# STUDIES ON PUBERTAL DEVELOPMENT IN BOARS AND RAMS: EFFECTS OF HEMICASTRATION AND ARTIFICIAL CRYPTORCHIDISM ON HORMONE SECRETORY PATTERNS AND DEVELOPMENT OF REPRODUCTIVE ORGANS

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ABSTRACT

STUDIES ON PUBERTAL DEVELOPMENT IN BOARS AND RAMS: EFFECTS OF HEMICASTRATION AND ARTIFICIAL CRYPTORCHIDISM ON HORMONE SECRETORY PATTERNS AND DEVELOPMENT OF REPRODUCTIVE ORGANS

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Experiments described in this thesis were designed to reinvestigate longitudinal patterns of secretion of LH and testosterone in boars and rams; also to determine the effects of hemicastration of boars and the effects of hemicastration and artificially induced cryptorchidism of rams on longitudinal and acute hormone secretion patterns, as well as some parameters of reproductive organ development.

Plasma LH and testosterone levels of spring and autumn born Large White x Landrace boars were relatively high at birth, but declined from about the 4th postnatal week. Autumn born boars showed a distinct prepubertal LH peak, but in spring born animals there was no such peak. LH concentrations of barrows were high throughout the period of study. Plasma testosterone levels were low between 4 and 12 weeks of age then increased progressively until the end of study. Season of birth had little influence on the longitudinal profiles of secretion of either hormone.

Intensive bleeding experiments with entire and hemicastrated boars confirmed that LH and testosterone were secreted in a pulsatile manner; hemicastration had no significant effect on pulsatile secretion, mean hormonal concentrations, nor LH or testosterone responses following injection of GnRH. On the other hand, while LH responses to GnRH administration were not affected by advancing age, the subsequent testosterone output increased with sexual maturation.

Compensatory hypertrophy in testicular and epididymal weights and in seminiferous tubular diameters was noted in organs recovered from hemicastrates. However, qualitative histological analyses of testicular samples revealed that the cellular changes observed in all animals were of maturational nature and entirely age-related. From these observations it was concluded that hemicastration of developing boars did not result in any acceleration of the onset of spermatogenesis nor any advancement of puberty.

In ram lambs LH concentrations were low at birth, increased to peak levels at around 10 weeks of age, then declined to low values between 16 and 30 weeks of age when the experiment ended. In contrast plasma testosterone values were low at birth but increased steadily, particularly from about 18 weeks of age.

Mean LH and testosterone concentrations recorded from longitudinal, acute profile and pre-GnRH plasma samples of hemicastrated ram lambs confirmed the observations recorded from hemicastrated boars that the remaining testes were capable of secreting near normal quantities of testosterone and hence maintaining virtually unchanged plasma LH levels. On the other hand, a transient but significant increase in plasma FSH levels was detected following hemicastration of ram lambs. Conversely, cryptorchidism caused an elevation of LH and FSH secretion throughout the period of study. Neither surgical treatment had any influence on longitudinal or acute prolactin or testosterone secretory patterns. Mean plasma prolactin levels recorded from all animals were high initially then declined steadily throughout the period of study. That decline in prolactin levels coincided with the seasonal decrease in daily photoperiod. Plasma testosterone levels recorded from all three treatment groups increased steadily from birth to reach peak concentrations at 30 weeks of age.

Short term profile studies with entires, hemicastrates and cryptorchids confirmed the episodic mode of secretion of LH, prolactin and testosterone, and to less extent FSH. Hemicastration had no significant effect on episodic secretion of any of these hormones. Cryptorchidism caused a significant increase in number of LH peaks and a decrease in number of testosterone peaks, but had no effect on patterns of prolactin or testosterone secretion.

GnRH administration caused an increase in plasma LH, FSH and testosterone secretion in entires and responses were unaffected by hemicastration. However, exaggerated gonadotrophin responses were noted from cryptorchids, while the testosterone responses recorded from these animals tended to be lower (but not significantly so) than those of entires and hemicastrates. Mean plasma gonadotrophin levels recorded from each group were reduced by testosterone propionate pre-treatment; that result gave support to the concept that hypoandrogenism may have been the major reason for the elevation of plasma LH levels in cryptorchids. While total LH responses declined with age and maximal FSH responses of all three treatment groups were noted at 24 weeks of age, testosterone responses increased with sexual maturation.

Testicular and epididymal weights and seminiferous tubular diameter data obtained from hemicastrate rams confirmed that this treatment resulted in compensatory hypertrophy of the remaining organs. As recorded from hemicastrated boars there was no major alteration in cell populations of the germinal epithelium or Leydig cells. The changes observed were entirely of a maturational nature and age-related. Testes and epididymides obtained from cryptorchids showed no increase in weight during the experiment and thus were smaller than those of age-matched entires. It was apparent that intra-abdominal temperature prevented normal development of these organs. Qualitative histological examination showed that there was complete arrest of all maturational changes, both in the germinal

epithelium and interstitial tissue of cryptorchid testes.

On the basis of hormonal and organ data obtained from experiments described in this thesis it was concluded that LH, FSH and possibly testosterone were responsible for compensatory hypertrophy of the remaining testes of hemicastrates. The transient elevation of plasma FSH levels which occurred following this treatment probably was due to an overall decrease in production of testicular inhibin, the major regulator of FSH secretion. Presumably the remaining testis subsequently produced sufficient inhibin to reduce FSH secretion back to normal levels. These observations add weight to the hypothesis that following hemicastration, the compensatory increase in testicular androgen secretion occurs more rapidly than does the increase in rate of secretion of FSH inhibitory products. In contrast the increased secretion of LH and FSH in the cryptorchids resulted from reduced testicular production of androgens and inhibin, respectively. Those changes in testicular secretion persist throughout the period of cryptorchidism.

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# CHAPTER I

## INTRODUCTION

Of the known several thousand species of mammals, reproductive biology has been studied intensively in less than twenty five, including rodents, rabbits, primates, farm animals, and a few marsupials.

Animals which have been domesticated by man to meet his needs for food, clothing, transport and companionship include cattle, sheep, goats, pigs, horses, cats, dogs and poultry. Though the process of domestication has been going on for centuries, these animals show considerable variations with respect to such reproductive phenomena as gestation length, sexual cycle, sexual season, litter size and lactation period. However these variations are less evident in domesticated than in wild species and less marked in tropical compared to temperate zones (Hafez, 1975).

Survival of a species depends on the ability of individual animals to reproduce. Attainment of the capacity to reproduce usually is reached after a transitional phase in development between sexual immaturity and adulthood. The biological processes of growth and maturation which make up this developmental phase constitute pubertal development.

Puberty is the period of adolescence when a male or female first becomes able to produce gametes. Occurrence of the first ovulation or oestrus indicates that the female has reached puberty, while it is more difficult to determine the exact time of puberty in males. The appearance of first spermatozoa is a criterion commonly used for determining puberty in males (Asdell, 1964).

Puberty is initiated in the brain but mediated and effected by a variety of hormones, in a complex series of neuroendocrine changes which still are incompletely under-

stood (Donovan and van der Werff ten Bosch, 1965).

This review of literature is concerned primarily with the current concepts of reproductive physiology and endocrinology of the male, with special reference to rams and boars. The respective roles of the hypothalamus, releasing hormones, anterior pituitary hormones and gonadal hormones, in relation to pubertal processes, will be discussed.

## 1. Neuroendocrine Control of Reproduction

In 1932 Moore and Price postulated a new concept of hormonal interactions involving the pituitary gland and gonads. This concept involved hypophyseal control of the gonads and reciprocal gonadal control of the hypophysis. However the authors failed to recognise any hypothalamic involvement.

Later Green and Harris (1947) demonstrated the hypothalamo-pituitary portal vascular link and reported the direction of blood flow as being towards the pituitary. Harris and Jacobsohn (1952) performed pituitary graft studies and established that secretion of anterior pituitary hormones was under hypothalamic control, mediated by factors transported via the hypothalamo-hypophyseal portal system.

Luteinising hormone (LH) releasing activity of crude hypothalamic extracts was first described by McCann et al. (1960) while evidence for follicular stimulating hormone releasing activity of the hypothalamus was described by Igarashi and McCann (1964).

### (a) Anatomy of the Hypothalamus

The anatomy of the hypothalamus has been reviewed by Donovan (1970) and Joseph and Knigge (1978). It consists of the ventral portion of the diencephalon with its anterior border marked by the rostral edge of the optic chiasma and its posterior border by the caudal tips of the mammillary bodies (Everett, 1978).

The adult hypothalamus is characterised by a diffuse array of nerve cells among which some are sufficiently aggregated to be recognized anatomically as nuclei. In general the borders of these nuclei are ill defined, showing only gradual transitions to relatively cell poor zones. In instances where there is no marked aggregation of cells the expression 'area' is preferred. The fine structure of the rat hypothalamus has been detailed by Clemmenti and Cecerelli (1970).

The anterior hypothalamic area (AHA) contains the anterior and suprachiasmatic nuclei (SCN), while medial basal hypothalamus (MBH) consists of the arcuate nucleus and ventromedial nucleus (VMN). Anatomical data regarding the hypothalamic areas which regulate gonadotrophin secretion mainly comes from intensive studies done in the rat. According to the "dual control" hypothesis two separate regions of the hypothalamus control the tonic and cyclic secretion of gonadotrophins (Gorski, 1971; Sharp and Fraser, 1978). The preoptic area (POA) and AHA have been shown by surgical isolation studies (Halasz and Gorski, 1967) to be specifically involved in regulating ovulation of female rats (and hence cyclic gonadotrophin secretion). However, similar studies performed on rhesus monkeys (Knobil, 1974) have shown that ovulatory cycles continue despite surgical isolation of the AHA and indicates probable species differences in localisation of control mechanisms. The MBH regulates tonic release of gonadotrophins (Donovan, 1970) and is the area considered to contain steroid negative feedback receptors (Davidson, 1974). When surgically isolated the MBH is capable of maintaining normal male pituitary and gonadal structure and function, whereas its removal abolishes normal sexual function (Halasz, 1969). Recent data from rats and sheep reconfirms the concept that the MBH is involved in maintaining tonic secretion of Gonadotrophic Releasing Hormone (GnRH) (Domanski et al., 1980).

Another hypothalamic area considered to be important in regulating gonadotrophin secretion is the median eminence

(ME) located immediately dorsal to the pituitary gland. It is considered to contain the final common pathway for the control of the adenohypophysis and contains more enzymes, neurotransmitters (established and putative) and hormones, of both central and peripheral origin, than any other region of the central nervous system (CNS) (Joseph and Knigge, 1978).

(b) Extra-hypothalamic Influences

The role of extra-hypothalamic structures in reproduction has been reviewed by Gorski (1974), Döcke (1976) and Ellendorff (1976). Among the structures involved in control and modulation of reproduction the most pronounced effects are exerted by the limbic system, the pineal gland and the olfactory system.

The limbic system consists of two parts: the subcortical and mesencephalic limbic regions. In the former, the basal amygdala and hippocampus have been found to be intimately involved in modulation of reproductive functions. The main pathways to and from the limbic lobe are provided by the fornix and medial forebrain bundle, in conjunction with the stria medullaris, stria terminalis and thalamocingulate projections (Donovan, 1970; Everett, 1978). The medial forebrain bundle, which is considered to be the major source of afferent input to the hypothalamus, also is connected with the olfactory apparatus, septum, amygdala, hippocampus and caudate nuclei.

(i) Amygdala

The amygdala is directly connected to the POA of the hypothalamus by the stria terminalis, which is part of the forebrain bundle and serves as the primary link which terminates in the POA and AHA. In turn the amygdala provides a functional link from the olfactory system, hippocampus, basal ganglia and septum to the hypothalamus (de Groot, 1966; Everett, 1978).

Amygdaloid lesions have been associated with male hypersexuality, seminiferous tubular degeneration and aspermia (Donovan, 1970). The changes that occur in the

amygdaloid nuclei during initiation of puberty may involve development of new receptor sites and changes in sensitivity of the feedback system (Döcke, 1976).

(ii) Hippocampus

The second most important limbic structure which influences the hypothalamus is the hippocampus and its major pathway to the hypothalamus is via the fornix (Everett, 1978). The hippocampus appears to exert an inhibitory influence on the hypothalamus and generally has functions inverse to those of amygdala (Donovan, 1970). In the male rat hippocampal stimulation has been shown to elicit erection of the penis, licking of genitalia and grooming behaviour (Donovan, 1970).

(iii) Olfactory system

The stria terminalis contains extensive reciprocal connections between the olfactory system, medial amygdala and the hypothalamus and the close association of the olfactory system with limbic structures suggests an important integration between the two systems in the transmission of olfactory stimuli.

The role of olfactory stimuli in mammalian reproduction has been the subject of a recent review by Aron (1979). Olfactory bulbectomy of lower species has been shown to abolish male sexual behaviour (Clegg and Doyle, 1967), while advancement of age at puberty seen in gilts exposed to boars, is said to be mediated by olfactory stimuli (Thompson and Savage, 1978).

(iv) Pineal gland

The epiphysis cerebri or pineal gland, so named because of its resemblance to a pineapple or pine cone, has been the subject of several comprehensive reviews (Wurtman, 1968; Relkin, 1976; Reiter, 1977). The pineal is considered to be an actively functioning neuroendocrine transducer which responds to photic stimuli, exhibits circardian rhythms, and influences the hypothalamo-pituitary-gonadal axis.

The main innervation of the pineal is by sympathetic postganglionic neurons from the cranial (superior) cervical ganglia, which enter the pineal paranchyma as the nervi conarii, or in company with blood vessels (Reiter, 1977).

Visual stimuli which may influence pineal activity originate in the retinae. Impulses traverse the optic nerves and eventually enter the hypothalamus. Some of these fibres, together with other axons from the SCN pass through the lateral hypothalamus as the inferior accessory optic system, to the medial terminal nucleus in the tegmentum of the midbrain. Unidentified pathways then project to the upper thoracic spinal cord from where the preganglionic neurons of the autonomic nervous system arise. From here fibres leave the cord and pass up the sympathetic trunk to the cranial cervical ganglia where they synapse with post-ganglionic neurons (Moore and Klein, 1974; Reiter, 1977). In addition a parasympathetic component arising from the superior salivary nuclei, probably exists in some species (Reiter, 1977).

Substances having both pro- and antigonadotrophic properties have been isolated from the pineal gland (Reiter et al., 1978). In addition to the antigonadotrophic melatonin and related indoleamines, a pineal peptide, arginine vasotocin, which has strong antigonadotrophic properties, has been identified in the pineal gland (Benson and Ebels, 1978; Pavel, 1978).

(v) Peripheral afferent inputs

Copulation induced gonadotrophin secretion and ovulation has been demonstrated in cats and rabbits (Clegg and Doyle, 1967; McDonald, 1975). However, very little information is available as to the role of tactile and other peripheral stimuli in influencing reproductive functions in domestic animals.

(c) Hypothalamic Releasing Hormones

Releasing hormones secreted by the hypothalamus have been the subject of many recent reviews (Vale et al., 1977; Schally, 1978; Guillemin, 1978), but to date only three peptides with hypophysiotrophic activity have been purified and characterised chemically. They are the thyrotrophin releasing hormone (TRH), GnRH, and somatostatin (Schally, 1978).

(i) Gonadotrophin releasing hormone

a. Chemistry and structure

GnRH is a decapeptide with the following amino acid sequence (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly ($NH_2$) (Matsuo et al., 1971; Schally et al. 1971). This structure, determined initially for porcine GnRH, has been confirmed for bovine (Currie et al., 1971) and ovine GnRH (Guillemin, 1978). Recent work using specific anti-sera against GnRH indicates that gonadotrophin secretion in a variety of species, including reptiles and birds, is controlled by peptides with amino acid sequences closely related or identical to that of porcine GnRH (Schally, 1978).

b. Synthesis

Synthesis of GnRH is assumed to take place in the perikarya of neurones of the MBH and the production rate has been estimated to be between 1.5 and 15 μg/day in man (Jeffcoate and Holland, 1974).

c. Release from storage sites

Appropriate neurochemical stimulation of the ME causes GnRH release (Sharp and Fraser, 1978). The role of neurotransmitters in the release of GnRH will be discussed in detail later (see page 12).

d. Metabolism and excretion

While it has been reported that peptides from the hypothalamus abolish the immunoreactivity and bioactivity of GnRH by splitting off the C-terminal amino acid (Griffiths

et al., 1974), the physiological significance of such non-specific enzymes is doubted (Reichlin et al., 1976).

The plasma half life of GnRH in man is relatively short, not exceeding 4 minutes (Redding et al., 1973), while in rats (Miyachi et al., 1973) and sheep (Crighton et al., 1973) similarly short half lives have been recorded. From its behaviour during gel filtration Virkkunen et al. (1974) concluded that GnRH was transported as a free molecule rather than bound to carrier proteins. Rapid accumulation of radioactivity in the liver, pituitary, kidney and bladder has been observed in rats following injection of radioactively labelled GnRH (Dupont et al., 1974). It is assumed that the major excretory pathway is via the kidneys since about 97% of total radioactivity has been recovered in urine of men injected with tritiated GnRH (Redding et al., 1973).

e. Distribution of GnRH in the CNS

Regional differences between sexes in the distribution and degree of development of GnRH positive neurones within the CNS have been seen in rats (Arimura, 1977). Within the hypothalamus the ME, arcuate nucleus, SCN and POA have been reported to contain greatest amounts of GnRH activity (Palkovitz et al., 1974; Sétáló et al., 1975). On the other hand no such activity has been detected in the cerebellum or cerebral cortex, but high concentrations were found in the pineal (White et al., 1974). The physiological significance of GnRH in extra-hypothalamic areas is not understood, although it has been suggested that it could influence sexual behaviour (Dyer and Dyball, 1974).

f. Mechanism of action

Current theories on the mechanisms by which GnRH exerts its effects on gonadotrophin release and synthesis have been reviewed by Jutisz et al. (1976) and Labrie et al. (1977; 1978). These hypotheses outline the following events:
(a) binding of GnRH to specific membrane receptors;
(b) this activates a receptor coupled adenyl cyclase system; (c) adenyl cyclase causes the formation of cyclic-adenosine monophasphate (cyclic AMP) from adenosine tri-

phosphate (ATP), which is said to be modulated by another intracellular enzyme, phosphodiesterase; (d) cyclic AMP which is formed causes the release of $Ca^{++}$ from cell membranes; (e) $Ca^{++}$ ions are necessary for promoting many intracellular enzymatic processes; (f) cyclic AMP also is found to activate a protein kinase, which promotes the activation of phosphorylase kinase participating in the phosphorylation process. The rest of the sequence of events which leads to release of GnRH remains unclear. Involvement of the microtubular and microfilament structures in the release of FSH and LH from pituitary gonadotrophs has been demonstrated by Ashok-Khar et al. (1979).

g. GnRH analogues and antagonists

Because the half life of GnRH is so short attempts have been made to synthesize analogues which not only have greater biological potency, but also longer durations of action. Generally such superactive analogues have been obtained by substitution of amino acids at positions 6 and/or 10 of the GnRH molecule (Schally and Coy, 1977). Successful use of one potent analogue (D-TRY-6-GnRH) to induce ovulation in infertile women was reported by Canales et al. (1980). Information gained from this approach also has resulted in the discovery of potent synthetic GnRH antagonists (devoid of any biological activity), which compete with endogenous GnRH for receptor sites in the pituitary. It is considered that such compounds may prove effective in the future as contraceptive agents. Reports on the use of such analogues as contraceptives in women have been published (e.g. D-SER(TBU)$^{6}$-EA$^{10}$-GnRH; Bergquist et al., 1979).

h. Clinical applications for GnRH

Economic pressures on animal husbandry practices have created demands for increased reproductive efficiency of farm animals and this has led to many investigations of the possible use of GnRH. Convey (1973) suggested that GnRH could have potential uses in advancing puberty, manipulation of spermatogenesis and libido, and possibly also in increasing the quantity of spermatozoa produced by animals used in

artificial insemination programmes.

GnRH caused ovulation in ewes (Convey, 1973) but subsequent luteal function was abnormal (Hoffman et al., 1975). GnRH also has been used in the treatment of reproductive disorders like cystic ovarian disease (Convey, 1973), delayed puberty in gilts (Edqvist et al., 1978) and anoestrus in ewes (Pelletier, 1976). It has also been used satisfactorily for inducing ovulation in gilts (Baker et al., 1973) and postpartum cows (Fonseca et al., 1980).

Research on the use of GnRH in treatment of various reproductive disorders in human males and females has been reviewed by Mortimer (1977). Syndromes which apparently have been treated successfully include: cryptorchidism in boys, delayed puberty in boys and girls, infertility in men with hypogonadotropic hypogonadism due to hypothalamic failure, and anovulation in adult women.

(ii) Control of prolactin secretion

Hypothalamic control of prolactin secretion appears to be organized along evolutionary lines such that it is predominantly inhibitory in amphibians, stimulatory in birds and both stimulatory and inhibitory in mammals (Ensor, 1978). Prolactin secretion control concepts have been the subject of many reviews (Horrobin, 1973; Reichlin et al., 1976; Ensor, 1978; Neill, 1980).

a. The hypothalamus and prolactin output

Knowledge of the hypothalamic nuclei controlling prolactin secretion is fragmentary. VMN and POA stimulation have been reported variously to increase or lower the output of prolactin (Meites and Clemens, 1972; Horrobin, 1973). Recently Malven (1975) produced an elevation of prolactin output in sheep after anterior ME stimulation and drop in plasma levels by posterior ME stimulation, indicating that at least in this species the ME probably is of prime importance in regulating prolactin secretion. Prolactin output also follows in vitro application of hypothalamic extracts to

the pituitary of rhesus monkeys, a result which supports the concept of the existence of a prolactin releasing factor in addition to an inhibitory factor (Horrobin, 1973).

Though it is clear that the hypothalamus regulates prolactin secretion, there is insufficient evidence to allow conclusions to be reached about the neurochemical mechanisms involved.

b. Prolactin inhibitory factor (PIF)

Dopamine (DA) inhibits the release of prolactin when administered directly to the hypothalamus or into the third ventricle (Meites and Clemens, 1972; Reichlin et al., 1976). Further evidence in favour of DA being the physiological inhibitor of prolactin release comes from the in vivo demonstration that DA blocking agents induce prolactin secretion in rats (Ojeda et al., 1974) and sheep (Davis and Borger, 1973a). Present research indicates that DA accounts for the major hypothalamic inhibitory control of prolactin secretion, but it is not regarded conclusively as the only prolactin inhibitory factor from the hypothalamus (Lancranjan and Friesen, 1978).

Another neurohormone, somatostatin, also has been reported to decrease prolactin release in pituitary cell cultures (Drouin et al., 1976). Recent reports of the isolation of a PIF-like tetra-decapeptide from porcine hypothalami needs further examination (Greibrokk et al., 1975).

c. Prolactin-releasing factor (PRF)

PRF, which provides the major control of prolactin secretion in birds, also has been suggested to exist in mammals (Ensor, 1978). The fact that at pharmacological doses TRH can stimulate prolactin release is widely accepted (Chen and Meites, 1975). However, whether or not it plays a major role in the regulation of prolactin under physiological conditions is still debatable. The most direct evidence that supports the concept of a physiological role for TRH was provided by the demonstration that administration

of anti-TRH serum to rats leads to a 50% decrease in serum PRL levels (Koch et al., 1977; Neill, 1980).

(d) Brain Monoamines and Pituitary Hormone Secretion

(i) Gonadotrophins

Neurosecretory elements which synthesize the hypothalamic hormones synapse with other neurons within the hypothalamus. Kordon et al. (1976), in a review of this subject, stated "the precise role of given transmitters in the regulation of adenohypophyseal hormones is still largely controversial".

DA has been reported to augment the release of FSH and LH when injected into the third ventricle in rats, but norepinephrine or epinephrine failed to produce similar results (Kamberi et al., 1970; 1971). However, other investigators have reported contrasting results. For example, Sawyer et al. (1974) found that norepinephrine, but not DA, could trigger a preovulatory LH surge in female rabbits, while Tima and Flerko (1974) observed similar results with female rats. Serotoninergic neurons appear to be inhibitory to LH release as was indicated by the observation that 5,6-dihydroxytryptamine (a drug which destroys serotoningergic neurones) elevated LH levels in both normal and gonadectomized male and female rats (Ladosky and Noronha, 1974).

(ii) Prolactin secretion

The influence of brain monoamines in the control of prolactin secretion has been the subject of many recent reviews (Fernstorm and Wurtman, 1977; Tindall, 1978; del-Pozo and Brownell, 1979; Johnathan, 1980). Data from these studies indicate that in addition to DA, which already has been mentioned as most likely being PIF, noradrenaline, serotonin, acetylcholine and histamine also can influence secretion of prolactin. Histamine and serotonin have been reported to be stimulatory, acetycholine mainly inhibitory, but results for noradrenaline still appear to be confused (Lancranjan and Friesen, 1978).

(e) Prostaglandins

Prostaglandins (PG) are ubiquitous substances which display a great variety of effects on almost all systems and functions of the body. Although the mechanisms of their actions are not well understood, PGs have been reported to cause the release of gonadotrophins, probably by acting at both hypothalamic and pituitary levels (Labhsetwar, 1975).

(i) PGs and gonadotrophin secretion

Prostaglandins have been postulated to play a physiological role in the hypothalamo-pituitary control of gonadotrophin secretion (Harms et al., 1973; 1974). This concept was based on the observation that release of gonadotrophins occurred when E series prostaglandins ($PGE_1$ and $PGE_2$) were administered systemically or directly into the third ventricle. Also it has been reported that the potent PG-inhibitor, indomethacin, causes a decrease in GnRH-induced LH release in rats (Ojeda et al., 1975). Most workers now believe that activation of E series PG's in the brain is an obligatory step in the neural mechanisms which lead to release of LH (Ojeda et al., 1975; 1979), though the exact mechanism of action is not clear.

2. Anterior Pituitary Hormones

The hypothalmo-pituitary portal vascular system carries the hypothalamic releasing and inhibiting factors responsible for regulating anterior pituitary gland function. Current concepts of pituitary control were the subject of an extensive review by Daniel and Pritchard (1975). This review concentrates on structure, metabolism and regulation of secretion of LH, FSH and prolactin only.

(a) Luteinising Hormone (LH)

(i) Structure and chemistry

LH is a glycoprotein with a molecular weight of approximately 30,000 and consists of two dissimilar polypeptide chains designated the α and β chains (Greep, 1973). Between-species antigenic cross-reactivity of LH molecules resides

in the β unit, whereas within-species cross-reactivity resides in the α unit. The α subunit of LH is common to FSH and TSH, but the β chain is hormone specific (Vaitukaitis et al., 1976). In dissociated form the subunits are devoid of any intrinsic biological activity, but when recombined they regain biological potency (Papkoff et al., 1973). The structure of ovine LH has been described in detail by Papkoff et al. (1973) and the structure of bovine LH has been shown to be very similar (Pierce et al., 1971).

(ii) Metabolism

Various estimates of the half life of ovine LH have been reported. Akbar et al. (1974) estimated a half life of 43 min in ewes, while a half life of 26-32 min was estimated by de Kretser et al. (1973). In foetal and prepubertal lambs a half life of about 26 min has been recorded (Foster et al., 1972c; 1975). Although the exact mode of metabolic degradation of LH is not known, most radio-labelled LH injected into sheep passes through the renal proximal convoluted tubules, from where it is excreted in the urine (de Kretser et al., 1973).

(iii) Site of action

The primary sites of action of LH in males are the testicular interstitial Leydig cells (de Kretser et al., 1969; Catt et al., 1980) to which it binds specifically and stimulates androgen production, both in vivo and in vitro (Moyle and Ramachandran, 1973; Catt et al., 1980). Very little or no binding of labelled LH has been detected in other cellular components of mammalian testes (Hansson et al., 1976a).

(iv) Mechanisms of action

As in the case of most protein hormones, the first stage in the action of LH involves its binding to specific receptors on the Leydig cells. It then enhances adenyl cyclase activity and the formation of cyclic AMP (Dorrington and Fritz, 1974; Catt et al., 1980). This elevation of cyclic AMP activity can be detected within minutes, but the subsequent testosterone secretory response always is

delayed by about 15-20 min, which indicated that a series of metabolic events were interposed between cyclic AMP formation and the release of testosterone in the testis (Setchell, 1978). Details of these obligatory steps are not clear. However, it appears that activation of a cyclic AMP-dependent protein kinase catalyses the formation of messenger proteins, resulting in the conversion of cholesterol to pregnenolone (Hall, 1966; Sandler and Hall, 1966).

(v) Actions of LH in males

a. Steroidogenesis

Testicular androgen formation is largely under the control of LH, and so far there is no definite evidence that any other factor directly affects the secretion of androgens, with the possible exception of reduced blood flow produced under experimental conditions (Eik-Nes, 1964). The mechanism by which LH regulates testosterone production is discussed more fully on page 20.

b. Testosterone release

Secretion of testosterone by the Leydig cells follows administration of LH *in vivo*, and also is induced by the rise in endogenous LH concentrations which results after GnRH administration. Trophic effects of LH in causing testosterone release are discussed on pages 20 and 34.

c. Feedback effects

It has been postulated that LH inhibits the production and/or release of GnRH by direct negative feedback inhibition of hypothalamic hypophysiotrophic centres (Greep, 1973). This topic is dealt with more fully on pages 27 and 28.

d. Spermatogenesis

A normally functioning pituitary gland is essential for optimal spermatogenic activity of the seminiferous epithelium in prepubertal and pubertal male animals (Steinberger and Duckett, 1967). However, the same authors also demonstrated that spermatogenesis in adult rats is entirely testosterone-dependent; the effect of LH

is mainly via stimulation of testosterone production. On the other hand Courot (1967; 1978) and Courot et al., (1970; 1979) found that LH was essential for initiation and maintenance of spermatogenesis in lamb and adult rams. The differentiation of gonocytes to type A spermatogonia was reported to be under the direct influence of LH (Courot et al., 1979) and hence there probably are species differences in endocrine control of spermatogenesis. This topic is discussed on pages 17 and 22.

(b) Follicle-Stimulating Hormone (FSH)

(i) Structure and chemistry

FSH is a glycoprotein hormone for which molecular weight estimates ranging from 32,000 to 52,000 have been reported. Like LH it consists of dissimilar $\alpha$ and $\beta$ subunits (Vaitukaitis et al., 1976).

(ii) Metabolism

The half life of FSH is much longer than that of LH and has been reported to be about 102 min in sheep (Akbar et al., 1974) and approximately 4 hr in humans (Coble et al., 1969). No information is available regarding its metabolic pathways.

(iii) Site of action

It is now clearly established that the main site of action of FSH in the male is within the seminiferous tubules (Means and Vaitukaitis, 1972; Bhalla and Reichert, 1974; Cheng, 1975; Means, 1977; Means et al., 1980). Further work with Sertoli cell-enriched tubules, both in vitro and in vivo, has shown that Sertoli cells are the primary testicular target cells for FSH (Dorrington and Fritz, 1974; Means et al., 1976; Dorrington and Armstrong, 1979).

(iv) Mechanism of action

Research on mechanisms of action of FSH in the testes has been reviewed by Dorrington and Armstrong (1979). FSH binds specifically to receptors located on Sertoli cell

membranes and activates a receptor linked adenyl cyclase system resulting in elevation of cyclic AMP levels (Dorrington and Armstrong, 1979; Means et al., 1980). Essentially no information is available to suggest how these biochemical reactions regulate Sertoli cell function. Nevertheless it has been reported that FSH can regulate the metabolism of Sertoli cells and their secretory products, such as androgen binding protein (ABP) (Hansson et al., 1976b; Means et al., 1980).

(v) Actions of FSH in the male

a. Spermatogenesis

FSH is recognised as being essential for the initiation of spermatogenesis and may also be important in adult animals for maintenance of optimal testicular function. The role of FSH in regulating spermatogenesis remains controversial. In immature rats lack of FSH causes disruption of spermatogenesis (Raj and Dym, 1976), but in adult male rats spermatogenesis is relatively insensitive to the withdrawal of FSH (Dym et al., 1979). On the other hand, in rams, spermatogenesis in pubertal and adult animals is severely disrupted by FSH withdrawal (Courot, 1967; Courot et al., 1979).

b. Androgen binding protein production

As mentioned earlier, FSH influences the production of ABP by Sertoli cells (Hansson et al., 1976b; Dorrington and Armstrong, 1979). ABP has a very high affinity for testosterone and 5 α -dihydrotestosterone and therefore has been suggested to be involved in maintaining high androgen levels within the seminiferous tubules, which is an essential component of the milieu required for the normal progress of spermatogenesis, at least in rats (Ritzen et al., 1974). In rams, goats and bulls ABP has been isolated from seminal fluid, but its presence in semen of men, stallions and boars has yet to be demonstrated (Courot, 1980; Waites, 1980).

ABP production in testes of rams varies with age and

season and its transport from testes to epididymides is specifically controlled by FSH (Courot, 1980).

c. Feedback effects

Evidence in favour of short loop feedback inhibition by FSH, of its own secretion, is discussed fully on page 27.

(c) Prolactin

Hormones similar to mammalian prolactin occur in all vertebrate groups, with the possible exception of cyclostomes (Ensor, 1978).

(i) Structure

Ovine prolactin is a protein hormone of molecular weight 23,000 (Ensor, 1978). It is composed of 198 amino acids with disulphide bonds between the 4th and 11th, 58th and 73rd, and 190th and 198th residues, while the amido terminal of the molecule has a looped appearance (Ensor, 1978).

(ii) Secretion and metabolism

Prolactin is synthesized and secreted by lactotroph cells of the anterior pituitary (Ensor, 1978). The biological half life of prolactin in sheep apparently has not been measured, however, the half life of human prolactin has been estimated at 10-20 min (Horrobin, 1973; 1974).

Prolactin is transported in plasma in an unbound state and very likely is inactivated in the liver (Ensor, 1978).

(iii) Actions

In mammals the primary reproductive function of prolactin is to promote growth of the mammary glands and secretion of milk, while it also is regarded as being luteotrophic in rodents. Other functions, such as roles in osmoregulation and influences on parental behaviour (in which prolactin has major roles in lower vertebrates), are of secondary importance (Horrobin, 1973; Ensor, 1978).

(iv) Prolactin and reproduction in males

Specific binding sites (receptors) have been detected in the testes and accessory sex glands of male rats

(Aragona and Friesen, 1975). Subsequently Aragona et al. (1977) reported that most testicular receptors for prolactin were located on the Leydig cells, and Leydig cell LH receptor activity tended to be modulated by serum prolactin levels. Prolactin also has been suggested to have a stimulatory effect on spermatogenesis in dwarf mice (Bartke and Lloyd, 1970) and enhances the availability of esterified cholesterol for steroidogenesis (Hafiez et al., 1972). In rodents prolactin generally appears to play a direct role in modulating Leydig cell function and sexual maturation (Bartke et al., 1978).

Recent work indicates that prolactin may play a vital role in regulating the seasonal cycles of regression and recrudescence of the testes in golden hamsters (Bex et al., 1978). In addition, studies with other seasonal breeders, such as goats and rams, have shown that increasing daily photoperiods during the summer months cause increases in plasma prolactin levels and that possibly is an important prelude to the increase in spermatogenic activity and libido, required during the breeding season (Buttle, 1974; Ravault, 1976, Wilson and Lapwood, 1978a; Barrell and Lapwood, 1979 a).

Possible roles played by prolactin during sexual maturation of ram lambs are discussed more fully on page 47 of this review; no comparable data appears to have been published for boars.

## 3. Testicular Steroids

The testes of mammals fulfil two major functions:
(a) production of male gametes (spermatozoa), and
(b) production of male sex hormones (androgens). Of the 20 or more steroids which have been isolated from testicular tissue, testosterone is one of the most potent androgens and is the major testicular androgen in a variety of species (Setchell, 1978), including rams and boars (Linder, 1961).

### (a) Testosterone

#### (i) Site of production of testosterone

Many research workers have demonstrated that Leydig

cells are the major source of androgens (Christensen and Mason, 1965; Hall et al., 1969; Gomes, 1970; Parvinen et al., 1970; Bubenik et al., 1975). It is secreted not only postnatally, but also by foetal testes: from approximately the 30th day of gestation in rams (Attal, 1969) and boars (Ford et al., 1980), well before the time of gonadal duct differentiation.

(ii) Synthesis and metabolism

Two major substrates for testicular steroid production have been suggested: cholesterol, synthesized elsewhere in the body and transported to the testes in the blood, and acetate, either derived from the blood as such, or formed from acetyl-coenzyme A during metabolism of glucose (Setchell, 1978). Acetate is converted to cholesterol (Hall, 1970) and that is present mainly in the free, but not the esterified form, in interstitial tissue.

In the testes LH binds to specific receptors on the cell membranes of Leydig cells and stimulates the adenyl cyclase system to produce cyclic AMP (Dorrington and Fritz, 1974; Catt et al., 1980). Cyclic AMP then converts cholesterol to pregnenolone (Eik-Nes, 1971) and testosterone is synthesized in the following steroidogenic chain:-

Pregnenolone ⟶ dehydroepiandrosterone ⟶ androstenediol (or androstenedione) ⟶ testosterone (Yanaihara and Treon, 1972).

The major route of distribution of testicular androgens is via the blood stream, although some may be carried in testicular lymph. Approximately 99% of circulating androgen is bound to one of three proteins: specific sex hormone binding globulin (SHBG), serum albumin or cortisol binding globulin (Ismail, 1976). SHBG is not present in plasma of rats or boars (Corvol and Bardin, 1973), thus the proportion of free testosterone is much higher, at least in rats, than in man (Setchell, 1978). Studies of the biological half life of testosterone often have involved experiments in which the rate of disappearance of exogenous hormone

has been measured; this is represented by two exponential curves: the initial curve presumably represents circulating endogenous testosterone and has a half life of 7 min in humans (Horton et al., 1965) and 8 min in bulls (Haynes et al., 1976), while the second curve represents injected hormone with a half life of 30 min for bulls and 34 min for humans.

Testosterone receptors are widely distributed in the body (Minguell and Sierralata, 1975; Gorski and Ganon, 1976). In androgen-sensitive tissues most testosterone is converted to more potent androgens, such as 5 α -dihydrotestosterone (DHT) and androstenediols, prior to exerting its physiological effects. That conversion is mediated by the enzyme 5 α- reductase which is present in the prostate (Oftner, 1968) and other androgen sensitive tissues, like seminal vesicles and skin (Minguel and Sierralata, 1975).

Though some authors believe DHT to be the neuroactive metabolite of testosterone (Martini, 1970), Naftolin et al. (1975) presented evidence supporting the hypothesis that oestrogen (probably oestradiol) is the active metabolite in neuroendocrine tissue. These authors demonstrated that testosterone-induced CNS sexual differentiation was mimicked by oestrogens. Furthermore, oestrogen has been reported to exert inhibitory effects on LH secretion in males of many species, including rams (Riggs and Malven, 1974; Schanbacher and Ford, 1977) and boars (Ford and Schanbacher, 1977).

Testosterone is largely metabolised in the liver to androstenedione and other weak androgens, though a second pathway, involving changes in the A-ring does occur, yielding more potent androgenic by-products (Ismail, 1976). These metabolites are then conjugated as glucuronides or sulphates and excreted in the bile. Extra-hepatic metabolism of androstenedione has been suggested to occur in humans, since its metabolic clearance rate exceeds the hepatic blood flow (Horton and Tait, 1966).

(iii) Mechanisms of action

Extensive discussion on the mechanisms of sex steroid action is outside the scope of the present review, but has been the subject of two comprehensive recent reviews (Minguell and Sierralta, 1975; Gorski and Gannon, 1976). Generally these events can be summarised as follows: the first step in the action of androgens is entry of the hormone into the cell. This process is mainly regulated by the concentration of free hormone in blood plasma, however, selective uptake and retention has been suggested to occur in some tissues. Once the active metabolite (testosterone or DHT) is bound to cytosol receptors, the androgen-cytosol complex migrates into the nucleus. The identity of the acceptor site(s) in the nucleus of the androgen-responsive cells is not clear, however there is general agreement that enhancement of ribonucleic acid synthesis occurs, and this probably is the key step in the action of the hormone.

The above summarised sequence of events is only applicable for some androgen-sensitive tissues. For example in bone marrow no androgen binding cytoplasmic receptors have been demonstrated, although the metabolism of this tissue is under androgenic influences (Minguell and Sierralta, 1975).

(iv) Actions of testosterone

The major effects of androgens in males have been reviewed by Setchell (1978). The following section summarises the actions of testosterone in males, with particular reference to effects in boars and rams.

a. Spermatogenesis

The importance of gonadotrophins in initiation of spermatogenesis in prepubertal rats and rams has already been discussed on pages 15 and 17, respectively.

However it often has been difficult to define the functions of gonadotrophins and testosterone in the maintenance of spermatogenesis in adult animals, particularly as considerable species differences apparently exist (Steinberger,

1971). For example, in male rats androgens have been reported to maintain spermatogenesis if replaced soon after hypophysectomy, that is before complete atrophy of the germinal epithelium occurs. Subsequent experiments have in fact revealed that testosterone alone can maintain spermatogenesis in adults of this species. FSH has been reported to be of little importance, while LH apparently influences the process only through its effects on testosterone production (Steinberger and Duckett, 1967; Ahmed et al., 1975; Harris et al., 1977; Dym et al., 1979). In contrast to these observations, Courot et al. (1979) found that in experiments on the hormonal requirements for spermatogenesis in adult hypophysectomised rams, LH, FSH and testosterone all were required. Differentiation of gonocytes to type A spermatogonia appeared to be LH-dependent, while the last stage of spermatogonial multiplication (to type B spermatogonia) was under FSH control. The subsequent events of the spermatogenic cycle were entirely testosterone-dependent.

b. Accessory reproductive structures

Castration and androgen replacement studies have been reported to affect functional and morphological aspects of all the accessory organs of reproduction and these effects have been reviewed extensively by Parkes (1966) and Orgebin-Crist et al., (1975). Androgen-dependent structures include: (i) accessory glands i.e. the seminal vesicles, prostate and bulbo-urethral glands, as well as (ii) organs such as the epididymides, scrotum and penis. In addition testosterone also is required for epididymal spermatozoal maturation (Dyson and Orgebin-Crist, 1973; Orgebin-Crist et al., 1975).

c. Feedback effects

The role of testosterone in feedback control of LH secretion in the male is discussed on pages 25 and 26.

d. Sexual differentiation

Differential development of male genital primordia occurs earlier than the corresponding changes in the female

and is induced at least partly by the foetal testicular androgens (Josso et al., 1977). The initiation, timing and influence of androgens in the process of sexual differentiation in rams and boars is dealt with fully on pages 35 and 40.

e. Sexual behaviour

The differences in behaviour produced by castration have been known since antiquity and are the main reason for the widespread use of castration of males in modern animal husbandry. Initiation and maintenance of male sexual behaviour is totally androgen dependent and the role of testosterone in this phenomenon has been discussed in many reviews (Davidson and Levine, 1972; Beach, 1974; Davidson, 1977). The most pronounced effects follow prepubertal castration, in which case the animal never acquires normal sexual behaviour. In contrast sexual activity generally persists to a variable extent after castration of adults, and can be restored by testosterone replacement therapy (Setchell, 1978). However, in intact males libido is poorly correlated with blood androgen levels (Phoenix et al., 1973; Robinson et al., 1975).

f. Miscellaneous

Most androgens have anabolic or myotrophic activity which is not necessarily related to their androgenic potency. Administration of testosterone to a castrate leads to nitrogen retention and muscular growth (Kochakian, 1976). The growth of horns in sheep (Parkes, 1966) and the change from 'velvet' to 'hard horn' antlers in deer (Lincoln et al., 1970) also are androgen dependent. Boar-taint in entire male pigs is caused by circulating androgenic compounds (Gower, 1972; Andresen, 1976), while odour glands in male goats (Parkes, 1966) are testosterone dependent.

## 4. Negative Feedback Control of the Hypothalamo-pituitary-testicular Axis

As has already been described regarding the function of GnRH and the gonadotrophins, the elements of the hypothalamo-hypophyseal-testicular axis act in an hierarchial arrangement in which the hypothalamus induces gonadotrophin secretion

and this in turn induces testicular endocrine secretion. Conversely to these trophic effects, the secretions of the hypothalamus and anterior pituitary are generally negatively inhibited by secretions from lower within this hierarchy. 'Long loop' feedback effects involve the inhibition of the hypothalamus and pituitary by testicular products, while 'short loop' effects include the negative feedback of anterior pituitary hormones on the hypothalamus. 'Ultra-short loop' mechanisms involve autoregulation of GnRH secretion by the GnRH itself.

(a) Testicular Hormones

Testicular hormones exert long loop negative feedback effects on the hypothalamus and pituitary to regulate gonadotrophin secretion (Barraclough, 1973). Direct evidence in favour of this concept comes from the work of Sar and Stumpf (1973) and Davies et al. (1976), who demonstrated steroid binding receptors in the pituitary and hypothalamus, respectively. Further it has been shown that testicular atrophy follows implantation of testosterone into the ME, while similar implants of the anti-androgen, cyproterone-acetate, causes enlargement of accessory sex organs and increases in testicular weights (Smith and Davidson, 1967; Davidson and Bloch, 1969). At the hypothalamic level testosterone appears to inhibit GnRH and then LH secretion, by at least two mechanisms: an oestrogenic effect after aromatisation (Naftolin et al., 1975) and a direct androgenic effect, due to testosterone or its metabolites (McCann, 1974). On the other hand testosterone's inhibitory effect at the pituitary level appears to be due solely to androgenic effects (Bogdanove et al., 1975).

Androgen replacement experiments in wethers (Pelletier, 1970; Crim and Geschwind, 1972a) and entire ram lambs (Schanbacher, 1980b) have shown that there was a biphasic decrease in plasma LH concentrations. The first decrease, which occurred about 12 h following testosterone replacement, has been attributed to a decrease in pituitary sensitivity to GnRH, while the second decline at about 72 h may

have been due to inhibition of GnRH synthesis (Pelletier, 1970; 1974).

Although the effects of testosterone on LH secretion are quite marked, its effects on FSH secretion are not yet well understood. Nevertheless current research suggests that testosterone has some influence on FSH secretion (Crim and Geschwind, 1972a; Main et al., 1978; Schanbacher, 1979b; 1980b), while another testicular hormone probably is more important for specific regulation of FSH. McCullagh (1932) termed this hormone inhibin.

Strong evidence in favour of differential feedback control of LH and FSH secretion comes from numerous pathological conditions in humans like germinal cell aplasia, cryptorchidism and testicular feminising syndrome (Lipsett, 1976). Further evidence in favour of differential feedback control is provided by results of such experimental treatments as GnRH administration (Hopkinson et al., 1974; Lincoln, 1978; 1979a) hemicastration (Walton et al., 1978; 1980; Leidl et al., 1980) and cryptorchidism (Blanc and Terqui, 1976; Schanbacher and Ford, 1977; Lee et al., 1978); usually plasma levels of LH and FSH change independently of one another.

The possible existence of a testicular hormone, inhibin, and evidence for its physiological role has been the subject of much recent research which has been reviewed by several authors (Setchell and Main, 1974; Franchimont et al., 1979; Blanc, 1980). Inhibin has been defined as a peptide factor of gonadal origin which specifically or selectively lowers the rate of secretion of FSH (Franchimont et al., 1979). Some of its physicochemical properties have been established; thus inhibin is said to be water soluble and destroyed by proteolytic enzymes and heat, but estimates of its molecular weight have varied from 5000 to more than 160,000 (Blanc, 1980). Inhibin has also been partially purified from human seminal fluid, bovine seminal fluid, ram rete testis fluid, extracts of spermatozoa and testes, and Sertoli cell culture media (Steinberger, 1979). Experiments

by Steinberger and Steinberger (1976) and Steinberger (1979) showed unequivocally that Sertoli cells were directly involved in the synthesis of inhibin in the male.

The sites at which inhibin exerts its negative feedback effects on FSH secretion have not been defined with certainty, however present evidence tends to favour the pituitary as the most likely site (Franchimont et al., 1979). For example in culture experiments pituitary gonadotrophs co-cultured with Sertoli cells released significantly less FSH than pituitary cells grown either alone or cultured with spleen or kidney cells (Steinberger and Steinberger, 1976; Franchimont et al., 1979). These authors suggested that inhibin may well have inhibitory effects on synthesis as well as release of FSH from pituitary gonadotrophs. On the other hand evidence in favour of the hypothalamus as a site of action of inhibin comes from in vitro studies in which incubation of hypothalami with inhibin significantly reduced amounts of immunoreactive GnRH (Franchimont et al., 1979).

Little work has been done on the topic of interactions between sex steroids and inhibin in the male. This is an important area of research since inhibin does not appear to be the sole regulator of FSH secretion (Main et al., 1978; Franchimont et al., 1979). Whether or not inhibin has any effect on LH secretion is another area which needs further investigation. Nevertheless, Blanc (1980) reviewing the research on this topic, suggested that inhibin may act on FSH and eventually on LH secretion in a dose-dependent manner (dualist theory), or that it may act on both FSH and LH secretions with different kinetics (unicist theory).

(b) Pituitary Hormones

A large amount of evidence has accumulated in support of the concept that gonadotrophins and prolactin regulate the secretion of their own respective releasing and inhibiting hormones via 'short' feedback loops or mechanisms (Motta et al., 1969; Robyn et al., 1977). For example, implants of LH, FSH and prolactin in the ME caused specific reductions

in pituitary and plasma levels of these hormones (Neill, 1974; McCann, 1974; Horrobin, 1974). Further evidence for short loop inhibition of FSH secretion was supplied by autoradiographic studies in rats injected with $I^{125}$-labelled FSH; maximal accumulation of radioactivity was seen in the ME and arcuate nucleus of the hypothalamus (Davies et al., 1975).

However, the relative importance of such feedback systems has often been questioned in view of the large increases in secretion of both LH and FSH which occur following castration (McCann, 1974).

(c) Hypothalamic Hormones

GnRH has been suggested as a possible regulator of its own secretion by an 'ultra-short' feedback system (Motta et al., 1973).

Micro-iontophoretic application of synthetic GnRH to the MBH area has been reported to decrease the electrical activity recorded from nuclei within this part of the hypothalamus (Kawakami and Sakuma, 1974). Even if ultra-short feedback regulation of GnRH secretion occurred, Jeffcoate (1975) considered it unlikely to be mediated via the systemic circulation. It has been suggested by Dyer and Dyball (1974) that the effect may be transynaptic: the authors suggested that GnRH may act as a neurotransmitter, being released from axon branches, not only terminating near portal vessels, but also synapsing on other hypothalamic neurones. Since there is evidence to show that cerebrospinal fluid (CSF) contains high levels of GnRH (Anand Kumar, 1973), tanycytes lining the third ventricle take up $^{3}H$-labelled GnRH (Vaala and Knigge, 1974), and these cells link the CSF and arcuate nucleus (Bleier, 1972), it has been concluded that GnRH can influence its own secretion.

There are no reports to date of any ultra-short feedback mechanism regulating the secretion of prolactin inhibiting or releasing factors.

## 5. Patterns of Hormone Secretion

Pituitary and gonadal hormones are secreted in a variety of patterns ranging from short term pulsatile or episodic releases, to twenty four hour circardian patterns and much more prolonged seasonal trends. The physiological rationale for episodic (pulsatile) output of hormones is not understood. As suggested by Oately (1971), pulsatile release of hormones demonstrates the dynamic nature of neuroendocrine systems and highlights the lack of known quantitative laws which describe such activities within an organism. However, Gallo (1980), reviewing the neuroendocrine mechanisms regulating pulsatile hormone secretion, concluded that this pattern of hormone secretion helps minimize refractoriness of target organs under physiological conditions.

### (a) Pulsatile Hormone Secretion

#### (i) LH

Pulsatile secretion of LH has been demonstrated to occur in ram lambs (Foster et al., 1978; Wilson and Lapwood, 1979a), adult rams (Sanford et al., 1974; Barrell and Lapwood, 1978; Wilson and Lapwood, 1978a), wethers (Riggs and Malven, 1974) and boars (Sanford et al., 1976b; Lapwood and FlorCruz, 1978), as well as in males of several other species. Generally it is assumed that episodic LH secretion is induced by pulsatile release of GnRH from the hypothalamus. However, due to the large degree of haemodilution that occurs between pituitary portal blood and the systemic circulation, it often has been difficult to observe a close relationship between peripheral plasma GnRH and LH concentrations (Gallo, 1980). Although this pattern of GnRH release in rams and boars has not been verified, Crighton et al., (1973) recorded a pulsatile pattern in GnRH levels in the jugular blood from ewes. Likewise GnRH levels in pituitary blood of Rhesus monkeys show pulsatile fluctuations (Carmel et al., 1976).

#### (ii) FSH

In sheep plasma FSH levels have generally been reported

to be relatively constant throughout intensive sampling periods, with only minor fluctuations in comparison to those recorded for plasma LH levels (Sanford et al., 1976a; Lee et al., 1976a). There has been only one report on plasma FSH levels of boars (Colenbrander et al., 1980; and that only in abstract form); whether or not plasma FSH levels showed episodic fluctuations was not mentioned. Details of factors responsible for this differential release of LH and FSH are not known. Assuming that only one hypothalamic hormone (GnRH) is responsible for the release of gonadotrophins, Lincoln (1978; 1979a) suggested that this effect was the result of separate testicular negative feedback controls over LH and FSH secretion. The different biological half lives of FSH and LH (103 minutes as compared to 43 minutes, Akbar et al., 1974), and inherent differences in secretory capacities of pituitary gonadotrophs, may be the other contributory factors.

(iii) Prolactin

Plasma prolactin levels recorded from rams also have been found to fluctuate over short periods when intensive sampling regimes were used (Chamley et al., 1974; Barrell and Lapwood, 1978; Davis et al., 1978; Wilson and Lapwood, 1979a). However, there have been no similar reports on plasma prolactin levels of boars.

(iv) Testosterone

Episodic fluctuations in plasma testosterone levels have been recorded in many species including rams (Barrell and Lapwood, 1978; Wilson and Lapwood, 1979a) and boars (Sanford et al., 1976b; Lapwood and FlorCruz, 1978; Kattesh et al., 1979). The temporal association between LH and testosterone secretory peaks will be discussed under hormonal inter-relationships (on page 34).

(b) Circadian Rhythms

A circadian or "about 24 hours" rhythm implies a consistent pattern from day to day (Boon et al., 1972). Although the significance of circadian rhythmicity in

hormone secretion is not clear, it may well be related to changes in reproductive states such as puberty or seasonality. For example it has been recorded that a sleep-related increase in episodic LH secretion in children is a characteristic of the pubertal period (Weitzman et al., 1975).

On the other hand probably the most clear indications of circadian hormone secretion patterns recorded from rams have been those reported by Lincoln and colleagues (1977; 1979b), who utilised exaggerated changes in artificial photoperiods and animals of the very photosensitive Soay breed. These workers were able to show circadian changes in plasma levels of LH, FSH and testosterone. However, in studies with rams of domestic breeds other workers generally have been unable to detect any circadian rhythm in LH, testosterone (Katangole et al., 1974; Purvis et al., 1974; Sanford et al., 1974; Barrell and Lapwood, 1978; Wilson and Lapwood, 1978a) or FSH (Sanford et al., 1976b) secretion. On the other hand there are some reports of nocturnally elevated plasma prolactin levels in sheep (Davis and Borger, 1973b; Ravault and Ortavant, 1977; Barrell and Lapwood, 1978; Lincoln et al., 1978; Brown and Forbes, 1980), while other workers have not found any such change (Chamley et al., 1974; Wilson and Lapwood, 1978a; Fisher and Lapwood, 1981).

Twenty four hour hormone profile studies with boars of domestic breeds have not shown any indication of circadian patterns in secretion of either LH or testosterone (Sanford et al., 1976b; Lapwood and FlorCruz, 1978). However, Ellendorff et al. (1975) sampled sexually mature Göttingen miniature boars and recorded significantly higher testosterone levels at 08.00 hr as compared to those recorded between 19.00-21.00 hr, but did not observe a comparable pattern in plasma LH levels. No similar data has been published regarding plasma FSH or prolactin levels of either domestic or Göttingen miniature boars.

(c) Seasonal

In sheep, short-term fluctuations in hormone concentrations are superimposed on seasonal variations and there have been many reports on seasonal variations of reproductive hormone secretion in rams during the last few years.

Changes in the length of the daily photoperiod are regarded as the major environmental factor involved in control of the seasonality of reproduction in animals (Marshall, 1937; Ortavant et al., 1964). Photoperiod not only affects the incidence of oestrous activity in ewes, but also libido in rams. Pelletier and Ortavant (1975), subjecting rams to 6 month duration 'annual' light rhythms, reported that patterns of plasma LH levels were related to light photoperiod; increasing photoperiod was found to be inhibitory or less stimulatory, while decreasing photoperiod was stimulatory. These changes were similar to those reported previously as occurring at the pituitary level (Pelletier and Ortavant, 1964). Sanford et al. (1978) utilized a similar experiment to demonstrate that plasma LH, FSH and testosterone secretion underwent similar changes with photoperiod. Similar results have been reported from rams reared under natural environmental conditions (Katongole et al., 1974; Sanford et al., 1974; Barrell and Lapwood, 1979a) and also in rams exposed to artificial lighting regimes corresponding to the natural daily photoperiod (Barrell and Lapwood, 1979b).

Katongole et al. (1974) suggested that changes in the frequency, amplitude and duration of pulsatile releases of LH, FSH and testosterone may be important parameters of reproductive seasonality in rams. This led to the suggestion that the hypothalamus regulates the seasonal changes in pituitary gonadotrophin secretion, and hence testicular secretion by changing the frequency of episodic GnRH release. Lincoln and Short (1980) reviewed this concept and concluded that changes occur both in frequency and magnitude of GnRH release.

On the other hand plasma prolactin levels exhibit the

most marked cycle of seasonal changes recorded for reproductive hormones in rams, including lambs. Length of the daily photoperiod is positively correlated with prolactin levels monitored in blood, thus peak and lowest levels are recorded in summer and winter, respectively (Pelletier, 1973; Ravault, 1976; Ravault et al., 1977; Fisher, 1978; Wilson and Lapwood, 1978a; 1979a; Barrell and Lapwood, 1979a).

There apparently have been no reports published on seasonality of hormone secretion in boars.

(d) Stress-induced Changes in Hormone Secretory Patterns

It is generally accepted that stressful stimuli can induce elevations of plasma prolactin levels in sheep (Davis, 1972; Wilson and Lapwood, 1979a), cattle (Raud et al., 1971) and probably other species. In contrast most researchers consider that gonadotrophin levels are not influenced by stress. For example, Roche et al. (1970) claimed that various sampling methods had no effect on LH concentrations in ewes. However, Sitarz et al. (1977) reported that handling and venepuncture sampling procedures induced LH elevations in young bulls. Also Wilson and Lapwood (1979a) detected elevated levels of LH in the first of four half-hourly plasma samples collected in profile studies with ram lambs. However, they concluded that for LH, the stress effects on plasma levels probably were induced by shepherding with dogs just before sampling commenced and effectively could be eliminated by multiple bleeding procedures extending over several hours. It also has been demonstrated that stressful handling procedures could cause lowering of plasma LH levels in boars, resulting in a reduction of testicular steroidogenesis (Liptrap and Raeside; 1968; Andresen, 1975).

In addition heat stress has been shown to cause an elevation of plasma prolactin levels in sheep (Hooley et al., 1979), while exposure of boars to elevated ambient temperatures was reported to cause disruption in spermatogenesis and decline in plasma testosterone levels (Wettemann and Desjardins, 1979).

(e) Interrelationships in Patterns of Hormone Secretion

Close relationships often exist between the secretory patterns recorded for hormones of the hypothalamo-pituitary-testicular axis.

(i) GnRH, LH and FSH

GnRH administration causes an immediate dose-related LH response from the pituitary (Schally et al., 1972; Wilson and Lapwood, 1978b; 1979b). However, the FSH response following GnRH administration is rather slow and of reduced intensity (Hopkinson et al., 1974; Lincoln, 1978; 1979a). Attempts to demonstrate a relationship between peripheral plasma levels of GnRH and subsequent LH elevations at oestrous in the ewe failed, possibly due to insensitivity of GnRH assay utilised in that experiment (Nett et al., 1974).

(ii) LH and testosterone

The trophic effects of LH on testosterone output are well established, but qualitative and quantitative relations between the two are not always observed. Katongole et al. (1971) observed that in bulls LH peaks were followed by testosterone peaks about 40 minutes later, but Smith et al. (1973) observed that only 64% of LH elevations (above 1 ng/ml) resulted in elevations of testosterone levels in the same species and the time interval between peaks approximated one hour.

Similar associations between LH and testosterone have been detected in rams (Sanford et al., 1974; Wilson and Lapwood, 1978a; 1979a) and boars (Sanford et al., 1976b; Lapwood and FlorCruz, 1978).

Occasional releases of testosterone, unassociated with LH peaks, have been reported in several species, for example in rams (Schanbacher and Ford, 1976; Wilson and Lapwood, 1978a), bulls (Smith et al., 1973; Welsh et al., 1979) and boars (Lapwood and FlorCruz, 1978). Furthermore, Wilson and Lapwood (1979a) observed no consistent relationship between LH and testosterone peaks in 6 weeks old ram lambs, while a 1:1 relationship between the two hormones was recorded from 14 weeks onwards.

(iii) Gonadotrophins, corticosteroids and prolactin

Welsh et al. (1979) examined interrelationships between peripheral blood concentrations of corticosteroids, LH and testosterone concentrations in bulls. They concluded that alterations in endogenous concentrations of corticosteroids influenced the peripheral concentrations of LH and testosterone and also that adrenal dysfunction could significantly influence testicular function in the bull. However, no relationships between plasma cortisol and prolactin levels were observed in dairy cattle (Fulkerson et al., 1980).

## 6. Physiology of Reproductive Development in Males

Puberty in males commonly is defined as the time at which reproduction first becomes possible, that is when spermatozoa are first released (Asdell, 1964). Male puberty may also be defined as a phase in development when the endocrine functions of the testes become clearly evident, and is terminated with the appearance of spermatozoa in the seminiferous tubules (Donovan and van der Werff ten Bosch, 1965). The latter definition was also ascribed to by Skinner and Rowson (1968a) and Skinner et al. (1968). However, attainment of puberty does not imply acquisition of full reproductive capacity: such capacity is reached only at sexual maturity, which occurs at a later stage in development. In addition, whereas puberty in female animals is marked by the occurrence of the first behavioural oestrus, there is no such obvious manifestation of puberty in males.

### (a) Development of Gonads in Rams and Boars

#### (i) Prenatal

Primordial germ cells, presumably derived from yolk sac endoderm, are present in sheep gonads as early as the 29 th day after conception and histological evidence of the differentiation of gonads to testes first becomes apparent at 34 to 35 days, with the appearance of the tunica albuginea (Sapsford, 1962). In boar foetuses formation of the tunica albuginea is identifiable at around the 27 th

day of gestation (Pelliniemi, 1975; Pelliniemi et al., 1979).

Development of androgenic secretory capacity by foetal testes has been described in rams (Attal, 1969) and boars (Moon and Raeside, 1972; Raeside and Sigman, 1975; Ford et al., 1980) to occur at around 30 days, well before gonadal duct differentiation, which is evident by the 40 th day of gestation in sheep (Sapsford, 1962) and around the 1.5 cm crown-rump stage in male pig foetuses (Raeside and Sigman, 1975).

By the 42 nd day of development of male sheep foetuses testis cords containing gonocytes and supporting cells (indifferent cells) are formed, while interstitial cells are prominent between the cords. At this stage gonocytes are located centrally within the testis cords and are surrounded by indifferent cells (Sapsford, 1962). Glandular interstitial cells become obvious shortly after sexual differentiation of the gonads and are quite prominent by the 42 nd day of gestation. From 56 days up to 70 days of gestation the interstitial cells increase in number (Sapsford, 1962). The histological changes which occur in male pig foetuses are very similar to those mentioned for sheep and Leydig cells become recognisable histologically around the 30 th day of gestation (Pelliniemi et al., 1979).

(ii) Postnatal

The testes increase in weight and volume approximately in parallel with the postnatal increase in body weight. Such increases in weight have been reported for rams (Watson et al., 1956; Steffert, 1971) and boars (Hausser et al., 1952; FlorCruz and Lapwood, 1978; Esbenshade et al., 1979) among other species. The consistency of the testes becomes harder with increasing age, levelling off and becoming softer in the adult animal due to fat deposition. These changes have been demonstrated in bulls (Coulter et al., 1975; 1977) as well as boars (Esbenshade et al., 1979) and the authors suggested using testicular consistency as an index of the different stages of puberty in the above species.

Histologically the seminiferous tubules of neonatal testes appear as solid cords in which only two types of cells are distinguishable (Setchell, 1978). These are the supporting cells, which undergo transformation to Sertoli cells later in life, and the prospermatogonia, which give rise to the spermatogenic cells. Histological structures of this type have been described both for rams (Sapsford, 1962; Skinner and Rowson, 1968a; Skinner, 1971a; Steffert, 1971; Wilson, 1977; Lee et al., 1981) and boars (Philips and Andrews, 1936; Hauser et al., 1952; McFee and Eblen, 1967). Germ cells are at first more centrally placed within the sex cords than are supporting cells. However, before spermatogenesis begins they migrate towards the basement membrane of the seminiferous tubules (Sapsford, 1962).

The interstitial cells of Leydig are the source of testicular androgens (Gomes, 1970). In rams clusters of Leydig cells are found scattered at random in abundant, loose, peritubular connective tissue, while in boars the interstitial space is almost filled with large quantities of closely packed Leydig cells (Setchell, 1978). Using histologic, histomorphometric and histochemical methods, van Straaten and Wensing (1978) demonstrated that Leydig cells from pig testes show two developmental and activity phases: one perinatally and the other from 13 weeks postpartum onwards. However, no such striking changes in relative numbers of glandular interstitial cells were observed at different stages of development in testes of rams (Sapsford, 1962).

(iii) Pre-pubertal and pubertal

Pre-pubertal development of the seminiferous tubules is marked by cell divisions and differentiation which coincide with the rapid increase in testis weights (Setchell, 1978). Both the length and diameter of seminiferous tubules increase with age. In Romney ram lambs a maximal seminiferous tubular diameter of 196.71 μm was recorded at 32 weeks of age by Wilson (1977), while in Large White x

Landrace boars a maximal diameter of 213.00 μm was reported by FlorCruz and Lapwood (1978) to occur at 216 days.

During prepubertal development gonocytes are transformed into prospermatogonia, the number of nucleoli is reduced to one and nuclear dimensions increase (Sapsford, 1962; Setchell, 1978). Prospermatogonia move to the basement membrane, transform into type A spermatogonia and then start multiplying rapidly, both by mitotic and meiotic divisions, giving rise to spermatocytes and spermatids, respectively (Courot et al., 1970; Setchell, 1978).

Detailed histological studies involving serial castration experiments with Romney ram lambs (Steffert, 1971; Wilson, 1977) have indicated that primary spermatocytes first appear in seminiferous tubules at around 14 weeks of age, while round spermatids and spermatozoa were identified by 18 weeks. Mature spermatozoa within the epididymal tubules were recognised at 22 weeks. However, it is clear that the timing of first appearances of these cell types is breed-dependent, for example spermatocytes and spermatozoa do not appear in testes of Merino/Corriedale ram lambs until between 31 and 36 weeks of age (Lee et al., 1976b; Lee et al., 1981). The ages at which various cell types of the spermatogenic cycle appear in seminiferous tubules of ram lambs of different breeds have been summarised by Dýrmundsson (1973b).

Similar between breed differences also have been reported to occur in boars. For example, Andrews and Warnick (1949) identified mature sperm in seminiferous tubules of Duroc boars at 110 days of age, while in White King and Land Roc boars mature sperm appeared only at 130 days. Similarly Hauser et al. (1952) found differences between Poland China and Hampshire boars.

As far as Landrace x Large White boars are concerned, FlorCruz and Lapwood (1978) used serial castration and qualitative histological methods to characterise the course of testicular development. In 43 day old boars only two types of cells were evident, prospermatogonia and supporting cells, but at 90 days of age spermatogonia and occasional primary spermatocytes were seen and the

supporting cells had started to assume adult Sertoli cell morphology. By 127 days a few elongated spermatids were identified, while spermatozoa were abundant at 146 days. The authors concluded that puberty had occurred in boars of that crossbreed at between 127 and 146 days of age.

General histological features of the spermatogenic process are outside the scope of this review, but have been reviewed extensively (Roosen-Runge, 1962; Courot et al., 1970; Clermont, 1972; Setchell, 1978). Specific cytological details of the course of spermatogenesis in rams and boars have been described by Courot et al. (1970) and Swierstra (1968), respectively, while the duration of the cycle of the seminiferous tubule is 14 to 15 days in rams (Courot et al., 1970) and 8 to 9 days for boars (Swierstra, 1968).

Two major structural changes occur prior to initiation of spermatogenesis in mammalian testes. These are: (a) formation of the blood testis barrier and (b) formation of the central lumina in the seminiferous tubules (Setchell, 1978). The blood testis barrier of rodents includes myoid cells surrounding the seminiferous tubules, but in primates it is built solely on tight Sertoli cell junctions (Fawcett, 1977).

The central lumina of the seminiferous tubules first appear at between 12 and 14 weeks of age in Romney ram lambs (Wilson, 1977) and approximately 13 weeks in Large White x Landrace boars (FlorCruz and Lapwood, 1978).

Except in extremely seasonal breeding species the spermatogenic process is uninterrupted after puberty. This continuity is provided by the cyclic renewal of stem (type A) spermatogonia all through the animal's life: each of them giving rise to further stem spermatogenic cells. In sheep, breed differences in the time of first appearance of spermatozoa in ejaculates have been summarized by Skinner and Rowson (1968a); these ages range from 103 days in Suffolk crossbreds to 213 days in Merinos. No such summary appears to have been prepared for boars.

Semen can be collected from the time of puberty onwards and the sperm output increases with age until sexual maturity. However, in domestic mammals the semen produced at puberty is of poor quality as motility is low and abnormal spermatozoa are more numerous than in ejaculates of normal adults (Courot, 1978).

(b) Development of Other Organs of Reproduction in Males

(i) Prenatal

Transformation of the indifferent urogenital tract to the male type is determined by secretions of the foetal testes. Foetal and neonatal castration studies have demonstrated that normal phenotypic development is programmed towards the female type, and male structural formation only occurs as a consequence of such secretions by the foetal testes (Jost, 1976).

Androgens, secreted by the Leydig cells, and/or anti-Müllerian hormone (AMH) synthesised by the Sertoli cells during foetal life, cause regression of potentially female duct system structures (Müllerian) and facilitates development of male precursors of the Wolffian duct system (Josso et al., 1977; 1980). This sexual duct differentiation occurs by the 40 th day of foetal life in sheep (Sapsford, 1962), while in boars it is completed by the 1.5 cm foetal crown-rump stage (Moon and Raeside, 1972). AMH is a glycoprotein secreted by Sertoli cells during foetal life and has a molecular weight of 124,000 (Josso et al., 1980).

(ii) Postnatal

a. Epididymis

Studies with rams (Watson et al., 1956; Skinner et al., 1968; Steffert, 1971) and boars (Hausser et al., 1952; Niwa and Mizuho, 1954) have demonstrated that epididymal weight increases during pubertal development are more closely related to testicular weights than to body weights or age. The major histological changes that occur in the epididymides are found to be similar in most species; they include an increase in epididymal tubular diameters and differentiation

of the epithelium from a simple columnar type to pseudo-stratified, with concurrent increases in epithelial height and the development of steriocilia on their luminal borders (Asdell, 1964; Setchell, 1978).

b. Accessory sex glands

The accessory sex glands include the prostate, seminal vesicles and the bulbourethral glands. Growth of these organs is more highly correlated with testicular weight than with body weight (Asdell, 1964). Histologically these glands have the same appearance in young animals as in adults, but they undergo changes in weight, secretory epithelial height and secretory capacity as the animal approaches puberty. In rams it has been reported that the citric acid and fructose contents of the seminal vesicular secretions increase with approaching puberty and are highly correlated with testicular androgen production (Skinner and Rowson, 1968a; Skinner et al., 1968). In hypophysectomised adult boars Morat et al. (1980) demonstrated that the secretory capacity of these organs also was androgen dependent.

c. External genitalia

Both the penis and scrotum exhibit rapid growth during puberty and their development is completed by the time of puberty in rams (Dun, 1955; Watson et al., 1956; Skinner and Rowson, 1968a) and boars (Cheng et al., 1964).

Separation of adhesions between the prepuce, penis and urethral process is another change which occurs during this period. The adhesions first disappear from the urethral process of rams at approximately 15 weeks of age (Johnstone, 1948; Skinner, 1971a) and finally from the penile shaft at around 20 weeks (Wiggins and Terrill, 1953; Dun, 1955; Skinner and Rowson, 1968a). Breakdown of adhesions is androgen-dependent since separation is not complete in wethers even at one year of age (Johnstone, 1948). On the other hand completion of separation between the penis and the penile part of the prepuce occurs in boars at approximately 5 months of age (Cheng et al., 1964), while penile growth is most rapid at 7-8 months (Niwa and Mizuho, 1954).

(iii) Factors affecting puberty in males

Factors affecting sexual development in ewe and ram lambs have been reviewed by Dýrmundsson (1973a; 1973b). Although the ontogeny of the process is largely determined genetically, the normal rate of maturation can be modified by environmental influences (Donovan and van der Werff ten Bosch, 1965; Land, 1978). Environmental factors which influence reproductive development are: daily photoperiod, temperature, stress, nutrition and social and sexual stimuli.

a. Genetic factors

Information on the influence of genetic factors in influencing reproductive development in rams and boars has been obtained from studies on pure breeds and their crosses. Some examples illustrate this point. Ram lambs of the Finnish Landrace breed mature more rapidly than do Merinos, while Southdown ram lambs mature a few weeks earlier than do Hampshire rams (Carmon and Green, 1952; Terrill, 1975). Similarly boars of the cross between Kirin and Berkshire breeds mature earlier than do those of the pure breeds (Cheng et al., 1964). Finally, Duroc boars attain puberty earlier than White-King or Land-Roc breeds (Andrews and Warnick, 1949).

b. Effects of 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, but not male rodents (Donovan and van der Werff ten Bosch, 1965). In ram lambs Skinner and Rowson (1968a) observed a delay in reproductive development in lambs born in late summer compared to those born in spring and suggested that seasonal factors such as environmental daylight (photoperiod) influenced sexual development.

Advancement of puberty, by exposing developing boars to extended daily lighting (15 hours/day), during naturally occurring short days was demonstrated by Mahone

et al. (1979). This concept was confirmed by Berger et al. (1980) who reported that extended photoperiods had a beneficial effect on age at initiation of behavioural puberty as measured by libido scores and percentage of animals from which semen could be collected.

Although a decline in fertility has been observed in adult rams (Van Demark and Free, 1970) and boars (Wettemann et al., 1979) following exposure to high temperatures, there apparently is no evidence to illustrate any direct effect of high temperatures on pubertal development in male domestic animals. Age at maturation and spermatogenic potential of boars does not differ much in the tropics as compared with temperate zones (Egbunike and Steinbach, 1972).

c. Nutrition

Reproductive capacity of adult male animals is affected by level of nutrition (Allden, 1970; Leathem, 1970). In addition very low energy intake delays the onset of sexual maturity (Moustgaard, 1969). This has been confirmed both in rams (Watson et al., 1956) and boars (Burger, 1952; Dutt and Barnhart, 1959). Studies of sexual development of ram lambs under controlled feeding conditions have demonstrated that those reared on higher planes of nutrition normally attain puberty at lower ages and heavier body weights than do rams on lower levels of feeding (Marincowitz, 1966). Under conditions of inadequate or poor nutrition, the androgenic function of the testes is retarded more markedly than is spermatogenesis (Skinner and Rowson, 1968a). Mann (1964) suggested that this effect was most likely due to lack of gonadotrophins from the pituitary, rather than inability of testes to produce androgens. However, from research on heifers reared under conditions of restricted energy intake Gombe and Hansel (1973) demonstrated that gonadal hypofunction was more evident than pituitary hypofunction.

d. Social and sexual stimuli

The effects of social environment and sexual stimuli

on reproductive development have been studied extensively in females (Parkes and Bruce, 1961; Whitten, 1966), but less so in males. Rearing ram lambs in different social environments such as: isolation, all male groups, and mixed sex groups, did not have any effect on plasma testosterone levels or maturational processes such as sexual behaviour (Illius et al. 1976a). This concept was further confirmed by research on effects of ewe proximity on plasma testosterone levels (Illius et al., 1976b); no significant alterations in plasma hormone levels were detected.

Similar results have been recorded from developing boars reared in different social conditions: isolation, all male groups, and mixed sex groups, from 3 to 30 weeks of age. Although the results did not show any significant effects of the treatments on physical or sexual development, or age at puberty, the behavioural patterns exhibited at courting and copulation were altered in animals reared in isolation (Hemsworth et al., 1977). The establishment of social hierarchies amongst boars (Fraser, 1974) has been suggested as a possible factor which influences the androgenic capacity of the testes, and subordinate boars in the group have lower plasma levels of pheromonal steroids and delayed onset of testicular steroidogenesis (Andresen, 1976). Exposure to boars during development also hastens the pubertal process in gilts (Mavrogenis and Robinson, 1976; Thompson and Savage, 1978), but no such data is available for boars.

## 7. Prepubertal Neuroendocrine Mechanisms

### (a) Foetal

#### (i) Rams

Foetal pituitary tissue contains an increasing amount of bioactive LH over the final 30 days of intrauterine life (Foster et al., 1972c). However despite these rising concentrations, plasma LH levels decrease late in gestation (Foster et al., 1972b). A similar pattern also was observed

for foetal plasma FSH levels (Gluckman et al., 1980), but no data appears to be available regarding pituitary levels of that hormone. The foetal pituitary is able to respond to GnRH by the 80 th day of gestation, and from that time onwards the magnitude of gonadotrophin responses to releasing hormone parallels the changes in plasma gonadotrophin levels (Foster et al., 1972b; Gluckman et al., 1980).

On the other hand Attal (1969) reported that foetal testicular androgen secretion reached peak levels around the 70 th day of gestation.

Lactotrophs were first identified in the ovine foetal pituitary by electron microscopy at about the 75 th day of gestation (Alexander et al., 1973), but with immunofluorescence techniques they were not detectable until around the 80 th day (Stokes and Boda, 1968). Plasma prolactin levels reached detectable levels by the 87 th day of gestation and remained low up till about the last month of pregnancy (Mueller et al., 1979). The same authors also recorded elevated plasma prolactin levels towards the end of gestation, but did not observe any difference in plasma concentrations between male and female foetuses.

(ii) Boars

Colenbrander et al. (1977) measured serum LH concentrations in male pig foetuses and found that they were very low up to the second trimester of pregnancy, then increased gradually up to the second week of postnatal life. However, there have been no reports on foetal pituitary responses to GnRH administration, foetal hypothalamic GnRH content, nor plasma FSH or prolactin levels in boar foetuses.

By way of contrast serum testosterone concentrations in male pig foetuses are elevated from day 30 of gestation to day 35, then decrease to low levels by the time of birth (Colenbrander et al., 1978; Ford et al., 1980).

The hormonal data for ram and boar foetuses clearly indicate that foetal testes in these species are capable of secreting testosterone during the period of sexual

differentiation. The probable roles of testicular androgens in sexual differentiation are dealt with more fully on pages 36 and 40.

## 8. Postnatal and Pubertal Endocrine Patterns in Rams and Boars

### (a) Rams

Details of hormonal secretory patterns in developing ram lambs have been reviewed by Wilson (1977), while information on factors affecting pubertal development in ram lambs have been reviewed by Dýrmundsson (1973b). In the following section current knowledge of the endocrine changes occurring in ram lambs from birth through puberty will be summarized.

#### (i) LH

Mean plasma LH levels during the first few weeks after birth are lower than those recorded in the latter stages of intrauterine life (Foster et al., 1972c). Plasma LH concentrations show a steady rise from birth until approximately 70 days, whereupon they become variable, but remain elevated until about the 15 th week of life (Courot et al., 1975; Lee et al., 1976a; Wilson and Lapwood, 1979a; Lee et al., 1981). This general pattern has been recorded in ram lambs, born both in spring as well as autumn (Courot et al., 1975). Pituitary LH content also has been observed to rise sharply at between 20 and 80 days of age and remain high thereafter (Foster et al., 1972c; Courot et al., 1975).

As far as the characteristics of LH secretion are concerned, Foster et al. (1978) recorded pulsatile release of LH as early as two weeks of age and this observation was recently confirmed by Savoie et al. (1979).

#### (ii) FSH

Relatively constant plasma levels of FSH have been recorded from ram lambs aged between 30 and 150 days of age (Crim and Geschwind 1972a). However, Lee et al. (1976a; 1981) observed an increase in plasma FSH levels from birth to 5 weeks of age, with little change thereafter. By way of

contrast Savoie et al. (1979) did not observe any specific trend in plasma FSH levels between birth and 2 months of age.

Episodic fluctuations in plasma FSH levels have been observed as early as the second week after birth, although they were not as marked as those for LH (Savoie et al., 1979).

(iii) Prolactin

Plasma prolactin concentrations in ram lambs born in spring tended to be higher than those recorded from autumn born animals (Ravault et al., 1977). In postnatal life concentrations of this hormone generally follow photoperiod-induced seasonal patterns as discussed below.

Ravault and Courot (1975) studied plasma prolactin levels of autumn born ram lambs, from birth to 17 weeks of age. Except for a peak recorded at around 10-12 weeks of age, plasma concentrations generally varied in phase with length of daily photoperiod. That peak coincided with the time of onset of a rapid increase in testis weights and the onset of spermatogenesis. Subsequently Ravault (1976) reported a longitudinal study of plasma prolactin levels from birth to 3 years of age. He observed a similar peak at about 10 weeks after which plasma prolactin levels varied in phase with daily photoperiod. In a study with spring born Romney rams Wilson and Lapwood (1979a) found that plasma prolactin levels increased from birth to highest levels during the summer months, then fell to low concentrations by 8 months of age (winter).

Subsequent experiments in the French research programme on the role of prolactin in sexual maturation of ram lambs were reported by Ravault et al. (1977) and Barenton and Pelletier (1980). They utilised lambs born both in spring and autumn, as well as treatment with the prolactin inhibitor - 2Br-$\alpha$ ergocryptine (CB 154). Depressed prolactin secretion had no effect on LH or testosterone secretion, testicular growth, onset of spermatogenesis or development of testicular LH receptors, however, seminal vesicular growth and function was retarded.

Recent work by Howles et al. (1980) has added weight

to the concept that prolactin apparently does not play a critical role in testicular development in ram lambs. These authors utilised constant short (8 hr light) or long (16 hr light) daily photoperiods, and despite widely divergent plasma prolactin levels there were no differences in testicular weights. However, the same authors suggested that prolactin may be involved in mediating sexual behavioural responses to changes in photoperiod.

Episodic prolactin secretory patterns have been detected from the second week of postnatal life (Savoie et al., 1979).

(iv) Testicular steroids

In rams testosterone is the dominant androgen from birth (Skinner et al., 1968; Attal et al., 1972). Crim and Geschwind (1972b) found an age-related rise in spermatic vein testosterone levels up to 30 weeks, with highest values occurring once initiation of spermatogenesis was complete. However, Lee et al. (1976a) found that plasma testosterone levels peaked at 40 weeks of age, while Williams et al. (1976) recorded higher androgen levels in yearling rams than in adults. Wilson and Lapwood (1979a) observed that testosterone levels of Romney ram lambs increased with age from birth to 32 weeks.

Pulsatile testosterone secretion has been detected as early as the first week of life (Savoie et al., 1979). However, no consistent relationship between LH and testosterone pulses has been observed prior to 6 weeks of age (Savoie et al., 1979; Wilson and Lapwood, 1979a); only after this age are most LH peaks followed by testosterone secretory spikes.

(v) Responses to GnRH

Since Amoss and Guillemin (1969) demonstrated the release of LH in rams in response to injection of purified hypothalamic extracts of GnRH, the efficiency of this hormone in eliciting pituitary gonadotrophin release in sheep has been widely documented (Convey, 1973; Pelletier, 1976).

In apparently the first report on the use of GnRH in ram lambs Foster et al. (1972c) recorded higher LH responses in 8 week old rather than two week old animals. Subsequently Galloway and Pelletier (1974) recorded an increase in LH output with age from 1 to 20 weeks. Lee et al. (1976b) reported the results of intracarotid infusions of GnRH to ram lambs and observed that maximal pituitary LH output occurred at 8-12 weeks of age. In contrast Wilson and Lapwood (1979b) recorded maximal LH secretory responses in ram lambs at 6 weeks of age and lowest responses at 32 weeks. That decline in LH secretory responses corresponded well with the linear decrease in basal plasma LH levels observed in a parallel study (Wilson and Lapwood, 1979a).

Divergent plasma FSH and LH responses have been reported following GnRH administration to lamb (Lee et al., 1976b; Lee et al., 1981) and adult rams (Hopkinson et al., 1974; Pelletier, 1976; Lincoln, 1978; 1979a). FSH responses recorded from such experiments always were less pronounced, but more prolonged, than those for LH (Lee et al., 1976b; Lincoln, 1978; 1979a; Lee et al., 1981)

In contrast total testosterone responses to exogenous GnRH have been shown to increase with age (Lee et al., 1976b; Wilson and Lapwood, 1979b). This effect probably is due to a combination of an increase with age in the number of secreting Leydig cells (Savoie et al., 1979) and an increase in Leydig cell receptor sites, which are associated with sexual maturation (Odell and Swerdloff, 1976).

(b) Boars

Compared to the situation with rams, knowledge of the endocrine control of sexual maturation in boars, particularly of hypothalamo-pituitary-testicular relationships, is still limited.

(i) LH

Data on plasma LH levels during sexual maturation of boars were first published by Pomerantz et al. (1974) who used Göttingen miniature boars. They recorded resting LH

levels of 0.6 ng/ml in pubertal boars, as compared to 0.7 ng/ml in adult boars and 3.0 ng/ml in castrates. The same group of workers further characterized the patterns of change in plasma LH levels from birth to 12 weeks of age, but found no significant change in LH levels during this period (Elsaesser et al., 1976).

Ford and Schanbacher (1977) monitored plasma LH levels during the first 9 weeks of life in boars of domestic breeds and recorded elevated LH levels from birth to 3 weeks of age; these then declined to levels observed in adult boars. Colenbrander et al. (1977) recorded elevated serum LH levels during the first 3 weeks postpartum, which subsequently decreased with age. FlorCruz and Lapwood (1978) reported data for plasma LH levels in Landrace x Large White boars: relatively low levels, ranging from 0.20 to 1.25 ng/ml, were recorded from 41 days to 82 days of age, but a marked prepubertal elevation was seen between 110 and 152 days of age, with mean levels ranging between 1.35 and 2.19 ng/ml; plasma LH levels then declined and stabilised at between 0.5 and 1 ng/ml. In the same study the testicular histological data indicated that puberty had occurred between the ages of 90 and 146 days.

(ii) FSH

Only one paper appears to have been published regarding the plasma FSH levels of young boars (Colenbrander et al., 1980). High serum FSH levels ($51.43 \pm 12.6$ ng/ml) were recorded during the first 14 days postpartum; these subsequently declined to $4.5 \pm 0.27$ ng/ml between 56 and 84 days, then after that age remained relatively constant at approximately 7 ng/ml. The high perinatal serum FSH levels occurred at a time when LH levels also are high, when there are increases in Leydig cell secretion, and also marked increases in lengths of seminiferous tubules (Colenbrander et al., 1977; 1978).

(iii) Prolactin

No data appears to have been published on plasma prolactin levels of boars.

(iv) Testicular steroids

In comparison with most other species boar testes have an enormous amount of interstitial tissue. This tissue produces a broad spectrum of steroids in three different classes: androgens, Δ-16 steroids and oestrogens (Claus and Hoffman, 1980). However, testosterone is the main biologically active androgen secreted in adult (Linder, 1961; Elsaesser et al., 1972) and foetal boars (Raeside and Sigman, 1975; Colenbrander et al., 1978).

Spermatic vein plasma levels of testosterone in boars aged 3 to 9 months were measured by Carlson et al. (1971) and Gray et al. (1971) using thin layer chromatography techniques. Both groups recorded increased concentrations of testosterone towards puberty.

Although Ford and Schanbacher (1977) did not observe any increase in plasma testosterone levels of boars between birth and five weeks, Meussey-Dessolle (1976) found that concentrations of this hormone increased up to 157 days after birth. Conversely Colenbrander and colleagues (1978) monitored serum testosterone levels until 117 days postnatally and found that in the perinatal period concentrations rose to 1.30 ng/ml by the 3rd week, then only gradually until 18 weeks, when they reached 1.77 ng/ml.

In contrast to this FlorCruz and Lapwood (1978) recorded uniformly low plasma testosterone levels in boars between 41 and 82 days of age, then from 110 days the values increased progressively to a peak of 8.0 ng/ml when 138 days old. After a period of decline, during which the levels averaged 1.58 ng/ml, a second peak of 7.73 ng/ml was observed at the 194 th day. Later concentrations fluctuated between 1.40 and 3.8 ng/ml until 216 days. Although no satisfactory explanation could be given for the second testosterone peak, the first peak coincided with the prepubertal LH peak reported to occur between 110 and 152 days.

In addition to testosterone and androstenedione, a number of other androgens have been reported to be secreted by boar testes. These include musk smelling 16-unsaturated

$C_{19}$ steroids, which are known to serve as pheromones (Gower, 1972), and dehydro-epiandrosterone (Huis in't Veld et al., 1964; Booth, 1975).

The 16-unsaturated $C_{19}$ steroids are weak androgens (Gower, 1972), one of which has been identified to be 5α-androstenone, frequently detected in boar fat and suggested to be the causative agent of 'taint' in boar meat (Patterson, 1968; Andresen, 1976). The production of 5α-androstenone increases with age, and the source of origin has been confirmed to be the testes, since castration reduced 5α-androstenone levels (Andresen, 1976).

Beside the androgens, boar testes secrete large amounts of oestrogens (Claus and Hoffman, 1980). These authors also demonstrated that castration reduced plasma oestrogen to undetectable levels. The physiological role of testicular oestrogens is not clear, although Joshie and Raeside (1973) have suggested a synergistic effect of oestrogens and testosterone in maintaining the functions of the accessory sex glands. The main oestrogenic compound in plasma of boars is oestrone (Claus and Hoffman, 1980).

(v) Responses to GnRH injection

Plasma LH responses to GnRH infusion in pubertal, intact adult and castrated miniature boars have been reported by Pomerantz et al. (1974). The results indicated that in all animals the LH responses were dependent on the dose of GnRH used, but age had no effect. In contrast testosterone secretory responses following GnRH treatment increased with sexual maturation (Elsaesser et al., 1973).

## 9. The Onset and Control of Puberty

As discussed previously puberty is a phase in sexual maturation during which there is accelerated growth and development of the gonads and accessory reproductive structures, and during which mature spermatozoa are first produced in males.

The endocrine control of the onset of puberty has been the subject of much research and many reviews (Critchlow

and Bar-Sela, 1967; Ramirez, 1973; Grumbach et al., 1974; McCann, 1976; Ramaley, 1979). A brief summary of current concepts, based largely on research with male rats, is presented below.

When anterior pituitary tissue from immature donor rats is grafted into adult recipients it is capable of inducing the onset of normal oestrous cycles and maintaining pregnancy (Harris and Jacobsohn, 1952). These results offered strong evidence for the view that maturation of the pituitary was not the primary trigger for sexual maturation; rather anterior pituitary hormones were under hypothalamic control and maturation of that structure was the critical prelude to onset of puberty.

In experiments aimed at determining the feedback effects of gonadal steroids on secretion of gonadotrophins, ME implants of testosterone were shown to delay puberty in males (Smith and Davidson, 1967). However, the dose of testosterone necessary for suppression of gonadotrophin output pre-pubertally was significantly less than that required in adults (Smith and Davidson, 1967; Davidson and Bloch, 1969).

In the light of similar work, the concept of a "change in sensitivity of the hypothalamus" to gonadal steroids was proposed by Ramirez and McCann (1965) as being the maturational prelude to the onset of puberty. According to this concept, prior to puberty the hypothalamic "gonadostat" is very sensitive to feedback inhibition by gonadal steroids. As a result circulating levels of gonadotrophins, similar to those typical of adulthood, are not attained despite low circulating gonadal steroid concentrations. At the onset of puberty the gonadostat is 'reset' at a higher level (i.e. less sensitive) and the increasing steroid levels seen in the circulation no longer suppress the release of GnRH. Thus, gonadotrophin secretion can occur at increased levels, resulting in further increases in steroid secretion, and these in turn induce sexual maturation.

The gonads generally were believed to play a passive role during sexual maturation. However, Odell and Swerdloff

(1976) reviewed research on changes in gonadal responsiveness to gonadotrophins associated with sexual maturation. The concept proposed by these authors indicated that the testicular responsiveness to LH not only increased with age, but also was very much influenced by synergistic effects of FSH. This was clearly illustrated by a ten-fold increase in testosterone secretion seen in male rats exposed to both FSH and LH, in contrast to only a two-fold rise elicited by administration of LH alone. Perhaps that augmentation of gonadal steroid output is due to FSH-induced LH receptor formation in the testes, as it appears that the receptor population per Leydig cell does change in response to FSH. It was concluded that this increase in LH receptor population causes a greater output of testosterone and represents a major component of the testicular processes responsible for sexual maturation (Odell and Swerdloff, 1976).

Increase in sensitivity of the pituitary to GnRH also has been suggested to be a factor involved in the pubertal mechanisms. Such changes in pituitary responsiveness have been reported to occur at least in the rat (Debeljuk et al., 1972) and ram lambs (Lee et al., 1976b; Wilson and Lapwood, 1979b; Lee et al., 1981).

The role of extra-hypothalamic structures in initiation of pubertal processes has been reviewed by Gorski (1974). Döcke (1976) demonstrated that changes in the extra-hypothalamic CNS could involve gradual modulation of physiological functions and the development of receptor sites, which may result in changing primarily inhibitory prepubertal nervous structures in such a way that they respond to positive feedback effects pubertally. Gorski (1974) suggested that the amygdala, hippocampus and pineal gland were by far the most important extra-hypothalamic structures which could influence puberty.

The role of the adrenal glands in the onset of puberty is not clear. The concentrations of adrenal steroids dehydroepiandrosterone (DHA), dehydroepiandrosterone sulphate (DHAS) and $\Delta^4$-androstenedione increase in man

before the pubertal rise in gonadal steroid secretion (Cutler et al., 1978). To date, adrenarche has been demonstrated only in man and chimpanzees, but not in Rhesus monkeys, rodents or farm animals (Cutler et al., 1978).

## 10. Purpose of the Present Study

Investigations described in this thesis involved the use of radioimmunoassay procedures for measurement of peripheral plasma hormone concentrations in studies of their secretion, possible physiological roles and feedback control, during the course of sexual development. Large White x Landrace boars and Romney ram lambs were utilised in these experiments.

Experiments 3.1, 3.2 and 5.1 were designed to reinvestigate the patterns of secretion of LH and testosterone from birth to sexual maturity in boars and ram lambs.

Experiments 4.1, 4.2, 5.2 and 5.3 were designed to investigate the effects of hemicastration on LH and testosterone production in boars and ram lambs. Organ weights and histological data also were collected to determine the effects of this treatment on compensatory hypertrophy of the remaining testes and epididymides. GnRH response studies also were performed at various ages in order to measure changes in pituitary and gonadal sensitivity.

This study was continued (experiments 6.1, 6.2 and 6.3) in an attempt to elucidate the endocrine basis of compensatory hypertrophy. Furthermore, the study on feedback control of these hormones in ram lambs was extended by inclusion of surgically induced cryptorchidism as a treatment, and also by measurement of plasma FSH and prolactin concentrations.

# CHAPTER II

## MATERIALS AND METHODS

### 1. Porcine Experiments

#### (a) Animals

Large White x Landrace boars were utilized in the porcine experiments described in this thesis. Twenty animals from four litters born in August, 1977 were utilized in experiment 3.1, while 24 boars born in 4 litters in March, 1978 were used in experiments 3.2, 4.1 and 4.2.

#### (b) Animal Management

The animals were reared at the Pig Research Centre, Massey University. Litter mates were grouped together in concrete pens provided with wooden platforms. Animals were weaned at 5 weeks of age when body weights ranged between 8 and 10 kg. From weaning until 3 months of age they were fed *ad libitum* with a standard weaner meal composed of the following ingredients: barley - 43.5%, maize - 32.0%, skimmed milk powder - 8.5%, fish meal - 4.0%, lupin seed meal - 6.0%, lucerne - 2.0%, bone flour - 3.0% and salt premix - 1.0%. Thereafter the feed was changed to grower meal, which was fed at approximately 3 kg/animal/day until the end of the experiment. The grower meal consisted of the following ingredients: barley - 75.0%, dried blood - 10.0%, bone flour 6.0%, fish meal - 8.0% and salt premix - 1.0%. Water was provided *ad libitum* through automatic waterers.

#### (c) Surgical Techniques

##### (i) Castration of day old animals

Day old animals were castrated for experiment 3.1 without using an anaesthetic agent. After the scrotal area had been clipped then sterilized with 'Hibitane' (I.C.I., U.K.) a primary skin incision was made along the median raphe of the scrotum. One testicle was exteriorized

through the opening made by incising the underlying fascia and parietal tunica vaginalis. After sectioning the tunica vaginalis the testis was withdrawn to expose the spermatic cord. This was ligated using chromic catgut and the testicle severed distal to the point of ligation. The second testis was removed using the same technique. The skin incision was not sutured in day old animals.

(ii) Castration and hemicastration of animals in experiment 4.1

For experiment 4.1 the experimental design determined that surgery had to be performed at a range of ages. Animals weighing under 30 kg were operated on under local anaesthesia. They were restrained manually by an assistant, while the scrotal sac and the perineal area were sterilized using 'Hibitane' prior to surgery. Approximately 5 ml of local anaesthetic solution (2% xylocaine, Astra, Australia) was injected into the neck of the scrotum and spermatic cord.

Animals weighing over 30 kg were operated on under general anaesthesia. These were pre-medicated with azaperone ('Stresnil', Ethnor Limited, N.Z.) at a dose rate of 2 mg/kg body weight, then general anaesthesia was induced by rapid injection of a 2.5% solution of thiopentone sodium ('Intraval Sodium', May and Baker, U.K.) into the lateral ear vein. Anaesthesia was maintained with halothane vapour ('Flouthane', I.C.I., U.K.) 3% (v/v) in oxygen, administered via a face mask.

The castration procedure employed was essentially the same as that described for day old animals. However, the skin incisions were closed with interrupted non-absorbable sutures.

(iii) Post-surgical care

After all surgical procedures, antibiotic therapy (procaine penicillin-streptomycin suspension, 'Streptopen', Glaxo, N.Z.) at a rate of 2 to 3 ml/animal/day was administered for one week as a prophylactic measure against infection.

(d) Blood Collection

Approximately 8 ml blood samples were collected into heparinised vacutainers by puncture of the anterior vena cava, following the method described by Carle and Dewhirst (1942). Animals weighing less than 30 kg were bled while restrained on their backs in a crate built for the purpose. A 3.8 cm long, 18 gauge 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. From this point the needle was directly dorso-caudally. Larger animals were bled standing while restrained by a pig halter applied around the upper jaw. Nineteen gauge needles, 9-11.5 cm long were used and in this position the needle was introduced at the same site as described above, but directed medio-dorso-caudally.

Blood samples were kept in an ice bucket for up to 1 hr until the plasma was separated by centrifugation at 3000 r.p.m. for 20 min. Plasma was stored at -18$^{o}$C until required for hormone assays.

(e) Hormone Assays

(i) LH

Plasma levels of porcine LH were measured by a double antibody radioimmunoassay based on that described by Niswender et al. (1970) and validated previously in this laboratory (FlorCruz and Lapwood, 1978). This assay utilized the following materials: rabbit anti-porcine LH serum (pool# 566, courtesy of Dr G.D. Niswender, Colorado State University, U.S.A.); solutions of purified porcine LH (LER 788-4 provided by Dr L.E. Reichert, Emory University, U.S.A.) in a range of concentrations corresponding to plasma levels of 0 to 16 ng/ml as standards; and highly purified porcine LH (LER 786-3, courtesy of Dr L.E. Reichert) for radioiodination. Donkey anti-rabbit precipitating serum (DARS, RD 17, Wellcome Reagents Limited, England) diluted 1:40 in 0.05 M ethylene-diamine tetra-acetic acid-disodium salt

(EDTA) in 0.01 M phosphate-buffered 0.14 M saline (PBS).

All standards and samples were assayed in triplicate, while each assay contained a triplicate of tubes with the first antibody excluded, as a check for non-specific binding. Assay sensitivity ranged from 0.09 to 0.12 ng/ml, while within- and between-assay coefficients of variation (C.V.) for three plasma samples are shown in table 2.1.

Table 2.1: Between- and within-assay CV for porcine LH radioimmunoassay based on repeated measurement of reference porcine plasma samples

| Replication factor | Reference plasma | Mean hormone concentration (ng/ml) | Within-assay C.V.(%) | Between-assay C.V.(%) |
|---|---|---|---|---|
| 5 assays, 3 replicates per assay | X | 3.30 | 15.38 | 19.69 |
| | Y | 8.94 | 12.86 | 20.81 |
| | Z | 2.00 | 13.00 | 31.23 |

(ii) Testosterone

Plasma testosterone levels were estimated using a radioimmunoassay described previously (Wilson and Lapwood, 1978a) and which was validated separately for measurement of testosterone in porcine plasma (FlorCruz and Lapwood, 1978). Assay sensitivity varied between 0.08 and 0.48 ng/ml, while within- and between-assay C.V. are shown in table 2.2.

Table 2.2: Between- and within-assay CV for testosterone radioimmunoassay based on repeated measurement of reference porcine plasma samples

| Replication factor | Reference plasma | Mean hormone concentration (ng/ml) | Within-assay C.V.(%) | Between-assay C.V.(%) |
|---|---|---|---|---|
| 5 assays, | $P_1$ | 1.78 | 4.88 | 12.19 |
| 3 replicates | $P_2$ | 4.76 | 3.96 | 7.12 |
| per assay | $P_3$ | 6.97 | 5.19 | 8.22 |

## 2. Sheep Experiments

### (a) Animals

Thirty two Romney ram lambs, born in August, 1977 were utilized in experiments 5.1, 5.2 and 5.3, while 48 animals born in August, 1978 were used in experiments 6.1, 6.2 and 6.3.

### (b) Animal Management

Lambs were identified individually soon after birth. They were maintained at pasture with their dams for the first three months of life, then weaned. Lambs were vaccinated with a multiple clostridial vaccine ('Covax-5', T.V.L., N.Z.) at two weeks of age and drenched with anthelmintic ('Nilverm', I.C.I., N.Z.) at weaning and periodically thereafter. Animals were shorn at approximately 4 months of age.

Ram lambs used in experiments in chapter 6 remained healthy throughout the experiments. However, a number of those used in experiments in chapter 5 suffered from ryegrass staggers during March, 1978 and consequently lost weight.

Prior to bleeding the animals were shepherded with sheep dogs and then allowed to settle for about half an hour before sampling commenced.

(c) Surgical Procedures

(i) Hemicastration

Hemicastrations for experiments 5.2, 5.3, 6.1, 6.2 and 6.3 were performed with the animals restrained in dorsal recumbency. After clipping and chemically sterilising the scrotal skin with 'Hibitane', about 6 ml of 2% Xylocaine solution was injected into the neck of the scrotum and spermatic cords. A caudo-ventral incision was made over the right testis which was then exteriorized after sectioning the parietal tunica vaginalis. Hemicastration was completed by severing the spermatic cord with emasculators. Each testis and epididymis was dissected free of extraneous tissue before weighing then sampling for histology.

(ii) Artificial cryptorchidism

Artificial cryptorchidism was induced in ram lambs utilised in experiments 6.1, 6.2 and 6.3 at two weeks of age, following the method described by Schanbacher (1978) for inducing cryptorchidism in bulls. General anaesthesia was induced with sodium thiamylal ('Surital', Park Davis, N.Z.) and maintained with approximately 1.5% (v/v) halothane vapour in oxygen, administered via endotracheal tube.

The scrotal sac and surrounding ventral abdominal area was clipped and sterilised with 'Hibitane' then a lateral incision was made high on the scrotal skin. After the right testicle was separated from the scrotum by division of the mesorchium, the inguinal canal was made patent and the testicle was pushed into the abdominal cavity. However, care was taken to avoid torsion of the spermatic cord. Interrupted sutures were used to close the inguinal canal and prevent later descent of the testis, or visceral herniation. Topical antiseptic powder ('Aureomycin', Cyanamid, U.S.A.) was sprayed within the scrotum and about the incision site to reduce the chances of secondary infection. Chromic catgut (2/0, Ethnor Limited, Australia) was used to close the wound, and 2% iodine solution was applied to the incision line. This procedure was then repeated for the left testicle.

(iii) Castration of animals in experiment 5.2

Castrations were performed using the same method as described for hemicastration, however in this instance skin incisions were made on either side of the scrotum for exteriorising each testis before severing the spermatic cord with emasculators.

(iv) Post-operative care

Following all ovine surgery antibiotic therapy ('Streptopen'), 2 to 3 ml/animal/day, was administered intramuscularly for one week as a prophylactic measure against infection. Cryptorchid animals were examined two weeks later but none had post-operative descent of testes at that time, nor at any other time during the experiment.

(d) Blood Collection

Eight ml blood samples were collected into heparinized vacutainers after jugular venepuncture. Samples were centrifuged at 3000 r.p.m. for 20 min before plasma was separated and stored at -18$^{o}$C until required for hormone assays.

(e) Hormone Assays

(i) LH

Plasma LH levels were measured by a double antibody radioimmunoassay based on that described by Niswender et al. (1969), as modified by Barrell and Lapwood (1979a). The following materials were used in this assay: ovine LH (NIH-LH-S18, courtesy of N.I.A.M.D.D., N.I.H., U.S.A.) solutions at concentrations corresponding to a range of plasma levels of 0 to 8 ng/ml, as standards; rabbit anti-ovine LH serum (pool # 15, courtesy of Dr G.D. Niswender, Colorado State University, U.S.A.); and highly purified ovine LH (LER-1374A, courtesy of N.I.A.M.D.D., N.I.H., U.S.A.) for radioiodination. All standards and samples were assayed in triplicate. Assay sensitivity ranged from 0.03 to 0.16 ng/ml, while within- and between-assay C.V. for 2 reference plasma samples are shown in table 2.3.

Table 2.3: Between- and within-assay CV for ovine LH radioimmunoassay based on repeated measurement of reference ovine plasma samples

| Replication factor | Reference plasma | Mean hormone concentration (ng/ml plasma) | Within-assay C.V.(%) | Between-assay C.V.(%) |
|---|---|---|---|---|
| 20 assays, 3 replicates per assay | LH 1 | 0.47 | 15.22 | 34.28 |
| | PRL 3 | 4.51 | 12.15 | 14.36 |

(ii) FSH

Ovine FSH levels were measured by a double antibody radioimmunoassay based on that described by Dr I.J. Clarke (Melbourne, Australia, pers. comm.).

a. Reagents

(i) PBS contained 0.01 M phosphate buffer and 0.14 M sodium chloride, with 0.01% (w/v) sodium merthiolate as a preservative. The pH of PBS was adjusted to 7.3.

(ii) PBS-EDTA-BSA (PEB) was a solution of PBS containing 0.05 M EDTA and 0.5% bovine serum albumin (BSA, Fraction V, Sigma Chemicals, U.S.A.), with pH adjusted to 7.3.

b. Radioiodination of ovine FSH

3.5 µg lactoperoxidase (Calbiochem, U.S.A.) in 3.5 ml distilled water and 6.5 µl, 0.5 M phosphate buffer were added to 2 µg highly purified ovine-FSH (G4-211B, supplied by Dr H. Papkoff, University of California, U.S.A.) dissolved in 20 µl 0.5 M phosphate buffer, pH 7.4. Two hundred µ Ci $I^{125}$ (Radiochemical Centre, Amersham, U.K.) was then added and mixed before 5 µl hydrogen peroxide (Ajax Chemicals, Australia) diluted 1:15,000 in distilled water, was added to start the reaction. After the reaction had proceeded for 3 min a further 5 µl hydrogen peroxide was added and allowed to react for a further 3 min. The

mixture was then transferred quantitatively to a 1 x 25 cm polyacrylamide gel column (Biogel P60, Biorad Laboratories, U.S.A.) which had previously been equilibrated with 20 ml PBS, pH 7.3, followed by 1 ml 3% BSA in PBS. One ml fractions were eluted from the column with PBS, pH 7.3 and collected into 11 x 75 mm polystyrene tubes, from which 10 µl aliquots were taken and counted for radioactivity. Samples with peak radioactivity were stored frozen until required.

c. Radioimmunoassay procedures

Radioimmunoassays were performed in polystyrene tubes (11 x 75 mm) and both unknown samples and standards were assayed in triplicate.

Solutions of ovine FSH (NIH-FSH-S9, N.I.A.M.D.D., N.I.H., U.S.A.) were prepared in 100 µl PEB to provide a standard curve corresponding to a range of plasma FSH concentrations of 0 to 1000 ng/ml. Hypophysectomised sheep plasma (200 µ l) was added to each standard tube, while sample tubes contained 200 µ l unknown plasma.

Both standards and samples were then adjusted to contain a volume of 400 µ l by adding 100 µ l non-immune rabbit serum NRS (1:800 in PEB) to standards and 200 µ l (NRS) (1:1600 in PEB) to samples. Two hundred µ l rabbit anti-ovine FSH serum (59# 7, courtesy of Dr V.W.K. Lee, Melbourne, Australia) diluted 1:6000 in PEB, was added to each tube. Tubes were vortexed then incubated at 4°C for 24 h. Radio-iodinated ovine FSH (approximately 20,000 cpm diluted to 100 µ l in PEB) was then added to each tube and the incubation allowed to proceed for 96 h at 4°C. After adding 100 µ l DARS diluted 1:80 in PBS, the mixture was incubated for a further 24 h at 4°C, before antibody precipitation was completed by centrifuging at 2000 r.p.m. for 30 min at 4°C. The supernatant was then aspirated and the precipitate counted for one min in a Nuclear Enterprises NE 1600 Gamma counter.

Each assay contained a triplicate of tubes from which the first antibody was omitted to provide a check on the

level of nonspecific binding of $I^{125}$-FSH. Computation of plasma FSH concentrations were made on a Sord 222 computer following the method of Burger et al. (1972), as modified by Professor R.E. Munford, Massey University. Counts obtained for nonspecific binding were subtracted from those recorded for standards and samples. A best-fit expression for the standard curve was calculated, then values for the samples (means $\pm$ standard deviations) were computed. A composite standard curve representing the mean values from 8 consecutive assays is shown fig 2.1.

d. Validation of ovine FSH radioimmunoassay

Specificity of the ovine FSH antiserum has been determined by Dr I.J. Clark (Melbourne, Australia, pers. comm.) who showed that radioimmunoassay estimates of FSH potency were not affected by high levels of ovine LH, prolactin or growth hormone.

Non-specific binding always was low (less than 11% of the counts bound in the 0 ng/ml standard), while assay sensitivity ranged from 8.53 to 18.35 ng/ml.

Reproducibility of assay results was estimated by assaying three samples, three times in each of eight assays. The within- and between-assay C.V. are shown in table 2.4.

Table 2.4: Between- and within-assay C.V. for ovine FSH radioimmunoassay based on repeated measurements of reference ovine plasma samples

| Replication factor | Reference plasma | Mean hormone concentration (ng/ml plasma) | Within-assay C.V.(%) | Between-assay C.V.(%) |
|---|---|---|---|---|
| 8 Assays, | $LH_1$ | 120.61 | 2.00 | 17.30 |
| 3 replicates | $PRL_3$ | 565.87 | 9.00 | 9.20 |
| per assay | $WP_3$ | 925.85 | 19.00 | 17.30 |

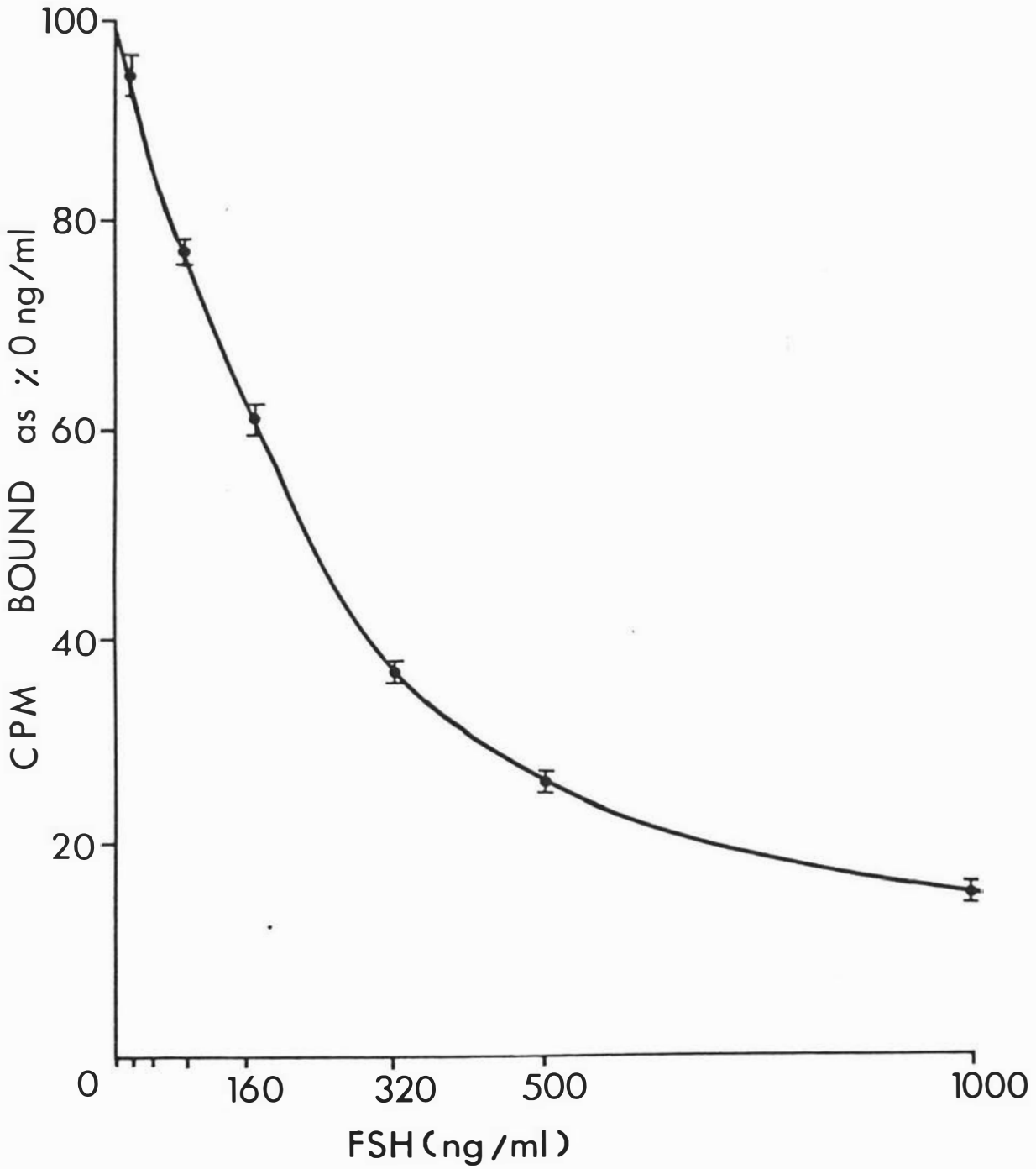



Figure 2.1: Composite standard curve (mean $\pm$SEM) derived from 8 consecutive ovine FSH radioimmunoassays.

Parallelism of the standard curve was checked by assaying two samples undiluted (541.50 and 922.90 ng/ml), diluted 1:1 (303.20 and 546.60 ng/ml) and diluted 1:3 (182.00 and 298.50 ng/ml) in hypophysectomized sheep plasma. These results demonstrated parallelism between dilutions of unknown samples and the standard curve.

On the basis of results from these validation tests it was considered that this assay gave reliable estimates of ovine plasma FSH concentrations.

(iii) Prolactin

Plasma ovine prolactin levels were estimated by a double antibody radioimmunoassay described previously by Wilson and Lapwood (1978a). This assay utilised rabbit antiserum to ovine prolactin (courtesy of Professor D.S. Flux, Massey University); solutions of sheep prolactin (NIH-P-S11, N.I.A.M.D.D., N.I.H., U.S.A.) corresponding to a range of plasma prolactin concentrations of 0 to 250 ng/ml as standards; and highly purified ovine prolactin (LER-860-2, courtesy of Dr L.E. Reichert, Emory University, U.S.A.) for radioiodination. Assay sensitivity ranged from 5.83 to 13.85 ng/ml, and within- and between-assay C.V. for 3 reference plasma pools are shown in table 2.5.

Table 2.5: Between- and within-assay C.V. for ovine prolactin radioimmunoassay based on repeated measurements of reference ovine plasma samples

| Replication factor | Reference plasma | Mean hormone concentration (ng/ml plasma) | Within-assay C.V.(%) | Between-assay C.V.(%) |
|---|---|---|---|---|
| 15 Assays, | $PRL_1$ | 13.58 | 13.83 | 18.40 |
| 3 replicates | $PRL_2$ | 28.09 | 9.65 | 12.74 |
| per assay | $PRL_3$ | 109.18 | 4.86 | 10.69 |

(iv) Testosterone

Estimates of testosterone concentrations in ovine plasma were made using the radioimmunoassay described previously by Wilson and Lapwood (1978a).

For plasma of this species, assay sensitivity varied from 0.06 to 0.48 ng/ml, while the within- and between-assay C.V. for 3 reference plasma pools are shown in table 2.6.

Table 2.6: Between- and within-assay CV for testosterone radioimmunoassay based on repeated measurement of reference ovine plasma samples

| Replication factor | Reference plasma | Mean hormone concentration (ng/ml plasma) | Within-assay C.V.(%) | Between-assay C.V.(%) |
|---|---|---|---|---|
| 40 Assays, | $PRL_3$ | 0.57 | 8.86 | 20.38 |
| 4 replicates | indole | 2.77 | 7.85 | 18.41 |
| per assay | $LH_3$ | 8.18 | 8.47 | 22.00 |
| | 18 | 11.18 | 5.86 | 15.56 |

3. Histological Procedures

5 mm equatorial sections of testicular tissue were fixed in Bouin's fluid for 24 h, before being transferred to 70% ethyl alcohol, then embedded in paraffin wax after automatic processing. Sections cut at 6 μm thickness were stained with haematoxylin and eosin, then examined under a light microscope for seminiferous tubular diameter estimations and qualitative histology. Estimates of seminiferous tubular diameters for each specimen in chapters 4, 5 and 6 were calculated as the mean recorded from 20 circular tubules, measurements being made on a microscope fitted with eyepiece micrometer.

## 4. Experimental Design and Analyses

Details of the experimental designs and statistical analyses used for individual experiments are given in the appropriate sections of chapters 3 to 6.

### (a) Missing Data

Values for missing hormone data were calculated as the mean estimate from preceding and succeeding plasma samples.

### (b) Transformations

All estimates of hormone concentrations, except those used in calculations of GnRH-induced LH, FSH and testosterone output curves, were transformed to logarithms prior to statistical analyses using the relationship: log hormone concentration = $100 \log_{10} (x + 1.1)$, where x is the hormone concentration in ng/ml. This relationship has been established on empirical grounds by the finding of a linear relationship between the estimated mean and its standard error, for subgroups of hormone data (Barrell, 1976).

### (c) Total Responses to GnRH Injection

The areas under individual LH, FSH and testosterone response curves in experiments 4.2, 5.3 and 6.3 were calculated as a measure of total hormone output in response to GnRH administration. Areas were calculated by integrating hormone concentrations in samples with the time intervals between samples and results were expressed as ng/ml.hr.

### (d) Statistical Analyses

The effect of treatments in each experiment were examined in analyses of variance. Main effects were tested by examining the significance of contrasts for individual degree of freedom, based on orthogonal coefficients (Cochran and Cox, 1960) which were taken from the tables of Fisher and Yates (1963), or which were constructed to test specific hypotheses. Where the contrasts were successive terms of a polynomial relationship, the mean squares for individual degrees of freedom were not calculated beyond the third power.

Levels of significance in all analyses are denoted thus:

| | |
|---|---|
| * | $P < 0.05$ |
| ** | $P < 0.01$ |
| *** | $P < 0.001$ |

# CHAPTER III

## A LONGITUDINAL STUDY OF PLASMA LUTEINIZING HORMONE LEVELS IN BOARS AND BARROWS, AND OF TESTOSTERONE LEVELS IN BOARS, FROM BIRTH TO 180 DAYS OF AGE

### 1. Introduction

Limited studies of the endocrine changes occurring from birth to sexual maturity in boars of domestic breeds are summarized on page 49 of this thesis. In general it has been shown that LH levels are high during the first 3 postnatal weeks, subsequently fall to low values and in some, but not all studies, a prepubertal peak also has been recorded. Plasma testosterone levels in boars also are relatively high during the neonatal period, subsequently decline, and then increase at about the time of puberty.

Castration and androgen replacement therapy experiments with boars have indicated that the hypothalamo-pituitary unit is relatively insensitive to the negative feedback effects of gonadal steroids in the first 2-3 weeks after birth (Ford and Schanbacher, 1977; Elsaesser et al., 1978; Colenbrander et al., 1977; 1978).

Because the previous longitudinal study of reproductive development in boars reported from this laboratory (FlorCruz and Lapwood, 1978) only commenced at 42 days of age (after weaning) and utilized a fortnightly blood sampling protocol, the present experiments were designed to obtain more detailed information regarding plasma hormone levels in boars from birth to sexual maturity. Effects of castration on the longitudinal hormone secretory pattern of LH were examined by including barrows in the first experiment.

The second experiment was designed as a study of the effects of season on longitudinal hormone secretion profiles in young boars and utilized boars born in autumn, rather than spring.

## 2. Materials and Methods

### (a) Animals

#### (i) Experiment 3.1

Twenty spring born boars were identified individually then allocated randomly to treatment groups: (i) castrated at one day of age, and (ii) maintained as entires.

#### (ii) Experiment 3.2

Eight autumn born boars (which were allocated randomly to groups E and F of experiment 4.1 and maintained as entires until 18 weeks of age) were utilized. However, because of the design of experiment 4.1 the number of animals had to be reduced to 4 at 18 weeks and the study was terminated at 24 weeks of age.

### (b) Sampling

Blood samples were obtained each Monday between 09.00 and 10.00 h. Sampling extended from day old to about 180 days of age in experiment 3.1, and from day old to about 168 days in experiment 3.2. All plasma samples were assayed for concentrations of LH and testosterone.

### (c) Body Weights

Animals were weighed immediately after each blood sampling as a check on body growth and the weights were recorded to the nearest kg.

### (d) Statistical Analyses

LH, testosterone and body weight data from studies 3.1 and 3.2 were subject to analyses of variance. Hormonal data from experiment 3.2 was examined only up to 18 weeks of age because of the reduction in number of animals thereafter.

Students *t*-tests were then used to examine the differences between mean LH levels in boars and barrows in experiment 3.2, and also to make informal (crude) between-experiment comparisons of mean plasma hormone levels recorded in the first 18 weeks of each experiment.

## 3. Results

### (a) Spring Born Boars and Barrows (Experiment 3.1)

#### (i) LH levels in boars

(Figure 3.1 and Table 3.1)

Relatively high mean plasma LH levels ($3.06 \pm 0.55$ to $4.90 \pm 1.97$ ng/ml) were recorded from birth to 5 weeks of age, after which they decreased and fluctuated between $0.82 \pm 0.14$ and $3.11 \pm 0.51$ ng/ml. Peaks in LH concentrations (above 2 ng/ml) were seen at 7, 9, 12, 13, 15, 16, 22 and 23 weeks, but no overall change in average plasma LH concentrations was seen near the onset of puberty, except that a peak of $3.11 \pm 0.51$ ng/ml was seen at the 16th week. In the analysis of variance of LH data the significant linear ($P < 0.001$), quadratic ($P < 0.05$) and cubic ($P < 0.05$) components of the effect of age were attributable to the above general pattern of change.

#### (ii) Testosterone levels in boars

(Figure 3.2 and Table 3.1)

Mean plasma testosterone levels recorded during the study varied between $0.16 \pm 0.04$ and $2.31 \pm 0.42$ ng/ml. However, values recorded during the first five weeks were relatively higher ($0.70 \pm 0.15$ to $1.33 \pm 0.17$ ng/ml plasma) than seen in the subsequent 9 weeks ($0.16 \pm 0.04$ to $0.89 \pm 0.39$ ng/ml). From 14 weeks of age onwards plasma testosterone concentrations began to increase and peaks above 2 ng/ml were recorded at 15, 16 and 22 weeks. These general changes were reflected by a significant cubic ($P < 0.001$) component in the analysis of variance.

Plasma testosterone concentrations in castrates were below the limit of sensitivity of the assay as from when the first samples were collected one week after castration.

#### (iii) LH levels in castrates

(Figure 3.3 and Table 3.1)

Mean plasma LH levels recorded from castrates ranged between $3.72 \pm 0.75$ and $5.92 \pm 0.70$ ng/ml but did not show any

significant pattern of change with age. Levels recorded from castrates in the first 3 weeks were in the same range as observed for boars, but did not show the subsequent reduction in values which was observed from the entires. Hence the overall mean LH level observed in castrates was significantly higher than that of entires ($t_{(50)} = 5.80$, ($P < 0.001$).

(iv) Body weights

(Figure 3.4 and Table 3.2)

Body weights of castrates and entires increased throughout the experiment and the rate of gain accelerated at the later weighings. Thus not only was the linear component of the age main effect in the analysis of variance significant ($P < 0.001$), but so also was the quadratic component ($P < 0.001$). Overall castrates were significantly ($P < 0.001$) heavier than boars.

(b) Autumn Born Boars (Experiment 3.2)

(i) LH levels

(Figure 3.1 and Table 3.3)

Plasma LH levels were high during the first 5 weeks ($1.66 \pm 0.43$ to $4.83 \pm 1.22$ ng/ml) after birth but subsequently declined to a value of $1.19 \pm 0.19$ ng/ml at 7 weeks. From 8 weeks to 17 weeks the values ranged between $1.30 \pm 0.28$ and $2.58 \pm 0.34$ ng/ml with a relatively higher mean value ($1.86 \pm 0.11$ ng/ml) compared to levels observed from 17 to 24 weeks (these ranged between $0.77 \pm 0.88$ and $1.92 \pm 0.22$ ng/ml with mean of $1.11 \pm 0.08$ ng/ml).

In the analysis of variance of LH data the most highly significant effect of age was a linear ($P < 0.001$) decline, but the quadratic ($P < 0.05$) and cubic ($P < 0.05$) components also were significant.

(ii) Testosterone levels

(Figure 3.2 and Table 3.3)

During the first 4 weeks plasma testosterone levels were higher (between $0.55 \pm 0.12$ and $0.76 \pm 0.16$ ng/ml than those generally recorded in the subsequent 7 weeks (values

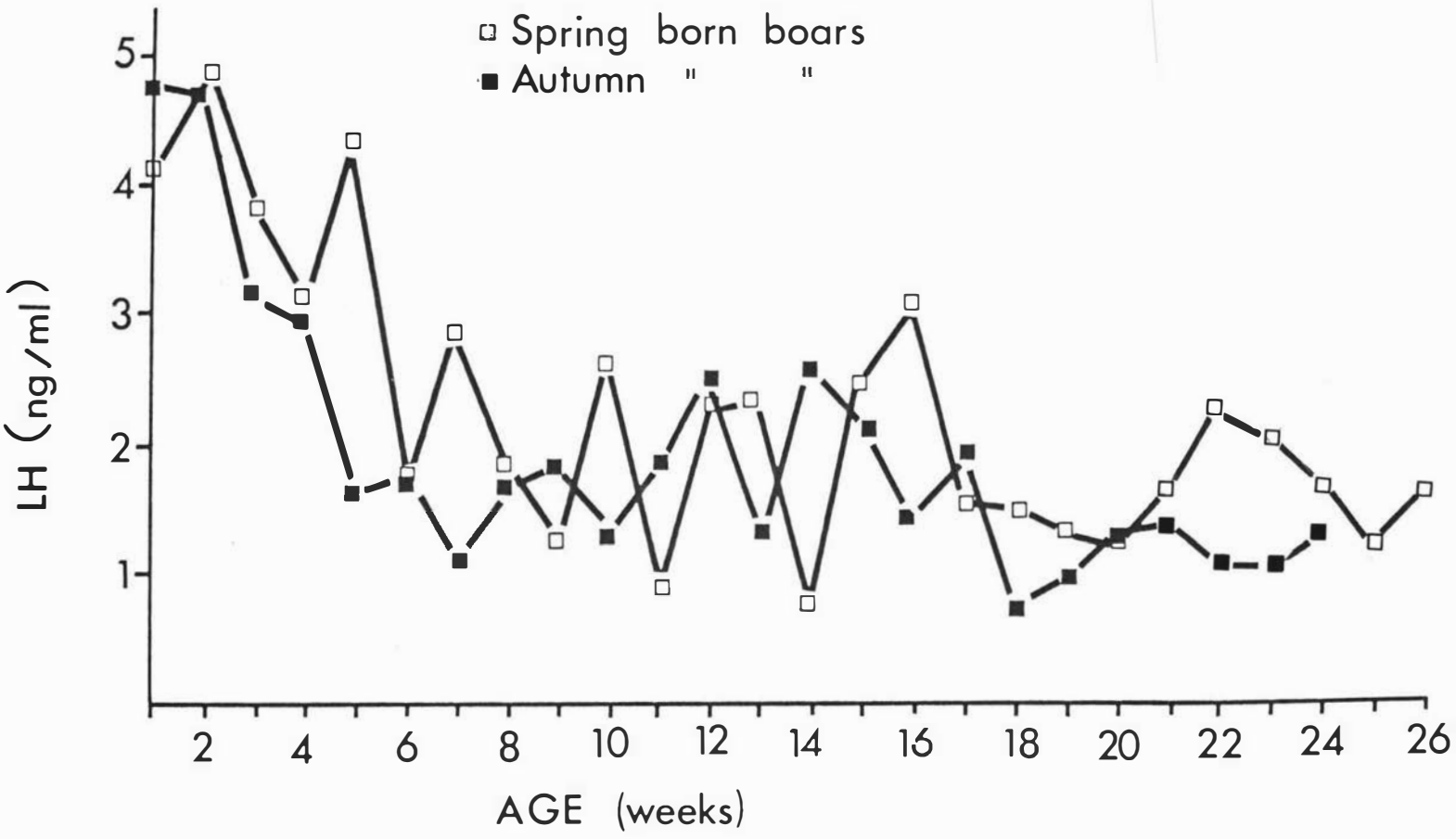



Figure 3.1: Mean plasma LH levels in boars from 1 to 26 (spring born) or 24 (autumn born) weeks of age (experiments 3.1 and 3.2, respectively; n=10).

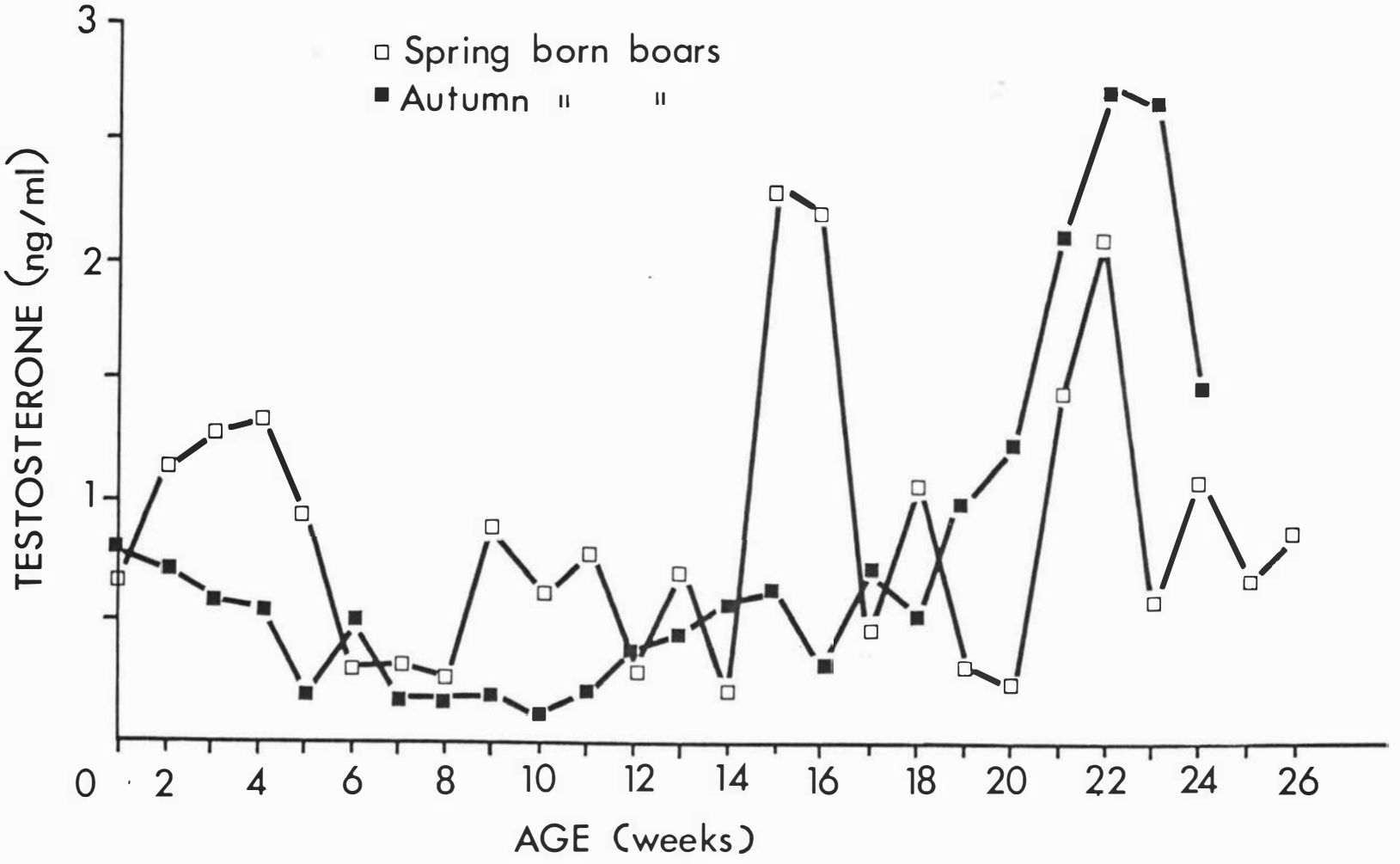



Figure 3.2: Mean plasma testosterone levels in boars from 1 to 26 (spring born) or 24 (autumn born) weeks of age (experiments 3.1 and 3.2, n=10).

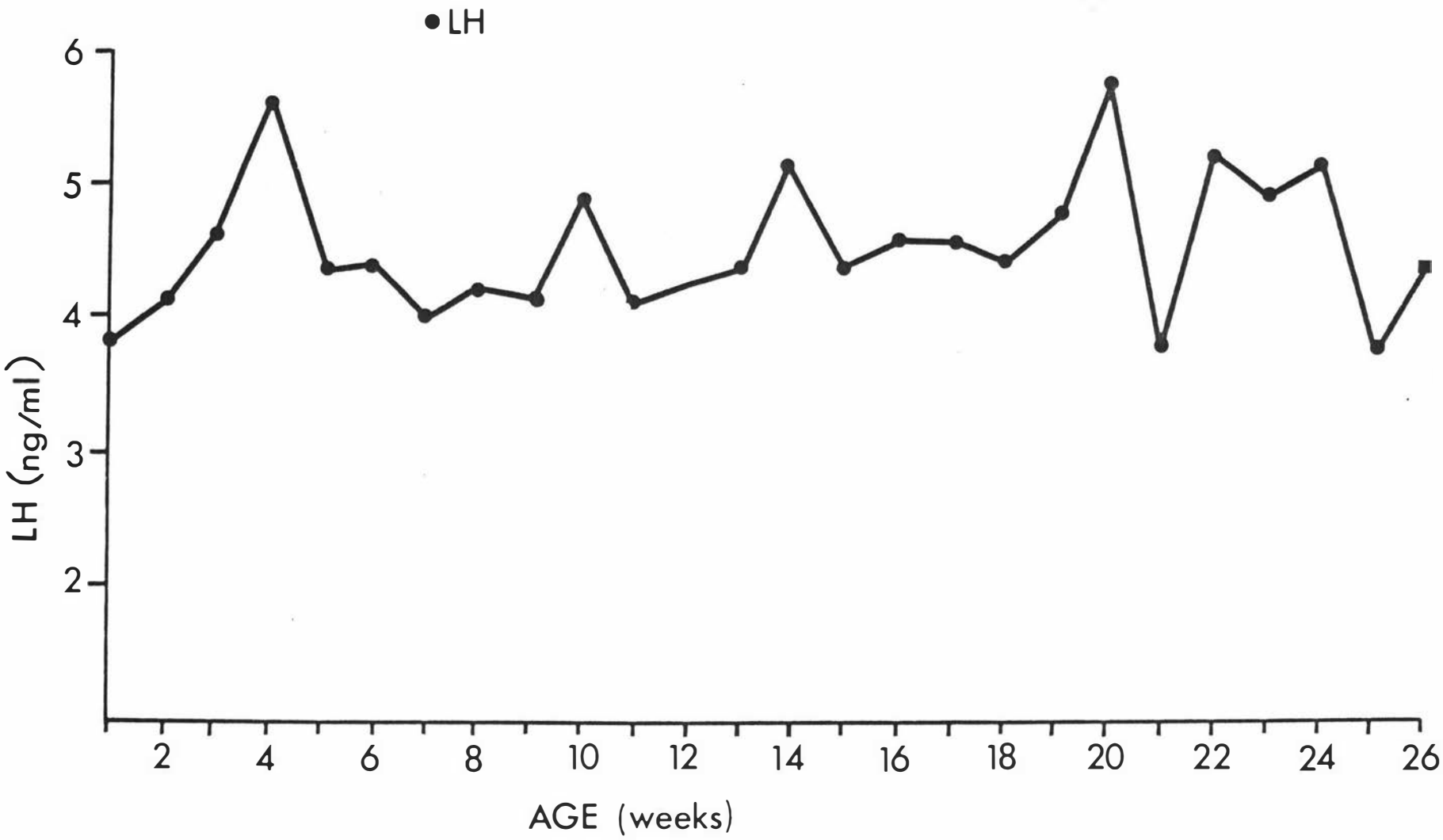



Figure 3.3: Mean plasma LH levels in spring born barrows from 1 to 26 weeks of age (experiment 3.1, n=10).

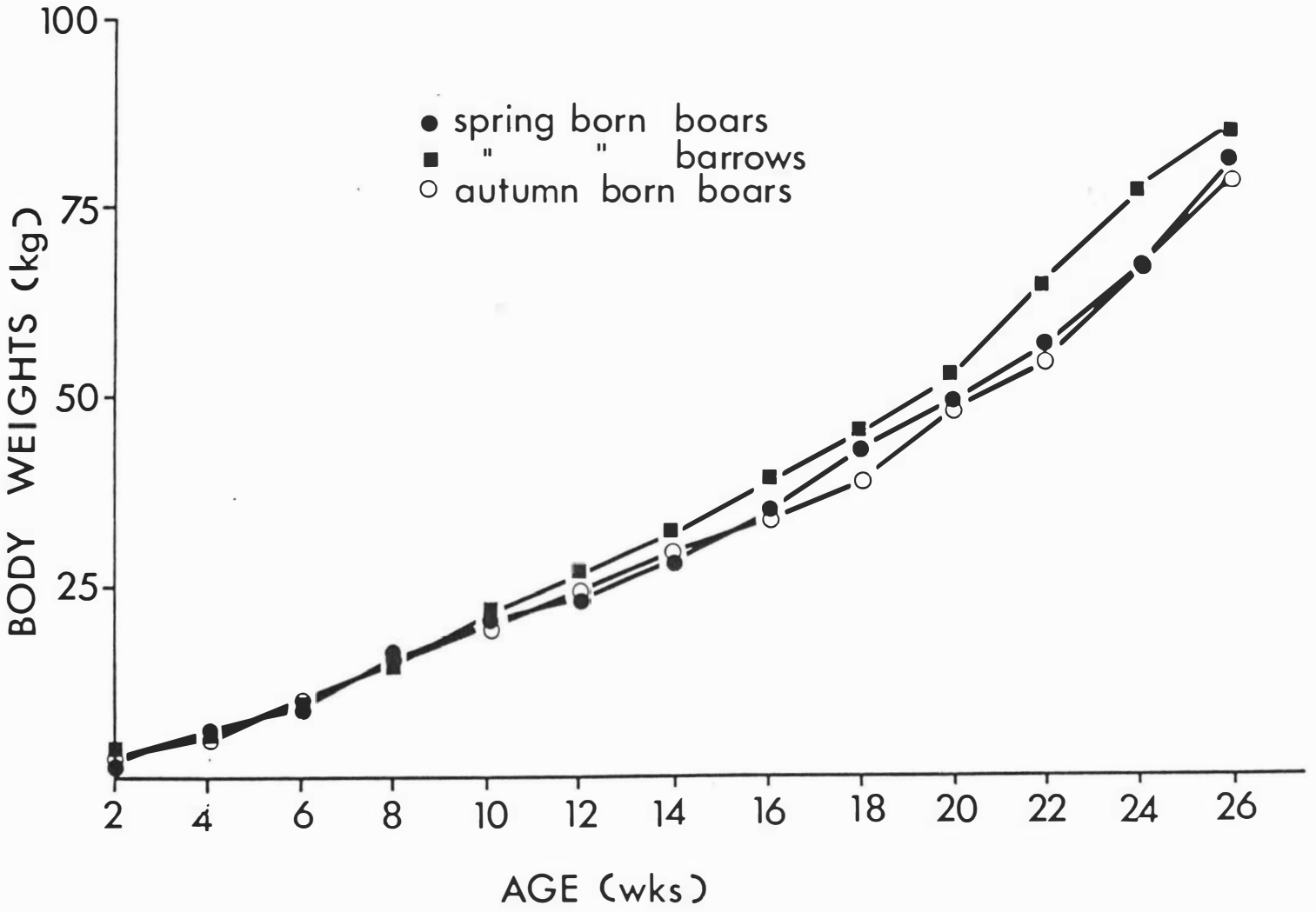



Figure 3.4: Mean body weights of boars and barrows (spring born) (experiment 3.1, n=10) and boars (autumn born) (experiment 3.2, n=8) from 2 to 26 weeks of age.

Table 3.1: Summary of analyses of variance of LH and testosterone data from spring born boars and barrows in experiment 3.1

| Source of Variation | DF | Variance Ratios | | |
|---|---|---|---|---|
| | | Boars | | Barrows |
| | | LH | Testosterone | LH |
| A. Age | 12 | | | 1.68 |
| (i) Linear | 1 | 20.51*** | 0.51 | - |
| (ii) Quadratic | 1 | 4.42* | 3.80 | - |
| (iii) Cubic | 1 | 4.59* | 10.79*** | - |
| Remainder | 9 | 1.21 | 0.04 | - |
| B. Animals | 9 | 0.74 | 3.64** | 5.44*** |
| Residual mean square | 108 | 233.95 | 139.32 | 165.07 |

Table 3.2: Summary of analysis of variance of body weight data from spring born boars and barrows in experiment 3.1

| Source of Variation | | DF | | Variance Ratios |
|---|---|---|---|---|
| A. Boars vs Barrows | | 1 | | 136.77*** |
| B. Age | | 12 | | |
| (i) | Linear | | 1 | 25,537.45*** |
| (ii) | Quadratic | | 1 | 758.54*** |
| | Remainder | | 10 | 0.50 |
| Residual mean square | | 247 | | 5.89 |

Table 3.3: Summary of analyses of variance of LH testosterone and body weight from autumn born boars in experiment 3.2

| Source of Variation | DF | | Variance Ratios | | |
|---|---|---|---|---|---|
| | | | LH | Testosterone | Body Weights |
| A. Age | 8 | | | | |
| (i) Linear | | 1 | 19.28*** | 0.19 | 680.19*** |
| (ii) Quadratic | | 1 | 5.80* | 27.00*** | - |
| (iii) Cubic | | 1 | 4.79* | 5.15* | - |
| Remainder | | 5 | 1.75 | 0.62 | - |
| Remainder | | 7 | - | - | 0.78 |
| B. Boars | 7 | | 1.06 | 1.97 | 6.88* |
| Residual mean square | 56 | | 151.16 | 66.67 | 14.41 |

ranged between 0.13±0.03 and 0.45±0.17 ng/ml). Thereafter concentrations tended to increase steadily to reach a maximal mean plasma concentration of 2.73±0.54 ng/ml at 22 weeks and only fell noticeably at the last sampling. Those changes in testosterone concentrations contributed to a very highly significant quadratic component in the orthogonal partition of age effects. A significant cubic component ($P < 0.05$) probably was of minor importance.

(iii) Body weights

(Figure 3.4 and Table 3.3 )

Body weights of boars increased throughout the period of study. Thus in the analysis of variance the age main effect was very highly significant ($P < 0.001$).

(c) Informal Comparisons Between Spring and Autumn Born Boars

There was no significant overall difference in mean plasma LH levels between spring (2.57±0.28 ng/ml) and autumn (2.21±0.27 ng/ml) born boars ($t_{(34)} = 0.04$, $P > 0.05$). Similarly the overall mean plasma testosterone level recorded from spring born boars (0.88±0.61 ng/ml) did not differ from that of autumn born boars (0.43±0.22 ng/ml) ($t_{(34)} = 0.73$, $P > 0.05$).

Graphical comparison of body weights of spring and autumn born boars indicated that there was no consistent difference in favour of one group or the other.

## 4. Discussion

### (a) Luteinizing Hormone

In experiments 3.1 and 3.2 generally similar patterns of change in plasma LH levels were recorded. Relatively high concentrations were recorded during the first 4 to 5 weeks of postnatal life and similar results have been published previously for boars of domestic breeds (Ford and Schanbacher, 1977; Colenbrander et al., 1977) as well as for Göttingen miniature boars (Elsaesser et al., 1976).

After neonatal castration and androgen replacement studies with domestic and Göttingen miniature breed boars, Ford and Schanbacher (1977) and Elsaesser et al. (1978), respectively, concluded that these high neonatal LH levels were the result of the hypothalamo-pituitary units being relatively insensitive to steroid negative feedback inhibition. Further evidence of such insensitivity of young boars to testosterone was provided by results from experiment 3.1 : in the first 2-3 weeks of life similarly high LH concentrations were recorded from boars as well as barrows, despite the recording of quite high androgen levels from the boars.

Although in the current experiments autumn born boars showed some evidence of a pubertal peak in LH levels, in the spring born animals the only indication of a pubertal elevation was an isolated peak of 3.11 ng/ml observed at 16 weeks. The irregular fluctuations in weekly plasma LH levels observed in the experiment with spring born animals may have masked any pubertal elevation in that study. Plasma LH levels fluctuate quite frequently in boars (Sanford et al., 1976b; Lapwood and FlorCruz, 1978), and in the present experiments such variations should have been compensated for by use of multiple blood samplings at each age. Differing patterns of gonadotrophin secretion have been reported in previous studies. Neither Colenbrander et al. (1977) nor Elsaesser et al. (1976) observed any significant changes in plasma LH levels from boars approaching puberty. However, FlorCruz and Lapwood (1978) recorded a distinct prepubertal peak in LH levels from boars of the same breed as utilized in the present study.

(b) Testosterone

High plasma testosterone levels were observed in the neonatal period of these experiments as well as in most other similar studies (Elsaesser et al., 1972; Booth, 1975; Meussey-Dessolle, 1976; Colenbrander et al., 1978). However in a paper by Ford and Schanbacher (1977), the reported levels of testosterone were low and remained

relatively constant during the first 9 weeks of life. The significance of high androgen levels during the neonatal period remains obscure, but they occur in a period in which there is a high degree of intertubular Leydig cell differentiation and an increase in Leydig cell volume (Van Straaten and Wensing, 1977a; 1978), as well as an increase in enzymic activity in Leydig cells (Wrobel et al., 1973; Van Straaten and Wensing, 1978). These changes were preceded by elevated LH levels in the present study and those of Colenbrander et al. 1977; 1978). The parallel high plasma concentrations of testosterone and LH during the neonatal period were compatible with the concept that the hypothalamus is very insensitive to the inhibitory feedback effects of steroids at this stage of development (Colenbrander et al., 1978).

The subsequent recording of generally lower plasma testosterone concentrations up to 13 weeks of age, confirmed similar data reported by Booth (1975), Meus ey-Dessolle (1976), Colenbrander et al. (1978) and FlorCruz and Lapwood (1978). It also corresponds well with the observation of low plasma LH levels, as well as the relative regression of the intertubular Leydig cell population reported to occur during this stage (Van Straaten and Wensing, 1977a; 1978).

Plasma testosterone concentrations recorded in both longitudinal studies showed a progressive increase from about the 13th week of postnatal life. Similar increases in plasma testosterone levels associated with sexual maturation in boars have been reported by Meussy-Dessolle (1976), Colenbrander et al. (1978) and FlorCruz and Lapwood (1978).

The progressive increase in plasma testosterone levels associated with the onset of puberty was accompanied by only a relatively small elevation in LH secretion. This apparent increase in testicular sensitivity to LH probably was related to a concurrent increase in steroid histochemical activity of the testis (Wrobel et al., 1973; Van Straaten and Wensing, 1978). Another contributing factor may have been the increase in relative volume of

the Leydig cells (Van Straaten and Wensing 1977a; 1978) and possibly an increase in Leydig cell receptor activity, as suggested by Odell and Swerdloff (1976).

Evidence of prepubertally increased LH secretion, despite rising plasma levels of testosterone, indicated that in addition to the above mentioned mechanisms, a reduction in sensitivity of the set-point of hypothalamic mechanisms which regulate secretion of gonadotrophins occurs with the onset of puberty (McCann et al., 1974).

Testosterone was not detectable in plasma from barrows in the present study nor in that of FlorCruz and Lapwood (1978), clearly indicating that the testes are the major source of androgens in this species.

(c) Body Weights

Castration of young male pigs is a common porcine husbandry practice, the principal benefit of which is reduction in sexual drive and aggressiveness. Other possible influences which may be important include effects on growth rates and carcase composition, as well as on the incidence of boar taint (Patterson, 1968; Prescott and Lamming 1964a; 1964b; Walstra and Kroeske, 1968; Walstra 1969).

The highly significant difference between the body weights of barrows and boars in the present study should be interpreted cautiously: because of inadequate numbers of pens the boars and barrows were not housed separately. Thus, even though feeding was at a restricted level of approximately 3 kg/animal/day from 3 months of age, the possibility existed that barrows may have consumed more food (and hence achieved a greater rate of growth) than boars, as has been recorded in previous studies of nutrition and body growth in male pigs (Walstra and Kroeske, 1968; Walstra, 1969).

(d) Season of Birth

There was no significant effect of season of birth on the LH or testosterone levels recorded in the present study.

However, more careful evaluation of seasonal effects on puberty is warranted in light of recent work published by Mahone et al. (1979) and Berger et al., (1980). These authors were able to induce puberty in growing boars by artificial extension of the natural photoperiod when day-light lengths were short. Although various testicular parameters indicated acceleration of pubertal processes in animals exposed to extended light periods, no hormonal data were reported in those papers, nor apparently in any other report on the effects seasons on sexual maturation in boars.

There was no significant effect of season of birth on body weights in the present study. Extended photoperiods did not exert any beneficial effect on rates of body weight gain or on feed conversion efficiency of boars or barrows in the study of Berger et al., (1980).

# CHAPTER IV

## EFFECTS OF HEMICASTRATION AT DIFFERENT AGES ON LUTEINIZING HORMONE AND TESTOSTERONE SECRETORY PATTERNS AND ON COMPENSATORY TESTICULAR HYPERTROPHY IN SEXUALLY MATURING BOARS

### 1. Introduction

In various species unilateral castration of young animals induces compensatory testicular hypertrophy (CTH). For example, CTH has been shown to occur in male rats (Hochereau-de Reviers, 1975; Cunningham et al., 1978), rams (Voglmayr and Mattner, 1968; Skinner, 1971b; Hochereau-de Reviers and Pelletier, 1971; Land and Carr, 1975; Hochereau-de Reviers et al., 1976; Walton et al., 1978; 1980), bulls (Linder and Rowson, 1961; Johnson, 1978; Barnes et al., 1980a, b, c; Leidl et al., 1980) and cockerels (Driot et al., 1979). Only one such study of boars has been reported and in that, CTH followed hemicastration of sexually mature animals (Hauser et al., 1952).

Likewise little research has been undertaken regarding hormonal responses following hemicastration of boars. Liptrap and Raeside (1971) in a study using two animals, found urinary levels of dehydroepiandrosterone (DHA) and oestrogen were unaffected following hemicastration. In Göttingen strain miniature boars, hemicastrated one week after birth, there were no effects of this treatment on plasma LH or testosterone levels during the subsequent five weeks (Elsaesser et al., 1978).

The present experiment was designed to examine the effects of hemicastration on hormone secretory patterns, testicular hypertrophy and hormonal responses to administration of exogenous GnRH at three ages during the course of sexual maturation of Landrace x Large White boars.

## 2. Materials and Methods

### (a) Animals

Twenty four boars from four litters born at approximately the same time in March, 1978 were used in these experiments.

### (b) Surgical Procedures (Experiments 4.1 and 4.2)

After identification the animals were randomly allocated to 3 pairs of groups (A and B, C and D, and E and F). Animals in groups A, C and E were hemicastrated at 4, 12 and 20 weeks of age, respectively. Those in groups B, D and F acted as age-matched controls and were bilaterally castrated at 8, 16 and 24 weeks. At those ages also, the second testes were removed from the corresponding hemicastrates. A summary of the experimental surgical protocol is given in Table 4.1.

Table 4.1: Ages at which surgical treatments were applied to boars in various treatment groups in experiments 4.1 and 4.2

| Age (weeks) / Treatment | 4 | 8 | 12 | 16 | 20 | 24 |
|---|---|---|---|---|---|---|
| 1. Hemicastration (HC) | A | | C | | E | |
| 2. Removal of Second Testes (RST) | | A | | C | | E |
| 3. Bilateral Castration | | B | | D | | F |

### (i) Acute profile studies (Experiment 4.1)

Four acute hormone secretion profile studies, in which blood samples were collected each half hour for 2 hours, were conducted for each pair of experimental groups, as

Table 4.2: Summary of ages (weeks) at which sampling procedures were undertaken in experiments 4.1 and 4.2

| Profile No. / Treatment Groups | 1 (Pre HC) | 2 (2 weeks post HC) | 3 (4 weeks post HC) | 4 (2 weeks post-castration) |
|---|---|---|---|---|
| A and B | 4 | 6 | 8 | 10 |
| C and D | 12 | 14 | 16 | 18 |
| E and F | 20 | 22 | 24 | 26 |

Hemicastration of animals in Groups A, C and E immediately after 1st profile bleeding.

GnRH study in hemicastrates and control entires followed profile 3, immediately before bilateral castration and removal of second testes.

indicated in Table 4.2. All plasma samples were assayed for concentrations of LH and testosterone.

(ii) GnRH response study (Experiment 4.2)

After each profile (see Table 4.2) blood sampling, at 8, 16 and 24 weeks of age, but before the subsequent castrations (Table 4.2) animals were injected intramuscularly with GnRH (0.5 µg/kg body weight) dissolved in 5.0 ml acidified (0.01 M acetic acid) 0.9% saline.

Blood samples were obtained immediately prior to injection of GnRH (preinjection) and 30, 60, 120 and 240 minutes after injection. All plasma samples were assayed for concentrations of LH and testosterone.

(c) Organ Weights and Qualitative Histology

Testes and epididymides were weighed, then testicular samples were taken for histological processing, estimation of seminiferous tubular diameters and qualitative histological examination.

(d) Statistical Analyses

Effects of treatments and age on LH and testosterone secretion in the profile and GnRH experiments were examined by analyses of variance. Effects of treatments on mean testicular and epididymal weights, and on mean seminiferous tubular diameters, were tested by Student's *t*-test comparisons.

3. Results

(a) Acute Profile Study (Experiment 4.1)

(i) LH*

(Figure 4.1, tables 4.3 and 4.4)

Pulsatile LH secretory patterns were evident at all ages in entires, hemicastrates and castrates during the two hour acute profile studies. No differences in frequency of secretory pulses were observed between the three groups, however, the magnitude of pulses was greater in castrates.

See inside back cover.

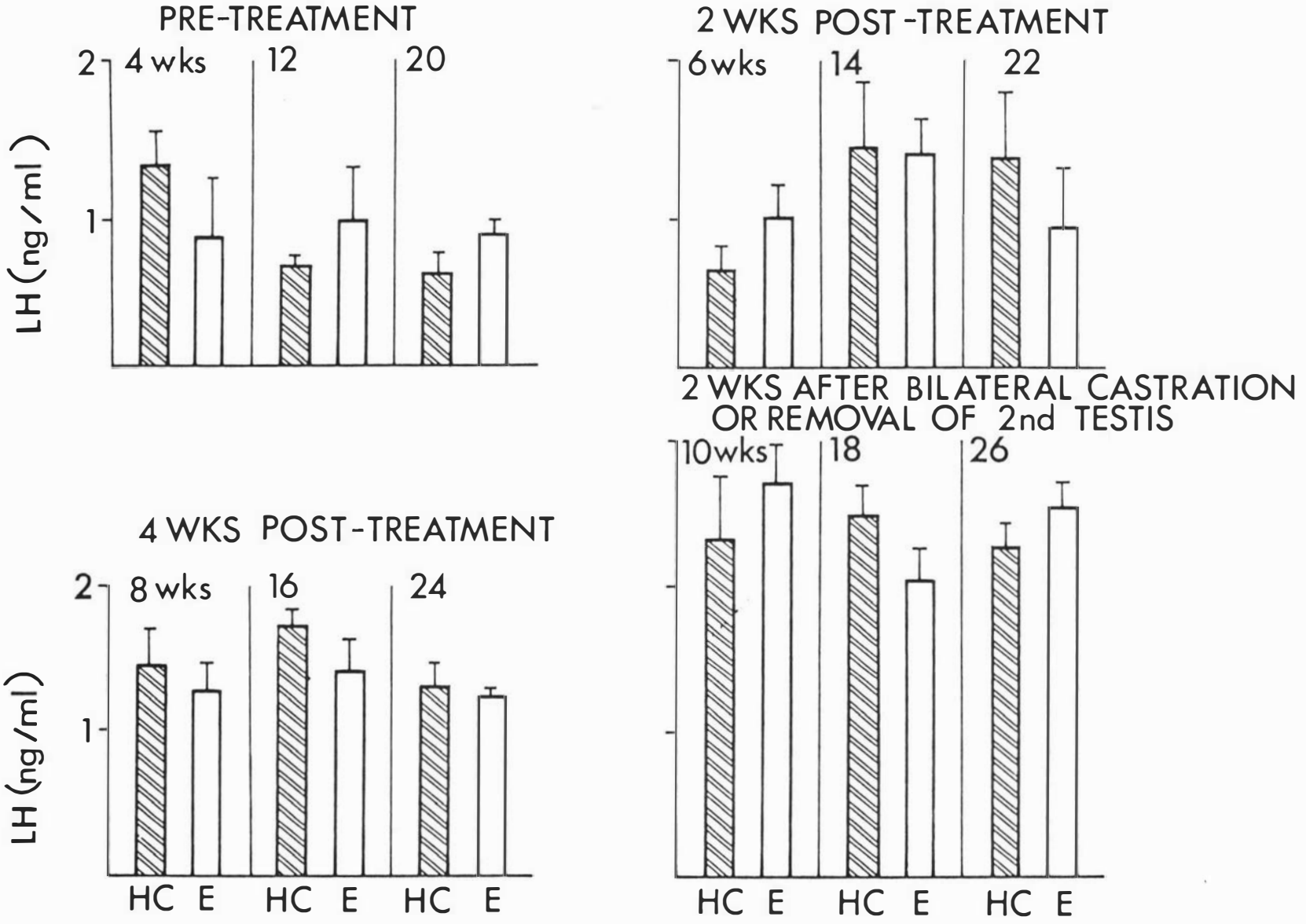



Figure 4.1: Mean (±SEM) plasma LH levels recorded from entire (E) and hemicastrate (HC) boars during acute profile studies conducted at the ages indicated (experiment 4.1, n=4).

Table 4.3: Mean (±SEM) LH concentrations (ng/ml) of hemicastrate and entire boars in experiment 4.1

| Group | Age (weeks) at Profile 1 † | Profile 1 | Profile 2 | Profile 3 | Profile 4 |
|---|---|---|---|---|---|
| | | | HEMICASTRATES | | |
| A | 4 | 1.37±0.24 | 0.68±0.15 | 1.42±0.29 | 2.33±0.45 |
| C | 12 | 0.69±0.08 | 1.60±0.45 | 1.72±0.11 | 2.48±0.18 |
| E | 20 | 0.63±0.13 | 1.42±0.40 | 1.30±0.16 | 2.28±0.14 |
| | Overall mean | 0.90±0.15 | 1.23±0.20 | 1.48±0.11 | 2.36±0.16 |
| | | | ENTIRES | | |
| B | 4 | 0.89±0.40 | 1.04±0.22 | 1.26±0.18 | 2.76±0.49 |
| D | 12 | 1.02±0.37 | 1.57±0.25 | 1.39±0.11 | 2.02±0.23 |
| F | 20 | 0.91±0.05 | 0.96±0.04 | 1.24±0.04 | 2.54±0.17 |
| | Overall mean | 0.91±0.18 | 1.19±0.11 | 1.29±0.07 | 2.44±0.18 |

† Subsequent profile samplings were undertaken at 2 weekly intervals

Table 4.4: Summary of analysis of variance of LH data from experiment 4.1

| Source of Variation | | DF | Variance Ratios |
|---|---|---|---|
| A. | Treatment & Sampling | 7 | |
| | (i) Pre vs Post-treatment | 1 | 50.39*** |
| | (ii) 2 and 4 weeks post-treatment vs 6 weeks post-treatment | 1 | 76.6*** |
| | (iii) 2 weeks vs 4 weeks post-treatment | 1 | 5.96* |
| | Remainder | 4 | 0.43 |
| B. | Age | 2 | 1.08 |
| | Interaction | | |
| | Treatment & Sampling x Age | 14 | |
| | (i) Pretreatment vs Post-treatment x Linear | 1 | 5.90* |
| | (ii) Pretreatment: allocated controls vs hemicastrates x Linear | 1 | 5.70* |
| | Remainder | 12 | 1.00 |
| | Residual mean square | 72 | 57.61 |

Pre-surgical LH levels recorded from boars allocated to the hemicastrate ($0.90 \pm 0.23$ ng/ml) and entire ($0.91 \pm 0.04$ ng/ml) treatment groups were not significantly different. Similarly at the fourth profile sampling, after castrations had been completed, there was no difference in the LH concentrations of these two groups of animals.

As judged by the non-significance of contrasts comparing LH levels recorded from hemicastrates and entires, both at 2 weeks and 4 weeks after hemicastration, surgical treatment had no effect on secretion of that gonadotrophin.

The combined mean LH level for hemicastrate and entire boars increased successively and significantly over the first three profile sample collections. Non-orthogonal contrasts revealed that while there was a significant increase, between profiles 2 and 3, in mean LH values recorded from hemicastrates ($1.23 \pm 0.28$ *vs* $1.48 \pm 0.13$ ng/ml, $F_{(1,72)} = 5.88$, $P<0.05$), the increase recorded from entires was not significant ($1.19 \pm 0.19$ *vs* $1.29 \pm 0.04$, $F_{(1,72)} = 1.04$, $P>0.05$). This effect in the treated animals presumably was a slowly developing consequence of hemicastration.

Age had no significant overall effect on mean LH levels. However, a significant ($P<0.05$) pretreatment x linear component of treatment x age interaction was due to a decrease with age in the pre-treatment LH levels recorded from animals destined to be in the hemicastrate group. This effect probably was due to a random sampling error because no such effect was recorded in the control group. Also it was clear that the pre-treatment result recorded from intended hemicastrates was responsible for the significant ($P<0.05$) pre-treatment *vs* post-treatment x linear component of the treatment x age interaction.

(ii) Testosterone

(Figure 4.2, tables 4.5 and 4.6)

Episodic testosterone secretion was recorded from entires and hemicastrates at all sampling ages, but pulses tended to be relatively smaller at the earlier samplings.

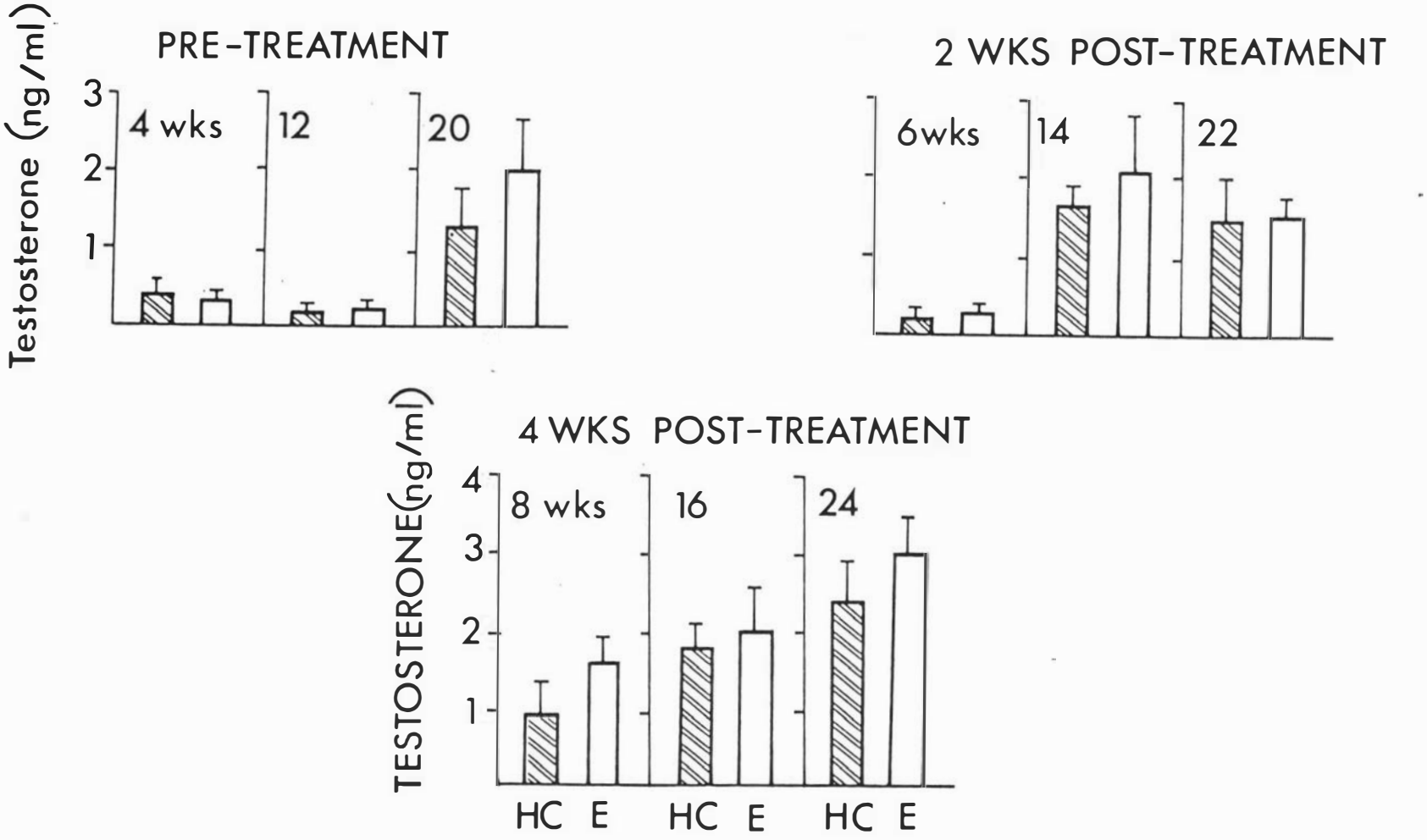



Figure 4.2: Mean ($\pm$SEM) plasma testosterone levels recorded from entires (E) and hemicastrate (HC) boars during acute profile studies conducted at the ages indicated (experiment 4.1, n=4).

Table 4.5: Mean (±SEM) testosterone concentrations (ng/ml) of hemicastrate and entire boars in experiment 4.1

| Group | Age (weeks) at Profile 1 ¶ | Profile 1 | Profile 2 | Profile 3 |
|---|---|---|---|---|
| | | | HEMICASTRATES | |
| A | 4 | 0.38±0.10 | 0.20±0.02 | 0.92±0.37 |
| C | 12 | 0.15±0.04 | 1.58±0.25 | 1.81±0.27 |
| E | 20 | 1.24±0.55 | 1.49±0.54 | 2.40±0.52 |
| Overall mean | | 0.59±0.18 | 1.09±0.19 | 1.71±0.23 |
| | | | ENTIRES | |
| B | 4 | 0.19±0.03 | 0.33±0.04 | 1.60±0.30 |
| D | 12 | 0.20±0.07 | 2.14±0.86 | 2.01±0.59 |
| F | 20 | 1.99±0.65 | 1.54±0.23 | 3.01±0.44 |
| Overall mean | | 0.79±0.21 | 1.34±0.29 | 2.20±0.26 |

¶ Subsequent profile samplings were undertaken at 2 weekly intervals.

Table 4.6: Summary of analysis of variance of testosterone data from experiment 4.1

| Source of Variation | DF | | Variance Ratios |
|---|---|---|---|
| A. Treatment and Sampling | 5 | | |
| (i) Pre vs Post-treatment | | 1 | 34.86*** |
| (ii) 2 weeks post-treatment vs 4 weeks post-treatment | | 1 | 10.05** |
| Remainder | | 3 | 1.06 |
| B. Age | 2 | | |
| (i) Linear | | 1 | 42.33*** |
| (ii) Quadratic | | 1 | 0.08 |
| Interaction | | | |
| Treatment and Sampling x Age | 10 | | 1.07 |
| Residual mean square | 54 | | 135.86 |

Another influence of age on testosterone secretion was that at 8 weeks and over, testosterone secretion followed most LH secretory peaks (82 out of 91), but at 4 and 6 weeks of age only about half (10 out of 21) the LH peaks were followed by testosterone secretion. Hemicastration had no obvious influence on any parameter of episodic testosterone secretion.

At each age similar pre-treatment testosterone levels were recorded from animals allocated to the entire and hemicastrate groups.

At both post-treatment samplings hemicastrates tended to have lower testosterone concentrations than entires, but this effect was not significant. By way of contrast, animals from both groups had higher ($P<0.01$) androgen levels at 4 weeks after sampling started than at 2 weeks (mean values were $1.71\pm0.43$ ng/ml at 4 weeks *vs* $1.09\pm0.54$ ng/ml at 2 weeks for hemicastrates and $2.20\pm0.42$ ng/ml *vs* $1.34\pm0.53$ ng/ml for entires).

In the analyses of variance the age main effect was very highly significant. Inspection of the data in table 4.5 invariably revealed that, both for boars and hemicastrates, lowest levels were recorded at each profile sampling from the animals first sampled at 4 weeks of age and highest from those introduced to the experiment at 20 weeks, intermediate values tended to be recorded from groups C and D.

(b) GnRH Responses (Experiment 4.2)

Both LH and testosterone secretory responses followed the injection of GnRH in entires and hemicastrates.

(i) LH

(Figures 4.3-4.5 and tables 4.7 and 4.8)

At each age mean pre-injection LH levels were similar to those observed in the acute profile studies. For hemicastrates levels were $1.22\pm0.29$ ng/ml at 8 weeks, $1.64\pm0.14$ ng/ml at 16 weeks and $1.26\pm0.09$ ng/ml at 24 weeks, while for entires the corresponding values were $1.03\pm0.43$,

$2.38 \pm 0.45$ and $1.07 \pm 0.18$ ng/ml. These patterns of change were represented by a significant ($P < 0.05$) quadratic component in the analysis of the effects of age on pre-injection LH concentrations.

As far as total LH responses were concerned those of entires and hemicastrates were not significantly different, nor was there any significant effect of age.

(ii) Testosterone

(Figures 4.3-4.5 and tables 4.7 and 4.8)

Mean pre-injection plasma testosterone levels recorded from hemicastrates were low at 8 weeks ($0.36 \pm 0.24$ ng/ml), peaked at $2.05 \pm 0.93$ ng/ml at 16 weeks, and then declined to $1.45 \pm 0.27$ ng/ml at 24 weeks. However, for entires plasma concentrations were $0.08 \pm 0.04$ ng/ml at 8 weeks, $1.19 \pm 0.58$ ng/ml at 16 weeks and $1.77 \pm 0.61$ ng/ml at 24 weeks. Consequently in the analysis of variance both the linear ($P < 0.001$) and quadratic ($P < 0.05$) components of the effects of age on pre-injection levels of testosterone were significant.

Treatment (hemicastrates vs entires) had no significant effect on pre-injection plasma testosterone levels, nor on total output of this hormone.

Total testosterone responses from hemicastrates were lowest at 8 weeks (14.66 ng/ml.hr), peaked at 16 weeks (49.54 ng/ml) and then declined to 45.23 ng/ml at 24 weeks. However, in entires the total hormone output increased progressively from 12.04 ng/ml.hr at 8 weeks, to 40.93 ng/ml.hr at 16 weeks and 47.31 ng/ml.hr at 24 weeks. These patterns of change were represented by significant ($P < 0.001$) linear and quadratic ($P < 0.05$) components in the analysis of the effects of age on total testosterone responses.

(c) Organ Weights

(Tables 4.9 and 4.10)

Data on testicular and epididymal weights and on seminiferous tubular diameters obtained from animals at the time of hemicastration, and from control entires at

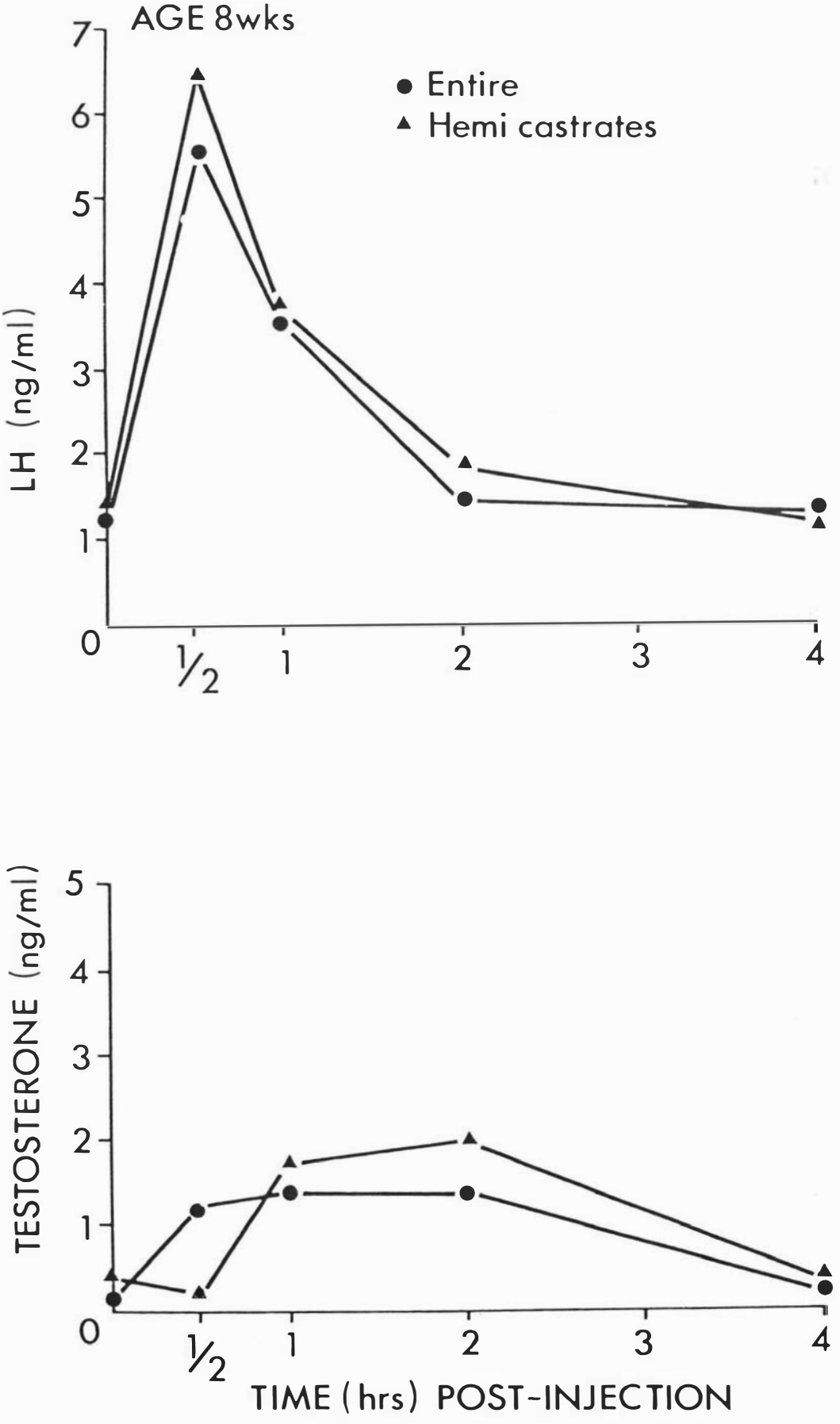



Figure 4.3: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate boars at 8 weeks of age (experiment 4.2, n=4).

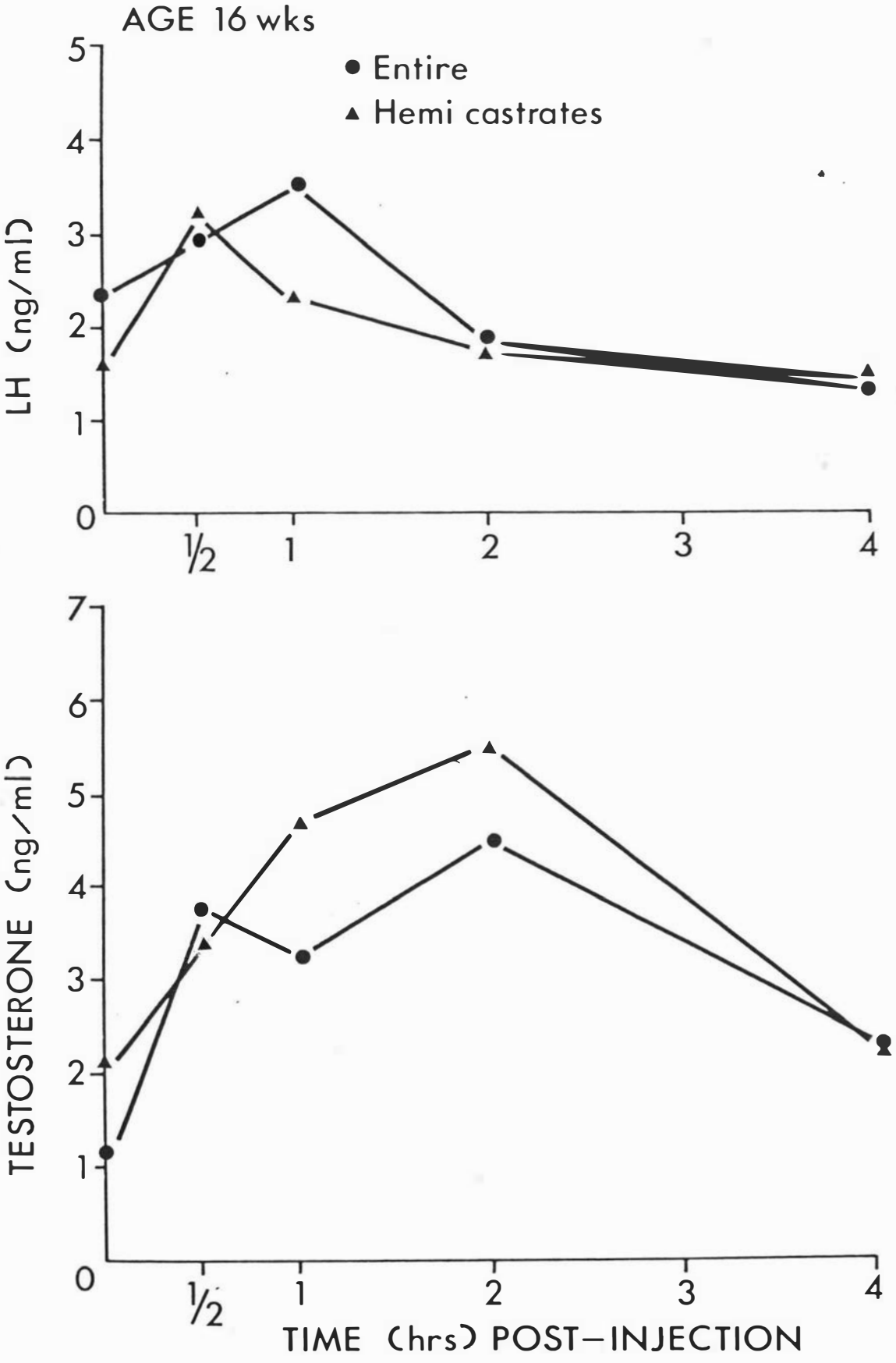



Figure 4.4: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate boars at 16 weeks of age (experiment 4.2, n=4).

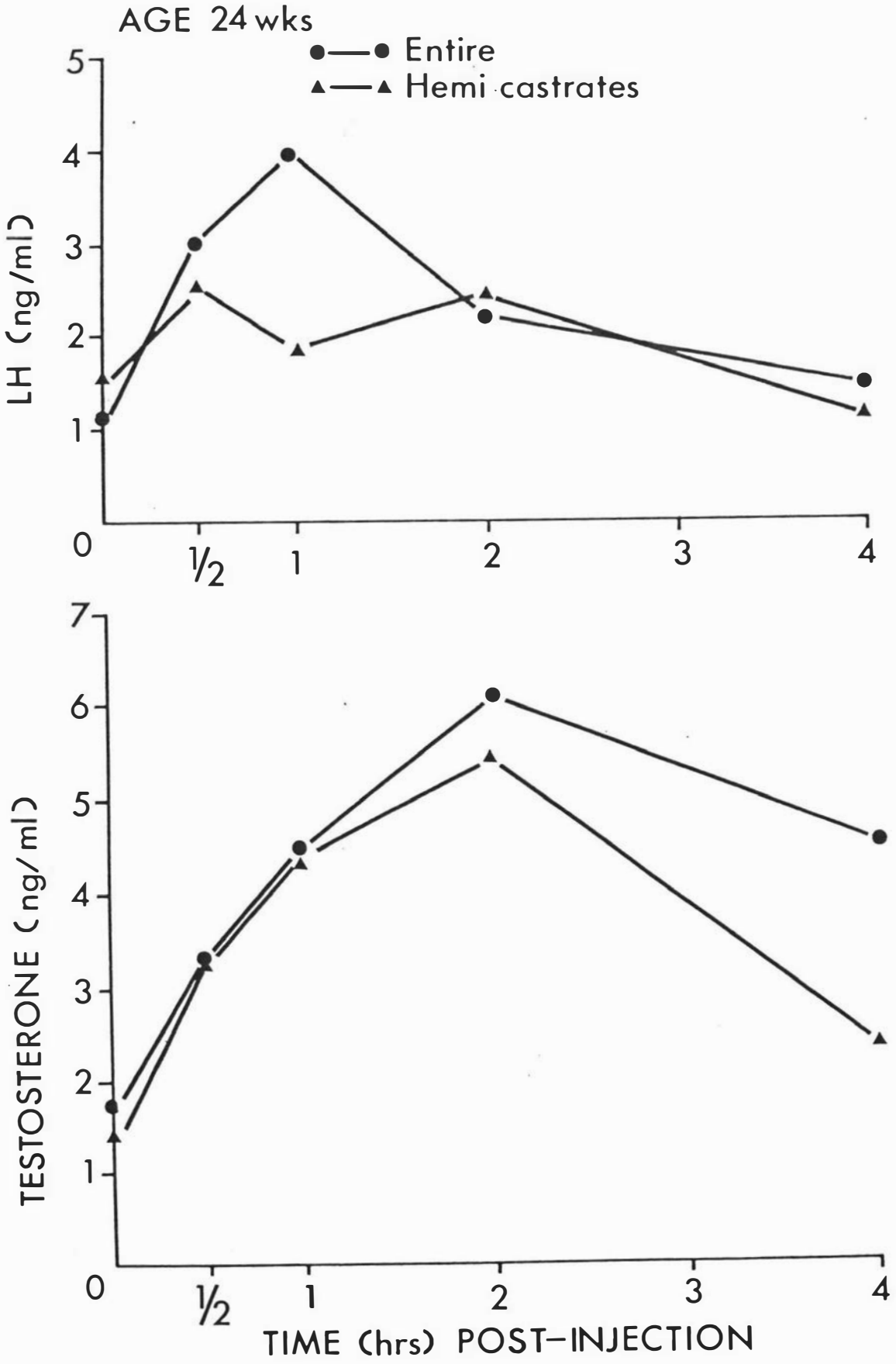



Figure 4.5: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate boars at 24 weeks of age (experiment 4.2, n=4).

Table 4.7: Mean (±SEM) pre-GnRH injection LH and testosterone levels (ng/ml) and total LH and testosterone responses (ng/ml.hr) from boars in experiment 4.2

| Age (weeks) | LH | | Testosterone | |
|---|---|---|---|---|
| | Entires | Hemicastrates | Entires | Hemicastrates |
| | PREINJECTION HORMONE LEVELS | | | |
| 8 | 1.03±0.43 | 1.22±0.29 | 0.08±0.04 | 0.36±0.24 |
| 16 | 2.38±0.45 | 1.64±0.14 | 1.19±0.58 | 2.05±0.93 |
| 24 | 1.07±0.18 | 1.26±0.09 | 1.77±0.61 | 1.45±0.27 |
| Overall Mean | 1.49±0.21 | 1.37±0.11 | 1.01±0.28 | 1.28±0.30 |
| | TOTAL HORMONE RESPONSES | | | |
| 8 | 32.21±1.18 | 30.72±1.24 | 12.04±1.41 | 14.66±0.81 |
| 16 | 29.04±0.53 | 28.78±0.55 | 40.93±0.85 | 49.54±2.83 |
| 24 | 31.77±1.19 | 24.23±0.73 | 47.31±0.81 | 45.23±2.27 |
| Overall Mean | 31.00±0.58 | 27.91±0.51 | 33.42± 0.61 | 36.47±1.23 |

Table 4.8: Summary of analyses of variance of basal and total LH and testosterone data from experiment 4.2

| Source of Variation | DF | Variance Ratios | | | |
|---|---|---|---|---|---|
| | | LH | | Testosterone | |
| | | Basal | Total | Basal | Total |
| A. Treatment | | | | | |
| (Control vs Hemicastrate) | 1 | 0.29 | 0.98 | 0.07 | 0.30 |
| B. Age | 2 | | | | |
| (i) Linear | 1 | 0.14 | 0.08 | 46.94*** | 23.54*** |
| (ii) Quadratic | 1 | 4.48* | 1.82 | 6.21* | 6.89* |
| Interaction | | | | | |
| Treatment x Age | 2 | 0.28 | 0.52 | 0.72 | 0.43 |
| Residual mean Square | 18 | 161.71 | 3.66 | 97.89 | 11.51 |

Table 4.9: Mean (±SEM) testicular and epididymal weights (g) from hemicastrate (HC) and entire boars, and t-test values from experiment 4.1.

| PARAMETER | Age (Weeks) 4 | 8 | 12 | 16 | 20 | 24 |
|---|---|---|---|---|---|---|
| | | | TESTICULAR | | | |
| Weight of 1st testes HC | 3.92±0.52 | | 13.78±1.25 | | 92.84±2.21 | |
| Weight of 2nd testes HC | | 15.52±0.75 | | 109.18±1.70 | | 245.63±3.38 |
| Mean weight of testes of entires | | 6.71±0.71 | | 64.03±2.83 | | 179.94±9.0 |
| Ratio of Weights: | | | | | | |
| Weight of 2nd testes HC / Mean weight of testes of entires | | 2.31 | | 1.71 | | 1.37 |
| t-test values (IO DF) † | | 8.15, P<0.001 | | 4.04, P<0.01 | | 2.77, P<0.05 |
| | | | EPIDIDYMAL | | | |
| Weight of 1st epididymides HC | 1.58±0.09 | | 5.40±0.44 | | 25.12±2.40 | |
| Weight of 2nd epididymides HC | | 6.48±0.29 | | 18.28±0.90 | | 52.30±0.89 |
| Mean weight of epididymides of entires | | 3.93±0.24 | | 18.08±0.90 | | 48.03±2.13 |
| Ratio of Weights: | | | | | | |
| Weight of 2nd epididymides HC / Mean Weight of epididymides of entires | | 1.65 | | 1.01 | | 1.09 |
| t-test values (IO DF) ¶ | | 3.85, P<0.01 | | 0.03, P>0.05 | | 0.45, P>0.05 |

† t-test values are for comparison between mean weights of hemicastrate 2nd testes vs mean weight of testes from entires.

¶ t-test values are for comparisons between mean weights of hemicastrate 2nd epididymides vs mean weight of epididymides from entires.

Table 4.10: Mean (±SEM) seminiferous tubular diameters (STD - μm) of hemicastrate (HC) and entire boars, and t-test values from experiment 4.1

| Age (weeks) / Parameter | 4 | 8 | 12 | 16 | 20 | 24 |
|---|---|---|---|---|---|---|
| STD 1st testes HC | 52.85±1.23 | | 65.17±2.94 | | 153.3±3.38 | |
| STD 2nd testes HC | | 65.87±2.30 | | 155.49±6.61 | | 192.48±0.24 |
| STD entires | | 58.82±1.91 | | 125.98±4.41 | | 172.87±1.60 |
| Ratio of STD's: | | | | | | |
| $\frac{\text{STD 2nd testes HC}}{\text{STD entires}}$ | | 1.12 | | 1.23 | | 1.11 |
| t-test values (10 DF) ¶ | | 1.55, P>0.05 | | 1.16, P>0.05 | | 3.40, P<0.01 |

¶ t-test values are for comparisons between mean STD of second testes of hemicastrates vs mean STD of entires.

castration (i.e. data relating to organs for which compensatory hypertrophy could not be expected), clearly indicated that these parameters increased linearly with age.

Testicular data obtained in the present experiment clearly showed that for each pair of groups, weights of testes from entires were greater than those of the first testes of hemicastrates, but significantly less than weights of second hemicastrate testes. However, the relative degree of compensatory hypertrophy decreased with age, from 131% at 8 weeks to 37% at 24 weeks of age. A similar trend in results was noted in the epididymal weight data (Table 4.9), but only at 8 weeks were epididymides from hemicastrates significantly ($P < 0.01$) heavier than those of entires.

Hemicastration also induced compensatory increases in seminiferous tubular diameters at all three ages (Table 4.10). However, those increases were statistically significant only at 24 weeks ($P < 0.01$).

(d) Qualitative Testicular Histology

Major qualitative histological features of testes collected at various ages are summarized below. In general maturational changes could be attributed entirely to age at castration and hemicastration had no influence.

(i) 4 and 8 weeks
(Figure 4.6)

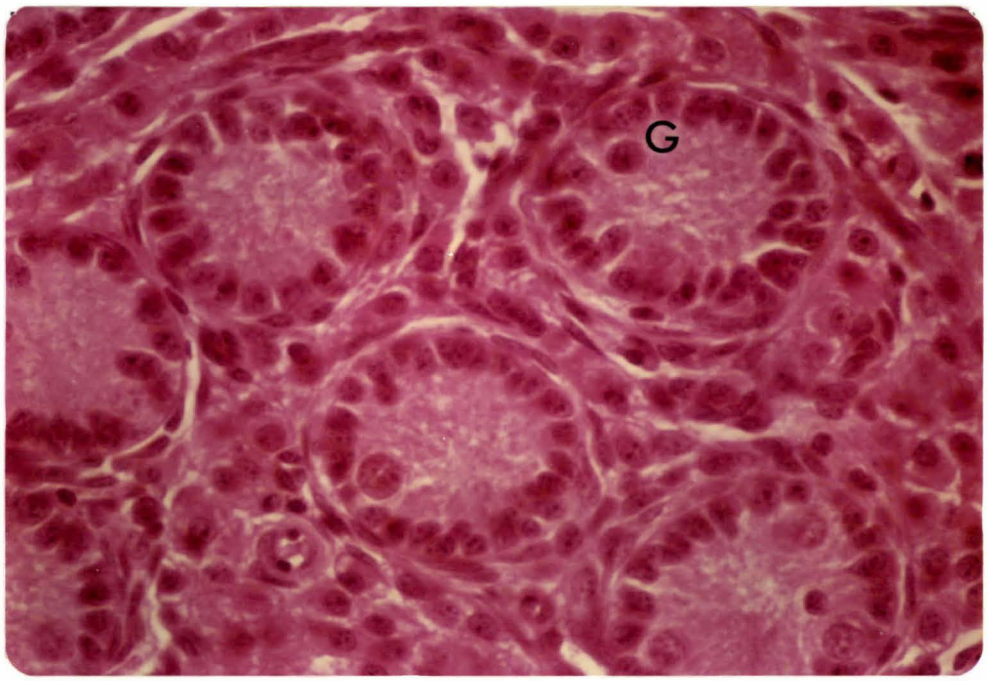
The histological appearance of testes removed at 4 weeks was very similar to that observed after removal of the second testes from hemicastrates at 8 weeks. Only two types of cells were evident in the seminiferous tubules: (a) supporting cells which formed a single basal layer of small cells with darkly stained nuclei; and (b) a few larger cells (gonocytes/prospermatogonia) with rounded highly stained nuclei located more centrally. Interstitial tissue formed the bulk of the testes and comprised a few fibroblasts and numerous Leydig cells; the latter were characterised by eosinophilic granular cytoplasm and basophilic nucleoplasm containing one or more nucleoli.

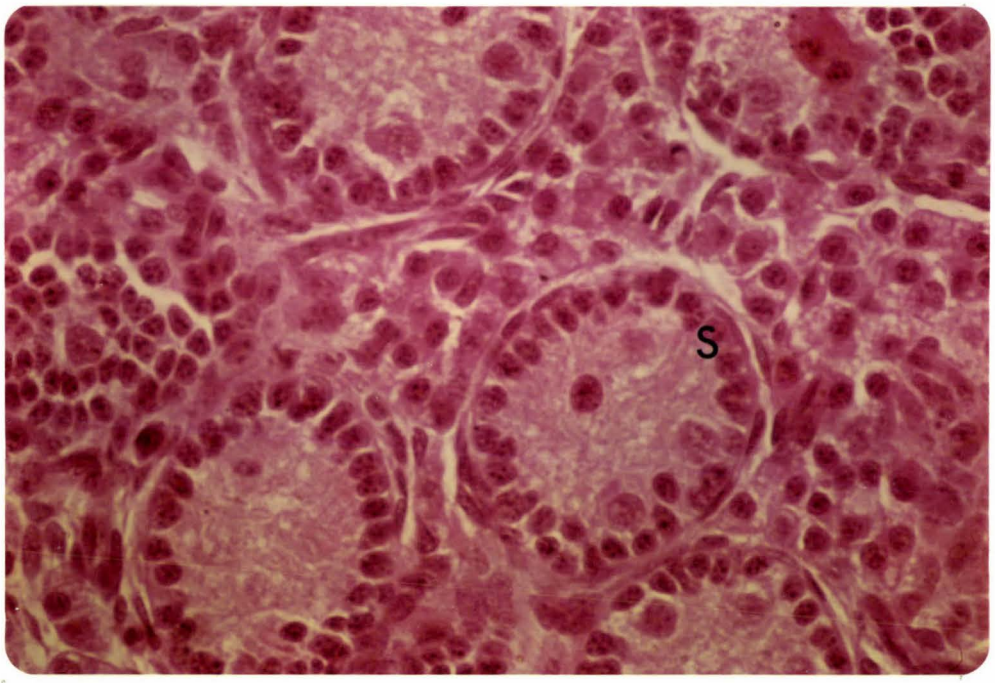
Figure 4.6: Testicular photomicrographs from 4 and 8 week old boars. Magnification x 650; Haematoxylin and Eosin stain. Gonocytes (G), supporting cells (S) and Leydig cells (L).

(a) 4 week old entire

(b) 8 week old entire

(c) 8 week old hemicastrate


G


S

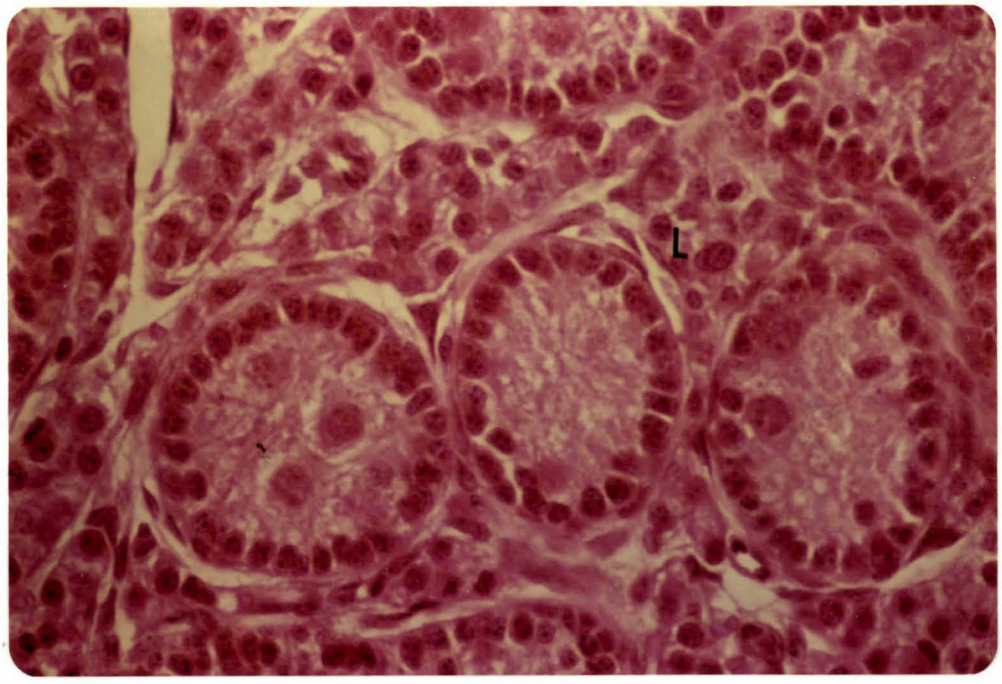
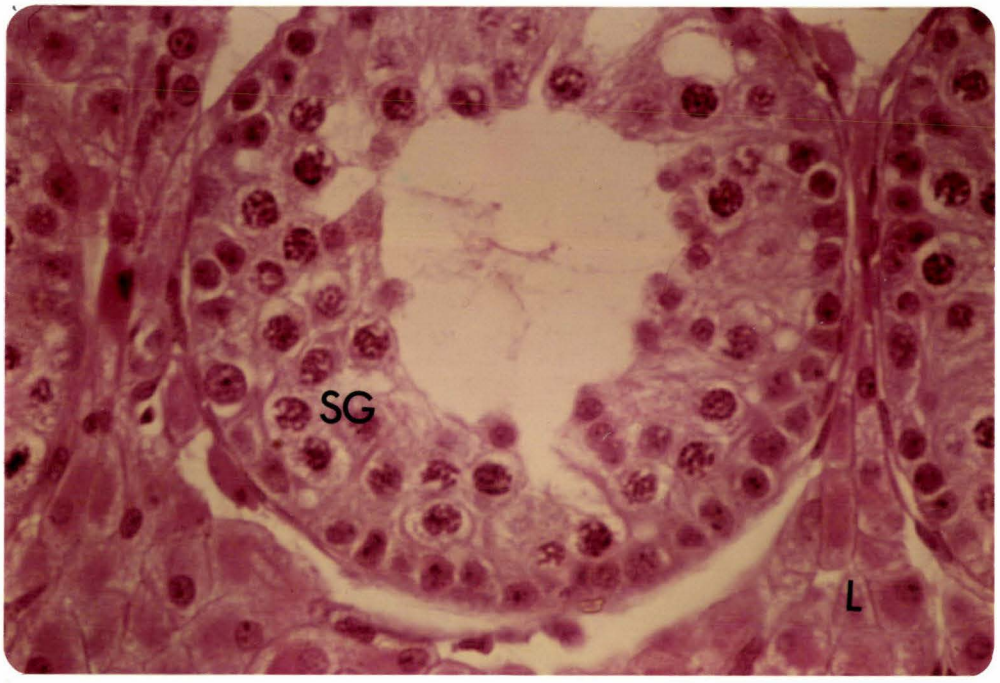

L

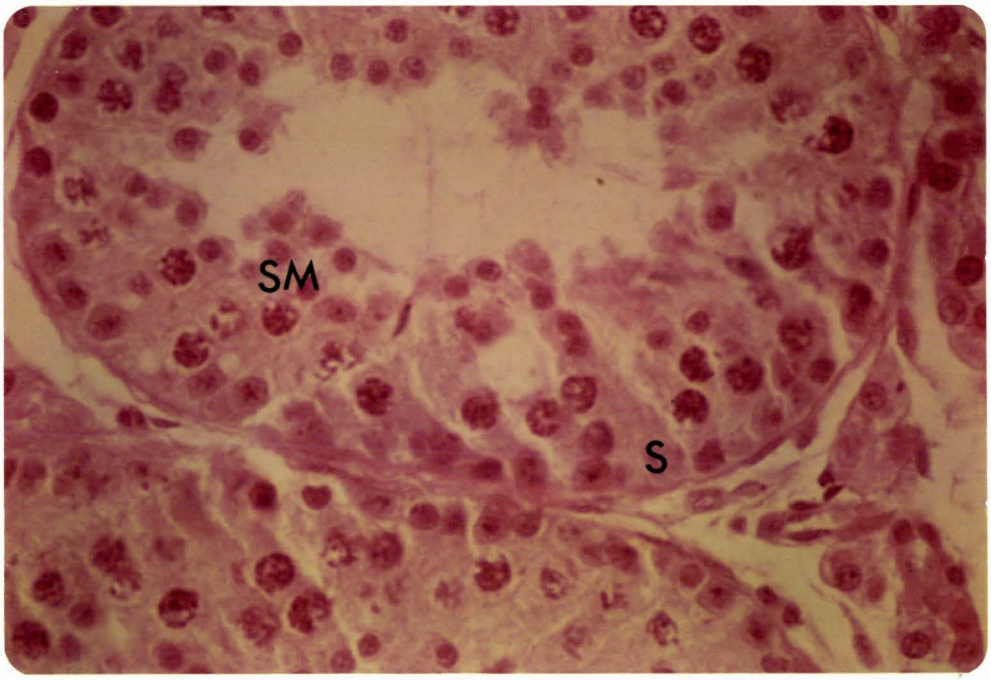
Figure 4.7: Testicular photomicrographs from 12 and 16 week old boars. Magnification x 650; Haematoxylin and Eosin stain. Spermatogonia (SG), spermatocytes (SM), round spermatids (RS), Sertoli cells (S) and Leydig cells (L).

(a) 12 week old entire

(b) 16 week old entire

(c) 16 week old hemicastrate


SG
L


SM
S

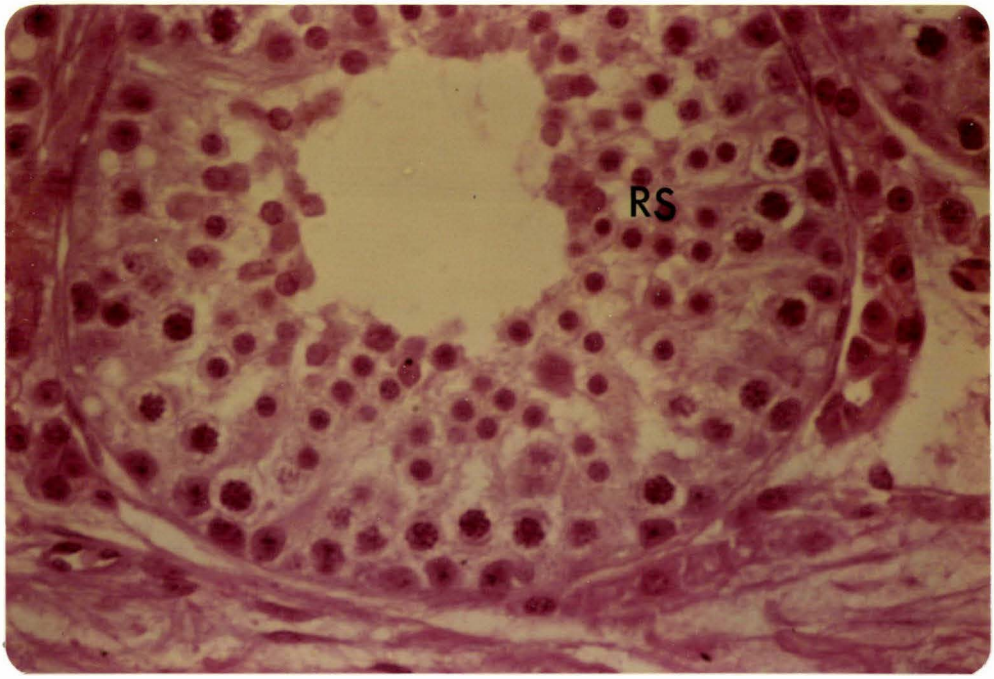
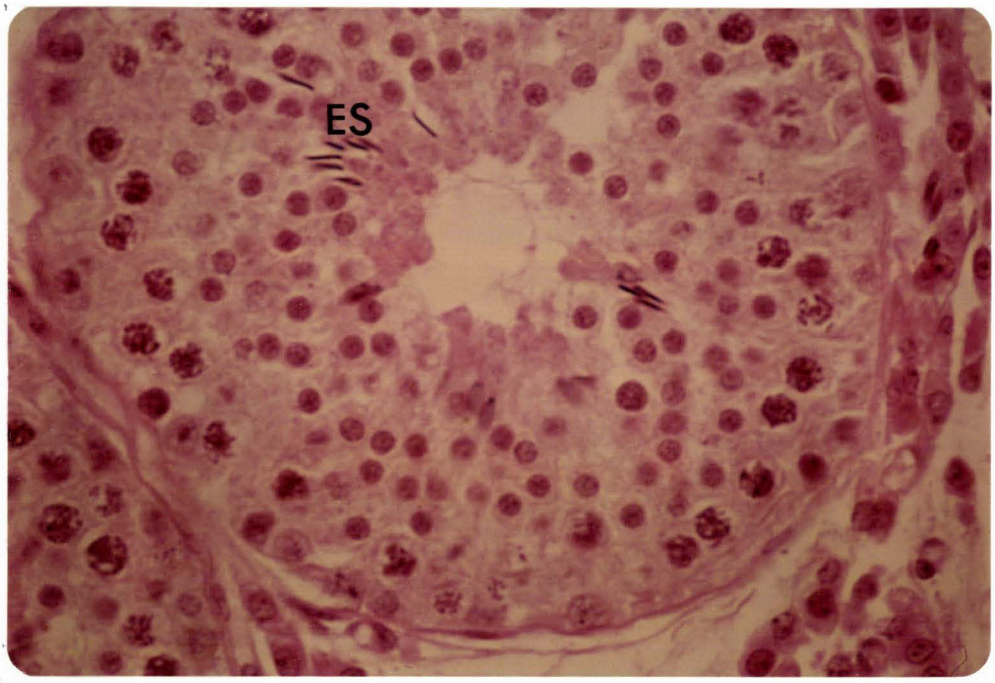

RS

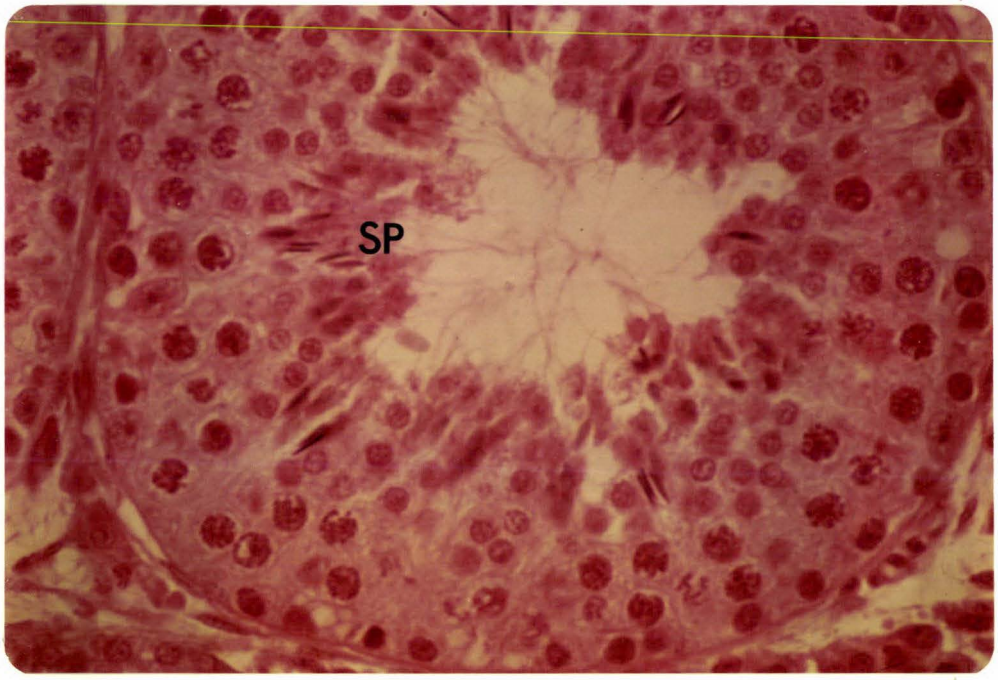
Figure 4.8: Testicular photomicrographs from 20 and 24 week old boars. Magnification x 650; Haematoxylin and Eosin stain. Round spermatids (RS), elongated spermatids (ES) and mature spermatozoa (SP).

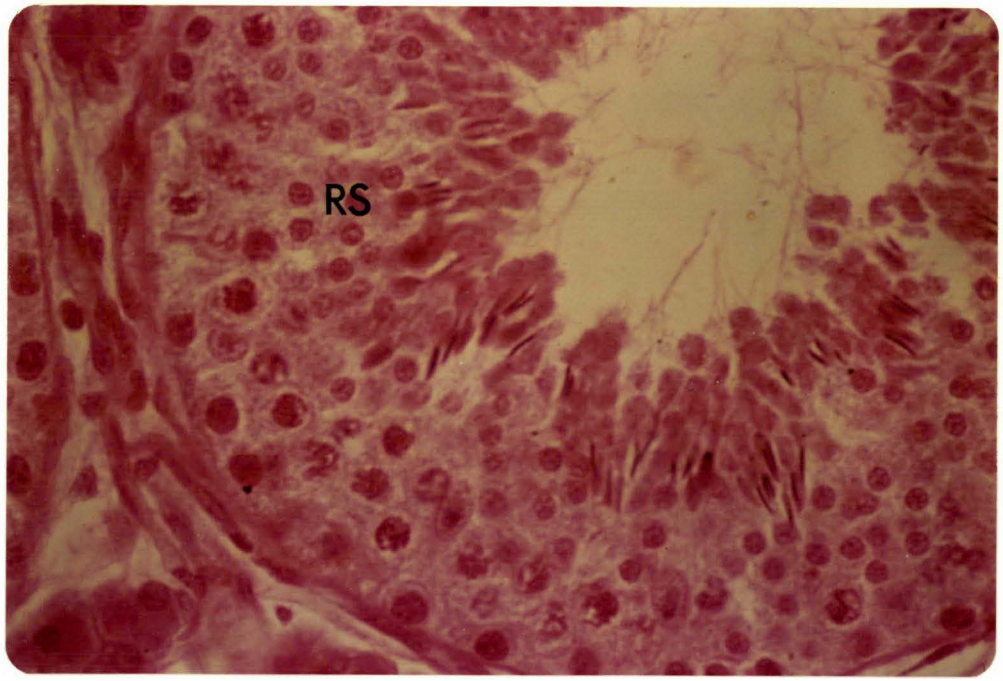
(a) 20 week old entire

(b) 24 week old entire

(c) 24 week old hemicastrate


ES


SP


RS

No qualitative differences in histological appearance were recorded from testes of age-matched control boars castrated at 8 weeks of age.

(ii) 12 and 16 weeks

(Figure 4.7)

In testes obtained at hemicastration at 12 weeks, the germinal cell epithelium showed signs of nuclear divisions and occasional spermatocytes were seen. Supporting cells had started to acquire adult Sertoli cell morphology, while tubular lumina were visible in some seminiferous tubules. The relative volume of interstitial tissue had declined when compared with that seen in testes of 4 and 8 week old boars.

At 16 weeks the remaining testes of hemicastrates and those from entires, displayed distinct seminiferous tubular lumina. In addition to the cell population observed at 12 weeks, primary spermatocytes and few elongated spermatids were seen. Numbers of spermatogonia had increased and mature Sertoli cells were identified in all testes. However, no obvious changes in Leydig cell morphology were evident.

(iii) 20 and 24 weeks

(Figure 4.8)

The microscopic appearance of the testicular tissue at 20 weeks of age was similar to that seen at 16 weeks, but the number of primary spermatocytes had increased and round spermatids were seen towards the lumina. Elongated spermatids were more numerous, while Sertoli cell nuclei had adult size and morphology.

In testes collected from hemicastrates and entires at 24 weeks, the germinal epithelium was much taller. The cell population also had 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 round nuclei. Some testes from both entires and hemicastrates contained spermatozoa.

## 4. Discussion

### (a) Acute Profile Studies

Experiments involving frequent blood sampling techniques have shown that in male animals of several species, including boars of various ages, LH is secreted into the blood in a pulsatile rather than continuous manner (Ellendorff *et al.*, 1975; Sanford *et al.*, 1976*b*; Lapwood and FlorCruz, 1978). In boars pulsatile variations in plasma LH levels have been detected within a few hours after birth (Colenbrander *et al.*, 1977), thus it was not surprising that evidence of episodic release of this hormone was recorded at each sampling age in the present experiment.

Pulsatile LH secretory patterns also were evident at each sampling of hemicastrates and bilateral castrates, but the fluctuations recorded from castrates were of greater magnitude than those of entires or hemicastrates. Similar exaggerated pulsatile variations of plasma LH levels have been reported previously for barrows (Colenbrander *et al.*, 1977) and castrate males of other species (for example, wethers; Riggs and Malven, 1974).

Most episodic releases of LH recorded from boars and hemicastrates aged 8 weeks or more were followed by peaks in plasma testosterone concentrations. A result that concurred with previous reports on secretion of these hormones in pubertal (Lapwood and FlorCruz, 1978) and post-pubertal (Sanford *et al.*, 1976*b*; Lapwood and FlorCruz, 1978) boars. Prior to 8 weeks only about half the LH peaks resulted in elevations in testosterone levels. No previous research appears to have been published on the inter-relationships between the secretion of these two hormones in such young boars. However, it is pertinent to note that while Wilson and Lapwood (1979*a*) observed that in ram lambs older than 14 weeks, LH peaks always were followed by increases in testosterone levels, no such consistent relationship was recorded at 6 weeks.

LH data from the present experiment did not show any

acute effects of hemicastration. The lack of significant differences in LH levels, between age-matched entires and hemicastrates 2 weeks after surgery, probably was an indication that within a short time the remaining testes became capable of secreting sufficient testosterone to maintain near normal LH levels, although those recorded from hemicastrates tended to be higher than those of entires. In the only other similar report on porcine research (Elsaesser et al., 1978) there was no significant effect of unilateral orchidectomy on plasma LH and testosterone levels of Göttingen miniature boars, although again the hemicastrates tended to have elevated LH and lower testosterone levels compared to entires. Similar results also have been recorded from ram lambs (Walton et al., 1978).

One result in the analysis of variance for LH data from the profile experiment provided some evidence that hemicastration influenced the secretion of that hormone. That evidence was the significant difference between LH concentrations of plasma collected 2 and 4 weeks post-treatment. However, it was concluded that this was as much an effect of age as hemicastration. In favour of this conclusion was the fact that at 4 weeks post-hemicastration there was no significant difference between mean LH levels recorded for hemicastrate and entire boars. Also LH concentrations recorded from entires at those two samplings increased, even though non-significantly.

The lack of any significant difference in plasma testosterone concentrations of entires and hemicastrates at 2 and 4 weeks after castration indicated that the remaining testes were secreting more androgens than each testis of entires. Further evidence for rapid compensatory hypersecretion by the remaining testis of hemicastrated boars comes from the studies of Liptrap and Raeside (1971) and Elsaesser et al. (1978). In the latter study near normal testosterone levels were recorded 24 hours after unilateral orchidectomy of Göttingen miniature boars, while in the former study normal dehydroepiandrosterone and oestrogen

levels were recorded in the first urine sample collected after unilateral castration of two adult boars.

Increases in plasma LH levels were recorded 2 weeks after bilateral castrations at 10, 18 and 26 weeks of age, confirming that the steroid negative feedback system was operative at least as early as 10 weeks of age. That result is compatible with the observations made by Ford and Schanbacher (1977) and Colenbrander et al. (1978).

(b) Hormone Responses to GnRH Injection

GnRH administration causes pituitary LH release followed by testicular androgen secretion in male animals of many species (Convey, 1973; Pelletier, 1976) including boars (Elsaesser et al., 1973; Elsaesser et al., 1974; Pomerantz et al., 1972; 1974). Typical gonadotrophic and androgenic secretory responses always followed releasing hormone injection in experiment 4.2.

(i) Pre-injection hormone levels

Pre-injection LH and testosterone concentrations recorded from both entires and hemicastrates corresponded well with hormone levels recorded in the longitudinal (experiment 3.2) and acute profile studies (experiment 4.1). The age-related changes in LH and testosterone levels detected in the present study also agreed with similar changes observed in those longitudinal and acute profile studies.

(ii) Total hormone responses

There was no significant difference in total LH output of hemicastrates and entires following GnRH administration, confirming the observations on LH secretion made in the acute profile study of the present experiment.

Total LH responses were not significantly affected by age in either group of animals. That result confirmed the research of Pomerantz et al. (1974), who reported that the LH responses of Göttingen miniature boars to GnRH stimulation were directly dose-dependent and not affected by age.

However, in ram lambs injected with a single dose of GnRH at 6, 10, 14, 18, 22, 26 and 32 weeks of age, Wilson and Lapwood (1979b) recorded a linear decrease with age in total LH output. Nevertheless, when repeated doses were used, the maximal response was recorded at 14 weeks. At that age the LH content of ram lamb pituitaries is maximal (Skinner et al., 1968; Courot et al., (1975). Collectively, the data discussed in this paragraph indicates the need for further research on variations in boar pituitary gonadotrophin content, and in responses to GnRH, during sexual development.

Hemicastration had no influence on total testosterone secretory responses following GnRH administration. Thus, it would appear that 4 weeks was a sufficient period for hemicastrate testes to increase their androgen secretory capacities up to the total secretory capacities of entire boars. This concept is discussed more fully in relation to similar results which were obtained from hemicastrate rams in the experiments recorded in chapters 5 and 6.

The significant effect of age on total testosterone output is compatible with results from experiments 3.2 and 4.1, which showed that plasma testosterone levels of boars increased with age. Similar effects of age on porcine testosterone responses to GnRH have been recorded in Göttingen miniature boars (Elsaesser et al., 1973; 1974). Likewise Wilson and Lapwood (1979b) found that the total testosterone output of ram lambs increased with age. Such increases with age in testicular steroid secretion may have resulted from increases in Leydig cell secretory activity, as recorded from sexually maturing boars (Wrobel et al., 1973; Van Straatten and Wensing, 1977a; 1978) and/or from increases in numbers of Leydig cell receptors as suggested by Odell and Swerdloff (1976).

(c) Testicular and Epididymal Weights

As cited in the introduction to this chapter, compensatory testicular hypertrophy in hemicastrates, such as observed in the present study, has been reported to occur in a wide range of species including postpubertal boars (Hauser

et al., 1952). No data appears to have been published regarding the effects of this treatment when applied to sexually maturing boars of domestic breeds.

Evidence of compensatory testicular hypertrophy also was provided by the fact that at 8, 16 and 24 weeks seminiferous tubule diameters (STD) of hemicastrates were greater than those of entires. However, despite the fact that the greatest difference in STD's was recorded at the 16 week sampling, it was only at 24 weeks that the difference in tubular diameters was statistically significant. The reason for this apparent contradiction in results at those 2 ages was that there was much greater heterogeneity in STD's at the 16 week sampling. No explanation for that greater variability can be offered although both samplings occurred within the phase of development in which STD's increase most rapidly (Philips and Andrews, 1936; FlorCruz and Lapwood, 1978).

Similarly in bull calves castrated at 1 week of age, significant increases in STD's were detected only in testes recovered 2 and 4 months after treatment (Barnes et al., 1980b). Only one previous paper appears to have been published on the effects of hemicastration on STD's in boars. In that report, Hauser et al. (1952) found that hemicastration of postpubertal boars had no effect on this parameter. However, for many of their experimental animals hemicastrations appear to have been performed at ages when seminiferous tubular diameters already were maximal, or nearly so.

Other testicular parameters which may have contributed to compensatory testicular hypertrophy include increases in seminiferous tubule length and in interstitial tissue volume. Hauser et al. (1952) recorded evidence of increased seminiferous tubule length following hemicastration of post-pubertal boars, but did not measure interstitial tissue volume. However, in young calves (Barnes et al., 1980b) and adult rams (Hochereau-de Reviers et al., 1976) compensatory hypertrophy of the remaining testes included significant increases in interstitial tissue volume.

At 8 weeks of age significant ($P < 0.01$) hypertrophy of epididymides from hemicastrates was evident, but there was no such significant difference at the other 2 sampling ages, nor in the study from postpubertal boars by Hauser et al. (1952). Similarly Johnson (1978) did not observe any significant increase in epididymal weights following hemicastration of pubertal bulls. However, in developing rams (Riesen et al., 1977) and calves (Barnes et al., 1980a) and in adult rams (Johnson et al., 1971; Barrell, G.K. 1980; pers. comm.) significant increases in epididymal weights have been observed following hemicastration.

The endocrine basis of compensatory epididymal hypertrophy in hemicastrates is not clear. Most similar experiments have indicated that testosterone levels in hemicastrates are similar to those of entires, so that hypertrophy cannot be explained in terms of elevated peripheral androgen levels. However, it is clear that hemicastration is followed by an increased flow of rete testis fluid (Voglmayr and Mattner, 1968) which contains high levels of androgens (Waites, 1980). Also it is apparent that the per testes production of testosterone must be higher in hemicastrates than entires. Thus the possibility exists that compensatory hypertrophy is due to increased local androgenic stimulation from rete testis fluid testosterone.

(d) Qualitative Testicular Histology

Only very subjective methods of qualitative histological analysis of testes were used in this experiment because such observations were regarded as being very much secondary to the main thrust of the endocrine study. Given the limitations of that approach, hemicastration and the subsequent compensatory testicular hypertrophy did not appear to cause any major changes in the composition of in seminiferous tubular cell populations. A similar conclusion was reached by Swierstra (1968) who used tritiated thymidine incorporation studies.

On the other hand researchers who have undertaken detailed and systematic histological analyses, and who

allowed much greater time periods between hemicastration and recovery of the second testes, have reported qualitative changes in the germinal cell population associated with compensatory hypertrophy. For example in ram lambs hemicastrated at 6 weeks of age, compensatory hypertrophy of the remaining testes was seen to be associated with increases in Sertoli and spermatogonial cell numbers (Hochereau-de Reviers and Courot, 1978; de Reviers et al. 1980). Similarly hemicastration of impuberal rats or calves resulted in an increase in numbers of Sertoli cells and stem spermatogonia (Hochereau-de Reviers and Courot, 1978; Barnes et al., 1980b) in the remaining adult testis. Hemicastration of adult rams and bulls also has been reported to induce changes in the germinal cell population, but not in Sertoli cell numbers (Hochereau-de Reviers et al., 1976).

Thus, it was apparent that hemicastration of developing boars induced significant compensatory increases in hypersecretion of androgens from the remaining testes and that in turn produced normal peripheral androgen levels in these animals.

# CHAPTER V

## EFFECTS OF HEMICASTRATION ON SECRETION OF LUTEINIZING HORMONE AND TESTOSTERONE, AND ON COMPENSATORY TESTICULAR HYPERTROPHY IN RAM LAMBS.

### 1. Introduction

As mentioned in the introduction to Chapter IV, compensatory testicular hypertrophy has been observed following hemicastration of male animals of a wide range of species, including rams.

Inconsistent results have been recorded in studies of changes in plasma gonadotrophin levels following hemicastration of rams. Some authors have observed an increase in plasma LH concentrations (Hochereau-de Reviers and Pelletier, 1971; Hochereau-de Reviers et al., 1976; Land and Carr, 1975), while others have recorded no change (Riesen et al., 1977; Walton et al., 1978; 1980). In apparently the only detailed accounts of the effects of hemicastration on FSH secretion, Walton et al. (1978; 1980) reported a transient elevation of plasma FSH levels from ram lambs hemicastrated early in life; they claimed that this was responsible for the hypertrophy of the remaining testis.

Compensatory androgen hypersecretion by the remaining testis of hemicastrated rams is well documented (Johnson et al., 1971; Walton et al., 1978; 1980). Similar androgen hypersecretion by the remaining testis of hemicastrates also has been recorded in bulls (Johnson, 1978), boars (experiments described in Chapter IV) and cockerels (Driot et al., 1979).

The major aim of experiments described in this chapter was to examine the effects of hemicastration on secretory profiles of LH and testosterone in developing ram lambs. The effects of this treatment on the pituitary-testicular

endocrine axis were further explored by conducting GnRH response studies four weeks after hemicastration at four different ages. A longitudinal study was undertaken in order to re-examine LH and testosterone secretory patterns in spring born Romney ram lambs between birth and 30 weeks of age.

## 2. Materials and Methods

### (a) Animals

32 ram lambs were identified and allocated randomly to four pairs of treatment groups (A and B, C and D, E and F, and G and H), each group having four animals.

### (b) Longitudinal Study (Experiment 5.1)

The eight ram lambs allocated to groups G and H were utilized in a longitudinal study of hormone secretion from birth to 30 weeks of age. From 28 weeks the number of animals in the experiment was reduced to 4 because these animals were part of another experiment (experiment 5.2).

#### (i) Sampling procedure

Blood samples were obtained every Monday from birth to 30 weeks of age. Sampling commenced at 09.00 h on each occasion. LH and testosterone estimations were performed on all plasma samples.

#### (ii) Body weights

Body weights were recorded each fortnight, from eight weeks to 32 weeks, as a check on body growth.

### (c) Surgical Treatment Protocol (Experiments 5.2 and 5.3)

Ram lambs in groups A, C, E and G were hemicastrated at 4, 12, 20 and 28 weeks of age, respectively, while those in groups B, D, F and H were maintained as age-matched control entires. Four weeks after hemicastrations had been performed the remaining testes of those animals were removed; at the same time animals in the appropriate age-matched control groups were bilaterally castrated. A protocol of surgical procedures is given in table 5.1.

See inside back cover.

Table 5.1: Summary of surgical procedures for experiment 5.2

| Age (weeks) | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|---|---|
| Treatment | | | | | | | | |
| 1. Hemicastration: (HC) | A | | C | | E | | G | |
| 2. Removal of the second testis (RST) | | A | | C | | E | | G |
| 3. Bilateral Castration | | B | | D | | F | | H |

Table 5.2: Summary of ages (weeks) at which blood sampling and surgical procedures were undertaken in experiments 5.2 and 5.3

| Profile No / Treatment Groups | 1 (Pre HC) | 2 (2 weeks POST HC) | 3 (4 weeks POST HC) | 4 (2 weeks post castration) |
|---|---|---|---|---|
| A and B | 4 | 6 | 8 | 10 |
| C and D | 12 | 14 | 16 | 18 |
| E and F | 20 | 22 | 24 | 26 |
| G and H | 28 | 30 | 32 | 34 |

Hemicastration of animals in Groups, A, C, E and G immediately after 1st profile bleeding.

GnRH study in Hemicastrates and control entires followed profile 3, immediately before bilateral castration and removal of second testes.

(d) Acute Profile Study (Experiment 5.2)

Four acute hormone secretion profile studies, in which blood samples were collected each half hour for four hours, were undertaken as indicated in table 5.2. All plasma samples were assayed for concentrations of LH and testosterone.

(e) GnRH Response Study (Experiment 5.3)

Immediately after each profile 3 blood sampling of experiment 5.2, at 8, 16, 24 and 32 weeks of age, but before the subsequent castrations (table 5.2), animals were injected intravenously with GnRH at a dose of 0.5 µg/kg body weight. The required dose was dissolved in 5.0 ml of acidified (0.01 M acetic acid) 0.9% saline and administered via jugular venepuncture as a single rapid injection. Blood samples were obtained immediately prior to injection (pre-injection) and 30, 60, 90, 120, 150, 180, 210 and 240 minutes after the injection. All plasma samples were assayed for concentrations of LH and testosterone.

(f) Organ Weights and Qualitative Histology

Testes and epididymides were weighed, and samples taken for histological processing and subsequent estimations of seminiferous tubular diameters and qualitative histological examination.

(g) Statistical Analyses

All hormonal and body weight data were examined by analyses of variance. Hormone data from experiment 5.1 were analysed only up to 28 weeks of age because of the reduction in number of animals thereafter.

Testicular weights, epididymal weights and seminiferous tubular diameter data from hemicastrates at 8, 16, 24 and 32 weeks were compared with similar data obtained from age-matched controls using Student's t-test.

3. Results

(a) Longitudinal Study (Experiment 5.1)

(i) LH

(Figure 5.1 and table 5.3)

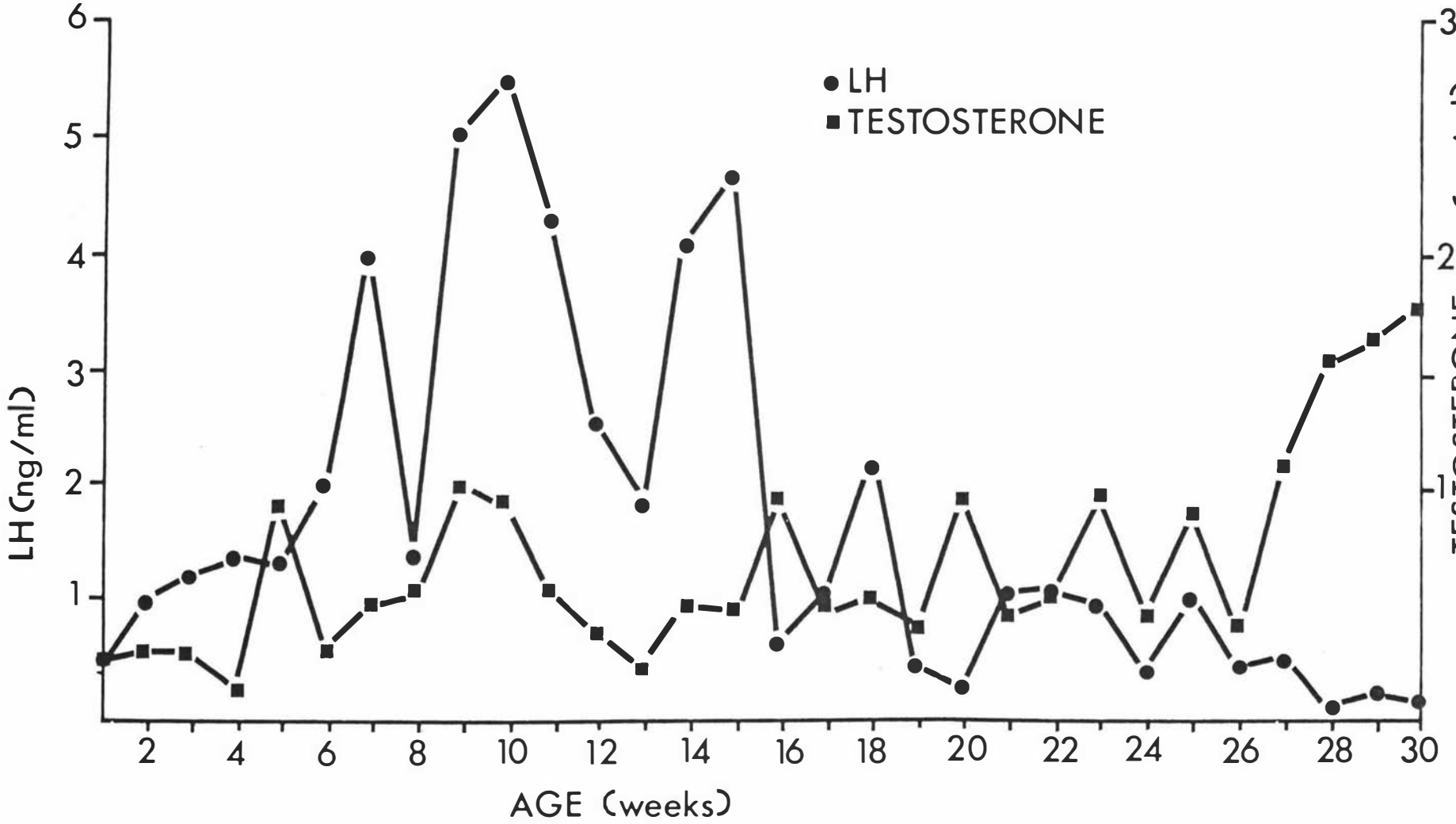



Figure 5.1: Mean plasma LH and testosterone concentrations in ram lambs between 1 and 30 weeks of age (experiment 5.1, n=8).

Table 5.3: Summary of analyses of variance of LH and testosterone data from experiment 5.1

| Source of Variation | DF | Variance Ratios | |
|---|---|---|---|
| | | LH | Testosterone |
| A. Age | 13 | | |
| (i) Linear | 1 | 2.25 | 3.95* |
| (ii) Quadratic | 1 | 20.16*** | 2.12 |
| Remainder | 11 | 0.30 | 0.54 |
| B. Rams | 7 | 0.32 | 2.12 |
| Residual mean square | 91 | 665.68 | 135.38 |

Table 5.4: Summary of analysis of variance of body weight data from experiment 5.1

| Source of Variation | DF | | Variance Ratios |
|---|---|---|---|
| A. Age | 12 | | |
| (i) Linear | | 1 | 1055.02*** |
| Remainder | | 11 | 0.39 |
| B. Rams | 7 | | 0.49 |
| Residual mean square | 84 | | 3.96 |

Mean plasma LH levels recorded during the first 5 weeks were relatively low and ranged between $0.47 \pm 0.14$ and $1.46 \pm 0.77$ ng/ml with a mean level of $1.11 \pm 0.38$ ng/ml. However, in the subsequent 10 weeks concentrations varied between $1.51 \pm 0.47$ and $5.58 \pm 1.88$ ng/ml with a mean of $3.59 \pm 1.45$ ng/ml. Peaks above 4 ng/ml were recorded at 7, 9, 10, 11, 14 and 15 weeks, during a period in which there was a prepubertal elevation of plasma LH levels. From 16 weeks onwards plasma values for LH were lower and relatively stable and ranged between $0.23 \pm 0.04$ and $2.21 \pm 1.09$ ng/ml. These patterns of change in hormonal levels contributed a very highly significant ($P < 0.001$) quadratic component in the analysis of variance.

(ii) Testosterone

(Figure 5.1 and table 5.3)

From birth to 4 weeks of age plasma testosterone levels were low and varied between $0.20 \pm 0.04$ and $0.32 \pm 0.09$ ng/ml with a mean of $0.27 \pm 0.03$ ng/ml. Between 5 and 26 weeks of age the levels fluctuated between $0.24 \pm 0.07$ and $1.04 \pm 0.39$ ng/ml with a higher mean concentration ($0.63 \pm 0.05$ ng/ml). The levels then increased progressively from $1.18 \pm 0.82$ ng/ml at 27 weeks of age to $1.74 \pm 0.27$ ng/ml at 32 weeks and the mean hormone concentration during this period was $1.52 \pm 0.12$ ng/ml. In the analysis of variance of age effects on testosterone levels this pattern of change showed up as a significant ($P < 0.05$) linear component.

(iii) Body weights

(Table 5.4)

Mean body weights increased progressively from $13.48 \pm 0.98$ kg at 8 weeks to $34.38 \pm 0.52$ kg at 32 weeks. The analysis of variance revealed a very highly significant ($P < 0.001$) linear effect of age on body weights.

(b) Acute Profile Study (Experiment 5.2)

(i) LH

(Figure 5.2, tables 5.5 and 5.6)

Evidence of pulsatile LH secretion was obtained from

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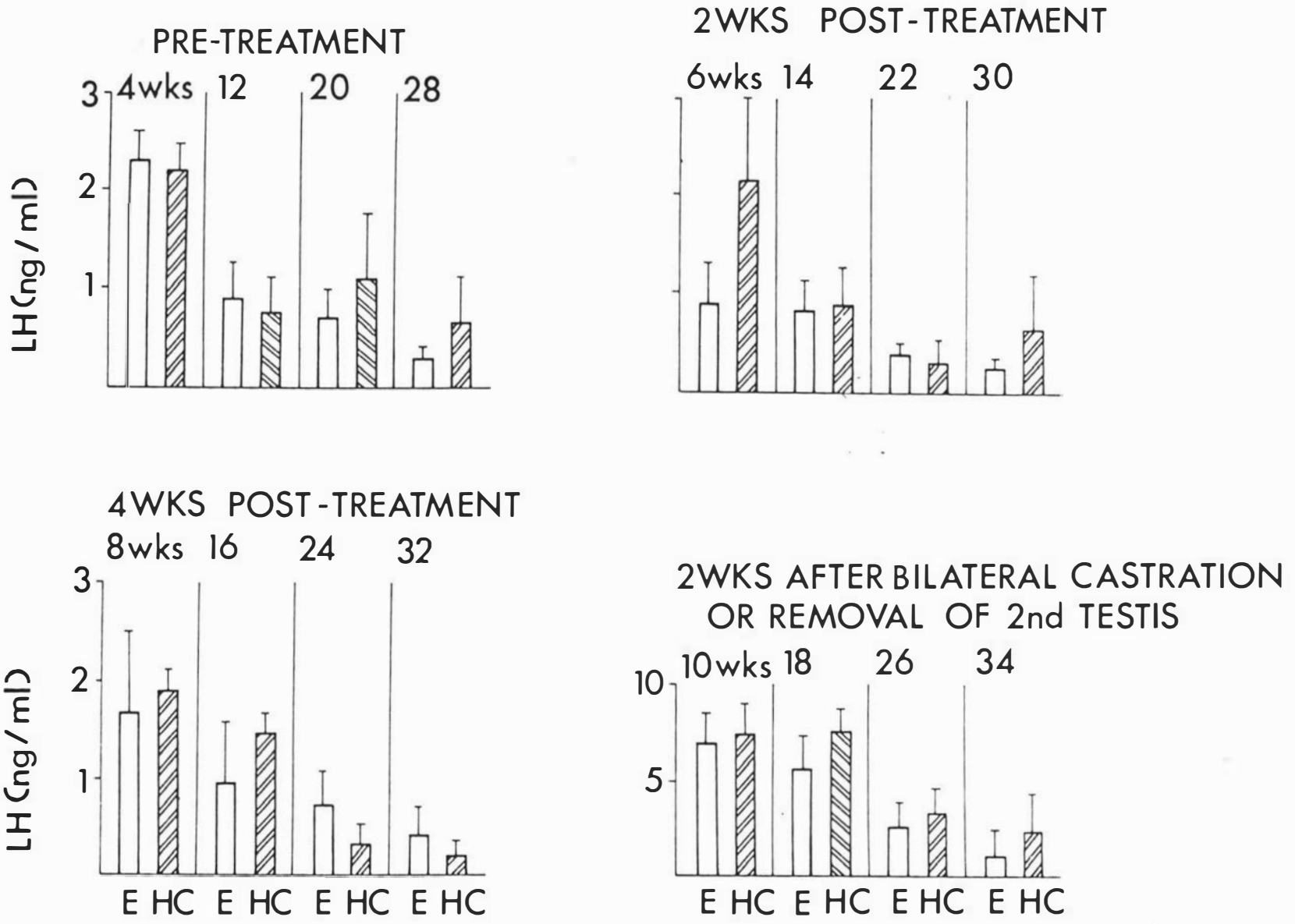



Figure 5.2: Mean (±SEM) plasma LH levels recorded from entire (E) and hemicastrate (HC) ram lambs during acute profile studies conducted at the ages indicated (experiment 5.2, n=4).

Table 5.5: Mean (±SEM) LH concentrations (ng/ml) of hemicastrate and entire lambs in experiment 5.2

| Group | Age (weeks) at Profile 1 ¶ | Profile 1 | Profile 2 | Profile 3 | Profile 4 |
|---|---|---|---|---|---|
| | | | HEMICASTRATES | | |
| A | 4 | 2.31±0.18 | 2.16±0.98 | 1.90±0.23 | 7.50±1.36 |
| C | 12 | 0.74±0.37 | 0.83±0.27 | 1.48±0.19 | 7.97±0.83 |
| E | 20 | 1.13±0.68 | 0.33±0.09 | 0.27±0.19 | 3.43±2.78 |
| G | 28 | 0.75±0.48 | 0.67±0.54 | 0.19±0.09 | 2.50±1.83 |
| Overall Mean | | 1.24±0.26 | 0.99±0.33 | 0.96±0.10 | 5.35±1.06 |
| | | | ENTIRES | | |
| B | 4 | 2.34±0.22 | 0.90±0.38 | 1.73±0.84 | 7.05±1.63 |
| D | 12 | 0.91±0.36 | 0.82±0.35 | 0.98±0.59 | 5.83±1.38 |
| F | 20 | 0.68±0.29 | 0.38±0.18 | 0.73±0.52 | 2.47±1.14 |
| H | 28 | 0.26±0.06 | 0.28±0.13 | 0.41±0.29 | 1.10±0.74 |
| Overall Mean | | 1.05±0.14 | 0.60±0.16 | 0.96±0.34 | 4.11±0.73 |

¶ Subsequent profile samplings were undertaken at 2 weekly intervals.

Table 5.6: Summary of analysis of variance of LH data from experiment 5.2

| Source of Variation | | DF | Variance Ratios |
|---|---|---|---|
| A. Treatment and Sampling | | 7 | |
| (i) | Pre vs Post-treatment | 1 | 11.12** |
| (ii) | 2 weeks & 4 weeks post-treatment vs 6 weeks post-treatment | 1 | 117.12*** |
| | Remainder | 5 | 0.52 |
| B. Age | | 3 | |
| (i) | Linear | 1 | 46.55*** |
| | Remainder | 2 | 1.71 |
| Interaction | | | |
| Treatment & Sampling x Age | | 21 | |
| (i) | Pretreatment vs Post-treatment x linear | 1 | 7.50** |
| (ii) | 2 & 4 weeks post-treatment vs 6 weeks post-treatment x linear | 1 | 9.72** |
| (iii) | 2 & 4 weeks post-treatment vs 6 weeks post-treatment x quadratic | 1 | 4.75* |
| (iv) | Control vs hemicastrate: 2 weeks post-castration or removal of second testes x linear | 1 | 4.50* |
| | Remainder | 17 | 0.37 |
| Residual mean square | | 96 | 235.17 |

entire, hemicastrate and castrate animals during the 4 hour profile studies. While the magnitude of secretory pulses observed in castrates was larger than for entires or hemicastrates, pulse frequency was not influenced by treatment.

At the pre-hemicastration sampling there was no significant difference between mean plasma LH levels recorded from ram lambs allocated to the hemicastrate ($1.24 \pm 0.37$ ng/ml) or entire ($1.05 \pm 0.45$ ng/ml) treatment groups. Likewise at the fourth profile sampling (i.e. after bilateral castration of entires and removal of the second testes of hemicastrates) mean plasma LH levels of the former entires and hemicastrates were not significantly different.

Hemicastration did not cause any significant alteration of average LH levels at either of the 2 or 4 week post-treatment bleedings. By way of contrast a very substantial elevation of LH concentrations was recorded following complete castration of entires (levels rose from $0.96 \pm 0.28$ to $4.11 \pm 1.39$ ng/ml) and hemicastrates (levels increased from $0.96 \pm 0.43$ to $5.35 \pm 1.39$ ng/ml).

Overall mean LH levels decreased linearly with age ($P < 0.001$). This effect was evident at each profile bleeding, but was much more pronounced after bilateral castration (profile 4) than after the first profile bleeding; that differential effect of age was the principal reason for significant components in the treatments x age interaction in the analysis of variance of LH data.

(ii) Testosterone

(Figure 5.3, tables 5.7 and 5.8)

Evidence of episodic testosterone secretion also was recorded from entires and hemicastrates at all ages in profiles 1, 2 and 3. Up to 8 weeks of age, a total of 43 LH peaks was recorded from hemicastrates and entires, but only 34 were followed by testosterone peaks. In contrast, after 8 weeks of age a total of 109 LH peaks was recorded and all were followed by testosterone peaks.

Overall mean pre-treatment testosterone levels in

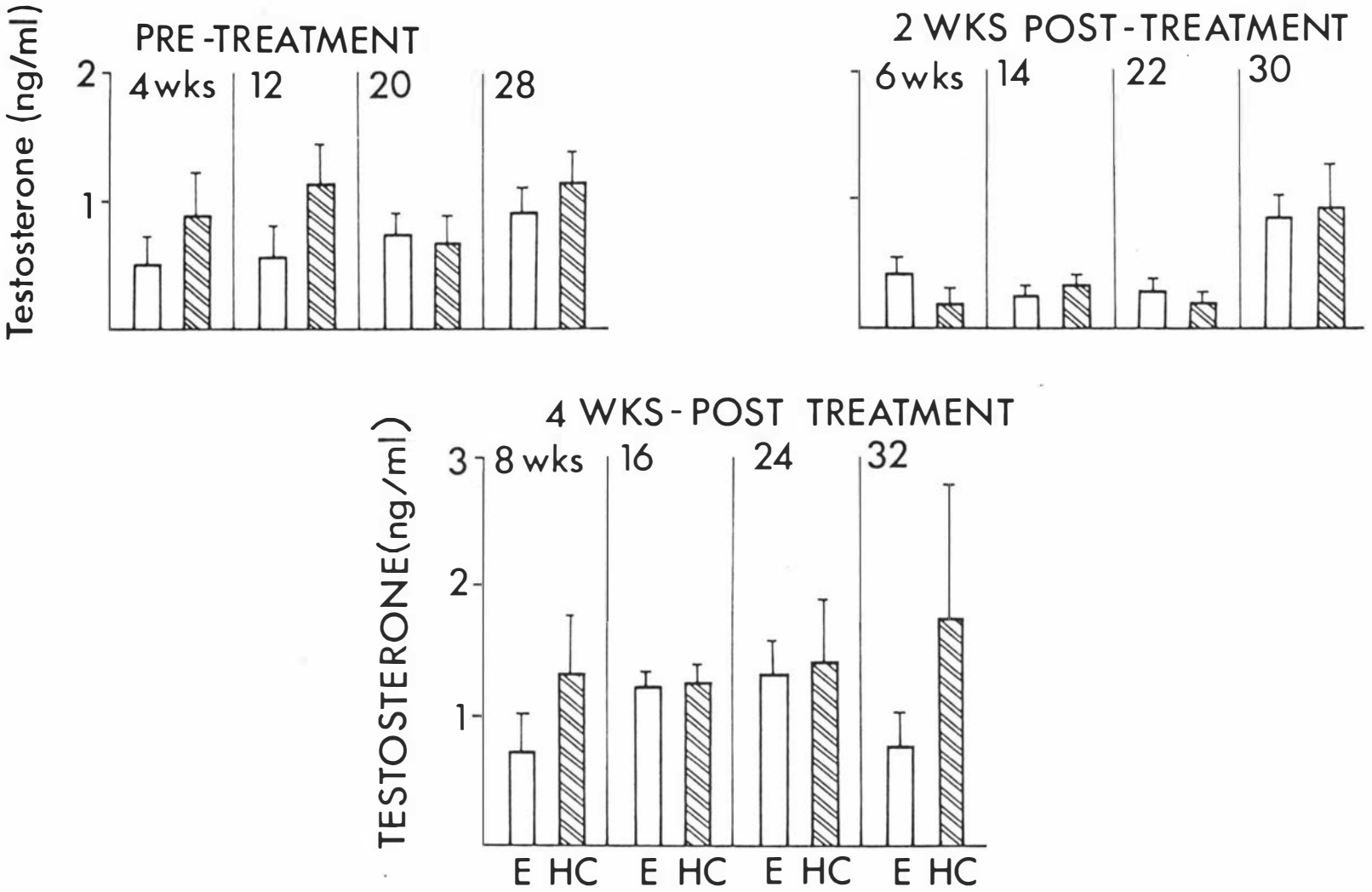



Figure 5.3: Mean (±SEM) plasma testosterone levels recorded from entire (E) and hemicastrate (HC) ram lambs during acute profile studies at the ages indicated (experiment 5.2, n=4).

Table 5.7: Mean (±SEM) testosterone concentrations (ng/ml) of lambs in experiment 5.2

| Group | Age (weeks) at Profile 1 ¶ | Profile 1 | Profile 2 | Profile 3 |
|---|---|---|---|---|
| | | | HEMICASTRATES | |
| A | 4 | 0.93±0.32 | 0.17±0.07 | 1.32±0.46 |
| C | 12 | 1.42±0.32 | 0.36±0.07 | 1.26±0.15 |
| E | 20 | 0.68±0.23 | 0.17±0.03 | 1.43±0.83 |
| G | 28 | 1.12±0.25 | 0.98±0.35 | 1.77±1.04 |
| Overall Mean | | 1.04±0.16 | 0.42±0.10 | 1.45±0.40 |
| | | | ENTIRES | |
| B | 4 | 0.51±0.18 | 0.41±0.18 | 0.74±0.26 |
| D | 12 | 0.53±0.30 | 0.07±0.05 | 1.25±0.09 |
| F | 20 | 0.73±0.18 | 0.29±0.07 | 1.36±0.22 |
| H | 28 | 0.90±0.17 | 0.86±0.22 | 0.78±0.25 |
| Overall Mean | | 0.68±0.12 | 0.41±0.07 | 1.03±0.12 |

¶ Subsequent profile samplings were undertaken at 2 weekly intervals.

Table 5.8: Summary of analysis of variance of testosterone data from experiment 5.2

| Source of Variation | DF | | Variance Ratios |
|---|---|---|---|
| A. Treatment and Sampling | 5 | | |
| (i) 2 weeks post-treatment vs 4 weeks post-treatment | | 1 | 30.69*** |
| (ii) Remainder | | 4 | 0.16 |
| B. Ages | 3 | | 0.76 |
| Interaction | | | |
| Treatment and Sampling x Age | 15 | | |
| (i) Pretreatment vs Post-treatment x quadratic | | 1 | 5.04* |
| (ii) 4 weeks post-treatment: Control vs hemicastrate x quadratic | | 1 | 4.96* |
| (iii) Remainder | | 13 | 0.76 |
| Residual mean square | 72 | | 88.06 |

animals allocated to hemicastrate and entire treatment groups were not significantly different.

As judged by the non-significance of comparisons between testosterone levels recorded from hemicastrates and entires, both at 2 weeks and also 4 weeks after treatment, it was evident that hemicastration had no effect on testosterone levels. By way of contrast animals from both treatment groups had significantly higher ($P < 0.001$) androgen levels 4 weeks after sampling started than at 2 weeks (mean values were $0.42 \pm 0.19$ ng/ml at 2 weeks and $1.45 \pm 0.11$ ng/ml at 4 weeks for hemicastrates, and $0.41 \pm 0.17$ and $1.03 \pm 0.16$ ng/ml for entires).

In the analyses of variance the 'age' main effect was not significant.

Two components of the treatment x age interaction were significant at the 5% level, but detailed examination of the data involved revealed that these components were of little, if any, physiological importance.

(c) GnRH Responses (Experiment 5.3)

Plasma concentrations of both LH and testosterone always increased markedly following the injection of GnRH into entires and hemicastrates.

(i) LH

(Figures 5.4-5.7 and tables 5.9 and 5.10)

At each age mean LH levels prior to GnRH injection were similar to those observed in experiments 5.1 and 5.2. For hemicastrates these levels were highest at 8 weeks ($0.93 \pm 0.28$ ng/ml), then declined progressively to $0.15 \pm 0.07$ ng/ml at 32 weeks. Comparable values for entires fell from $0.41 \pm 0.08$ ng/ml at 8 weeks to $0.10 \pm 0.01$ ng/ml at 32 weeks. These patterns of change were represented by a significant ($P < 0.05$) linear component in the analysis of the effects of age on pre-injection LH concentrations.

Total LH output of hemicastrates was significantly ($P < 0.05$) higher than that of entires. In both groups of animals total responses were maximal at 16 weeks of age, then

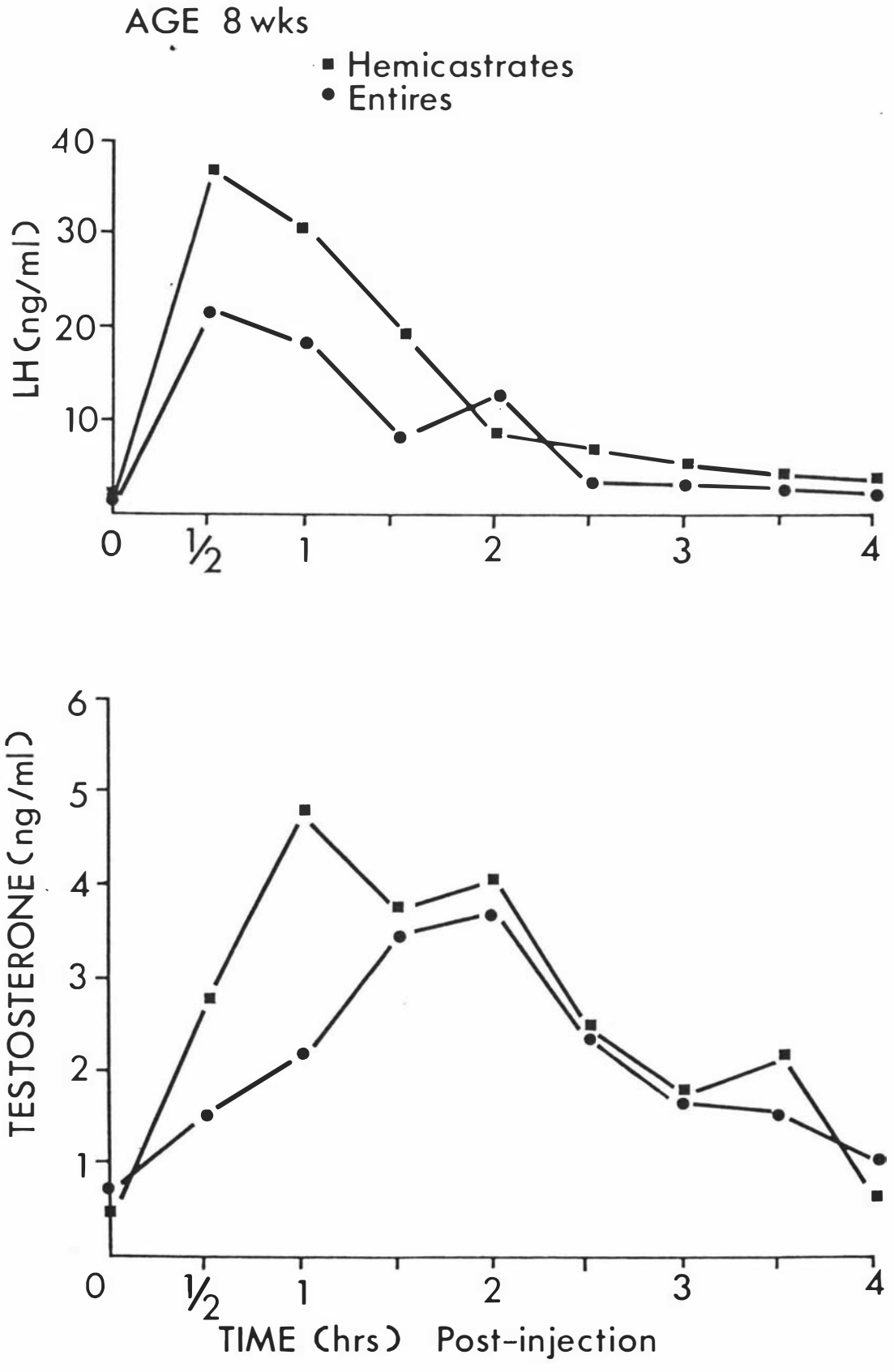



Figure 5.4: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate ram lambs at 8 weeks of age (experiment 5.3, n=4).

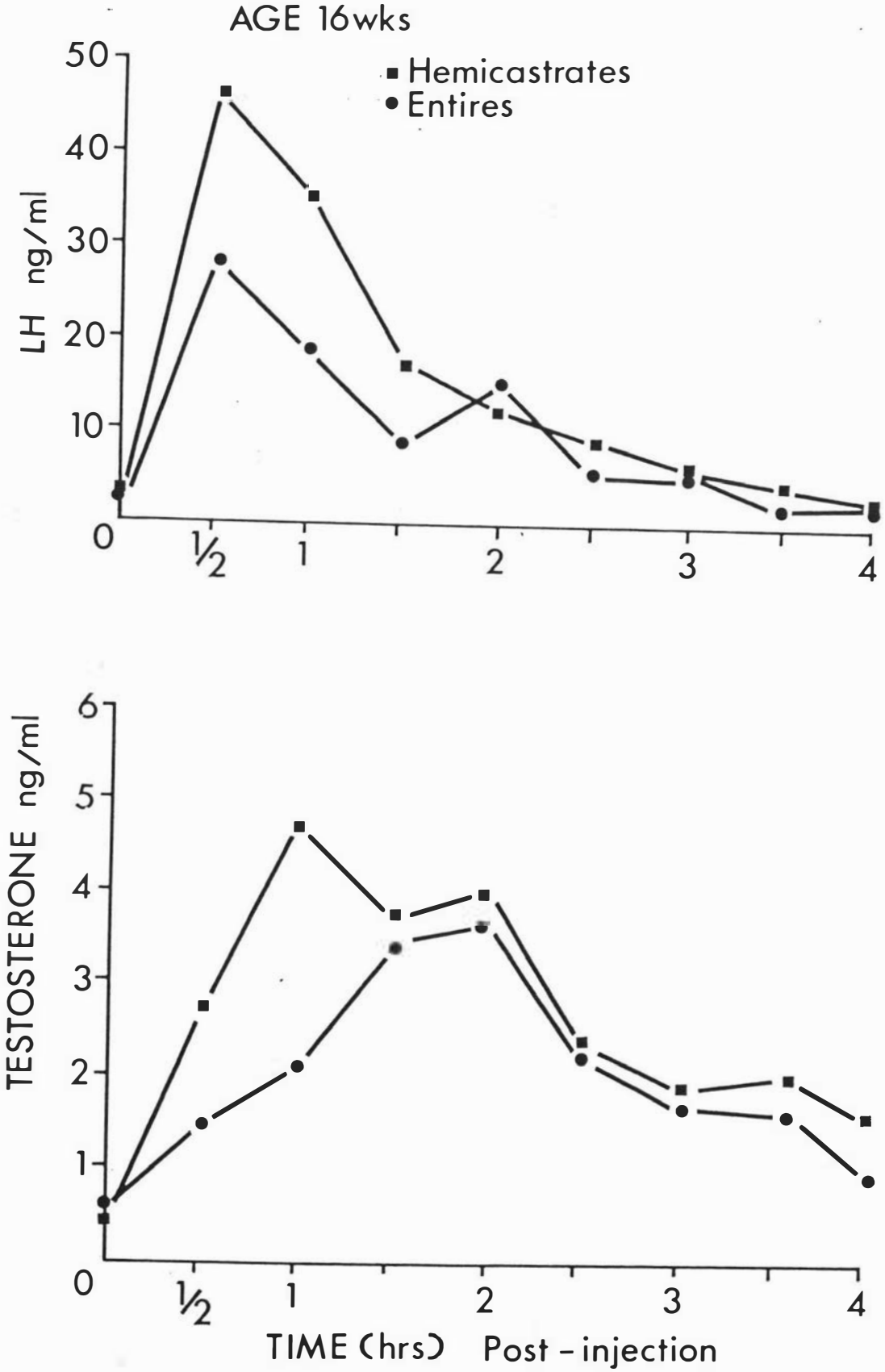



Figure 5.5: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate ram lambs at 16 weeks of age (experiment 5.3, n=4).

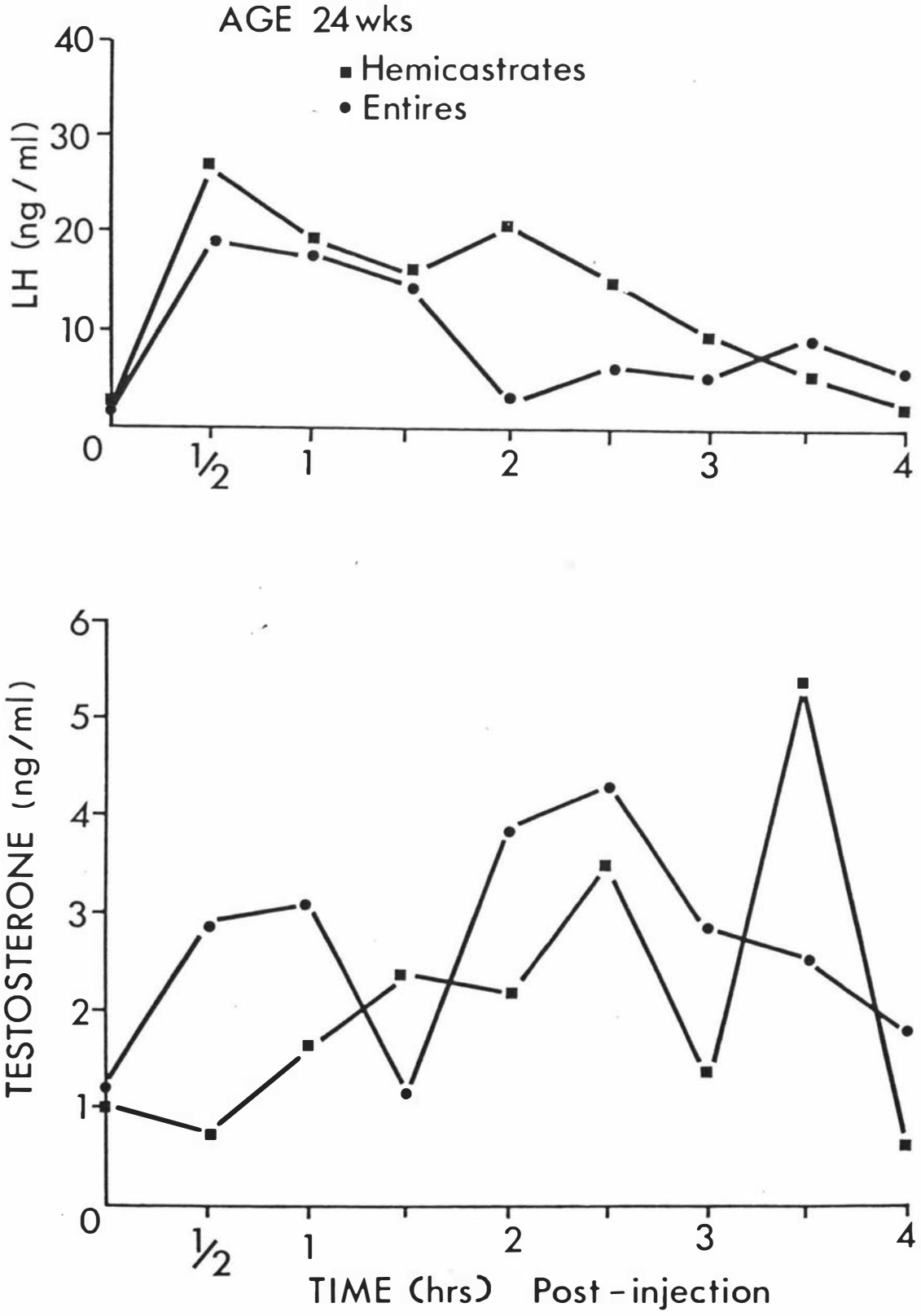



Figure 5.6: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate ram lambs at 24 weeks of age (experiment 5.3, n=4).

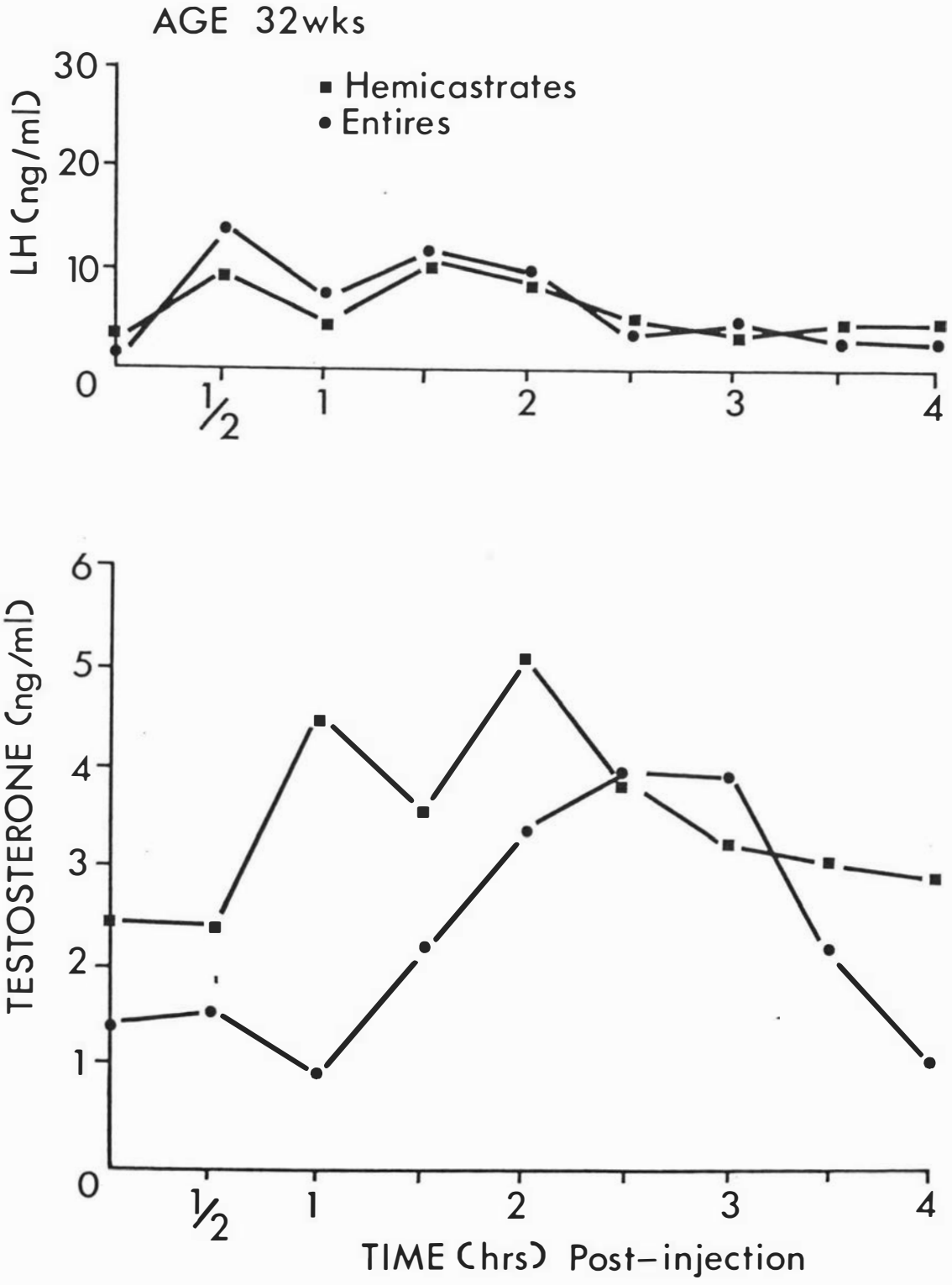



Figure 5.7: Plasma LH and testosterone responses following administration of GnRH (0.5 µg/kg) to entire and hemicastrate ram lambs at 24 weeks of age (experiment 5.3, n=4).

Table 5.9: Mean (±SEM) pre-GnRH injection LH and testosterone levels (ng/ml), and total LH and testosterone responses (ng/ml.hr) in experiment 5.3

| Age (weeks) | LH | | Testosterone | |
|---|---|---|---|---|
| | Entires | Hemicastrates | Entires | Hemicastrates |
| | | PREINJECTION HORMONE LEVELS | | |
| 8 | 0.41±0.08 | 0.93±0.28 | 0.65±0.29 | 0.50±0.05 |
| 16 | 0.17±0.06 | 0.83±0.39 | 0.54±0.24 | 0.43±0.18 |
| 24 | 0.17±0.16 | 0.20±0.01 | 1.16±0.14 | 1.00±0.19 |
| 32 | 0.10±0.01 | 0.15±0.07 | 1.38±0.51 | 2.45±1.13 |
| Overall Mean | 0.21±0.05 | 0.53±0.14 | 0.93±0.18 | 1.09±0.33 |
| | | TOTAL HORMONE RESPONSES | | |
| 8 | 149.35±11.19 | 251.90±3.76 | 32.25±1.16 | 37.92±1.89 |
| 16 | 172.48±4.83 | 267.80±7.20 | 33.57±3.11 | 43.06±1.25 |
| 24 | 156.25±5.15 | 221.44±12.70 | 45.67±2.14 | 39.77±3.93 |
| 32 | 89.19±2.86 | 86.21±4.47 | 48.38±4.28 | 63.70±3.90 |
| Overall Mean | 141.81±3.90 | 206.84±4.53 | 39.96±1.68 | 46.11±1.73 |

Table 5.10: Summary of analyses of variance of basal and total LH and testosterone data from experiment 5.3

| Source of Variation | DF | | Variance Ratios | | | |
|---|---|---|---|---|---|---|
| | | | LH | | Testosterone | |
| | | | total | basal | total | basal |
| A. Treatment | | | | | | |
| (Control vs Hemicastrate) | 1 | | 7.25* | 2.69 | 2.96 | 0.01 |
| B. Age | 3 | | | | | |
| (i) Linear | | 1 | 11.73** | 4.27* | 5.08* | 4.46* |
| (ii) Quadratic | | 1 | 4.34* | 0.58 | 0.20 | 2.01 |
| (iii) Cubic | | 1 | 0.96 | 0.26 | 0.58 | 0.63 |
| Interaction | | | | | | |
| Treatment x Age | 3 | | 0.99 | 0.98 | 0.90 | 0.09 |
| Residual mean square | 24 | | 291.58 | 288.38 | 12.67 | 476.38 |

declined with advancing sexual maturation, particularly at 32 weeks of age (table 5.9). This pattern of changes with age for total LH responses was represented by significant linear ($P < 0.01$) and quadratic ($P < 0.05$) components in the analysis of variance.

(ii) Testosterone

(Figures 5.4-5.7 and tables 5.9 and 5.10)

Mean pre-injection testosterone levels were low at 8 and 16 weeks of age: values for hemicastrates were $0.50 \pm 0.05$ ng/ml at 8 weeks and $0.43 \pm 0.18$ ng/ml at 16 weeks, while those for entires were $0.65 \pm 0.29$ ng/ml at 8 weeks and $0.54 \pm 0.24$ ng/ml at 16 weeks. At 24 and 32 weeks of age concentrations for hemicastrates increased from $1.00 \pm 0.19$ to $2.45 \pm 1.13$ ng/ml, while those for entires increased from $1.16 \pm 0.14$ to $1.38 \pm 0.51$ ng/ml. Thus in the analysis of variance the linear component of the age main effect was significant ($P < 0.05$). Hemicastration had no effect on total testosterone responses, however this parameter increased significantly ($P < 0.05$) with age.

(d) Organ Weights

(Tables 5.11 and 5.12)

Analysis of testicular and epididymal weights and of seminiferous tubular diameter data, from organs obtained at the time of removal of first testes of hemicastrates and bilateral castration of entires, indicated that these parameters increased linearly with age.

The second testes of hemicastrates were significantly heavier ($P < 0.001$) than those from age-matched control entires. This compensatory increase in testicular weights was much higher at 8 and 16 weeks (increases of 486% and 271.71%, respectively) than at 24 and 32 weeks (increases of 116.16% and 56.65%, respectively) of age.

Epididymal weights recorded from hemicastrates at the time of removal of second testes were greater than those of control entires. However, this difference reached statistically significant levels only at 8 weeks ($P < 0.05$). The

Table 5.11: Mean (±SEM) testicular and epididymal weights (g) from hemicastrates (HC) and entire rams, and t-test values from experiment 5.2.

| PARAMETER \ Age (wks) | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|---|---|
| **TESTICULAR** | | | | | | | | |
| Weight of 1st testes HC | 2.83±0.36 | | 20.44±0.42 | | 48.08±3.24 | | 95.96±4.18 | |
| Weight of 2nd testes HC | | 16.61±1.23 | | 75.99±2.89 | | 103.93±1.99 | | 150.33±3.45 |
| Mean weight of testes of entires | | 6.70±0.35 | | 38.25±2.66 | | 83.54±3.36 | | 106.50±6.94 |
| Ratio of Weights: | | | | | | | | |
| $\frac{\text{Weight of 2nd testes HC}}{\text{Mean weight of testes of entires}}$ | | 2.48 | | 1.98 | | 1.24 | | 1.41 |
| t-test values (10 DF) † | | 5.81,P<0.001 | | 8.13,P<0.001 | | 4.78,P<0.001 | | 5.26,P<0.001 |
| **EPIDIDYMAL** | | | | | | | | |
| Weight of 1st epididymides HC | 1.41±0.39 | | 4.74±0.56 | | 8.90±0.84 | | 15.67±0.96 | |
| Weight of 2nd epididymides HC | | 4.70±0.91 | | 10.99±1.41 | | 13.04±1.18 | | 17.98±2.42 |
| Mean weight of epididymides of entires | | 2.72±0.51 | | 7.98±1.21 | | 10.16±1.44 | | 17.14±1.08 |
| Ratio of weights: | | | | | | | | |
| $\frac{\text{Weight of 2nd epididymides HC}}{\text{Mean weight of epididymides of entires}}$ | | 1.73 | | 1.37 | | 1.28 | | 1.05 |
| t-test values (10 DF) ¶ | | 2.82,P<0.05 | | 1.00,P>0.05 | | 1.39,P>0.05 | | 0.47,P>0.05 |

† t-test values are for comparisons between mean weights of hemicastrate 2nd testes vs mean weight of testes from entires.

¶ t-test values are for comparisons between mean weights of hemicastrate 2nd epididymides vs mean weight of epididymides from entires.

Table 5.12: Mean (±SEM) seminiferous tubular diameter (STD-μm) from hemicastrates (HC) and entires, and t-test values from experiment 5.2

| Age (wks) / PARAMETER | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|---|---|
| STD 1st testes HC | 46.96±2.13 | | 83.62±5.35 | | 132.88±4.01 | | 146.26± 7.59 | |
| STD 2nd testes HC | | 89.55±3.90 | | 155.50±3.60 | | 165.50±3.60 | | 173.30±1.17 |
| STD entires | | 50.97±0.69 | | 118.7 ±4.18 | | 140.29±3.85 | | 160.13±1.52 |
| Ratio of STD's: | | | | | | | | |
| STD of 2nd testes HC / STD entires | | 1.76 | | 1.31 | | 1.18 | | 1.08 |
| t-test values (10 DF) † | | 5.37, P<0.001 | | 5.92, P<0.001 | | 2.66, P<0.05 | | 3.12, P<0.05 |

† t-test values are for comparisons between mean STD of second testes of hemicastrates vs mean STD of entires.

compensatory hypertrophy of the remaining epididymides was maximal at 8 weeks ( 72.79%) and least at 32 weeks ( 4.90%).

Similarly, hemicastration induced significant increases in seminiferous tubular diameters at each age. Increases in seminiferous tubular diameters also were maximal at 8 weeks (75.69%) and minimal ( 8.22%) at 32 weeks.

(e) Qualitative Testicular Histology

In general maturational changes could be attributed entirely to age at castration or hemicastration; hemicastration had no effect in accelerating the initiation of spermatogenesis. The principal qualitative histological features of testes collected at each age are summarized below. Those features corresponded closely with those of testes from the entire and hemicastrate rams utilized in chapter 6 and illustrated in photomicrographs in figures 6.29-6.32.

(i) 4 weeks

At four weeks of age seminiferous tubules consisted of solid sex cords. Supporting cells lined the basement membrane, while some gonocytes and a few dividing prospermatogonia were present in both central and eccentric locations. Interstitial spaces were filled with Leydig cells, some of which contained eosinophilic cytoplasmic granules. Fibroblasts and blood vessels also were identified in the interstitial spaces.

(ii) 8 weeks

Testes obtained by the removal of second testes from hemicastrates, and by bilateral castration of entires, were very similar in qualitative histological structure. Seminiferous tubular lumina had not formed in either group of animals, but nuclei of supporting cells were starting to acquire the shape typical of adult Sertoli cells. Actively dividing prospermatogonia were seen in both groups and the majority of these cells were located close to the basement membrane. Interstitial spaces were still packed with Leydig cells, but the volume of that tissue appeared relatively less than at four weeks.

(iii) 12 weeks

At 12 weeks of age most tubules had well defined lumina and germ cells were all in peripheral positions. In most tubules the germinal cell layer showed signs of active cell division. Some tubules contained primary spermatocytes. Sertoli cells were interspaced between germ cells and cell boundaries were ill-defined. Leydig cells formed the major part of the interstitial spaces which now contained greater numbers of blood vessels.

(iv) 16 weeks

Testes from hemicastrates and entires contained large numbers of primary spermatocytes which were undergoing nuclear division. Most seminiferous tubules had lumina, but there were a few tubules from both groups that lacked lumina. In some tubules from both groups a few darkly stained elongated spermatids were identified. Leydig cells were larger and contained deeply stained nucleoli.

(v) 20 weeks

Most of the tubules contained both early and late primary spermatocytes. Darkly stained elongated spermatids were more frequent than at 16 weeks, while some tubules also contained spermatozoa. Sertoli cells had triangular shaped nuclei and pale staining cytoplasm. Leydig cells were large and had deeply staining nucleoli. The interstitial space had a relatively large number of blood vessels.

(vi) 24 weeks

Testes from both hemicastrates and entires had tubules containing spermatozoa. A wide variety of cell types were seen within each histological section, but not all tubules contained all cell types. Sertoli cells had adult cell morphology, while no changes were seen in Leydig cells.

(vii) 28-32 weeks

The histological appearance of all testicular sections of ram lambs older than 28 weeks was similar. All elements of the spermatogenic cycle were present. Spermatozoa were

abundant in most tubules and were arranged in characteristic streaming fashion, with tails directed towards the central lumen of seminiferous tubules.

## 4. Discussion

### (a) Longitudinal Study

Marked fluctuations in weekly plasma LH and testosterone levels recorded in the longitudinal study may have been caused by the episodic secretion of these hormones such as is discussed on the next two pages. A major deficiency of the longitudinal study was that it was based on only single weekly plasma samples, but this was offset by the supplementary data from the acute study and the pre-GnRH data.

#### (i) LH

The patterns of change in plasma LH concentrations observed in the longitudinal study, profile studies and pre-GnRH samplings corresponded closely with each other. They indicated that the major changes in LH concentrations during sexual maturation were: relatively low neo-natal LH levels which increased prepubertally and subsequently declined to low values in the post-pubertal period. Similar general patterns of change have been reported by Crim and Geschwind (1972a), Courot et al. (1975) and Wilson and Lapwood (1979a). Furthermore Courot et al. (1975) found that longitudinal LH profiles of ram lambs were not affected by season of birth. In contrast Lee et al. (1976a) recorded maximal LH levels in Merino x Corriedale lambs at 5 weeks of age, but also observed peaks at 11 and 33 weeks. However the authors indicated that in these lambs puberty (first appearance of spermatozoa in the testes) only occurred after 30 weeks of age.

The pattern of changes in plasma LH levels corresponded well with data which showed that in ram lambs pituitary LH contents and concentrations increased up to approximately 14 weeks of age, then tended to decline to low levels (Skinner et al., 1968; Courot et al., 1975). Thus the post-pubertal decline in plasma LH concentrations observed in

this and other studies (Crim and Geschwind, 1972a; Wilson and Lapwood, 1979a), may have been caused partly by reduced pituitary LH secretory capacity, but the concurrent increases in plasma testosterone concentrations almost certainly contributed as well.

(ii) Testosterone

Longitudinal patterns of testosterone secretion, with levels increasing similarly to those observed in the present longitudinal, acute profile and pre-GnRH samplings have been reported to occur in ram lambs of various breeds: Ile-de France (Courot, 1974; Cotta et al., 1975), Merino x Corriedale (Lee et al., 1976a) and Romney (Wilson and Lapwood, 1979a). During this period testicular androgen content increases (Skinner et al., 1968), undoubtedly as a result of testicular growth as well as increases in Leydig cell numbers (Sapsford, 1962) and Leydig cell receptor activity (Odell and Swerdloff, 1976).

(b) Pulsatile Hormone Secretion

(i) LH

Frequent blood sampling at each age, which gave clear evidence of pulsatile LH secretion, confirmed observations made previously for ram lambs (Foster et al., 1978; Savoie et al., 1979; Wilson and Lapwood, 1979a). Foster et al. (1978) and Savoie et al. (1979) also demonstrated that pulsatile secretion of LH was detectable within one week after birth, so it was not surprising that this type of secretion was evident at the sampling 4 weeks after birth. Episodic LH secretion also was detected at each sampling of hemicastrates and bilateral castrates. Fluctuations in plasma LH levels in castrates were of greater magnitude than those from the other two groups, but there was no effect of treatment on the number of peaks recorded. Walton et al. (1978; 1980) recorded similar results for hemicastrates and entires but did not include castrates. However, pulsatile variations in plasma LH levels of wethers have been reported previously (Riggs and Malven, 1974). Similarly, in a study with young bulls, Haynes

et al. (1976) did not observe any significant effect of hemicastration on frequency of LH peaks, nor on peak heights.

(ii) Testosterone

Hemicastration did not have any significant effect on the magnitude or frequency of testosterone peaks when compared with those recorded from entires. However, in hemicastrated bulls a significant reduction in peak plasma testosterone levels, and in numbers of peaks, was reported by Haynes et al. (1976). The authors concluded that the testosterone peaks appeared more regularly in hemicastrates and that this may have been due to lack of synchrony in the secretion of testosterone from two testes, rather than the one of hemicastrates.

(iii) LH/testosterone interrelationships

In the present study many LH peaks noted prior to 8 weeks of age were not followed by testosterone secretion. However, that disparity disappeared in subsequent samplings. Likewise Wilson and Lapwood (1979a) observed consistent 1:1 relationships between LH and testosterone peaks at 14 and 22 weeks, but not at 6 weeks of age. Savoie et al. (1979) observed many LH peaks which were not followed by testosterone elevations at 8 weeks of age but did not sample older animals.

(c) Effects of Hemicastration

(i) LH

As indicated in the introduction to this chapter previously reported changes in plasma LH concentrations following hemicastration of ram lambs have been inconsistent. For example, as noted in the current experiment, Walton et al. (1978; 1980) found no significant increase in LH levels following hemicastration, but Riesen et al. (1977) observed a decline in LH levels in hemicastrates, although the difference was not significant. Conversely Land and Carr (1975) and de Reviers et al. (1980) registered increases in plasma LH (and FSH) levels after this treatment. Clarification of the effects of this treatment in rams probably requires investigation of the influences of such experimental conditions and procedures as breed of rams,

age, post-operative period and seasons. The use of hormone secretion stimulation and inhibition tests, such as in experiment 5.3, may also be helpful.

In adult rams increases in plasma LH levels have been recorded from animals hemicastrated in spring as well as autumn (Hochereau-de Reviers and Pelletier, 1971; Hochereau-de Reviers et al., 1976). In contrast Barrell, G.K. (1980, pers. comm.) did not observe any elevation of LH levels in adult rams hemicastrated in autumn.

(ii) Testosterone

Since there was no significant difference between hemicastrates and entires in mean plasma testosterone levels in the present study, it can be concluded that the remaining testes of hemicastrates produced more testosterone than each single testis of intact rams. Similar evidence of compensatory androgen hypersecretion by the remaining testes of hemicastrated ram lambs has been noted by Walton et al. (1978), while Johnson et al. (1971) recorded this effect in adult rams.

(d) Effects of Bilateral Castration

(i) LH

Substantial increases in plasma LH levels were recorded at each of the profile samplings performed at 10, 18, 26 and 34 weeks of age, 2 weeks following bilateral castrations. That result confirmed that the steroid negative feedback system already was operative by 10 weeks of age. In fact Foster et al. (1972a) showed that this feedback system was operative in ram lambs as early as 2 weeks of age.

From plasma LH levels recorded following bilateral castrations in the present experiment it became clear that there was a differential effect of age at castration on LH rebound responses. Animals from hemicastrate as well as entire groups showed higher LH responses following castration at 10 and 18 weeks compared to 26 and 34 weeks. Similar differential effects of age on plasma LH rebound following

castration of ram lambs have been reported by Crim and Geschwind (1972a) and Pelletier et al. (1977). Although the reasons for these decreases with age in LH rebound responses were obscure, Pelletier et al. (1977) hypothesised that some elements of the testicular tubules secreted a factor, which in synergy or not with testosterone, induced ultimate maturation of the hypothalamo-hypophyseal system; lack of this substance in younger animals caused much greater LH rebound after castration. Other factors which may have contributed were the decreases in basal LH levels, and also in pituitary LH content and concentration which have been reported to occur during the course of sexual maturation in ram lambs (Courot et al., 1975).

(e) Hormonal Responses to GnRH: Effects of Hemicastration

(i) LH

GnRH administration causes pituitary LH release followed by testicular androgen secretion in young (Savoie et al., 1979; Wilson and Lapwood, 1979b) and adult rams (Hopkinson et al., 1974; Galloway and Pelletier, 1975; Pelletier, 1976; Wilson and Lapwood, 1978b). Typical gonadotrophic and androgenic secretory responses always followed releasing hormone injection into entire ram lambs in experiment 5.3.

Hemicastration significantly increased the total pituitary LH secretory responses to GnRH injection, thus confirming a non-significant trend observed in the only similar published work with hemicastrated ram lambs (Walton et al., 1980). The reasons for such effects on LH responses were not clear: similar mean plasma LH concentrations were recorded in the immediately preceding profile study and there was no difference between androgen levels of entires and hemicastrates in either the profile or GnRH experiments. The decreases with age in total LH responses noted in the present study corresponded well with results of similar previous research (Wilson and Lapwood, 1979b).

(ii) Testosterone

The total testosterone responses recorded from hemicastrates and entires were not significantly different from each other. That result confirmed the concept that the remaining testes of hemicastrates were capable of secreting as much androgens as both testes of entires. Total testosterone responses increased with age in both groups of animals as has been observed in earlier experiments with ram lambs (Lee et al., 1976b; Wilson and Lapwood, 1979b). However, no such data has been published from hemicastrated ram lambs.

(f) Testicular and Epididymal Weights

Compensatory testicular hypertrophy, such as that observed in the present experiment, has been recorded in similar research with young rams (Renfro and Dutt, 1970; Land and Carr, 1975; Riesen et al., 1977; Walton et al., 1978; de Reviers et al., 1980) as well as with adults (Voglmayr and Mattner, 1968; Hochereau-de Reviers and Pelletier, 1971; Skinner, 1971b). Hochereau-de Reviers et al. (1976) also demonstrated that in adult rams hemicastration in spring induced immediate testicular hypertrophy during that season; similar treatment in autumn caused hypertrophy only after a delay of six months (i.e. the following spring). Also after autumnal hemicastration the weight of remaining testes was maintained: hemicastration counteracted the normal seasonal testicular regression.

Relative hypertrophy of the remaining testis following hemicastration has been reported to decrease with age. This concept has been demonstrated in serial hemicastration experiments with rats (Cunningham et al., 1978; Putra and Blackshaw, 1980) and cockerels (de Reviers et al., 1980). However, no comparable serial hemicastration studies on ram lambs appear to have been published. Nevertheless, hemicastration performed at different ages in the present experiment was followed by compensatory hypertrophy of the remaining testes.

Whether the compensatory hypertrophy was attributable more to increases in tubular or peritubular testicular components was not assessed in this experiment. However, the increases in seminiferous tubular diameters which were recorded at all ages gave some measure of their hypertrophy. This is in agreement with observations made by Walton et al. (1980), but Riesen et al. (1977) did not observe any significant change in tubular diameters following unilateral castration. In adult animals similar discrepancies in results appear in the literature. For example Voglmayr and Mattner (1968) and Skinner (1971b) observed significant increases in seminiferous tubular diameters after hemicastration, while Hochereau-de Reviers et al. (1976) did not observe any such change in either autumn or spring born adult rams, but instead noticed significant increases in tubular length.

Although compensatory hypertrophy of epididymides was noted at all sampling ages of the present experiment the difference reached significant levels only at 8 weeks of age. Since the mean plasma androgen concentrations of hemicastrates and entires were not significantly different, the compensatory hypertrophy of the epididymides can not be explained in terms of peripheral testosterone concentrations, but may have resulted from an increased local androgen stimulation as discussed in Chapter IV (Page 117) of this thesis. Similar hypertrophy of epididymides following hemicastration of ram lambs has been reported previously by Riesen et al. (1977).

(g) Qualitative Testicular Histology

Apart from increases in seminiferous tubular diameters, no other histological feature of the testes showed any change due to the effects of hemicastration. All qualitative changes noticed in the seminiferous tubules were age-related. Similarly Walton et al. (1980) did not report any effects of hemicastration on spermatogenic cellular components of the testes in spring born lambs treated at 1 week of age. However, in spring and autumn born Ile-de France lambs

hemicastration induced increases in total number of round spermatids produced/testis/day, and in total numbers of Sertoli cells (de-Reviers et al., 1980). Similar changes in total number of spermatids in hemicastrated calves were detected by Barnes et al. (1980b).

One probable reason for this apparent divergence in results was the variable amount of time which different authors have allowed to elapse between hemicastration and recovery of the second testes. Since Walton et al. (1980) did not observe any marked changes in cellular composition in hemicastrate testes, even allowing 9 to 17 weeks before recovery of the remaining organs, it was not surprising that no changes were detected in the present study in which the time lapse was only 4 weeks. Nevertheless in none of the above cited reports on research with immature animals were there any signs of accelerated initiation of spermatogenesis as a consequence of hemicastration. This topic is discussed in chapters 6 and 7.

In contrast with the results in immature animals, increases in efficiency of spermatogenesis have been reported in adult rams hemicastrated in spring or in autumn (Hochereau-de Reviers et al., 1976). The changes included: (i) increases in number of spermatogonia, and (ii) increases in daily production of round spermatids/testis. Similar changes in the spermatogonia have been reported following hemicastration of adult bulls (Johnson, 1978).

Increases in intertubular tissue volume following hemicastration have been observed during the breeding and non-breeding seasons in rams, but the relative increase in intertubular volume was insufficient to counteract the tubular regression which normally occurs during the non-breeding season (Hochereau-de Reviers et al., 1976).

Results of the experiments described in this chapter indicated that compensatory testicular hypertrophy followed hemicastration in ram lambs, but did not induce any

acceleration in initiation of spermatogenesis. There was no consistent increase in LH secretion during this period of compensatory hypertrophy. Whether the increase in weights of the remaining testes resulted from local increases in androgen concentrations, or from increased FSH secretion was not investigated, however, the second possibility was examined in experiments described in chapter VI of this thesis. However, at the time chapter 5 experiments were designed there was no access to assays to measure plasma FSH or prolactin.

# CHAPTER VI

## EFFECTS OF HEMICASTRATION AND CRYPTORCHIDISM ON HORMONE SECRETION PATTERNS IN MALE LAMBS

### 1. Introduction

Hemicastration and induced cryptorchidism are accepted techniques for studying the hypothalamo-pituitary-testicular axis. Changes in reproductive organ and endocrine parameters resulting from hemicastration of boars and rams have been discussed in Chapters IV and V of this thesis. However, comparable data on gonadotrophin levels in cryptorchid rams has been rather inconsistent. Thus in this species cryptorchidism has been found to cause elevations in blood concentrations of LH (Hillard and Bindon, 1975), FSH (Blanc and Terqui, 1976) and both FSH and LH (Schanbacher and Ford, 1977). In contrast Lee et al (1978) did not detect any change in gonadotrophin levels of cryptorchid ram lambs prior to 13 months of age. Subsequently plasma FSH, but not LH concentrations increased in these animals. Bilateral cryptorchidism in bulls has been reported to be associated with elevated plasma LH and FSH levels (Schanbacher, 1978; 1979a).

Androgen levels recorded from cryptorchid ram lambs were not significantly different from those of age-matched entires (Pelletier et al., 1977; Lee et al., 1978). Similar results have also been reported from adult rams (Schanbacher and Ford, 1977).

GnRH administration causes elevation of plasma LH, FSH and testosterone content in ram lambs (Galloway and Pelletier, 1974; Lee et al., 1976b; Wilson and Lapwood, 1979b). However, there is only one such report from hemicastrated ram lambs: no significant differences were found in the gonadotrophic responses of hemicastrates and entires (Walton et al., 1980). On the other hand GnRH injection into cryptorchid rams resulted in exaggerated gonadotrophic responses (Lee et al.,

1979; 1980) accompanied by subnormal testicular androgen secretion (Lee et al., 1980; Schanbacher, 1980a).

The present experiments were designed to examine the effects of induced cryptorchidism and hemicastration on longitudinal and acute hormonal secretory patterns in ram lambs, from 4 to 32 weeks of age. The hemicastration treatment was different from that used in experiments 5.2 and 5.3 in which surgery was performed at varying ages, with a constant 2 week post-operative sampling period; for these experiments surgery was performed at 2 weeks of age while the ages of sampling varied. The effects of these surgical treatments on the pituitary-testicular axis were further examined by eliciting GnRH responses at four different ages. Organ weights and testicular histological data of these animals were compared with those from entires, in order to help elucidate the physiological mechanisms responsible for any endocrine changes.

## 2. Materials and Methods

### (a) Animals and Surgical Treatments (Experiments 6.1, 6.2 and 6.3)

Forty eight ram lambs were identified individually then allocated randomly to groups designated A, B and C. Animals in group A were maintained as entires, while those in groups B and C were rendered hemicastrate and cryptorchid, respectively, at two weeks of age using surgical methods described in chapter II of this thesis.

### (b) Longitudinal Hormone Profiles (Experiment 6.1)

Two blood samples collected 30 min apart were obtained from 6 animals randomly selected from each group every second Monday. Sampling was conducted between 4 and 32 weeks of age and commenced at 09.00 hr on each occasion. LH, FSH, prolactin and testosterone estimations were performed on all plasma samples.

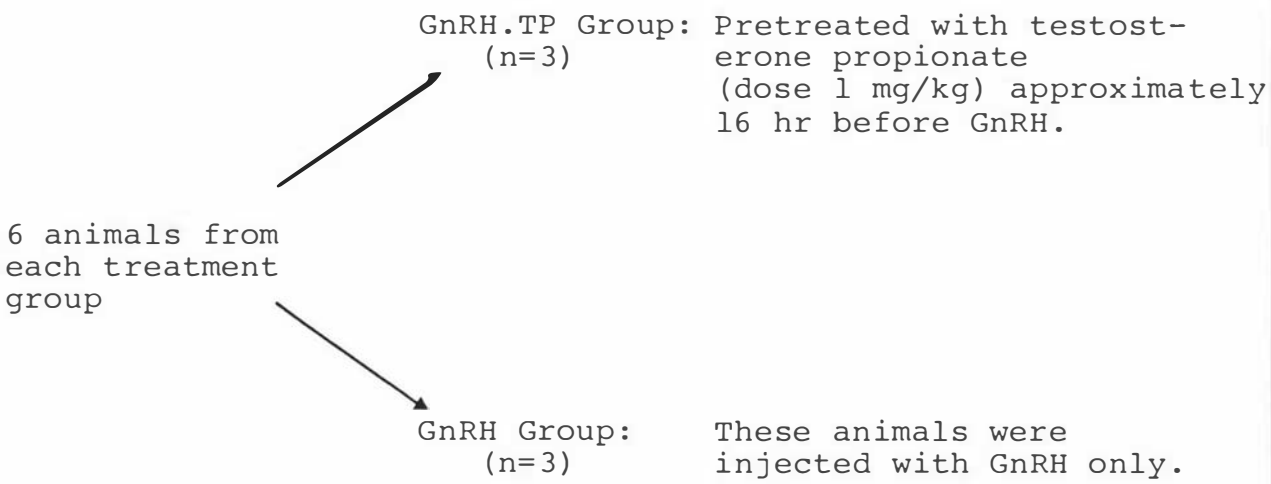
### (c) Acute Hormone Secretion Study (Experiment 6.2)

Six animals from each group were randomly selected for sampling at 8, 16, 24 and 32 weeks of age. Blood samples

were obtained at ½ hr intervals for 4 hr, commencing at 09.00 hr. Again LH, FSH, prolactin and testosterone levels were determined for all plasma samples.

(d) Responses to GnRH or Testosterone Propionate (TP) and GnRH (Experiment 6.3)

This study was conducted on the day after each of the acute profile samplings of experiment 6.2. The six animals from each major treatment group which were subjected to acute profile study at each age were utilized in the present study. The experimental protocol was:




TP pretreatment consisted of intramuscular injection of testosterone propionate in ethyl oleate ('Virormone', Paines and Byrne Ltd, U.K.).

GnRH was injected at a dose rate of 0.5 µg/kg body weight and the required dose was dissolved in 5.0 ml of acidified (0.01 M acetic acid) 0.9% saline and administered via jugular venepuncture, as a single rapid injection.

Two blood samples, collected ½ hour apart, were obtained before GnRH injection, while further samples were obtained 15, 30, 45, 60, 90, 120, 180 and 240 min post-injection. All plasma samples were assayed for LH, FSH and testosterone concentrations.

(e) Organ Weights and Qualitative Histology

The animals subjected to the GnRH.TP treatment at each age in experiment 6.3 were sacrificed in order to obtain data on the effects of the surgical treatments on testicular and

epididymal development. Testicular samples were taken for histological processing then estimation of seminiferous tubular diameters and qualitative histological examination.

(f) Statistical Analyses

All hormonal data were analysed by analyses of variance. Hormone data from experiment 6.1 were analysed only up to 30 weeks, because of the reduction in number of animals thereafter. Testosterone response data from TP treated animals were not included in the analysis of results from experiment 6.3.

Between-group differences in numbers of hormonal peaks in experiment 6.2 were tested for significance by Chi-square analysis. Testicular weights, epididymal weights and seminiferous tubular diameter data from hemicastrates and cryptorchids, at 8, 16, 24, 32 weeks of age, were compared with similar data obtained from age-matched controls using Student's t-tests.

3. Results

(a) Longitudinal Study (Experiment 6.1)

(i) LH

(Figure 6.1 and tables 6.1 and 6.5)

In the longitudinal study the overall mean plasma LH level recorded from entires was $1.00 \pm 0.19$ ng/ml, but concentrations over 2 ng/ml were noted at 6 and 18 weeks. LH levels tended to decrease from 18 weeks of age reaching $0.32 \pm 0.04$ ng/ml at 32 weeks. A similar overall mean plasma LH level was noted for hemicastrates ($1.04 \pm 0.13$ ng/ml). However, fortnightly mean plasma LH values frequently were higher than 1 ng/ml. The levels generally tended to decrease from 24 weeks of age and a plasma concentration of $0.60 \pm 0.26$ ng/ml was recorded at 32 weeks. In contrast a much higher overall mean plasma LH concentration was recorded from cryptorchid ram lambs ($6.11 \pm 0.78$ ng/ml) and fortnightly LH concentrations tended to increase throughout the study, reaching a maximal value of $11.08 \pm 4.64$ ng/ml at 28 weeks.

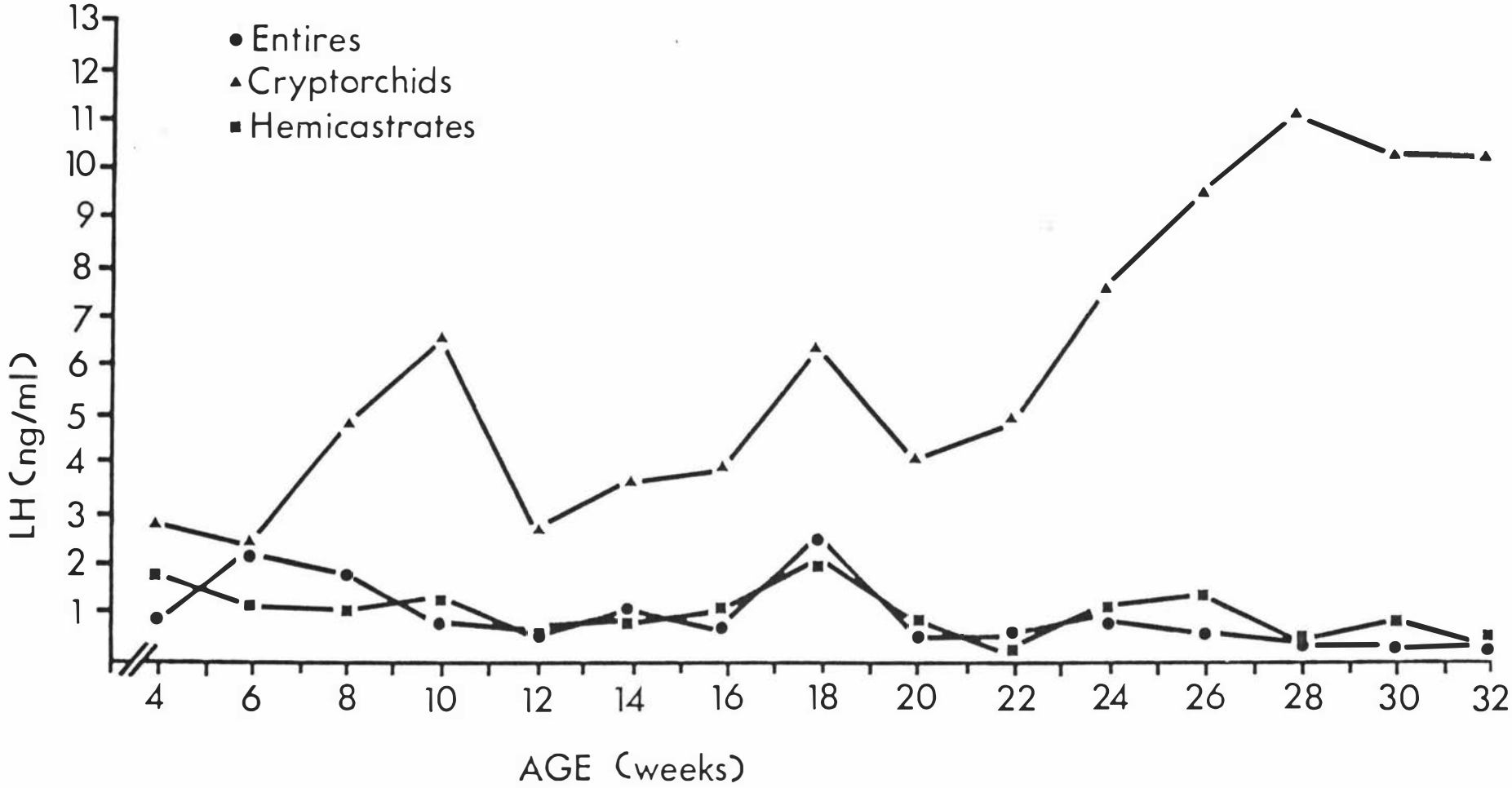



Figure 6.1: Mean plasma LH levels in entire, hemicastrate and cryptorchid ram lambs from 4 to 32 weeks of age (experiment 6.1, n=6).

Table 6.1: Mean (±SEM) fortnightly plasma LH levels (ng/ml) for lambs in experiment 6.1

| Age (weeks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 4 | 0.81±0.33 | 1.91±0.79 | 2.81±1.13 |
| 6 | 2.29±1.57 | 1.22±1.69 | 2.43±0.56 |
| 8 | 1.98±0.54 | 1.16±0.50 | 4.97±1.27 |
| 10 | 0.97±0.37 | 1.44±0.56 | 6.64±1.60 |
| 12 | 0.52±0.13 | 0.45±0.15 | 2.75±0.52 |
| 14 | 1.04±0.62 | 0.89±0.22 | 3.78±1.30 |
| 16 | 0.79±0.32 | 1.08±0.53 | 3.82±1.26 |
| 18 | 2.69±1.15 | 2.18±0.64 | 6.41±1.59 |
| 20 | 0.60±0.20 | 0.82±0.19 | 4.08±1.00 |
| 22 | 0.60±0.29 | 0.31±0.14 | 4.91±2.51 |
| 24 | 0.93±0.27 | 1.15±0.65 | 7.53±1.60 |
| 26 | 0.71±0.23 | 1.04±0.31 | 9.54±2.66 |
| 28 | 0.41±0.10 | 0.54±0.18 | 11.08±4.64 |
| 30 | 0.39±0.15 | 0.91±0.23 | 10.43±2.53 |
| 32 | 0.32±0.04 | 0.60±0.26 | 10.45±2.05 |

In the analysis of variance of hormone concentrations it was evident that cryptorchidism, but not hemicastration, resulted in a significant ($P < 0.001$) elevation of plasma LH levels. Overall mean plasma LH levels increased linearly with age ($P < 0.01$), however, this increase was caused entirely by the marked increase in plasma LH values recorded from cryptorchid ram lambs. Thus the entire and hemicastrate *vs* cryptorchid x linear component of the treatments x age interaction was highly significant ($P < 0.01$).

(ii) FSH

(Figure 6.2, tables 6.2 and 6.5)

Plasma FSH levels recorded from entires and hemicastrates showed no distinct pattern of change during the experiment. In contrast, cryptorchids had a much higher overall mean plasma FSH level ($286.30 \pm 40.79$ ng/ml) and values tended to increase throughout the period of study from $124.00 \pm 10.46$ ng/ml at 4 weeks to a peak of $542.31 \pm 104.83$ ng/ml at 28 weeks.

Fortnightly mean plasma FSH levels of hemicastrates often were higher than those recorded from entires, this effect being most marked at the first two samplings; however, the overall difference between results for these two groups ($72.38 \pm 6.97$ *vs* $57.99 \pm 4.21$ ng/ml) just failed to reach significance ($F_{(1,210} = 3.80$, $0.05 < P < 0.10$). Further analysis using Student's *t*-tests confirmed that at 4 and 6 weeks of age FSH levels recorded from hemicastrates were significantly higher than those from entires (at 4 weeks $\pm t_{(10)} = 5.38$, $P < 0.001$, and at 6 weeks $\pm t_{(10)} = 3.73$, $P < 0.01$). In contrast, throughout the experiment FSH levels recorded from cryptorchids were greatly ($P < 0.001$) elevated compared to results from the other two groups.

In the analysis of variance the linear ($P < 0.001$) and quadratic ($P < 0.01$) components of the age main effect for plasma FSH levels were significant. These effects were attributable mainly to the very substantial increase in plasma FSH levels recorded from cryptorchids. That change in cryptorchid FSH values also was responsible for the significant entire and hemicastrate *vs* cryptorchid x linear

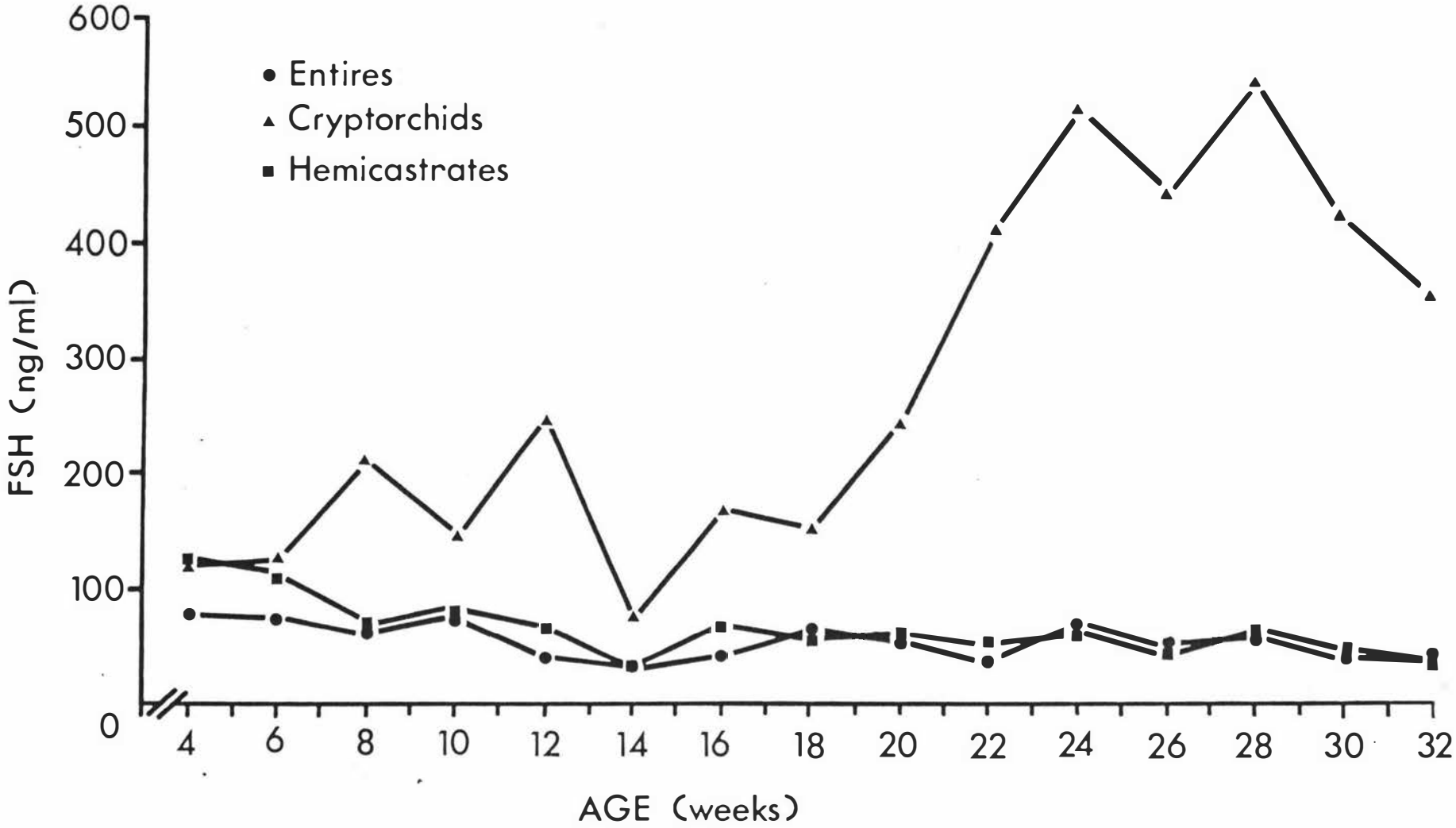



Figure 6.2: Mean plasma FSH levels in entire, hemicastrate and cryptorchid ram lambs from 4 to 32 weeks of age (experiment 6.1, n=6).

Table 6.2: Mean (±SEM) fortnightly plasma FSH levels (ng/ml) for lambs in experiment 6.1

| Age (weeks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 4 | 80.57± 3.53 | 134.43± 2.87 | 124.00±10.46 |
| 6 | 77.93± 4.00 | 116.41± 2.43 | 126.00±16.38 |
| 8 | 62.91±13.52 | 79.89± 9.09 | 212.00±25.02 |
| 10 | 77.39±11.42 | 86.40±11.84 | 151.67±25.84 |
| 12 | 41.62± 6.06 | 68.28±13.62 | 255.79±50.91 |
| 14 | 34.87±14.79 | 34.30±12.12 | 79.81±13.75 |
| 16 | 44.64± 8.59 | 95.32±20.74 | 170.50±22.15 |
| 18 | 67.41±14.94 | 63.92± 9.40 | 164.19±39.23 |
| 20 | 59.98± 8.63 | 61.69±10.04 | 247.52±34.83 |
| 22 | 40.99± 2.27 | 58.65± 9.76 | 414.19±125.58 |
| 24 | 80.99±12.88 | 74.50±18.20 | 523.84±50.62 |
| 26 | 57.82±10.48 | 52.87±11.53 | 456.14±82.32 |
| 28 | 59.00±15.53 | 67.33±14.68 | 542.31±104.83 |
| 30 | 41.32± 7.98 | 45.20± 7.35 | 427.64±75.65 |
| 32 | 42.36± 9.50 | 46.28± 4.49 | 399.28±99.95 |

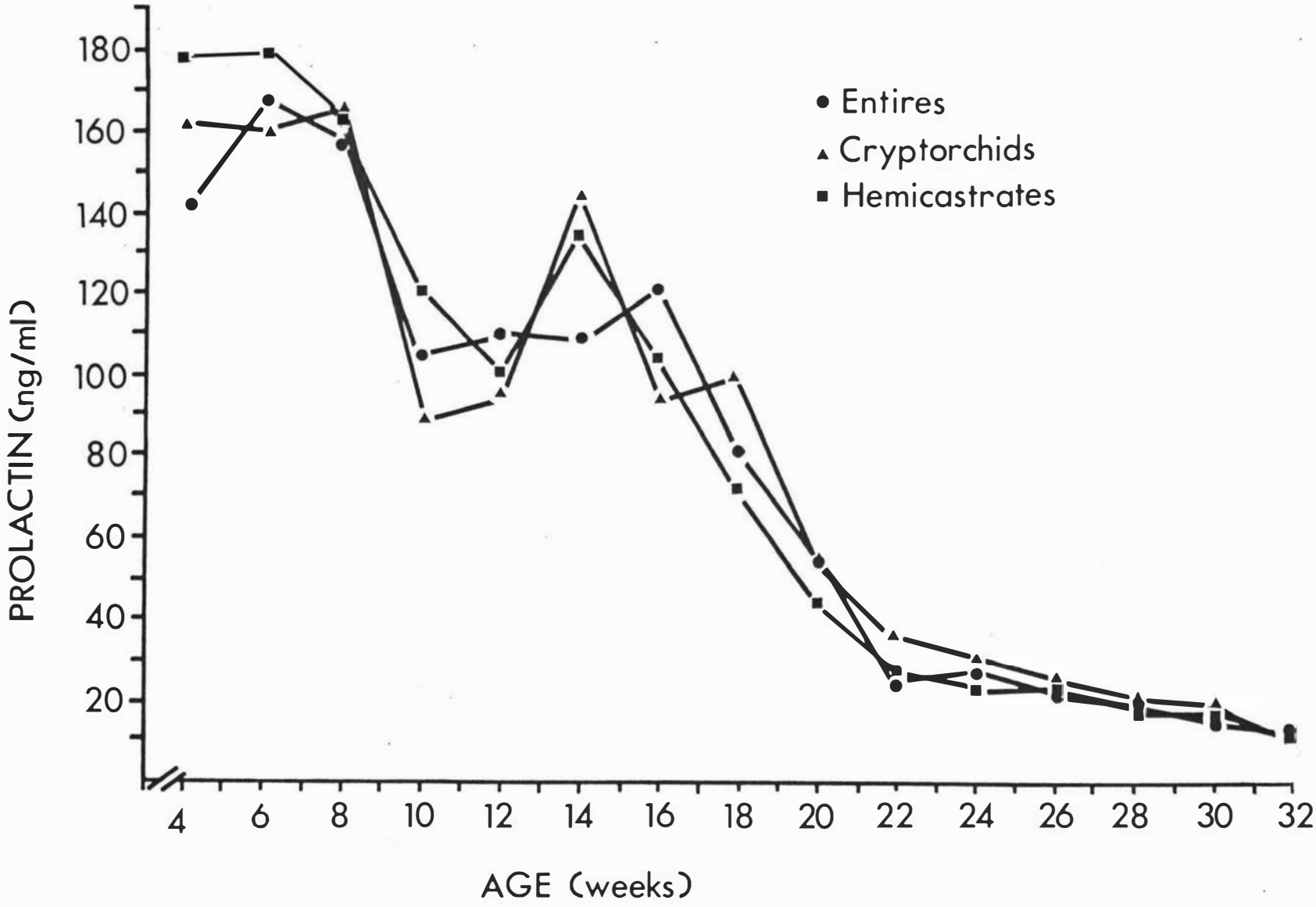



Figure 6.3: Mean plasma prolactin levels in entire, hemicastrate and cryptorchid ram lambs from 4 to 32 weeks of age (experiment 6.1, n=6).

Table 6.3: Mean (±SEM) fortnightly plasma prolactin levels (ng/ml) for lambs in experiment 6.1

| Age (wks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 4 | 143.86±27.64 | 178.15±24.56 | 163.90±25.70 |
| 6 | 169.87±16.11 | 179.38±30.90 | 161.20±27.49 |
| 8 | 159.02±32.18 | 161.88±13.97 | 165.86±17.82 |
| 10 | 106.34±24.46 | 121.47±34.90 | 88.74±15.23 |
| 12 | 110.70±35.73 | 100.50±21.20 | 93.96±19.10 |
| 14 | 109.94±11.24 | 135.46±23.92 | 146.95±25.40 |
| 16 | 121.54±20.76 | 104.57± 7.97 | 93.91±14.52 |
| 18 | 81.66±19.91 | 71.24±12.77 | 99.93±25.16 |
| 20 | 50.74±10.88 | 44.90±13.29 | 54.57±15.86 |
| 22 | 25.76± 8.25 | 26.79± 5.56 | 35.16± 3.46 |
| 24 | 28.62± 6.68 | 23.24± 4.33 | 31.74± 7.73 |
| 26 | 23.43± 4.18 | 23.03± 4.92 | 26.01± 3.14 |
| 28 | 19.75± 2.90 | 19.84± 1.39 | 21.91± 4.06 |
| 30 | 15.09± 1.72 | 16.98± 2.11 | 19.44± 1.46 |
| 32 | 14.72± 0.91 | 14.45± 0.91 | 13.73± 4.14 |

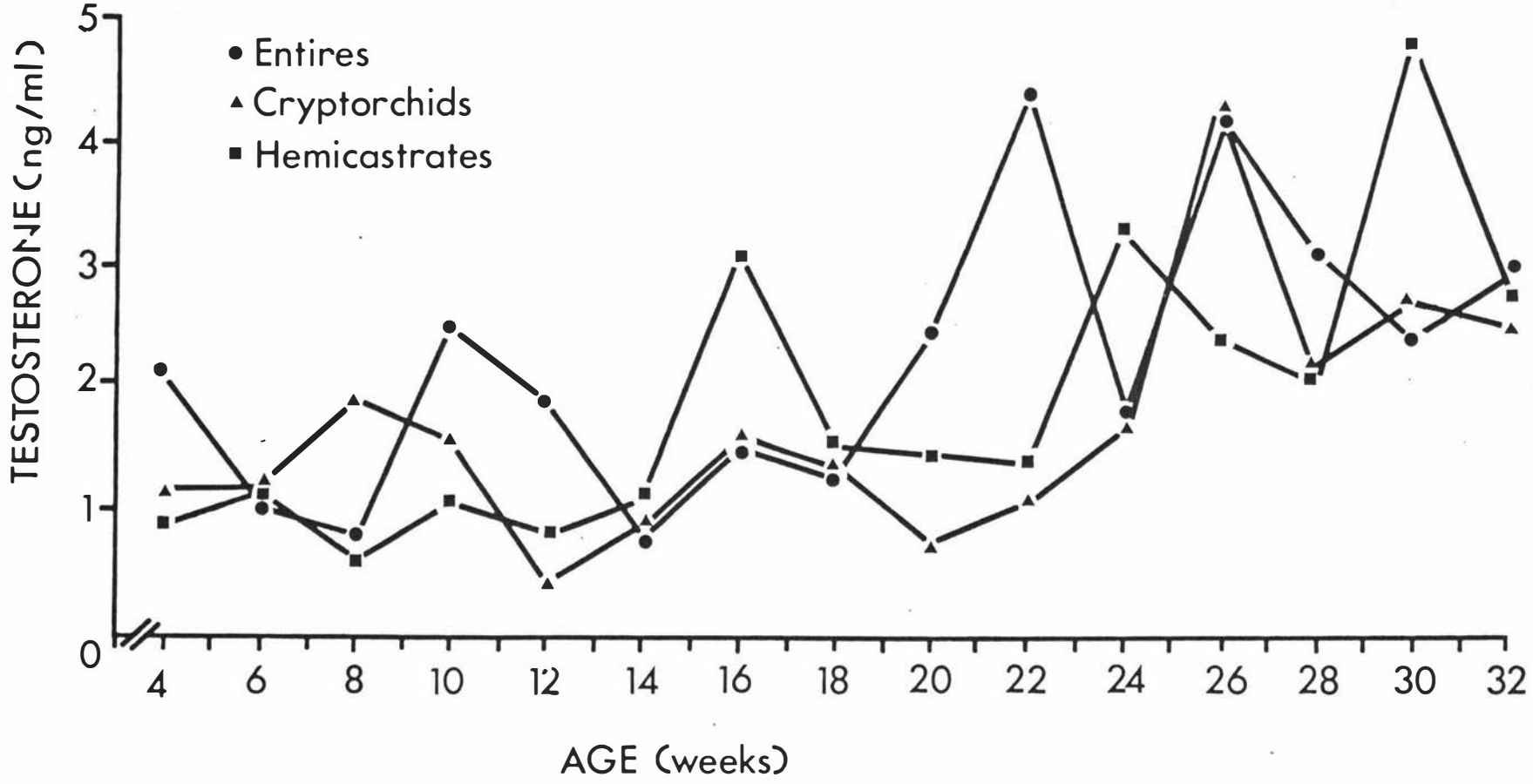



Figure 6.4: Mean plasma testosterone levels in entire, hemicastrate and cryptorchid ram lambs from 4 to 32 weeks of age (experiment 6.1, n=6).

Table 6.4: Mean (±SEM) fortnightly plasma testosterone levels (ng/ml) for lambs in experiment 6.1

| Age (wks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 4 | 2.07±0.88 | 0.99±0.35 | 1.19±0.36 |
| 6 | 1.05±0.39 | 1.07±0.26 | 1.14±0.27 |
| 8 | 1.01±0.42 | 0.69±0.28 | 1.97±0.36 |
| 10 | 2.56±1.28 | 1.07±0.52 | 1.67±0.29 |
| 12 | 1.98±0.52 | 0.90±0.33 | 0.43±0.10 |
| 14 | 0.87±0.41 | 1.18±0.24 | 0.92±0.33 |
| 16 | 1.63±0.54 | 3.08±1.19 | 1.69±0.60 |
| 18 | 1.36±0.66 | 1.69±0.58 | 1.46±0.42 |
| 20 | 2.54±1.05 | 1.52±0.34 | 0.83±0.27 |
| 22 | 4.20±1.58 | 1.48±0.98 | 1.15±0.58 |
| 24 | 1.93±0.48 | 3.35±1.72 | 1.79±0.90 |
| 26 | 4.23±1.87 | 2.48±0.79 | 4.41±2.16 |
| 28 | 3.15±1.15 | 2.05±0.61 | 2.13±0.27 |
| 30 | 2.27±0.73 | 4.92±0.83 | 2.84±0.98 |
| 32 | 3.08±0.55 | 2.81±0.61 | 2.52±0.29 |

Table 6.5: Summary of analyses of variance of LH, FSH, prolactin and testosterone data from experiment 6.1

| Source of Variation | | DF | | Variance Ratios | | | |
|---|---|---|---|---|---|---|---|
| | | | | LH | FSH | Prolactin | Testosterone |
| A. Treatment | | 2 | | | | 0.73 | 0.67 |
| (i) | Entires vs Hemicastrates | | 1 | 2.32 | 3.80 | - | - |
| (ii) | Entires & Hemicastrates vs Cryptorchids | | 1 | 224.32*** | 355.24*** | - | - |
| B. Age | | 13 | | | | | |
| (i) | Linear | | 1 | 10.92** | 40.46*** | 615.09*** | 33.72*** |
| (ii) | Quadratic | | 1 | - | 7.97** | 10.72** | 5.94* |
| (iii) | Cubic | | 1 | - | - | 8.34** | - |
| | Remainder | | 11-12 | 0.74 | 0.40 | 1.62 | 0.62 |
| Interaction | | | | | | | |
| Treatments x Age | | 26 | | | | 0.31 | 0.91 |
| (i) | Entires & Hemicastrates vs Cryptorchids x Linear | | 1 | 7.35** | 109.27*** | - | - |
| (ii) | Entires & Hemicastrates vs Cryptorchids x Quadratic | | 1 | - | 5.96* | - | - |
| | Remainder | | 24-25 | 0.82 | 0.27 | - | - |
| Residual mean square | | 210 | | 427.79 | 504.01 | 454.01 | 415.77 |

($P < 0.001$) and quadratic ($P < 0.05$) components of the treatments x age interaction.

(iii) Prolactin

(Figure 6.3, tables 6.3 and 6.5)

Treatments had no effect on plasma prolactin levels. An overall mean plasma prolactin concentration of $78.80 \pm 14.53$ ng/ml was recorded from entires, while values of $81.46 \pm 16.12$ ng/ml and $81.13 \pm 14.75$ ng/ml were recorded from hemicastrates and cryptorchids, respectively.

In all three groups hormone concentrations tended to fall throughout the experiment, except for a sharp increase in levels recorded at 14 and 16 weeks of age. Thus in the analysis of variance for prolactin data the linear ($P < 0.001$), quadratic ($P < 0.01$) and cubic ($P < 0.01$) components of the age main effect were significant.

(iv) Testosterone

(Figure 6.4, tables 6.4 and 6.5)

Treatments did not have any significant effect on mean plasma testosterone levels. Overall mean concentrations of $2.26 \pm 0.27$ ng/ml, $1.96 \pm 0.30$ ng/ml and $1.74 \pm 0.58$ ng/ml were recorded from entires, hemicastrates and cryptorchids, respectively. In all three groups relatively low concentrations were recorded during the first 12 weeks of life, but subsequently values tended to increase with age. In the analysis of variance these patterns of change with age, for plasma testosterone data, contributed to highly significant linear ($P < 0.001$) and quadratic ($P < 0.05$) components.

(b) Acute Profile Study (Experiment 6.2)

(i) LH

(Figure 6.5, tables 6.6 and 6.10)

At each age of sampling in the four hour profile studies, pulsatile LH secretion patterns were clearly seen in all three treatment groups. Although there was no difference between total number of LH peaks recorded from entires (30) and hemicastrates (30), a greater number (55) was noted from cryptorchids. Chi-square analysis indicated that this

difference (entire and hemicastrates vs cryptorchids) was significant ($\chi^2_{(2)} = 10.86$, $P < 0.001$).

Mean plasma LH levels recorded from each treatment group during the 4 hr acute profile studies are summarised in table 6.6.

Table 6.6: Mean ($\pm$SEM) LH levels (ng/ml) during 4 hr profile studies (experiment 6.2)

| Age (wks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 8 | $1.43 \pm 0.43$ | $0.66 \pm 0.18$ | $3.74 \pm 0.67$ |
| 16 | $0.90 \pm 0.27$ | $0.50 \pm 0.06$ | $2.75 \pm 0.34$ |
| 24 | $1.29 \pm 0.43$ | $1.60 \pm 0.70$ | $7.30 \pm 0.96$ |
| 32 | $0.68 \pm 0.20$ | $0.60 \pm 0.15$ | $4.95 \pm 0.83$ |

Analysis of variance revealed that cryptorchids had a significantly higher ($P < 0.001$) overall mean plasma LH concentration ($4.69 \pm 0.98$ ng/ml) than hemicastrates ($0.84 \pm 0.25$ ng/ml) and entires ($1.07 \pm 0.17$ ng/ml). The cubic component of the effect of age on LH levels was significant ($P < 0.01$); that was a result of relatively higher mean LH levels being recorded at 8 and 24 weeks compared to those noted at 16 and 32 weeks.

(ii) FSH *

(Figure 6.6 and tables 6.7 and 6.10)

FSH levels recorded during the acute profile studies did not fluctuate very much, thus only 9 secretory peaks were recorded from entires compared to 12 and 11 registered from hemicastrates and cryptorchids, respectively. However, that difference was not significant.

Mean plasma FSH levels recorded from each group during the 4 hr acute profile studies are summarised in Table 6.7.

See inside back cover.

Table 6.7: Mean (±SEM) FSH levels (ng/ml) during 4 hr profile studies (experiment 6.2)

| Age (wks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 8 | 39.55±4.37 | 57.00±9.67 | 73.24±6.01 |
| 16 | 42.33±7.57 | 39.12±6.79 | 262.88±72.71 |
| 24 | 66.18±14.30 | 79.91±9.35 | 419.46±76.94 |
| 32 | 74.38±7.80 | 66.00±6.86 | 393.18±62.24 |

Analysis of hormone data revealed that cryptorchidism caused a significant elevation ($P < 0.001$) of overall mean plasma FSH levels (287±79.10 ng/ml) compared to those recorded from hemicastrates (60.50±8.54 ng/ml) and entires (55.61± 8.65 ng/ml). Overall mean hormone levels increased linearly ($P < 0.001$) with age. This effect was due mostly to the marked increase with age in FSH levels recorded from cryptorchids. The same result was responsible for the significant entires and hemicastrates vs cryptorchids x linear ($P < 0.01$) and quadratic ($P < 0.05$) components of the treatments x age interaction.

(iii) Prolactin

(Figure 6.7 and tables 6.8 and 6.10)

Pulsatile prolactin secretory patterns were observed in all three groups. A total of 19 peaks was recorded from entires, while hemicastrates and cryptorchids had 21 and 20 peaks, respectively; that difference was not statistically significant.

Mean plasma prolactin levels recorded during the 4 hr acute profile studies are summarised in table 6.8.

Table 6.8: Mean ($\pm$SEM) prolactin levels (ng/ml) during 4 hr profile studies (experiment 6.2)

| Age (wks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 8 | $175.91 \pm 9.35$ | $160.78 \pm 16.54$ | $180.51 \pm 7.49$ |
| 16 | $85.08 \pm 3.91$ | $90.92 \pm 8.62$ | $93.96 \pm 1.57$ |
| 24 | $32.18 \pm 11.84$ | $29.34 \pm 6.95$ | $46.62 \pm 10.43$ |
| 32 | $15.72 \pm 1.59$ | $13.45 \pm 1.13$ | $14.73 \pm 1.78$ |

Analysis of variance of hormone levels revealed that overall mean plasma prolactin levels were not affected by surgical treatments, but decreased ($P < 0.001$) linearly with age.

(iv) Testosterone

(Figure 6.8 and table 6.9 and 6.10)

Plasma testosterone concentrations recorded in the present study indicated that the surgical treatments had no significant effect on the secretion of this hormone. Pulsatile secretory patterns were evident in all three groups at all sampling ages. A total of 39 peaks were recorded from entires while hemicastrates and cryptorchids had 36 and 35 peaks, respectively. Testosterone peaks generally were preceded by LH peaks, but in both entires and hemicastrates spontaneous testosterone elevations, which apparently were not preceded by LH peaks, were noted. On the other hand in cryptorchids 18 out of 55 LH peaks (32.7%) were not followed by testosterone elevations.

Mean plasma testosterone levels recorded from each treatment group during 4 hr acute profile studies are summarised in table 6.9.

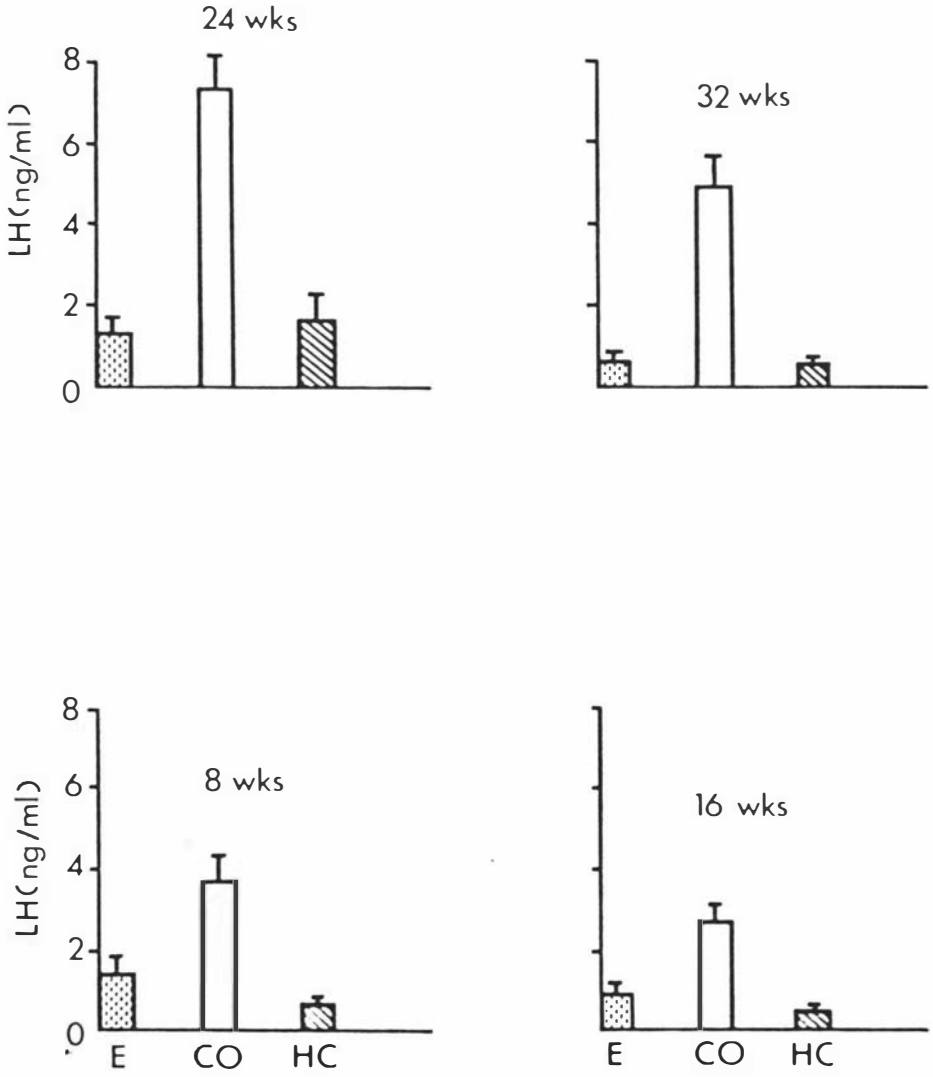



Figure 6.5: Mean (±SEM) plasma LH levels recorded from entire (E), hemicastrate (HC) and cryptorchid (CO) ram lambs during acute profile studies at 8, 16, 24 and 32 weeks of age (experiment 6.2, n=6).

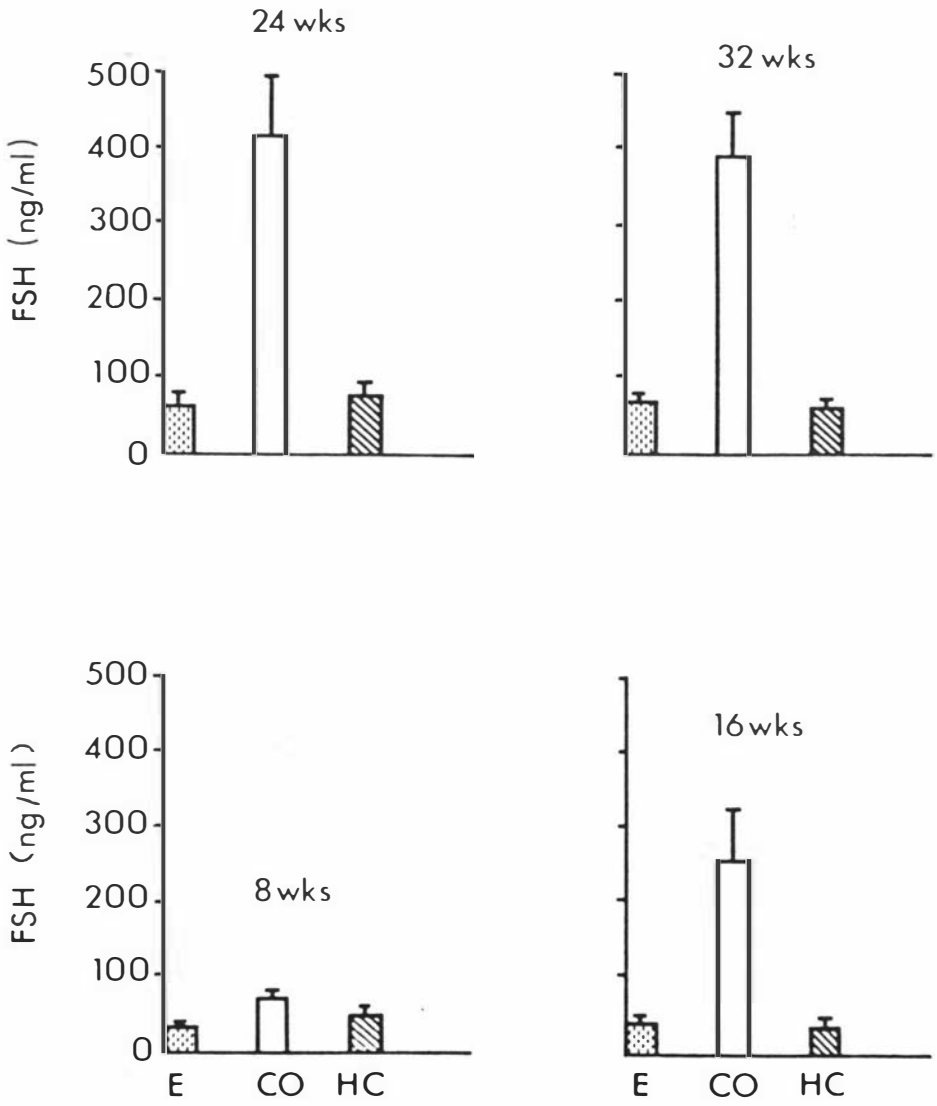



Figure 6.6: Mean ($\pm$SEM) plasma FSH levels recorded from entire (E) hemicastrate (HC) and cryptorchid (CO) ram lambs during acute profile studies at 8, 16, 24 and 32 weeks of age (experiment 6.2, n=6).

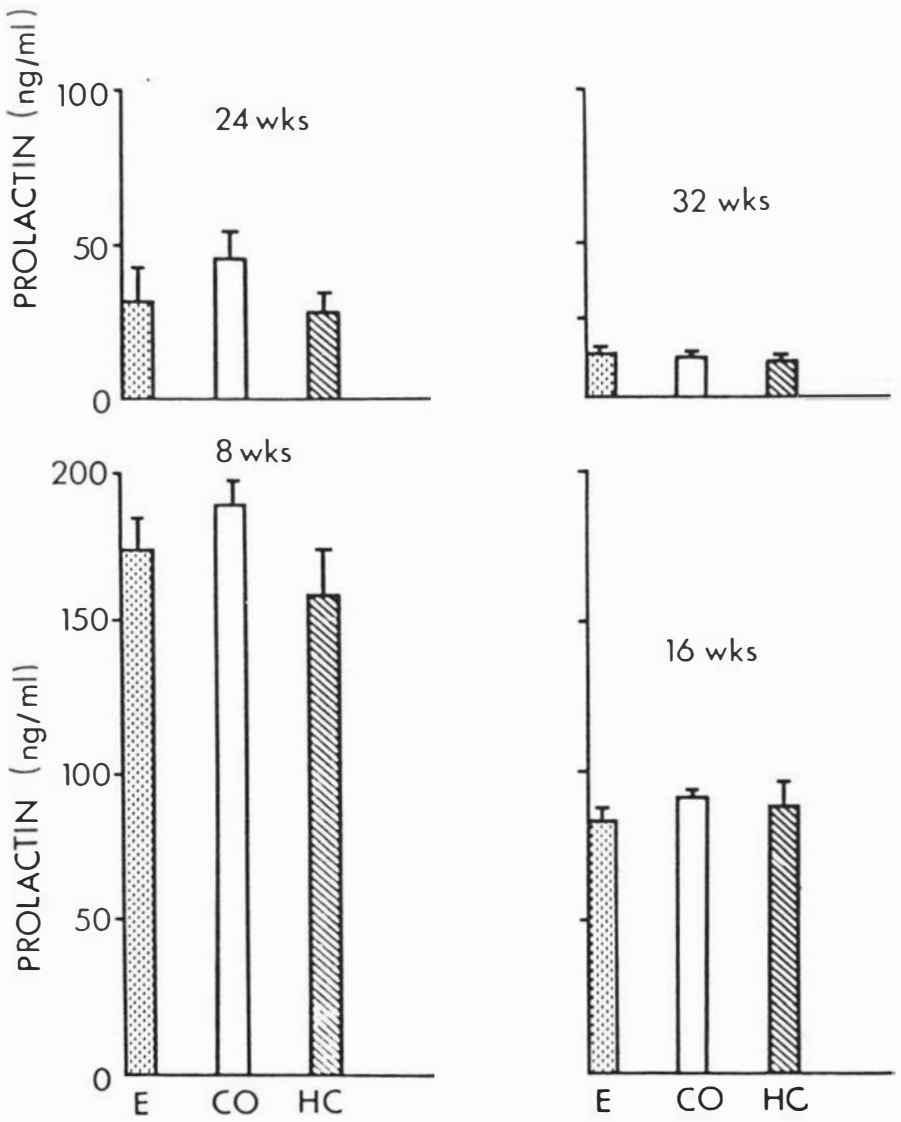



Figure 6.7: Mean (±SEM) plasma prolactin levels recorded from entire (E), hemicastrate (HC) and cryptorchid (CO) ram lambs during acute profile studies at 8, 16, 24 and 32 weeks of age (experiment 6.2, n=6).

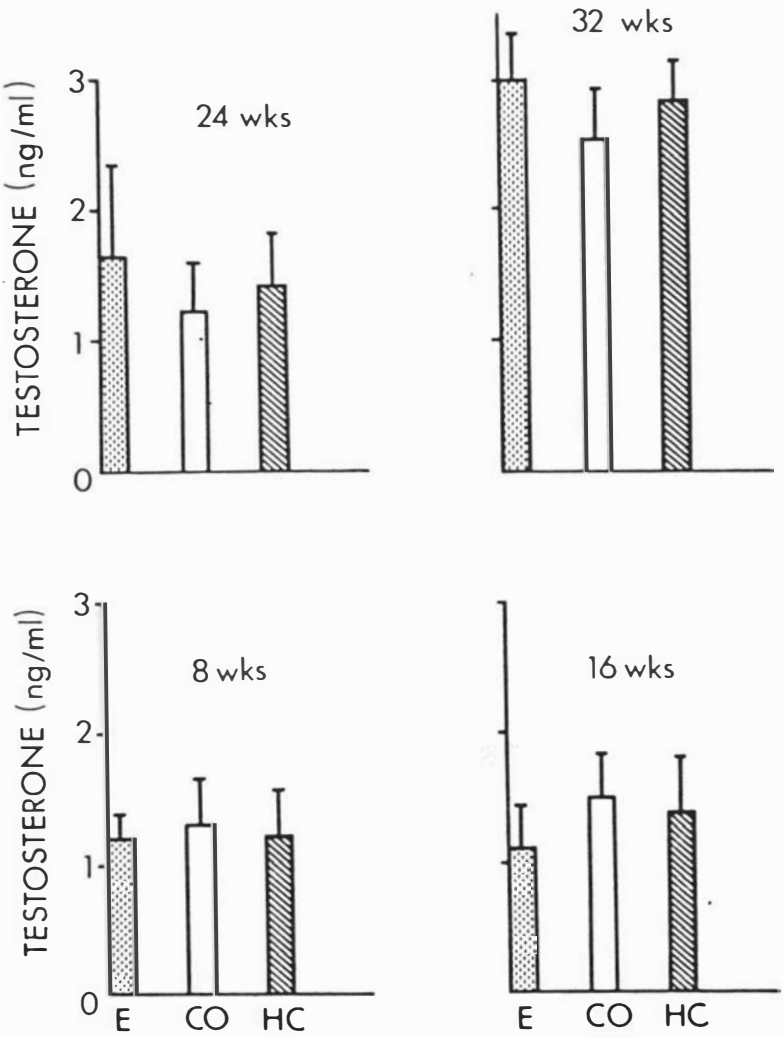



Figure 6.8: Mean ($\pm$SEM) plasma testosterone levels recorded from entire (E) hemicastrate (HC) cryptorchid (CO) ram lambs during acute profile studies at 8, 16, 24 and 32 weeks of age (experiment 6.2, n=6).

Table 6.10: Summary of analyses of variance of LH, FSH, prolactin and testosterone data from experiment 6.2

| Source of Variation | DF | | Variance Ratios | | | |
|---|---|---|---|---|---|---|
| | | | LH | FSH | Prolactin | Testosterone |
| A. Treatments | 2 | | | | 2.02 | 0.65 |
| (i) Entires vs Hemicastrates | | 1 | 0.94 | 1.09 | - | - |
| (ii) Entires & Hemicastrates vs Cryptorchids | | 1 | 115.86*** | 120.59*** | - | - |
| B. Age | 3 | | | | | |
| (i) Linear | | 1 | - | 33.48*** | 577.33*** | 34.45*** |
| (ii) Quadratic | | 1 | - | - | - | - |
| (iii) Cubic | | 1 | 9.44** | - | - | - |
| Remainder | | 2 | 1.08 | 1.79 | 0.89 | 1.21 |
| Interaction | | | | | | |
| Treatments x Age | 6 | | 0.96 | - | 0.38 | 0.13 |
| (i) Entire & Hemicastrates vs Cryptorchids x Linear | | 1 | - | 8.13** | - | - |
| (ii) Entires & Hemicastrates vs Cryptorchids x Quadratic | | 1 | - | 7.03* | - | - |
| Remainder | | 4 | - | 0.86 | - | - |
| Residual mean square | 60 | | 226.62 | 536.92 | 204.14 | 158.14 |

Table 6.9: Mean (±SEM) testosterone levels (ng/ml) during 4 hr profile studies (experiment 6.2)

| Age (wks) | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|
| 8 | 1.20±0.19 | 1.23±0.39 | 1.32±0.37 |
| 16 | 1.12±0.35 | 1.39±0.46 | 1.50±0.34 |
| 24 | 1.65±0.79 | 1.42±0.43 | 1.24±0.36 |
| 32 | 2.98±0.35 | 2.81±0.30 | 2.52±0.39 |

Analysis of variance revealed that mean plasma testosterone levels increased linearly ($P < 0.001$) with age, but surgical treatments had no significant influence on concentrations of this hormone.

(c) GnRH Responses (Experiment 6.3)

(i) LH

(Figures 6.9-6.16 and tables 6.11 and 6.13)

a. Pre-GnRH

At each age of sampling, pre-injection LH levels were higher in cryptorchids than in the other groups. Concentrations for them were 5.70±2.62 ng/ml compared to 1.80±0.71 and 0.44±0.17 ng/ml recorded from hemicastrates and entires, respectively. However, TP treatment resulted in a reduction of pre-GnRH injection LH levels in androgenized animals. Overall mean values for androgenized animals were 1.58±0.59, 0.49±0.20 and 0.29±0.08 ng/ml for cryptorchids, hemicastrates and entires, respectively.

b. Post-GnRH

Plasma LH levels increased following GnRH injection both in TP treated and non-androgenized animals. Mean total LH responses of non-androgenized cryptorchids declined from 504.48±16.73 ng/ml.hr at 8 weeks to 398.65±22.60 ng/ml.hr at 32 weeks. Similar patterns were obtained from non-androgenized hemicastrates and entires for which responses declined from 270.34±19.92 ng/ml.hr and 314.95±32.2 ng/ml.hr

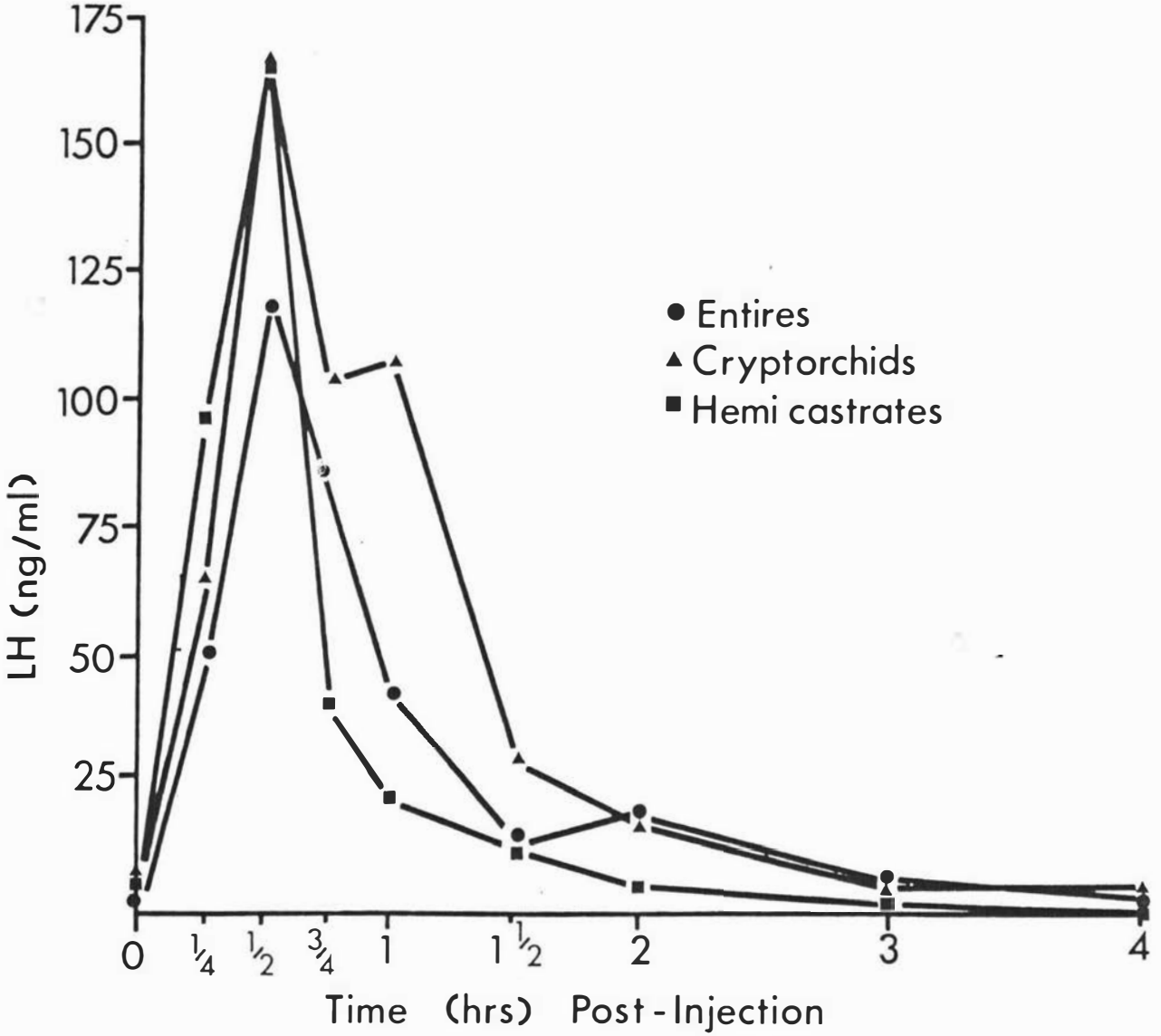



Figure 6.9: Plasma LH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 8 weeks of age (experiment 6.3, n=3).

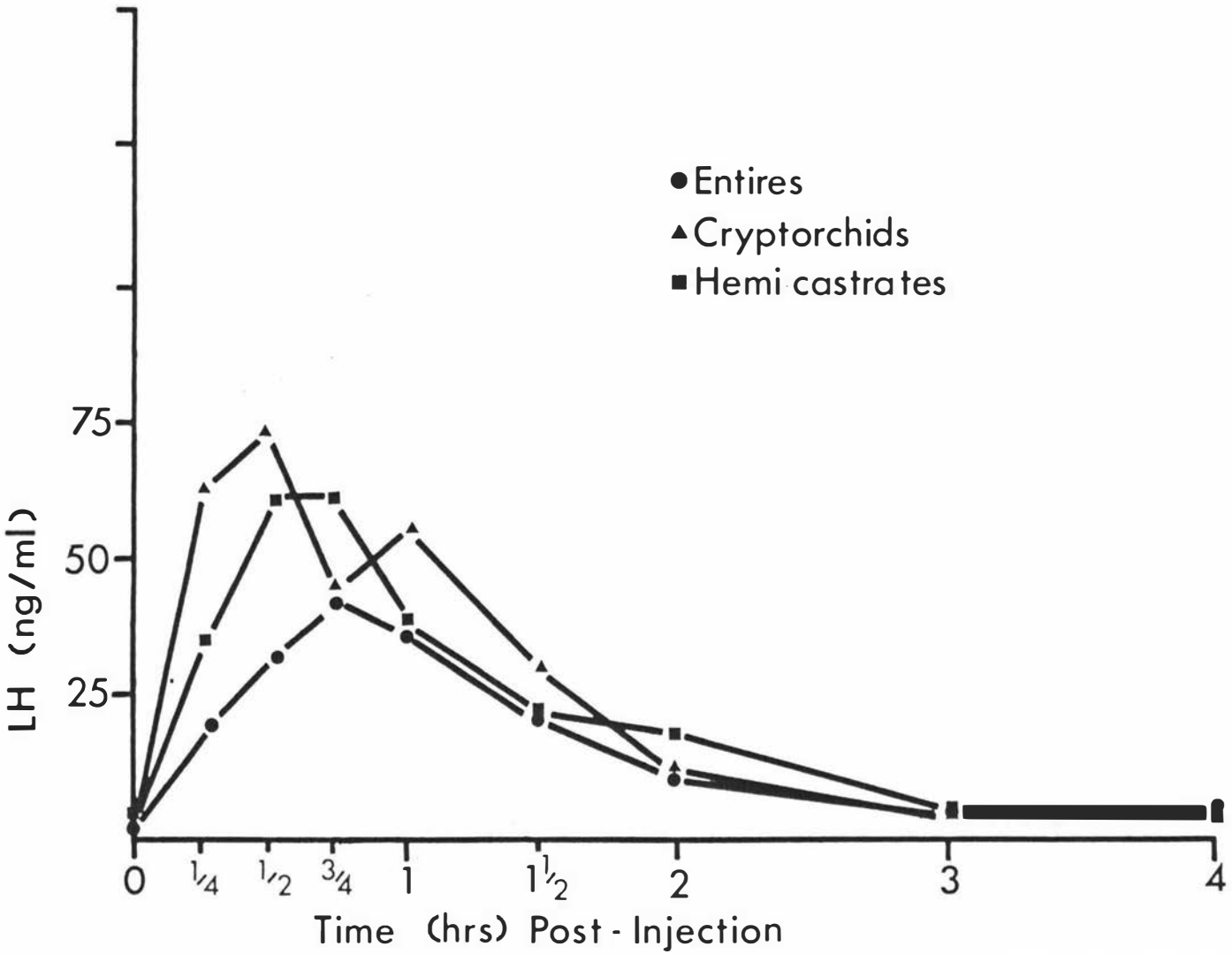



Figure 6.10: Plasma LH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 16 weeks of age (experiment 6.3, n=3).

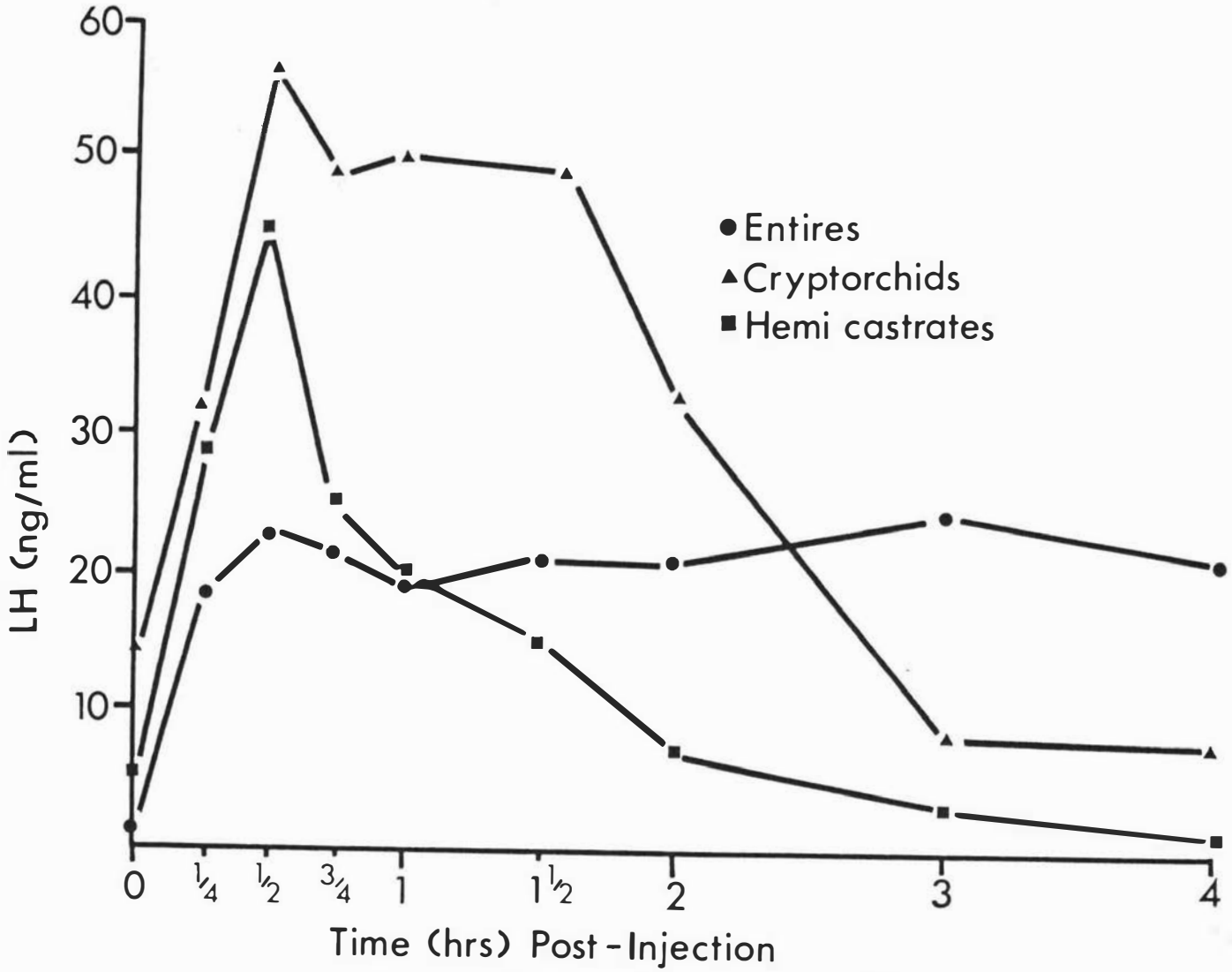



Figure 6.11: Plasma LH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 24 weeks of age (experiment 6.3, n=3).

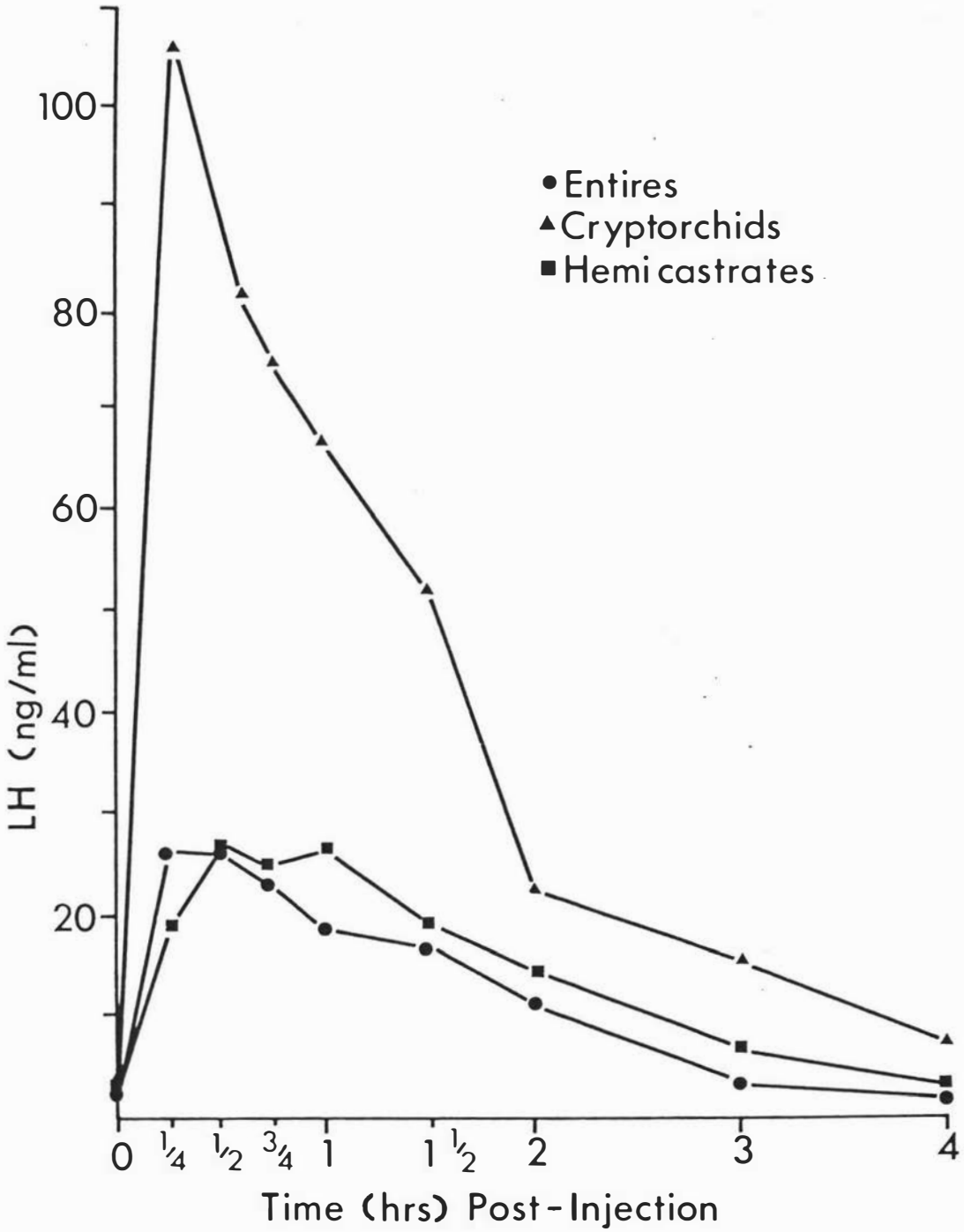



Figure 6.12: Plasma LH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 32 weeks of age (experiment 6.3, n=3).

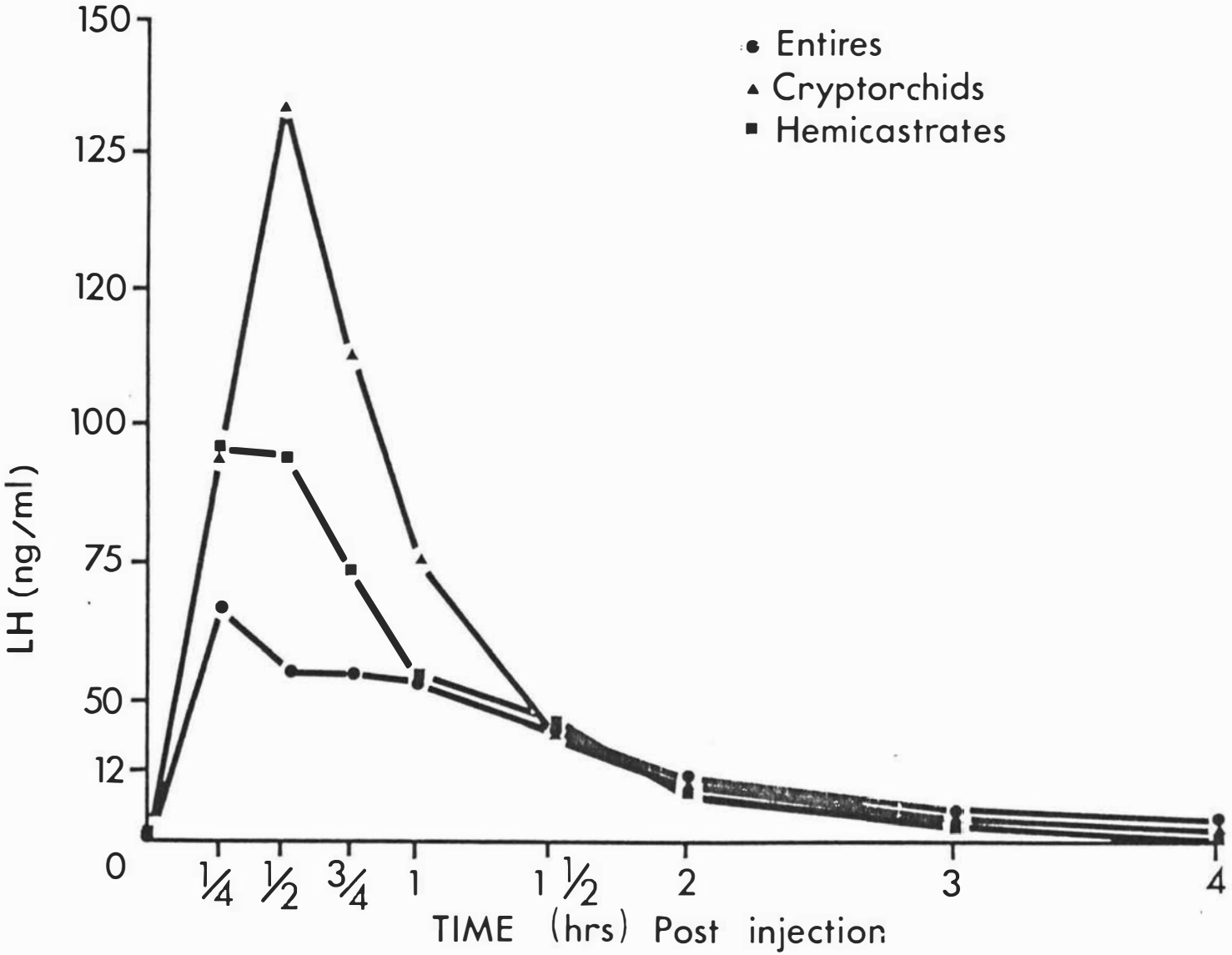



Figure 6.13: Plasma LH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 μg/kg) at 8 weeks of age (experiment 6.3, n=3).

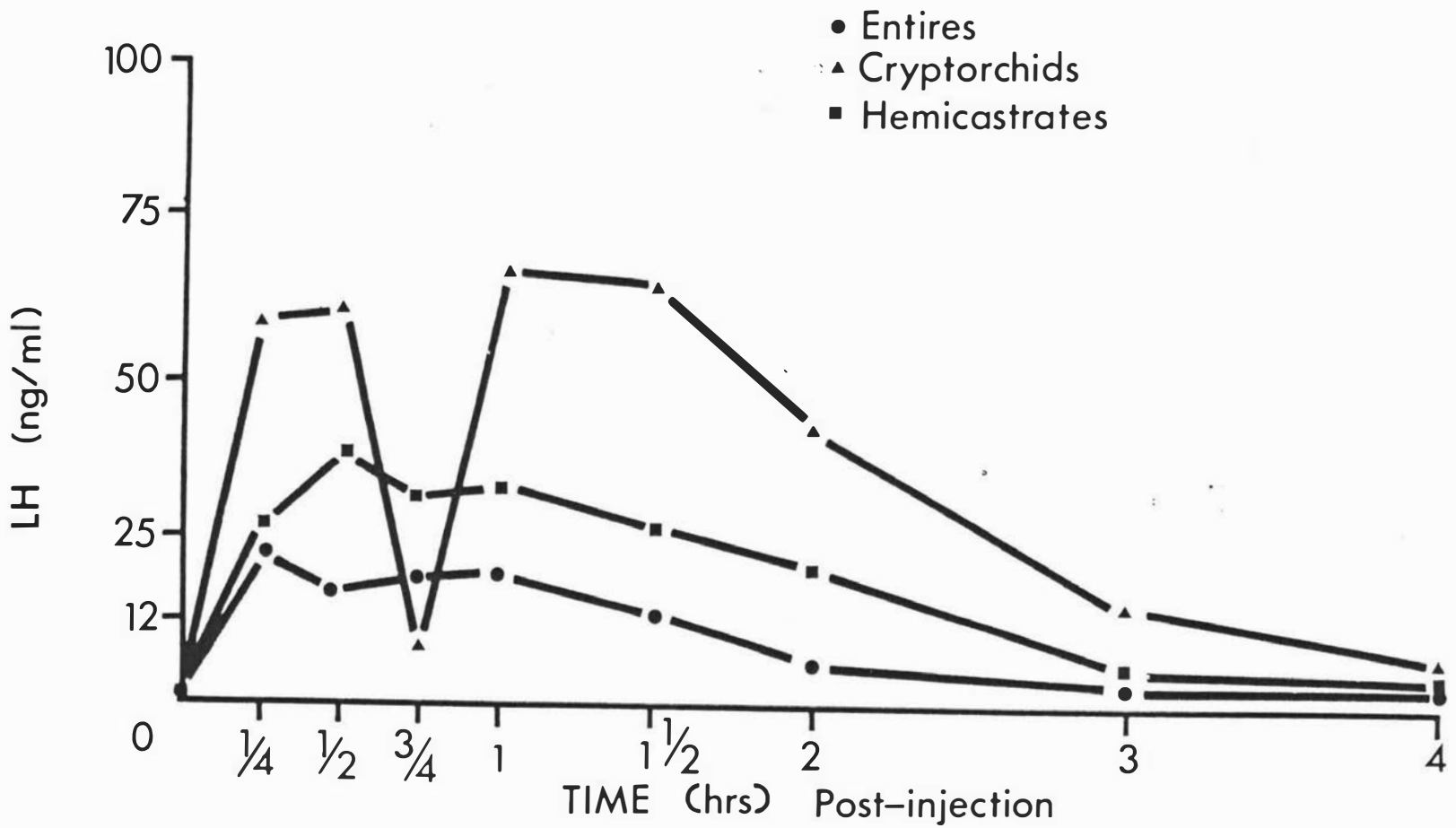



Figure 6.14: Plasma LH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 µg/kg) at 16 weeks of age (experiment 6.3, n=3).

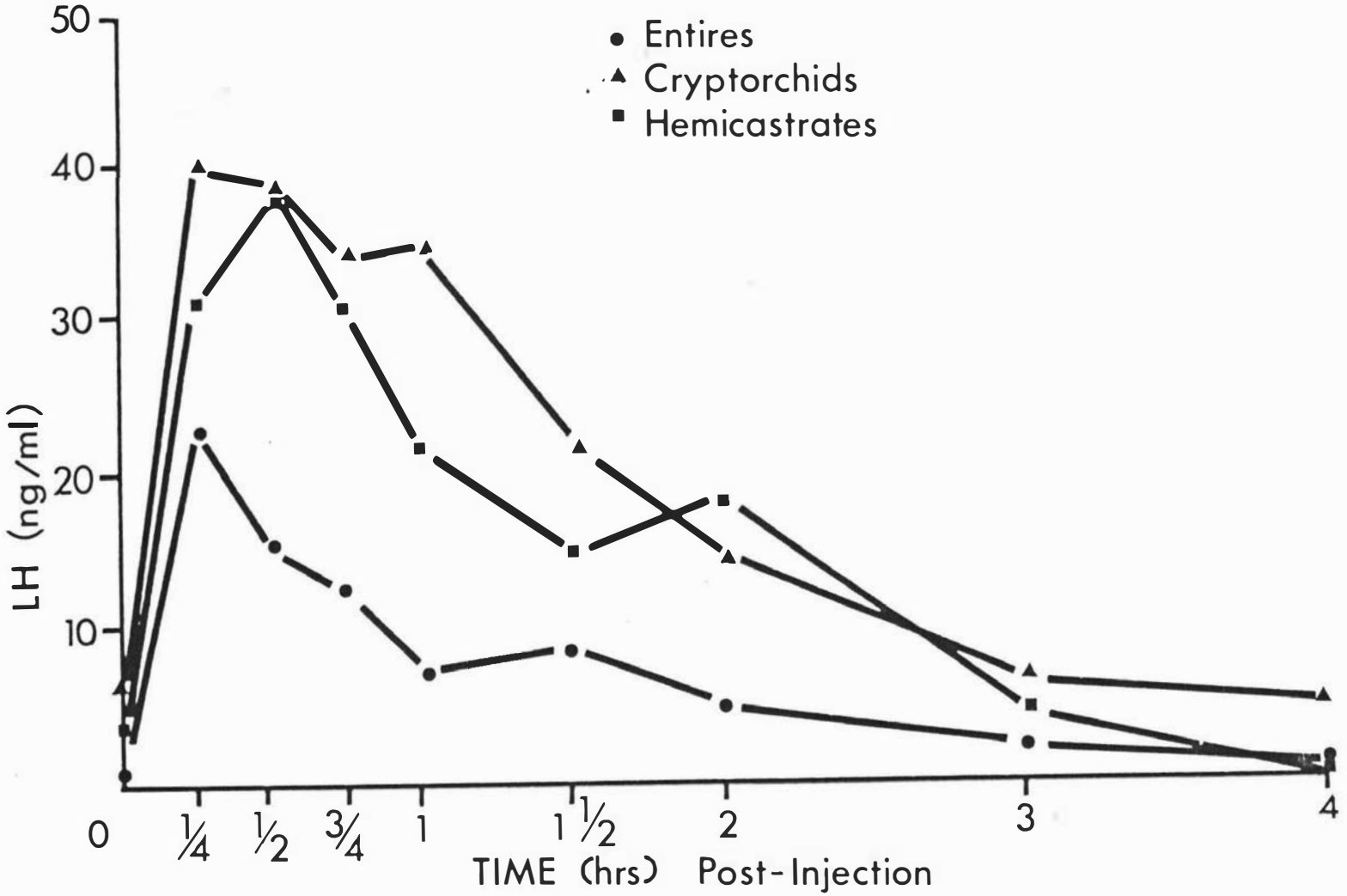



Figure 6.15: Plasma LH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 µg/kg) at 24 weeks of age (experiment 6.3, n=3).

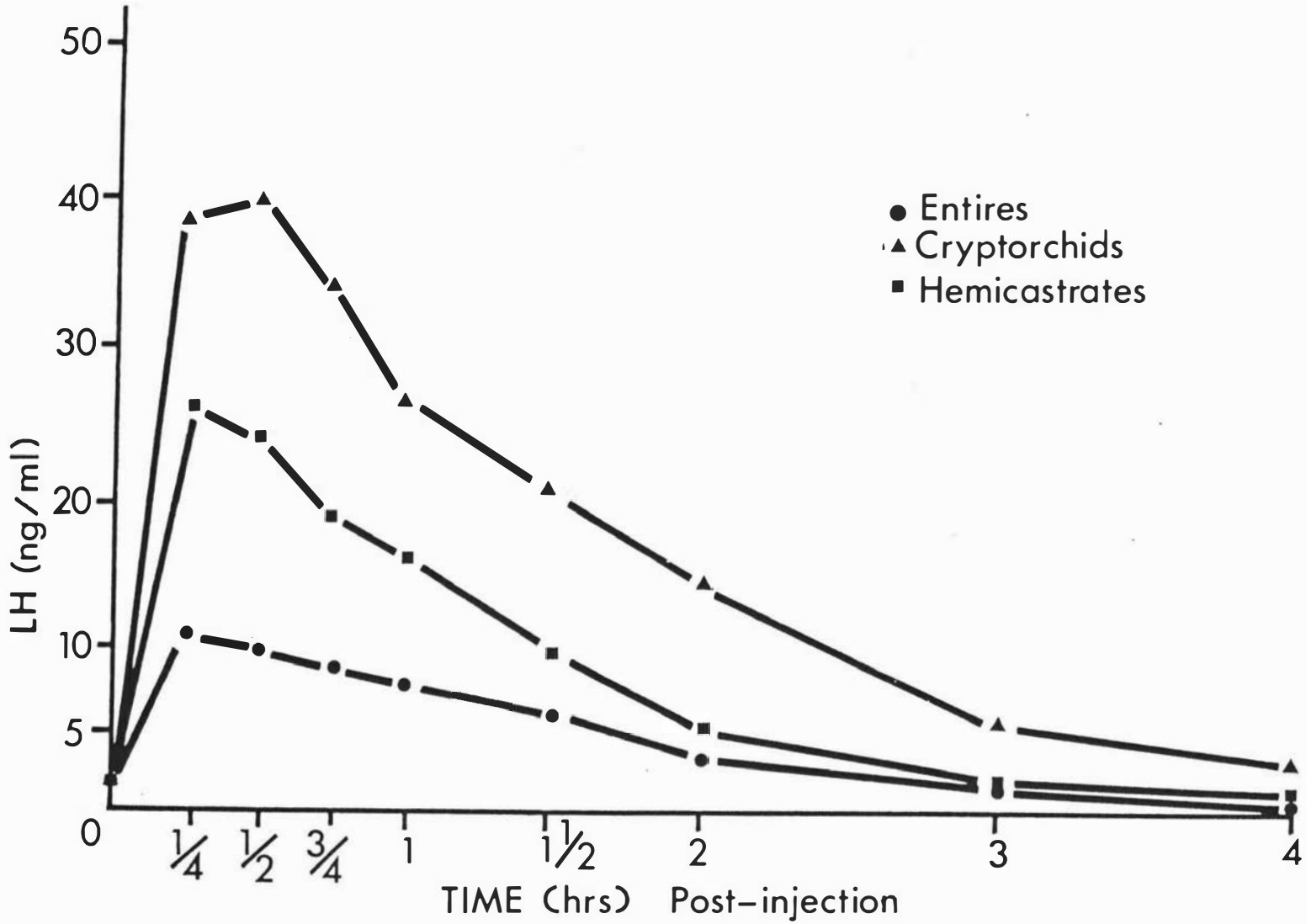



Figure 6.16: Plasma LH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 µg/kg) at 32 weeks of age (experiment 6.3, n=3).

Table 6.11: Mean (±SEM) pre-GnRH LH levels (ng/ml) and total hormone responses (ng/ml.hr) in experiment 6.3

| Surgical Treatment | | ENTIRES | | HEMICASTRATES | | CRYPTORCHIDS | |
|---|---|---|---|---|---|---|---|
| | Hormonal treatment | GnRH | GnRH.TP | GnRH | GnRH.TP | GnRH | GnRH.TP |
| Age (wks) | | | | | | | |
| 8 | Pre | 0.50±0.15 | 0.18±0.07 | 2.60±1.98 | 0.63±0.12 | 7.28±1.43 | 2.70±1.12 |
| | Total | 314.95±32.2 | 205.22±12.42 | 270.34±19.92 | 165.21±6.61 | 504.48±16.73 | 426.76±51.90 |
| 16 | Pre | 0.47±0.30 | 0.11±0.37 | 0.27±0.12 | 0.09±0.02 | 0.50±0.20 | 0.59±0.24 |
| | Total | 233.47±12.86 | 116.47±11.75 | 310.27±5.47 | 171.90±6.69 | 385.64±16.82 | 335.09±8.33 |
| 24 | Pre | 0.20±0.02 | 0.50±0.07 | 2.06±1.96 | 1.00±0.13 | 12.31±2.30 | 2.52±1.10 |
| | Total | 131.84±3.29 | 84.91±6.78 | 178.11±12.57 | 155.25±7.62 | 337.38±21.88 | 215.84±31.00 |
| 32 | Pre | 0.60±0.21 | 0.35±0.09 | 2.28±1.53 | 0.25±0.20 | 2.75±1.10 | 0.50±0.21 |
| | Total | 126.43±11.39 | 73.20±11.69 | 168.45±15.44 | 105.68±2.98 | 398.65±22.60 | 168.87±24.22 |

at 8 weeks to 168.45±15.44 ng/ml.h and 126.43±11.39 ng/ml.hr at 32 weeks.

TP treatment resulted in reductions in total LH responses in all groups ($P < 0.01$). Thus for cryptorchids mean total responses were 426.76±51.90 ng/ml.hr at 8 weeks, declining to 168.87±24.22 ng/ml.hr at 32 weeks. Comparable values for androgenised hemicastrates were 165.21±6.61 ng/ml.hr at 8 weeks and 105.68±2.98 ng/ml.hr at 32 weeks, while for entires total responses were 205.22±12.42 ng/ml.hr at 8 weeks and 73.20±11.69 ng/ml.hr at 32 weeks. In the analysis of variance of LH response data cryptorchid ram lambs had significantly higher ($P < 0.001$) total responses than the other two groups. Also the decrease with age for total responses was statistically significant ($P < 0.001$).

(ii) FSH

(Figures 6.17-6.24 and tables 6.12 and 6.13)

a. Pre-GnRH

The pattern of changes in pre-injection hormone levels concurred with the general pattern of hormonal data recorded in experiments 6.1 and 6.2. The overall mean pre-injection FSH level of cryptorchids was 312.55±119.45 ng/ml, which was considerably higher than the values of 58.61±27.12 ng/ml and 35.17±8.53 ng/ml recorded from hemicastrates and entires.

TP pre-treatment caused a reduction in basal FSH levels in all animals. For these animals the overall mean concentration recorded from cryptorchids was 200.88±69.02 ng/ml while concentrations of 27.47±12.34 ng/ml and 28.08±9.59 ng/ml were recorded from hemicastrates and entires respectively.

b. Post-GnRH

Graphical examination of FSH responses following GnRH administration indicated that the responses were less dramatic than those observed for LH. Peak levels were recorded within 1-2 hours of GnRH injection.

The total hormone responses recorded from cryptorchids

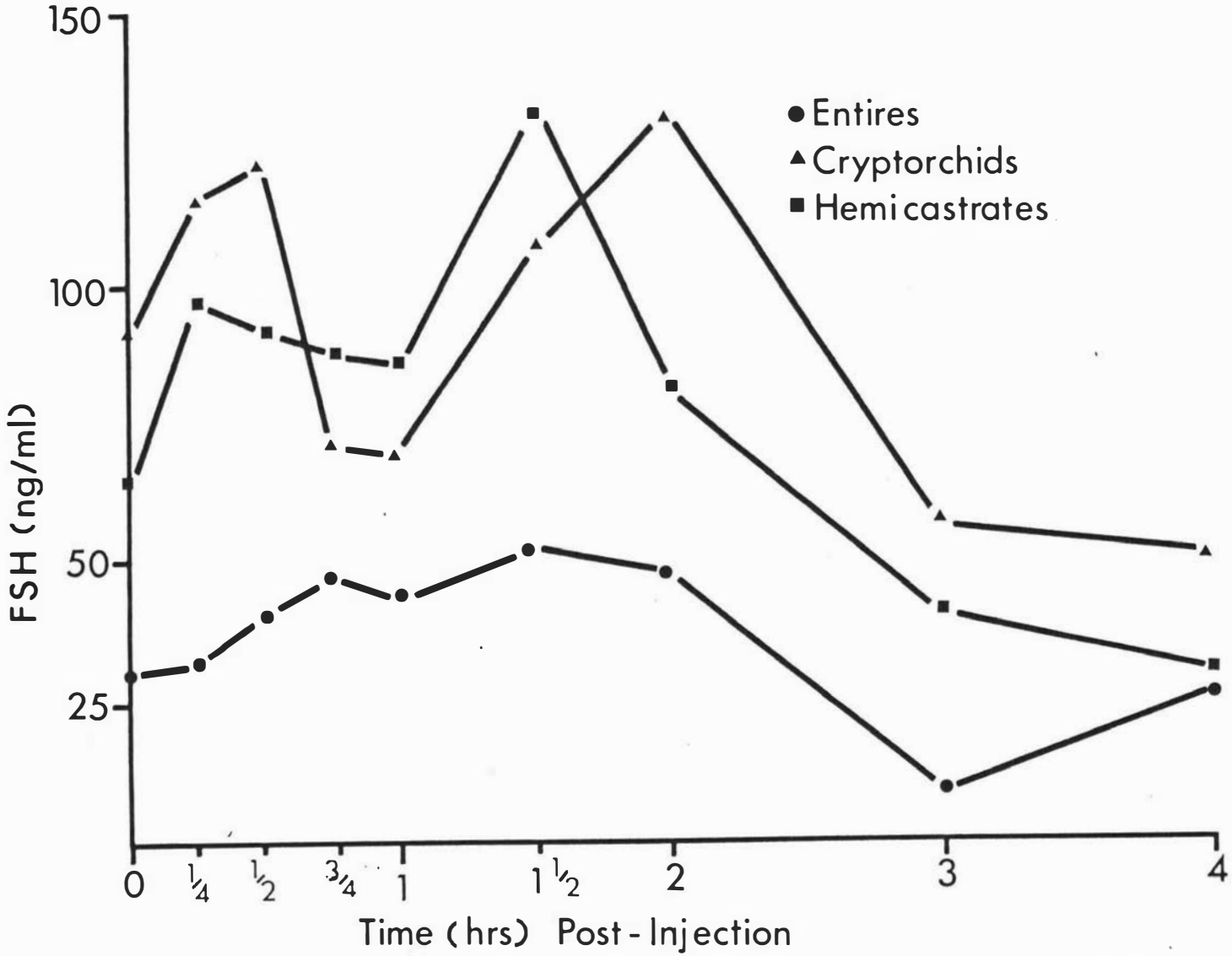



Figure 6.17: Plasma FSH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 8 weeks of age (experiment 6.3, n=3).

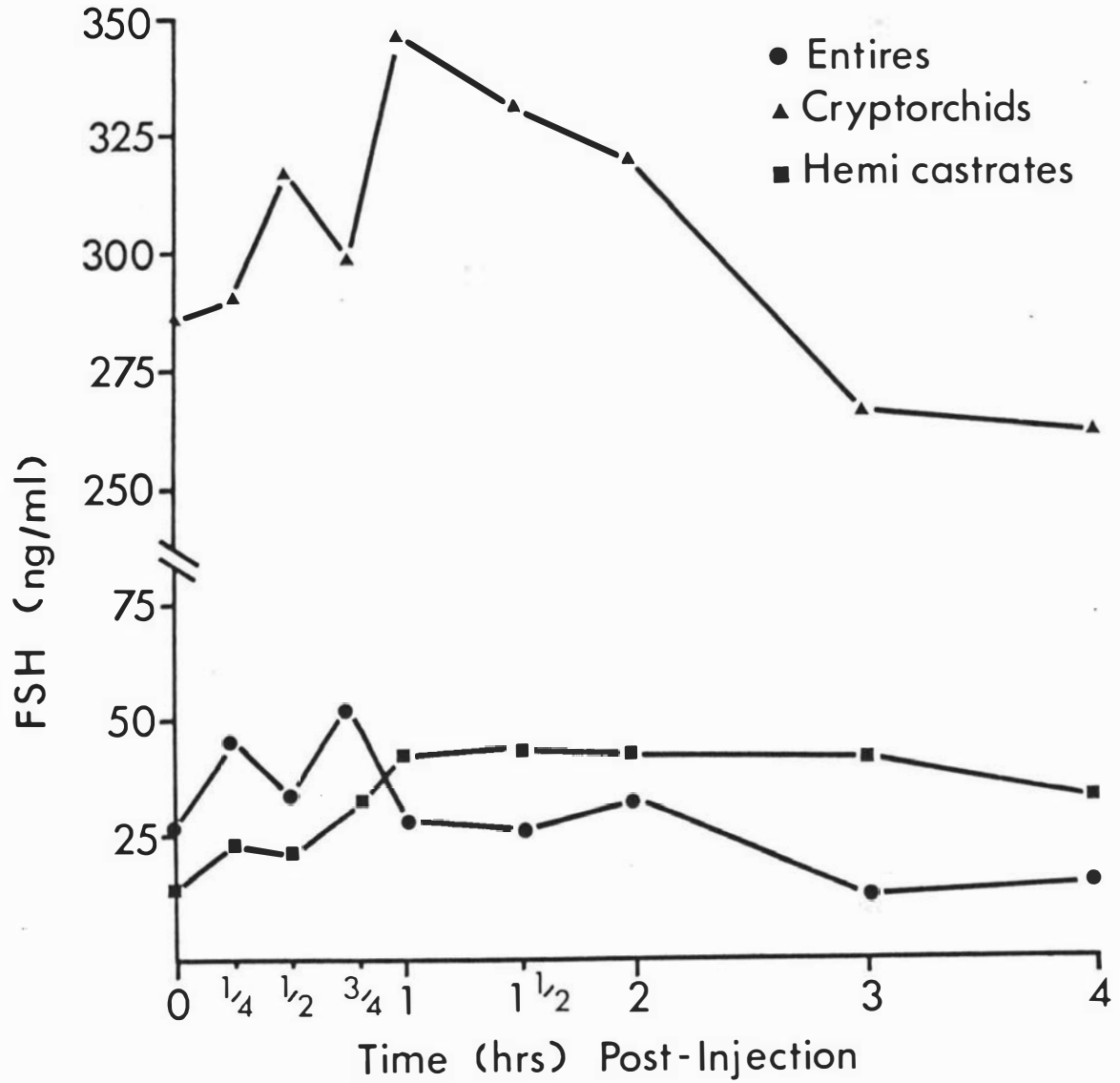



Figure 6.18: Plasma FSH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 16 weeks of age (experiment 6.3, n=3).

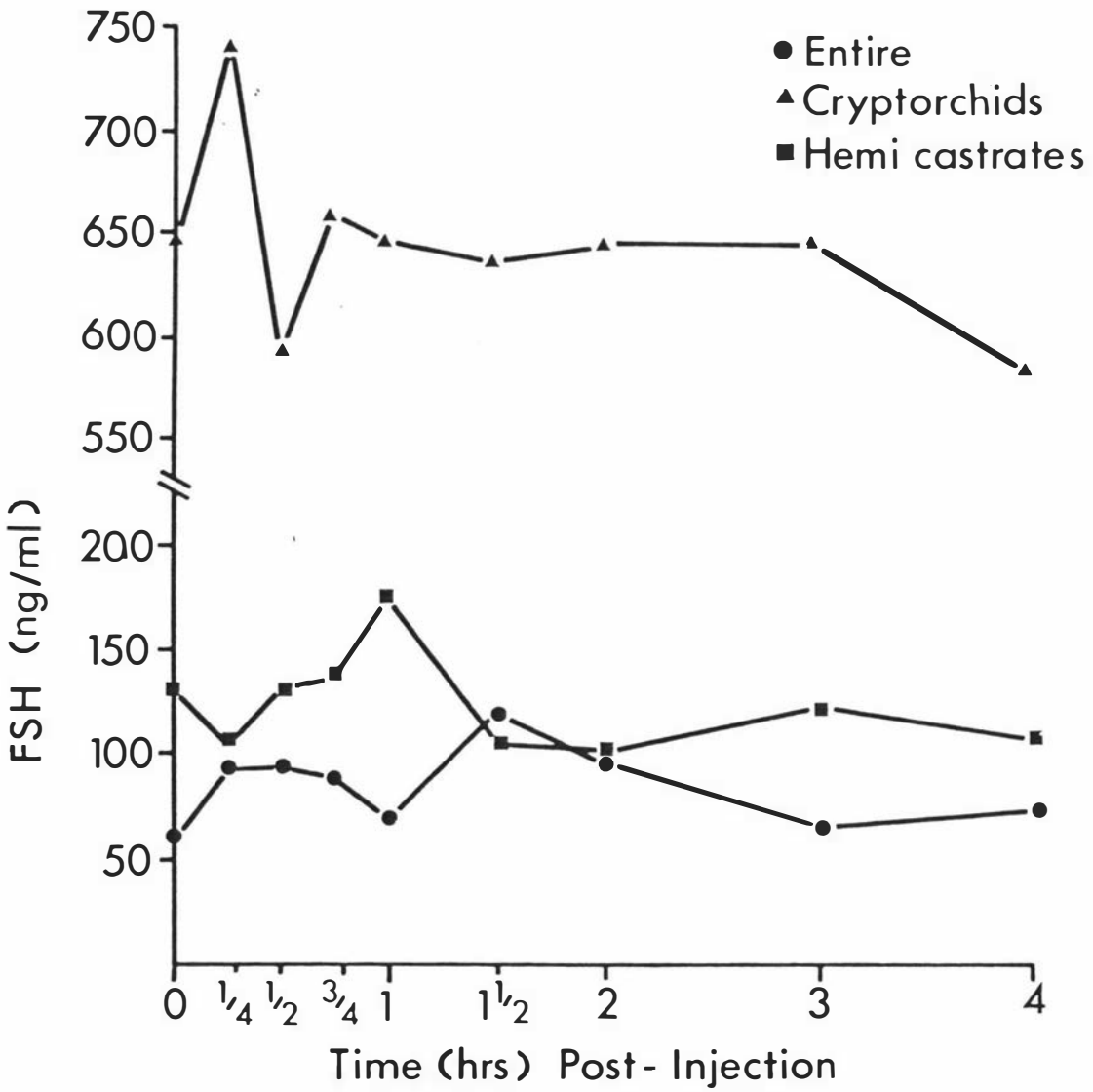



Figure 6.19: Plasma FSH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 24 weeks of age (experiment 6.3, n=3).

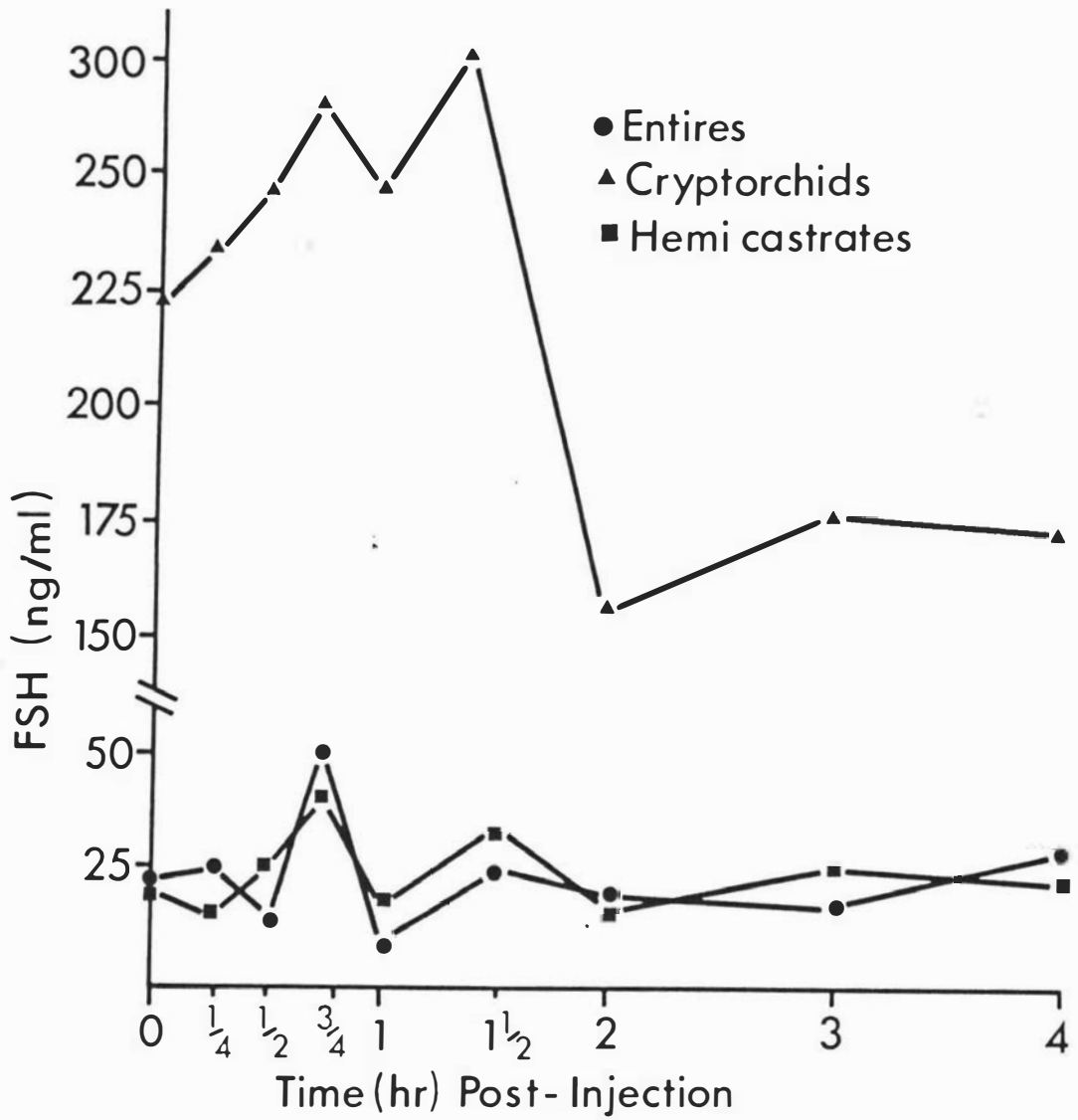



Figure 6.20: Plasma FSH responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 32 weeks of age (experiment 6.3, n=3).

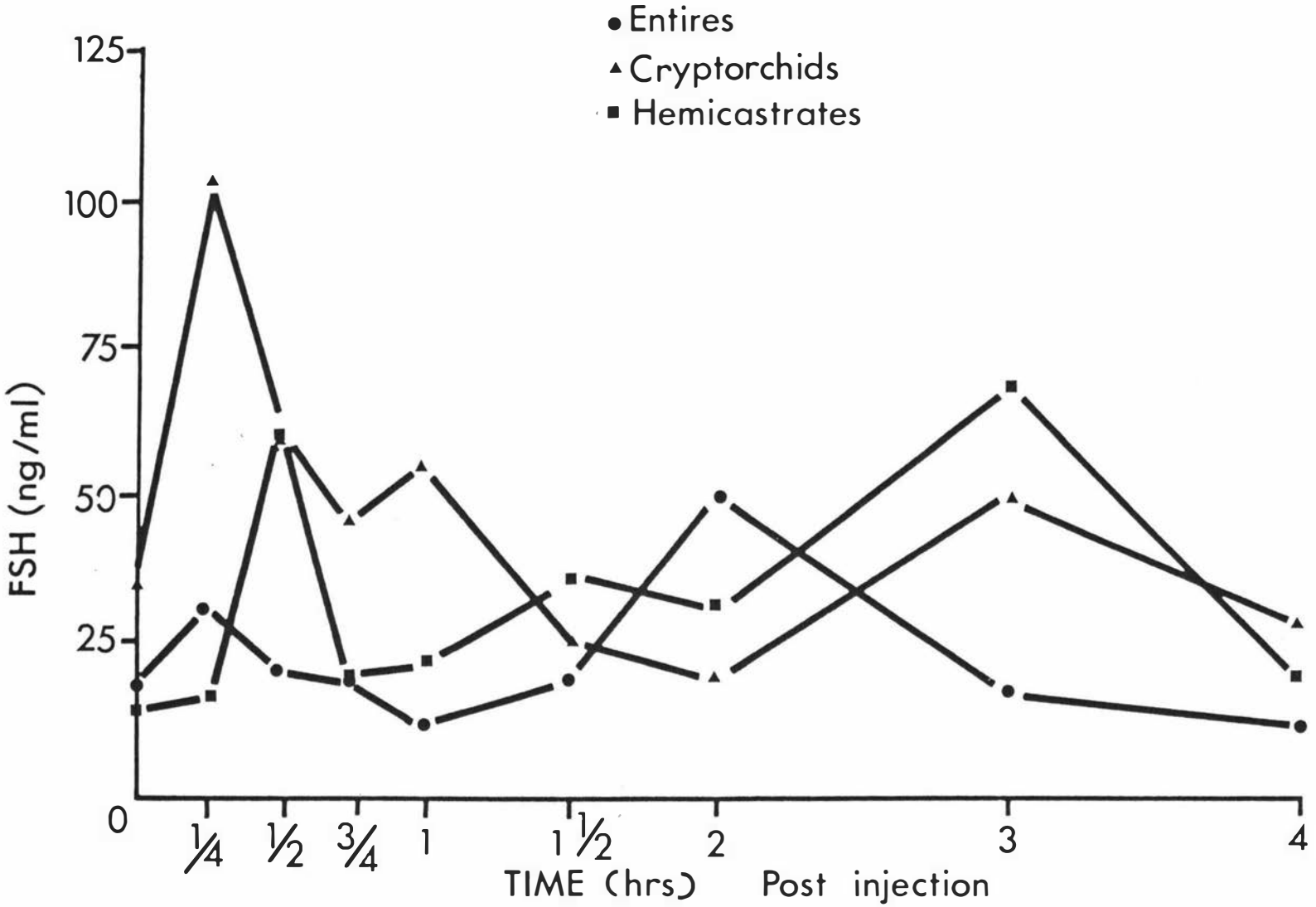



Figure 6.21: Plasma FSH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 µg/kg) at 8 weeks of age (experiment 6.3, n=3).

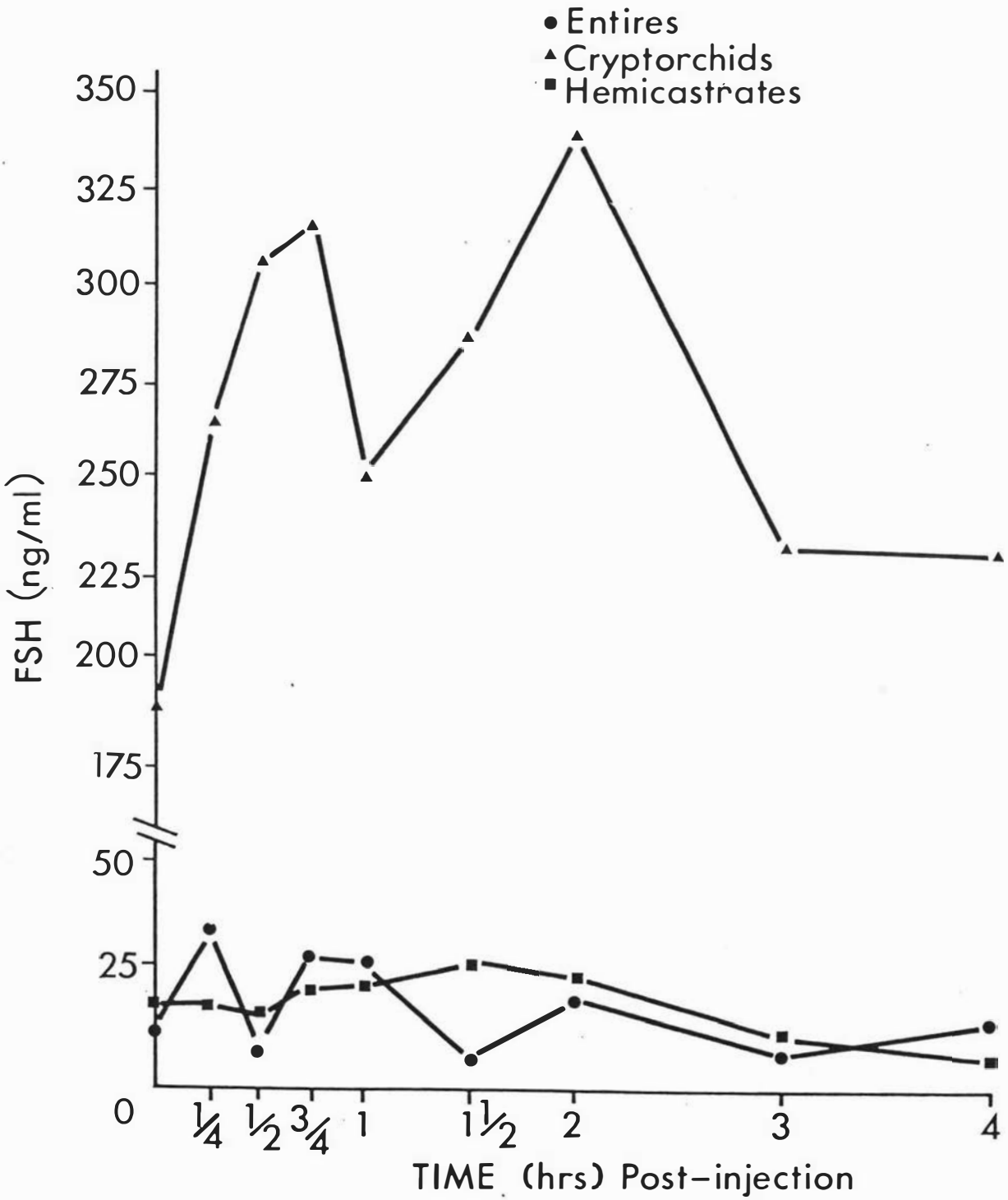



Figure 6.22: Plasma FSH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 µg/kg) at 16 weeks of age (experiment 6.3, n=3).

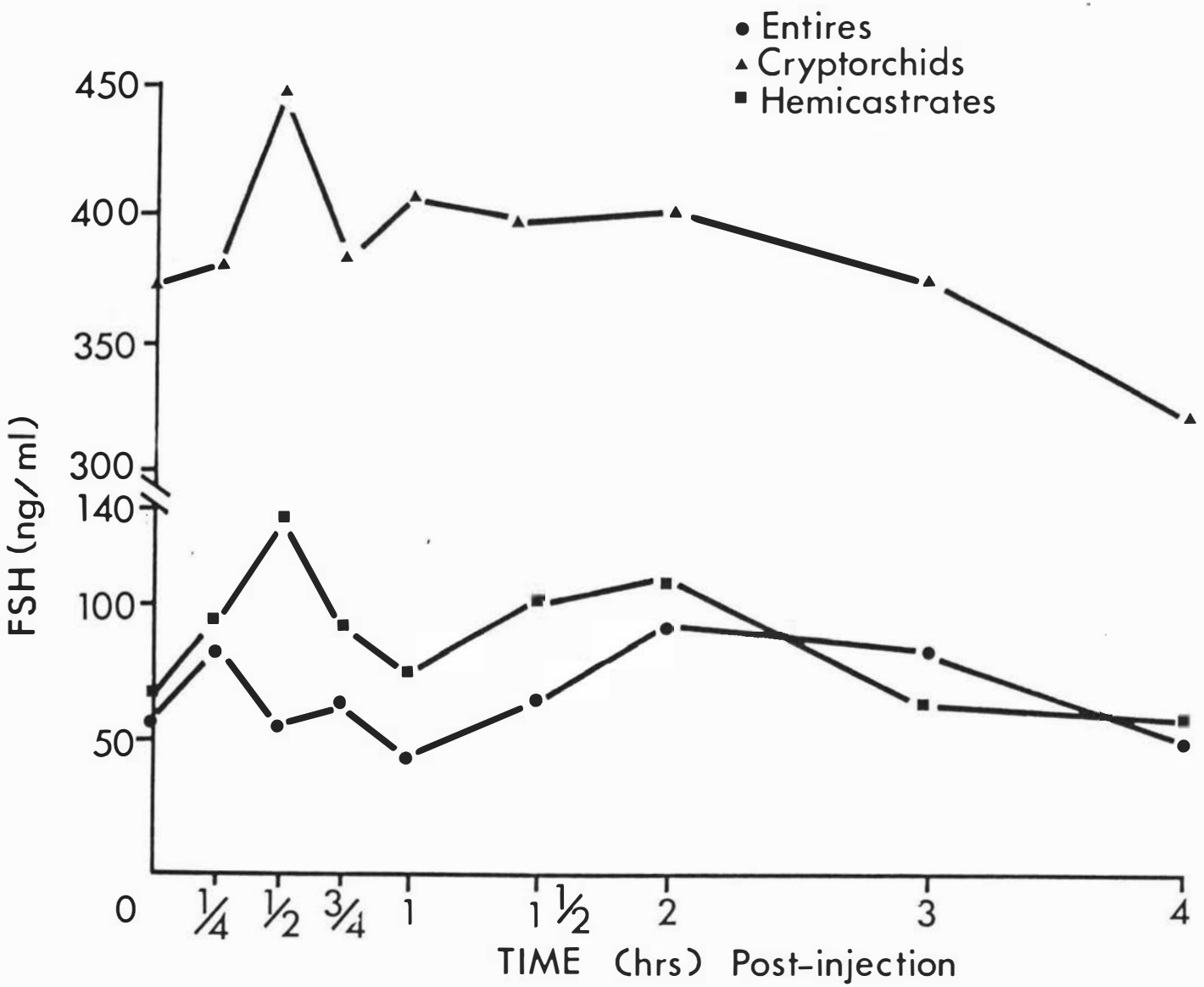



Figure 6.23: Plasma FSH responses of androgenised (testosterone propionate, 1 mg/kg) rams following administration of GnRH (0.5 µg/kg) at 24 weeks of age (experiment 6.3, n=3).

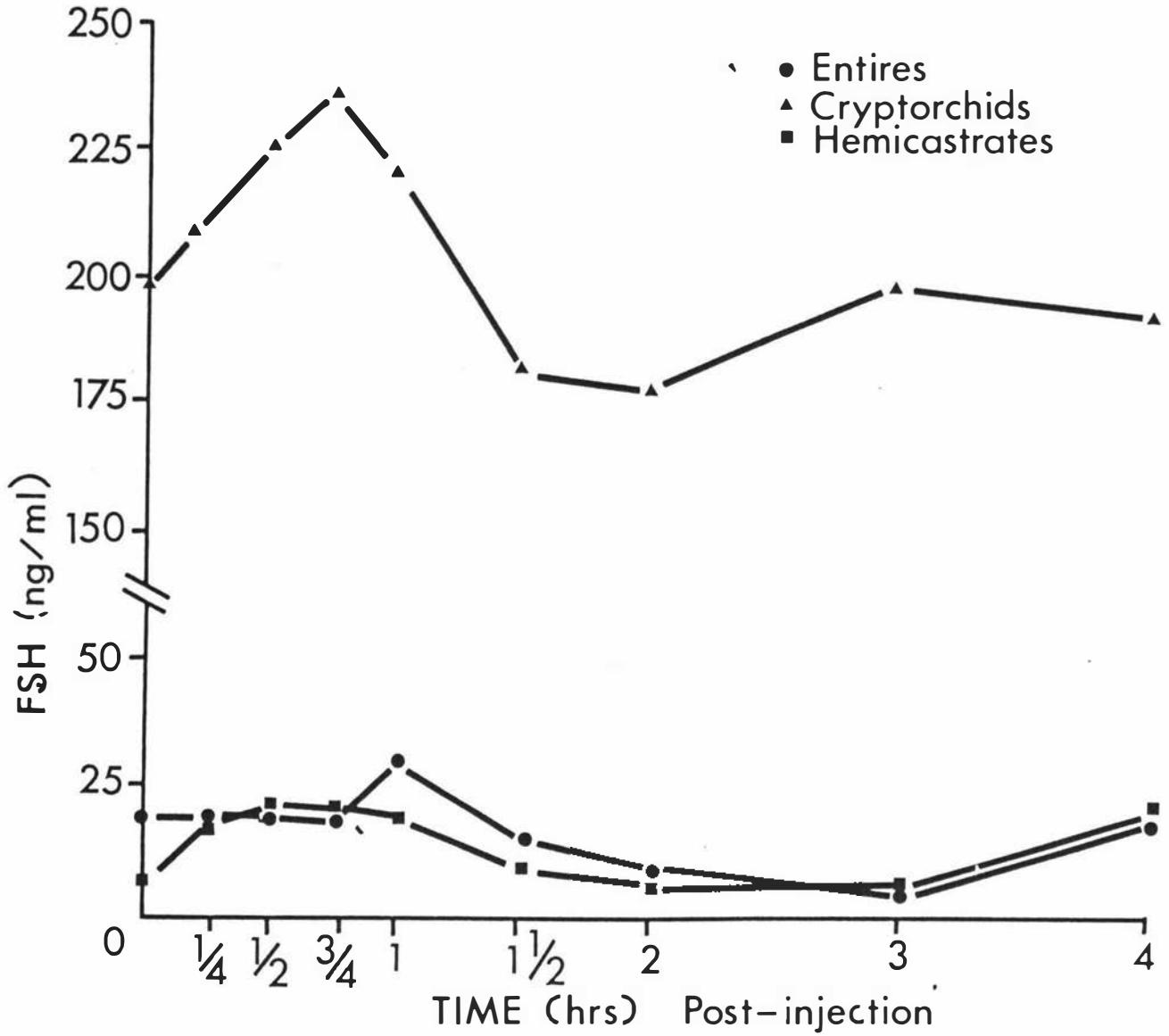



Figure 6.24: Plasma FSH responses of androgenised (testosterone propionate, 1 mg/kg) ram lambs following administration of GnRH (0.5 µg/kg) at 32 weeks of age (experiment 6.3, n=3).

Table 6.12: Mean (±SEM) pre-GnRH FSH levels (ng/ml) and total hormone responses (ng/ml.hr) in experiment 6.3

| Surgical Treatment | | ENTIRES | | HEMICASTRATES | | CRYPTORCHIDS | |
|---|---|---|---|---|---|---|---|
| Age (wks) | Hormonal treatment | GnRH | GnRH.TP | GnRH | GnRH.TP | GnRH | GnRH.TP |
| 8 | Pre | 30.60±15.70 | 19.66±7.51 | 64.93±0.55 | 16.31±9.53 | 91.01±24.02 | 35.47±4.68 |
| | Total | 512.57±21.66 | 335.27±17.08 | 737.00±0.04 | 342.55±15.79 | 1041.01±74.2 | 533.52±27.31 |
| 16 | Pre | 26.86±11.95 | 14.99±2.53 | 14.76±6.71 | 20.75±2.86 | 285.64±59.7 | 195.33±56.55 |
| | Total | 370.56±30.99 | 216.45±30.18 | 606.40±22.50 | 182.62±20.48 | 3564.6 ±162.30 | 3227.18±307.2 |
| 24 | Pre | 61.09±9.54 | 56.58±18.47 | 132.58±18.75 | 63.81±11.36 | 649.54±10.1 | 373.40±110.52 |
| | Total | 1270.11±200.15 | 720.33±8.94 | 1461.59±127.02 | 1045.57±125.6 | 7727.2 ±305.03 | 3848.57±664.50 |
| 32 | Pre | 24.48±7.58 | 21.06±4.03 | 21.88±11.26 | 9.04±3.02 | 224.0 ±19.01 | 199.33±61.72 |
| | Total | 342.40±30.10 | 184.16±5.02 | 335.49±35.65 | 185.16±26.88 | 2288.75±35.09 | 2076.45±271.0 |

Table 6.13: Summary of analyses of variance of total LH and FSH output data from experiment 6.3

| Source of Variation | | DF | Variance Ratios | |
|---|---|---|---|---|
| | | | LH | FSH |
| A. Treatments | | 2 | | |
| (i) | Entires vs Hemicastrates | 1 | 1.15 | 0.14 |
| (ii) | Entires & Hemicastrates vs Cryptorchids | 1 | 44.06*** | 107.93*** |
| B. Age | | 3 | | |
| (i) | Linear | 1 | 21.79*** | 5.77* |
| (ii) | Quadratic | 1 | - | 30.00*** |
| (iii) | Cubic | 1 | - | 12.14** |
| | Remainder | 2 | 1.02 | - |
| C. Testosterone | | 1 | 12.52** | 6.74* |
| Interactions | | | | |
| A X B | | 6 | 0.42 | - |
| (i) | Entire & Hemicastrate vs Cryptorchid x linear | 1 | - | 10.58** |
| (ii) | Entire & Hemicastrate vs Cryptorchid x quadratic | 1 | - | 29.88*** |
| | Remainder | 4 | - | 0.06 |
| A X C | | 2 | 0.49 | 1.02 |
| B X C | | 3 | 0.02 | 1.14 |
| Residual mean square | | 54 | 1121.49 | 105286.41 |

were significantly higher ($P < 0.001$) than those recorded from the other two groups. Mean total responses recorded from non-androgenized cryptorchids increased from $1040.01 \pm 74.22$ ng/ml.hr at 8 weeks to $7727.22 \pm 30.00$ ng/ml.hr at 24 weeks and then declined to $2288.75 \pm 125.63$ ng/ml.hr at 32 weeks. Comparable values from non-androgenized hemicastrates and entires were $737.04 \pm 48.97$ ng/ml.hr and $512.57 \pm 21.66$ ng/ml.hr at 8 weeks, $1461.59 \pm 127.02$ ng/ml.hr and $1270.11 \pm 200.15$ ng/ml.hr at 24 weeks and $335.49 \pm 35.65$ ng/ml.hr and $342.40 \pm 30.10$ ng/ml at 32 weeks, respectively.

TP treatment resulted in a reduction of total FSH responses in all groups ($P < 0.05$). Thus mean total responses observed from testosterone treated cryptorchids increased from $533.52 \pm 27.31$ ng/ml.hr at 8 weeks to $3848.57 \pm 664.50$ ng/ml at 24 weeks and then declined to $2076.45 \pm 271.10$ ng/ml at 32 weeks. Responses of hemicastrates and entires increased from $342.55 \pm 15.79$ ng/ml.hr and $335.27 \pm 17.08$ ng/ml.hr at 8 weeks, to $1045.57 \pm 125.67$ ng/ml.hr and $720.33 \pm 8.94$ ng/ml.hr at 24 weeks and then subsequently declined to $185.16 \pm 26.88$ ng/ml.hr and $184.16 \pm 5.02$ ng/ml.hr at 32 weeks, respectively.

The overall patterns of changes with age for total FSH responses were represented not only by a highly significant quadratic ($P < 0.001$) component, but also by significant linear ($P < 0.05$) and cubic ($P < 0.01$) components. Significant entire and hemicastrate *vs* cryptorchid x linear ($P < 0.01$) and quadratic ($P < 0.001$) components of the treatments x age interaction indicated that these effects of age were more marked in cryptorchids than in the other two groups.

(iii) Testosterone

(Figures 6.25 - 6.28 and tables 6.14 and 6.15)

a. Pre-GnRH

Overall mean plasma testosterone concentrations increased from $1.82 \pm 0.63$ ng/ml at 8 weeks to $3.54 \pm 1.02$ ng/ml at 32 weeks. In contrast, pre-GnRH plasma testosterone levels recorded from TP treated animals ranged between 17 and 22 ng/ml.

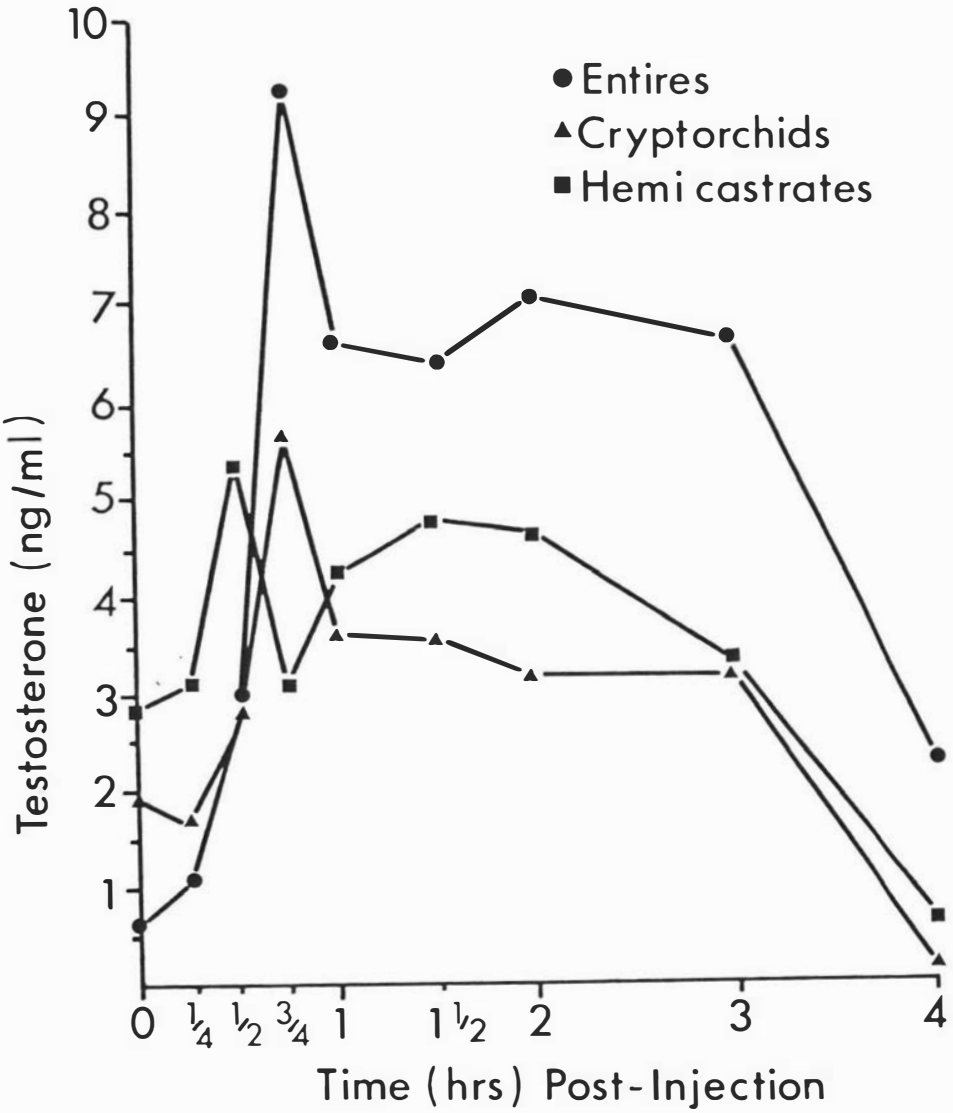



Figure 6.25: Plasma testosterone responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 8 weeks of age (experiment 6.3, n=3).

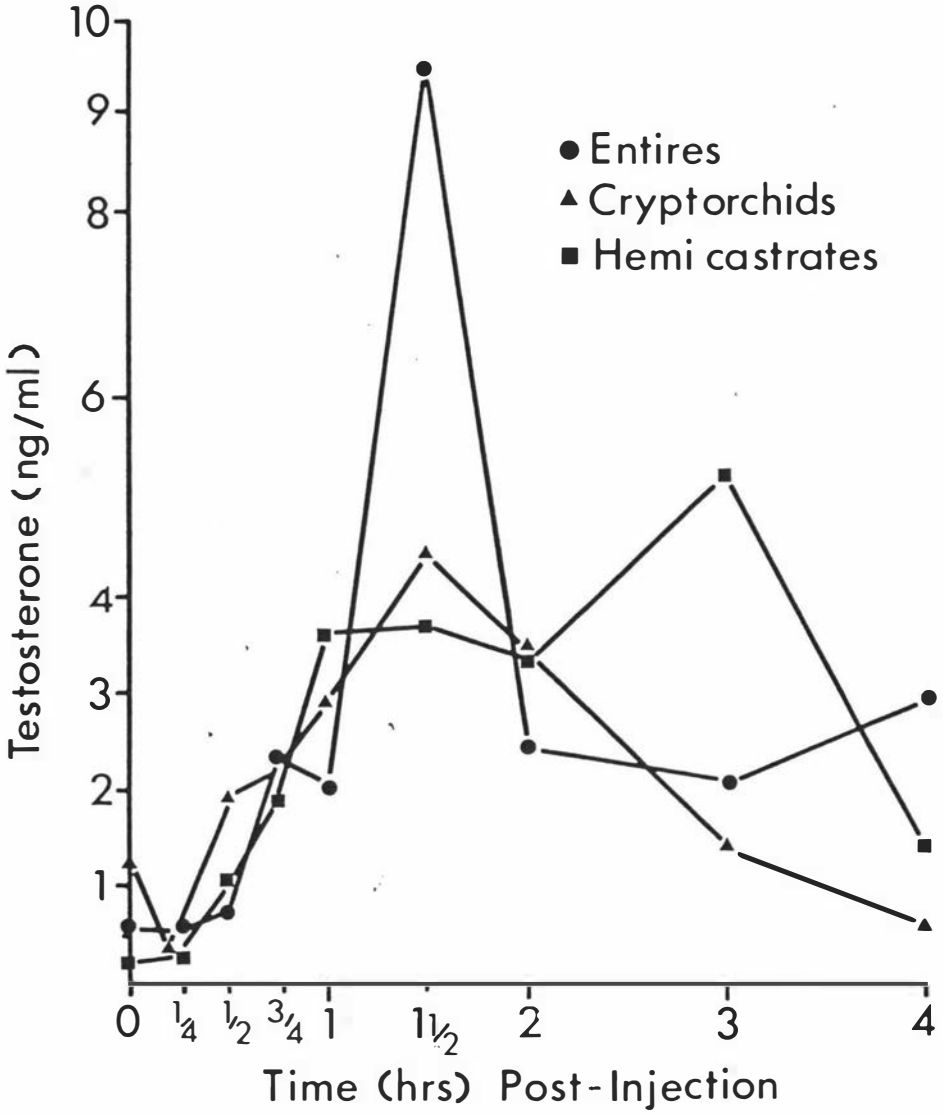



Figure 6.26: Plasma testosterone responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 16 weeks of age (experiment 6.3, n=3).

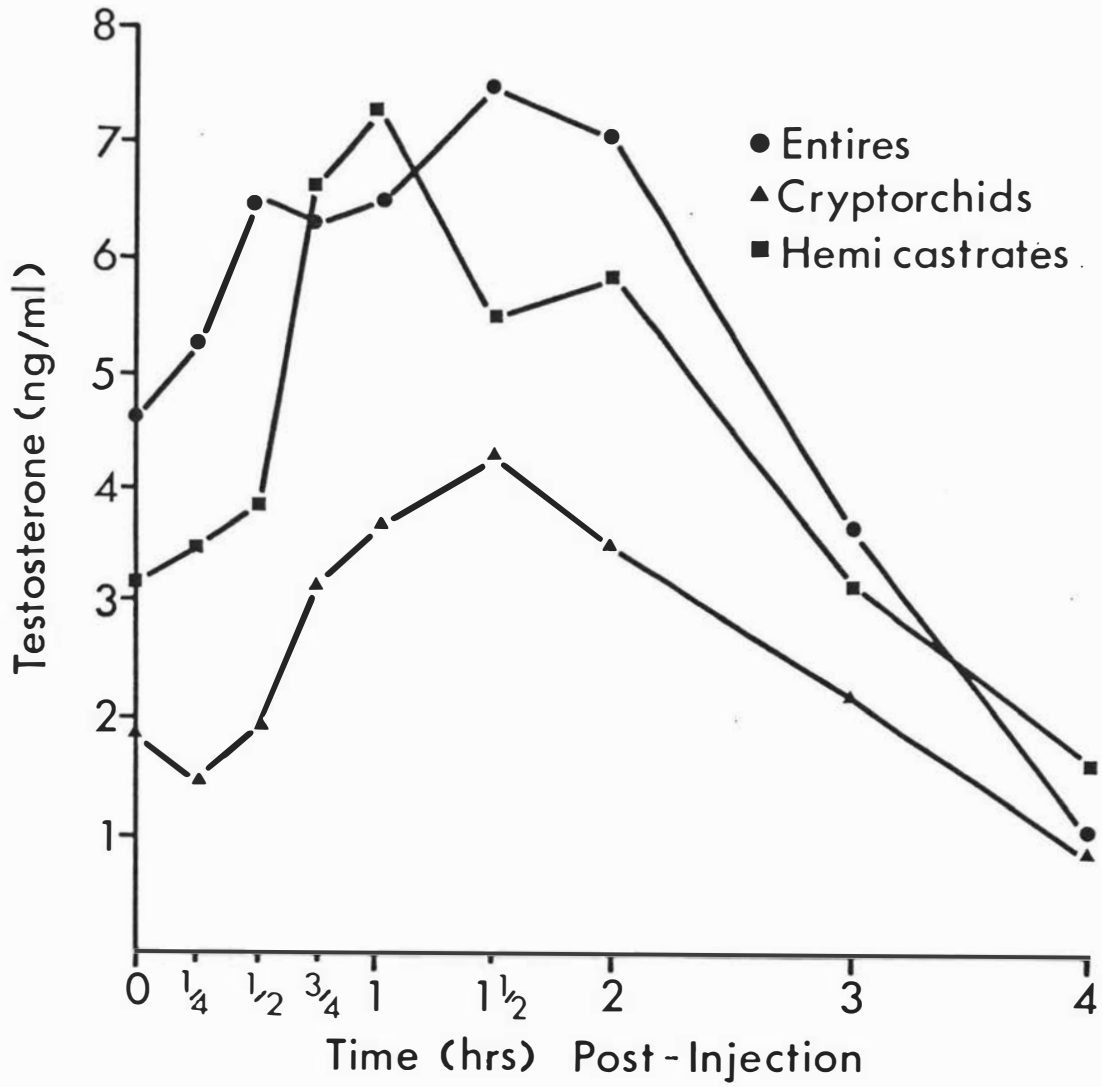



Figure 6.27: Plasma testosterone responses of non-androgenised ram lambs following administration of GnRH (0.5 µg/kg) at 24 weeks of age (experiment 6.3, n=3).

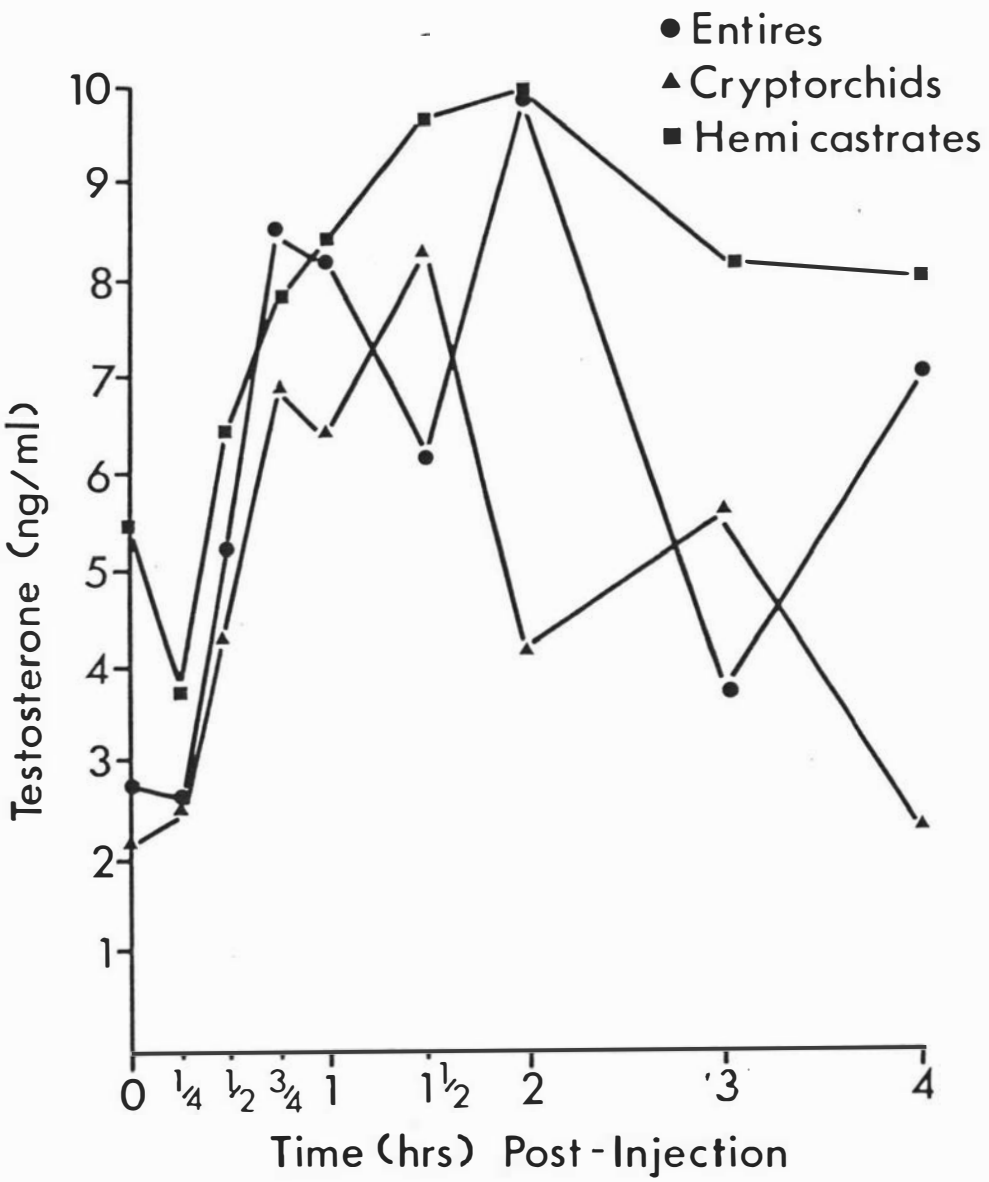



Figure 6.28: Plasma testosterone responses of non-androgenised rams following administration of GnRH (0.5 µg/kg) at 32 weeks of age (experiment 6.3, n=3).

Table 6.14: Mean (±SEM) pre-GnRH testosterone levels (ng/ml) and total hormone responses (ng/ml.hr) in non-androgenized animals from experiment 6.3

| Surgical Treatment | | Entires | Hemicastrates | Cryptorchids |
|---|---|---|---|---|
| Age (wks) | Hormonal Treatment | GnRH | GnRH | GnRH |
| 8 | Pre | 0.66±0.46 | 2.85±1.16 | 1.96±1.01 |
| | Total | 67.90±2.12 | 39.91±2.62 | 35.35±1.83 |
| 16 | Pre | 0.58±0.21 | 0.23±0.01 | 1.38±1.08 |
| | Total | 39.99±2.84 | 38.54±3.13 | 30.81±2.12 |
| 24 | Pre | 4.62±2.63 | 3.11±1.20 | 1.92±0.85 |
| | Total | 62.37±7.27 | 53.42±4.25 | 38.85±2.94 |
| 32 | Pre | 2.98±1.10 | 5.54±3.16 | 2.12±1.10 |
| | Total | 90.99±6.50 | 98.76±3.20 | 84.84±8.59 |

Table 6.15: Summary of analysis of variance of total testosterone response data from non-androgenized animals in experiment 6.3

| Source of Variation | DF | | Variance Ratios |
|---|---|---|---|
| A. Treatments | 2 | | 2.03 |
| B. Age | 3 | | |
| (i) Linear | | 1 | 17.22*** |
| (ii) Quadratic | | 1 | 11.57** |
| (iii) Cubic | | 1 | 0.01 |
| Interaction | | | |
| Treatments x Age | 6 | | 0.41 |
| Residual mean square | 24 | | 60.50 |

b. Post-GnRH

Because pre-GnRH testosterone levels in androgenized animals were so high, and also because there was no consistent pattern of increasing testosterone concentrations after GnRH administration, total testosterone response data from the TP treated group were not included in the statistical analysis.

Plasma testosterone concentrations increased rapidly following GnRH administration in all non-androgenized animals. In the analysis of variance of total hormone responses surgical treatments did not have any significant effect. However the responses recorded from cryptorchids were low compared to those of entires and hemicastrates and this difference just failed to reach statistical significance ($F_{(1,24)} = 3.43$, $0.05 < P < 0.10$).

Overall mean testosterone responses were high at 8 weeks ($47.72 \pm 10.18$ ng/ml.hr), but declined to $36.44 \pm 2.85$ ng/ml.hr at 16 weeks and then subsequently increased to $91.53 \pm 4.03$ ng/ml.hr at 32 weeks. This pattern of changes was responsible for the significant linear ($P < 0.001$) and quadratic ($P < 0.01$) components of the overall effects of age in the analysis of variance of total testosterone response data.

(d) Organ Weights

(Tables 6.16 and 6.17)

Weights of testes and epididymides from entires increased linearly with age. Mean testicular and epididymal weights increased from $13.49 \pm 0.29$ g and $3.74 \pm 0.16$ g at 8 weeks to $123.81 \pm 0.82$ g and $15.48 \pm 0.08$ g at 32 weeks, respectively. Similar, but more marked increases in weights of remaining testes (from $26.01 \pm 0.42$ g to $186.66 \pm 0.98$ g) and epididymides (from $12.58 \pm 0.18$ g to $21.31 \pm 0.53$ g) were noted from hemicastrates at these ages. At each age these differences between organ weights of entires and hemicastrates were very highly significant (table 6.17). In contrast, the weights of cryptorchid testes ($6.10 \pm 0.12$ g at 8 weeks and $6.96 \pm 0.27$ g at 32 weeks) and epididymides

Table 6.16: Mean (±SEM) testicular and epididymal weights (g) and seminiferous tubular diameters (STD-μm) from experiment 6.3.

| Age (Wks) | ENTIRES | | | HEMICASTRATES | | | CRYPTORCHIDS | | |
|---|---|---|---|---|---|---|---|---|---|
| | TESTES | EPIDIDYMIDES | STD | TESTES | EPIDIDYMIDES | STD | TESTES | EPIDIDYMIDES | STD |
| 8 | 13.49±0.29 | 3.74±0.16 | 81.12±1.16 | 26.01±0.42 | 12.58±0.18 | 90.86±0.71 | 6.10±0.12 | 2.34±0.05 | 54.51±1.16 |
| 16 | 34.41±0.82 | 6.60±0.08 | 118.00±1.03 | 88.23±1.58 | 16.80±0.40 | 138.16±0.50 | 6.96±0.08 | 3.55±0.04 | 60.97±2.17 |
| 24 | 84.40±0.12 | 10.20±0.13 | 150.23±0.71 | 147.96±1.52 | 17.74±0.09 | 165.86±0.47 | 7.23±0.16 | 3.75±0.03 | 61.87±0.58 |
| 32 | 123.81±0.82 | 15.48±0.08 | 172.18±0.15 | 186.66±0.98 | 21.31±0.53 | 190.13±1.11 | 6.96±0.27 | 3.61±0.05 | 58.97±0.46 |

Table 6.17: Summary of t-test values and significance levels for testicular and epididymal weights and seminiferous tubular diameters (STD)

| Age (wks) | | 8 | 16 | 24 | 32 |
|---|---|---|---|---|---|
| t-test contrasts | | | | | |
| Entires vs Cryptorchids (10 DF) | Testes | 10.58, P<0.001 | 12.09, P<0.001 | 130.79, P<0.001 | 76.87, P<0.001 |
| | Epididymides | 8.23, P<0.001 | 14.52, P<0.001 | 21.50, P<0.001 | 12.96, P<0.001 |
| | STD | 9.85, P<0.001 | 14.29, P<0.001 | 50.20, P<0.001 | 108.86, P<0.001 |
| Entires vs Second organ of hemicastrates (7 DF) | Testes | 27.34, P<0.001 | 17.58, P<0.001 | 40.74, P<0.001 | 27.93, P<0.001 |
| | Epididymides | 18.41, P<0.001 | 27.56, P<0.001 | 16.75, P<0.001 | 53.95, P<0.001 |
| | STD | 5.80, P<0.001 | 2.83, P<0.05 | 9.19, P<0.001 | 17.09, P<0.001 |

($2.34 \pm 0.05$ g at 8 weeks and $3.61 \pm 0.05$ g at 32 weeks) indicated that these organs did not undergo any significant growth during the period of study and thus weighed significantly less than organs recovered from entires and hemicastrates.

Similar patterns of results were evident in the seminiferous tubular diameter data. Thus hemicastrates had significantly greater seminiferous tubular diameters compared to the other two groups. The values recorded from entires increased from $81.12 \pm 1.16$ µm at 8 weeks to $172.18 \pm 0.15$ µm at 32 weeks, while those from hemicastrates increased from $90.86 \pm 0.71$ µm to $190.13 \pm 1.11$ µm at the corresponding ages. However, no such increase in seminiferous tubular diameters was detected from cryptorchids. For these animals the seminiferous tubular diameters at 8 weeks had a mean value of $54.51 \pm 1.16$ µm, while at 32 weeks the mean was $58.97 \pm 0.46$ µm. Thus, the seminiferous tubular diameters recorded from cryptorchids were significantly less than those of age-matched control animals.

(e) Qualitative Histology
(Figures 6.29-6.32)

As illustrated in figures 6.29-6.32 on pages 210 to 213 it was evident that testicular histological changes observed from entires and hemicastrates were essentially age-related and corresponded well with those described in Chapter 5; no further detailed description will be given here. At each age of sampling the histological picture of testes from entires and hemicastrates was very similar: although seminiferous tubular diameters of the latter group were greater, the tubules did not contain more mature spermatogenic cells than seen in testes of entires. Thus it was concluded that hemicastration did not cause an acceleration in onset of spermatogenesis and mature spermatozoa were identified in testicular samples of both groups at 24 weeks of age.

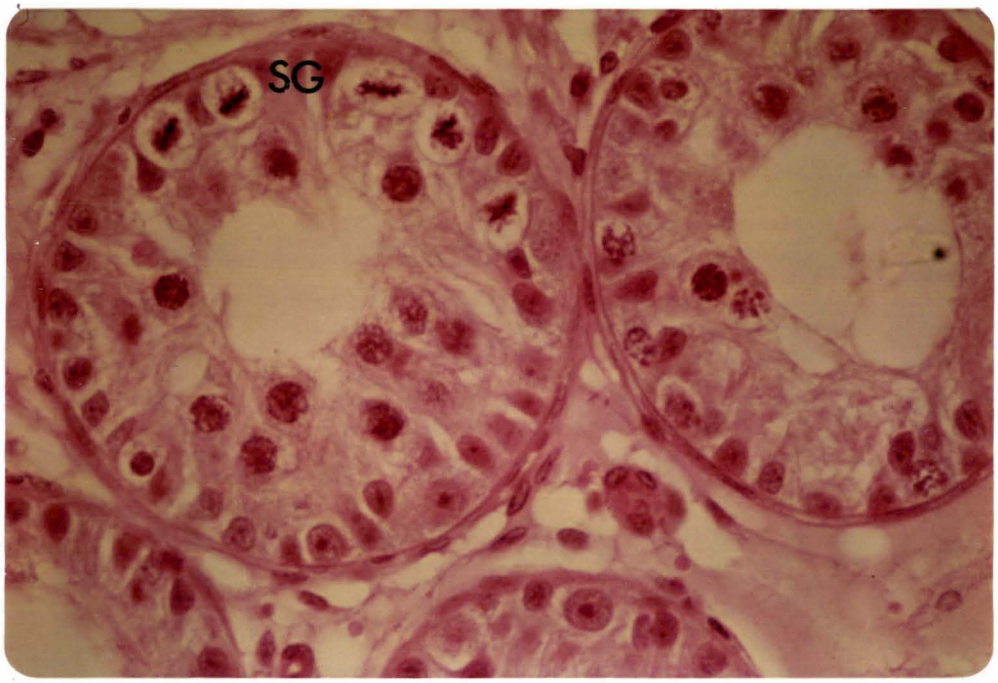
In contrast, the histological changes observed in cryptorchid testes were minimal. At 8 weeks of age most tubules had well defined lumina and germ cells were all located in peripheral positions. Supporting cells had not

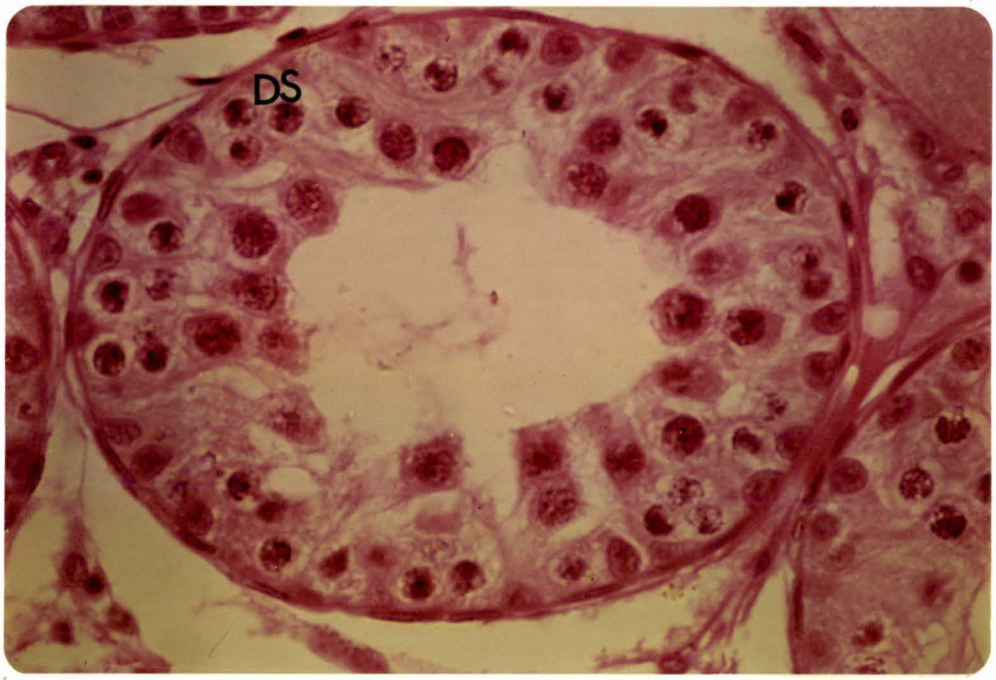
Figure 6.29: Photomicrographs of testes from 8 week old rams. Magnification x 650; Haematoxylin and Eosin stain. Spermatogonia (SG), actively dividing spermatogonia (DS) and Leydig cells (L).

(a) Entire

(b) Hemicastrate

(c) Cryptorchid


SG


DS

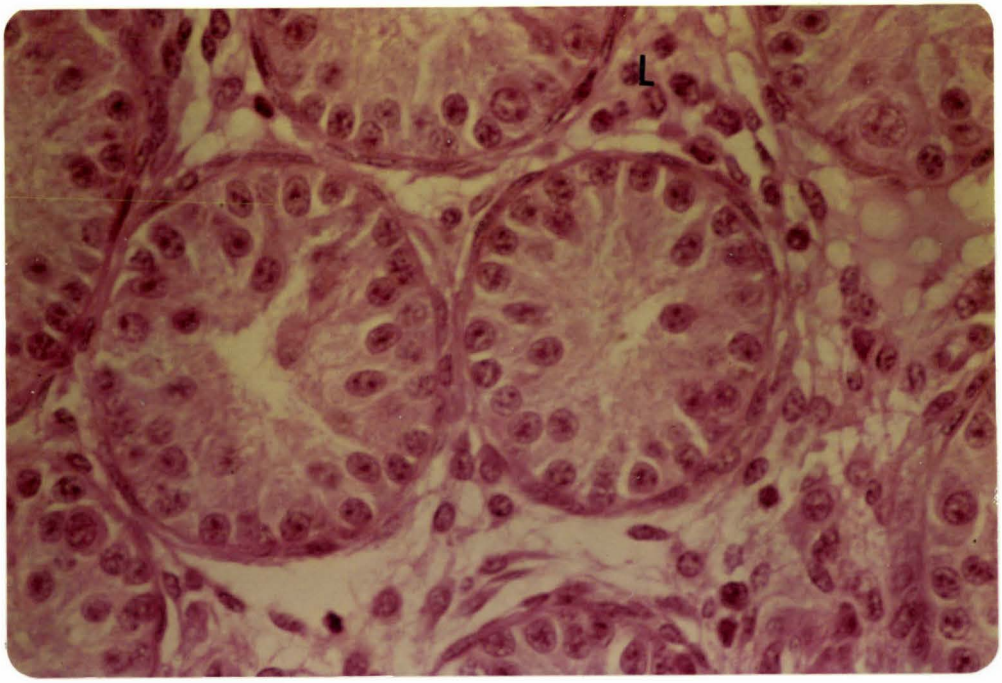
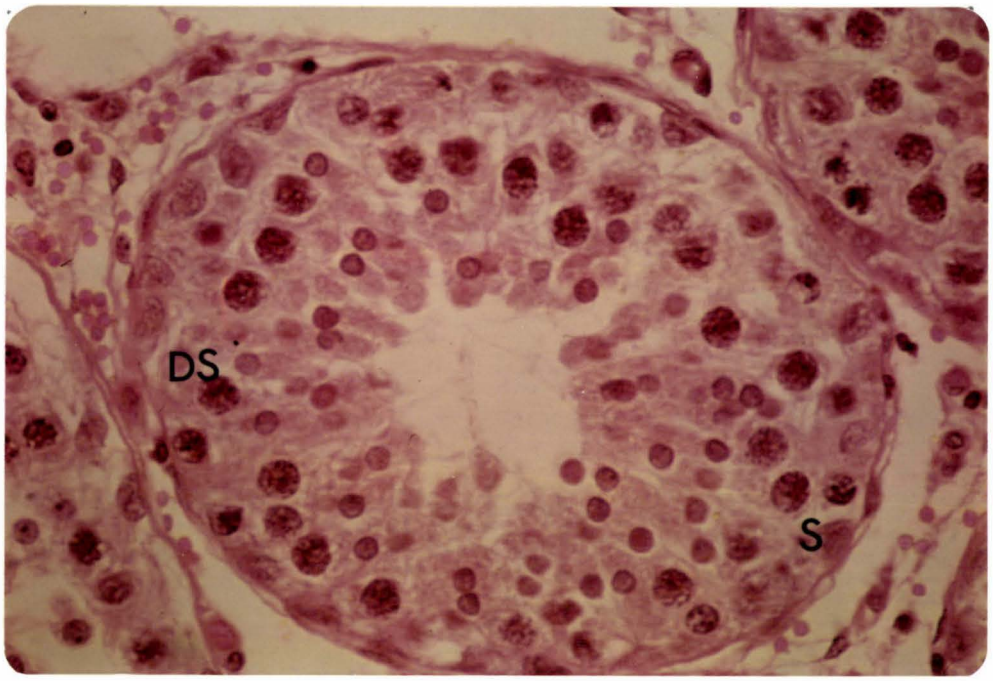

L

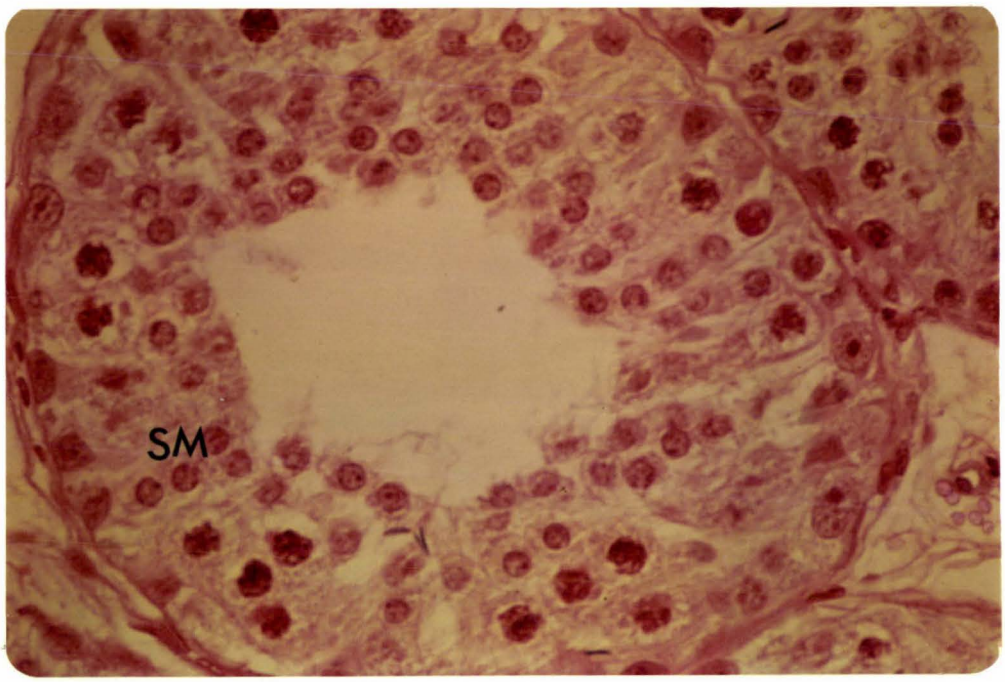
Figure 6.30: Photomicrographs of testes from 16 week old rams. Magnification x 650; Haematoxylin and Eosin stain. Spermatogonia (SG), dividing spermatogonia (DS), spermatocytes (SM), Sertoli cells (S) and Leydig cells (L).

(a) Entire

(b) Hemicastrate

(c) Cryptorchid


DS
S


SM

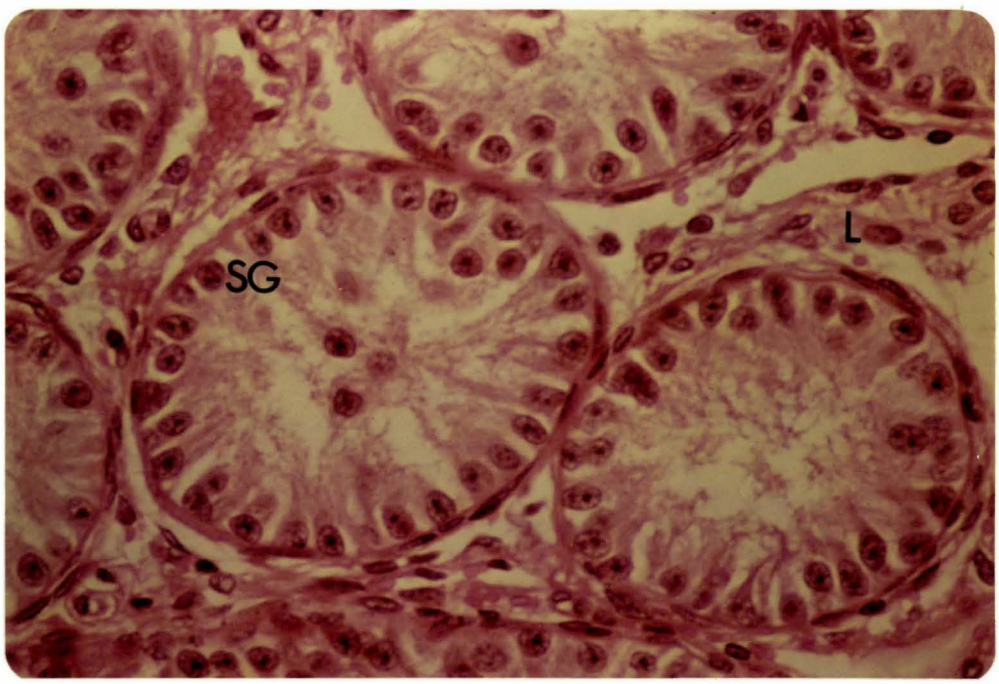
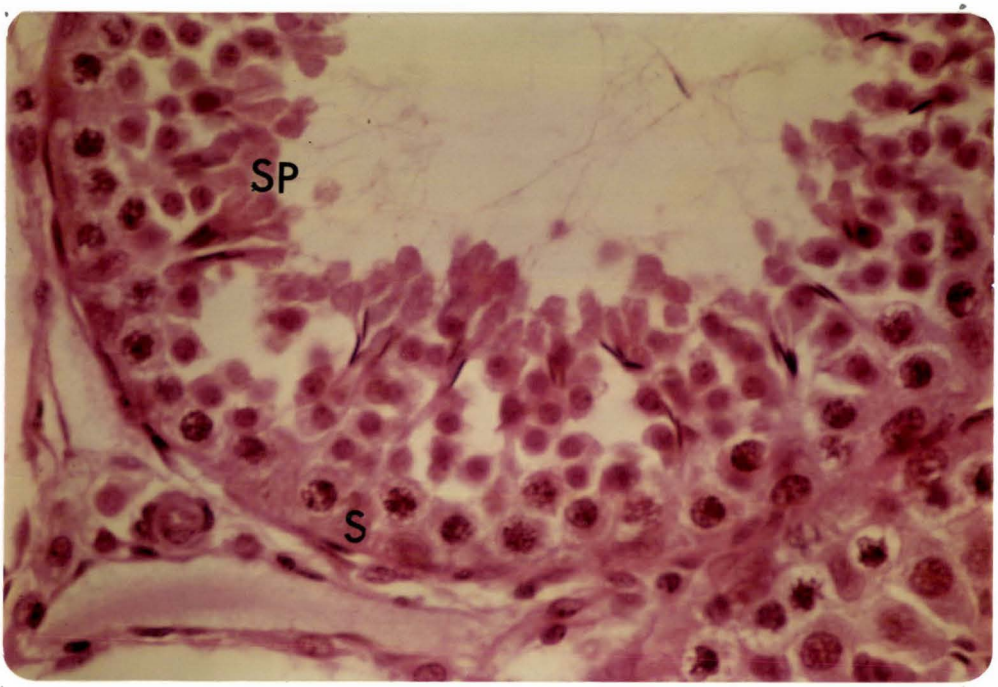

SG
L

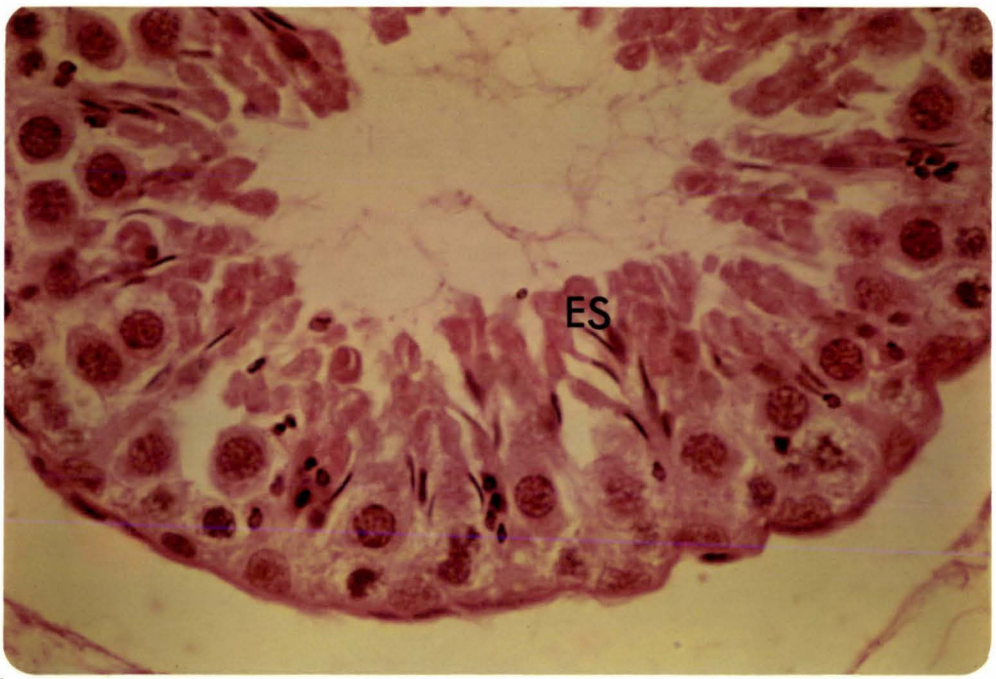
Figure 6.31: Photomicrographs of testes from 24 week old rams. Magnification x 650; Haematoxylin and Eosin stain. Spermatogonia (SG), elongated spermatids (ES), spermatozoa (SP), Sertoli cells (S) and Leydig cells (L).

(a) Entire

(b) Hemicastrate

(c) Cryptorchid


SP
S


ES

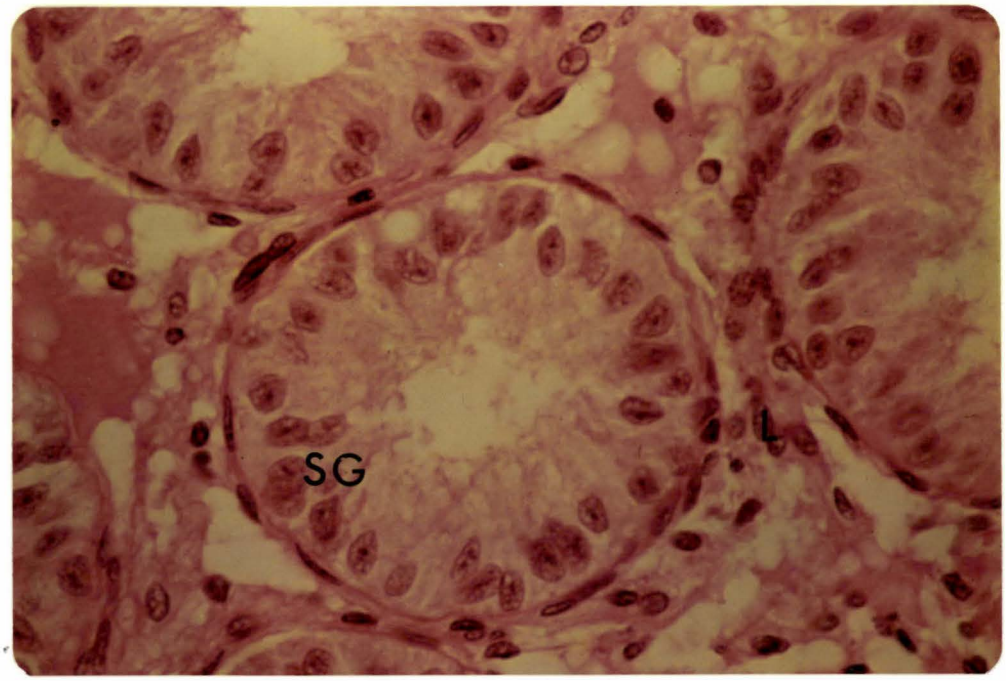
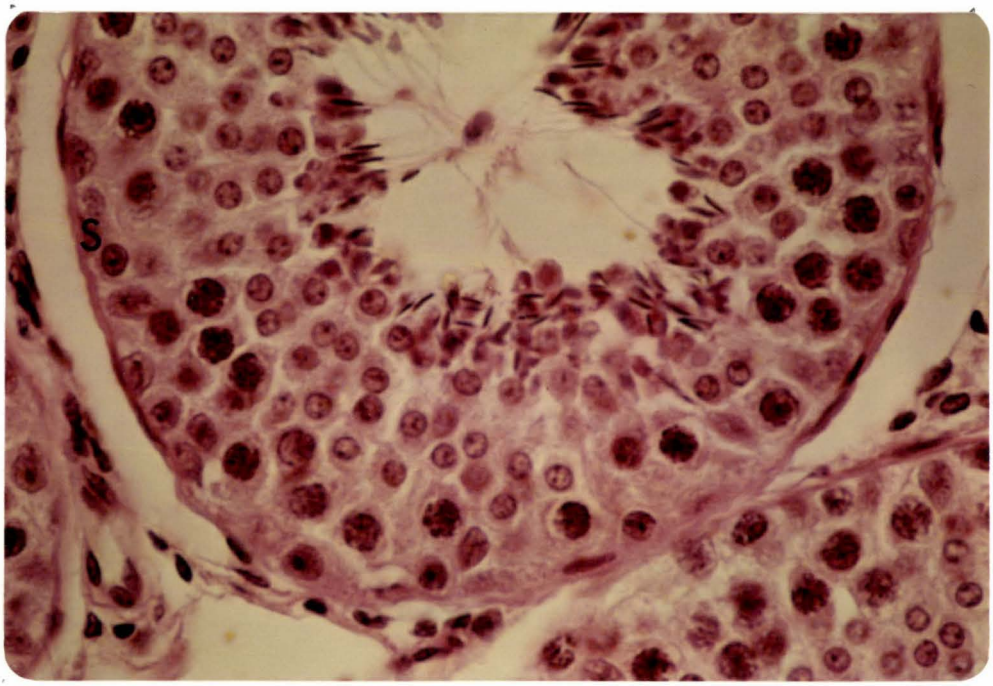

SG
L

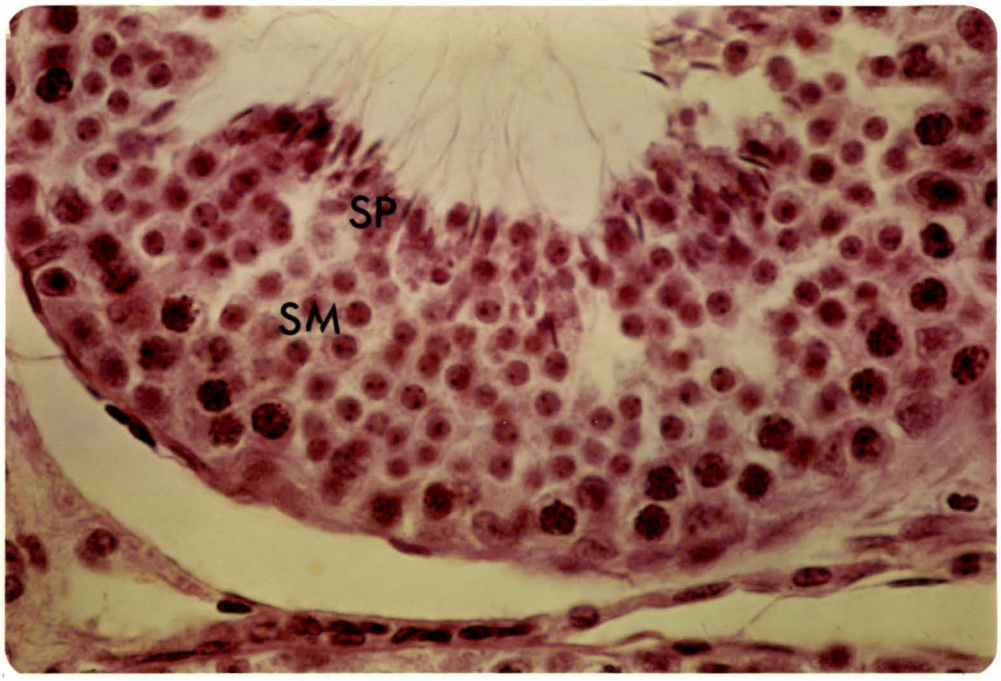
Figure 6.32: Photomicrographs of testes from 32 week old rams. Magnification x 650; Haematoxylin and Eosin stain. Spermatogonia (SG), spermatocytes (SM), spermatozoa (SP), Sertoli cells (S), and Leydig cells (L).

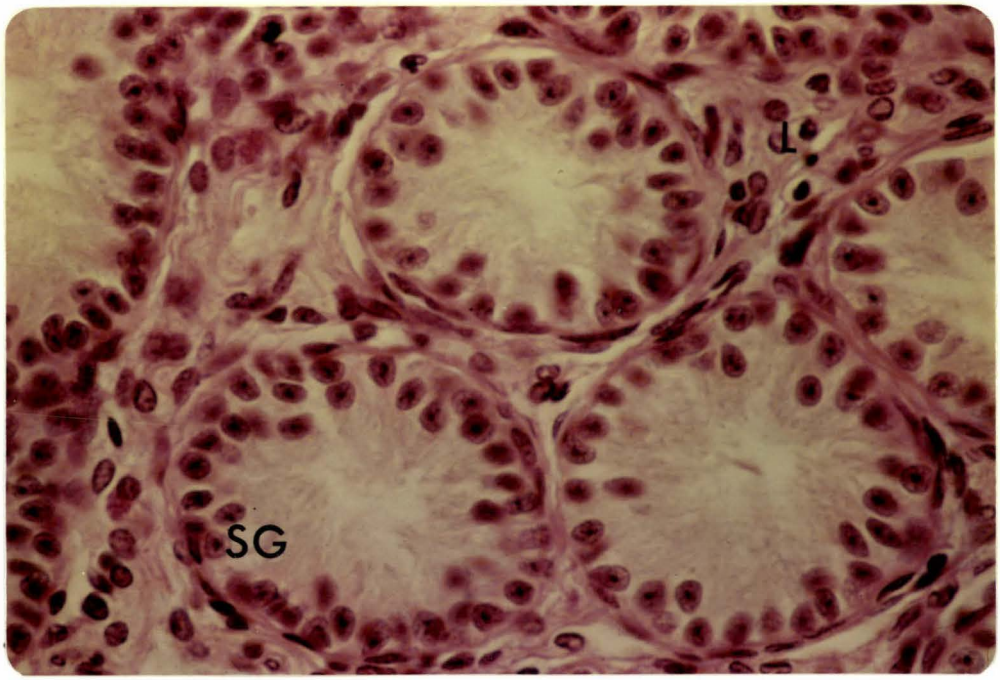
(a) Entire

(b) Hemicastrate

(c) Cryptorchid


S


SP
SM


T
SG

acquired adult cell shape. Actively dividing prospermatogonia were seen in most animals and these cells were located close to the basement membranes. Interstitial spaces were packed with Leydig cells, but fibroblasts and blood vessels also were present.

The histological structures of cryptorchid testes recovered at 16, 24 and 32 weeks of age were identical to those seen at 8 weeks. No spermatogenic activity was detected, neither were spermatocytes, spermatids, nor mature spermatozoa seen in cryptorchid testes. Mature Sertoli cells were very rare even at 32 weeks of age, while the content of the interstitial spaces also was unaffected by age.

## 4. Discussion

### (a) Basal Hormone Levels

Data discussed in this section is that derived from the longitudinal study, (experiment 6.1) mean hormone values from experiment 6.2 and pre-GnRH concentrations of non-androgenized lambs from experiment 6.3. For animals in each surgical treatment group the general patterns of change in data for each hormone, and in each of the above experiments, were similar.

#### (i) LH and FSH

Patterns of change in basal LH levels recorded from entire animals were similar to those recorded in previous research with developing ram lambs (Crim and Geschwind, 1972a; Lee et al., 1976a; Wilson and Lapwood, 1979a; Savoie et al., 1979; Walton et al., 1980; experiment 5.1 of this thesis).

Hemicastration did not result in any overall change in LH levels in experiments 6.1 or 6.2, nor in basal levels in experiment 6.3. This observation concurred with results of experiment 5.2 and those of Walton et al. (1978; 1980). However, plasma FSH concentrations recorded from the first two samplings of experiment 6.1 (at 4 and 6 weeks of age)

were significantly elevated compared to those of entires. Walton et al., (1978; 1980) also recorded a transient elevation of plasma FSH levels in hemicastrated ram lambs, and this persisted up to approximately 10 weeks of age.

On the other hand, in all three experiments cryptorchidism resulted in a marked elevation of basal plasma concentrations of LH and FSH. These results concurred with previously published results from cryptorchid ram lambs (Blanc and Terqui, 1976; Blanc et al., 1978), while elevated plasma levels of LH (Hillard and Bindon, 1975), and LH and FSH (Schanbacher and Ford, 1977) have been detected in adult cryptorchid rams. In pubertal bulls artificially induced cryptorchidism also has been noted to result in elevation of LH and FSH concentrations (Schanbacher, 1978; Schanbacher, 1979 a). By way of contrast, not only did Lee et al. (1978) not detect any increase in plasma LH levels of cryptorchid ram lambs, but also an elevation of plasma FSH content only occurred after 13 months of age.

(ii) Prolactin

Data from experiments 6.1 and 6.2 indicated that neither hemicastration nor cryptorchidism had any significant effect on plasma prolactin levels. It appears that no previous data has been published on the effects of these treatments on plasma prolactin levels in ram lambs, nor in males of any other species. Also it is difficult to imagine any physiological basis by which either hemicastration or cryptorchidism might affect prolactin secretion.

Mean plasma prolactin levels recorded from entires, hemicastrates and cryptorchids changed in phase with the length of daily photoperiod, as has been recorded in previous research with spring born ram lambs (see page 47, Introduction). In contrast, Courot (1974) and Ravault (1976) using autumn born ram lambs, showed that in winter mean plasma prolactin levels were low and steady apart from a distinct peak at around 10-12 weeks of age; subsequently values increased with daily photoperiod. Those experiments were aimed at

revealing effects of age on plasma prolactin levels, unimpeded by seasonal influences.

In further research on the possible role of prolactin during sexual maturation of ram lambs, Ravault et al. (1977) used 2-Br-α-ergocryptine (CB-154) treatment of lambs born either in spring or autumn. Low plasma prolactin levels, which resulted from the treatment, did not affect LH, FSH or testosterone concentrations, testis weights, nor the onset of spermatogenesis. However, weights of seminal vesicles and vesicular fructose concentrations were significantly diminished in CB-154 treated animals. Also Howles et al. (1980) demonstrated that developing ram lambs, exposed either to constant long or short photoperiods, did not experience any major difference in patterns of testicular growth, although the prolactin levels of the two groups were markedly different. Thus it appears that prolactin probably has only a minor role in influencing reproductive development in ram lambs.

(iii) Testosterone

Basal plasma testosterone levels of lambs in all three treatment groups increased with sexual maturation. These results concurred with similar observations made from ram lambs in experiment 5.1 and those reported by other workers (Crim and Geschwind, 1972a; Courot, 1974; Cotta et al., 1975; Lee et al., 1976a; Wilson and Lapwood, 1979a).

Hemicastration had no significant effect on plasma testosterone levels. This was evident from results of experiments 6.1 and 6.2, and also pre-GnRH plasma testosterone concentrations of experiment 6.3. Similar observations were made in experiments 5.2 and 5.3, as well as in previous research on testosterone secretion in hemicastrated ram lambs (Walton et al. 1978), adult rams (Johnson et al., 1971), bull calves (Barnes et al., 1980c) and adult bulls (Johnson, 1978; Leidl et al., 1980).

Cryptorchidism had no significant effect on plasma testosterone levels in either experiment 6.1 or 6.2, and

these results concurred with previously published data from cryptorchid ram lambs (Blanc and Terqui, 1976; Lee et al., 1978) and adult rams (Schanbacher and Ford, 1977). However, in bulls (Kellaway et al., 1971; Bass et al., 1976; Schanbacher, 1978) and stallions (Cox et al., 1973; Ganjam and Kenny, 1975) cryptorchidism always has been reported to be associated with a reduction in plasma testosterone levels.

(b) Pulsatile Hormone Release

During the 4 hr acute profile studies pulsatile secretory patterns were clearly evident for LH, prolactin and testosterone, but less so for FSH.

As in chapter 5, testosterone peaks generally were preceded by LH peaks in all three groups, but both entires and hemicastrates showed some spontaneous testosterone spikes which were not associated with elevations of plasma LH levels. However, with the ½ hourly sampling schedule there was a distinct possibility that some gonadotrophin peaks may not have been detected. In contrast cryptorchidism resulted in an increase in number of LH peaks, compared to those recorded from entires and hemicastrates, and also a reduction in number of testosterone elevations which followed LH peaks: for a total of 55 LH peaks only 18 testosterone elevations were noted. However, the reasons for this difference remain obscure. The increase in LH peaks may have resulted from increased pituitary LH release occurring under conditions of reduced negative feedback effects from testicular androgens. On the other hand the decrease in testosterone peaks may well have been due to a reduction in androgenic secretory capacity of cryptorchid testes, resulting from disruption of steroidogenic enzymes (Van Straaten et al., 1978) and/or depletion of LH receptors in the cryptorchid testes (down-regulation of receptors - Gallo, 1980) as a result of being exposed to continuously elevated LH levels.

The surgical treatments had no effect on number of peaks in plasma FSH levels, although the episodic fluctuations

in plasma FSH levels were less marked than those observed for LH. Likewise, neither hemicastration nor cryptorchidism had any effect on pulsatile secretion of prolactin. There appears to have been no reports in the literature on the effects of these treatments in influencing the acute pattern of secretion of either FSH or prolactin.

(c) Pre-GnRH Hormone Levels in Androgenised Lambs

Pre-GnRH LH levels in all TP treated animals were significantly reduced compared to those in untreated animals, at all ages and in all three surgical treatment groups. These results ratified those of Crim and Geschwind (1972a), Hopkinson et al. (1974), Galloway and Pelletier (1975), Pelletier (1976) and Schanbacher (1980b), all of whom showed that exogenous testosterone injection reduced basal LH levels in entires and wethers. In contrast, the reduction in basal LH levels of cryptorchid ram lambs in the present study differed from results published by Schanbacher and Ford (1977). In the latter study, only injected oestrogens, but not testosterone propionate or dihydrotestosterone, had any effect on basal LH levels of cryptorchids. However, this discrepancy in results may have arisen from the fact that a higher dose rate of TP was utilised in the present study (1 mg/kg body weight vs total dose of 25 mg).

Pre-GnRH FSH levels also were reduced by exogenous testosterone, at all ages and in all three treatment groups. However, the degree of secretory inhibition was much less than that recorded for LH and also varied in extent between ages, and between individual animals. Similar animal-to-animal variations in degree of inhibition of FSH secretion have been reported following treatment of rams with exogenous testosterone (Hopkinson et al., 1974).

Decreases in FSH levels observed in the present experiment may have been due to pharmacological effects of testosterone, rather than to physiological effects, because plasma testosterone levels in excess of 17 ng/ml were recorded from these animals. Such levels are well in excess of the usual physiological range: plasma testosterone levels in developing Romney ram lambs have been reported to be

below 7 ng/ml (Wilson and Lapwood, 1979a). However, in experiments where replacement testosterone treatments have been designed to mimic physiological levels, either by long term treatment with smaller testosterone doses (Crim and Geschwind, 1972a), or by the use of implanted silastic capsules containing crystalline testosterone (Schanbacher , 1980b), inhibition of both LH and FSH levels has been reported. Nevertheless, the extent to which constant plasma testosterone levels resemble the physiological situation is unknown. For example, Resko et al. (1977) were unable to suppress LH and FSH in castrated male monkeys with testosterone capsules alone, whereas Plant et al. (1978) were successful when they supplemented capsules with pulsatile (injected) testosterone.

(d) Responses to GnRH

(i) Non-androgenised lambs

a. LH and FSH

In all animals LH and FSH release followed injection of GnRH, the LH responses being more rapid and dramatic than those for FSH. These patterns of gonadotrophin response were similar to those recorded in research with ram lambs (Lee et al., 1976b; Wilson and Lapwood, 1979b) and adult rams (Hopkinson et al., 1974; Lee et al., 1976b; Lincoln, 1978; 1979a).

Gonadotrophin secretory responses to administration of GnRH generally confirmed previous observations made in experiments 6.1 and 6.2 that cryptorchidism, but not hemicastration caused significant increases in gonadotrophin secretion. Exaggerated plasma LH and FSH responses have previously been reported following GnRH administration to cryptorchid rams (Lee et al., 1979; 1980) and bulls (Schanbacher, 1979a).

Differences between LH responses recorded from entires and hemicastrates did not reach significant levels, although the total LH responses recorded from hemicastrates tended to be higher than those of entires. However, these results

did not agree with LH response data recorded from entires and hemicastrates (experiment 5.3), in which case hemicastrates had significantly higher overall total LH responses. This discrepancy in results probably arose because the post-operative time period, between hemicastration and the GnRH response study was only 4 weeks in experiment 5.3, compared to the 6, 14, 22 or 30 week time lapses in experiment 6.3. Evidence in favour of that concept was provided by data published by Walton et al. (1980). In their experiment hemicastrations were performed at one week of age and GnRH responses were examined at 10, 13 and 18 weeks. Although hemicastrated lambs tended to have higher LH responses than entires, the difference was not statistically significant.

Total LH responses of animals in all three treatment groups decreased linearly with age and similar effects of age have been observed in GnRH response data from developing ram lambs (Wilson and Lapwood, 1979b) but apparently no data regarding age effects on LH responses has been published for hemicastrated or cryptorchid ram lambs.

In contrast maximal total FSH responses were recorded in all three treatment groups at 24 weeks of age. That observation coincided well with relatively higher plasma FSH levels observed in all three groups at 24 weeks, in both experiments 6.1 and 6.2. These data indicated a divergence with age in LH and FSH responses to exogenous GnRH.

Nevertheless, divergences between LH and FSH responses to GnRH have been reported previously for ram lambs (Lee et al., 1976b) and also adult rams (Lincoln, 1978; 1979a). Androgens and/or inhibin may have been responsible for these divergent responses. The possibility of an influence of inhibin was suggested by the fact that the difference in gonadotrophic responses was greatest in cryptorchids, in which the production of inhibin may be impaired (Blanc and Terqui, 1976). Interactions at the pituitary level, between inhibin and androgens, may also have been a contributing factor. However, as yet it has not been demonstrated

convincingly that testosterone or inhibin affect pituitary responsiveness to GnRH directly in ram lambs or in adults. Other possible reasons for this difference in LH and FSH responses include intrinsic differences in the secretory mechanism of the anterior pituitary gonadotrophs, compounded by the slower clearance rate of FSH from the blood (Lincoln 1979a).

b. Testosterone

Plasma testosterone levels increased following GnRH injection in entires, hemicastrates and cryptorchids. Lack of any significant difference in the total testosterone responses of entires and hemicastrates indicated that the remaining testes of hemicastrates were capable of secreting as much androgen as the two testes of entires; similar results were recorded from hemicastrate and entire ram lambs in experiment 5.3. In contrast cryptorchids tended to have reduced testosterone responses compared to the other two groups, although that difference just failed to reach statistical significance. Similarly reduced androgen responses have been recorded from adult cryptorchid rams (Lee et al., 1979; 1980; Schanbacher, 1980a) and bulls (Schanbacher, 1979a) following GnRH injection.

Total testosterone responses increased with age in entires, hemicastrates and cryptorchids. These observations were in accordance with similar results reported from entire ram lambs (Lee et al., 1976b; Wilson and Lapwood, 1979b) and hemicastrates (experiment 5.3), but no comparable research has been published for cryptorchid ram lambs. This observation adds further weight to the concept that Leydig cells of cryptorchids secrete androgens at their maximum capacity (Schanbacher and Ford, 1977; Schanbacher, 1980a). Hypersecretion by the existing Leydig cells, rather than new LH receptor development may have been responsible for the increase in testosterone levels, since there was no apparent increase in testicular weights of cryptorchids in the present study nor in that of Skinner and Rowson (1968b). Observations on ultra-structural

changes in organelles of Leydig cells from cryptorchid rat testes also favour this concept (Kerr et al., 1979a).

(ii) Androgenised lambs

GnRH injection caused increases in plasma LH and FSH levels in all testosterone pre-treated animals, but total and peak responses for both LH and FSH were smaller than in non-androgenised animals. Similar reductions in gonadotrophin responses to GnRH in rams and wethers pre-treated with testosterone, have been reported (Hopkinson et al., 1974; Galloway and Pelletier, 1975; Pelletier, 1976), but there is no comparable published data for hemicastrates or cryptorchids.

(e) Organ Weight Data

(i) Entires

Testicular and epididymal weight data obtained at 8, 16, 24 and 32 weeks of age clearly indicated that these organs underwent normal growth, as described in the previous chapter.

(ii) Hemicastrates

Remaining testes and epididymides recovered from hemicastrates in the present experiment were significantly heavier than organs recovered from age-matched control entires. These results confirmed the observations made in chapter 5 of this thesis and also corroborated similar research reported previously from hemicastrated ram lambs (Renfro and Dutt, 1970; Land and Carr, 1975; Riesen et al., 1977; Walton et al., 1978; 1980; de Reviers et al., 1980). Seminiferous tubular diameters of the remaining testes recovered from hemicastrates also showed compensatory enlargement, as described in chapter 5.

(iii) Cryptorchids

Not only were testes and epididymides from cryptorchids significantly lighter than those of age-matched controls, but their weights showed no increase with age. Among similar results reported in the past were those of Skinner and Rowson (1968b) who worked with unilateral cryptorchid

ram lambs and calves. Also Hochereau-de Reviers et al. (1979) noted a drastic decrease in weights of testes and epididymides in ram lambs made cryptorchid at 15 weeks of age and recovered 12 months later.

Lack of any significant increase in seminiferous tubular diameters in cryptorchid testes in the present study also confirmed results of similar previous reports (Skinner and Rowson, 1968b; Hochereau-de Reviers et al., 1979).

(f) Qualitative Testicular Histology

The qualitative histological changes in testicular samples recovered from entires and hemicastrates were entirely age-related and similar to those reported and discussed more fully in chapter 5 of this thesis. No further discussion is warranted.

Histological changes detected in cryptorchid testes in the present study were similar to those reported by Skinner and Rowson (1968b). The absence of spermatogenic activity in cryptorchid testes clearly indicated that intra-abdominal temperature was detrimental to most cellular elements of the spermatogenic cycle. However, gonocytes and prospermatogonia were more resistant to elevated temperatures; that observation agreed with similar reports from rams (Waites and Ortavant, 1968) and bulls (Kellaway et al., 1971). The fact that elevated temperature was the major factor detrimental to elements of the spermatogenic cycle was clearly established in recent research by Frankenhuis and Wensing (1979) who demonstrated that artificial cooling of cryptorchid testes in boars resulted in induction of normal spermatogenesis.

Research involving more precise histological analytical methods and specific enzyme staining techniques (Van Straaten et al., 1978; Hochereau-de Reviers et al., 1979) has shown that cryptorchidism caused a reduction in volume (Van Straaten and Wensing, 1977b) and number/testis of Leydig cells (Hochereau-de Reviers et al., 1979). Decreases in activity of enzymes involved in steroidogenesis

(3β-hydroxysteroid dehydrogenase and 17β -hydroxysteroid dehydrogenase) in Leydig cells of cryptorchid boar testes also were reported by Van Straaten et al., 1978. These observations were compatible with the concept that cryptorchidism causes a decrease in efficiency of Leydig cell androgen secretion; this only became evident in Leydig cell stimulation (GnRH response) studies (Lee et al., 1979; 1980; Schanbacher, 1980a; experiment 6.3), but not in longitudinal hormone (Lee et al., 1978; experiment 6.1) or acute profile secretion (Schanbacher and Ford, 1977; experiment 6.2) studies.

Cryptorchidism also caused a reduction in number of Sertoli cells when the treatment was induced in younger animals, but not in adults (Hochereau-de Reviers et al., 1979). The immaturity and paucity of Sertoli cells in cryptorchid testes of the present study, in which rams were made cryptorchid at 2 weeks of age, concurred with the above observations and those of Skinner and Rowson (1968b).

(g) Endocrine Sequelae to Surgical Treatments

(i) Endocrine basis of compensatory testicular hypertrophy

As yet the hormonal mechanisms responsible for compensatory testicular hypertrophy in hemicastrates are not fully understood. However, following hemicastration in early life, ram lambs generally experience increased plasma concentrations of LH and FSH (Land and Carr, 1975; Walton et al., 1978; 1980). However, mean LH levels in the longitudinal, acute profile and pre-GnRH samplings (of experiments 6.1, 6.2 and 6.3, respectively) were not significantly increased after hemicastration. While that was so, LH concentrations recorded from hemicastrates in the present experiments, including those described in chapters 4 and 5, were consistently, even though non-significantly increased. Also the LH responsiveness of hemicastrates was significantly greater than that of entires in experiment 5.3, however, a similar trend in post-GnRH responses in experiment 6.3 was not significant.

By way of contrast FSH secretion tended to be increased as a result of hemicastration, although this increase in FSH levels was statistically significant only at the first two samplings of the longitudinal study (experiment 6.1). Probably, the increase in plasma FSH levels at that stage was a major factor in initiation of the compensatory hypertrophy of the remaining testes. Similar transient increases in plasma FSH levels have been recorded from hemicastrated ram lambs (Walton et al., 1978; 1980).

Presumably the remaining testes subsequently produced sufficient amounts of feedback regulator (inhibin) to reduce FSH secretion back to normal levels. The increase in inhibin production may have been achieved either by hypersecretion of existing Sertoli cells or an increase in Sertoli cell numbers/testis, which followed hemicastration (de Reviers et al., 1980). Perhaps the continuous elevation of plasma FSH levels observed in hemicastration experiments with adult rams (Walton et al., 1978) and bulls (Leidl et al., 1980) resulted from inadequate inhibin production by the Sertoli cells, since Sertoli cells do not undergo mitosis in adult animals (Hochereau-de Reviers and Courot, 1978). Thus it is appropriate to conclude that following hemicastration of ram lambs the feedback regulatory mechanism(s) controlling FSH secretion (inhibin) appear to adapt at a slower rate than that regulating LH (androgens).

The possibility of compensatory hypertrophy of the remaining testes occurring partly as a result of local actions of androgens can not be excluded and this concept was discussed on page 117 of chapter 4, particularly in relation to hypertrophy of epididymides of hemicastrates. In addition, the endocrine data presented above suggests that gonadotrophins also may be involved in causing compensatory hypertrophy of the remaining testes.

(ii) Endocrine consequences of cryptorchidism

Mean hormonal concentrations obtained from experiments 6.1, 6.2 and pre-GnRH hormone levels (experiment 6.3) clearly indicated that this treatment resulted in marked

elevation of plasma gonadotrophin levels.

As discussed on pages 215 of this chapter the major reason for elevation of FSH probably was a disruption in production of FSH negative feedback regulator (inhibin). However, as mentioned on page 239 of chapter 7, one major handicap of the present study was the unavailability of an assay method to determine plasma inhibin levels. Nevertheless, other research workers (Kerr et al., 1979a; 1979b; Aumüller et al., 1980) who used the same surgical treatment in male rats recorded elevated plasma FSH levels and a decrease in ABP production by Sertoli cells. They claimed that the latter observation indicated a decrease in Sertoli cell function. Further evidence in favour of alterations in Sertoli cell metabolism comes from ultrastructural studies of Sertoli cells and plasma hormone levels of cryptorchid rats (Aumüller et al., 1980; Damber and Bergh, 1980). In addition to changes in amounts of endoplasmic reticulum, elevation of plasma oestradiol content was noted, which they claimed to indicate alterations in the metabolism of Sertoli cells. This concept is further discussed on page 241 of chapter 7.

Pre-GnRH gonadotrophin levels (androgenised) and decreased testosterone response data from (non-androgenised) cryptorchid animals tended to indicate that the major reason for elevation of plasma LH levels was hypoandrogenism (see page 218 of this chapter).

However, one aspect that was not considered in this experiment was the feedback effects of inhibin on plasma LH levels. There has been one report on the effects of inhibin preparations in lowering plasma LH levels in cryptorchid rams (Blanc et al., 1978).

The testicular data from the present experiment added weight to the concept that hemicastration causes compensatory hypertrophy of the remaining testes of ram lambs, but had no influence on the time of onset of spermatogenesis. Despite the differences in post-operative time lapses

before sampling animals in experiments 5 and 6, similar general patterns of hormonal and organ data were recorded from hemicastrate lambs in those two experiments. The trend to immediate post-operative increases in plasma FSH levels in hemicastrates indicated that this hormone plays a major role in mediating compensatory testicular hypertrophy. On the other hand cryptorchidism resulted in complete arrest of maturational changes of the testes, as well as disruption of androgen (and inhibin) production.

# CHAPTER VII

## GENERAL DISCUSSION AND CONCLUSIONS

Puberty is a phase in the development of an individual during which it is transformed from a state of sexual immaturity to one of maturity; acquisition of that capacity for gamete production occurs at a specific stage in growth which is characteristic of each species (Levasseur, 1977). Mechanisms that have been suggested to be responsible for initiation of pubertal processes were summarised on pages 52 to 55 of this thesis.

Experiments described in this thesis were designed as an investigation of the normal course of pubertal maturation in boars and rams, and also as a study of hypothalamo-pituitary-testicular hormonal interactions during the post-natal period of sexual development. Three approaches were adopted in investigations of reproductive hormone secretion: longitudinal studies of hormone output, acute secretion profile studies, and studies of pituitary and subsequent gonadal responsiveness to GnRH. In addition, the surgical treatments hemicastration and cryptorchidism were employed, since these are accepted techniques used for altering the interactions between androgens and gonadotrophins in the male.

### (a) Longitudinal Hormone Secretion Patterns

#### (i) Boars

Experiment 3 utilized a weekly sampling schedule, started with day old animals, and was designed to augment information from previous studies in this laboratory (FlorCruz and Lapwood, 1978). By replicating the study over two seasons, an attempt was also made to evaluate effects of birth season on LH and testosterone production, and on sexual development in young boars. The influence of the gonads on LH secretion was examined by monitoring hormone profiles in a group of

castrates.

The general pattern of hormone secretion was similar to that observed by FlorCruz and Lapwood (1978), except that the prepubertal LH peak recorded from spring born boars was less prominent in the present study. Season of birth, the effects of which apparently have not been examined previously, did not have any significant influence on LH or testosterone secretion. Earlier sexual maturation has been reported to result from provision of supplemental lighting during naturally occurring short days (Mahone et al., 1979; Berger et al., 1980), but these reports made no reference to hormonal changes.

Plasma LH levels recorded from castrates were elevated throughout the study, whereas those of entires declined to low levels by the fourth postnatal week. This longitudinal pattern of hormone secretion indicated the development of hypothalamo-hypophyseal sensitivity to feedback effects of testosterone by the 4th week of age, as noted in earlier reports (Ford and Schanbacher, 1977; Elsaesser et al., 1978).

In a recent paper Colenbrander et al. (1980) presented data on the longitudinal changes in FSH levels in developing boars. Levels were relatively high during the first 2 weeks of the postpartum period ($51.4 \pm 12.6$ ng/ml), then declined during the following 7 to 10 weeks to $7.32 \pm 0.47$ ng/ml. That pattern of change is similar to those which generally have been recorded for plasma LH levels during the neonatal period in boars (Colenbrander et al., 1977; experiments 3.1 and 3.2). However, the reliability of this information on FSH has yet to be substantiated, partly because of the close coincidence of LH and FSH secretory patterns; also because details of validation of the FSH assay have not been published and no comparable porcine FSH data has been published from other laboratories. While these reservations may prove unfounded, it is pertinent to note, for example, that similar coincidence of plasma LH and FSH levels was not evident in maturing ram lambs (e.g. Lee et al., 1976b; and experiment 6.1). The other doubts may well be nullified upon formal publication of the Dutch research and on publication

of similar research from other institutions. Lack of data on plasma FSH and prolactin levels in the porcine experiments described in this thesis was due to non-availability, at this laboratory, of suitable assay methods.

(ii) Rams

Basal plasma levels and the general patterns of secretion of LH and testosterone recorded in experiment 5.1, and plasma LH, FSH, prolactin and testosterone levels observed from entire ram lambs in experiment 6.1, were consistent with previously published data from developing ram lambs (see pages 46 to 49, Introduction). However, the FSH data in chapter 6 is the first such report on plasma FSH levels in Romney ram lambs.

Longitudinal hormonal secretory patterns recorded from hemicastrates and cryptorchids in experiment 6.1 also were in accord with results reported by Blanc and Terqui (1976) and Walton et al. (1978; 1980), but the FSH results differed from those reported for crossbred Merino-Corriedale cryptorchid ram lambs (Lee et al., 1978). The latter workers recorded no increase in plasma FSH levels of cryptorchid ram lambs until after 13 months of age, while in the present study (experiment 6.1) and in that of Blanc and Terqui (1976) cryptorchidism resulted in an elevation of plasma FSH levels within a few weeks of the treatment being induced. This discrepancy in results could either have resulted from breed differences (V.W.K. Lee; Melbourne, pers. comm.) or differing methods of inducing cryptorchidism. For example Merino/Corriedale ram lambs used in the experiment described by Lee et al. (1978) were reported to mature late: first spermatozoa in the seminiferous tubules appear around 39-42 weeks of age (Lee et al. 1981), while in Romney ram lambs the first appearance of spermatozoa occurs at 18-22 weeks of age (Wilson, 1977). Lee et al. (1978) used surgical as well as elastrator ring methods for inducing cryptorchidism in ram lambs. However, the validity of the elastrator ring method must be questioned after reports of discrepancies in results which have followed its use to produce cryptorchid bulls

(Kellaway et al., 1971; Bass et al., 1976). The technique adopted for experiments in chapter 6 (surgical induction of intra-abdominal cryptorchidism) was that recommended by Schanbacher (1978) as an attempt to standardize experimental procedures for this avenue of research.

(b) Acute Hormone Profiles

(i) Pulsatile secretion

Prior to acceptance of the concept of episodic hormone secretion (revealed by frequent sampling methods), widely divergent hormone levels often were rejected or regarded as unimportant.

As mentioned in the literature review on page 29, the physiological importance of episodic hormone secretion is not fully understood, but intermittent GnRH stimulation of adenohypophyseal gonadotrophs probably is important in allowing regeneration of GnRH receptors. Currently, episodic release of GnRH is considered to be induced by hypothalamic and associated areas of the brain (Gallo, 1980).

Acute profile studies described in this thesis provided further evidence of pulsatile secretion of: LH and testosterone in boars; LH, prolactin and testosterone in rams; and to a lesser extent FSH in rams. These results coincided well with similar reports from other workers as reviewed on pages 29 and 30 of chapter one.

Also it was noted that prior to 8 weeks of age there was no quantitative (1:1) relationship between LH and testosterone peaks in boars (experiment 4.2), or ram lambs (experiment 5.2); these observations were similar to data reported by Wilson and Lapwood (1979a). In fact the latter authors suggested that establishment of a 1:1 relationship, which they recorded from rams at ages greater than 8 weeks, probably was an important factor in sexual maturation. No previous research appears to have been published on the influence of age on the quantitative relationship between LH and testosterone secretory episodes in young boars.

(ii) Effects of hemicastration on hormone secretion patterns and GnRH responses in boars

Acute hormone profiles obtained from experiment 4.1 and pre-GnRH hormone levels of experiment 4.2, showed that the remaining testes of hemicastrates were capable of secreting sufficient testosterone to maintain near normal plasma testosterone, and hence LH, levels. These results were consistent with those reported from hemicastrated Göttingen miniature boars (Elsaesser et al., 1978). There have been no reports of similar endocrine studies of boars of domestic breeds.

Pulsatile LH secretory patterns were evident at all ages of sampling of entires, hemicastrates and castrates. In addition episodic testosterone secretory patterns were recorded from entires and hemicastrates at each sampling age. Although hemicastration had no obvious influence on the magnitude or frequency of LH or testosterone secretory spikes, bilateral castration caused much more marked fluctuations in plasma LH levels compared to those observed from entires. Similarly exaggerated pulsatile variations in plasma LH levels in barrows have been reported previously (Colenbrander et al., 1977).

To date very little information on GnRH responses of boars has been published. It appears that the LH and testosterone data from experiment 4.2 is the first such data from domestic boars, although some has been published on the GnRH responses of Göttingen miniature boars (Elsaesser et al., 1974; Pomerantz et al., 1974). Rapid elevations of plasma LH and testosterone levels followed GnRH injection, but the total LH responses did not decline with sexual maturation. While that observation was in contrast with those recorded from developing ram lambs (Wilson and Lapwood, 1979b; experiments 5.3 and 6.3), the progressive increases with age in GnRH-induced testosterone responses were similar to those recorded from ram lambs (Lee et al., 1976b; Wilson and Lapwood, 1979b; experiments 5.3 and 6.3). Undoubtedly the increase in testicular responses resulted from a variety

of causes, such as increases in steroid histochemical activity volume of Leydig cells and in Leydig cell receptor activity, which have been discussed on page 115 in chapter IV.

(iii) Effects of hemicastration and cryptorchidism on hormone secretion patterns and GnRH responses of ram lambs

Acute hormone profile data from experiments 5.2 and 6.2 and pre-GnRH data from experiments 5.3 and 6.3 confirmed the observations made in the longitudinal study, that cryptorchidism, but not hemicastration, resulted in an overall elevation of plasma gonadotrophin concentrations.

Hemicastration did not have any effect on pulsatile secretion of any hormone measured, while the effects of cryptorchidism in increasing the number of LH peaks and decreasing the frequency of testosterone peaks, were discussed on page 217 in chapter VI. Conversely the latter treatment did not have any effect on the frequency of prolactin secretory pulses. Even though hemicastration had short-term effects in raising mean FSH levels, and cryptorchidism had prolonged influences, the frequency and magnitude of episodic fluctuations of this hormone were little influenced by these surgical treatments.

GnRH responses recorded from hemicastrated ram lambs generally confirmed the observations made from the longitudinal and profile studies. In experiment 5.3 hemicastration resulted in a significant increase in total LH responses compared to those of entire animals. However, analysis of GnRH responses of hemicastrates and entires in experiment 6.3 showed that while hemicastrates tended to have higher LH responses, the difference did not reach statistically significant levels. This discrepancy between the experiments may have resulted from the differences in design (i.e. only 4 weeks lapsed between hemicastration and the GnRH response study at each age in experiment 5.3, while the comparable time lapses in experiment 6.3 were 6, 14, 22 and 30 weeks).

In contrast cryptorchid ram lambs had greatly

exaggerated gonadotrophin responses, further confirming the observations made in experiments 6.1 and 6.2, as well as those reported by Lee et al. (1979; 1980) from cryptorchid rams, and by Schanbacher (1979a) from cryptorchid bulls.

Even in spite of the exaggerated gonadotrophin responses, testosterone responses recorded tended to be reduced: probably this decrease in testicular androgen production ($0.05<P<0.10$) would have been significant if a larger number of animals had been available for experiment 6.3 (n=3). For example Lee et al. (1979; 1980) and Schanbacher (1980a) used larger numbers of cryptorchid rams in their experiments and demonstrated that the testicular responses of these animals were lower than those of age-matched entires.

Neither Schanbacher and Ford (1977) nor Lee et al. (1978) observed any difference in the testosterone levels of entires and cryptorchids until GnRH response studies were conducted. Further discussion on the potential use of trophic compounds in diagnosis of endocrine disorders is presented on page 241 in this chapter.

Pre-GnRH plasma LH and FSH concentrations recorded from androgenised cryptorchid ram lambs further confirmed that hypoandrogenism was the major reason for elevated plasma LH levels in cryptorchids. This observation was different from those made by Schanbacher and Ford (1977) who found that exogenous testosterone did not have any effect on elevated plasma LH levels in cryptorchids. A reduction in FSH responses observed from androgenized animals was suggestive of an effect of testosterone on FSH secretion, as proposed by Crim and Geschwind (1972a) and Schanbacher (1979b; 1980b). However these results need cautious evaluation because of the supraphysiological pre-GnRH testosterone levels recorded from the androgenized animals and the limited number of animals.

Schanbacher (1980b) reviewed the methods used in androgen replacement studies, and questioned whether or not those generally used (single or series of testosterone

injections) were of any value in mimicking physiological androgen levels in treated animals. However, the alternative method suggested by him (use of implanted Silastic capsules containing crystalline testosterone) produced constant plasma testosterone levels in treated animals, a situation which also is unlikely to be physiological.

(c) Organ Weights and Histological Data

(i) Testicular changes in hemicastrates

Compensatory testicular hypertrophy always occurred following hemicastration of both boars and rams. These results were consonant with similar published work from those species, as discussed in chapters 4, 5 and 6. However, the relative importance of the various testicular components in contributing to this hypertrophy (apart from STD) was not determined. This was mainly because histological changes were regarded as being secondary to the endocrine investigations reported in this thesis.

Given the limitations of that approach and the subjective histological analytical methods employed, hemicastration did not appear to cause any major change in the composition of seminiferous tubular cell populations in either species. These results agree with similar reports of porcine (Hauser et al., 1952; Swierstra, 1968) and ovine research (Voglmayr and Mattner, 1968; Walton et al., 1980). Undoubtedly the compensatory increase in seminiferous tubular diameters was a major contributor to this hypertrophy (Voglmayr and Mattner, 1968; and experiments in chapters 4, 5 and 6). Nevertheless, compensatory increases in lengths of the seminiferous tubules resulting from hemicastration, also have been reported to occur in boars (Hauser, et al., 1952) as well as rams (Hochereau-de Reviers et al., 1976).

The endocrine basis of compensatory testicular hypertrophy in relation to altered secretion of gonadotrophins and testosterone was described on pages 117 and 224 in chapters 4 and 6. However, an intriguing question to consider here is why hemicastration did not induce acceleration of

pubertal spermatogenic maturation, even though it resulted in marked compensatory hypertrophy of the remaining testes. Likewise other workers have found no evidence of earlier initiation of spermatogenesis in hemicastrated ram lambs (Hochereau-de Reviers and Courot, 1978; Walton et al., 1980; de Reviers et al., 1980) or bull calves (Barnes et al., 1980a; c).

Superficially it would appear that the intratesticular hormonal environment of hemicastrates should have favoured advanced initiation of spermatogenesis because of the increased circulating concentrations of LH (Land and Carr, 1975), FSH (Walton et al., 1978; 1980; experiment 6.1) and testosterone (Walton et al., 1978; experiments 4.1, 5.2, 6.1 and 6.2). Thus it appears that other factor(s) are important determinants of the time of earliest onset of spermatogenesis. For example a critical body weight undoubtedly is one of them, or it may be an inherent characteristic of a species which is relatively refractory to manipulation.

On the other hand local factors may also have an influence on time of onset of spermatogenesis. Sertoli cell numbers and their maturity may be critical determinants. Evidence in favour of this idea comes from the correlation between Sertoli cell stocks and those of spermatogonia reported in pubertal rams (Hochereau-de Reviers et al., 1978; de Reviers et al., 1980). Perhaps establishment of this cell association may be decisive, since hemicastration of prepubertal ram lambs resulted in increases in Sertoli cell numbers (de Reviers et al., 1980). However, as yet there is no evidence to indicate that hemicastration hastens Sertoli cell maturation. Mature Sertoli cells may determine the time of onset of spermatogenesis by producing sufficient ABP, which helps to produce the required high testosterone concentrations inside the seminiferous tubules (Ritzen et al., 1974; Waites, 1980).

(ii) Testicular changes in cryptorchids

As discussed on page 223 in chapter 6, the histological changes recorded in the germinal cell epithelium, Sertoli and Leydig cells of cryptorchid testes in the present study undoubtedly resulted from increased intratesticular temperature.

Despite the decrease in efficiency of Leydig cell androgen secretion following this treatment (see page 222 of chapter 6), the peripheral testosterone levels in cryptorchid ram lambs (Blanc and Terqui, 1976; Lee et al., 1978; experiments 6.1 and 6.2) were not significantly different from those of entires. Deficiencies in androgen output were recognised only following the GnRH response studies in experiment 6.3 and those described by Lee et al. (1979; 1980) and Schanbacher (1980a). The potential for use of such trophic hormones in diagnosis of subtle changes in hormone secretion is discussed more fully on page 241 of this chapter.

(d) Possible Applications of the Present Study and Avenues for Future Research

Knowledge of the basic endocrine mechanisms controlling reproduction in the male, including those responsible for sexual maturation, is a necessary prelude to development of techniques for manipulating pubertal processes, as well as spermatogenesis in the adult males.

Expanded knowledge of the neuroendocrine control of reproduction in sheep will assist the development of pharmacological techniques which have practical agricultural benefits. For example the use of steroids, prostaglandins and gonadotrophins in such techniques as: induction of parturition or lactation, ovarian stimulation and super-ovulation, and treatment of infertility has generally followed basic scientific studies on the endocrine control of reproduction. Likewise research leading to discovery of methods for advancing puberty in domestic animals would be an advantage in animal husbandry. Such data may not

be of immense value for increasing production in porcine husbandry (because of shorter inter-litter intervals, lack of seasonality and relative early puberty), however would be of great interest in sheep husbandry. For example advancement of breeding age would permit more rapid genetic turnover, by allowing more mating of ram lambs of higher genetic merit (Dýrmundsson, 1973b), particularly in late maturing breeds such as Merinos (Land, 1978).

On the other hand information on basal hormonal levels and their secretory patterns during puberty is an essential requirement for successful treatment of endocrine disorders like hyperprolactinaemia infertility (Besser, 1978) and associated subfertility conditions (Schill, 1979), and development of successful contraceptive agents in the human male (Wong, 1980).

Most of the preliminary information in this type of research is obtained by experimentation either with laboratory or domestic animals. However, such basic data often is lacking for farm animals. For example information on hormonal secretory patterns during sexual maturation of boars is very limited.

It is evident from the different profiles of hormone secretion recorded during reproductive development, and their advanced stage of sexual maturity (puberty in males is around 12-15 weeks of age - Ellendorff et al., 1977), that Göttingen strain miniature boars provide a model of limited value for the study of pubertal development of domestic breeds of boars. This concept was previously reported by FlorCruz and Lapwood (1978). On the other hand advantages from use of mini-pigs undoubtedly could accrue from reduced feed costs, relative ease of handling, and factors such as more rapid genetic turnover. To this end they may be better suited for use in studies of female reproductive physiology as well as research on other aspects of endocrinology.

Because of unavailability of assay methods for determining plasma FSH levels in boars, experiments involv-

ing cryptorchid animals were not included in the present study. However, once such assays become available surgical treatments such as hemicastration and induced cryptorchidism would be valuable experimental tools in the investigation of feedback mechanisms controlling FSH and LH secretion in this species.

As mentioned on page 85 of chapter 3 the significant difference in body weight data between barrows and boars should be interpreted cautiously. The higher body weights of barrows was not surprising because of their greater appetites (Walstrá and Kroeske, 1968). However, from studies in which carcase analyses have been done it has been noted that carcases recovered from barrows contain more fat and less lean meat and thus, smaller percentages of primal cuts (Prescott and Lamming, 1964a;b; Walstra and Kroeske, 1968). One disadvantage of rearing boars for meat is that boar 'taint' may become evident. Boar taint is caused by the presence of the steroid 5 α -androst-16 ene-3one (5 α-androstenone) in meat from adult boars (Patterson, 1968), resulting in a characteristic odour which may cause consumer rejection of such meat. Nevertheless, Booth (1975) and Andresen (1976) reported that levels of 5 α -androstenone in adipose tissue and peripheral plasma reached critical levels only in animals older than approximately 140 days, while Rhodes (1972) demonstrated that boar 'taint' was negligible in meat from boars less than 200 days old.

Use of techniques such as immunising boars and rams against GnRH, gonadotrophins, androgens or oestrogens individually, with concurrent estimations of plasma hormone levels, could yield more information regarding hormone feedback regulatory mechanisms in these species. Although steroid replacement studies have been attempted it is essential to maintain physiological levels of these hormones in order to ascertain their physiological functions. Although the use of slow releasing hormone depots in silastic capsules is now becoming common (Schanbacher, 1980b), the constant hormone levels produced in such replacement studies do not mimic the natural secretory patterns of the hormone under investigation.

Although the results of the experiments involving ram lambs in the present study did not yield information of direct and immediate practical benefit to the agricultural industry it has provided a foundation of knowledge about feedback control of gonadotrophin secretion in ram lambs and on the endocrine basis of compensatory testicular hypertrophy in this species. The basic hormonal data obtained could be used as a foundation for designing further experiments for evaluating feedback mechanisms controlling LH and FSH.

Investigation of the effects of cryptorchidism in inducing the ultrastructural and biochemical changes which occur in Leydig and Sertoli cells in the testes of rams would be a logical extension in this field of study. To date no studies have been attempted on those lines with rams and the available data comes mainly from the work with rats (Kerr et al., 1979a; b; Aumüller et al., 1980).

Adaptation of this experimental approach to extreme seasonal breeders such as Soay rams, would also be an interesting extension of the current research. Soay rams are unique in respect to their response to photoperiod; the gonads becoming quite inactive in the non-breeding season and perhaps the feedback mechanisms required to maintain such low levels of gonadotrophins may also be different from those of less highly seasonal domesticated breeds (Lincoln and Short, 1980). Those authors in fact likened the seasonal testicular recrudescence, which occurs in Soay rams (and stags), to an annual puberty. Whether or not information derived from such animals would be of benefit to research on puberty, has yet to be investigated.

Unavailability of assays for estimating inhibin, which is now considered to be the feedback regulator of FSH, was a definite disadvantage in this study. However the recent report on an in vitro bioassay for inhibin described by Scott et al. (1980) may be of great value in research in this area. Using this assay procedure the authors concluded that elevated plasma FSH levels recorded from infertile

men with germinal epithelial damage was due to reduced inhibin production from Sertoli cells (Scott et al., 1980). Another parameter which often has been used by research workers to estimate Sertoli cell function was to estimate the rate of production of ABP (Kerr et al. 1979a; Aumüller et al., 1980).

The observation that oestrogen production is enhanced in cryptorchid testes also needs further evaluation (Damber and Bergh, 1980). The same authors hypothesised that increased synthesis of oestrogen may be a factor responsible for the Leydig cell malfunction. Whether this abnormality lies primarily in Leydig cells or in Sertoli cells is not known. Since Sertoli cells in the testes have been shown to have the capacity to synthesise oestrogens (Dorrington and Armstrong, 1979), enhanced oestrogen production by cryptorchid testes may well result from an alteration in metabolic pathways in Sertoli cells. Hence further in vitro and in vivo studies are needed to elucidate the site of synthesis and functional significance of oestrogens produced in cryptorchid testes.

The fact that GnRH treatment revealed reduced testosterone secretion (experiment 6.3) in cryptorchids, which was not evident in the longitudinal or profile studies (experiments 6.1 and 6.2), adds weight to the concept of using such stimulation studies for the diagnosis of subtle endocrine disorders in human and veterinary medicine. Similarly neither Schanbacher and Ford (1977), nor Lee et al. (1978) observed any deficiency in androgen secretory capacity of cryptorchid ram testes in acute or longitudinal studies, until GnRH responses were undertaken (Lee et al., 1979; 1980; Schanbacher 1980a). Cox et al. (1973) and Cox and Williams (1975) recommended the use of GnRH/HCG stimulation tests for differential diagnosis of cryptorchidism in male horses, while adrenocorticotropic hormone (ACTH) stimulation tests are performed to confirm diagnosis of such adrenocortical disorders as Cushing's syndrome (Ling et al., 1979). The latter condition cannot be diagnosed from basal plasma cortisol data.

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