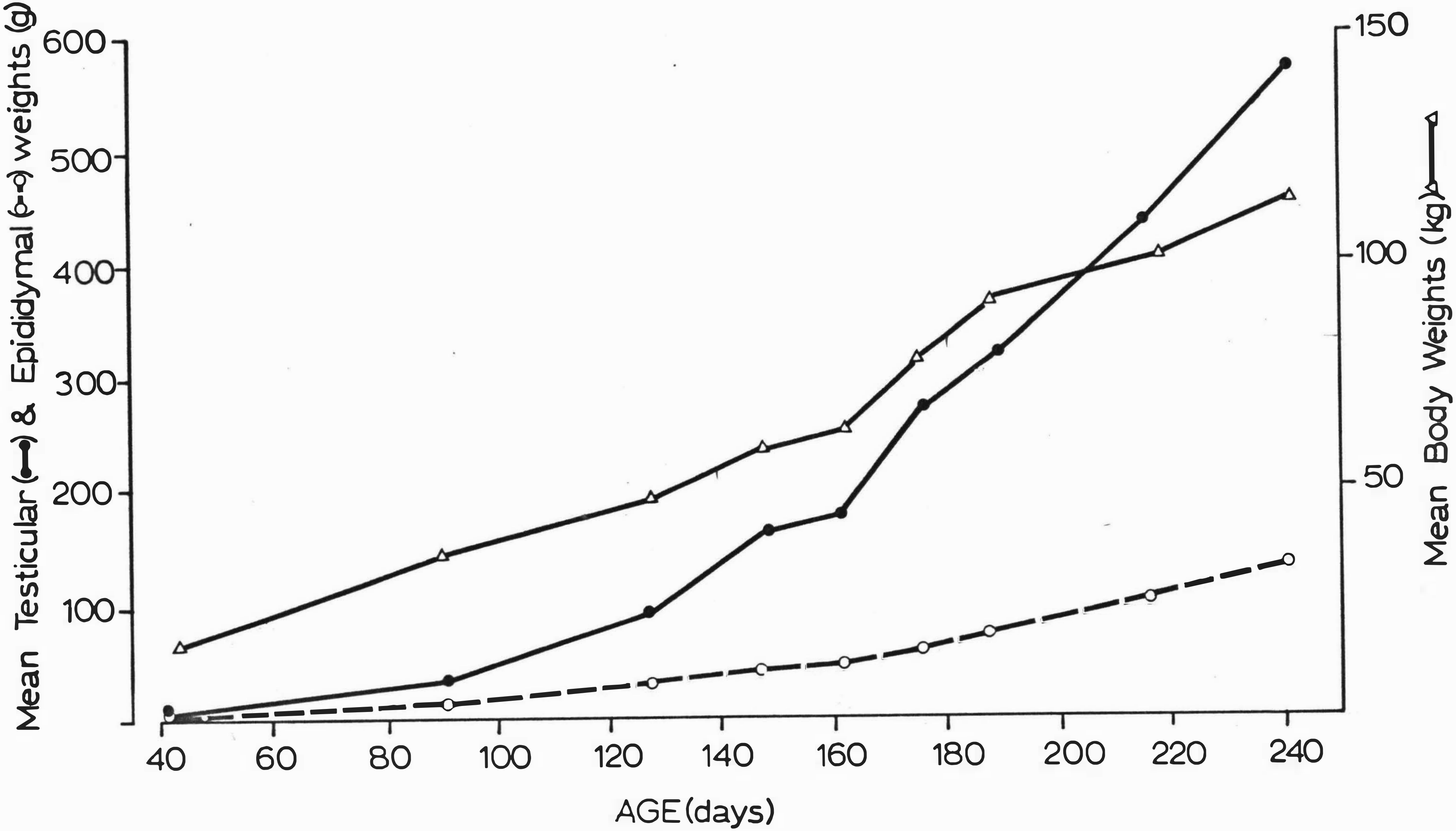


Figure 3.1 : Body weights, testicular weights, and epididymal weights of boars during sexual maturation.

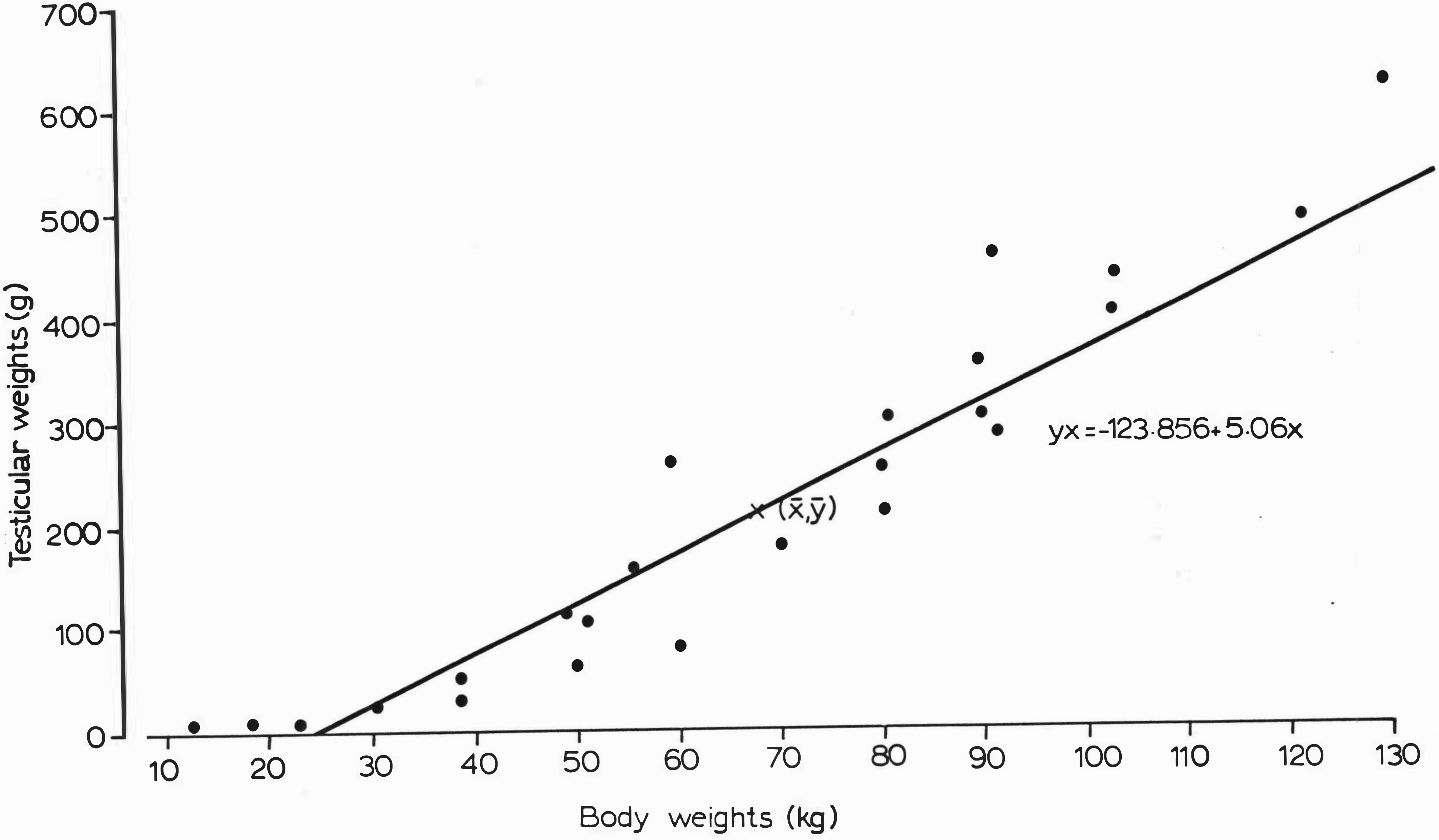


Figure 3.2 : Relationships between testicular weights and body weights of boars during sexual maturation.

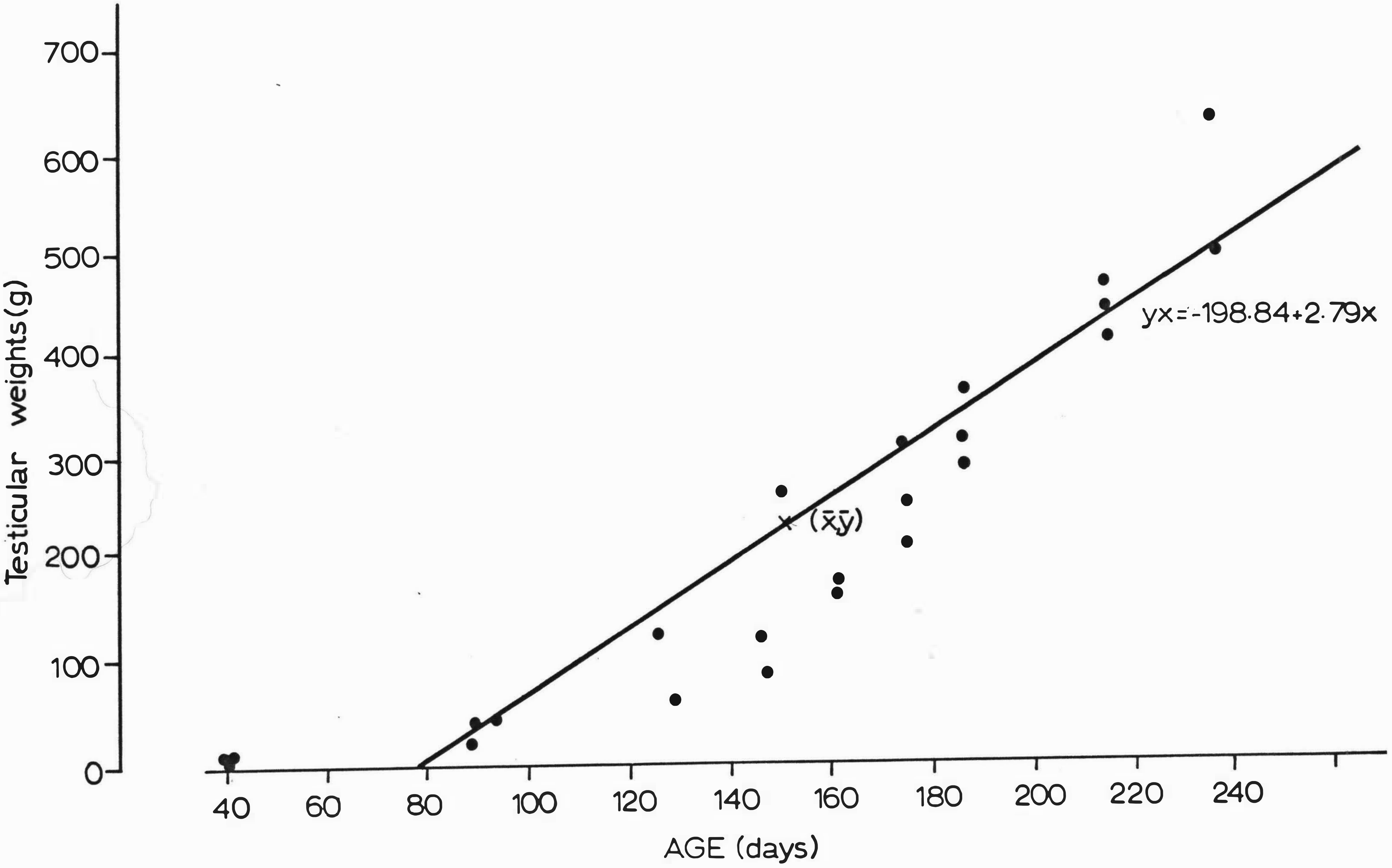


Figure 3.3 : Relationships between testicular weights and age of boars during sexual maturation.

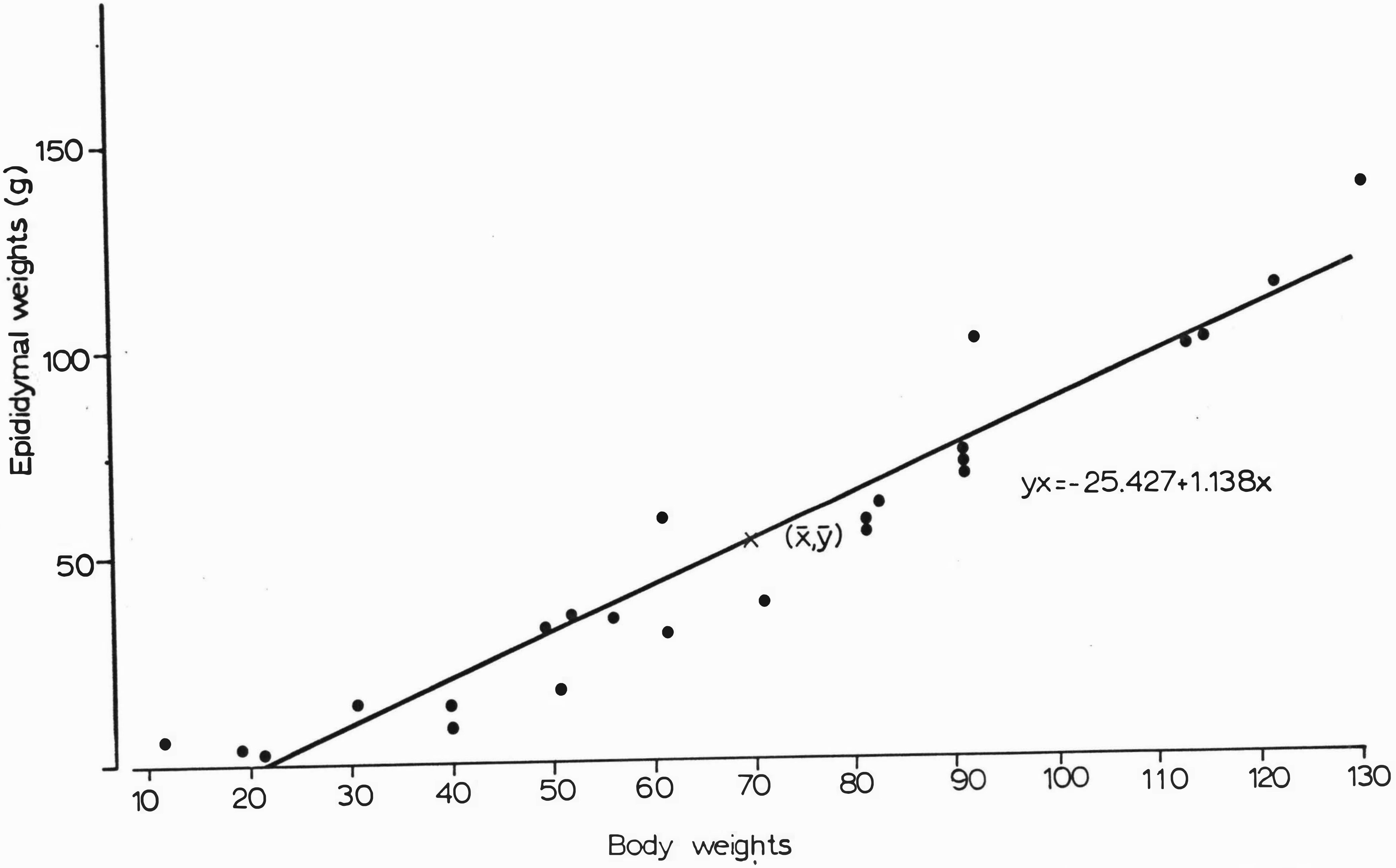


Figure 3.4 : Relationships between epididymal weights and body weights of boars during sexual maturation.

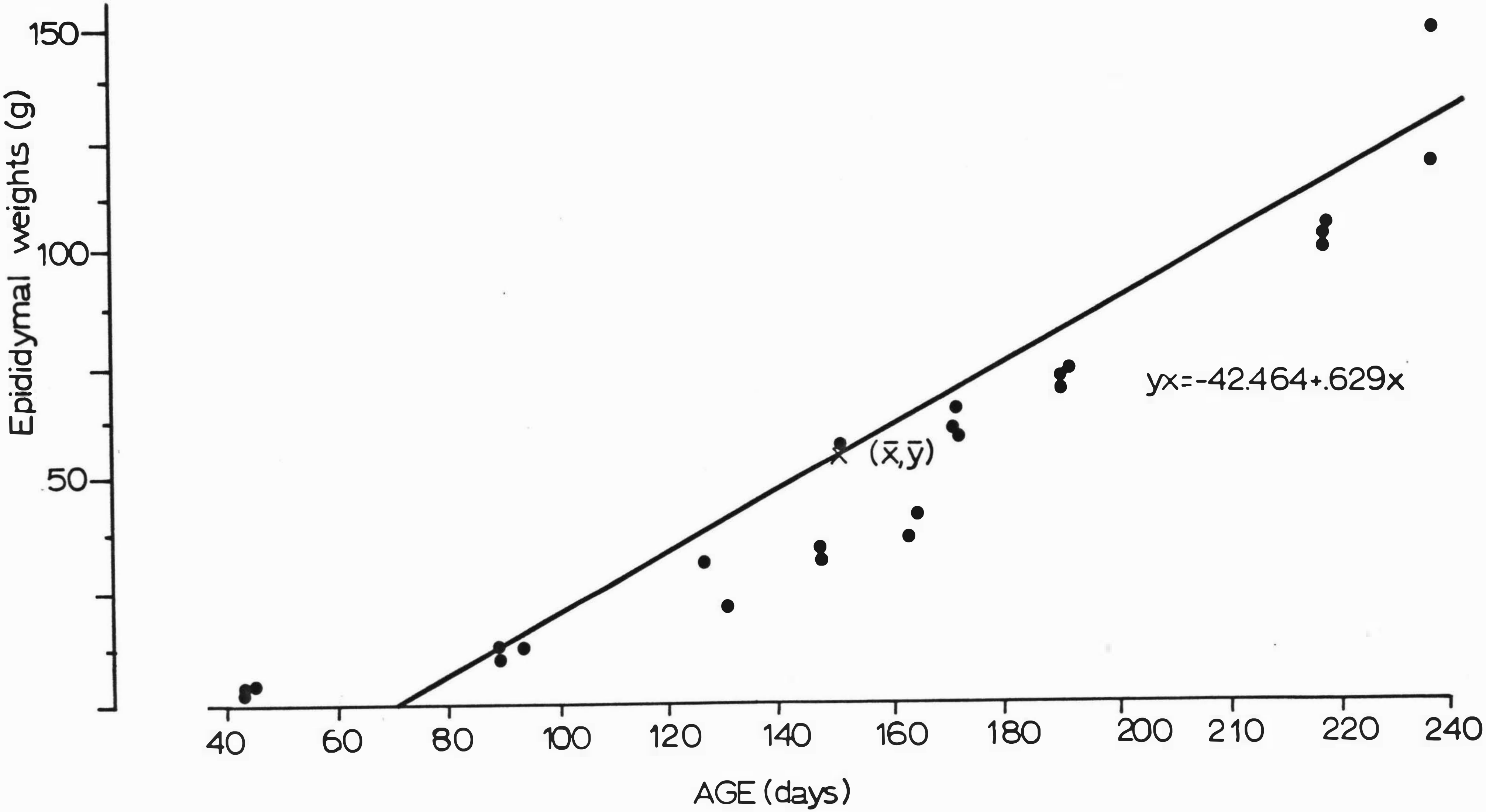


Figure 3.5 : Relationships between epididymal weights and age of boars during sexual maturation.

of age. The course of testicular growth was slightly more highly correlated with body weight ($r = 0.953$, $P < 0.001$) than with age ($r = 0.919$, $P < 0.001$). The regression of testicular weight on body weight (Figure 3.2) was described by the equation $y = -123.85 + 5.06x$, while the regression of testicular weight on age (Figure 3.3) was given by the equation $y = -198.84 + 2.79x$. Compared to testicular weight, epididymal weight was not so highly correlated with body weight ($r = 0.558$, $P < 0.01$) or age ($r = 0.593$, $P < 0.01$). The equation $y = -25.427 + 1.138x$ described the regression of epididymal weight on body weight (Figure 3.4), while the relationship between epididymal weight and age (Figure 3.5) was described by the equation $y = -42.464 + 0.629x$.

2. Epididymal Sermatozoal Reserves

For epididymides collected from boars aged between 43 and 127 days epididymal spermatozoal reserves (ESR) were zero. At 146 days of age, one boar (21/8) with testicular development more advanced than others from its group, had an ESR count which even exceeded those from the 161 and 175 day old boars; the other two animals sampled at 146 days had no epididymal sperm. Mean ESR's from 146 days of age until the last sampling were: 5.54×10^9 at 146 days; 1.85×10^7 at 161 days; 8.14×10^9 at 175 days; 27.96×10^9 at 187 days; 45×10^9 at 216 days; and 130×10^9 at 241 days.

3. Testicular and Epididymal Histology

a. Seminiferous Tubule and Epididymal Tubule Diameters

See Tables 3.1 and 3.2 and Figures 3.6 and 3.7.

Both seminiferous tubule and epididymal tubule diameters increased with age in parallel with the increase in testicular and epididymal weights, respectively. However, after reaching

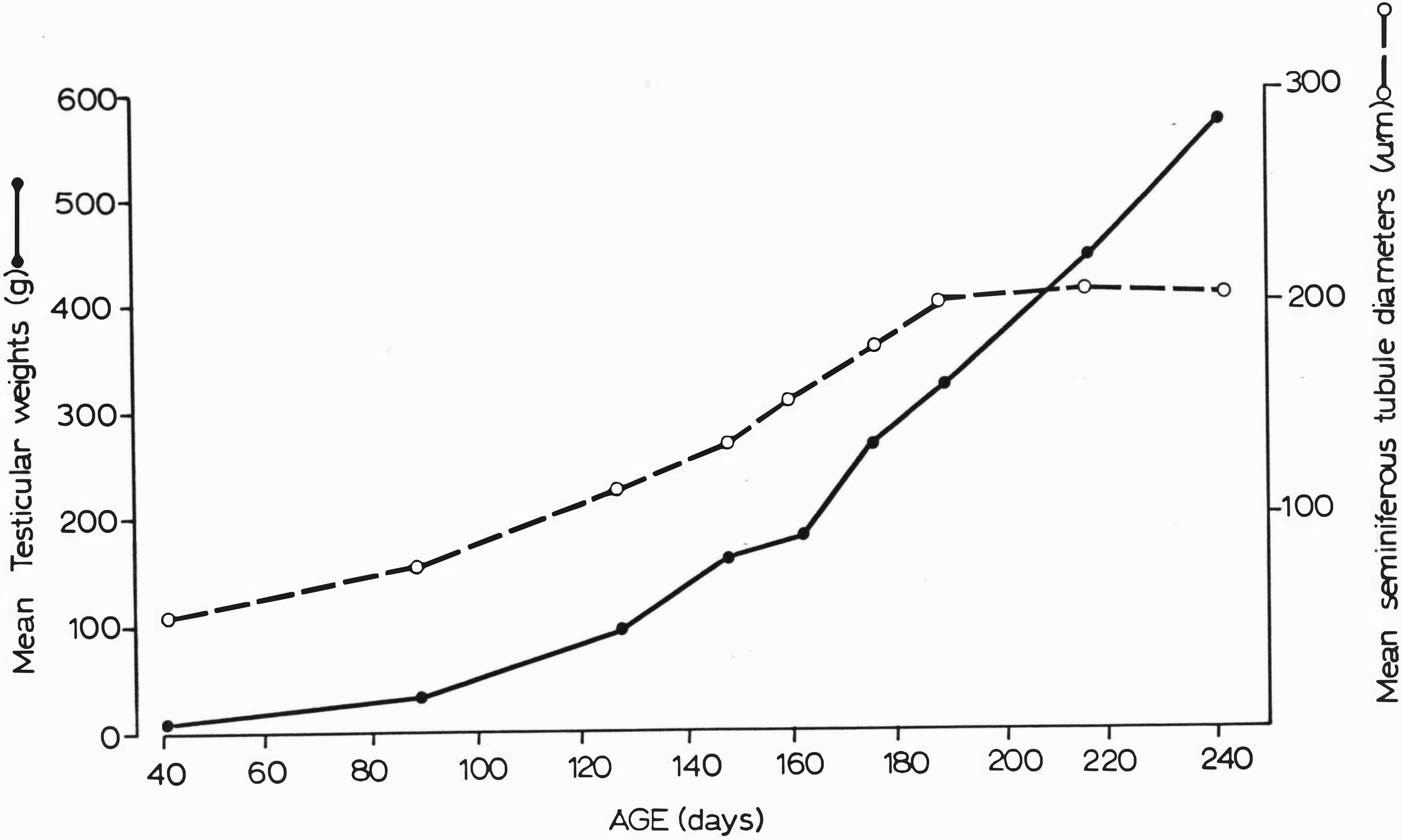


Figure 3.6 : Testicular weights and seminiferous tubule diameters during sexual maturation.

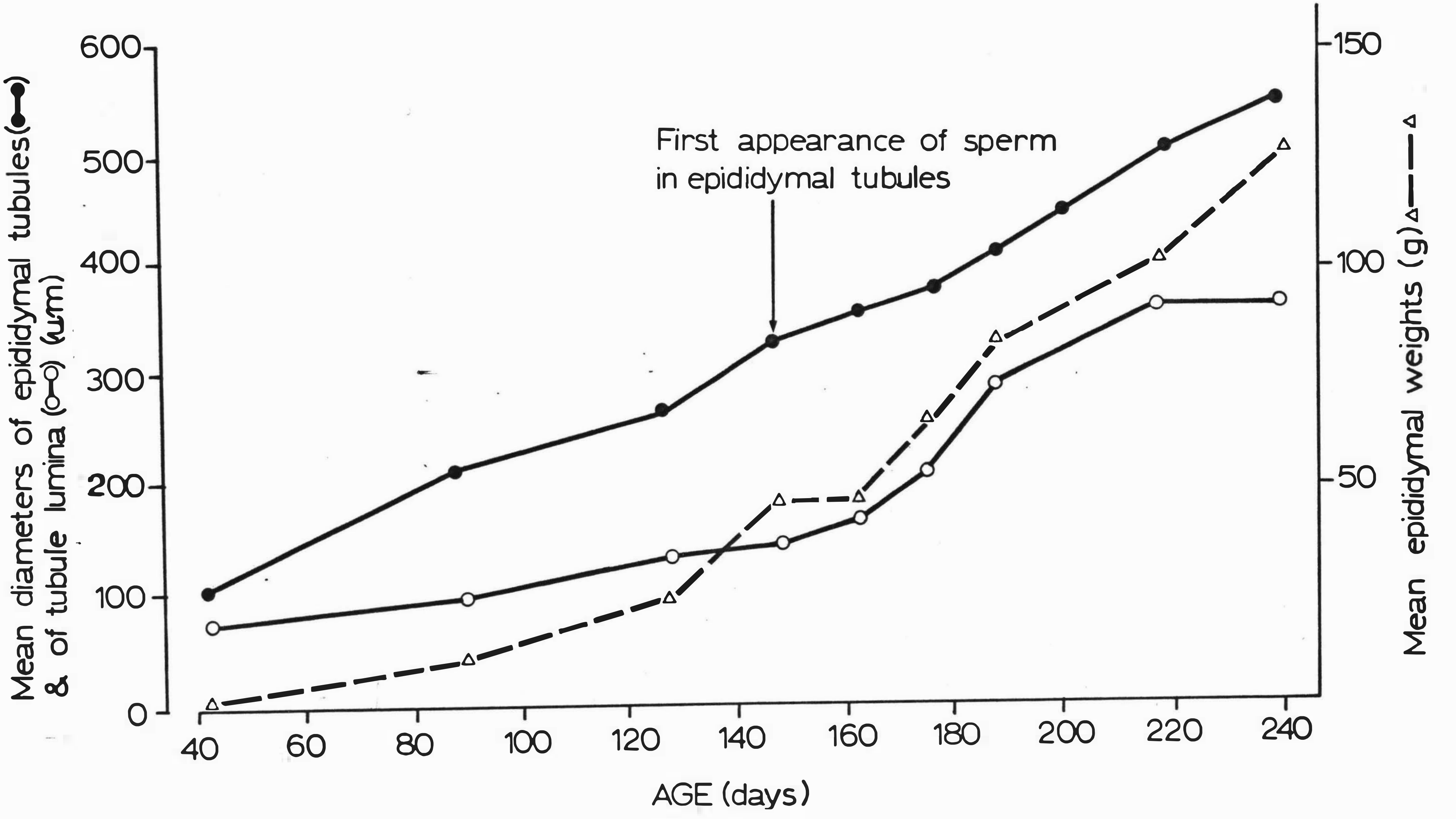


Figure 3.7 : Epididymal weights and diameters of epididymal tubule and of tubule lumina during sexual maturation

a maximum diameter of 213 µm at 216 days of age, no further increase in seminiferous tubule diameters occurred even though testicular growth continued until the last sampling (241 days). In contrast to that pattern, epididymal tubules continued to increase in diameter throughout the study, but there was no increase in diameter of epididymal tubule lumina between the samplings at 216 and 241 days (348.6 vs 345.0 µm).

b. Qualitative Testicular and Epididymal Histology

See Figures 3.8 - 3.17.

Microscopic examination of testicular and epididymal sections revealed very distinct histological differences between prepubertal, pubertal, and sexually mature boars.

In 43 day old boars (Figure 3.8) seminiferous tubules were of small diameter ($\bar{x}$ = 52.12 µm) and lacked lumina. Only two types of cells were noted in the seminiferous tubules: (i) adjacent to the basement membrane, the supporting cells formed a single layer of small, irregularly shaped cells with darkly staining nuclei and, (ii) a few larger cells (gonocytes/prospermatogonia) with rounded lightly staining nuclei were located more centrally. At this age interstitial tissue comprised the bulk of the testes and consisted of a few fibroblasts and numerous interstitial Leydig cells, which were characterized by eosinophilic granular cytoplasm and basophilic nucleoplasm containing one or two nucleoli. Epididymal sections at this age (Figure 3.9) showed few tubules with narrow diameters ($\bar{x}$ = 107.5 µm) but patent lumina. These tubules were lined by a simple cuboidal epithelium.

At 90 days of age (Figure 3.10) the following developmental changes were observed: (i) mean seminiferous tubule diameter had increased to 76.95 µm; (ii) spermatogonia and occasional primary

Figure 3.8 : Photomicrograph of a testicular section from a 43 day old boar. H. & E. stain. X 400. Note: predominance of interstitial tissue (I); presence of gonocytes (g) and single row of supporting cells (sc) in seminiferous tubules.

Figure 3.9 : Photomicrograph of an epididymal section from a 43 day old boar. H. & E. stain. X 400. Note simple cuboidal to low columnar epithelium lining the epididymal tubule.


g
sc
l

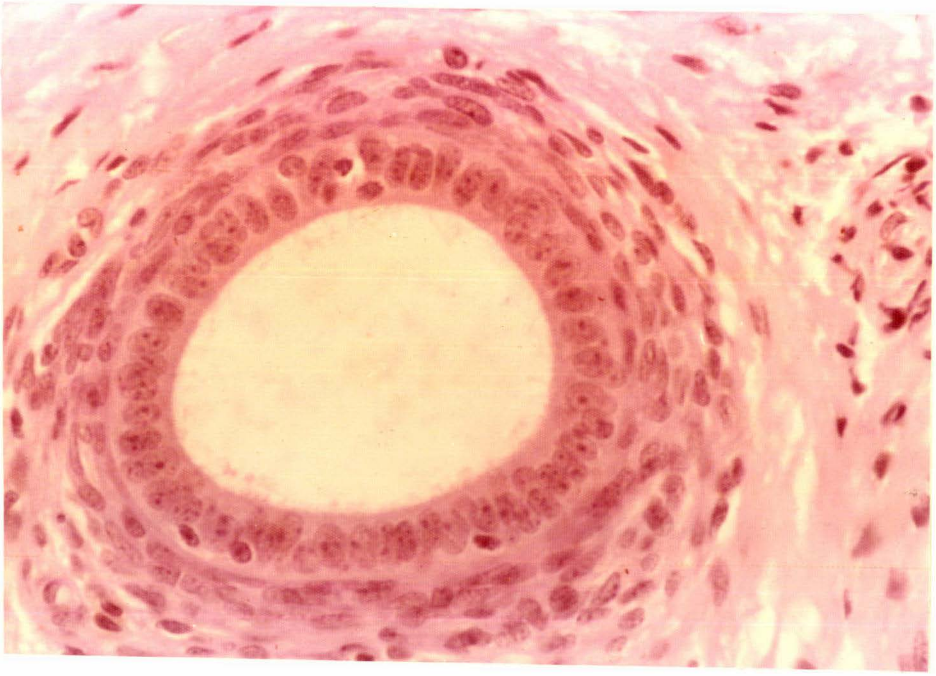
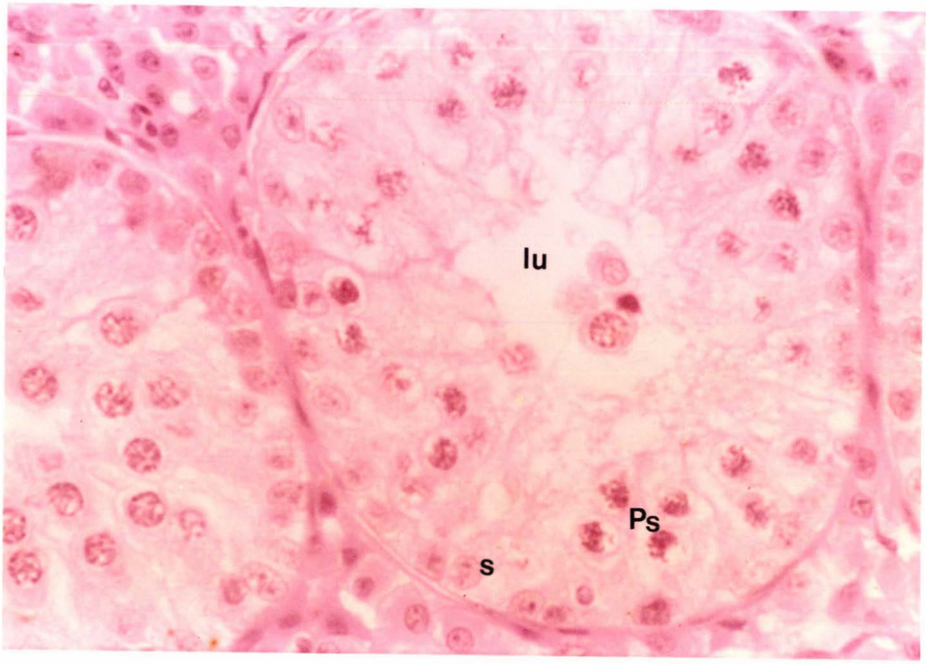


Figure 3.10 : Photomicrograph of a testicular section from a 90 day old boar. H. &. E. stain. X 400. Note the increase in diameter of seminiferous tubules and appearance of central lumen (lu), spermatogonia (s), and primary spermatocytes (Ps).

Figure 3.11 : Photomicrograph of an epididymal section from a 90 day old boar. H. & E. stain. X 400. Note the development of pseudostratified columnar epithelium and the stereocilia along the luminal border.

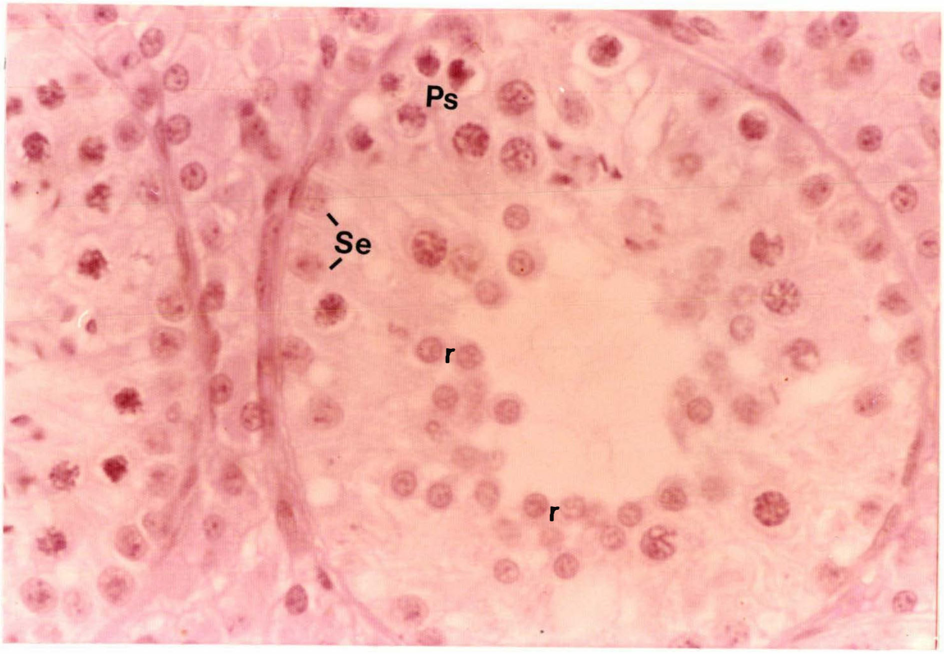

lu
Ps
s

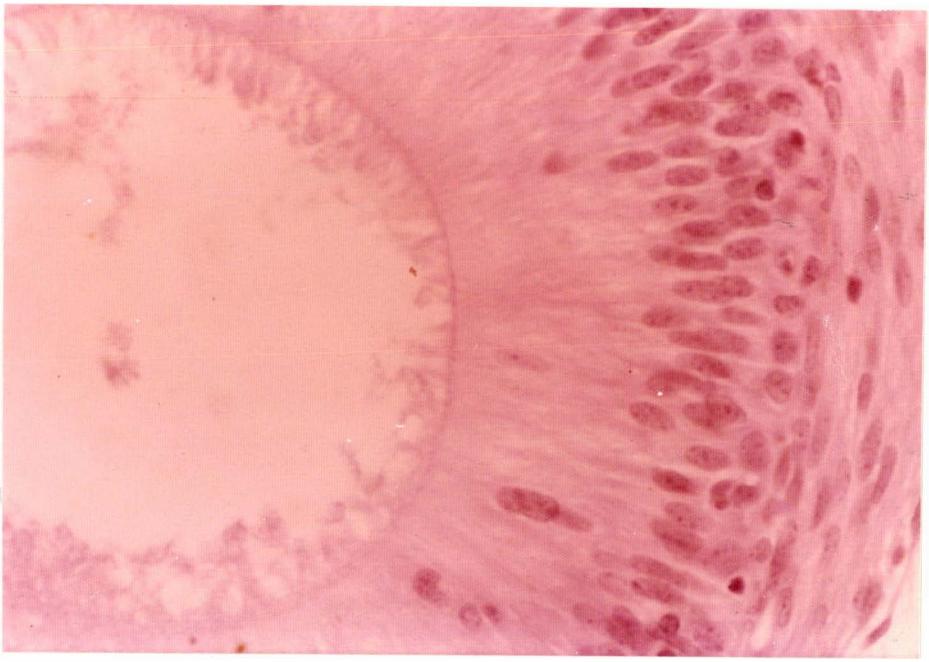
spermatocytes had appeared; (iii) supporting cells had started to assume Sertoli cell morphology (with irregular nuclei and lightly staining nucleoplasm); (iv) lumina had begun to form in some tubules; (v) the relative volume of interstitial tissue had declined; and (vi) interlobular septa had developed and divided the testis into distinct lobules. One boar from this group showed relatively advanced testicular development with the appearance of seminiferous tubule lumina, increased numbers of spermatogonia and a greater number of primary spermatocytes. The testicular weight of this particular boar (49.79 g) was well above that of the other animals castrated at this age (24.29 and 33.48 g). Epididymal tubule diameters at 90 days of age (Figure 3.11) had increased to 214.8 µm. The pseudostratified columnar epithelial cells bordering the basement membrane showed a four fold increase in mean height as compared to the 43 day old group (55.00 vs 13.33 µm).

Seminiferous tubules in sections from the 127 day old boars (see Figure 3.12) had increased in size to a mean diameter of 113.55 µm and all exhibited patent lumina. Increased numbers of spermatogonia and a few mature Sertoli cells were identified in all boars. More centrally were found primary spermatocytes and a few elongating spermatids, while one boar had mature spermatozoa in some of its seminiferous tubules. There also was a further reduction in the proportion of interstitial tissue, however Leydig cells were still numerous. The epididymal tubules exhibited a nearly mature appearance at this stage (Figure 3.13): the height of the pseudostratified columnar cells increased to a mean of 64.31 µm and tufts of long stereocilia projecting towards the luminal surface were prominent; likewise, large increases in epididymal tubule and epididymal tubule lumen diameters were noted

Figure 3.12 : Photomicrograph of a testicular section from a 127 day old boar. H. & E. stain. X 400. Note presence of round spermatids (r), Sertoli cells (Se) along with primary spermatocytes (Ps) and spermatogonia (s).

Figure 3.13 : Photomicrograph of an epididymal section from a 127 day old boar. H. & E. stain. X 400. Note further increase in epididymal tubule lumen diameter and well-developed pseudostratified ciliated columnar epithelium.


Ps
Se
r
r



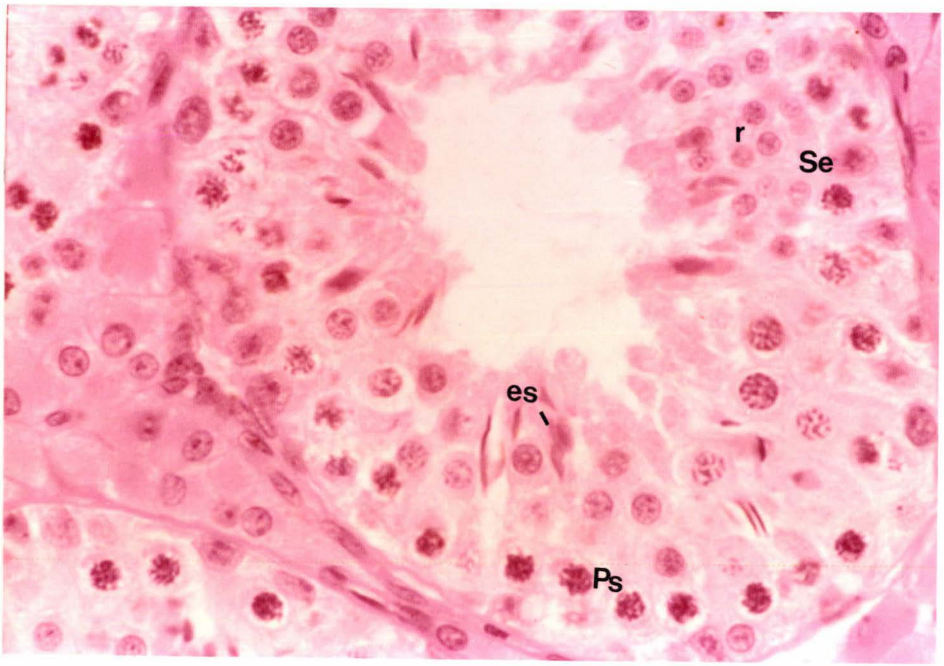
($\bar{x}$ = 266.9 and 117.9 µm, respectively).

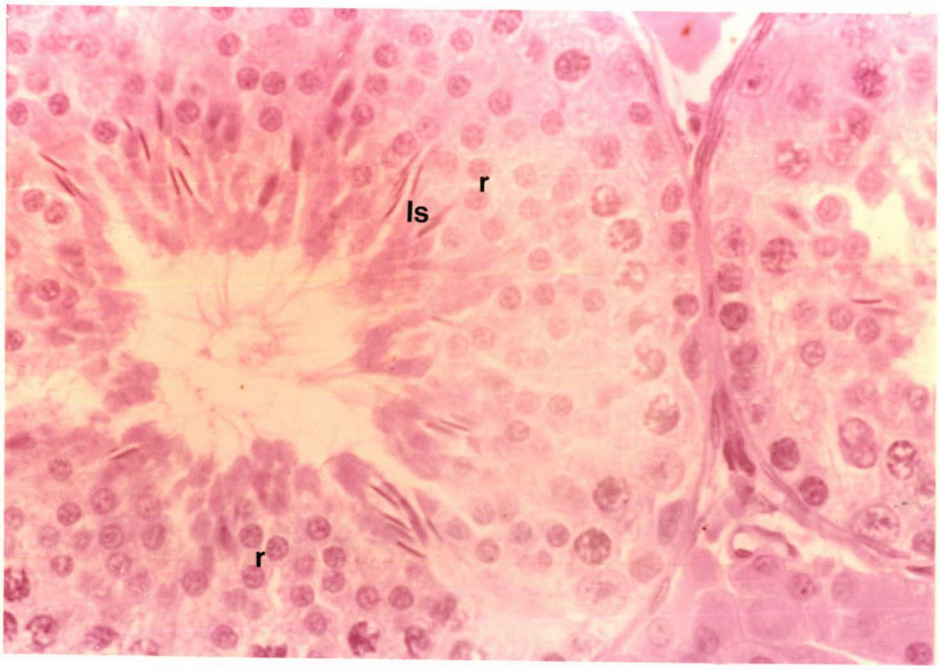
At 146 days of age (Figure 3.14) the mean seminiferous tubule diameter was 113.32 µm, while the most noteworthy qualitative changes were the appearance of more mature Sertoli cells and an increase in the proportion of primary spermatocytes and spermatids. This great increase in seminiferous tubule diameters had reduced the relative size of spaces between the tubules and made the interstitial cells appear more compact, however no obvious change in the Leydig cell morphology was observed. There was a considerable variation in the degree of testicular development between the individual boars. One animal clearly was more advanced than the others (combined testicular weight = 268.00 g <u>vs</u> 87.60 and 119.20 g) and showed the presence of spermatozoa in the majority of its epididymal tubules; no other animal had epididymal spermatozoa. Evidence of secretory activity in epididymal tubules also was noted: lipid droplets and secretory granules could be seen passing out of the epithelial cells into the lumen. Mean diameters of epididymal tubules and of tubule lumina had increased to 303.4 and 139.9 µm, respectively.

At 161 days the mean seminiferous tubule diameter had increased further to 154.52 µm, while the cell population also increased greatly and consisted mostly of spermatids and primary spermatocytes. No further reduction in the volume of interstitial tissue was noted. Leydig cells appeared larger with well-defined eosinophilic cytoplasm and rounded nuclei. Nucleoplasm contained basophilic coarse chromatin grunules and one or two nucleoli. In epididymal sections, increases in diameters of tubules (320.0 µm) and of tubule lumina (152.0 µm) were apparent. Except for one boar, all animals showed spermatozoa in the epididymal tubules.

Figure 3.14 : Photomicrograph of a testicular section from a 146 day old boar. H. & E. stain. X 400. Note increasing number of primary spermatocytes (Ps), round spermatids (r), and elongating spermatids (es), and mature Sertoli cells (Se).

Figure 3.15 : Photomicrograph of a testicular section from a 187 day old boar. H. & E. stain. X 400. Note presence of immature spermatozoa (Is), and greater number of round spermatids (r).


r
Se
es
Ps


r
ls
r

By 187 days (Figure 3.15) mean seminiferous tubule diameter was virtually maximal (200.34 µm), and the associations of different generations of germinal cells within the tubules were similar to those seen in adult animals. Sertoli cells as well as spermatogonia could be seen along the basement membrane. Primary spermatocytes, spermatids in various stages of nuclear transformation, and spermatozoa were located successively more centrally in the tubules. Aggregations of interstitial cells were disposed in the angular spaces between the seminiferous tubules. Mean epididymal tubule diameter (427.9 µm) and mean epididymal tubule luminal diameter (272 µm) showed further increases, but the height of the pseudostratified epithelium had regressed slightly (from 80.00 µm at 175 days of age to 73.33 µm). Dense concentrations of spermatozoa within the epididymal tubule lumina were found in all boars (Figure 3.16).

At 241 days of age (Figure 3.17) mean seminiferous tubule diameter was 205.33 µm and the cellular organization within the tubules was typical of the adult testis, as described above. No apparent change in the morphology and distribution of Leydig cells was observed. Mean epididymal tubule diameter had increased slightly (492.0 µm) and all lumina were filled with dense masses of spermatozoa separated from the surface of the epithelium by tufts of long stereocilia.

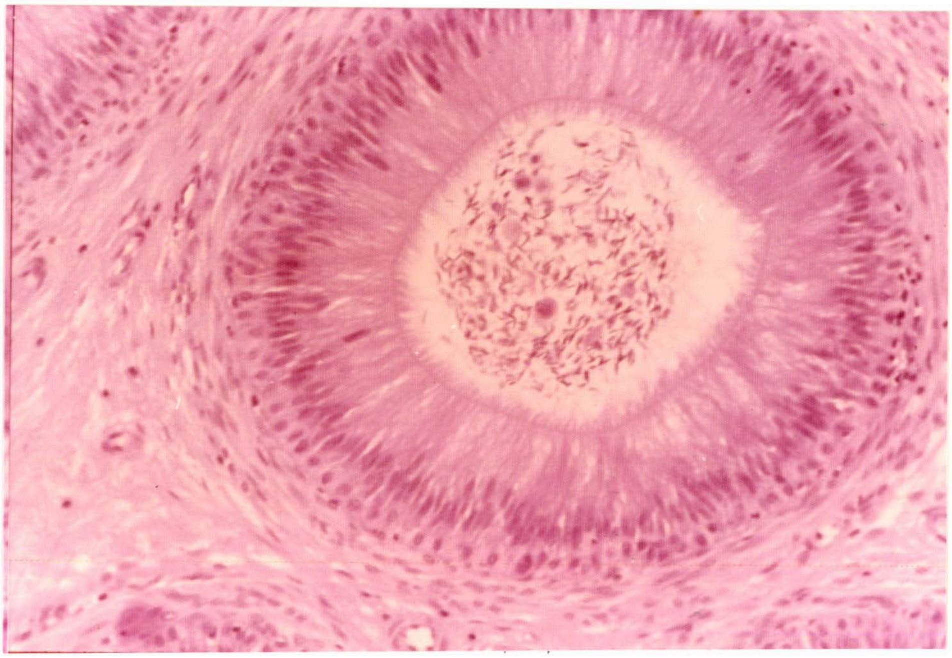
4. Plasma LH Concentrations

See Table 3.3 and Figure 3.18.

Mean plasma LH concentrations during the sampling period ranged from 0.20 to 2.19 ng/ml plasma. Relatively low mean levels (0.20 - 1.25 ng/ml plasma) were recorded between 41 and 82 days of age, then between 110 and 152 days higher mean levels (1.35 -

Figure 3.16 : Photomicrograph of an epididymal section from a 187 day old boar. H. & E. stain. X 400. Note the presence of spermatozoa in epididymal tubule lumen.

Figure 3.17 : Photomicrograph of a testicular section from a 241 day old boar. H. & E. stain. X 400. Note mature appearance of the testis at this age.



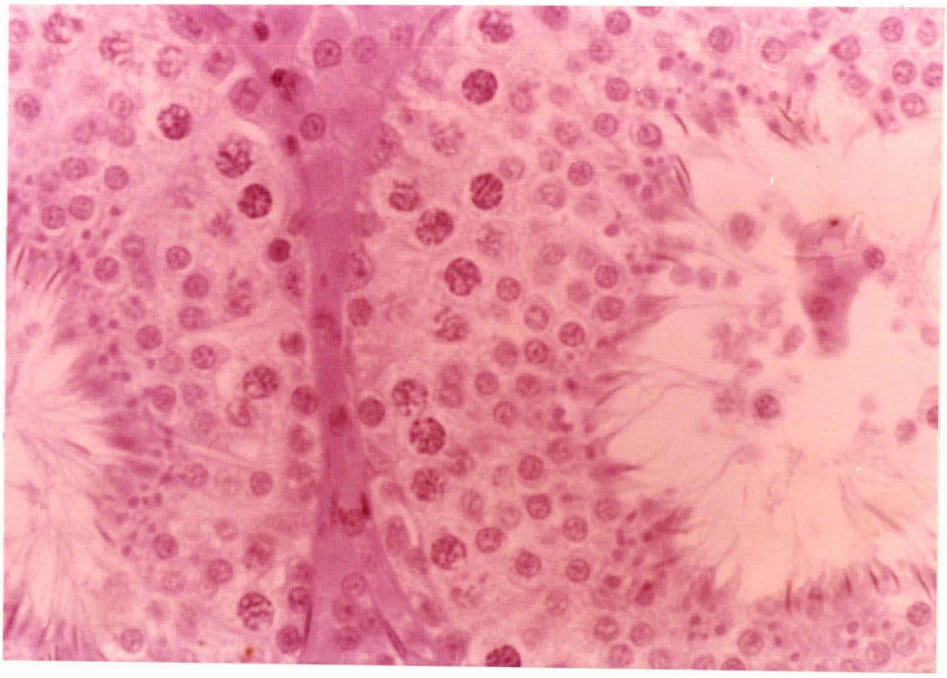


Table 3.3

Plasma LH Levels of Boars Aged 41 to 236 Days and of Barrows Aged 222 and 236 Days.

AGE	BOAR NUMBER										Mean ± S.E.
(Days)	20/5	20/9	20/6	19/5	19/57	20/58	20/60	19/8	19/567	19/67	
41	1.05	2.41	1.95	0.46	1.46	1.45	1.08	0.25	0.64	1.75	1.25 ± 0.21
54	0.12	0.00	0.15	0.21	0.00	0.10	0.04	0.68	0.00	0.03	0.20 ± 0.06
68	0.25	0.59	1.13	0.92	1.08	1.30	0.57	0.89	1.54	1.16	0.94 ± 0.12
82	0.91	0.41	1.06	1.22	1.11	1.53	0.69	1.17	1.22	0.83	1.01 ± 0.10
110	1.08	1.27	4.46	0.90	2.74	1.33	1.58	4.17	1.60	2.85	2.19 ± 0.40
124	2.30	2.33	0.91	1.43	3.29	1.08	3.23	2.00	1.00	1.46	1.90 ± 0.27
138	0.74	1.27	1.12	1.18	0.66	1.67	0.85	3.14	1.20	1.67	1.34 ± 0.22
152	2.79	1.32	1.84	0.69	1.50	1.17	1.72	3.03	1.12	1.16	1.63 ± 0.23
166	0.26	0.74	0.59	0.86	1.21	0.94	0.94	0.95	0.60	0.90	0.79 ± 0.08
180	0.36	0.25	1.16	0.41	0.51	0.66	0.51	0.36	0.35	0.47	0.50 ± 0.08
194	1.38	0.71	1.36	0.81	1.84	1.01	0.94	0.84	0.69	0.93	1.05 ± 0.11
208	1.07	1.01	0.97	0.54	0.79	0.88	0.72	0.47	0.82	0.89	0.81 ± 0.06
222	0.38	0.97	0.57	0.53	0.35						0.56 ± 0.11
236	0.49	1.05	0.96	0.30	1.31						0.82 ± 0.18

BOARS CASTRATED AT 215 DAYS OLD

AGE (Days)											Mean ± S.E.
222						2.12	2.02	1.66	1.27	1.02	1.61 ± 0.21
236						2.21	1.43	0.71	0.79	1.09	1.24 ± 0.27

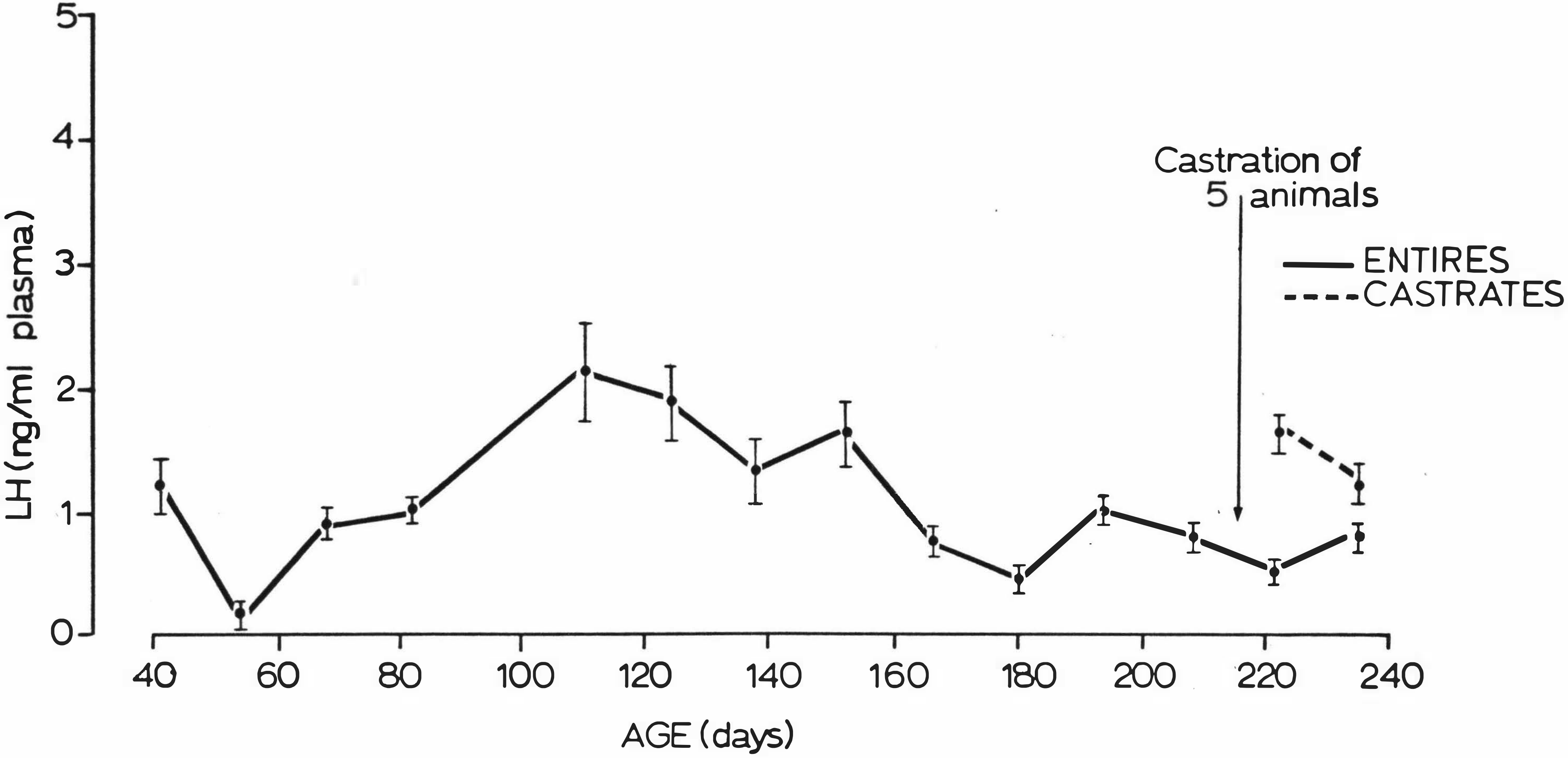


Figure 3.18 : Profile of plasma LH levels in boars during maturation.

(values are means ± S.E.; n = 10)

2.19 ng/ml) were obtained. Subsequently mean LH levels declined to between 0.5 and 1.0 ng/ml.

When the longitudinal LH secretory profiles from individual animals were inspected, the above general pattern was seen to occur in all boars. There were however, slight variations in the ages at which the pubertal peak of secretion was recorded, and also in the relative magnitude of peak. The longitudinal patterns of LH secretion for boars derived from each litter showed similar profiles (Figure 3.19). Comparison of the overall mean LH levels of the two litter groups revealed no significant difference ($\underline{t}_{(18)}$ = 0.09; P > 0.05).

5. Plasma Testosterone Concentrations

See Table 3.4 and Figure 3.20.

Mean testosterone levels were uniformly low (0.10 - 0.27 ng/ml plasma) until 82 days old. At 110 and 124 days testosterone levels progressively increased and a maximal mean value of 8.00 ng/ml plasma was recorded at 138 days old. Following this peak testosterone levels declined during the 3 subsequent samplings to a value of 1.58 ng/ml plasma at 180 days. A second testosterone peak (7.73 ng/ml plasma) was obtained only at the 194 day sampling, then subsequently, levels fell and fluctuated from 1.40 to 3.80 ng/ml plasma during the last 3 samplings (208 to 236 days of age). Except for one boar (20/68) for which only the first peak was noted, the above general pattern of testosterone secretion was exhibited by all the boars. When testosterone levels of the two litters were compared (Figure 3.21) similar profiles but different plasma concentrations were observed. Boars from litter 19 consistently had higher mean testosterone levels than those from litter 20 (overall mean levels were 3.24 <u>vs</u> 1.91 ng/ml;

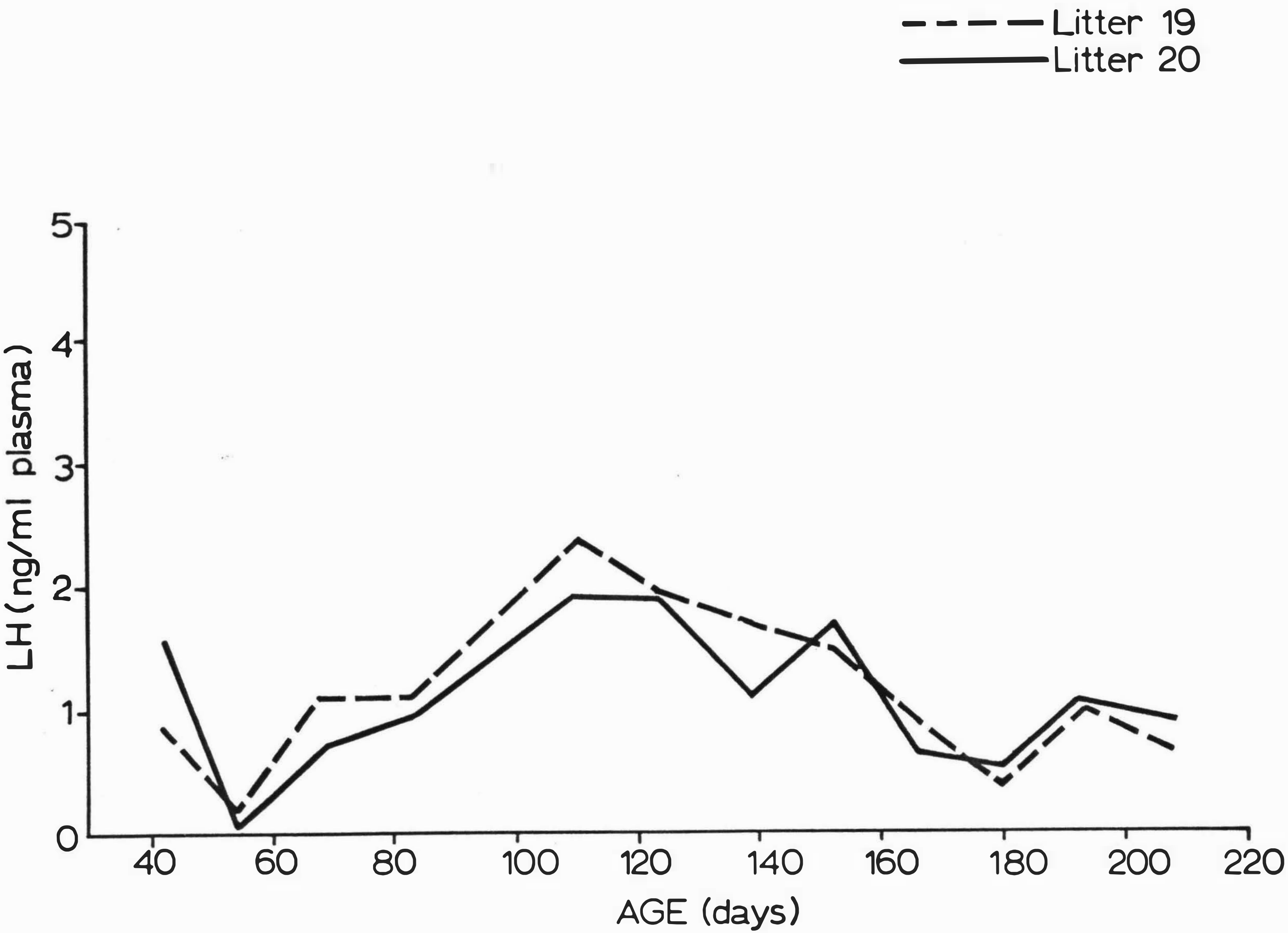


Figure 3.19 : Comparison of profiles of plasma LH levels between two litters of boars.

(values represent the means recorded from 5 boars)

Table 3.4

Plasma Testosterone Levels of Boars Aged 41 to 236 Days and of Barrows Aged 222 and 236 Days.

AGE (Days)	BOAR NUMBER 20/5	20/9	20/6	19/5	19/57	20/58	20/68	19/8	10/567	19/67	MEAN ± S.E.
41	0.32	0.25	0.29	0.46	0.08	0.00	0.61	0.09	0.96	0.95	0.40 ± 0.10
54	1.15	0.37	0.35	0.00	0.00	0.47	0.07	0.25	0.18	0.46	0.33 ± 0.10
68	0.20	0.20	0.54	0.65	0.18	0.00	0.01	0.35	0.46	0.23	0.28 ± 0.06
82	0.41	0.00	0.23	0.22	0.21	0.48	0.00	0.09	1.08	0.00	0.27 ± 0.11
110	1.23	0.00	0.83	0.90	0.53	0.05	0.02	0.03	2.25	0.75	0.60 ± 0.25
124	0.81	2.34	0.27	2.15	6.85	0.44	0.92	2.07	2.31	2.52	2.06 ± 0.59
138	3.49	5.35	5.37	22.86	5.65	6.96	0.52	8.35	17.60	3.85	8.00 ± 2.17
152	2.82	5.21	4.01	7.66	8.66	5.28	1.29	7.49	3.13	3.17	4.87 ± 0.76
166	0.91	5.04	0.22	1.86	4.97	0.38	0.59	3.34	8.54	0.27	2.61 ± 0.88
180	2.22	0.78	2.30	0.38	2.04	0.01	3.23	1.68	2.53	0.70	1.58 ± 0.03
194	5.75	7.81	13.18	3.41	9.47	4.27	6.00	6.10	5.28	16.03	7.73 ± 1.23
208	1.05	1.05	1.08	4.26	1.94	3.11	1.00	3.05	1.40	1.30	1.92 ± 0.36
222	0.88	2.54	0.36	0.82	2.41						1.40 ± 0.44
236	3.20	4.54	3.16	6.42	1.68						3.80 ± 0.79

BOARS CASTRATED AT 215 DAYS OLD

AGE (Days)											Mean ± S.E.
222						0.07	0.28	0.10	0.25	0.24	1.18 ± 0.04
236						0.01	0.00	0.00	0.10	0.29	0.08 ± 0.05

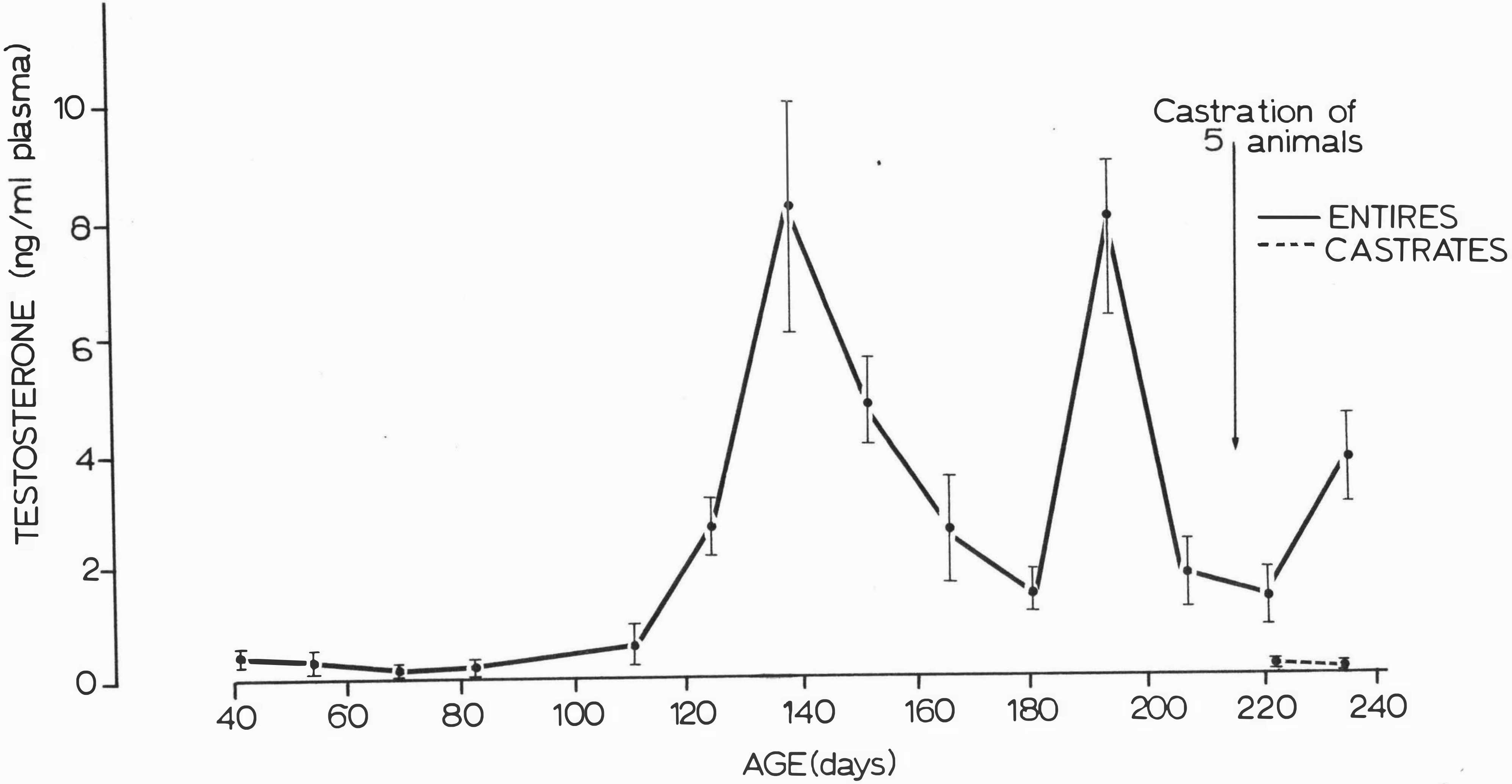


Figure 3.20 : Profile of plasma testosterone levels in boars during sexual maturation. (values are means ± S.E.; n = 10)

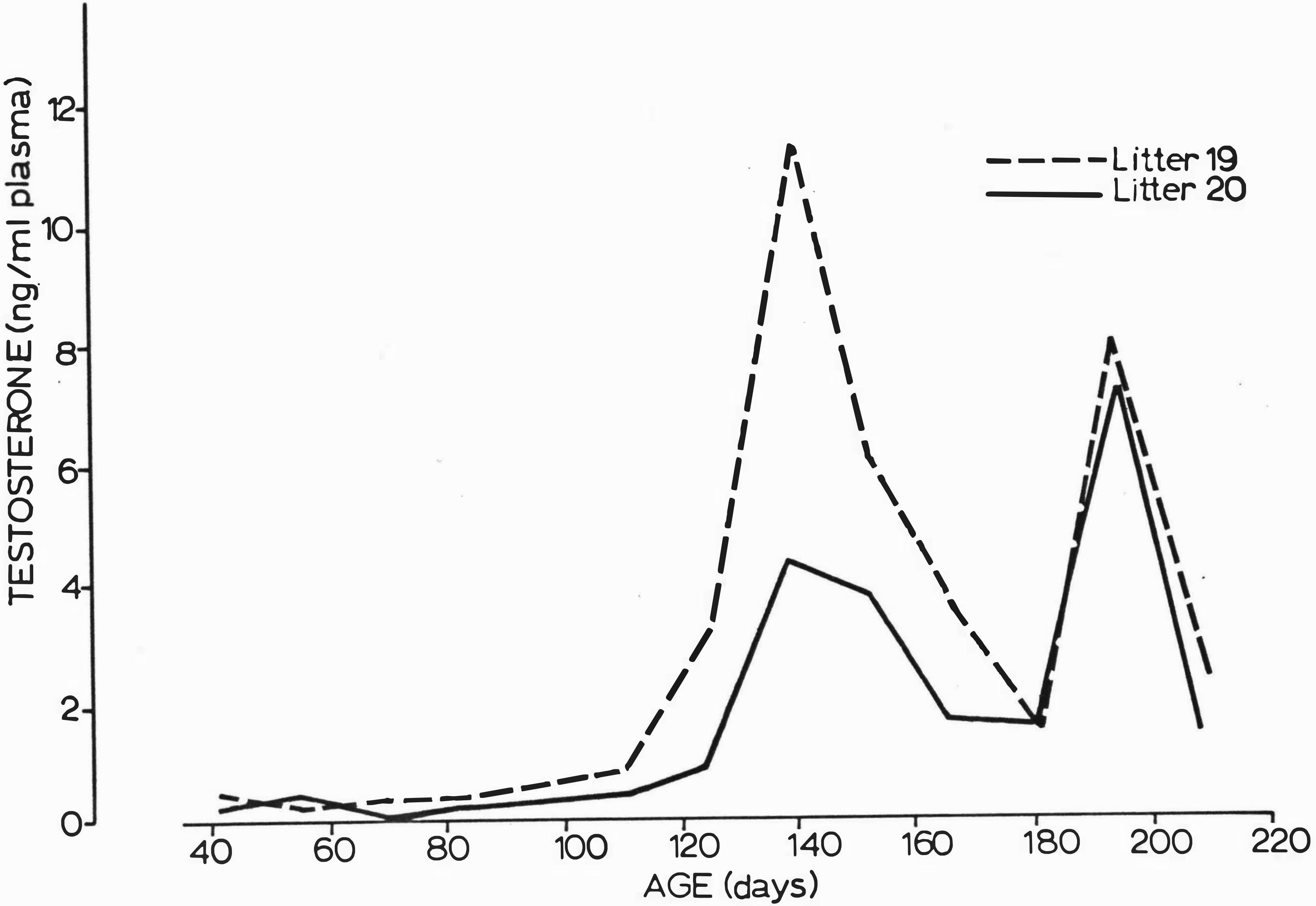


Figure 3.21 : Comparison of profiles of plasma testosterone levels between two litters of boars. (values represent the means recorded from 5 boars)

$\underline{t}_{(18)} = 3.76$; $P < 0.001$).

6. Effects of Castration on Plasma LH and Testosterone Concentrations (See Table 3.3 and 3.4 and Figures 3.18 and 3.20)

After castration of 5 boars at 215 days of age the mean plasma LH levels at 222 and 236 days in castrates (1.43 ng/ml) was significantly higher ($\underline{t}_{(18)} = 2.26$; $P < 0.05$) than the mean recorded from the 5 entire boars (0.69 ng/ml). Testosterone levels fell markedly following castration so that the mean value obtained at the last two samplings from the boars (2.60 ng/ml) was significantly higher than that from the barrows (0.13 ng/ml) ($\underline{t}_{(18)} = 4.19$; $P < 0.001$). In fact, the mean plasma testosterone level recorded from the barrows was below the limit of the sensitivity of the testosterone assay, as defined in Chapter II.

D. DISCUSSION

For the present study, puberty has been defined as the period during which there is rapid gonadal development associated with the establishment of spermatogenesis and testicular androgen secretion. This is in line with the concept of Skinner and Rowson (1968), Donovan and van der Werff ten Bosch (1965) and Lincoln (1971), who also referred to puberty as a period of development rather than as a particular point in time.

Results from the serial castration experiment showed that progressive increases in testicular weight occurred throughout the period of sampling, but with the greatest rate of increase being noted between 127 and 241 days of age. Except for the fact that the present study terminated before a definite plateau was reached, these results corresponded with those of Niwa and Mizuho

(1954), McFee et al. (1967), and Egbunike and Steinbach (1972), all of whom noted sigmoid curves of testicular growth. Parallel increases in other parameters studied (e.g. seminiferous tubule diameter, epididymal weight, epididymal tubule diameter and epididymal tubule lumen diameter) were observed during the period of rapid testicular growth, indicating that development of these structures probably was coordinated by the same or related endocrine mechanisms. Testicular growth continued in the period after the maximal seminiferous tubule diameter had been reached, presumably due to a continuing increase in length and tortuosity of the tubules. An increase in the amount of interstitial tissue probably also occurred because in the later stages of the experiment the relatively large proportion of this tissue was maintained, even when the seminiferous tubules were growing rapidly.

Generally, it was observed that boars with advanced spermatogenesis had greater body and testicular weights. This observation, as well as the highly significant correlation obtained between body weight and testicular weight, implied that boars with greater body and greater testicular weights have an earlier onset of spermatogenesis. Similarly, the investigation of Niwa (1954) indicated that boars growing at normal rates had greater testicular weights and an earlier onset of spermatogenesis than did boars which were growing subnormally. Also, Wiggins et al. (1951) reported that heavier prepubertal boars reached puberty earlier than lighter unthrifty boars.

Epididymal spermatozoal reserves (ESR) were zero prior to 146 days of age which was to be expected since the first appearance of sperm in the epididymal histological sections was first noted only at this age. The higher mean ESR count recorded from epididymides of the 146 day old boars, rather than the 161 day old ones, was due to the exceptionally advanced reproductive development of one of the younger boars. ESR values recorded from individual boars all were compatible with their respective epididymal weights and epididymal histological data. Values obtained from the boars aged 241 days agreed with the findings of Egbunike et al.(1975), and also were closer to the theoretical ESR values (calculated using the formula of Dott and Skinner (1967) for rams: ESR = testicular weight x 0.0122 x 10^9 x 13 to 15) than were those for younger boars. Between 146 and 216 days of age the actual ESR values were much lower than the theoretical ESR calculated from the above formula; these results were not surprising since full sexual maturity in domestic boars is not attained until beyond 8-10 months of age (Niwa and Mizuho, 1954; Egbunike and Steinbach, 1972), hence testes of animals younger than 8 months would not have had a full spermatogenic yield. Leman and Rodeffer (1976) further claimed that sperm production in domestic boars increased progressively until 18 months of age.

Qualitative histological analysis of the testes showed that at 43 days the testes were immature; seminiferous tubules lacked lumina and possessed only the precursor cells of spermatogonia and Sertoli cells. From 90 days onwards germ cells started to evolve in a progression which generally agreed with the findings of Niwa and Mizuho (1954) and McFee et al. (1967): on the average, spermatogonia and primary spermatocytes were noted at 90 days coincident with the formation of lumina in the seminiferous tubules, while spermatids and spermatozoa were observed at 127 and 146 days of age, respectively. The boars at these ages had respective mean body weights of 35.3, 49.0 and 58.6 kg. Individual animals showed wide variations in the timing of appearance of particular generations of spermatogenic cells. For instance, the ages of appearance of sperm in seminiferous tubules ranged from 127 to 161 days and in epididymal tubules from 146 to 187 days.

The morphogenesis of Sertoli cells was evident at 90 days when the nuclei of supporting cells lost their deeply staining basophilic granules and nuclear chromatin became dust-like in appearance. However, the typical adult appearance of the Sertoli cells (with folded nuclear membranes and reticular fibrils extending towards the lumen) became evident only at 146 days of age when elongating spermatids had increased in numbers and aggregated around the Sertoli cells.

The exact role played by Sertoli cells is not well known but their cytological structure (Vilar *et al.*, 1970), and their particular position in relationship to the germ cells were considered to be indicative of secretory, nutritive and endocrine roles. Recent evidence indicates that Sertoli cells are the specific target cells of FSH in the testes; it is claimed that with the mediation of cyclic AMP, FSH induces the production of androgen binding protein which in turn facilitates the accumulation of androgen in the germinal epithelium (Hansson *et al.*, 1976; Facunding *et al.*, 1976). Presumably, the rise in FSH prior to puberty, as reported in other species (Lee *et al.*, 1976a; Payne *et al.*, 1977), is necessary to induce the maturation of the Sertoli cells which in turn facilitates the onset of spermatogenesis.

A remarkably large proportion of interstitial tissue containing numerous Leydig cells was particularly observed in the prepubertal testes sampled in the castration experiment. This attribute is considered to be characteristic of the porcine species

(Sisson and Grossman, 1953). If the common assumption that the level of androgen varies with the number of Leydig cells is true, one might expect to record exceptionally high plasma levels of testosterone even at this early stage of reproductive development. However, this assumption is valid only for mature functioning Leydig cells, which could not be demonstrated by the simple Hematoxylin-Eosin staining technique utilized. Special staining techniques (e.g. Threadgold's method for demonstrating lipids, Threadgold, (1957) or histochemical localization of androgen biosynthetic enzymes (Hay and Deane, 1966; Baillie *et al*., 1966) are required to demonstrate the functional integrity of Leydig cells. Numerous Leydig cells and a few fibroblasts were observed in 43 day old testes and comparison of these cells with those from testes at pubertal and post-pubertal ages revealed no conspicous morphological difference as far as staining affinity of the cytoplasm and nuclei were concerned. The presence of vacuoles, which has been regarded as a morphological indicator of secretory activity also was only minimally manifested in the Leydig cells of pubertal and adult boars. Similar observations were noted by other investigators (reviewed by Hooker, 1970). Hooker (1970) also reported no conspicous change in the number and structure of Leydig cells during puberty in bulls, despite the increase in plasma testosterone levels occuring at this stage (Rawlings *et al*., 1972; Secchiari *et al*., 1976). In this experiment, the low testosterone levels observed prepubertally indicated that the numerous Leydig cells during this immature stage were not fully functional.

Profiles of LH and testosterone secretion displayed distinct elevations during puberty. The rise in plasma LH concentrations at this stage contrasted with the finding of Elsaeser *et al*.(1976 *b*) who recorded relatively constant prepubertal LH levels in miniature boars. Neverthless, in humans (Yen _et al._, 1969; August _et al._, 1972), ram lambs (Courot _et al._, 1975) and rats (Swerdloff _et al._, 1973; Ramirez, 1973), distinct increases in LH secretion at or before the onset of puberty have been observed.

The longitudinal profile of testosterone secretion generally conformed with the findings of Carlson _et al._ (1971), Gray _et al._ (1971); Elsaesser _et al._ (1976 _b_); and Andresen (1976). The first two groups of investigators observed maximum testosterone levels in spermatic vein blood during puberty and lower levels at 8-9 months of age. Radioimmunoassay measurement of testosterone levels in peripheral plasma by Elsaesser _et al._ (1976 _b_) and Andresen (1976) also confirmed a marked increase in secretion of the hormone at puberty. Also, the latter author noted a decline of testosterone secretion between 170 and 205 days of age, but no such decline was reported by Elsaesser _et al._ (1976 _b_). This variation between papers may have been due to age differences since the study of Elsaesser _et al._ (1976 _b_) terminated when the boars were 12 weeks of age; however they claimed that miniature boars have already attained sexual maturity by this age. Probably this difference in age of sexual development should be interpreted as indicating that miniature swine are of limited value for studies of puberty in domestic breeds.

Meussy Dessole (1976) described a testosterone profile which was characterized by large rises at 5 and 17 days after birth, declined prepubertally, then increased progressively during puberty with high values being maintained in the adult boars; this pattern of androgen secretion conformed with profiles reported in human males (Frasier _et al._, 1969; Roth _et al._, 1973) but is at variance with the present results and with the other porcine reports mentioned with respect to the post-pubertal decline of testosterone concentration. Also, it conflicts with findings of Elsaesser et al. (1976 b) and Ford and Schanbacher (1977) who noted low and constant testosterone levels during the first 5 weeks of post-natal life.

A feature of the testosterone profile described in the present study which was difficult to understand was the isolated peak at 194 days of age. Sexual stimuli from estrous sows or gilts during that particular sampling could have been a factor as Andresen (1976) reported a marked increase in testosterone levels a few minutes after mating. However, the effect of sexual stimuli and copulation on testosterone secretion remains unclear in boars; Ellendorff et al. (1975) observed no significant change in plasma testosterone levels after copulation although LH levels were significantly elevated.

The histological data and the profiles of LH and testosterone obtained in the present study supported the view that rapid testicular development during puberty is due to increased gonadotrophin and testosterone secretion. The role of LH in synergizing with FSH to induce spermatogenic activity appears to be secondary to its direct stimulatory action on the secretion of androgens, which in turn induces important physiological and behavioural changes during puberty. Androgens stimulate the development of the accessory organs of reproduction which in this study was indicated by the notable increases in epididymal weight, epididymal tubule diameter, epididymal tubule lumen diameter and height of epididymal pseudostratified epithelium. These parameters have been shown to be highly androgen dependent in other species

(Skinner _et al_., 1968; Lincoln, 1971). The increased levels of androgens undoubtedly also had a major role in the establishment of spermatogenesis, acting in synergy with FSH (Courot _et al_., 1970; Steinberger, 1971). The critical requirement for androgen in the meiotic division of primary spermatocytes and during the formation of spermatids has been described (Lostroh, 1963; Steinberger, 1971), hence the greater levels of androgen during puberty presumably are required for induction of spermatogenic activity.

Post-pubertal castration of 5 boars clearly demonstrated the existence of a dynamic negative feedback relationship between LH and testosterone. Testosterone levels fell to below the limit of sensitivity of the assay while LH levels rose significantly. This finding was in conformity with the concept that testosterone negative feedback is the predominant regulator of LH secretion (Lee _et al_., 1972; Stewart-Bentley _et al_., 1974).

Between-litter comparisons of mean of LH and testosterone levels revealed a significant, ($P < 0.001$) difference in testosterone levels, but not in LH concentrations ($P > 0.05$). The importance of this finding is not obvious as there was no evidence that animals with higher androgen levels reached puberty or sexual maturity at an earlier age than those from the other litter.

CHAPTER IV

TWENTY-FOUR HOUR PLASMA PROFILES OF LUTEINIZING HORMONE AND TESTOSTERONE IN PUBERTAL AND POST-PUBERTAL BOARS

A. INTRODUCTION

Since the advent of radioimmunoassays to measure plasma hormone levels, experiments involving frequent blood sammpling techniques have shown that for several species, LH and testosterone are secreted into the blood in a pulsatile rather than continuous manner (Naftolin et al., 1973; Katongole et al., 1974; Ellendorff et al., 1975; Sanford et al., 1976). The existence of diurnal cyclicity in plasma levels of gonadotrophins is still disputed, but it is generally agreed that no diurnal rhythm of LH secretion occurs in adult human males (Boyar et al., 1972), bulls (Smith et al., 1973), rams (Sanford et al., 1974; Barrell, 1976) or boars (Ellendorff et al., 1975; Sanford et al., 1976). However, studies with pubertal human males have revealed a nocturnal augmentation of LH and testosterone secretion during the (rapid eye movement) REM stage of sleep (Boyar et al., 1974).

Data on testosterone 24 hr secretory profiles in adult males of several species also has been conficting; while Ellendorff et al. (1975) noted a diurnal variation in boars, no such variation was observed by Sanford et al. (1976). Similar discrepancies have been reported for men; some investigators claimed the presence of diurnal periodicity (Rowe et al., 1974) while others could not demonstrate any such cyclicity (Boon et al., 1972; Hudson et al., 1967). Significant diurnal variations in testosterone secretion also were reported in adult male monkeys

(Goodman et al., 1974) and stallions (Sharma, 1976), but not in rams (Sanford et al., 1974; Katongole et al., 1974; Barrell, 1976).

To date no report has been published describing the 24 hr profiles of LH and testosterone secretion in pubertal boars. The experiment described in this chapter was an investigation of the secretory profiles of LH and testosterone in both pubertal and post-pubertal boars, throughout 24 hr periods. In particular, it was designed as an investigation of the effects of stage of sexual development on: mean plasma levels of LH and testosterone, patterns of secretion of these hormones , and their temporal relationships within the 24 hr periods.

B. MATERIALS AND METHODS

Four pubertal (118 days old) and three post-pubertal (241 days old) boars were subjected to 24 hr blood sampling on July 29 and November 29, 1976, respectively. These boars were selected at random from the group of animals utilized in Experiment 1.2. A fourth post-pubertal boar could not be restrained adequately to allow the jugular cannulae to be maintained, and had to be excluded from this experiment.

The animals were fitted with indwelling jugular catheters (as described in Chapter II) through which eight milliliters of blood were withdrawn every 20 min for 24 hours. Plasma samples were assayed for LH and testosterone concentrations.

Prior to statistical analysis, all estimates of hormone concentrations were transfromed to logarithms using the formula -

log hormone concentration = 100 $\log_{10} (x + 1.1)$,

where x = plasma hormone concentration (ng/ml).

The transformed values for each boar were progressively summed in the order of sampling to form a series of cumulative totals. The regression coefficients of this cumulative distribution against time were tested for linearity by analysis of variance, then used as parameters of hormone secretion rates, for comparing the plasma hormone levels of the pubertal and post pubertal boars. Between-age comparisons of the gradients of cumulative hormone distributions were performed using Student's 't' test.

C. RESULTS

1. LH

At the pubertal sampling, all boars exhibited distinct fluctuations in circulating LH levels which were consistent with an episodic secretory mechanism (See Figure 4.1). The frequency and magnitude of peaks (defined as an elevation in hormone level of greater than 1 ng/ml) varied widely between boars. Four peaks were recorded in boars 19/5, 20/58, and 20/6 with maximal values ranging from 2.44 - 3.68 ng/ml, while boar 19/8 exhibited 3 peaks which ranged in magnitude from 2.05 to 4.38 ng/ml. Most of these peaks occurred as a single pulse and declined to approximately half the peak value within 20 minutes. Their occurrence was randomly distributed throughout the sampling period with no evidence of any circadian periodicity nor was there any effect of environmental lighting. The overall mean plasma concentrations of LH in the pubertal boars was 0.82 ng/ml.

LH profiles of post pubertal boars (Figure 4.2) also showed an episodic nature of hormone secretion, but the secretory episodes were very much suppressed and no elevation of LH levels greater than 1 ng/ml was noted. The overall mean LH concentration

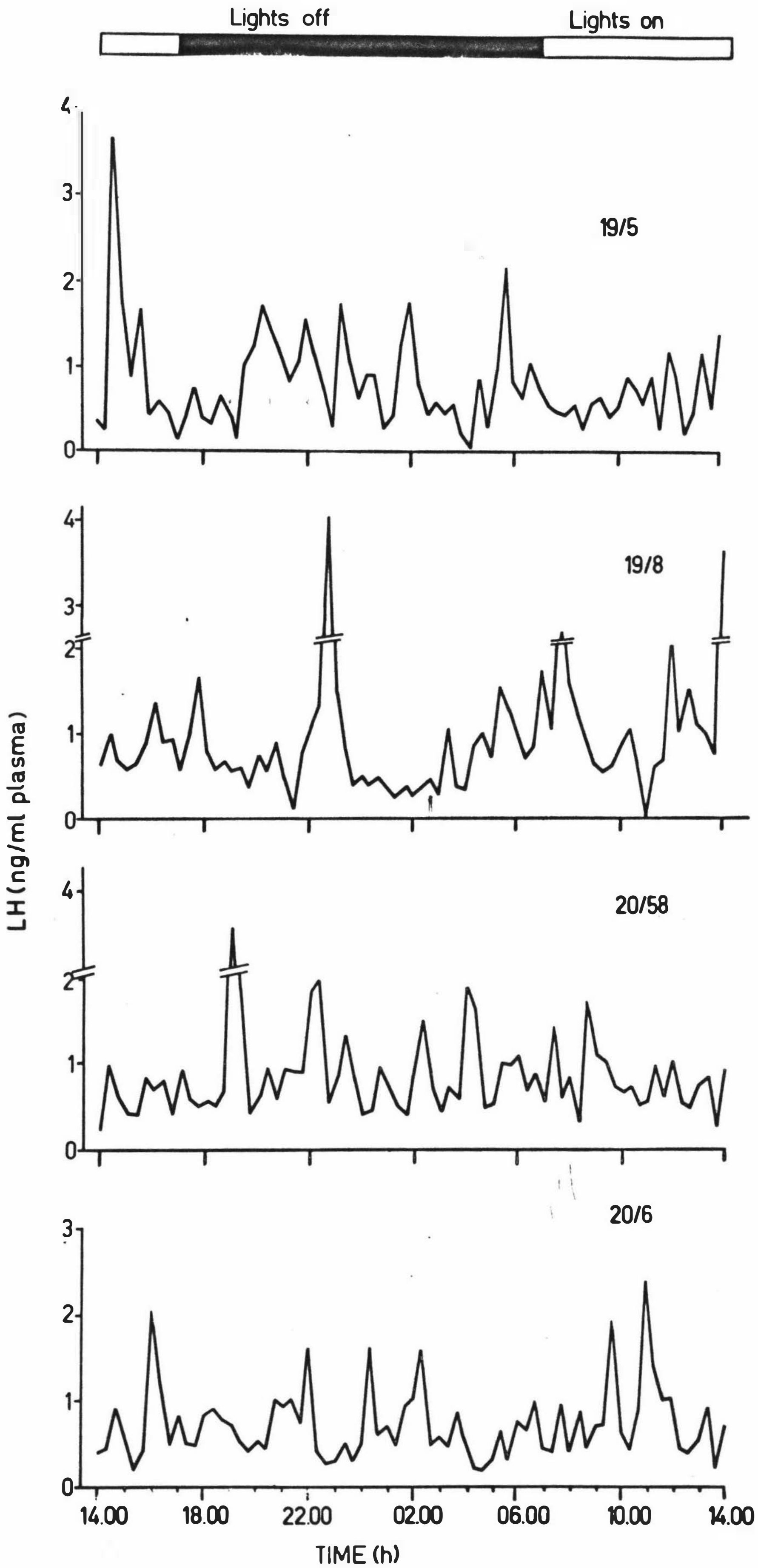


Figure 4.1 : LH secretory profiles recorded from plasma samples collected from four pubertal boars sampled at 20 min intervals for 24 hours.

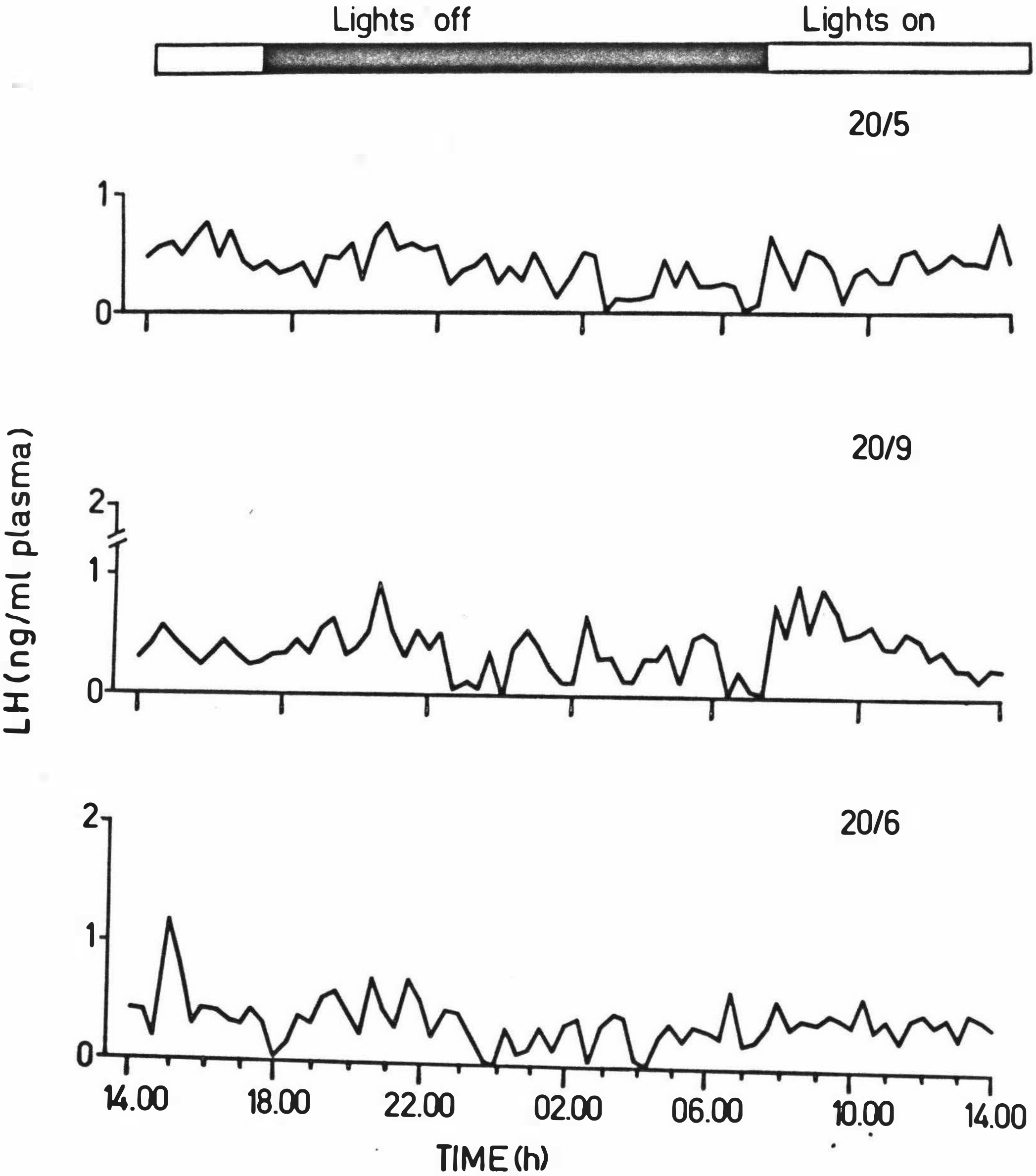


Figure 4.2 : LH secretory profiles recorded from plasma samples collected from three post-pubertal boars sampled at 20 min intervals for 24 hours.

throughout the sampling period was 0.39 ng/ml.

Comparison of mean LH levels, by testing the significance of differences in cumulative LH distributions against time, confirmed that the gonadotrophin levels were higher in pubertal than in post-pubertal boars ($\underline{t}_{(5)} = 8.85$, $P < 0.001$), (Figure 4.3 and Table 4.1).

2. Testosterone

Twenty-four hr patterns of testosterone secretion in the pubertal boars (Figure 4.4) also were of a pulsatile naure, but the testosterone pulses were not as frequent as those of LH. Two boars, 19/5 and 19/8, displayed major testosterone peaks (5.45 and 9.44 ng/ml, respectively) along with other pulses of smaller amplitude. These two major peaks did not occur as a single pulse but as a prolonged elevation which continued over three to four samplings. Boars 20/58 and 20/6 exhibited smaller peaks only, with maximum values of 2.39 and 2.97 ng/ml, respectively. Overall, the mean plasma level of testosterone in pubertal boars was 1.04 ng/ml.

The post-pubertal boars exhibited testosterone profiles characterized by minor episodic fluctuations throughout the 24 hr period (Figure 4.5). The highest values recorded from individual animals ranged between 2.21 and 3.37 ng/ml, while the overall mean testosterone level was 0.81 ng/ml.

Comparison of mean testosterone levels, based on gradients of cumulative testosterone distributions against time (Figure 4.6), showed that testosterone levels in pubertal boars were significantly higher than those of post-pubertal boars ($\underline{t}_{(5)} = 3.16$, $P < 0.05$). There was no evidence of a circadian pattern of testosterone secretion in boars of either age.

Table 4.1

Gradients of Cumulative LH and Testosterone Distributions and Mean Hormone Concentrations Recorded from Plasma Samples Collected Over a 24 hr Period from Boars Aged 118 and 241 Days.

	LH		Testosterone	
	Distribution Gradients	Mean Plasma Levels (ng/ml)	Distribution Gradients	Mean Plasma Levels (ng/ml)
118 Days Old	0.2984	0.89	0.2784	0.81
(Pubertal)	0.2622	0.79	0.3538	1.39
	0.2689	0.88	0.3566	1.50
	0.2577	0.75	0.1844	0.49
Mean ± S.E.	0.2712 ± 0.01	0.82 ± 0.05	0.2933 ± 0.04	1.04 ± 0.41
241 Days Old	0.1662	0.39	0.2989	0.92
(Post-pubertal)	0.1605	0.39	0.2910	0.83
	0.1604	0.39	0.2674	0.69
Mean ± S.E.	0.1623 ± 0.004	0.39 ± 0.00	0.2857 ± 0.01	0.81 ± 0.12

$\underline{t}_{(5)}$ = 8.85 ($P < 0.001$)

$\underline{t}_{(5)}$ = 3.16 ($P < 0.05$)

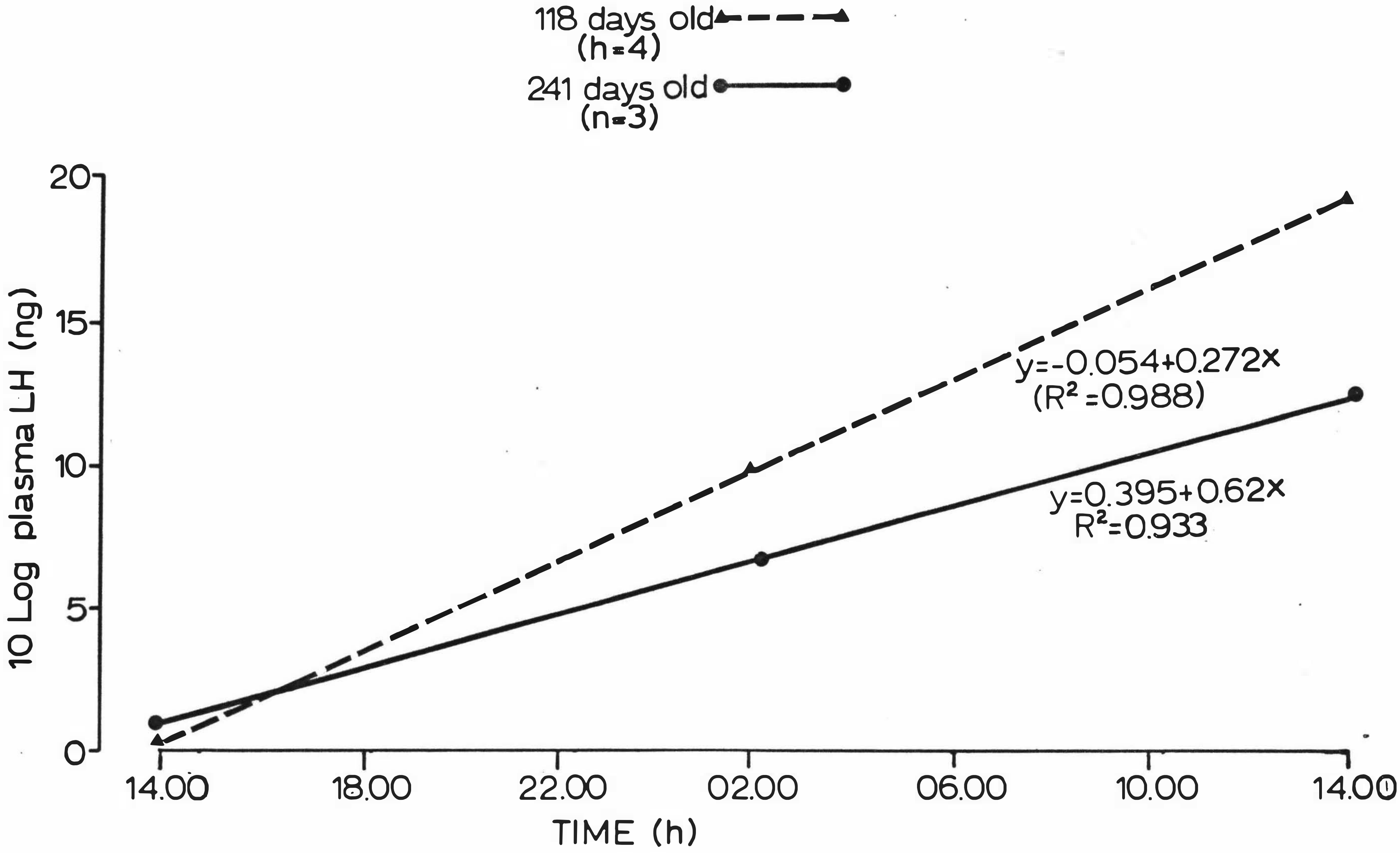


Figure 4.3 : Twenty-four hr cumulative LH levels in pubertal (118 day old) and post-pubertal (241 day old) boars showing pooled regression lines.

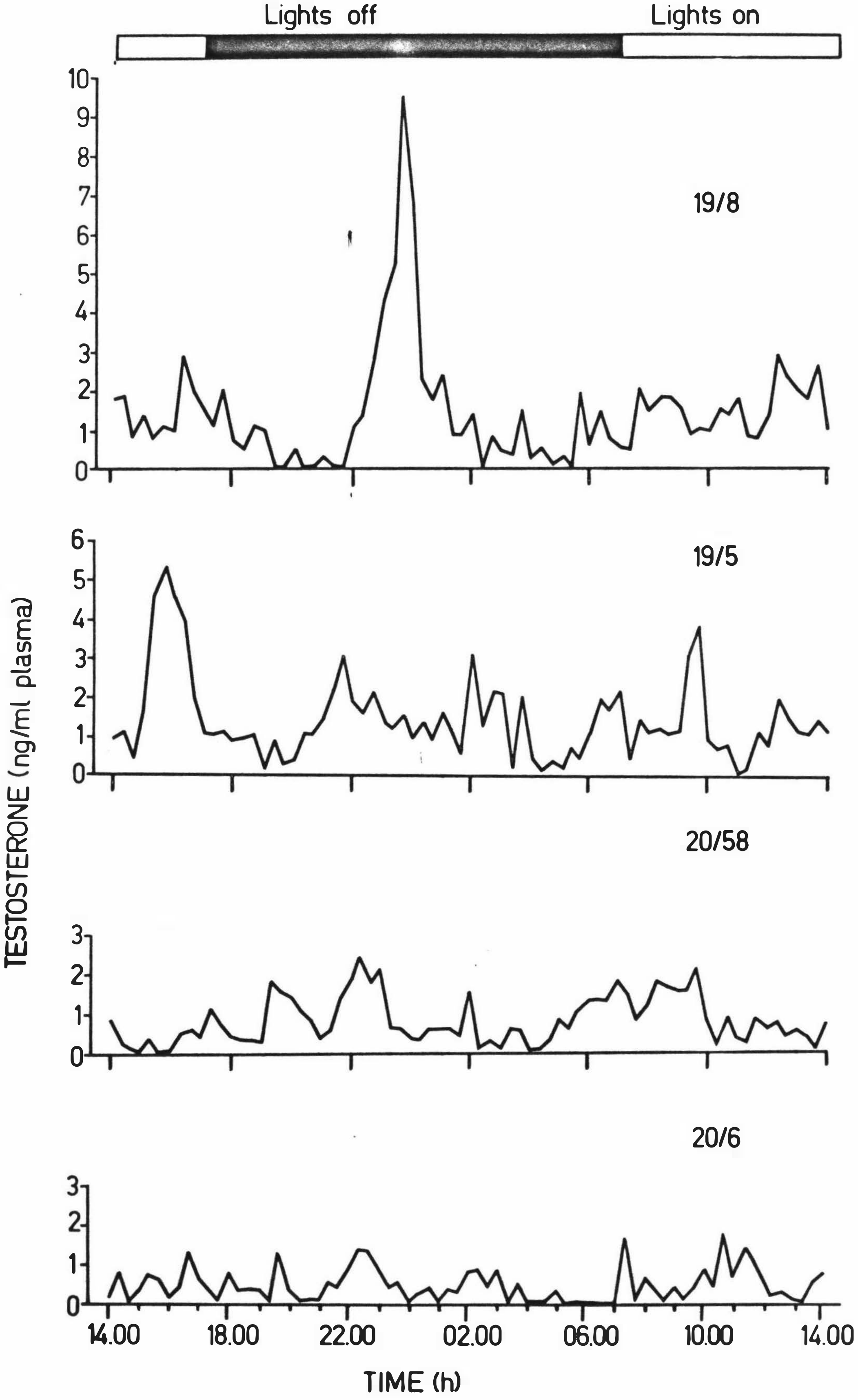


Figure 4.4 : Testosterone secretory profiles recorded from plasma samples collected from four pubertal boars sampled at 20 min intervals for 24 hours.

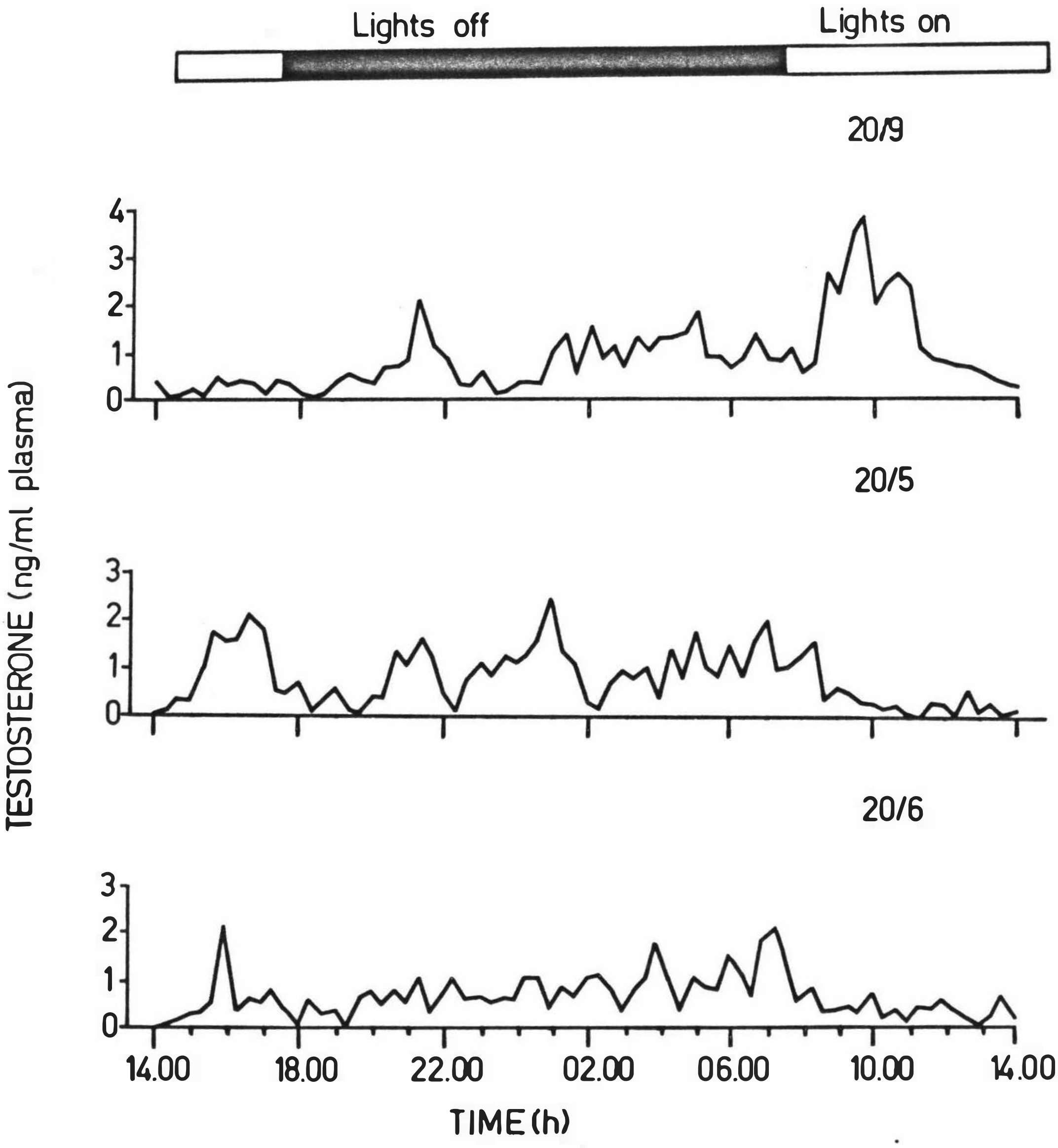


Figure 4.5 : Testosterone secretory profiles recorded from plasma samples collected from three post-pubertal boars sampled at 20 min intervals for 24 hours.

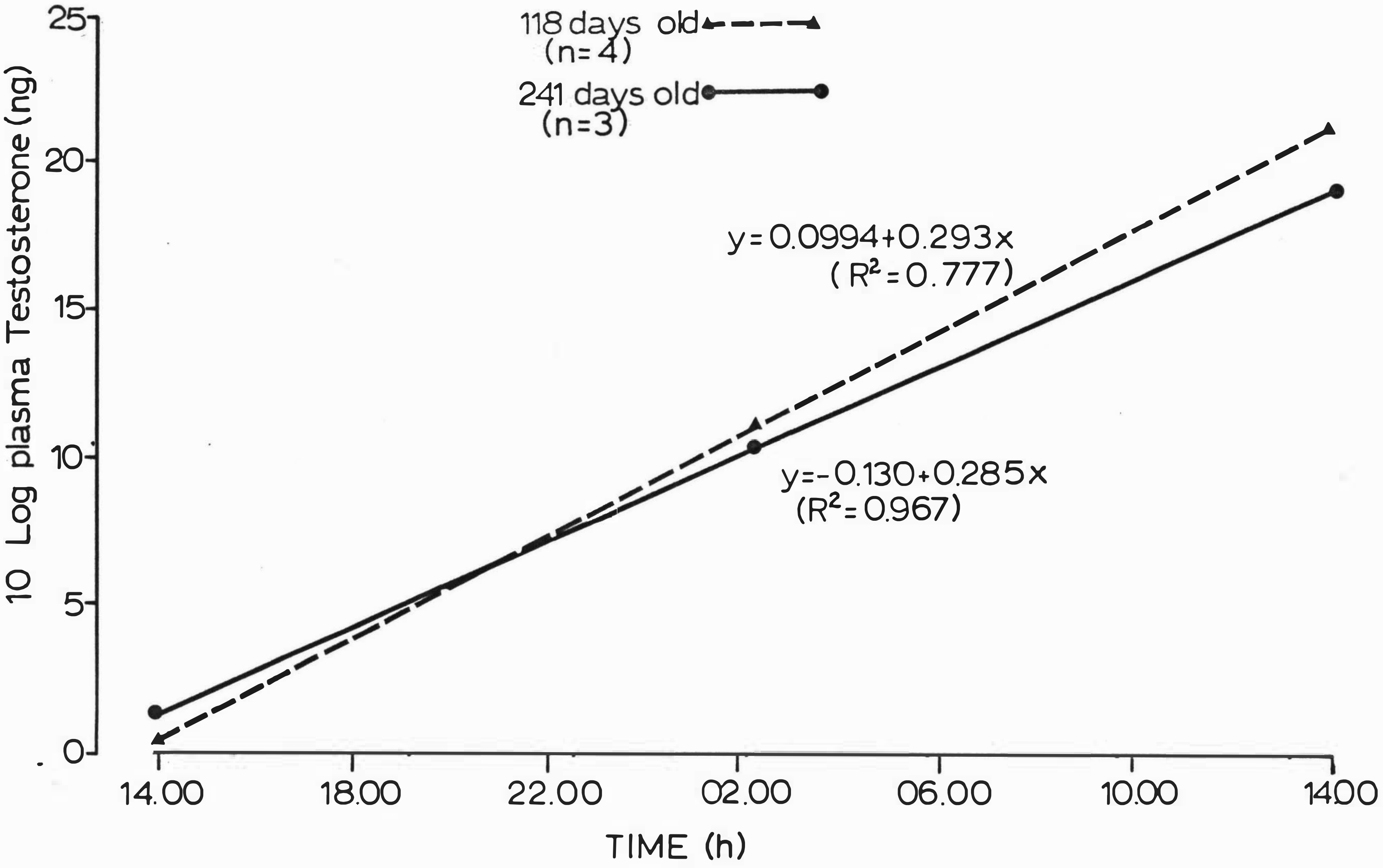


Figure 4.6 : Twenty-four hr cumulative testosterone levels in pubertal (118 day old) and post-pubertal (241 day old) boars showing pooled regression lines.

3. LH-Testosterone Relationships

All major peaks of testosterone secretion recorded from the pubertal boars followed large LH discharges. This relationship was particularly evident in boars 19/8 and 19/5 whose major testosterone peaks occurred 60 min after LH peaks. Other minor pulses of testosterone also were apparently related to LH discharges, however, the relationship was not consistent since not all LH pulses were followed by testosterone elevations. A similar lack of consistent LH-testosterone relationships was shown by the other two boars for which elevations of both hormones sometimes occurred simultaneously.

In post-pubertal boars, most of the noticeable elevations of testosterone also were preceeded by LH pulses, while other smaller pulses of LH and testosterone did not exhibit such associations. Age at sampling had no apparent influence on the usual time interval between LH and testosterone peaks.

D. DISCUSSION

1. Hormone Profiles in Pubertal Boars

No study of the profiles of LH and testosterone secretion during puberty has yet been published for boars. Overall mean plasma LH and testosterone levels in the 24 hr experiment with pubertal boars corresponded well with the elevated values for these hormones obtained in the longitudinal study described in chapter III. A greater output of hormones prior to puberty also was described for female pigs by Chakraborty et al. (1975) who found that prepubertal gilts had higher basal LH levels than do sexually mature sows. Similarly, studies with sheep during sexual maturation have revealed that blood levels of both FSH and LH were very high prepubertally and both were released into the blood episodically (Thimonier et al., 1972; Foster et al., 1975; Bindon and Turner, 1974).

Twenty-four hour profiles of LH and testosterone secretion for pubertal boars in the present experiment clearly indicated highly pulsatile modes of hormone release. However, episodic releases occurred erratically without any evidence of a circadian pattern. In pubertal humans of both sexes, not only is gonadotrophin secretion highly pulsatile (Boyar et al., 1974) but the secretory episodes follow a distinct circadian pattern with notably higher pulses during night time than during the day. Monitoring of brain activity revealed that the higher nocturnal pulses of gonadotrophin and testosterone secretion in pubertal boys were closely associated with the rapid eye movement (REM) phase of sleep. This finding was interpreted as one of the major physiological mechanisms involved in the initiation of puberty in humans. As periods of increased Growth Hormone and prolactin secretion also were shown to sleep-related, Boyar et al. (1974) and Weitzman et al. (1975) suggested that important synchronized metabolic relationships were being established during sleep in pubertal children, which induced the accelerated somatic growth and skeletal changes associated with puberty. In pubertal boars, no association of LH or testosterone secretion pulses with sleep could be deduced since brain activity was not monitored to identify periods of sleep. Also, the sleep process in boars appears not to be similar to that of humans; pigs undergo frequent day time sleep periods and it remains to be determined whether they pass into a continuous nocturnal sleep.

2. Hormone Profiles in Post-Pubertal Boars

The episodic nature of LH and testosterone secretion and the lack of any diurnal variation in their levels in post-pubertal boars, all were results consistent with previous findings from adult boars published by Sanford et al. (1976). However, secretory pulses of the hormones were relatively suppressed at this stage which presumably was due to the increased sensitivity of the hypothalamo-pituitary unit to the inhibitory feedback of testosterone. Functional maturation of the LH-testosterone feedback relationship post-pubertally was confirmed by the effects of castration of 215 day old boars as described in Experiment 1.2 and by the findings of Ford and Schanbacher (1977). Overall mean levels of LH and testosterone in post-pubertal boars were much lower than those of pubertal boars, which corresponded with the post-pubertal decline in secretion of these hormones noted in the longitudinal experiment.

No circadian rhythmicity in testosterone secretion occurred in the post-pubertal boars, a result which confirmed those in the earlier report by Sanford et al. (1976). However, Ellendorff et al. (1975) reported that plasma levels were significantly lower during night time than in the morning. This apparent discrepancy may have been due to the difference in sampling frequency as the hourly sampling interval utilized by Ellendorff et al. (1975) probably was not adequate to assess the actual frequency of secretory episodes. Similar discrepancies of results were reported for adult men (Rowe et al., 1974; Boon et al., 1972), but in adult rams and bulls no diurnal pattern of testosterone secretion was observed (Sanford et al., 1974; Katogole et al., 1974; Smith et al., 1973).

3. LH-Testosterone Relationships

The relationship between LH and testosterone secretory pulses, (LH preceeding T) was in accordance with previous reports of research with humans (Boyar et al., 1974), bulls (Katongole et al., 1971), rams (Sanford et al., 1974) and adult boars (Sanford et al., 1976). Although it was not always possible to relate LH peaks to subsequent elevations in testosterone levels, it was clearly demonstrated that large LH pulses were followed by large peaks of testosterone after approximately 60 min. Also, similar associations of LH and testosterone secretory pulses were shown for the majority of the smaller pulses. Sanford et al. (1976) also recorded an approximately 60 min time-lag between LH and testosterone peaks in boars.

The status of the LH-testosterone negative feedback relationship during puberty has not been investigated previously in domestic boars. Findings of Ford and Schanbacher (1977) showed that the steroid negative feedback mechanism is relatively insensitive during the first week of post-natal life but is functionally sensitive at 5 months of age. In miniature boars (Elsaesser et al., 1975 and 1976 a) and male rodents (Ramirez and McCann, 1965; Negro-Vilar, 1973 b), an apparently declining sensitivity of the hypothalamo-pituitary system to steroid negative feedback was reported to occur prior to and during puberty and almost certainly this neuroendocrine mechanism accounted for the higher pulses of LH observed in the pubertal rather than the post-pubertal boars.

Despite the difference in LH output, it is clear that at both pubertal and post-pubertal ages, LH release into the circulation is pulsatile and not continuous. What actually generates the episodic secretion of LH is not known, but probably it is induced by centrally-controlled pulsatile releases of hypothalamic GnRH, which in turn stimulates the pulsatile release of LH (Schally *et al*., 1972).

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

Puberty is recognized to be a distinct phase of maturation which involves both reproductive organ development and concurrent hormonal changes. Hence, comprehensive studies of the process must involve investigation of both anatomical and hormonal changes. The experiments described in this thesis were the first systematic studies of pubertal development in boars in which there has been an evaluation of the interrelationships of bodily growth with testicular and epididymal development, and with the plasma profiles of LH and testosterone. These experiments were designed to produce data on the normal course of puberty in boars and thus provide a sound basis for intended future studies of factors influencing this process, and of the neuroendocrine mechanisms which regulate pubertal development.

A. LONGITUDINAL STUDY

The highly significant correlation between body and testicular weights, and the observation that boars with heavier testicles had an earlier onset of spermatogenesis, indicated that age at puberty could be influenced markedly by the rate of bodily growth. However, age was found to be highly correlated with testicular weight as well, which implied that both body weight and chronologic age were important parameters in determining pubertal attainment in boars. This of course is not surprising, as under normal circumstances the age and weight of young animals will be highly correlated, and the relative importance of each factor as a regulator of the onset of puberty could only be assessed by conducting experiments specifically designed to examine the individual importance of these two factors (e.g. studies such as those of Allen and Lamming (1961) which showed that age at puberty could be advanced or delayed by controlling rate of body growth through nutrition).

The histological and hormonal profile studies clearly indicated that the rapid gonadal development during puberty coincided with the elevation of plasma LH and testosterone levels. However, it was difficult to relate precisely the timing of the onset of the elevation of hormone levels with the onset of spermatogenesis for several reasons: testicular and epididymal histological data and the hormone profile data were obtained from different groups of animals; serial castration intervals were not sufficiently frequent to allow accurate definition of the timing of appearance of the more advanced cell types; and considerable individual variations in terms of gonadal development (which appeared to be related to heterogeneity of body weights) and hormonal profile, were observed. Despite these limitations it is clear that the development of the testes and epididymides in boars was accompanied by, or induced by, notable increases in plasma LH and testosterone levels. Further experiments will be required to elucidate the neuroendrocrine maturational changes which induced this increased hormonal secretion.

Apart from body weight and age,other factors which may affect puberty were not investigated, although nutrition and genetics undoubtedly contributed to some of the observed differences in rates of bodily and testicular growth. Any possible effect of season of birth was eliminated as an experimental factor since all boars were born at approximately the same time. Preliminary investigations by Wiggins _et al_. (1951) revealed no significant effect of season of birth on age at puberty in boars. However, it has been reported that gilts born in fall attain sexual maturity earlier than those farrowed in spring (Robertson _et al_., 1951; Mavrogenis and Robinson, 1976). Further investigations along this line are needed to clarify the relationships between the season of birth and age at puberty, and also to define which particular climatic factors (daily photoperiod / temperature) are involved.

A major deficiency of the present study was the lack of data for plasma FSH levels. There is growing evidence that the principal function of FSH is in the initiation of both spermatogenic and androgenic functions of the prepubertal testes (Ortavant _et al_., 1969; Courot, 1970; Swerdloff _et al_., 1971; Odell _et al_., 1973; Chen _et al_., 1976). Hence a longitudinal profile of FSH secretion in relation to the LH and testosterone profiles and the onset of spermatogenesis, would have provided a clearer picture of the endocrine events occurring during puberty in boars. To date no papers appear to have been published on plasma FSH levels in pigs, nor on the development of specific radioimmunoassay procedures for measuring porcine FSH. A similar deficiency of porcine prolactin data apparently is related to the lack of suitable assay methods.

B. TWENTY-FOUR HOUR HORMONE PROFILE STUDY

The two short term studies revealed distinct differences in plasma hormone profiles between pubertal and post-pubertal boars. This difference was particularly marked for LH, for which a highly pulsatile mode of secretion at puberty contrasted with a relatively suppressed secretory profile post-pubertally. It was not possible to identify the precise age at which this highly pulsatile mode of LH secretion commenced, nor whether this type of secretory mechanism was functional from birth. Perhaps an earlier 24 hr sampling, or frequent intensive bleeding studies, would have been desirable to help elucidate the onset of such highly episodic releases. The existence of highly pulsatile LH secretion has been recorded as early as 30 days of age in ram lambs (Bindon and Turner, 1974).

A pronounced pubertal elevation of both LH and testosterone levels was observed in both the longitudinal and short term experiments and indicated a change in the neuroendocrine mechanisms which regulate their secretion; this change probably is important in the induction of the onset of puberty. The relative importance of the factors which have been postulated to be involved in the initiation of puberty (i.e. a declining sensitivity of the hypothalamus to steroid negative feedback, an increasing responsiveness of the pituitary gonadotrophs to GnRH stimulation, a greater responsiveness of the testes to gonadotrophin stimulation, or some combination of these factors) could not be deduced from the data reported in this thesis.

As in the longitudinal study, an unfortunate but unavoidable deficiency was the lack of data for plasma FSH levels.

C. POSSIBLE APPLICATIONS OF THE PRESENT FINDINGS

Early puberty is a desirable characteristic for breeder animals not only for females but also for males (Coulter and Foote, 1976). Boars attaining puberty early would attain full reproductive capability at a younger age (Niwa and Mizuho, 1954), hence they could be trained and used for artificial insemination or natural breeding programmes much earlier than those with a delayed pubertal onset. However for males, early identification of pubertal animals poses difficulties due to the lack of external manifestations such as the behavioural estrus in females. The present findings provided some basic information on the assessment of puberty in boars. For instance, observation of the advanced onset of spermatogenesis in boars with heavier testicular weights than others of the same age, indicated that testicular size could be a good criterion of early sexual maturity and presumably of the potential capacity of boars as future breeders. This criterion is being utilized in the identification of young bulls with high spermatozoal producing potential, based on the finding of a high correlation between testicular size and spermatozoal output (Amann, 1970; Coulter and Foote, 1976). In lambs, Carr and Land (1975) reported that highly prolific breeds (Finnish Landrace) have higher mean plasma LH levels than less prolific breeds (Border Leicester and Cheviot). These investigators also showed a significant correlation between testis diameter and plasma LH concentrations,and suggested that a combination of rate of testis growth and plasma LH estimations may form a good basis on which to select animals for high fecundity. The potential usefulness of plasma LH concentration measurement (or other endocrine parameters) as an indication of potential future reproductive performance in boars, deserves further investigation.

Monitoring of plasma testosterone levels during sexual maturation in relation to other steroids, particularly the 16-androstenes which causes boar taint, may also have some practical implications. It is a routine farm practice to castrate male pigs within a few weeks of birth to avoid the occurrence of boar taint. However, this practice sacrifices the potential advantage that could be derived from the anabolic effect of testosterone, as indicated by findings that boars have better feed conversion and higher growth rates than barrows (Hansson, 1974). It would be of practical benefit for pork producers if male animals could be raised as entires until such an age that 16-androstene levels began to increase to the point that they may cause taint in meat, so that advantage could be taken of the anabolic effect of testosterone. Rhodes (1972) observed that taint is negligible in meat from boars less than 200 days old, which agrees with the report of Booth (1975) who reported that concentrations of 16-androstenes in the testes were low before 148 days of age. Andresen (1976) also found that levels of 5α-androstenone (one of the 16-androstenes) in peripheral plasma and adipose tissue only increased markedly between 135 and 170 days of age. Thus it appears that castration could be avoided for boars slaughtered before 135 days of age without meat taint occurring; such animals could be expected to produce carcasses of 45 - 50 kg, which is in the middle of the New Zealand porker grade weight range.

Knowledge of the longitudinal and short-term profiles of LH and testosterone secretion may be a useful reference for future studies on the endocrinology of puberty in boars, particularly if supplemented by data which will be produced when RIA techniques for FSH and prolactin become available. A radioimmunoassay for 5α-androstenone has been developed (Andresen, 1976) and its plasma profiles during sexual maturation have already been published. Such data on the normal course of pubertal development in boars should assist the monitoring of progress in likely future studies on the advancement of puberty by environmental manipulation and/or by nutritional or pharmacological techniques.

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