EFFECTS OF CRANIAL CERVICAL GANGLIONECTOMY AND CASTRATION ON ENDOCRINE AND MORPHOLOGICAL CHARACTERISTICS OF MALE LAMBS

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ABSTRACT

EFFECTS OF CRANIAL CERVICAL GANGLIONECTOMY AND CASTRATION ON ENDOCRINE AND MORPHOLOGICAL CHARACTERISTICS OF MALE LAMBS

By Mark Warren Fisher

In a study of the effects of the pineal gland on reproductive development and hormone secretion, male lambs were incorporated into an overall 2 x 2 factorial design in which the experimental factors were:

(1) cranial cervical ganglionectomized or non-ganglionectomized, and

(2) castrated or entire.

The first experiment described was a longitudinal study of endocrine and morphological parameters recorded from these animals between 7 and 37 weeks of age. Plasma LH levels in entire lambs usually were very low throughout the experiment, although non-ganglionectomized entires did display a small elevation in levels between 8 and 13 weeks of age, which was not evident in ganglionectomized entires. At all ages plasma LH levels were elevated significantly in castrated animals. Neither ganglionectomy nor its interaction with castration had any significant effect on LH levels. In entire lambs plasma testosterone concentrations increased from 7 weeks to highest concentrations between 31 and 37 weeks of age. Overall, ganglionectomy reduced testosterone secretion,

but this probably was due to the lower body and testicular weights recorded from that group. The normal photoperiod-induced seasonal pattern of prolactin secretion in non-ganglionectomized lambs, with high levels during the summer months and low during winter, was markedly disrupted by ganglionectomy. Castration had no effect on prolactin levels and the interaction of castration and ganglionectomy also was non-significant.

Bodyweight was reduced significantly by ganglionectomy and this effect was accentuated in the ganglionectomized castrates. At autopsy, testicular weights and epididymal weights as well as epididymal sperm reserves were reduced, but not significantly, by ganglionectomy; these results probably reflected the bodyweight of those animals. Neither ganglionectomy nor castration had any significant effect on pineal weights, however the interaction of these two factors was significant due to the very large pineal of one of the non-ganglionectomized castrates.

A second experiment involved measurement of LH, prolactin and testosterone profiles in plasma obtained during hourly blood samplings which were conducted for 24 hours when lambs were both approximately 100 and 300 days of age. At both ages pulsatile secretion of LH and testosterone was confirmed, but no circadian rhythms of LH, testosterone or prolactin secretion were detected. Castration elevated LH levels significantly at both ages. Ganglionectomy and its interaction with castration had no effect on LH secretion at 100 days, but at 300 days these

factors were significant largely due to elevated levels being recorded from ganglionectomized castrates. Ganglionectomy did not affect testosterone levels in entire animals at either age while castrates had no detectable testosterone. Ganglionectomy reduced prolactin concentrations at 100 days of age (summer) and prevented the normal winter decline at 300 days of age. Castration and the interaction of castration with ganglionectomy had no significant influence on plasma prolactin levels at either age.

Pituitary LH and gonadal testosterone responses to 10 μg synthetic GnRH were tested at 100 days and 300 days of age in a third experiment. In all animals, GnRH elevated LH levels and in entires this in turn resulted in increased testosterone levels. Castration significantly increased basal and peak LH levels together with total LH output. At both ages the LH and testosterone responses to GnRH were not influenced significantly by ganglionectomy, nor did the interaction of castration and ganglionectomy have any significant effect on LH secretory responses.

These studies confirm the concept that the pineal gland can influence the secretion of prolactin, and probably also LH and testosterone, and thus may be involved in the regulation of pubertal development in ram lambs.

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CHAPTER I

INTRODUCTION

Survival of a species depends on the ability of individual animals to reproduce and bring forth their young. Reproduction in mammals is a sexual process that requires the union of sperm and ova and their subsequent development in a favourable environment. This process is a complex of physiological and behavioural characteristics which ensure that gamete maturation and mating are co-ordinated, and that offspring are produced during a season when survival is enhanced by favourable environmental conditions.

Attainment of the capacity to reproduce usually is reached after a transitional phase in development between sexual immaturity (characteristic of juvenility) and full reproductive competence (characteristic of adulthood). The biological processes of growth and maturation which make up this developmental phase constitute pubertal development. The term puberty is derived from the Latin 'pubes' which referred to the presence of pubic hair as a criterion of whether girls and boys had reached maturity.

Puberty is a process initiated by the brain, mediated and effected by the reproductive hormones, and influenced by the environment. In the experiments described in this thesis the possible influence of the pineal gland on the pubertal process in rams was investigated. Where possible, the review of the literature has been confined to sheep.

A. ENDOCRINOLOGY OF MALE REPRODUCTION

1. General

Mammalian reproduction is a complex interaction of physiological and behavioural processes involving interactions between the environment and the reproductive axis. Stimuli can influence any level of the hypothalamic-pituitary-gonadal axis, but mainly act at the level of the hypothalamus.

2. The Hypothalamus

The hypothalamus (reviewed by Donovan, 1970) is the most ventral portion of the diencephalon and is bounded anteriorly by the optic chiasma and caudally by the mammillary bodies. A semi-lunar shaped 'hypophysiotropic area', comprising the suprachiasmatic, paraventricular, periventricular, anterior hypothalamic, arcuate, and premammillary nuclei and the medial halves of the ventromedial nuclei, is capable of maintaining nearly normal anterior pituitary function even in the absence of contact with higher brain areas. This area is thought to synthesize the hypothalamic releasing and inhibiting factors or hormones.

As gonadotrophin and prolactin secretion in the male are tonic in nature, there must be some differences in hypothalamic organization compared with that of females which display cyclical gonadotrophin release and surges in prolactin secretion. Probably there are also important species differences between the frequently studied rat and the sheep (Domański, 1976).

Investigation into the exact site of

gonadotrophin-releasing hormone (GnRH) synthesis within the hypothalamus has mainly been confined to the female rat. Discrete lesion and stimulation experiments suggested that separate specific areas were involved in follicle stimulating hormone (FSH), and luteinizing hormone (LH) releasing activity (Motta et al., 1970; McCann et al., 1973; Mess et al., 1973). In contrast, both Crighton et al. (1970) and Quijada (1971) found that the areas containing LH and FSH releasing factors were completely coextensive, extending from the suprachiasmatic region to the arcuate-median eminence region. Palkovits et al. (1974) concluded that in female rats the cell bodies most likely to synthesize GnRH were in the medial basal hypothalamus and most probably the arcuate nucleus, while in ewes ovulation can be induced by electrical stimulation of the medial and basal parts of the hypothalamus (Radford, 1967; Przekop and Domański, 1970). The greatest concentration of GnRH is found in the median eminence region (Palkovits, 1974).

In ewes the anterior medial basal hypothalamus has been found to have prolactin release-stimulating activity, while the caudal medial basal hypothalamus has prolactin release-inhibiting activity (Wolińska et al., 1977).

From the cell bodies within these regions, the hypothalamic factors are transported down the axons of the tubero-infundibular tract to the median eminence from where they are released into the portal capillaries for transport to the anterior pituitary (Donovan, 1970). The cerebrospinal fluid has been suggested (Rodriguez, 1976)

as an alternate route between the synthesizing neurons and the portal vessels; evidence for this mechanism has been produced, at least in rats (Ben-Jonathan et al., 1974) and mice (Zimmerman et al., 1974).

3. Extrahypothalamic Influences

The hypothalamus has extensive bilateral connections with those forebrain structures such as the pyriform cortex, septum, hippocampus, and amygdala, etc., known as the limbic system, as well as connections with the midbrain reticular formation (Raisman, 1970; Raisman and Field, 1971). Evidence for a role of extrahypothalamic structures in reproduction has been reviewed by Ellendorff (1976) and Domański (1976). Experiments involving destruction, transection and electrical and electrochemical stimulation of various limbic structures, particularly the amygdala, have altered the timing of the onset of puberty. Also limbic structures have the ability to concentrate radioactive steroids, while acquisition of their ability to retain these steroids appears to be a characteristic of pubertal maturation. Steroid implants in the limbic area alter pituitary gonadotrophin levels, as well as limbic electrical activity (Ellendorff, 1976).

4. Hypothalamic Hormones

McCann and Porter (1969), Blackwell and Guillemin (1973) and Vale et al., (1977) have reviewed the hypothalamic hormones which stimulate or inhibit the release of the anterior pituitary hormones. To date, only three peptides with hypophysiotrophic activity (viz., thyrotrophin releasing factor (TRF), GnRH, and

somatostatin) have been purified and characterized chemically. GnRH is also termed LH-releasing factor/FSH-releasing factor (LH-RF/FSH-RF). Only LH, FSH and prolactin regulating hormones will be discussed.

(i) GnRH

It is now generally accepted that the decapeptide GnRH stimulates the release of both FSH and LH from the anterior pituitary (Jeffcoate, 1975; Schally et al., 1976a), even despite some reports to the contrary (e.g. the inability of GnRH to stimulate FSH release in all physiological states, the mapping of separate hypothalamic FSH and LH releasing activity areas, and the report of a rat hypothalamic fraction capable of preferential FSH release - Bowers et al., 1973). Differential release of LH and FSH by GnRH may be due to selective steroid and inhibin feedback, different half-lives of FSH and LH, and/or a difference in the intrinsic mechanism by which the pituitary LH and FSH gonadotroph cells respond to GnRH.

(ii) Prolactin-inhibiting and -releasing Factors

When the mammalian pituitary is separated from the brain by stalk section or autotransplant procedures, it secretes increased quantities of prolactin, whereas output of other trophic hormones diminishes. This, and results of other studies utilizing hypothalamic extracts demonstrates that the predominant control of prolactin secretion is by a prolactin-inhibiting factor (PIF) (Meites and Nicoll, 1966; Tindal, 1974). The identity of PIF is still not completely resolved. Dopamine accounts for much of the inhibitory action of the hypothalamus on prolactin secretion, but the

existence of dopamine-free PIF hypothalamic fractions has also been reported (Schally et al., 1976b; Kordon et al., 1977).

Evidence also suggests that a prolactin releasing factor or factors might exist (Vale et al., 1977), which perhaps is involved in the minor, more acute, pulsatile releases of prolactin, such as occur in response to ether stress (Tindal, 1974). Synthetic TRF also is capable of releasing prolactin (Fell et al., 1973) but its physiological role in the control of prolactin secretion is unknown.

(iii) Influence of Brain Monoamines and Prostaglandins

Neurosecretory elements which synthesize the hypothalamic hormones synapse with other neurons within the hypothalamus, thus much work has centred on the involvement of synaptic transmitters in the synthesis and release of hypothalamic hormones. 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." Studies utilizing intraventricular and systemic injections of pharmacological doses of amines, mainly in the female rat, have demonstrated that catecholeamines, cholines and indoleamines can influence the release of gonadotrophin- and prolactin-regulating hypothalamic hormones (Kamberi et al., 1970, 1971a,b; Kamberi, 1973; Kordon et al., 1976, 1977). For example prolactin release can be stimulated by noradrenaline and serotonin (Kamberi et al., 1971a,b) while

noradrenalin can stimulate, and melatonin inhibit, LH and FSH secretion (Kamberi et al., (1970). Biogenic amines appear to be involved in regulation of the oestrous cycle in ewes (Wheaton et al., 1972).

Also there is some evidence indicating that prostaglandins may stimulate gonadotrophin release, probably by acting mainly at the level of the hypothalamus (Carlson et al., 1973; Labhsetwar et al., 1974; Labrie et al., 1976; McCann et al., 1976). Warberg et al. (1976) suggested that in rats the increase in LH release following intraventricular prostaglandin infusion was mediated by a specific brain receptor. Similar work suggests that prolactin secretion may be regulated in part by prostaglandins (McCann et al., 1976).

(iv) Mechanism of Action of Hypothalamic Hormones

Two main theories have evolved for explanation of the mechanism of action of hypothalamic hormones at the pituitary level; they involve either stimulus-secretion coupling or adenyl cyclase activation (McCann, 1974). The former suggests that releasing hormones alter cell membrane permeability leading to membrane depolarization and uptake of calcium ions, the Ca^{++} then activating the release process. The latter theory assumes that releasing hormones combine with specific receptors on the cell membrane causing an activation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). The cyclic AMP then alters hormone release in some way. Jutisz et al. (1976) have presented a hypothetical model of GnRH action which incorporated both hypotheses; they suggested

that a cyclic AMP stimulated protein kinase-phosphorylation process and calcium ions interacted to activate a cytoplasmic microtubular system involved in extrusion of secretory granules. Prolactin release may also involve cyclic AMP although the evidence is conflicting (Horrobin, 1975).

Although the principal action of GnRH is induced by extrusion of stored secretory granules, it probably also stimulates synthesis either primarily or secondarily to release (Schally et al., 1971).

Prolactin release and synthesis also appear to be two distinct processes, as under some circumstances synthesis can be stimulated while release is inhibited (e.g. in rats prolactin synthesis continues after the suckling stimulus for release has been removed (Convey and Reece, 1969; Neill, 1974)).

(v) Effects of GnRH in Sheep

Subsequent to the isolation, characterization and synthesis of the porcine decapeptide GnRH (Matsuo et al., 1971a,b), and the demonstration that it is identical to ovine GnRH (Burgus et al., 1972), it was shown (Arimura et al., 1972) that the synthetic polypeptide could release LH in rams. Recently Pelletier (1976) reviewed literature on the effects of GnRH on LH and FSH release in sheep, cattle and pigs. Although GnRH can cause release of FSH in rams, the response is not as dramatic as the LH response to the same GnRH dose (Hopkinson et al., 1974; Bremner et al., 1976; Lee et al., 1976b). Spona (1973) suggested that two distinct GnRH binding sites existed in the rat pituitary:

a high affinity one mediating LH release and a low affinity receptor mediating FSH release; this concept is consistent with a lower FSH response to GnRH than is seen with LH.

In rams pituitary LH responses to GnRH can be influenced by the daily photoperiod (Lincoln, 1977) and by the stage of reproductive development with maximal responses at 6-8 weeks of age (Galloway and Pelletier, 1974; Lee et al., 1976b; Wilson and Lapwood, 1978c); also they are greater and quicker in wethers than in rams (Reeves et al., 1970; Hopkinson et al., 1974; Galloway and Pelletier 1975; Pelletier, 1976).

5. Anterior Pituitary Hormones

(i) LH and FSH

a) Chemistry and Metabolism

LH and FSH (reviewed by Greep, 1973) are glycoprotein hormones consisting of two non-identical, non-covalently linked polypeptide chains called α and β subunits. Separately, these subunits have little biological activity, but when recombined activity is restored (Vaitukaitis et al., 1976). The α subunit is identical for LH and FSH (and also thyroid stimulating hormone) but the β subunit is different and determines the activity of the hormone.

Immunological studies in primates, using antisera raised against the intact hormones (Phifer et al., 1973; Robyn et al., 1973) or against the β subunits (Herbert, 1976), have shown that LH and FSH may sometimes be present in the same cell type.

Estimates of metabolic clearance rates and half-lives of LH and FSH have varied. For example Akbar et al. (1974)

estimated that FSH and LH had half-lives of 102 and 43 minutes, respectively, in ewes; Geschwind and Dewey (1968) and de Kretser et al. (1973) found LH to have a half-life of about 30 minutes in rams and ewes, and Foster et al. (1972, 1975) recorded half-lives of 20-28 minutes for LH in foetal and prepubertal lambs. LH, at least, probably is excreted after being secreted into the urine by the kidney proximal convoluted tubule (de Kretser et al, 1973).

b) Actions

Hansson et al. (1976a) have reviewed research on the target cells of LH and FSH in the testes, and also the role of gonadotrophins in regulating spermatogenesis (Hansson et al., 1976b). LH is concerned primarily with stimulation of androgen secretion by the interstitial cells of the testes; through this effect it has an indirect role in regulating spermatogenesis (see page 13). LH binds to membrane receptors of the Leydig cells and subsequently stimulates androgen secretion by an adenyl cyclase-mediated increase in the first step of cholesterol conversion to pregnenolone (Hall and Young, 1968).

The exact function of FSH in maintenance of spermatogenesis is not clear although it appears to influence the maturation of spermatids (Steinberger, 1971, 1974), but there may be important species differences. It is suspected that FSH also is necessary for initiation of spermatogenesis prior to puberty (Courot, 1967, 1976; Lostroh, 1969, Means, 1974). Available evidence indicates that Sertoli cells are the primary testicular targets for FSH although a role in increasing

testicular LH receptor formation before puberty has also been suggested (Odell and Swerdloff, 1976). FSH stimulates testicular protein synthesis (androgen binding protein) by binding to membrane bound receptors and subsequently increasing RNA synthesis via the stimulation of adenyl cyclase and protein kinase activities (Means, 1974). Lee et al. (1976c) stated that it would "be in character" if FSH also stimulated the production of inhibin.

(ii) Prolactin

a) Chemistry and Metabolism

Prolactin is a single protein molecule which probably is transported in the plasma in an unbound state. The existence in plasma of a 'big-prolactin' which contains a normal prolactin component, is also being investigated (Horrobin, 1975).

Only prolactin appears to be secreted by the lactotroph cells of the anterior pituitary, although the possibility that some cells may secrete both prolactin and growth hormone can not be excluded (Horrobin, 1973). The finding that cup-shaped prolactin secreting cells can contain gonadotroph cells within their cups (Nakane, 1970) suggests that gonadotrophin and prolactin secretion could be related.

A plasma half-life of 23 minutes for prolactin has been reported in the ewe (Akbar et al., 1974) while Davis and Borger (1973) observed different metabolic clearance rates between lambs and ewes in different physiological states. Prolactin appears to be bound and inactivated rapidly in the liver.

b) Actions

Prolactin has a very wide array of actions in vertebrates (Nicoll and Bern, 1972), but its best known actions in mammals are mammotrophic and luteotrophic; definite evidence of the latter function has been confined to rodents. In males the possibility of a gonadotrophic function also is becoming apparent. For example, hyperprolactinaemia in human males frequently is associated with hypogonadism and abnormal puberty (Koenig et al., 1977; McKenna et al., 1978).

In hamsters induction of testicular regression by a short daily photoperiod appears to be associated with a decline in peripheral plasma prolactin levels and with refractoriness of the testes to gonadotrophins (Bartke et al., 1978).

Specific prolactin binding sites have been identified in the rat prostate, testes, epididymides and seminal vesicles (Aragona and Friesen, 1975; Kledzik et al., 1976). Also Bartke and colleagues (see review by Bartke et al., 1978) have injected various hormones into hypophysectomized rats and shown that prolactin or prolactin and FSH act to increase the sensitivity of Leydig cells to LH by promoting LH receptor formation, and also by synergizing with LH in promoting testicular steroidogenesis by increasing the amount of esterified cholesterol available for conversion to steroid hormones (Hafiez et al., 1972a,b; Bartke and Dalterio, 1976). After injecting prolactin antiserum into immature male rats and measuring circulating hormone levels and reproductive organ weights, Hostetter and Piacsek

(1977) suggested that prolactin may have an inhibitory effect on LH secretion during sexual development, but a stimulatory effect on the development of the secondary sex tissues. In rodents, at least, the testicular action of prolactin probably reflects a direct action on the Leydig cells and may be important in sexual maturation (Bartke et al., 1978).

Autumn born ram lambs show a peak in prolactin secretion around ten to twelve weeks of age, at the same time as the beginning of the rapid increase in testicular weight and spermatogenic activity (Ravault and Courot, 1975). Subsequently Ravault et al. (1977b) showed that administration of the prolactin secretion inhibitor, 2- bromo-α-ergocryptine, in both spring and autumn born ram lambs between 10 and 21 weeks of age resulted in an initial decrease in testosterone levels and a significant decrease in seminal vesicle weights and fructose concentrations. Plasma LH levels, body, testes, albuginea, and epididymal weights and seminiferous tubule diameters all were unaffected. Thus, prolactin may synergize with both LH and testosterone at the gonadal and/or accessory sex gland levels.

6. Testicular Steroids

Mammalian testes have both gametogenic and steroidogenic functions. Androgens serve to develop and maintain spermatogenesis, accessory reproductive organs, and secondary sexual characteristics, while oestrogens may regulate the secretion of FSH (Hall, 1970). The role of testicular steroids in the regulation of spermatogenesis

was reviewed recently by Hansson et al. (1976b).

(i) Synthesis, Secretion and Metabolism

Leydig or interstitial cells function principally as producers of androgens. These hormones, mainly testosterone, androstenedione and dihydroepiandrostenedione, are synthesized from the initial precursor, plasma fatty acids, via acetyl CoA and cholesterol (Christensen, 1975). In turn, androgens can be precursors in the synthesis of oestrone and oestradiol-17β. Germinal cells appear to contain steroidogenic enzymes and Sertoli cell tumours can secrete large amounts of oestrogens. Oestrogens synthesized within the germinal cells may function locally in the control of differentiation of germ cells during prepubertal sexual development and also in the local regulation of Leydig cell sensitivity to LH (Hall, 1970; Fawcett, 1975; Dorrington et al., 1978).

Androgens are secreted into the interstitial fluid from where they are either taken up by the seminiferous tubules and transported into the efferent duct to the epididymis, or channelled into lymph and venous capillary blood; the latter route is the major pathway for entry of androgens into the systemic circulation (Eik-Nes, 1975).

In blood testosterone may be free, conjugated as glucuronide, or bound by such proteins as albumin, corticosteroid-binding globulin, and sex-steroid binding globulin (Liao and Fang, 1969), but only the unbound fraction is biologically active (Minguell and Sierralta, 1975). Haynes et al. (1976) reported that circulating

testosterone had a half-life of 8 minutes in prepubertal bulls.

High concentrations of androgens within the seminiferous tubules and epididymides depend on the presence and distribution of a specific androgen binding protein (ABP) (Hansson et al., 1975). ABP has been demonstrated in ram rete testis and epididymal fluid (Jegou et al., 1976) and seminal plasma (Jegou et al., 1978a), and particularly binds testosterone and dihydrotestosterone, and much to a lesser extent oestradiol-17β. Functions of ABP may be: (i) to concentrate or accumulate androgens within the seminiferous tubules during the initiation of spermatogenesis when testosterone secretion has not reached adult levels; (ii) to concentrate androgens in close proximity to the spermatozoa and the androgen dependent cells of the caput epididymis (Hansson et al., 1975); (iii) to protect the androgens from enzymic degradation; or (iv) to regulate androgen incorporation into testicular and epididymal intracellular receptors (Jegou et al., 1978b).

(ii) Actions

Testosterone probably is converted to its more active metabolites, dihydrotestosterone and androstenediols in androgen sensitive tissues such as the prostate, seminal vesicles and epididymides (Williams-Ashman, 1975). Androgens can also be converted into oestrogens in the peripheral tissues (Wilson, 1975).

It appears that within the testes androgens act: (i) on the germinal epithelium to maintain spermatogenesis; (ii) on the Sertoli cells to increase the effect of FSH on ABP synthesis; (iii) on the peritubular or myoid cells to regulate tubular contractions; and (iv) possibly on the Leydig cells sensitizing them to LH (Hansson et al., 1976a). Hansson et al. (1976b) concluded that there was only indirect evidence indicating that germ cells contain androgen receptors, the response of the seminiferous epithelium to androgen most likely is mediated entirely by testicular somatic cells.

Other major actions of testosterone include the feedback control of synthesis and secretion of pituitary gonadotrophins, sexual differentiation and the development and maintenance of secondary sexual organs and characteristics, behavioural patterns, and a general protein anabolic effect (Williams-Ashman, 1975).

7. Feedback Control of the Hypothalamo-Hypophyseal-Gonadal Axis

All hormones can be regulated by the actions of other hormones operating in a feedback system. Thus hormones of the hypothalamo-hypophyseal-testicular axis can influence their own secretion via feedback control of the synthesis and release of hypothalamic and pituitary hormones.

(i) Hypothalamic Hormones

Although there is a possibility that hypothalamic hormones may regulate their own secretion by an ultrashort feedback system (Motta et al., 1973), this field remains largely unexplored. Even if ultrashort feedback regulation

of GnRH secretion occurs, Jeffcoate (1975) considered it unlikely to be mediated via the systemic circulation but rather, as has been suggested by Dyer and Dyball (1974), the effect may be transynaptic: GnRH may be a neurotransmitter, being released from axon branches not only terminating near the portal vessels, but also synapsing on other hypothalamic cells.

There are no reports of an ultrashort feedback mechanism regulating the secretion of prolactin inhibiting or releasing factors.

(ii) Pituitary Hormones

A large amount of evidence has established the concept that LH, FSH and prolactin can regulate the secretion of their own respective releasing/inhibiting hormones via short feedback loops or mechanisms (Motta et al., 1973; Robyn et al., 1977). For example implants of LH, FSH and prolactin in the median eminence can reduce pituitary and blood levels of those hormones (Neill, 1974; McCann, 1974; Horrobin, 1974); radioactive gonadotrophins can be concentrated by the hypothalamus (Davies et al., 1975); and LH, possibly in synergy with the testicular steroids, stimulates the activity of the GnRH-destroying hypothalamic peptidases which may be involved in feedback regulation (Kuhl and Taubert, 1975; Griffiths et al., 1974, 1975).

However, the relative importance of such feedback mechanisms has been questioned in view of the large increases in secretion of LH and FSH following castration (McCann, 1974). In any case, a short feedback loop would

normally operate in conjunction with gonadal steroid and possibly inhibin feedback.

(iii) Testicular Hormones

Testicular hormones exert long loop negative feedback effects on the hypothalamus and pituitary to regulate gonadotrophin secretion (see review by Barraclough, 1973). Thus testicular atrophy follows implantation of testosterone into the median eminence while similar implants of the anti-androgen, cyproterone acetate, causes enlargement of accessory sex organs and a slight increase in testicular weight (Davidson, 1967). In turn, castration enhances the synthesis and release of pituitary gonadotrophins in rams (Pelletier, 1968; Crim and Geschwind, 1972b; Pelletier and Ortavant, 1975a). Injection of testosterone propionate into wethers causes a biphasic decrease in LH levels. The first decrease, 12 hours after injection, has been attributed to a decrease in pituitary sensitivity to GnRH, while the second reduction in LH levels at 72 hours may be due to inhibition of GnRH synthesis (Pelletier, 1970,1974). Steroid-binding receptors have been identified in the hypothalamus and pituitary (Davies et al., 1976).

It is now accepted that LH secretion is controlled by negative feedback effects of testosterone, possibly after its conversion to its α-reduced metabolites and oestrogens in the target tissues. In rams and wethers this is indicated by the fact that administration of testosterone and testosterone propionate lowers blood LH levels (Bolt, 1971; Crim and Geschwind, 1972b; Hopkinson et al., 1974;

Galloway and Pelletier, 1975) while oestradiol also suppresses LH secretion in rams (Bolt, 1971) and wethers (Riggs and Malven, 1974a; Karsch and Foster, 1975).

FSH secretion in rams also is influenced by the testicular steroids. Testosterone propionate replacement therapy reduced the elevated plasma FSH levels which resulted following castration (Crim and Geschwind, 1972b). The possibility of a positive feedback effect of testosterone on FSH secretion has been suggested since intrapituitary implants of testosterone increased serum FSH levels in adult castrated male rats (Martini, 1976).

FSH secretion probably also is regulated at both the hypothalamus and pituitary by inhibin, a product of the seminiferous epithelium, most likely the Sertoli cells (Setchell and Main, 1974; Lee et al., 1976c). Inhibin-like activity of ram rete testis fluid has been shown to suppress FSH secretion selectively, in rats (Setchell and Jacks, 1974; Franchimont et al., 1976) and rams (Blanc and Dacheux, 1976), while Chari et al. (1978) isolated a steroid-free protein with similar activity from bull seminal plasma.

A role of testicular steroids in regulation of prolactin secretion has been suggested following experiments in a number of species. Work mainly with rats suggests that the pattern of prolactin secretion in adult animals depends on the presence or absence of androgens shortly after birth: in females, or castrate males, the absence of androgen exposure at birth induces susceptibility to surge secretion of prolactin in response to oestrogen

administration, while males or androgenized females do not show any such prolactin response to oestrogen stimulation (Neill, 1974). An inverse relationship between plasma levels of prolactin and testosterone has been reported in adult men (Rubin et al., 1976), but Pelletier (1973) injected testosterone propionate into rams and found no interference with prolactin secretion. Also Ravault et al. (1977a) have found that the pre-pubertal peak of prolactin levels seen in autumn born ram lambs was not testis- or testosterone-dependent.

8. Patterns of Hormone Secretion

Hormone secretion displays a number of variable patterns ranging from short-term pulsatile or episodic release to circadian and seasonal trends. Seasonal patterns of hormone secretion will be discussed in a later section (page 25).

(i) Pulsatile Hormone Secretion

Several studies have shown that LH is released in a pulsatile or episodic manner in rams, with peak values being many times greater than basal levels; subsequent to LH pulses peaks of testosterone secretion occur (Katongole et al., 1974; Sanford et al., 1974c; Lincoln, 1976a; Barrell and Lapwood, 1978e; Wilson and Lapwood, 1978d). Pulsatile LH release also has been recorded in ram lambs (Foster, 1974; Carr and Land, 1975; Wilson and Lapwood, 1978a; Foster et al., 1978) and wethers (Riggs and Malven, 1974a) as well as in males of several other species.

Although the pattern of GnRH release in rams is unknown, Crighton et al., (1973) recorded a pulsatile

pattern in GnRH levels in the jugular blood of ewes, but Nett et al. (1974) could not relate peripheral GnRH levels to LH release in ewes. The large degree of haemodilution that occurs between the pituitary portal vessels and the general circulation probably makes it unlikely that there is a close relationship between peripheral plasma GnRH and LH concentrations. However, GnRH release has been found to be pulsatile in the Rhesus monkey pituitary portal vessels (Carmel et al., 1976).

In contrast, plasma FSH levels generally have been shown to remain relatively constant throughout periods of intensive sampling, with small elevations occurring independently of LH and testosterone fluctuations (Sanford et al., 1976, 1977; Lee et al., 1976a), however Lincoln (1978b) did correlate LH and FSH pulses in Soay rams.

Plasma prolactin levels also have been found to fluctuate over short periods when intensive sampling regimes were used (Chamley et al., 1974; Barrell and Lapwood, 1978e; Wilson and Lapwood, 1978d; Davis et al., 1978).

(ii) Circadian Rhythms

The present status of circadian cycles of plasma reproductive hormone levels is confused. Many studies have failed to show any such cyclicity while others have. These 'contradictory' results may reflect differing experimental conditions and sampling methods (ranging from jugular venepuncture to remote catheterization), different reproductive states, seasons, environmental conditions, species and breeds.

In the photosensitive Soay ram, Lincoln and colleagues, utilizing 'short-day' and 'long-day' artificial photoperiods, have been able to show circadian changes in plasma levels of LH, FSH, prolactin and testosterone (Lincoln et al., 1977, 1978). However in studies with rams of domestic breeds other workers generally have been unable to detect any circadian rhythm in LH or testosterone secretion (Purvis et al., 1974; Wilson and Lapwood, 1978d; Barrell and Lapwood, 1978e) although Wettemann and Desjardins (1973) did record nocturnally elevated plasma levels of testosterone.

In contrast, circadian rhythms in plasma prolactin levels, with peaks at 20-22.00 hours, have been recorded from growing wether lambs (Forbes et al., 1975) and adult rams (Barrell and Lapwood, 1978e). Higher plasma levels of prolactin also have been reported during dark periods in adult rams (Ravault and Ortavant, 1977). On the other hand, neither Chamley et al. (1974) nor Wilson and Lapwood (1978d) could detect any circadian pattern of prolactin secretion.

Several French workers have recorded the existence of a photosensitive phase in the 24 hour period in rams (Ravault et al., 1976; Garnier et al., 1977a; Ravault and Ortavant, 1977): in this period, exposure to light decreases pulsatile gonadotrophin release while light deprivation stimulates release. Similarly, prolactin secretion was maximal with light exposure during the photosensitive phase, and minimal in the absence of light. This may be a determinant of circadian rhythms and may be

involved in the regulation of seasonality.

Integrity of the suprachiasmatic nucleus appears to be essential for the generation of circadian rhythms, at least in rodents (Moore, 1978). This nucleus may function in the measurement of photoperiod, and thus in the regulation of seasonality (Rusak and Morin, 1976; Stetson and Watson-Whitmyre, 1976; Menaker et al., 1978).

In adult humans a circadian rhythm of prolactin secretion appears to be closely related to sleep (Parker et al., 1973; Rubin et al., 1974) while Lincoln et al. (1977) suggested that the daily activity cycle of rams affected gonadotrophin secretion.

In female rats, changes in the phase and amplitude of circadian rhythms of gonadotrophin secretion may be involved in the timing of the onset of puberty (MacKinnon et al., 1978) but it is not known if this has any such role in males. Human studies have suggested that nocturnal increases in LH secretion are characteristic of pubertal maturation (Weitzman et al., 1975).

A most striking example of a circadian rhythm of hormone secretion is that of melatonin. In many species including sheep the transition from light to dark is associated with an abrupt increase in plasma melatonin levels (Rollag and Niswender, 1976; Rollag et al., 1978a,c; Kennaway et al., 1977, 1978). This response to the circadian variations in lighting probably involves the pineal gland, and is discussed in a later section (page 40)..

(iii) Social and Sexual Stimuli

Sexual arousal and stimulation do not appear to stimulate gonadotrophin secretion in rams (Sanford et al., 1974b, 1976), although in both these studies LH and FSH levels were higher in the 12-hour mating period than in the 12-hour non-mating period immediately following. Similarly, the presence of oestrous ewes and differing social environments during development did not influence hormone levels (Sanford et al., 1974b; Illius et al., 1976a,b).

Testosterone levels were unaffected by copulation in adult rams (Purvis et al., 1974) and young rams (Illius et al., 1976a) but a post-copulatory testosterone peak can occur in older animals (Illius et al., 1976b; Moore et al., 1978).

(iv) Stress

It is generally accepted that stressful stimuli elevate prolactin levels in sheep (Davis, 1972; Wilson and Lapwood, 1978a) and cattle (Raud et al.,1971), while effects on LH and testosterone output have only recently been suggested. Although Roche et al. (1970b) recorded no effect of different sampling methods on LH levels in ewes, Wilson and Lapwood (1978a) showed elevated levels in the first of four half-hourly samples; those probably were induced by shepherding and handling procedures. Also Sitarz et al. (1977) recorded an effect of the stress of capture, restraint and venepuncture of young bulls on plasma LH and testosterone levels, although they

interpreted their data as a stress-induced reduction in hormone levels.

9. Seasonality of Reproduction

Seasonal breeding is apparent in many mammals, and although domestication generally has masked the necessity for seasonality, it persists to varying degrees in many domestic species (Marshall, 1937; Ortavant et al., 1964; Clegg and Ganong, 1969; Lodge and Salisbury, 1970). The ability of an animal to give birth to its young under optimal conditions for survival, particularly with respect to nutrient availability, depends on the timing of breeding and conception and is synchronized by the complex of climatic, nutritional and biotic factors which make up the environment. Of these environmental factors, daily photoperiod, particularly in higher latitudes, provides potentially the most accurate, constantly changing annual environmental cue (Menaker, 1971). In sheep the autumnal decrease in daily photoperiod stimulates reproductive activity (Yeates, 1949).

(i) Indications

Although male animals do not exhibit such obvious indications of reproduction as oestrus, gestation and parturition, which are characteristic of the female, seasonal changes in reproductive parameters are apparent in some species. The stag exhibits marked seasonal reproductive changes with the most obvious manifestations being antler growth and rutting behaviour (Lincoln, 1971). Several male rodents also exhibit seasonal changes, for example Racey (1978) has detailed seasonal changes in

plasma and testicular testosterone levels, body, testis and adrenal weights, epididymal sperm reserves and the weights of some androgen dependent accessory sex organs in the mole.

Although rams retain the capacity for sperm production throughout the year, fertility often is depressed during the spring and summer months (Clegg and Ganong, 1969) and seasonal trends in gonadal and accessory gland activity and sexual performance have been described. For example, Lemay and Corrivault (1973) reported a close relationship between the month of the year, testicular weights and seminiferous tubule diameters in rams. Spermatogenesis was maximal during the autumn breeding season and fell away during winter months.

Peaks in ejaculate volume and seminal fructose levels have been reported in rams during the breeding season (Sanford et al., 1977; Barrell and Lapwood, 1978a). There are similar increases in testicular capillary blood flow (Courot and Joffre, 1977) and in the concentration of ABP in rete testis fluid (Jegou et al., 1978b). Johnson et al. (1973) also found that spermatogenesis and testicular steroidogenic activity correlated well with changes in photoperiod and ambient temperature. Peaks in sexual activity and mating performance coinciding with the breeding season have also been reported in rams (Schanbacher and Lunstra, 1976; Lincoln and Davidson, 1977; Sanford et al., 1977; Shackell et al., 1977).

(ii) Gonadotrophins and Testicular Steroids

Seasonal changes in plasma hormone levels have been reported over the last few years. Elevated plasma levels

of LH (Sanford et al., 1974a; Schanbacher and Ford, 1976; Davies et al., 1977; Barrell and Lapwood, 1978a) and FSH (Sanford et al., 1976, 1977; Davies et al., 1977) have been recorded in summer and autumn, while peaks in plasma testosterone levels have coincided with the height of the autumn breeding season (Katongole et al., 1974; Sanford et al., 1974a; Schanbacher and Ford 1976; Davies et al., 1977; Barrell and Lapwood, 1978a). On the other hand Schanbacher and Ford (1976) found no seasonal cycle in plasma oestradiol concentrations.

Katongole et al. (1974) suggested that changes in the frequency, amplitude and duration of pulsatile hormone release may be important parameters of reproductive seasonality in rams. More frequent pulsatile LH and testosterone releases generally have been recorded during the summer and autumn photoperiods (Sanford et al., 1974c; Lincoln, 1976a; Lincoln et al., 1976; Wilson and Lapwood, 1978a). Recently, Sanford et al. (1977) reported that as the autumn breeding season progressed there was an increase in frequency of LH pulses while peak height and mean basal levels declined; there also was an increase in baseline and peak levels of testosterone suggesting that the testes become more sensitive to LH as the season progressed. Pituitary sensitivity to synthetic GnRH also has been shown to vary with the stage of the photoperiod-induced seasonal sexual cycle (Lincoln, 1977, 1978b), supporting the concept that seasonal variations in sensitivity of the hypothalamus and pituitary to testicular steroid feedback may be related to the corresponding changes in episodic hormone release (Katongole et al., 1974; Lincoln, 1976a). Seasonal variation in

gonadotrophin secretion may occur because, as Pelletier and Ortavant (1975b) suggested, "the decreasing light photoperiod has two effects:

1) stimulation of gonadotrophin release
2) lowering the intensity of the negative feedback of testicular androgens.

Conversly, increasing light photoperiod

1) is inhibitory or at least less stimulatory to LH release
2) increases the negative feedback effect of androgens on hypothalamo-hypophyseal activity."

(iii) Prolactin

Plasma prolactin levels show the most marked seasonal cycle of changes of the reproductive hormones in rams. Length of the daily photoperiod is closely related to the levels monitored in blood, with peak and lowest concentrations being recorded at the summer and winter solstices, respectively (Pelletier, 1973; Ravault, 1976; Barrell and Lapwood, 1978a,c). Ambient temperature may play a role in regulating prolactin levels, as has been shown in cattle (Wetteman and Tucker, 1974; Smith et al., 1977), but experiments with rams in photoperiod-controlled, constant-temperature rooms have shown that length of daylight is the major influence (Pelletier, 1973; Barrell and Lapwood, 1978c).

(iv) Photoperiod

Experiments involving manipulation of artificial photoperiods have confirmed the importance of daily photoperiod in regulating reproductive function in rams.

Lincoln and his colleagues have done much work on the photosensitive Soay breed which is thought to resemble the ancestral wild sheep from which domestic breeds have evolved (Jewell et al., 1974). These animals have more pronounced seasonal reproductive changes than sheep of domestic breeds. Abrupt lighting changes from "long-day" photoperiods of 16 hours light : 8 dark (16L:8D) to "short-day" photoperiods (8L:16D) reduce plasma prolactin levels, stimulate FSH and LH secretion and increase testosterone levels, testicular size and sexual activity. A change back to "long-day" photoperiods induces testicular regression, a decline in gonadotrophin levels and a rise in prolactin secretion (Lincoln, 1976b, 1978b; Lincoln and Peet, 1977; Lincoln and Davidson, 1977; Lincoln et al., 1977, 1978). Similar observations have been recorded by Pelletier and Ortavant (1975a,b) using Ile-de-France rams and wethers and by Sanford et al. (1978) using Finnish Landrace rams on six-monthly reduced "annual" photoperiodic cycles. Barrell and Lapwood (1978c,d), utilizing N.Z. Romney rams on normal, even (12L:12D) or reversed seasonal light cycles, found that the normal seasonal patterns in seminal fructose and plasma testosterone levels were disrupted in animals on the reversed light cycle, while prolactin levels were closely related to daylight length in all animals. Thus in rams, photoperiod appears to stimulate reproductive function during short photoperiods and suppress it during longer photoperiods.

The way in which animals perceive seasonal changes in photoperiodic cycles has been speculated upon by Menaker

(1971)..."In most of the photoperiodic systems which have been investigated in any detail the organism appears to measure daylength not by measuring the length of the light period, nor by measuring the length of the dark period nor again by measuring the ratio of the light period to the dark period. Rather, organisms distinguish between inductive and non-inductive daylengths by assessing whether or not light is present at a particular phase point on an endogenous circadian rhythm of sensitivity to its inductive effects." This also appears to be the case in rams, for example Lincoln (1978a) indicated that a change in the total quantity of light over 24 hours in the Soay ram was not a prerequisite for the photoperiodic response. Also by using a one hour light pulse to interrupt the dark phase Ravault et al. (1976), Garnier et al. (1977b) and Ravault and Ortavant (1977) have shown that the response to light varies over 24 hours with a photosensitive phase existing 16 hours after the "subjective dawn." However, the possibility that the timing of this sensitive phase alters throughout the season has been suggested (Ravault and Ortavant, 1977). It was proposed that during such a photosensitive phase the presence of light (corresponding to long photoperiods) would stimulate prolactin and suppress gonodatrophin secretion, while the absence of light (corresponding to short photoperiods) would have the opposite effect. This suggests that circadian rhythms may be involved in mediating seasonal cycles. Finally, Lincoln and Davidson (1977) commented: "it may therefore be necessary to envisage the role of photoperiod in sheep as

being to control the time of the sexual cycle rather than the cause of it. In natural conditions with a seasonally changing photoperiod, the stimulatory (autumn) and inhibitory (spring) effects of daylength described above would operate to entrain the cycle to the changes in the environment."

(v) Transmission of Photoperiodic Stimuli

The pathway by which light is transmitted to influence hypothalamic-hypophyseal function has not been established in rams, however much work has been done in rodents. Research into the effects of photoperiod on hypothalamic function has involved two main pathways, one being direct retino-hypothalamic tracts and the other via the pineal gland.

Although Ganong et al. (1963) found that a small amount of environmental light could penetrate the brain of sheep and other animals, and stimulate a photocell, the only definitely known photoreceptors in mammals are those of the retinae (Wurtman, 1967).

The Harderian glands, located behind and around the eyes, have been suggested to contain extra-retinal photo-receptors influencing both the suprachiasmatic area of the hypothalamus and the pineal gland in rats (Wetterberg et al., 1970; Bubenik et al., 1976) but there is no evidence suggesting that these glands have any physiological significance in mediating the effects of light on reproduction.

Photic impulses from the retinae can travel to the suprachiasmatic nucleus of the hypothalamus by way of

direct retino-hypothalamic tracts (Wurtman, 1967; Moore, 1973).

The visual pathways from the retinae to the pineal are thought to include the inferior accessory optic tract, medial forebrain bundle, medial terminal nucleus of accessory optic system, preganglionic sympathetic tracts in the spinal cord, superior cervical ganglia and post-ganglionic sympathetic fibres (Moore et al., 1968; Axelrod, 1974; Moore and Klein, 1974). The role of the pineal in regulating reproduction will be discussed in a later section (page 40).

B. REPRODUCTIVE DEVELOPMENT

1. Foetal

The sex of an individual is determined at conception and at an early age, genetic factors control differentiation of the gonadal primordia into either testes or ovaries (Jost, 1976).

Histological evidence has shown that differentiation of testes in sheep foetuses takes place 35 days after conception, while the scrotum can be distinguished at 45 days (Sapsford, 1962; Attal et al., 1972). Androgens have been detected in foetal gonads as early as 30 days after mating (Attal, 1969) and this early production probably is responsible for morphological sexual differentiation. Testicular androgenic activity declines by mid-gestation, while androgen production by the foetal adrenal probably increases as gestation proceeds (Pomerantz and Nalbandov, 1975). Experiments involving administration of testosterone implants to pregnant ewes have shown that differentiation

of Wolffian ducts and masculine development of the foetus depends on the presence of testosterone early in foetal life, while regression of the Mullerian ducts and feminine development is not influenced by testosterone (Short, 1974; Alifakiotis et al., 1976; Wilson and Tarttelin, 1978). Jost et al. (1973) suggested that male development may depend on a "Mullerian inhibitor" as well as the masculinizing influence of androgen. During the first half of gestation in sheep, foetal androgens also establish in the hypothalamus the non-cyclical male type of gonadotrophin secretion and the basis of male sexual behaviour (Short, 1974).

Studies by Foster and colleagues (Foster, 1974) have shown that the foetal pituitary can synthesize LH from day 60 of gestation with circulating levels being highest at mid-term and lowest at birth. The pituitary could respond to exogenous GnRH before birth but little GnRH activity could be detected in the hypothalamus. Castration at birth showed that gonadal steroids already exerted negative feedback control on the pituitary in the ram (Foster, 1974), whereas establishment of an inhibin-FSH feedback mechanism probably does not occur until 5-8 weeks after birth (Blanc and Terqui, 1976).

2. Post-natal

(i) Bodyweight, Gonads and Reproductive Tract

Reproductive development in New Zealand Romney rams has been studied by Steffert (1971) and Wilson and Lapwood (1978a,b,c). Bodyweight is a more important determinant of reproductive development in rams than is age (Dýrmundsson, 1973). Bodyweight increases rapidly from birth then begins

to level off from about 120 days of age. Testicular weights increase gradually from birth, then more sharply from about 70 days of age, corresponding to the time of first appearance of primary spermatocytes. With the onset of rapid testicular growth between 2 and 6 months, supporting cells proliferate and develop into Sertoli cells while the gonocytes differentiate into type A spermatogonia, and eventually spermatozoa are produced (Sapsford, 1962). The progressive development of spermatogonia, spermatocytes, spermatids and spermatozoa is correlated with age and testis weight (Courot, 1962; Ortavant et al., 1969)

Spermatogenesis is established by 22 weeks after birth, but testicular weights, steroidogenesis and both the quality and quantity of sperm production continue to increase until full adulthood has been reached (Steffert, 1971; Wilson and Lapwood, 1978c). Similar patterns of development in Suffolk rams have been recorded by Skinner et al. (1968), although there are breed differences in the age of first appearance of spermatozoa (Dýrmundsson, 1973). Associated with the increase in testis weights is an increase in testicular steroidogenesis, accessory sex gland activity, general reproductive tract growth, sperm motility and a decrease in the percentage of abnormal sperm (Skinner et al., 1968; Skinner and Rowson, 1968; Wilson and Lapwood, 1978a,c).

(ii) LH and FSH

Plasma LH levels increase from birth to about 70 days of age and remain elevated until the 15th week of life

(Courot et al., 1975; Lee et al., 1976a; Wilson and Lapwood, 1978a). Lambs born out of season also show this pattern of changing LH levels (Cotta et al., 1975; Courot et al., 1975).

Foster et al. (1978) found that pulsatile release of LH can occur as early as 1-3 weeks of age in rams. These authors also reported that LH pulses resulted in testosterone secretion from the age of onset of pulsatile LH release onwards, whereas Wilson and Lapwood (1978a) showed that LH pulses did not always result in consistent increases in testosterone secretion until after 2 months of age. With advancing age, lower LH levels are required to maintain increasing testosterone secretion (Foster, 1974; Wilson and Lapwood, 1978a).

Plasma FSH levels also increase to a peak at 5 weeks of age after which they show a small decline and then plateau (Lee et al., 1976a). The decline after 5 weeks may be due to the establishment of an inhibin-FSH feedback mechanism (Blanc and Terqui, 1976). Both the plasma LH and FSH level increases during the early postnatal period are paralleled by similar increases in pituitary gonadotrophin content (Skinner et al., 1968; Courot et al., 1975).

(iii) Prolactin

Spring born lambs show a rapid increase in plasma prolactin levels from birth and then secretion follows the photoperiod-induced seasonal patterns seen in adults (Wilson and Lapwood, 1978a). Lambs born in autumn have very low plasma prolactin concentrations, but there is a distinct peak at 12 weeks of age, coinciding with the onset

of the rapid increase in testicular growth and spermatogenic activity (Ravault and Courot, 1975; Ravault, 1976). Photoperiod-induced high plasma prolactin levels in spring born animals probably mask changes which otherwise may be associated with the stage of reproductive development.

(iv) Testicular Steroids

In rams, testosterone is the dominant androgen from birth (Skinner et al., 1968; Attal et al., 1972). Testicular androgen content has been shown to decrease from birth until 20 days of age, then subsequently increase with age from the 25th day, with highest levels being recorded in 3 year old animals (Attal et al., 1972). Similarly spermatic and jugular vein plasma testosterone levels increase gradually from birth and then more rapidly from about 150 days of age with high levels being attained in the adult (Crim and Geschwind, 1972a, Lee et al., 1976a; Wilson and Lapwood, 1978a). 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. Oestrone and oestradiol have been detected in the testes of newborn lambs, but it was considered that they were of maternal origin, rather than being a product of testicular oestrogen synthesis, since oestrogens were measured in the testes only from 20 days of age onwards (Attal et al., 1972).

3. Onset of Puberty

"Puberty is a phase in development in lambs and spermatozoa appear towards the end of this phase. It is that

time when the secretion of androgen, in response to pituitary gonadotrophins, accelerates the development of their target organs and the secondary sexual characteristics develop" (Skinner et al., 1968). That the initiation of puberty was induced by the brain rather than the pitutiary was first shown by Harris and Jacobsohn (1952), who transferred pituitaries from immature rats to below the median eminence of adult animals and found that they maintained adult function.

A popular view of the role of the brain in pubertal initiation is the gonadostat theory proposed by Ramirez and McCann (1963). According to this theory puberty occurs when the hypothalamic gonadostat, very sensitive to steroid negative feedback, gradually becomes less sensitive, thus allowing gonadotrophic secretion to increase and that in turn stimulates testicular steroidogenesis and the onset of spermatogenesis. However, it is becoming apparent that sexual maturation involves more than a mere decrease in the sensitivity of the hypothalamus to gonadal steroid feedback.

Male rats, which reach puberty at about 50 days of age, have been utilized as models of reproductive maturation in two recent reviews (McCann, 1976; Odell and Swerdloff, 1976). It is postulated that the gonads of neonatal rats are unresponsive or poorly responsive to LH and LH is ineffective in causing androgen secretion. However, the gonads are responsive to FSH from birth and a rise in plasma FSH levels some 10 days before the onset of puberty (McCann, 1976), possibly in synergism with prolactin and growth hormone, induces increased

testicular responsiveness to LH by increasing the number of testicular receptors. FSH secretion is gradually restrained as gonadal steroid (and possibly inhibin) secretion increases. The pattern of pituitary responses to GnRH also changes as the relative FSH to LH responses are greater in immature animals and less in adults. Hypothalamic pituitary sensitivity to steroid feedback suppression of LH may decrease with maturation but its importance in the control of pubertal onset has been questioned (Donovan, 1974; McCann, 1976; Odell and Swerdloff, 1976; Levasseur, 1977); this has occurred because a decrease in pituitary gonadotrophin secretion and an increase in testicular steroid secretion do not occur at the time when puberty is initiated. However, the absolute amounts of steroids "presented" to the brain may be regulated by the concentration of steroid binding proteins which increase prior to the onset of puberty (Plapinger and McEwen, 1973; Kato, 1976; Nunez et al., 1976). Pubertal development also is associated with an increase in responsiveness of accessory sex organs to androgens (Ojeda and Ramirez, 1973/74).

Foetal lambs experience a period of increased pituitary and testicular activity (Attal, 1969; Foster 1974) which decreases soon after birth (Attal et al., 1972, Foster, 1974). Further development takes place during the second month after birth as seen by: an increase in pituitary and peripheral plasma gonadotrophin levels (Skinner et al., 1968; Courot et al., 1975; Lee et al., 1976a; Wilson and Lapwood 1978a); a higher frequency and

greater amplitude of pulsatile LH releases (Foster et al., 1978); the development of a greater quantitative relationship between LH and testosterone releases (Foster 1974; Wilson and Lapwood, 1978a); and an increase in the sensitivity of the pituitary to GnRH (Galloway and Pelletier, 1974; Lee et al., 1976b; Wilson and Lapwood, 1978b). Levasseur (1977) suggested that this increase in pituitary function was related to a gradual lessening of central nervous system inhibition of the hypothalamic-pituitary unit which occurred after birth. Hyyppä (1974) suggested that the neuroendocrine control of puberty may involve hypothalamic maturation since hypothalamic neuroamines increase to adult levels some days before puberty in rats. The onset of puberty can be influenced by extrahypothalamic areas such as the limbic system, midbrain and pineal gland, as well as by the environment. It may also depend on the attainment of a critical body weight and a certain level of basal metabolism (Donovan and van der Werff ten Bosch, 1965; Critchlow and Bar-Sela, 1967; Frisch, 1974; Levasseur, 1977).

4. Influences of the Genotype and Environment

Dýrmundsson (1973) summarized data from various sources which showed that there were wide differences in the ages and bodyweights at which ram lambs of various breeds exhibited puberty. Subsequently Land (1978) showed that while Finnish Landrace rams and ewes will breed in their first season as lambs, very few Merinos will breed until the next breeding season. Dýrmundsson and Lees (1972a,b,c) suggested that photoperiod influenced the onset

of breeding activity following the observation that ram and ewe lambs born during the earlier part of the lambing season attained maturity at greater ages and heavier bodyweights than those born during the latter part of the lambing season. Land (1978) also found a seasonal effect on the age of puberty, with breed differences in the response to photoperiodic influences on sexual development.

Autumn born lambs show precocious testicular development following a larger gonadotrophin stimulus attributable to the more favourable photoperiod, compared with that to which spring born animals were exposed (Courot et al., 1975). Similarly, spring born lambs subjected to a reversed (decreasing) lighting cycle showed greater sperm production than lambs maintained on a normal (increasing) photoperiod (Alberio and Colas, 1976). However, little is known of the physiological effects of season on the onset of puberty in rams.

C. THE PINEAL GLAND

The epiphysis cerebri or pineal gland, so named because of its resemblance to a pineapple or a pine cone, has been the subject of several comprehensive reviews (Wurtman et al., 1968; Wolstenholme and Knight, 1971; Relkin, 1976; Reiter, 1977).

1. Anatomy and Innervation

The anatomy of ovine and bovine pineals has been extensively described by Anderson (1965). Embryologically, the pineal arises as a median evagination of the diencephalic roof of the third ventricle, between the

anlagen of the habenular commissure rostrally and the posterior commissure caudally. Although it develops from the brain, it is an end organ of the peripheral autonomic system with no nervous connections with the brain proper (Kappers, 1971).

The pineal lies in close proximity to the cerebrospinal fluid as the pineal recess of the third ventricle lies in the lower extremity of the gland and reaches into the lower most part of the pineal parenchyma. On the dorsal side, extensions of the third ventricle form the suprapineal recess. The pineal gland is well vascularised and roughly divided into indistinct lobules by trabeculae from the capsule. Two main cell types constitute the pineal parenchyma, secretory pinealocytes and supporting glial cells.

The pineal is mainly innervated by sympathetic post-ganglionic neurons from the cranial (rostral or superior) cervical ganglia which enter the pineal parenchyma as the nerve conarii, or in company with blood vessels (Kappers, 1971; Reiter, 1977). Preganglionic neurons which may enter the pineal synapse on post-ganglionic fibres within the gland.

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 suprachiasmatic nucleus neurons 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 (Kappers, 1965, 1971; Moore et al., 1968; Moore and Klein, 1974; Reiter, 1977).

In addition to this sympathetic innervation, a parasympathetic component arising from the superior salivary nuclei, probably exists in some species, entering the pineal along with blood vessels or the conary nerves (Kappers, 1971; Reiter, 1977). Their functional significance is unknown although Wartman et al. (1969) suggested that they mediated pineal hydroxyindole-O-methyltransferase (HIOMT) responses to light.

2. Biochemistry

Biochemically the pineal gland is very active and probably the most well known of its actions is the conversion of the amino acid tryptophan to the indoleamines serotonin and melatonin. Pineal biochemistry has been reviewed by Quay (1974).

(i) Indoleamines

Melatonin (reviewed by Cardinali, 1974) was first isolated from bovine pineals by Lerner et al. in 1958 and has since been isolated and identified in sheep pineals (van de Veerdonk, 1965; Ebels and Horwitz-Bresser, 1976) as well as those from other species. The pathway by which the pineal synthesizes melatonin from tryptophan, has been established as:

tryptophan
↓ tryptophan hydroxylase
5-hydroxytryptophan
↓ 5-hydroxytryptophan decarboxylase
serotonin (5-hydroxytryptamine)
↓ serotonin-N-acetyl transferase (NAT)
N-acetylserotonin
↓ HIOMT
melatonin (N-acetyl-5-methoxytryptamine)

Circadian rhythms have been demonstrated in the activity of pineal serotonin (high during the day and low at night), NAT, N-acetylserotonin and melatonin (all low during the day and high at night) (Axelrod, 1975) and in pineal blood flow, which also is greater at night (Rollag et al., 1978b). Similarly, a circadian rhythm in noradrenalin content has been reported, higher levels being recorded during the night than the day; this rhythm can be abolished by constant lighting or by removal of the cranial cervical ganglia (see references in Axelrod, 1975; Relkin, 1976). The mechanisms by which circadian variations in lighting, mediated by sympathetic nerves, are thought to influence pineal indoleamine synthesis have been summarized as follows by Axelrod (1975): "More norepinephrine is released at night, presumably via circadian activation of the suprachiasmatic nucleus. The released neurotransmitter stimulates the β-adrenergic receptor in the pineal cell, which turns on the synthesis of new N-acetyltransferase molecules by a still unknown mechanism involving cAMP. The increase in N-acetyltransferase results in a fall in its substrate

serotonin and an elevation of its product N-acetylserotonin. The latter compound is then O-methylated to melatonin by the pineal specific enzyme hydroxyindole-O-methyltransferase. The reverse process occurs during the daytime." Some serotonin is either further metabolized to hydroxyindole acetic acid or taken up by the sympathetic nerves within the pineal. Little melatonin is retained or metabolized by the pineal and there is some controversy over whether it is secreted into the cerebrospinal fluid and/or the blood (Cardinali, 1974). Melatonin has been identified in the cerebrospinal fluid of sheep (Rollag et al., 1978a), calves (Hedlund et al., 1977), and humans (Smith et al., 1976) with a distinct increase in night-time levels reported in calves. Rollag et al. (1978c) concluded that in ewes melatonin was secreted predominantly into the blood, although the small quantity secreted into the third ventricle could result in significant concentrations in the cerebrospinal fluid.

A circadian rhythm of melatonin concentrations has been detected in the peripheral blood of several species including sheep (Rollag and Niswender, 1976; Kennaway et al., 1977, 1978; Rollag et al., 1978a,c), with higher levels being associated with periods of darkness. However, melatonin persists in the plasma and urine of rats and ewes after pinealectomy, although at much lower levels in rats and without significant circadian variations (Ozaki and Lynch, 1976; Kennaway et al., 1977). The retinae, Harderian glands and intestinal mucosa may be supplemental sources of melatonin (Raikhlin, et al., 1975; Pang et al.,

1976; Bubenik et al., 1976) and the melatonin-forming enzyme HIOMT has been identified in the retinae and Harderian glands of rats, although Harderian gland HIOMT has a different reaction rate constant (Km) than pineal and retinal HIOMT (Cardinali and Wurtman, 1972).

Melatonin is taken up and concentrated by the hypothalamus and mid-brain and rapidly metabolized there (Cardinali, 1974). In plasma, 60-80% of melatonin is bound to serum albumin although it retains it's biological activity and has a half-life of 3-10 minutes in sheep (Rollag et al., 1978c). Melatonin is inactivated by the liver and excreted in the urine (Cardinali, 1974).

Many investigators have demonstrated antigonadotrophic effects of melatonin which may occur at the hypothalamic (Kamberi et al., 1970; Sorrentino, 1975) or pituitary levels (Martin and Klein, 1976; Ellis 1976). In addition, a direct effect on the testes has been suggested (Ellis, 1976). Antigonadotrophic effects of melatonin in sheep have also been described. Roche et al. (1970a) found that intravenous melatonin infusion did not affect ovulation in ewes but did prevent the post-castration increase in LH levels 15-30 days after castration. Also, Riggs and Malven (1974b) found a depression in spontaneous LH release in wethers following intraventricular infusion of serotonin, while the oestrous cycle was prolonged and ovulation delayed in ewes similarly infused with serotonin or melatonin (Domański et al., 1975).

However, several studies have reported little or no

antigonadotrophic effect of melatonin treatment, while in some circumstances progonadotrophic effects have resulted (Reiter, 1977). Interpretation of results is made complex by the inability of most techniques (injection, infusion or implants of melatonin) to reproduce the endogenous circadian patterns. If melatonin is not the primary pineal antigonadotrophin then it almost certainly plays an important role in the synthesis, secretion and/or actions of the definitive hormone (Reiter, 1977).

(ii) Polypeptides

Pineal peptides which may have a physiological role include "a neurohypophysial hormone, vasotocin, certain hypothalamic releasing factors and a host of unidentified, presumptive polypeptides" (Benson, 1977). A number of non-melatonin pineal fractions have been reported as having antigonadotrophic properties (Reiter, 1977). To date only arginine vasotocin, a potent antigonadotrophin (Vaughan et al., 1976) has been structurally identified and its presence confirmed in the pineal gland (Bowie and Herbert, 1976). Other structurally unidentified presumptive polypeptides and proteins have been isolated and reported to inhibit some reproductive parameters (Reiter and Vaughan, 1977). Pavel (1973) suggested that melatonin may function by stimulating the release of arginine vasotocin which in turn was the definitive pineal antigonadotrophic principle.

TRH, GnRH and principles with prolactin inhibiting and releasing activities have been isolated and identified from the pineals of several species (White et al., 1974;

Blask et al., 1976). However, other researchers could not find any evidence for the presence of GnRH (Gradwell et al., 1976; Carson et al., 1977).

Although various polypeptides with gonadotrophin-influencing activity have been identified in the pineal, and administration of these exogenous polypeptides can have profound effects on various reproductive parameters, there is as yet little evidence suggesting that these substances actually are secretory products of the pineal, and as such, regulate reproductive function (Reiter, 1977).

(iii) Influence of Pituitary and Testicular Hormones on the Pineal

Many experiments have shown that seasonal and sexual cycles, as well as modification of gonadal steroid levels, can influence morphological and biochemical parameters in the pineal, suggesting a gonadal-pineal feedback relationship (Relkin, 1976, Reiter, 1977). Cardinali and colleagues (see review by Cardinali and Vacas, 1978) have shown that the rat pineal has the properties of a steroid target organ which is able to take up and bind testosterone on specific cytoplasmic receptors, then convert it to androgen metabolites and oestrogens, which induce changes in protein synthesis (Cardinali et al., 1974a,b, 1976a; Nagle et al., 1975). Also it has been reported that the pinealocyte sex-steroid receptors may be regulated by catecholamines via a β-adrenergic receptor (Cardinali et al., 1975a,b). This group also has reported that FSH, LH and prolactin treatment of castrated rats increased pineal melatonin synthesis in sham-operated, but not in superior

cervical ganglionectomized animals (Cardinali et al., 1976b). However, the physiological role of pituitary and testicular hormones in regulating pineal function is unknown.

3. Pineal, Photoperiod and Seasonality

Length of the daily photoperiod is the single most important factor regulating the antigonadotrophic function of the pineal gland (Reiter, 1977). Well defined seasonal changes in the morphology of the pineal have been described in the hare (Lincoln, 1976c) while rams on long daily photoperiods had smaller pineals than those on shorter daily lighting (Forbes, 1975).

Reiter (1975) claimed that restricting light to several animal species "induces sexual collapse and reproductive incompetence." For example, in hamsters, which have been especially well studied, light restriction results in a decrease in testicular weights, aspermatogenesis, involution of the secondary sex organs and a lowering of circulating testosterone and gonadotrophin levels.

The mechanism by which changing photoperiods are entrained to regulate reproductive activity have only recently been postulated. Both Stetson and Watson-Whitmyre (1976) and Rusak and Morin (1976) working with hamsters, suggested that the suprachiasmatic nucleus is involved in the perception of photoperiods of different length. They found that destruction of the suprachiasmatic nucleus resulted in failure of the animals to entrain circadian cycles with the photoperiod and they suggested that this

was associated with suppression of the pineal; full gonadal activity in animals treated this way was independent of the length of the daily photoperiod. This resulted in the hypothesis that light perceived during a photosensitive phase of the 24 hour period is interpreted as a "long-day" and gonadal function is maintained. The absence of light during this photosensitive phase is interpreted as a "short day" and results in activation of the pineal by the suprachiasmatic nucleus and thus promotion of gonadal involution. As sheep are autumn breeders reproductive activity is stimulated by decreasing daily photoperiods. Based on the peripheral melatonin levels, Rollag et al. (1978a) put forward the hypothesis that seasonality in the ewe is dependent on circadian and circannual rhythms in the sensitivity of the hypothalamo-hypophyseal axis to melatonin stimulation. They suggested that the long nights of winter months resulted in high melatonin levels during a melatonin-sensitive phase of the circadian cycle, whereas in summer the presence of light resulted in low melatonin levels during this phase. It was proposed that the ensuing response of the reproductive system depended on the stage of the seasonal cycle.

Studies by Barrell and Lapwood (1978b,c,d) provide strong evidence in favour of a role of the pineal in mediating the effects of the annual photoperiodic cycle on the seasonality of reproduction in rams. They showed that seasonal effects of lighting on plasma LH, testosterone and prolactin levels, and on semen production, were disrupted by pineal denervation and pinealectomy. The

only other report of the role of the pineal in seasonality in sheep, is that of Roche et al. (1970c) who found no effect of pinealectomy on seasonal breeding in ewes. However, Herbert (1972) suggested that this may have been due to failure of those authors to continue the experimental observations for a sufficient period after pinealectomy; he reached that conclusion following the observation that pinealectomy did not disrupt seasonality of breeding in ferrets until the second breeding season after treatment.

4. Pineal and Puberty

In view of the inhibitory effects of the pineal on the hypothalamo-hypophyseal axis, an influence on the timing of the onset of puberty might be anticipated. Many clinical cases have been reported which have related early or delayed puberty to pineal malfunction in humans (Kitay, 1954; Wurtman, 1968). Parenchymatous pinealomas or tumors tend to be associated with delayed gonadal development, whereas nonparenchymatous lesions, such as gliomas or teratomas which destroy the pineal, tend to advance puberty (Relkin, 1976). Thus pineal hyperfunction often results in delayed puberty and hypofunction in precocious puberty.

Pinealectomy of post-natal male and female rats consistently advanced puberty by up to ten days (Kincl and Benagiano, 1967; Relkin 1970a,b, 1971) while blinding and the administration of pineal extracts delays sexual development (Reiter, 1968, 1974a).

Numerous reports provide indirect evidence that photoperiod may influence the timing of the onset of

puberty in lambs (see page 39) but it is not known if the pineal is involved. On the other hand, there have been no previous reports of the influence of the pineal on the pubertal process in sheep.

D. PURPOSE OF THE PRESENT STUDY

The study described in this thesis was undertaken as an examination of possible influences of the pineal gland on reproductive development in rams.

The experiments utilized the measurement of reproductive hormone levels in the plasma of entire and castrate animals to examine the effects of pineal denervation, achieved by cranial cervical ganglionectomy, on reproductive development by investigating the longitudinal patterns of hormone secretion (Experiment 1, Chapter 3), 24 hour hormone secretion profiles (Experiment 2, Chapter 4), and responses to exogenous GnRH (Experiment 3, Chapter 5).

CHAPTER II

MATERIALS AND METHODS

1. ANIMALS

New Zealand Romney ram lambs, born in August or September 1976, were used for the experiments described in this thesis.

2. ANIMAL MANAGEMENT

(i) On Pasture

Lambs were born and maintained on pasture under typical N.Z. farming management. They were weaned at 3 months of age, shorn in December, and dipped to control ectoparasites in January. Regular drenching with the anthelmintics "Thibenzole" (Merck Sharp and Dohme, N.Z. Ltd.) and "Nilverm" (I.C.I., N.Z. Ltd.) provided good control of internal parasites.

A few animals displayed symptoms of ryegrass staggers during April, 1977. However, apart from one animal (Lamb 52), which died in May 1977, the lambs maintained good health throughout the experiment; the reason for that death was not diagnosed.

(ii) Indoors

For the 24 hour profile and GnRH response blood collections (Experiments 2 and 3), the lambs were transferred from pasture to individual metabolism crates in climate-controlled rooms 2-3 days before sample collection began. Temperature was maintained at 22^{o}C and artificial lighting corresponding to the natural daily photoperiods

at the times of the 24 hour samplings was provided. Animals were provided with freshly cut grass and water ad libitum.

3. SURGICAL METHODS

(i) Cranial Cervical Ganglionectomy

Bilateral cranial cervical ganglionectomy, following the method described by Appleton and Waites (1957), was performed under halothane general anaesthesia at 5 weeks of age.

(ii) Castration

Castration was performed by the application of rubber rings to the neck of the scrotum at 5 weeks of age.

(iii) Jugular Cannulation

2-3 days before 24 hour samplings, animals were anaesthetized with halothane then silastic tubing cannulae were inserted into both jugular veins after surgical exposure of the vessels. The distal ends of the cannulae were exteriorized at the dorsum of the neck by passing them through a subcutaneous tunnel made with a 6 mm (o.d.) trochar. Retention ligatures were placed at the points of entry of the cannulae to the subcutaneous tunnels.

Cannulae were then connected to an infusion pump, and patency was maintained before and during the sampling periods by constant infusion of heparinized saline (25 I.U./ml of sodium heparin) at a rate of 1 ml/hr.

4. BLOOD COLLECTION

(i) Longitudinal Study

For the longitudinal component of this study, 10 ml

jugular blood samples were collected into heparinized vacutainer tubes. Two samples were taken once weekly, the second being collected approximately one hour after the first. Blood samples were centrifuged immediately then the plasma was separated and stored at -20°C until required for hormone assays.

(ii) 24 Hour Profiles and GnRH Responses

For the 24 hour profile and GnRH response studies, blood was collected via the indwelling jugular catheters. An hourly sampling regime was utilized in the 24 hour study, while in the GnRH experiment blood was collected immediately prior to, and 30, 60, 90, 120, 180, 240, 360, and 480 minutes after, GnRH administration. Seven ml samples of blood were withdrawn into heparinized tubes, centrifuged and the plasma was immediately frozen to -20°C until required for hormone assays.

All sampling and infusion took place from a remote sampling station outside the animal room. Also the room was locked during the 24 hour sampling period, thus the lambs were not disturbed during this time.

5. BODYWEIGHT DATA

After each weekly blood sampling, the animals were weighed before being returned to pasture.

6. AUTOPSY METHODS

At the completion of the experiment, lambs were sacrificed by administration of sodium pentobarbitone. The pineal glands, gonads and epididymides were dissected

free of surrounding tissue, weighed and processed further where appropriate.

(i) Histology

5 mm thick equitorial sections of testicular tissue were fixed in Bouin's fluid for 24 hours before automatic processing and embedding in paraffin wax. Two 5 µm sections of each specimen were stained with haematoxylin and eosin. Seminiferous tubule diameters were measured under a light microscope with an eyepiece micrometer by taking the mean of 20 observations from 2 sections.

(ii) Epididymal Sperm Reserves

Total epididymal sperm reserves were estimated by a method similar to that described by Lino (1972). Epididymides were sectioned into small pieces, homogenized and diluted to 250 mls with 0.9% saline, then duplicate aliquots were counted on the red cell grid of a haemocytometer.

7. HORMONE ASSAYS

(i) LH

Plasma LH levels were measured by a double-antibody radioimmunoassay based on that described by Niswender et al. (1969) and reported by Barrell and Lapwood (1978a). This assay used the following materials: rabbit anti-ovine LH serum (pool #15, courtesy Dr. G.D. Niswender), NIH-LH-S18 as assay standards, and highly purified ovine LH (LER-137A, courtesy Dr. L.E. Reichert) for radioiodination. All standards and samples were assayed in triplicate, while hypophysectomized sheep plasma was added to the standard

curve tubes. Assay sensitivity was 0.05 - 0.18 ng/ml and the within- and between-assay coefficients of variation (C.V.) for 2 reference plasma samples are shown in Table 2.1.

(ii) Prolactin

Plasma prolactin levels were estimated by a double antibody radioimmunoassay described previously by Wilson and Lapwood (1978d). This assay utilized rabbit antiserum to ovine prolactin (courtesy Professor D.S. Flux), NIH-P-S11 as assay standards, and highly purified ovine prolactin (LER-860-2, courtesy Dr. L.E. Reichert) for radioiodination. Assay sensitivity was 5 - 7.0 ng/ml and the within- and between-assay C.V. for 3 reference plasma pools are shown in Table 2.1.

(iii) Testosterone

Estimates of plasma testosterone concentrations were made using a radioimmunoassay described by Wilson and Lapwood (1978d) utilizing testosterone antiserum (S250) provided by Dr. G.D. Niswender. The limit of sensitivity was 0.06 ng/ml while within- and between-assay C.V. data are shown in Table 2.1.

8. EXPERIMENTAL DESIGN AND ANALYSIS

The animals utilized in these experiments were incorporated into an overall 2 x 2 factorial design in which the treatment factors were: (a) cranial cervical ganglionectomized (gangX) or non-ganglionectomized (non-gangX) and (b) castrated or entire. Within that design experiments consisted of: a longitudinal study of reproductive hormone secretion in these animals, between 7 and 37 weeks of age which was supplemented by autopsy data

TABLE 2.1

Between- and within-assay coefficients of variation for hormone radioimmunoassays based on repeated estimates of reference plasma samples.

Replication Factors	Reference Plasma	Mean Hormone Concentration (ng/ml plasma)	Within-Assay C.V. (%)	Between-Assay C.V. (%)
		Luteinizing Hormone		
16 assays, 4 replicates per assay	LH 1	0.47	17.22	24.26
	PRL 3	3.17	10.15	26.31
		Prolactin		
16 assays, 4 replicates per assay	PRL 1	13.50	12.21	29.79
	PRL 2	28.99	8.32	20.15
	PRL 3	100.39	3.37	12.38
		Testosterone		
27 assays, 3 replicates per assay	PRL 3	0.49	10.84	30.85
	Indole	2.53	8.18	21.03
	LH 3	7.78	8.47	19.84
	18	11.55	8.84	21.25

(Experiment 1, Chapter 3); a 24 hour hormone profile study when the animals were 100 days of age (pubertal) and again at 300 days of age (post-pubertal) (Experiment 2, Chapter 4); and a study of responses to exogenous GnRH, also at both 100 and 300 days of age (Experiment 3, Chapter 5).

For statistical analyses, estimates of hormone concentrations were transformed into logarithms using the formula: log hormone concentration = 10 $\log_{10}(x + 1.1)$ where x is the hormone concentration in ng/ml plasma. Missing data were calculated as the mean of preceding and succeeding measurements.

Details of the statistical analyses of each experiment are described in the appropriate chapters. Results have been expressed as the mean $\pm$ standard error of the mean (S.E.M.).

CHAPTER III

A LONGITUDINAL STUDY OF THE EFFECTS OF CRANIAL CERVICAL GANGLIONECTOMY AND CASTRATION ON ENDOCRINE AND MORPHOLOGICAL MATURATION OF MALE LAMBS

A. INTRODUCTION

Attainment of reproductive function in rams is associated with an increase in endocrine activity and the onset of spermatogenesis. This phase in development is known as puberty, while at a later age sexual maturity (acquisition of full reproductive capacity) is reached (Dýrmundsson, 1973). These developmental processes are regulated by the secretion of gonadotrophic and gonadal hormones; longitudinal patterns of some reproductive endocrine parameters during postnatal development of the ram have been reported recently (Skinner et al., 1968; Skinner and Rowson, 1968; Crim and Geschwind, 1972a,b; Courot, 1974; Courot et al., 1975; Ravault and Courot, 1975; Lee et al., 1976a; Wilson and Lapwood, 1978a,c).

A number of genetic and environmental factors can influence the attainment of puberty as well as sexual function in sheep; the most important of these appear to be the level of nutrition (Dýrmundsson, 1973) and the length of the daily photoperiod (Yeates, 1949; Ortavant et al., 1964). Although there is no direct evidence indicating a relationship between seasonality/photoperiodism and pubertal development in sheep, the studies of Ortavant et al. (1964), Skinner and Rowson (1968) and Dýrmundsson

and Lees (1972a,b,c) suggest that such a relationship does exist.

Modification of the hypothalamo-hypophyseal-gonadal axis by photoperiodic stimuli is thought to be mediated by the pineal gland (Reiter, 1973, 1974b, 1977). Although Roche et al. (1970c) reported that pinealectomy had no influence on some characteristics of the oestrous cycle and seasonal breeding patterns in ewes, Cardinali et al. (1974c) showed that variations in pineal indoleamine content and synthesis were a function of the stage of the ovarian cycle. In rams Barrell and Lapwood (1978c,d,e) have shown that pinealectomy diminished the effects which annual lighting changes had on plasma testosterone and prolactin levels. Pinealectomy also disrupted the lighting-induced seasonal rhythm in some semen parameters and abolished a circadian rhythm of prolactin secretion. Similarly, pinealectomy abolished the influence of daily photoperiod on prolactin secretion in growing male lambs (Forbes, 1975; Brown et al., 1977).

The afferent nerve supply to the pineal gland includes the cranial cervical ganglia (Moore et al., 1968; Moore and Klein, 1974; Reiter, 1977) and their removal from the adult ram produces results similar to those of pinealectomy, including disruption of the seasonal cycle of plasma LH and prolactin levels (Barrell and Lapwood, 1978b).

The experiment described in this chapter was designed as an investigation of the effects of cranial cervical ganglionectomy and castration on the longitudinal profiles of LH, prolactin and testosterone secretion in male lambs

up to approximately 9 months of age. In addition, where appropriate, the effects of these treatments on gonadal, epididymal and pineal gland parameters, were assessed.

B. MATERIALS AND METHODS

The experiment utilized 28 N.Z. Romney male lambs, prepared as described in Chapter 2; 9 animals were non-ganglionectomized entires, 7 non-ganglionectomized castrates, 6 ganglionectomized entires, and 6 ganglionectomized castrates. Between 7 and 37 weeks of age, two blood samples were collected, an hour apart between 9.00 and 11.00 h, once a week. After assay of plasma hormone levels, mean values for the two samples were taken as the weekly level. Animals were weighed weekly, and at the completion of the experiment lambs were sacrificed and autopsy results obtained as described in Chapter 2. Plasma LH and prolactin data, and body and pineal weights were analyzed by Student's *t*-test to examine the effects of castration, ganglionectomy and the interaction of castration with ganglionectomy. On the other hand plasma testosterone, and gonadal and epididymal data were examined only for the influence of ganglionectomy in entire animals; again *t*-tests were used. Hormone and bodyweight data at each age were averaged for each animal to give a fortnightly value for each parameter which was then transformed to logarithms and used in the analyses. Analyses of autopsy results were performed on the logarithm transformed data for each animal.

C. RESULTS

1. Longitudinal Hormone Secretion Profiles

(i) LH

Mean weekly LH concentrations for each treatment group of animals are shown in Figure 3.1 and Table 3.1, with results of statistical analyses summarized in Table 3.6. Mean plasma LH levels in entire lambs were very low throughout the experiment with values usually being less than 1.0 ng/ml. However, the non-ganglionectomized entire animals did display an increase in mean levels, up to about 2.70 ng/ml, between 8 and 13 weeks of age, which was not evident in the ganglionectomized entires. At all ages plasma LH levels were elevated in castrated animals ($P < 0.001$) with mean levels ranging between 5.5 and 20.0 ng/ml throughout the duration of the experiment. Although LH levels varied erratically in castrates there tended to be an overall increase in mean levels between 7 and 37 weeks of age. No overall differences were apparent between ganglionectomized and non-ganglionectomized animals, nor was there any overall interaction between the effects of castration and ganglionectomy.

(ii) Testosterone

See Figure 3.2 and Tables 3.2 and 3.6.

Mean testosterone levels in entire animals increased from levels below the limit of assay sensitivity at 7 weeks of age to about 1.0 ng/ml plasma at 26 weeks. Subsequently, plasma concentrations increased more rapidly to reach levels fluctuating from 2 to 4 ng/ml between 31 and 37 weeks of age. Ganglionectomy resulted in a general decrease

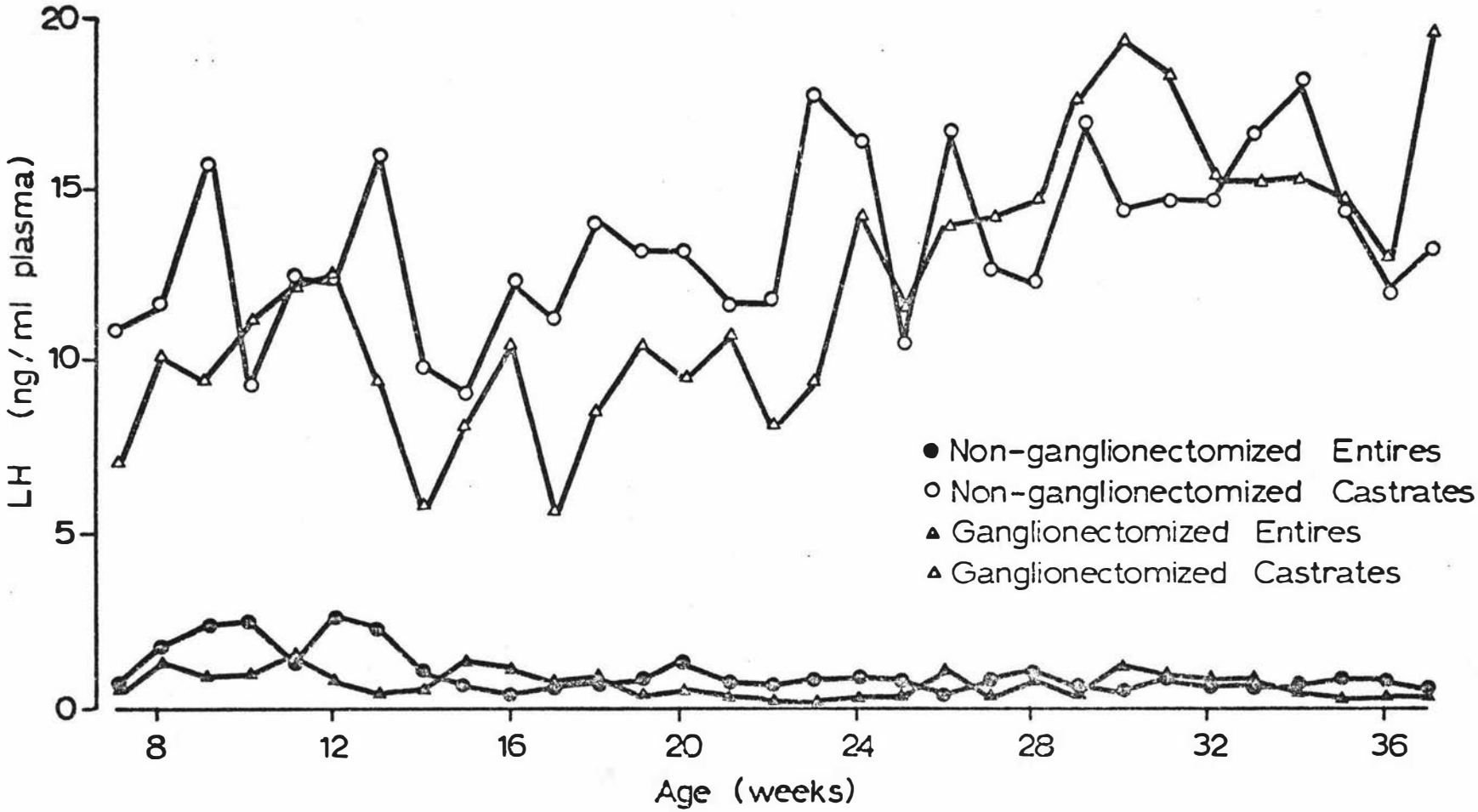

Figure 3.1 : Mean plasma LH concentrations recorded from lambs between 7 and 37 weeks of age.

Table 3.1 Mean (± S.E.M.) weekly plasma LH (ng/ml) concentrations.

Age (weeks)	Non-gangX Entires	Non-gangX Castrates	GangX Entires	GangX Castrates
7	0.53 ± 0.26	11.02 ± 3.70	0.36 ± 0.26	6.97 ± 3.08
8	1.75 ± 0.60	11.73 ± 2.83	1.26 ± 0.49	10.14 ± 2.38
9	2.25 ± 0.50	15.85 ± 3.52	0.80 ± 0.12	9.49 ± 1.47
10	2.38 ± 0.57	9.23 ± 1.21	0.93 ± 0.19	11.38 ± 2.41
11	1.12 ± 0.30	12.38 ± 1.62	1.19 ± 0.37	12.23 ± 1.87
12	2.68 ± 1.16	12.27 ± 1.80	0.77 ± 0.16	12.32 ± 1.93
13	2.13 ± 0.61	16.06 ± 3.05	0.47 ± 0.16	9.38 ± 1.81
14	0.92 ± 0.28	9.81 ± 1.95	0.62 ± 0.17	5.86 ± 2.11
15	0.67 ± 0.17	8.99 ± 2.17	1.26 ± 0.37	7.98 ± 3.12
16	0.39 ± 0.12	12.27 ± 1.60	1.07 ± 0.79	10.65 ± 1.85
17	0.53 ± 0.13	11.18 ± 1.75	0.58 ± 0.26	5.51 ± 1.54
18	0.66 ± 0.15	14.14 ± 3.06	0.76 ± 0.26	8.71 ± 1.88
19	0.76 ± 0.23	13.20 ± 1.71	0.45 ± 0.18	10.52 ± 2.48
20	1.14 ± 0.68	13.22 ± 1.53	0.53 ± 0.26	9.55 ± 1.67
21	0.68 ± 0.24	11.81 ± 1.19	0.36 ± 0.12	10.86 ± 2.50
22	0.59 ± 0.11	11.86 ± 2.22	0.21 ± 0.08	7.95 ± 1.25
23	0.75 ± 0.28	17.82 ± 2.69	0.18 ± 0.06	9.51 ± 2.37
24	0.83 ± 0.29	16.34 ± 5.11	0.35 ± 0.11	14.60 ± 2.73
25	0.77 ± 0.19	10.51 ± 1.83	0.30 ± 0.12	11.50 ± 2.33
26	0.33 ± 0.07	16.75 ± 2.40	1.09 ± 0.58	14.03 ± 2.98
27	0.83 ± 0.26	12.69 ± 1.28	0.33 ± 0.11	14.29 ± 2.32
28	1.00 ± 0.32	12.31 ± 2.77	0.98 ± 0.39	14.85 ± 2.52
29	0.57 ± 0.17	16.96 ± 3.31	0.47 ± 0.14	17.59 ± 2.25
30	0.42 ± 0.06	14.50 ± 2.11	1.27 ± 0.31	19.36 ± 1.80
31	0.70 ± 0.15	14.87 ± 1.96	0.89 ± 0.17	18.45 ± 2.18
32	0.62 ± 0.17	14.79 ± 2.27	0.67 ± 0.20	15.23 ± 1.94
33	0.61 ± 0.20	16.58 ± 3.27	0.70 ± 0.33	15.17 ± 2.23
34	0.64 ± 0.12	18.08 ± 3.69	0.55 ± 0.20	15.36 ± 2.72
35	0.78 ± 0.15	14.45 ± 2.18	0.42 ± 0.10	14.87 ± 2.26
36	0.74 ± 0.13	12.17 ± 1.47	0.43 ± 0.09	12.96 ± 1.55
37	0.45 ± 0.08	13.35 ± 2.28	0.39 ± 0.08	19.86 ± 2.03

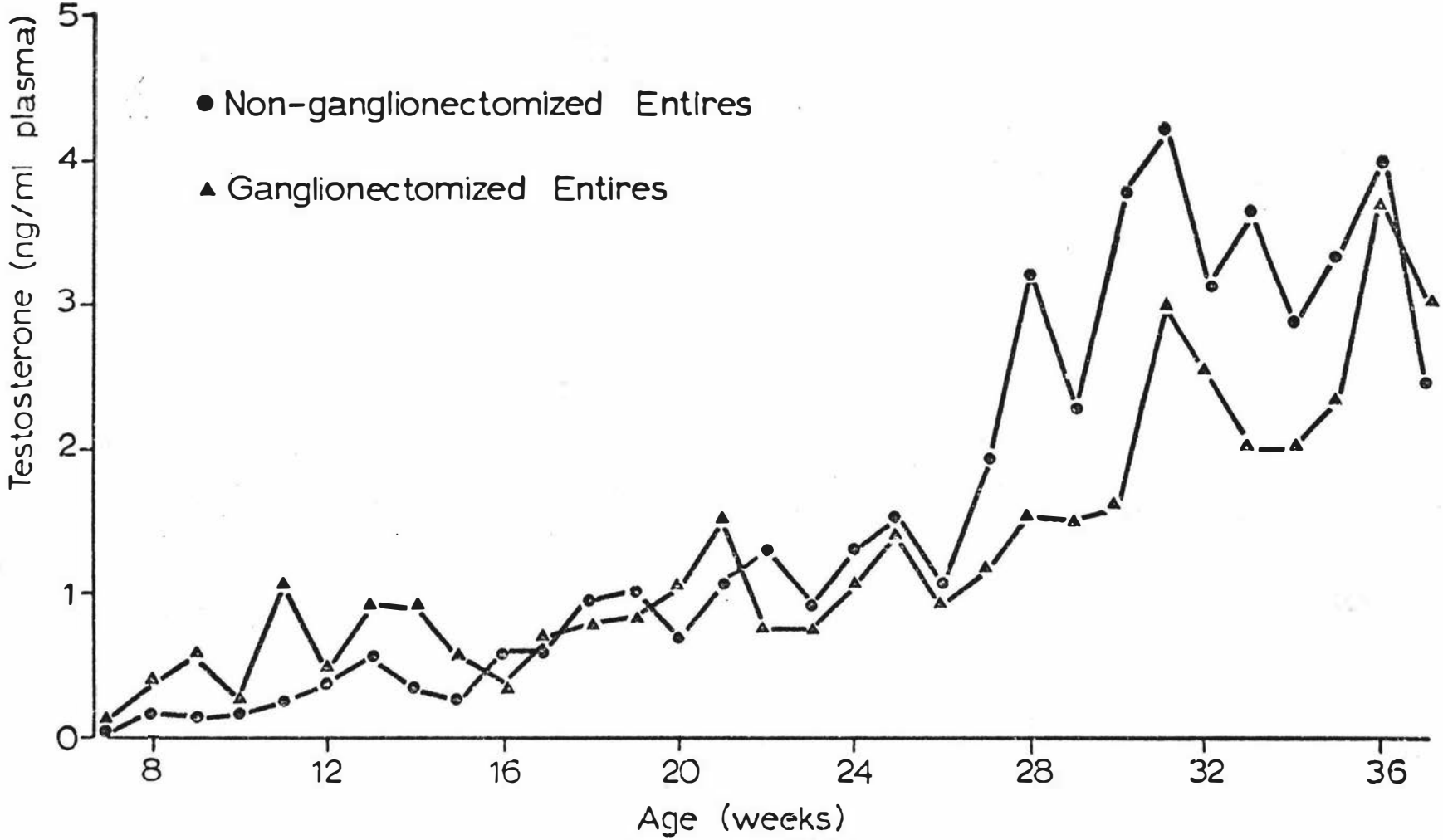

Figure 3.2 : Mean plasma testosterone concentrations recorded from lambs between 7 and 37 weeks of age.

Table 3.2 Mean (±S.E.M.) weekly plasma testosterone (ng/ml) concentrations.

Age (weeks)	Non-gangX Entires	GangX Entires
7	0.01 ± .01	0.11 ± .03
8	0.17 ± .08	0.37 ± .19
9	0.13 ± .04	0.54 ± .15
10	0.17 ± .04	0.27 ± .08
11	0.23 ± .06	1.03 ± .31
12	0.37 ± .12	0.48 ± .18
13	0.55 ± .18	0.93 ± .38
14	0.34 ± .09	0.90 ± .47
15	0.25 ± .10	0.54 ± .21
16	0.60 ± .26	0.37 ± .11
17	0.60 ± .22	0.66 ± .24
18	0.95 ± .30	0.79 ± .33
19	1.01 ± .32	0.82 ± .50
20	0.67 ± .19	1.03 ± .45
21	1.10 ± .30	1.52 ± .58
22	1.29 ± .27	0.78 ± .32
23	0.89 ± .26	0.77 ± .30
24	1.29 ± .29	1.09 ± .45
25	1.53 ± .38	1.42 ± .56
26	1.04 ± .24	0.91 ± .50
27	1.92 ± .50	1.19 ± .51
28	3.25 ± 1.01	1.57 ± .34
29	2.40 ± .46	1.53 ± .67
30	3.78 ± .86	1.61 ± .20
31	4.20 ± .60	3.02 ± .73
32	3.15 ± .64	2.56 ± .80
33	3.63 ± .71	2.05 ± .67
34	2.91 ± .46	2.04 ± .48
35	3.33 ± .45	2.31 ± .61
36	3.97 ± .67	3.71 ± 1.12
37	2.47 ± .53	3.01 ± 1.15

in mean testosterone concentrations ($P < 0.05$) which was due to that group having lower levels from about 26 weeks of age onwards. None of a representative number of plasma samples from castrate animals contained any detectable testosterone.

(iii) Prolactin

See Figures 3.3 and Tables 3.3 and 3.6.

Mean plasma prolactin levels in non-ganglionectomized animals increased from 70 - 110 ng/ml at 7 weeks of age, to reach a peak of 95 - 125 ng/ml at 9 - 12 weeks (summer), after which levels decreased then fluctuated at about 80 ng/ml until 22 weeks. Concentrations subsequently fell to reach values below 10 ng/ml between 30 and 37 weeks (winter). Ganglionectomized animals had mean prolactin levels fluctuating between 60 and 110 ng/ml plasma from 7 - 12 weeks, but they had decreased by 14 weeks and then varied between 15 and 65 ng/ml for the remainder of the experiment. Overall ganglionectomy reduced plasma prolactin concentrations significantly ($P < 0.001$) but neither the effects of castration, nor the interaction of castration with ganglionectomy, was significant.

In view of reports that the length of the daily photoperiod is the most important regulator of prolactin secretion in rams (Pelletier, 1973; Barrell and Lapwood, 1978c), prolactin data were rearranged according to the time of year (Figure 3.4) in order to examine seasonal influences. This was necessitated by a 4 week spread of the birth dates among these animals. Again the effect of summer photoperiods in elevating prolactin levels, and of

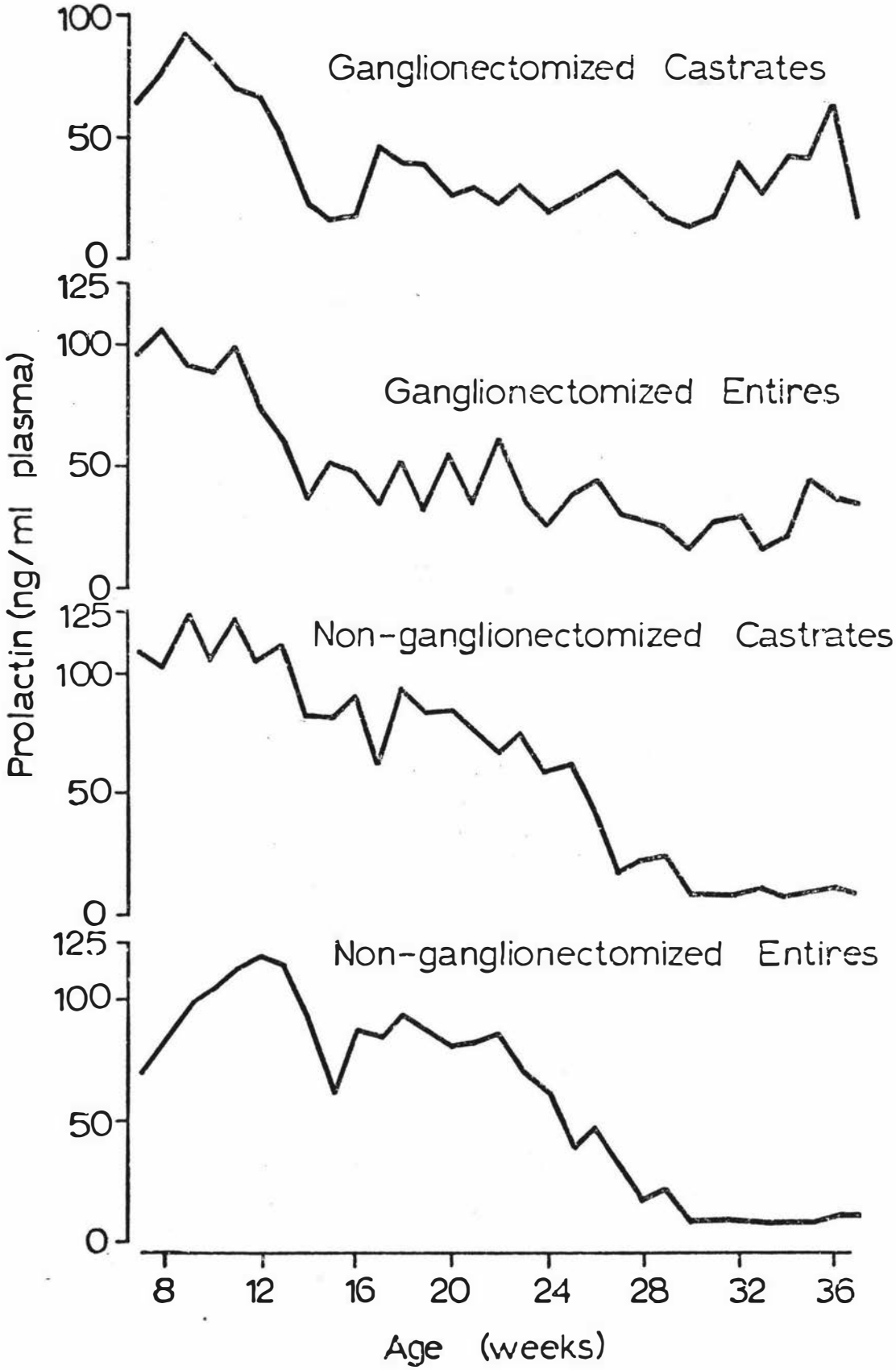

Figure 3.3 : Mean plasma prolactin concentrations recorded from lambs between 7 and 37 weeks of age.

Table 3.3 Mean (± S.E.M.) weekly plasma prolactin (ng/ml) concentrations.

Age (weeks)	Non-gangX Entires	Non-gangX Castrates	GangX Entires	GangX Castrates
7	69.2 ± 18.8	108.2 ± 9.6	96.7 ± 18.6	64.9 ± 11.8
8	83.3 ± 9.0	101.2 ± 9.1	107.3 ± 8.0	76.5 ± 14.0
9	97.7 ± 2.9	125.0 ± 4.6	92.5 ± 13.1	93.2 ± 12.4
10	102.2 ± 4.7	105.3 ± 6.0	89.1 ± 7.9	84.4 ± 11.1
11	111.2 ± 4.2	122.3 ± 4.2	99.5 ± 14.1	71.1 ± 12.4
12	116.7 ± 7.7	104.7 ± 7.2	75.7 ± 13.4	67.4 ± 12.5
13	112.1 ± 5.2	111.0 ± 4.9	60.8 ± 17.4	50.5 ± 12.6
14	92.4 ± 7.6	80.5 ± 14.7	35.5 ± 8.1	24.7 ± 10.4
15	61.0 ± 13.2	80.4 ± 15.5	52.8 ± 17.5	17.4 ± 7.9
16	87.2 ± 9.9	91.2 ± 8.7	48.8 ± 12.9	19.0 ± 3.5
17	84.3 ± 9.3	59.2 ± 13.3	33.2 ± 11.0	47.2 ± 12.5
18	94.5 ± 6.3	93.7 ± 9.6	52.8 ± 13.0	40.5 ± 13.1
19	87.6 ± 9.8	84.0 ± 10.2	32.6 ± 10.3	39.7 ± 11.2
20	80.3 ± 7.8	85.0 ± 10.1	55.6 ± 18.4	27.5 ± 9.1
21	82.6 ± 8.5	74.5 ± 8.8	36.8 ± 11.9	31.3 ± 8.9
22	86.0 ± 8.5	65.7 ± 11.8	63.5 ± 19.6	22.7 ± 9.5
23	69.9 ± 8.5	75.3 ± 11.6	37.0 ± 17.7	30.7 ± 6.7
24	62.2 ± 8.0	58.4 ± 11.6	25.8 ± 8.9	20.6 ± 5.6
25	38.1 ± 7.2	62.8 ± 13.6	39.6 ± 13.8	26.2 ± 7.9
26	47.2 ± 8.7	42.4 ± 8.5	46.8 ± 13.4	32.2 ± 8.8
27	33.8 ± 5.6	16.8 ± 3.4	30.0 ± 15.6	38.8 ± 8.8
28	18.3 ± 4.2	22.8 ± 7.8	28.8 ± 10.3	28.1 ± 7.3
29	22.0 ± 6.5	23.9 ± 8.0	25.7 ± 9.4	19.6 ± 3.3
30	8.7 ± 2.2	8.3 ± 1.7	16.9 ± 7.8	15.6 ± 3.5
31	9.2 ± 1.6	9.2 ± 1.1	27.2 ± 12.2	18.9 ± 3.6
32	8.5 ± 1.4	8.4 ± 1.5	30.3 ± 14.0	40.3 ± 8.9
33	6.9 ± 1.3	10.5 ± 1.7	15.6 ± 3.5	27.0 ± 8.3
34	8.0 ± 1.6	7.2 ± 1.4	21.7 ± 5.3	45.3 ± 12.2
35	8.5 ± 1.1	9.8 ± 1.6	46.8 ± 12.0	44.4 ± 13.1
36	11.4 ± 3.2	10.5 ± 1.5	39.2 ± 9.6	66.2 ± 14.2
37	11.4 ± 3.1	8.9 ± 1.5	36.5 ± 8.8	17.5 ± 1.7

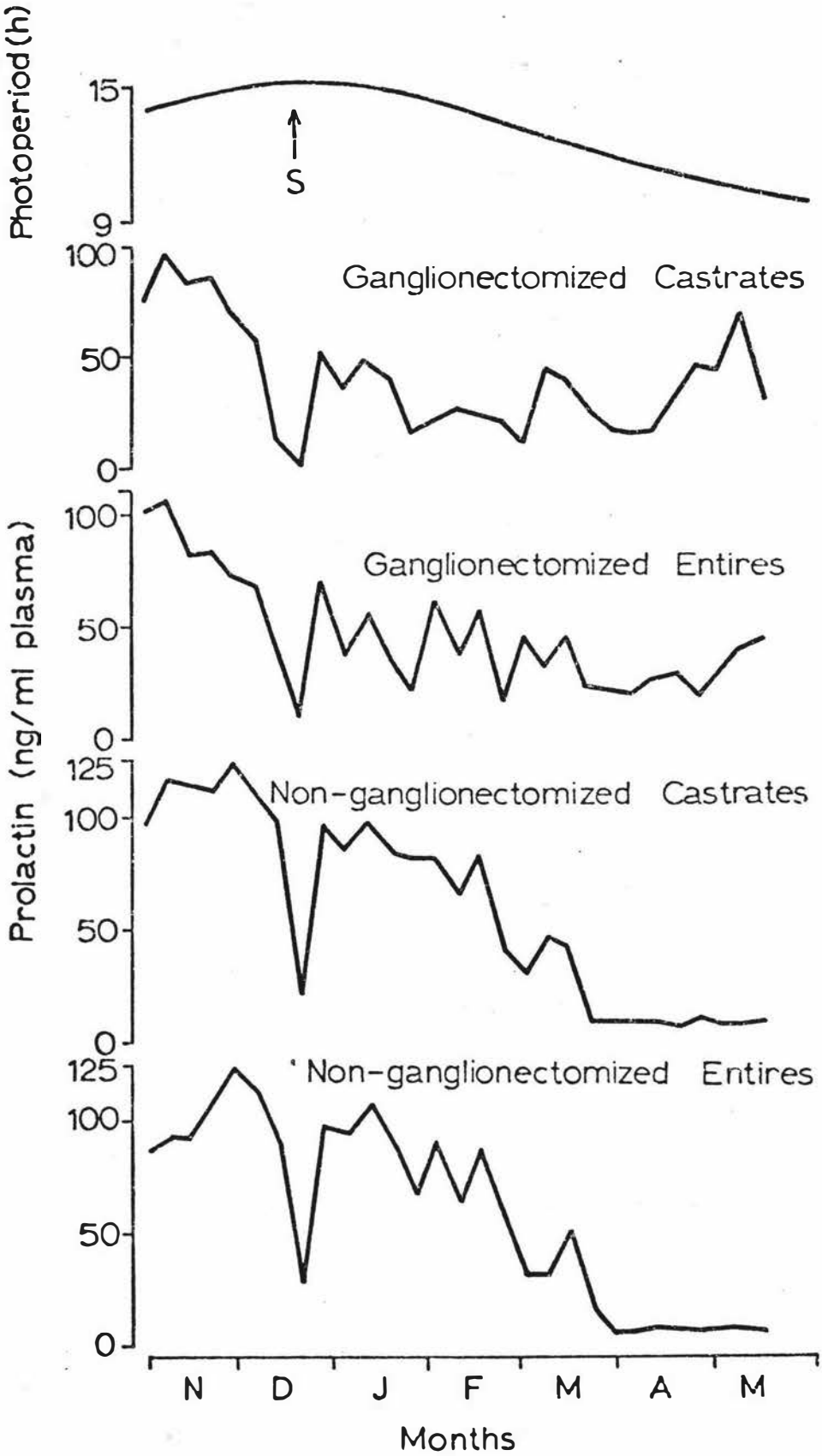

Figure 3.4 : Mean plasma prolactin concentrations recorded from lambs between November 1976 and May 1977. S = summer solstice.

short daily lighting in suppressing secretion, was evident. In addition, on the 22nd of December prolactin levels were very low in plasma collected from all except 8 animals; this date was the day of the summer solstice and four days after the animals were shorn. The eight animals which did not show decreased prolactin levels missed shearing at that time because they were being utilized for Experiments 2 and 3.

2. Bodyweight

See Figure 3.5 and Tables 3.4 and 3.6.

Mean liveweights of non-ganglionectomized lambs increased progressively from 14 - 17 kg at 8 weeks of age to 33 - 35 kg at 35 weeks. Both groups of ganglionectomized lambs showed reduced bodyweights over the course of the experiment. Ganglionectomized entires had a mean bodyweight of 21.3 kg at 8 weeks and increased to only 30.4 kg by 35 weeks of age, while ganglionectomized castrates increased from 15.4 to 26.0 kg over the same period.

Statistical analysis revealed that bodyweight was significantly reduced by ganglionectomy ($P<0.001$), while the significant interaction of ganglionectomy with castration ($P<0.001$) showed that this reduction in weights was much greater in the ganglionectomized animals which also had been castrated.

3. Autopsy Data

See Tables 3.5 and 3.6

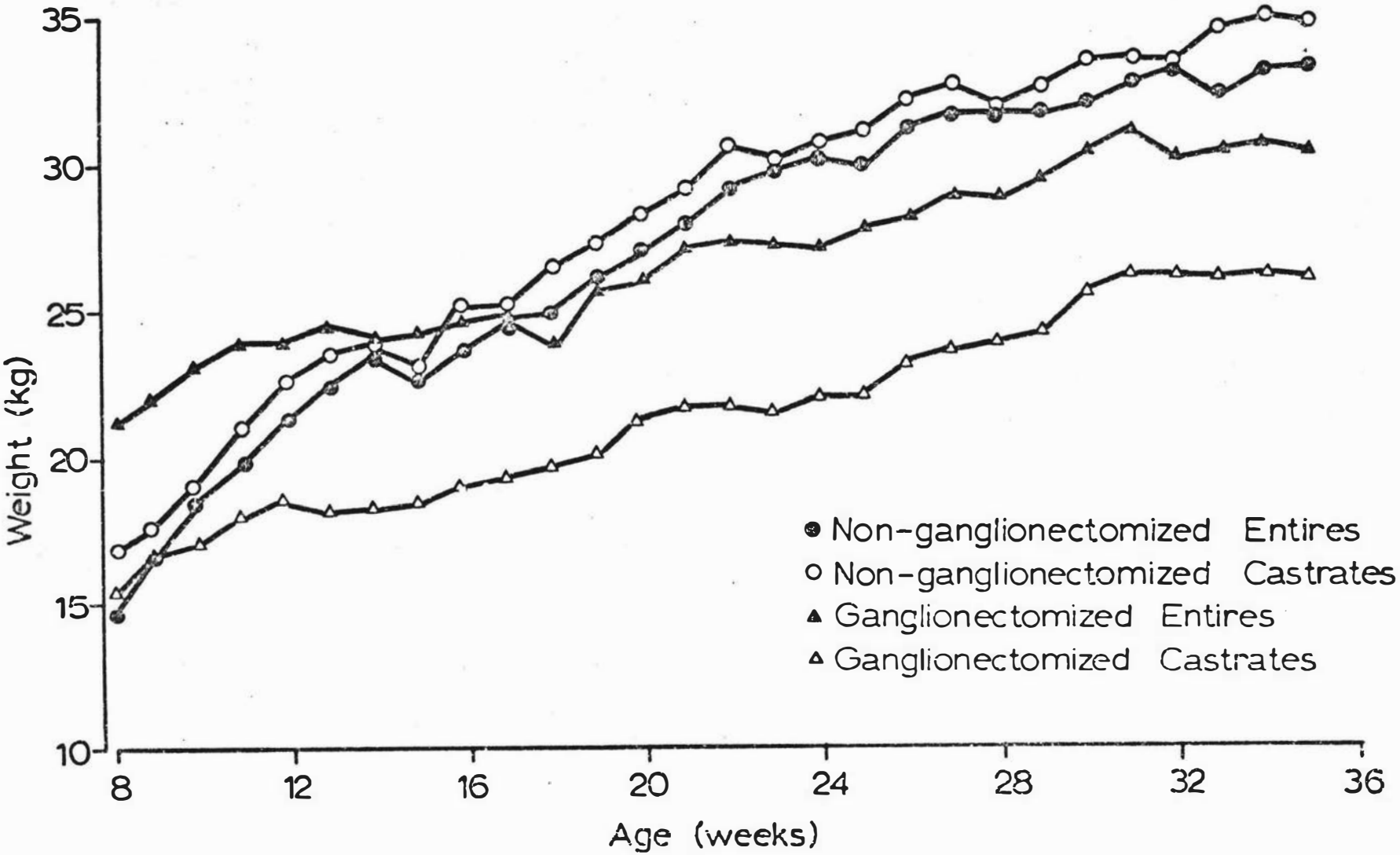

Figure 3.5 : Mean bodyweights recorded from lambs between 8 and 36 weeks of age.

Table 3.4 Mean (± S.E.M.) weekly bodyweight (kg) data.

Age (weeks)	Non-gangX Entires	Non-gangX Castrates	GangX Entires	GangX Castrates
8	14.7 ± 0.2	17.0 ± 0.2	21.3 ± 1.4	15.4 ± 1.9
9	16.6 ± 1.0	17.8 ± 0.9	22.3 ± 1.0	16.6 ± 1.1
10	17.8 ± 1.0	19.3 ± 1.1	23.4 ± 1.0	17.2 ± 0.9
11	20.0 ± 1.1	21.3 ± 0.9	24.2 ± 1.2	18.1 ± 0.9
12	21.5 ± 1.1	22.6 ± 1.2	24.2 ± 1.3	18.9 ± 1.0
13	22.6 ± 1.1	23.6 ± 1.1	24.7 ± 1.2	18.4 ± 0.9
14	23.6 ± 1.1	23.9 ± 1.4	24.0 ± 1.5	18.4 ± 1.1
15	22.6 ± 0.9	23.3 ± 0.9	24.4 ± 1.7	18.6 ± 1.1
16	23.9 ± 1.0	25.3 ± 1.2	24.9 ± 1.9	19.3 ± 1.1
17	24.9 ± 0.9	25.3 ± 0.9	25.1 ± 2.0	19.5 ± 0.8
18	25.1 ± 0.9	26.6 ± 0.9	24.9 ± 1.8	19.8 ± 0.8
19	26.3 ± 0.9	27.4 ± 0.8	25.7 ± 2.1	20.2 ± 0.8
20	27.0 ± 0.9	28.4 ± 0.9	26.1 ± 1.8	21.5 ± 0.7
21	28.0 ± 1.0	29.2 ± 0.9	27.3 ± 2.4	21.8 ± 0.8
22	29.2 ± 1.1	30.7 ± 1.0	27.5 ± 2.6	21.7 ± 1.0
23	29.5 ± 1.3	30.1 ± 1.5	27.4 ± 2.2	21.5 ± 0.7
24	30.2 ± 1.3	30.7 ± 1.4	27.2 ± 2.1	22.1 ± 0.9
25	30.0 ± 1.3	31.1 ± 1.5	27.9 ± 2.2	22.1 ± 1.2
26	31.4 ± 1.2	32.2 ± 1.3	28.2 ± 2.7	23.2 ± 1.1
27	31.7 ± 1.3	32.6 ± 1.4	29.0 ± 2.2	23.6 ± 1.0
28	31.7 ± 1.2	31.9 ± 1.5	28.8 ± 1.7	23.9 ± 0.8
29	31.6 ± 1.5	32.6 ± 1.8	29.5 ± 1.7	24.4 ± 0.7
30	32.0 ± 1.2	33.5 ± 1.5	30.5 ± 1.8	25.8 ± 0.8
31	32.9 ± 1.3	33.6 ± 1.5	31.2 ± 1.7	26.3 ± 0.6
32	33.2 ± 1.3	33.5 ± 1.6	30.2 ± 1.4	26.2 ± 0.9
33	32.3 ± 1.5	34.6 ± 1.4	30.5 ± 1.4	26.2 ± 0.7
34	33.3 ± 1.2	35.2 ± 1.7	30.7 ± 1.6	26.4 ± 0.6
35	33.2 ± 1.6	34.7 ± 2.2	30.4 ± 0.7	26.0 ± 1.0

Table 3.5 Data* (means ± S.E.M.) collected following autopsy of lambs used in Experiment 1.

	Testicular weights (g)	Seminiferous tubule diameters (μm)	Epididymal weights (g)	Epididymal Sperm Reserves ($\times 10^9$)	Pineal weights (mg)
Non-ganglionectomized Entires	215.0 ± 23.2	184 ± 5	33.9 ± 3.7	17.26 ± 4.74	37.0 ± 2.4
Non-ganglionectomized Castrates	-	-	-	-	57.8 ± 14.0
Ganglionectomized Entires	188.8 ± 22.0	186 ± 11	27.6 ± 3.1	12.93 ± 3.70	36.0 ± 3.8
Ganglionectomized Castrates	-	-	-	-	34.2 ± 9.7

* Data from paired organs have been summed.

(i) Testicular Weights

The difference in testicular weights between non-ganglionectomized (215.0 ± 23.2 g) and ganglionectomized (188.8 ± 22.0 g) animals was not statistically significant.

(ii) Epididymal Weights

Although non-ganglionectomized lambs had higher mean epididymal weights (33.96 ± 3.76 g vs 27.52 ± 3.11 g in ganglionectomized lambs) this difference was not significant.

(iii) Epididymal Sperm Reserves

Ganglionectomized animals had reduced mean epididymal sperm reserves (12.93 ± 3.70 x 10^9 vs 17.26 ± 4.74 x 10^9 in non-ganglionectomized rams) but this difference was not significant.

(iv) Seminiferous Tubule Diameters

Mean seminiferous tubule diameters were virtually the same in non-ganglionectomized (184 ± 5 μm) and ganglionectomized (186 ± 11 μm) lambs.

(v) Pineal Weights

There were no significant differences in mean pineal weights between non-ganglionectomized (47.4 ± 6.5 mg) and ganglionectomized (35.2 ± 4.6 mg) lambs, nor between entire (36.6 ± 2.0 mg) and castrate (47.1 ± 9.2 mg) animals. However, the interaction of castration with ganglionectomy was significant ($P < 0.01$) because of the greater mean pineal weight (57.8 ± 14.0 mg) recorded from the non-ganglionectomized castrates.

Table 3.6 Summary of t-test analyses of plasma LH, prolactin and testosterone data, and of bodyweight and autopsy data from Experiment 1.

Treatment	LH	Prolactin	Bodyweight	Pineal Weight
A. Entire vs Castrate	48.474*** (390)	1.096 (390)	1.669 (390)	0.744 (23)
B. Non-gangX vs GangX	0.204 (390)	5.025*** (390)	5.479*** (390)	1.688 (23)
C. Interaction A x B	0.670 (388)	0.972 (388)	5.105*** (388)	3.246** (21)

	Testosterone	Testicular Weights	Seminiferous Tubule Diameters	Epididymal Weights	Epididymal Sperm Reserves
D. Non-gangX Entires vs GangX Entires	2.430* (208)	0.796 (12)	0.136 (12)	1.186 (12)	0.866 (12)

***P<0.001, **P<0.01, *P<0.05

Note : Figures in brackets are the numbers of degrees of freedom for each contrast.

D. DISCUSSION

1. LH and Testosterone

(i) Non-ganglionectomized Entires

Elevated plasma LH levels during the first 3 - 4 months of life have been recorded in Grade Targhee (Crim and Geschwind, 1972b), Ile-de-France (Courot et al., 1975), Merino/Corriedale crossbred (Lee et al., 1976a) and Romney (Wilson and Lapwood, 1978a) ram lambs. Pituitary LH content also increases from birth to reach peak levels at about 3 months of age (Skinner et al., 1968; Courot et al., 1975).

Plasma testosterone levels also have been shown to increase gradually from birth onwards (Crim and Geschwind, 1972a; Cotta et al., 1975; Lee et al., 1976a; Wilson and Lapwood, 1978b) paralleling similar increases in testicular androgen content (Skinner et al., 1968; Attal et al., 1972).

(ii) Treated Lambs

The elevation in LH levels recorded from both non-ganglionectomized and ganglionectomized castrate lambs undoubtedly was due to the absence of the normal negative feedback regulation of LH secretion by testicular androgens.

Cranial cervical ganglionectomy had no influence on LH levels in castrate lambs but in entires it appeared to abolish the elevation in LH levels, between 8 and 13 weeks of age, seen in non-ganglionectomized entires. The possibility that elevated levels may have occurred in ganglionectomized entires before 7 weeks of age, when

sample collection commenced, cannot be overlooked.

Reduced plasma testosterone levels in ganglionectomized entires was unexpected since LH concentrations were not influenced by this treatment. Probably the lower bodyweight of the ganglionectomized entires contributed to their reduced testicular weights, and hence plasma testosterone levels, compared with values recorded from the non-ganglionectomized entires.

Plasma LH concentrations did not differ between non-ganglionectomized and ganglionectomized rams, apart from the early elevation in levels in non-ganglionectomized rams, while testosterone secretion was reduced in ganglionectomized lambs: these results contrasted with those of Barrell and Lapwood (1978b). Those workers reported that ganglionectomy of adult rams not only reduced the regular seasonal trends seen in plasma LH levels, but also elevated mean concentrations, especially in winter. However, in that study there were no significant differences in plasma testosterone levels due to ganglionectomy. Barrell and Lapwood (1978c) also reported that pinealectomy reduced the effects of photoperiod-induced seasonal testosterone secretion patterns, but an influence on LH secretion could not be assessed because concentrations were low in all groups of rams. Although Roche et al. (1970c) found that pinealectomy had no effect on some oestrous cycle parameters, nor on reproductive seasonality in ewes, Herbert (1972) has suggested that this result may have been

due to the failure to continue the study for several breeding seasons after the operation.

2. Prolactin

(i) Non-ganglionectomized Entires

The longitudinal profile of plasma prolactin levels recorded from the control lambs, showed fluctuations in levels of this hormone which were in phase with the length of the daily photoperiod and was remarkably similar to the profile recorded in previous work with Romney lambs at this laboratory (Wilson and Lapwood, 1978a).

In contrast, autumn born ram lambs showed a distinct peak in prolactin levels at about 10 - 12 weeks of age (Ravault and Courot, 1975; Ravault, 1976) which coincided with the beginning of the rapid increase in testicular weights and spermatogenic activity. The high prolactin levels seen in spring born animals, which apparently are related to the longer daily photoperiods compared to those to which autumn born lambs are exposed, probably mask any changes in prolactin secretion associated with stage of reproductive development. Photoperiodic influences on plasma prolactin concentrations were clear in this study and in those by Pelletier (1973), Forbes et al. (1975), Ravault (1976), Barrell and Lapwood (1978a,c) and Lincoln et al. (1978).

Further implications of the possible role of prolactin in reproductive maturation in rams was provided in a report by Ravault et al. (1977b). They found that administration of the prolactin secretion inhibitor 2-bromo-α-ergocryptine to both spring and autumn born lambs

resulted in an initial depression in testosterone levels and a significant reduction in seminal vesicular weights and fructose concentrations; there were no influences on LH secretion, body, testes, albuginea or epididymal weights, nor on the establishment of spermatogenesis.

(ii) Treated Lambs

Castration had no effect on plasma prolactin concentrations. Work mainly with rats and humans has shown that steroid hormones can influence prolactin secretion (Horrobin, 1974). Also it has been shown that administration of oestradiol-17β to ewes and cattle results in an increase in plasma prolactin concentrations (Fell et al., 1972; Schams and Karg, 1972; Schams and Reinhardt, 1973). In previous work with male sheep Pelletier (1973) found that wethers had greater prolactin concentrations than rams on both short (8 h) and long (16 h) daily photoperiods, but this result was not significant. Also he found no difference in prolactin levels between testosterone propionate treated and control rams and wethers on either photoperiod, and thus concluded that testicular androgens had little influence on prolactin secretion. Similarly, the prepubertal prolactin peak seen in autumn born ram lambs was not affected by castration nor by castration with testosterone replacement therapy (Ravault et al., 1977a). Although Davis et al. (1978) reported a difference in the prolactin secretory profiles of rams and wethers, their experimental design did not eliminate likely seasonal influences, since rams were sampled in September and wethers in December. However, those workers did find that large-

doses of androgen or oestrogen increased prolactin secretion in wethers.

In the present study ganglionectomy resulted in a disruption of the seasonal pattern of plasma prolactin levels, similar to that reported in ganglionectomized adult rams by Barrell and Lapwood (1978b). That result was attributed to altered pineal gland function since reduced levels of pineal HIOMT activity were recorded from treated animals. Confirmation of this hypothesis was given by the fact that pinealectomy abolished seasonal trends in prolactin secretion in rams (Barrell and Lapwood, 1978c). In contrast to the marked effects of ganglionectomy on seasonality of plasma prolactin levels in rams and wethers obtained from studies at this laboratory, Buttle (1977) failed to show a similar effect of ganglionectomy in adult male castrate goats, although animal numbers were low (n = 3 in each group) and sampling frequency low. However, he did report that ganglionectomy accelerated the normal seasonal increase in levels between winter and summer.

There was no apparent interaction of castration with ganglionectomy, and apparently there are no reports in the literature of direct evidence suggesting such an interaction in domestic animals. Evidence from work with rodents is discussed in Chapter 4 (page 128).

(iii) Shearing and Plasma Prolactin Levels

An interesting aspect of the present study was the precipitate decline in plasma prolactin levels in samples collected on 22nd December, 1976 (the day of the summer

solstice), four days after the lambs were shorn. Eight animals which were not shorn at that time; due to their involvement in 24 hour profile sample collection (Experiment 2, Chapter 4), did not show this effect. Ravault (1976) reported a similar effect of shearing on prolactin levels in rams in both the 2nd and 3rd years of life, but this effect was not apparent in the first year, although prolactin levels were below those recorded in the subsequent two years. As stress is assumed to increase prolactin secretion (Raud et al., 1971; Davis, 1972; Sitarz et al., 1977; Wilson and Lapwood, 1978a) Ravault suggested that the effect of shearing may have been due to changes in sodium and aldosterone concentrations occurring, with a disruption of hydro-mineral metabolism. There is in fact evidence which indicates that prolactin may have an indirect effect on electrolyte homeostasis in sheep (Burstyn et al., 1972; Horrobin et al., 1973). Also human studies have indicated that prolactin levels are sensitive to shifts in plasma osmolality (Horrobin, 1974), with an inverse relationship between plasma prolactin levels and electrolyte excretion (Auty et al., 1976).

A second possible explanation for the reduced prolactin levels is that shearing results in a lower body temperature, presumably by increasing the rate of heat loss (Dutt and Hamm, 1957). A direct relationship between ambient temperature and prolactin secretion has been established in cattle (Smith et al., 1977; Wettemann and Tucker, 1974), but no reports of comparable work in sheep have been noted.

3. Bodyweight Data

The significantly lower bodyweights of ganglionectomized entire and ganglionectomized castrate lambs, and the non-significant decrease recorded by Barrell and Lapwood (1978b) in ganglionectomized adult rams, suggests a stimulatory influence of the pineal on growth. Information in the literature regarding growth and the pineal gland is difficult to interpret. Work, almost exclusively with rodents, has suggested that pineal activity may be associated with an overall decrease in synthesis and release of pituitary growth hormone (Relkin, 1976; Reiter, 1977). However, a recent report (Rønnekleiv and McCann, 1978) indicated that pinealectomy significantly reduced daytime, but not night-time secretion of growth hormone in rats. In growing wethers, a long photoperiod stimulated faster growth and greater prolactin secretion compared with a short daily photoperiod (Forbes, 1975; Forbes et al., 1975), perhaps implying that the pineal and/or prolactin may be involved in mediating the effects of daylength on growth rate. However, in another report from that laboratory (Brown et al., 1977) pinealectomy did not influence growth rate or secretion of growth hormone in lambs; unfortunately removal of the gland was not complete in some animals so the importance of that result is difficult to assess. Neither daylength nor season appear to regulate growth hormone levels in goats or lambs (Hart and Buttle, 1975; Brown et al., 1977).

Interpretation of the results of ganglionectomy on body growth in this study would perhaps have been more

meaningful if supplemented by plasma growth hormone concentration data, but no assay was available in this laboratory for that hormone.

Previous studies have shown that rams and wethers have similar bodyweights over the period studied in this experiment, or that rams may be heavier, particularly if reared under favourable conditions (Dýrmundsson, 1973; Price, 1975; Ahmed et al., 1978).

4. Autopsy Data

Testicular and epididymal weights and epididymal sperm reserves all tended to be lower, although non-significantly, in ganglionectomized rams. This result probably reflected the lower mean bodyweights of those lambs since testicular weight, which is closely related to output of spermatozoa, is related to bodyweight (Skinner et al., 1968, Dýrmundsson, 1973). Seminiferous tubule diameters did not show this trend, being the same in both groups of rams. This result may have been related to the observation made by Courot (1962) that seminiferous tubule diameters could not be related to testicular weights. Any significant differences in plasma LH and testosterone concentrations may have been expected to influence the testes and secondary sexual organs; Barrell and Lapwood (1978b) reported that ganglionectomy-induced higher LH levels probably accounted for the greater seminiferous tubule diameters, and testicular and epididymal weights recorded at autopsy. An influence of ganglionectomy on the accessory sexual organs, secondary to an influence on prolactin secretion, cannot be precluded since seminal

vesicular weights and fructose concentrations, which may be regulated by prolactin in the developing ram lamb (Ravault et al., 1977b), were not recorded.

There was no overall effect of either ganglionectomy or castration on pineal weights at autopsy. The interaction of ganglionectomy and castration was significant, due to higher mean weights being recorded from non-ganglionectomized castrates than from other groups. The importance of this result is difficult to assess since it was due largely to data from one animal with a pineal weighing 125 mg. Previous ovine work has shown that ganglionectomy of adult rams resulted in a non-significant decrease in pineal weights, but a reduction in pineal HIOMT activity and an increase in pineal cell nuclear density (Barrell and Lapwood, 1978b).

5. General Discussion

In ram lambs, puberty is a period of reproductive development in which the secretion of pituitary gonadotrophins, regulated by the brain, stimulates testicular growth and steroidogenesis, and thus the onset of spermatogenesis and development of secondary sexual characteristics. In several studies the attainment of puberty has been taken as the age of first appearance of spermatozoa in the epididymal tubules, usually between 4 and 5 months of age (Dýrmundsson, 1973; Wilson and Lapwood, 1978c).

On the basis of the hormone and autopsy results presented in this experiment, both ganglionectomized and non-ganglionectomized rams reached similar stages of

reproductive development to those recorded at comparable ages in Romney rams (Wilson and Lapwood, 1978a,c). Possibly ganglionectomy is more likely to influence the timing of the pubertal process and a more detailed study of the influence of the pineal on the onset of spermatogenesis is needed to examine this point. Such a study should include hormonal as well as reproductive organ data, and quantitative and qualitative histological observations, made during the period of sexual maturation, from both control and pinealectomized or ganglionectomized rams.

There are no reports on the effects of pinealectomy on reproductive organ growth in lambs although the prolactin response to photoperiod is disrupted by this operation (Forbes, 1975; Brown et al., 1977). However, both ganglionectomy and pinealectomy of adult rams influenced pituitary, testicular and accessory sex organ functions (Barrell and Lapwood, 1978b,c,d,e), while pinealectomy advances the age of puberty in rats (Kincl and Benagiano, 1967; Relkin, 1970a,b, 1971). Also in humans, clinical cases of early and delayed puberty related to pineal hypofunction and hyperfunction respectively, have been reported (Kitay, 1954; Wurtman, 1968; Relkin, 1976). Thus an influence of the pineal of reproductive maturation in the ram is not inconceivable.

CHAPTER IV

THE EFFECTS OF CRANIAL CERVICAL GANGLIONECTOMY AND CASTRATION ON 24 HOUR PLASMA PROFILES OF LH, TESTOSTERONE AND PROLACTIN IN PUBERTAL AND POST-PUBERTAL MALE LAMBS

A. INTRODUCTION

In adult rams LH and testosterone are secreted in pulses at irregular intervals (Katongole et al., 1974; Sanford et al., 1974c, 1977; Barrell and Lapwood, 1978e; Wilson and Lapwood, 1978d). Both the timing and magnitude of testosterone pulses are related to those of the preceding LH pulses, while the magnitude and temporal relationships between episodic LH and testosterone releases vary over the photoperiod-induced seasonal cycle (Lincoln, 1976a; Lincoln et al., 1977; Sanford et al., 1977, 1978; Wilson and Lapwood, 1978d).

Pulsatile LH release is initiated in the first few weeks after birth (Foşter et al., 1978), but development of a consistent quantitative relationship between LH and testosterone secretory pulses does not occur until later; probably it results from testicular maturational changes (Foster, 1974; Wilson and Lapwood, 1978a).

Prolactin also probably is secreted in a pulsatile manner in rams, at least in winter (Wilson and Lapwood, 1978d) and autumn (Davis et al., 1978).

Research cited in Chapter 1 was inconclusive as to

whether or not the secretion of LH, FSH, prolactin and testosterone showed any circadian cyclicity.

Recent evidence indicates that the pineal gland of rams is involved in mediating the effects of photoperiod on seasonal patterns of hormone secretion and semen production (Barrell and Lapwood, 1978b,c,d). In addition, acute secretion profile studies have revealed that pinealectomy elevated mean LH levels and abolished a nocturnal surge of prolactin secretion (Barrell and Lapwood, 1978e).

This experiment was undertaken to examine the effects of cranial cervical ganglionectomy and castration on the 24 hour secretory profiles of LH, testosterone and prolactin in male lambs of pubertal and post-pubertal ages. Between these two ages Romney rams normally are expected to attain the capacity to produce fertile spermatozoa (Wilson and Lapwood, 1978c), even though there probably are subsequent improvements in both quantitative and qualitative aspects of spermatogenesis (Courot, 1962; Dýrmundsson, 1973).

B. MATERIALS AND METHODS

Initially, 6 animals were assigned to each treatment group, but owing to the death of one animal and some failures of cannula patency, complete sets of data could be collected during the post-pubertal sampling only from 6 non-ganglionectomized entire lambs, 5 non-ganglionectomized castrates, 4 ganglionectomized entires and 5 ganglionectomized castrates.

The pubertal sampling took place in December 1976 when the animals were approximately 100 days of age and exposed to an artificial photoperiod of 15 hours light: 9 hours dark. The post-pubertal collection period, when the animals were approximately 300 days old, occurred during May-June 1977 with a 9½ hours light: 14½ hours dark artificial photoperiod.

For ease of presentation not only have individual hormone profiles been included, but so also have mean profiles for each treatment group. Mean profiles were derived by calculating mean hormone levels for all lambs in each treatment group at each hourly sampling, then graphing these data. Effects of experimental treatments on the concentrations of each hormone were analyzed by Student's t-tests, using the mean logarithm transformed hormone levels from each lamb as parameters. In addition for animals in each treatment group the effect of sampling age on mean hormone levels was examined by t-test. Sampling frequency was not adequate to permit accurate estimation of hormone secretion peak frequency or peak height data.

C. RESULTS

See Tables 4.1 and 4.2

1. LH Secretory Profiles

Individual LH profiles for the pubertal 24 hour sampling period are shown in Figures 4.1 to 4.4 with the mean secretory profiles for each treatment group shown in Figure 4.5. Post-pubertal LH profiles are shown in like manner in Figures 4.6 to 4.10. Mean 24 hour plasma LH

Table 4.1 Mean (± S.E.M.) plasma hormone concentrations (ng/ml) obtained from the 24 hour profile study of pubertal (100 day old) and post-pubertal (300 day old) male lambs.

	LH		Testosterone		Prolactin	
Age (days)	100	300	100	300	100	300
Treatment						
Entires						
Non-ganglionectomized	2.30 ± 0.26	0.41 ± 0.04	0.55 ± 0.06	1.47 ± 0.17	100.6 ± 1.7	9.0 ± 0.8
Ganglionectomized	1.92 ± 0.32	0.41 ± 0.06	0.47 ± 0.05	0.90 ± 0.11	35.1 ± 2.9	50.7 ± 4.2
Castrates						
Non-ganglionectomized	11.16 ± 0.57	10.38 ± 0.62	-	-	102.7 ± 1.5	8.5 ± 0.5
Ganglionectomized	9.22 ± 0.71	14.06 ± 0.56	-	-	51.8 ± 3.0	50.7 ± 3.0

Table 4.2 Summary of the t-test analyses of LH, testosterone and prolactin data from Experiment 2.

	LH		Testosterone		Prolactin	
Age (days)	100	300	100	300	100	300
Comparison						
A. Castration vs Entire	7.332*** (22)	18.479*** (18)	-	-	0.692 (22)	0.988 (18)
B. GanglionX vs Non-gangX	1.435 (22)	3.332** (18)	0.358 (10)	0.762 (8)	3.823** (22)	4.656*** (18)
C. Interaction A x B	0.964 (20)	3.271** (16)	-	-	1.034 (20)	0.612 (16)

D. Effects of Age (100 vs 300 days)	LH	Testosterone	Prolactin
(i) Non-ganglionectomized entires	3.596** (10)	2.081 (10)	10.428*** (10)
(ii) Non-ganglionectomized castrates	0.356 (9)	-	12.626*** (9)
(iii) Ganglionectomized entires	3.767** (9)	2.080 (9)	0.810 (9)
(iv) Ganglionectomized castrates	1.913 (9)	-	0.520 (9)

***P<0.001, **P<0.01

Note: Figures in brackets are the numbers of degrees of freedom for each contrast

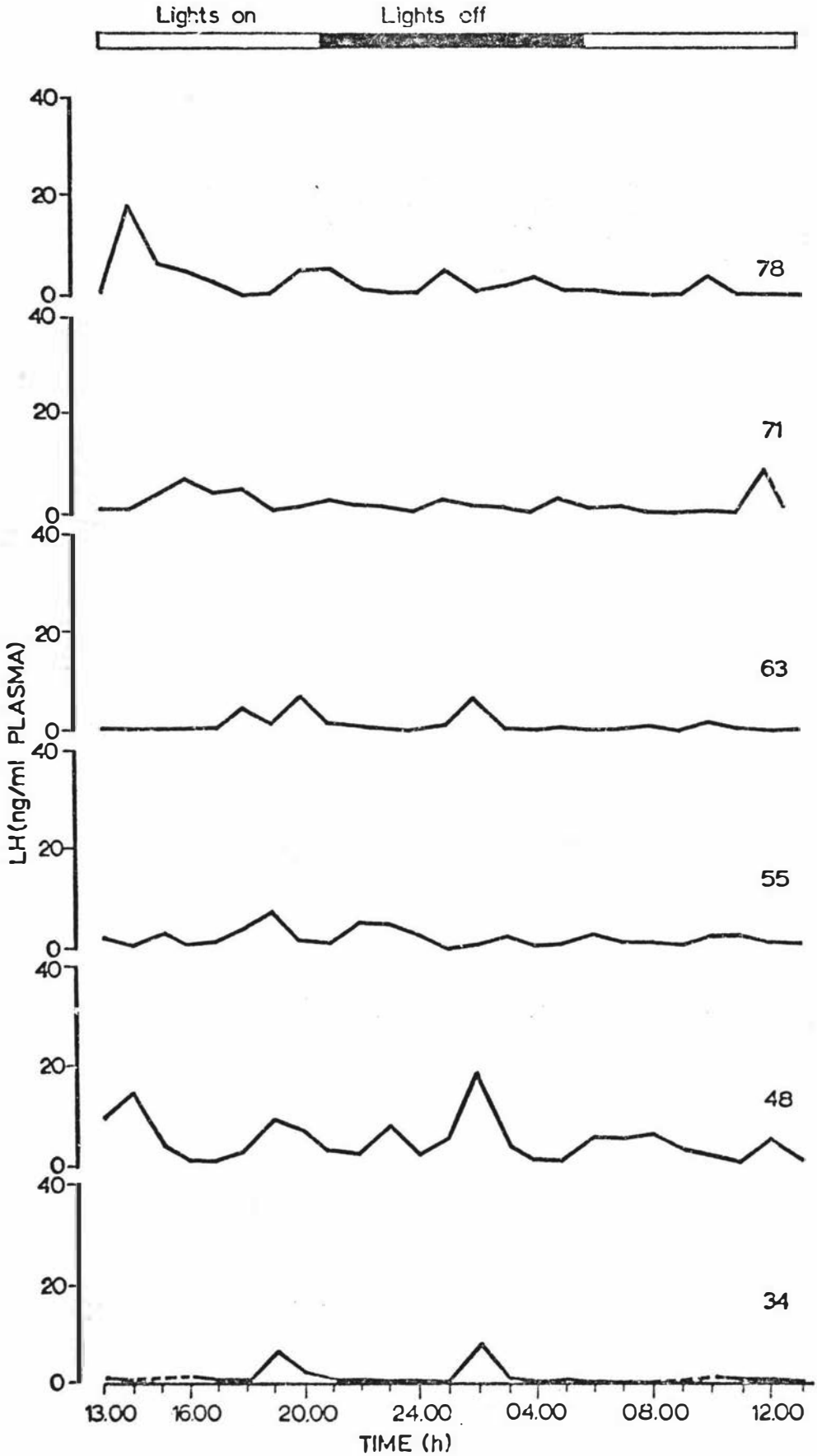

Figure 4.1 : Individual 24 hour profiles of plasma LH levels recorded from 6 non-ganglionectomized entire lambs at 100 days of age.

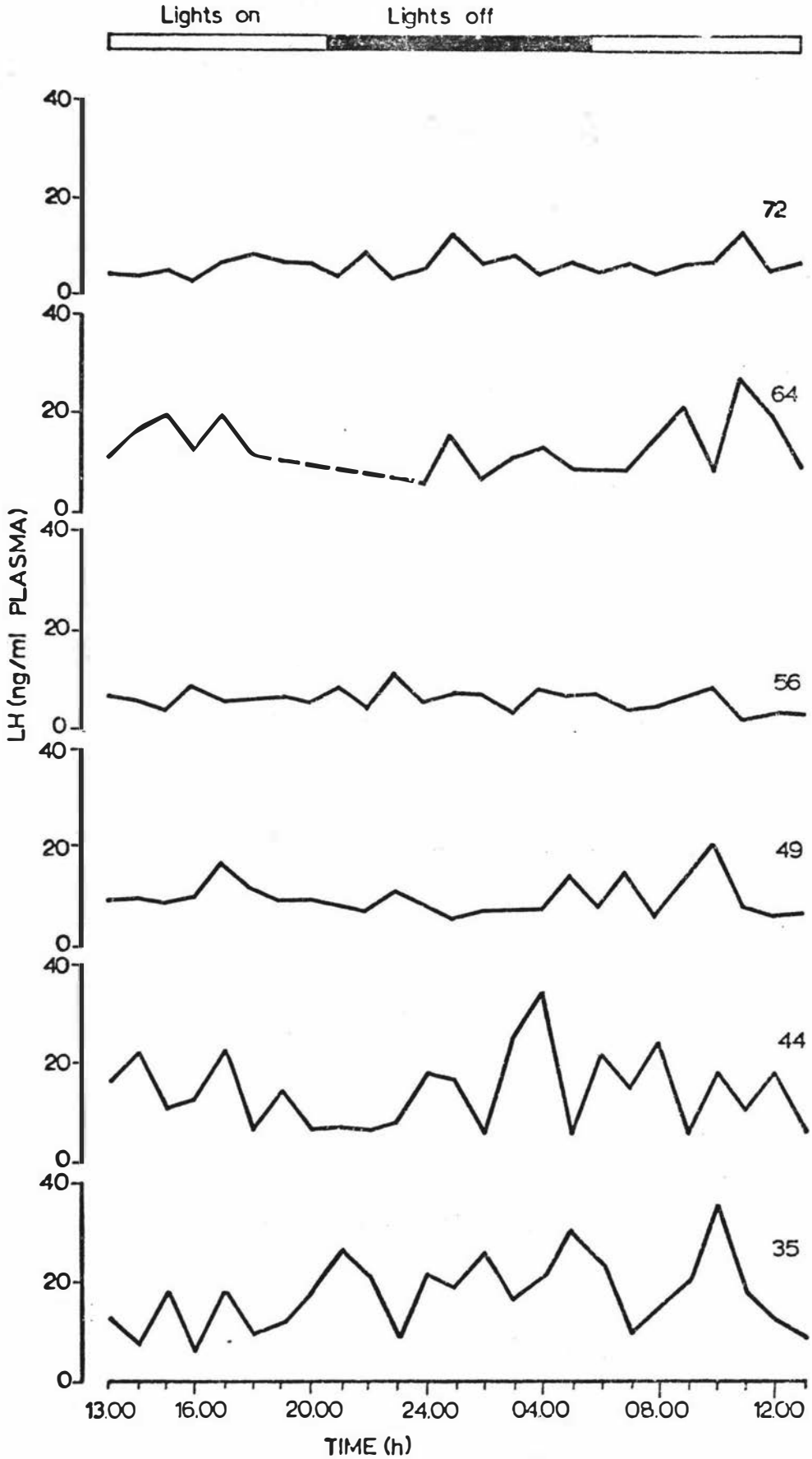

Figure 4.2 : Individual 24 hour profiles of plasma LH levels recorded from 6 non-ganglionectomized castrate lambs at 100 days of age.

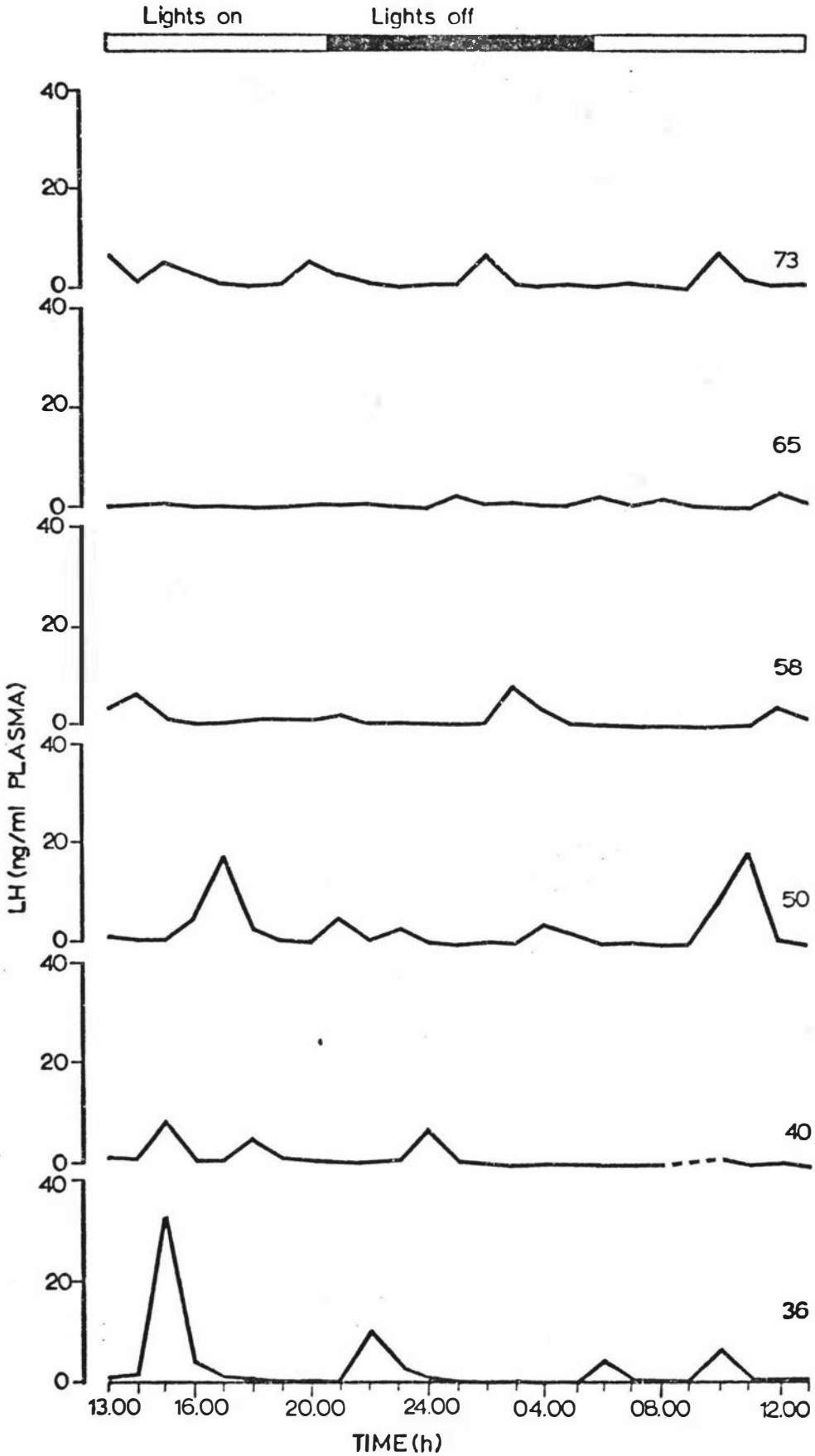

Figure 4.3 : Individual 24 hour profiles of plasma LH levels recorded from 6 ganglionectomized entire lambs at 100 days of age.

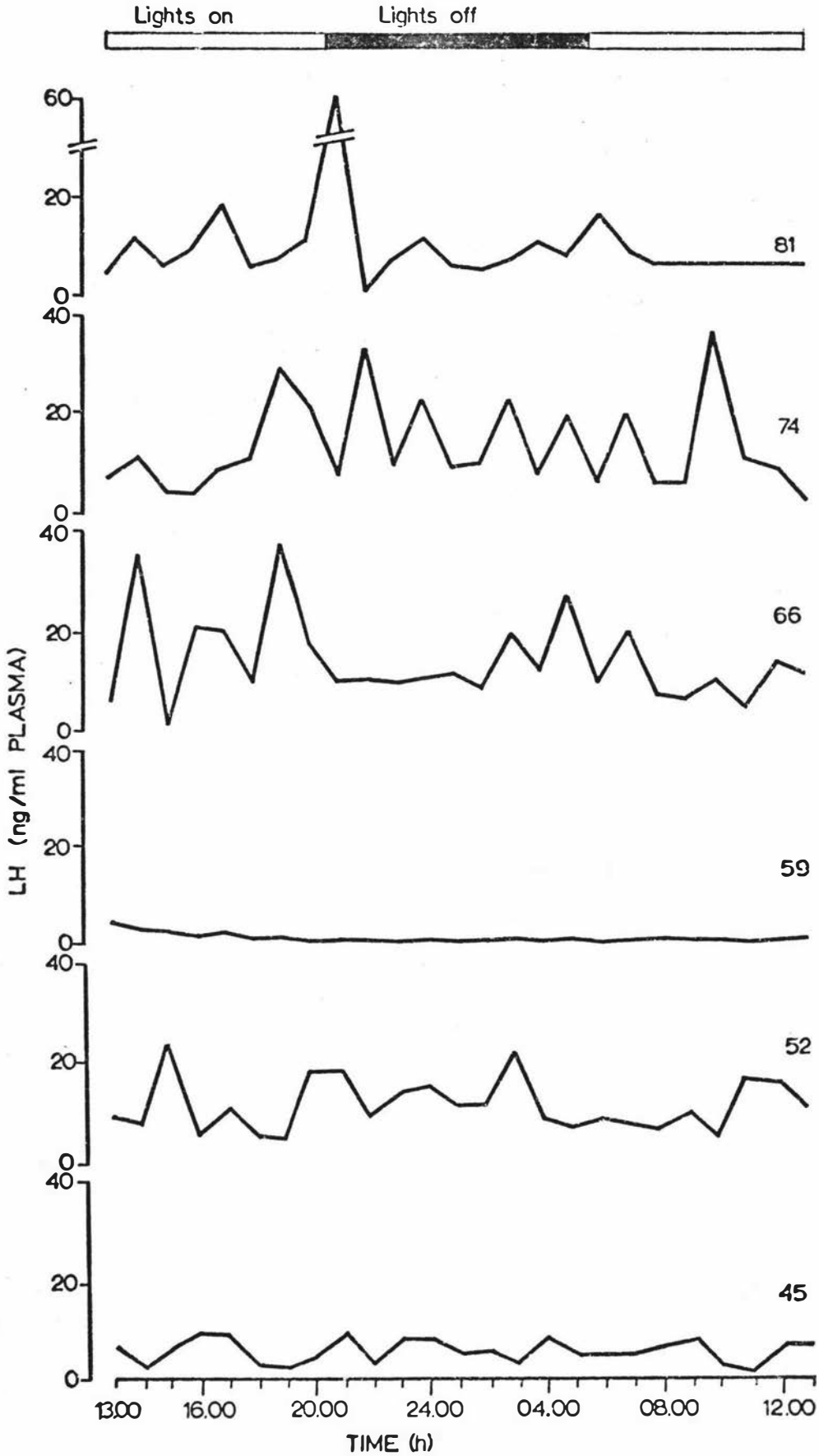

Figure 4.4 : Individual 24 hour profiles of plasma LH levels recorded from 6 ganglionectomized castrate lambs at 100 days of age.

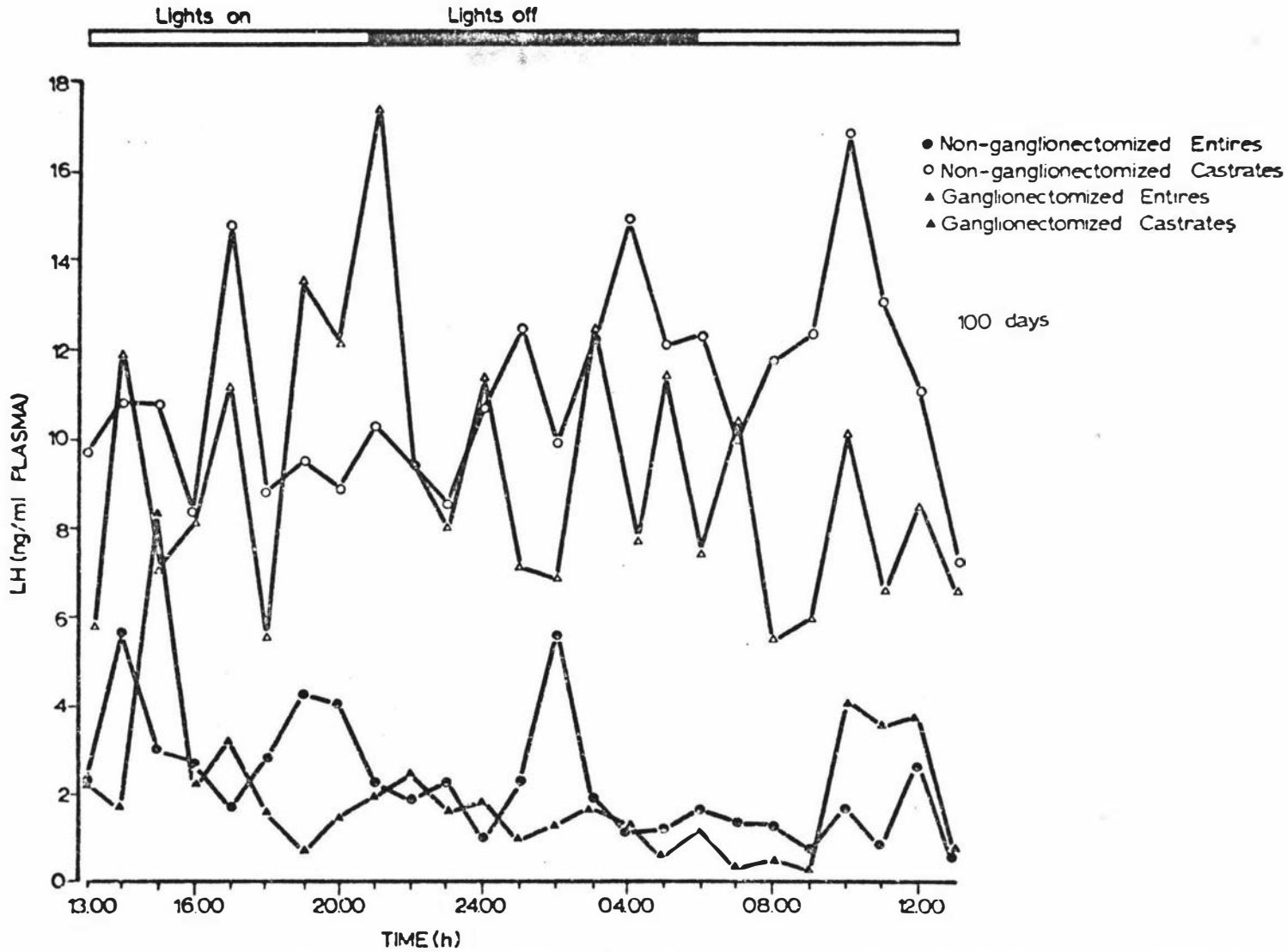

Figure 4.5 : Mean 24 hour plasma LH profiles recorded from lambs at 100 days of age.

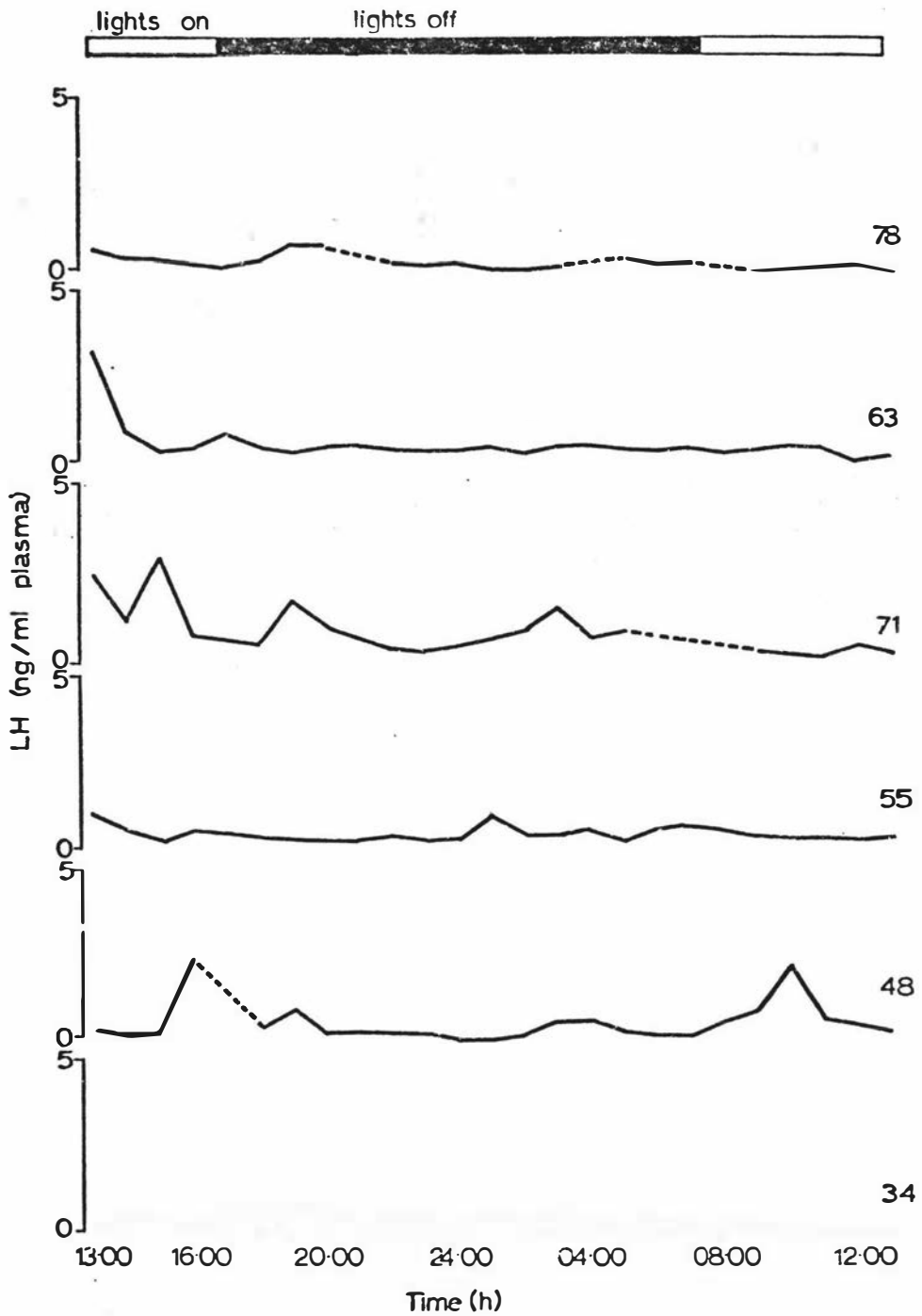

Figure 4.6 : Individual 24 hour profiles of plasma LH levels recorded from 6 non-ganglionectomized entire lambs at 300 days of age.

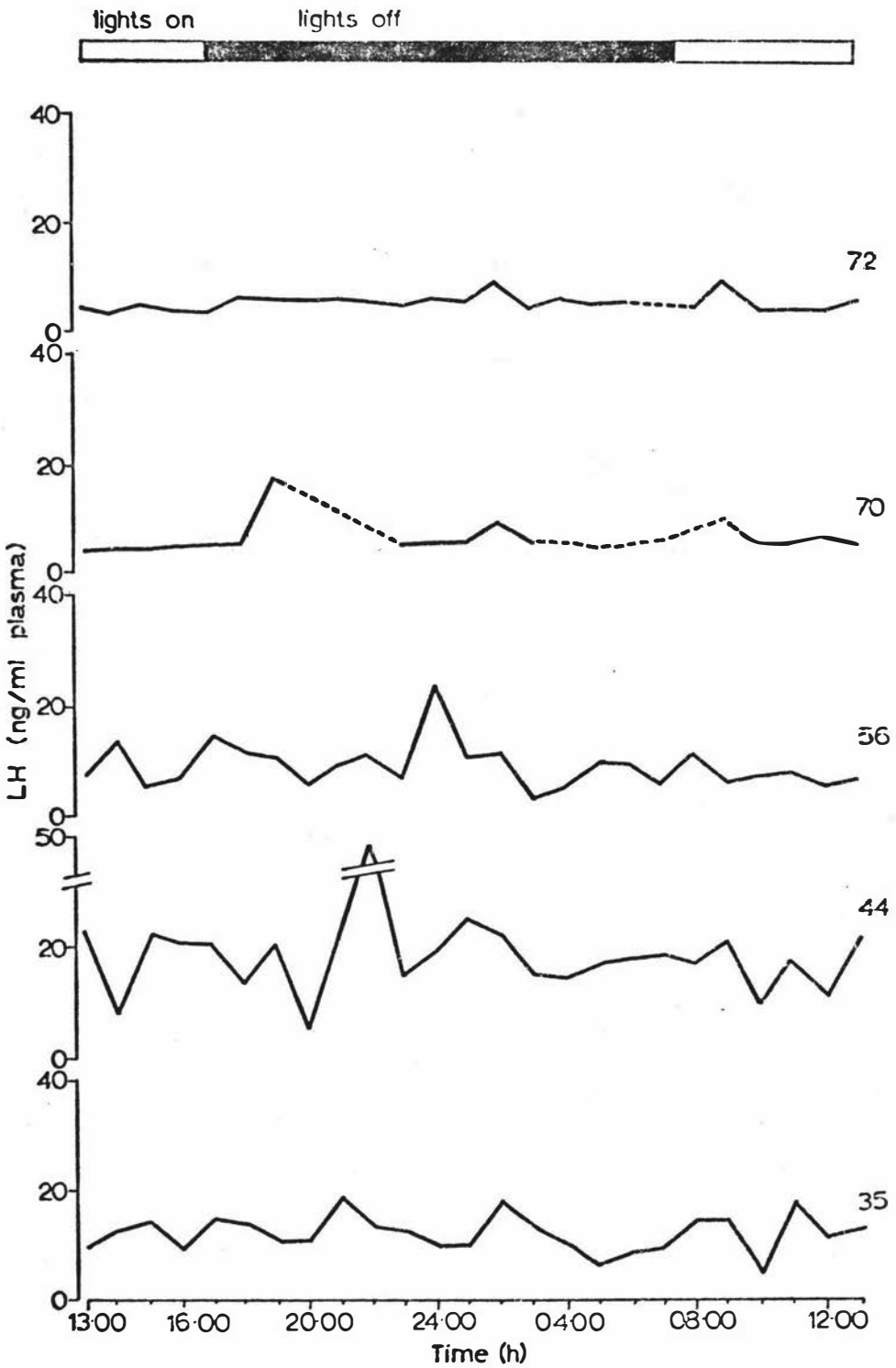

Figure 4.7 : Individual 24 hour profiles of plasma LH levels recorded from 5 non-ganglionectomized castrate lambs at 300 days of age.

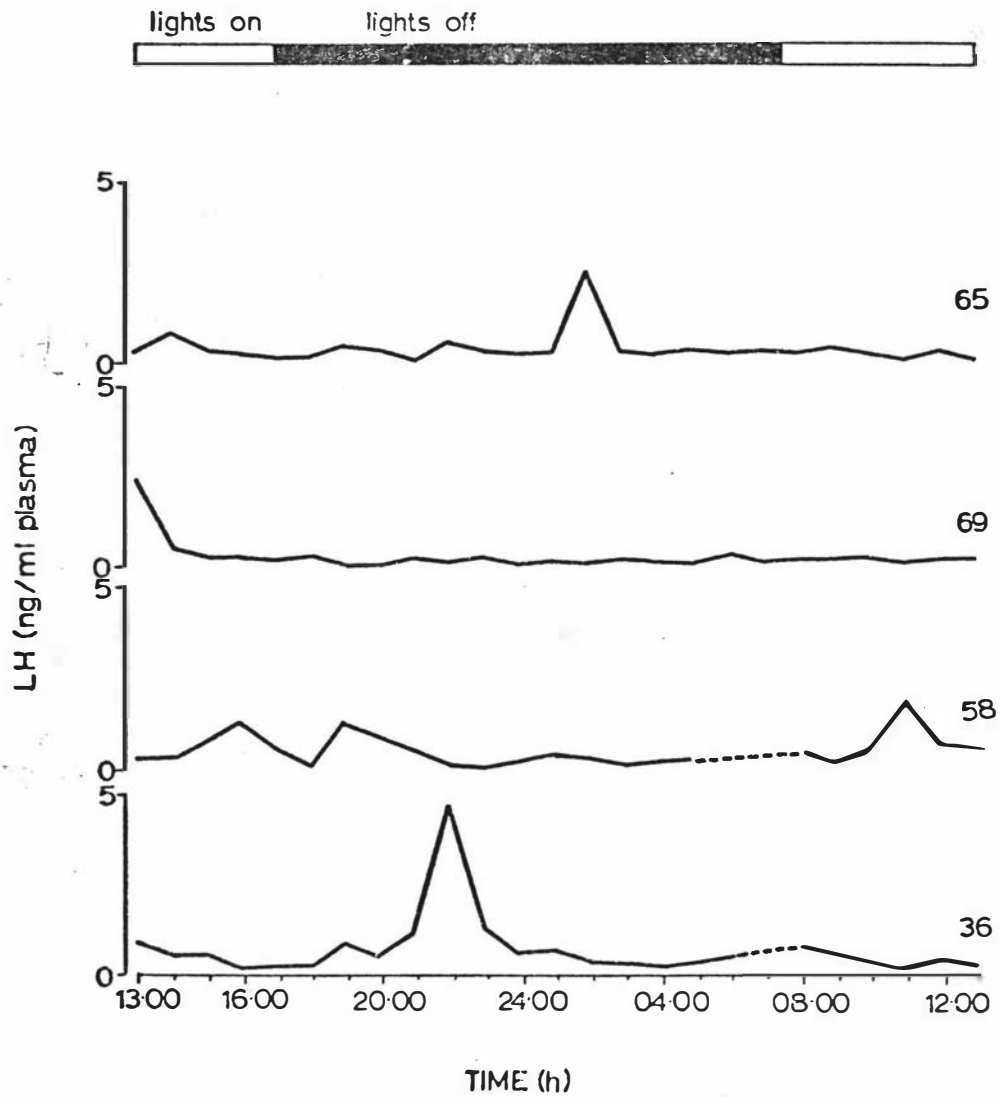

Figure 4.8 : Individual 24 hour profiles of plasma LH levels recorded from 4 ganglionectomized entire lambs at 300 days of age.

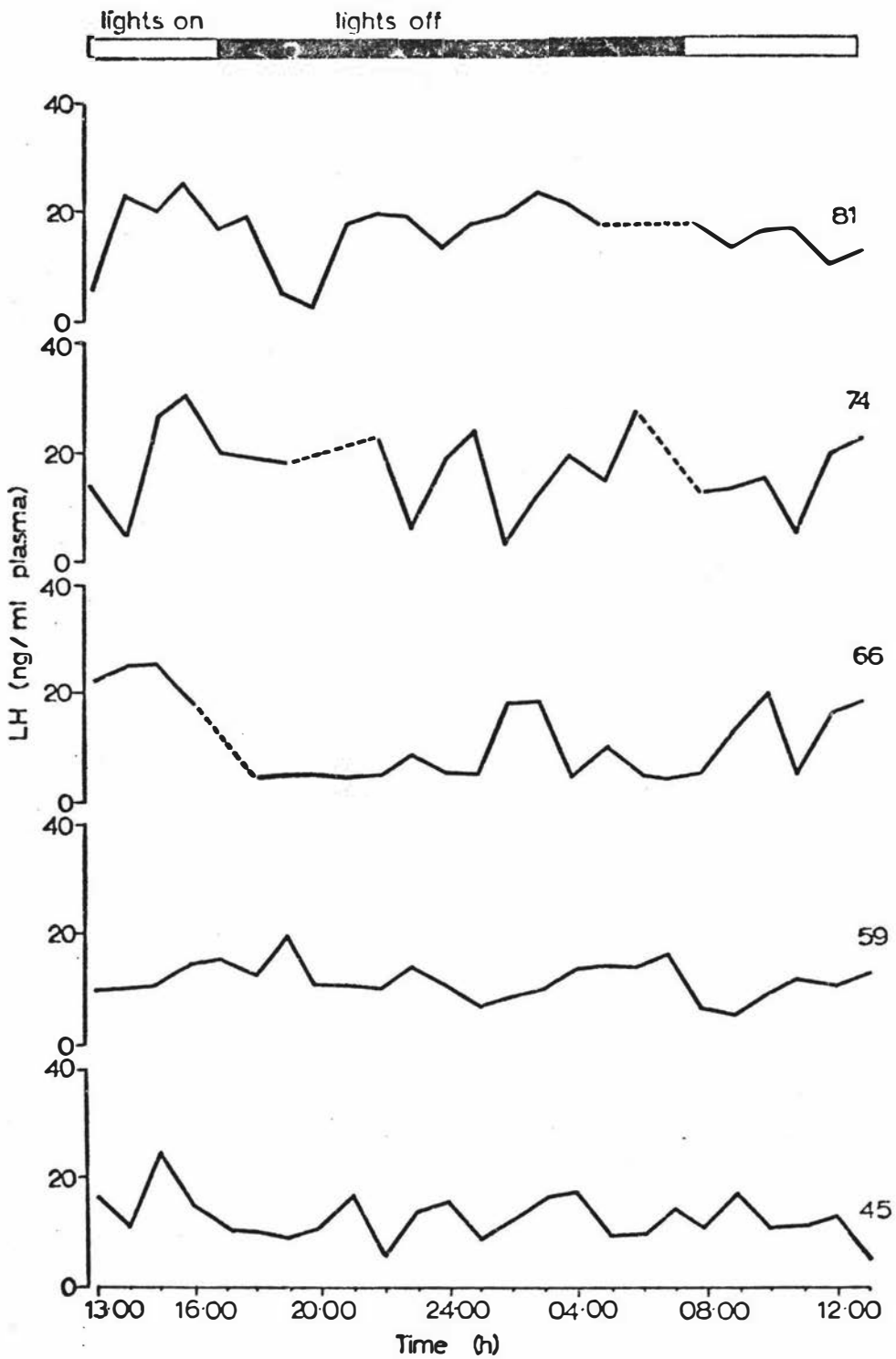

Figure 4.9 : Individual 24 hour profiles of plasma LH levels recorded from 5 ganglionectomized castrate lambs at 300 days of age.

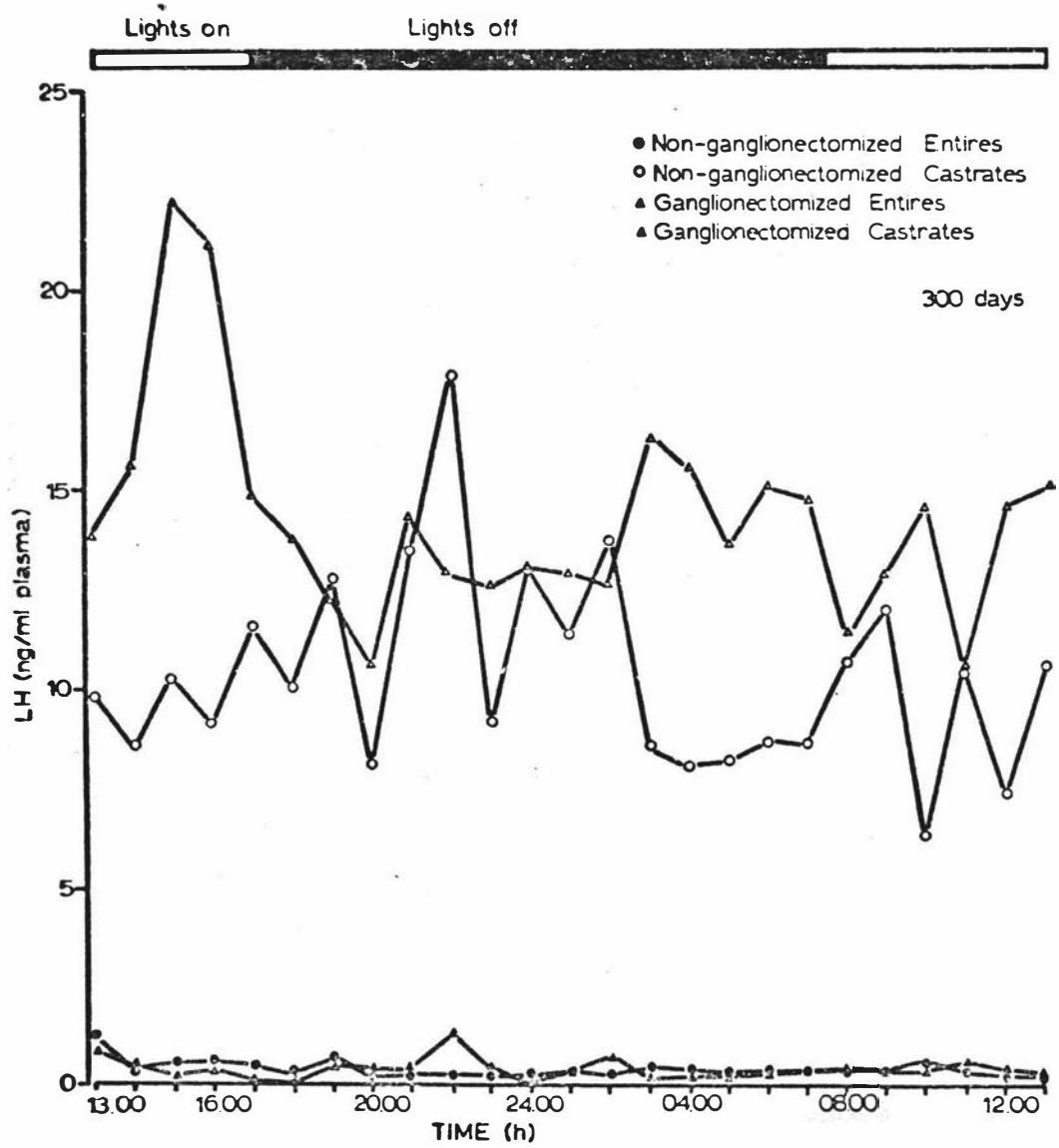

Figure 4.10 : Mean 24 hour plasma LH profiles recorded from lambs at 300 days of age.

concentrations are presented in Table 4.1 with the results of statistical analyses in Table 4.2.

No circadian fluctuations in LH levels were evident in individual animals nor in any treatment group at either the pubertal or post-pubertal samplings. LH levels in castrated animals tended to fluctuate over a wider range and were higher than those from entires, at both the pubertal (24 hour mean levels were 10.19 ± 0.46 ng/ml in castrates *vs* 2.11 ± 0.20 ng/ml in entires, $P < 0.001$) and post-pubertal (12.22 ± 0.44 ng/ml in castrates *vs* 0.41 ± 0.03 ng/ml in entires, $P < 0.001$) collection periods.

At 100 days of age, ganglionectomy had no significant influence on LH secretion with 24 hour mean levels being 5.52 ± 0.44 ng/ml in ganglionectomized lambs *vs* 6.68 ± 0.41 ng/ml in non-ganglionectomized animals. However, at 300 days of age ganglionectomy caused a significant increase in LH concentrations from a mean of 5.40 ± 0.42 ng/ml in non-ganglionectomized lambs to 7.93 ± 0.56 ng/ml in ganglionectomized animals ($P < 0.01$). Also, the interaction of castration with ganglionectomy had no significant effect on LH levels at the pubertal sampling but significantly elevated levels ($P < 0.01$) at the post-pubertal sampling. These effects of ganglionectomy, and the interaction of castration and ganglionectomy, on post-pubertal LH secretion were due to high levels recorded in the ganglionectomized castrates (mean level 14.06 ± 0.56 ng/ml *vs* 10.38 ± 0.62 ng/ml in non-ganglionectomized castrates).

Comparison of pubertal and post-pubertal 24 hour profiles showed that LH levels in entire animals were significantly higher at the pubertal sampling (mean levels were 2.30 ± 0.26 ng/ml at 100 days *vs* 0.41 ± 0.04 ng/ml at

300 days, $P < 0.01$, in the non-ganglionectomized entires; and 1.92 ± 0.32 ng/ml at 100 days vs 0.41 ± 0.06 ng/ml at 300 days, $P < 0.01$, in the ganglionectomized entires). Even though the mean LH level in ganglionectomized castrates at 100 days (9.22 ± 0.71 ng/ml) was lower than at 300 days (14.06 ± 0.56 ng/ml), there was no significant effect of age on LH levels in either group of castrates.

2. Testosterone

Testosterone data for entire lambs is graphed in Figures 4.11 to 4.13 (pubertal sampling) and Figures 4.14 to 4.16 (post-pubertal sampling) and summarized in Tables 4.1 and 4.2.

Plasma testosterone concentrations in entire lambs at both ages showed irregular pulsatile variations with no apparent circadian rhythmicity. Testosterone levels in representative plasma samples from castrate animals collected at both sampling ages, were below the limit of assay sensitivity.

Ganglionectomy had no significant effect on testosterone levels in entire animals: at 100 days of age mean 24 hour levels were 0.55 ± 0.06 ng/ml plasma in non-ganglionectomized lambs vs 0.47 ± 0.05 ng/ml in ganglionectomized animals; and at 300 days non-ganglionectomized animals had 1.47 ± 0.17 ng/ml vs 0.90 ± 0.11 ng/ml in ganglionectomized lambs.

Testosterone levels were higher at the 300 day sampling than at 100 days in both groups of entires, but these differences just failed to reach significance.

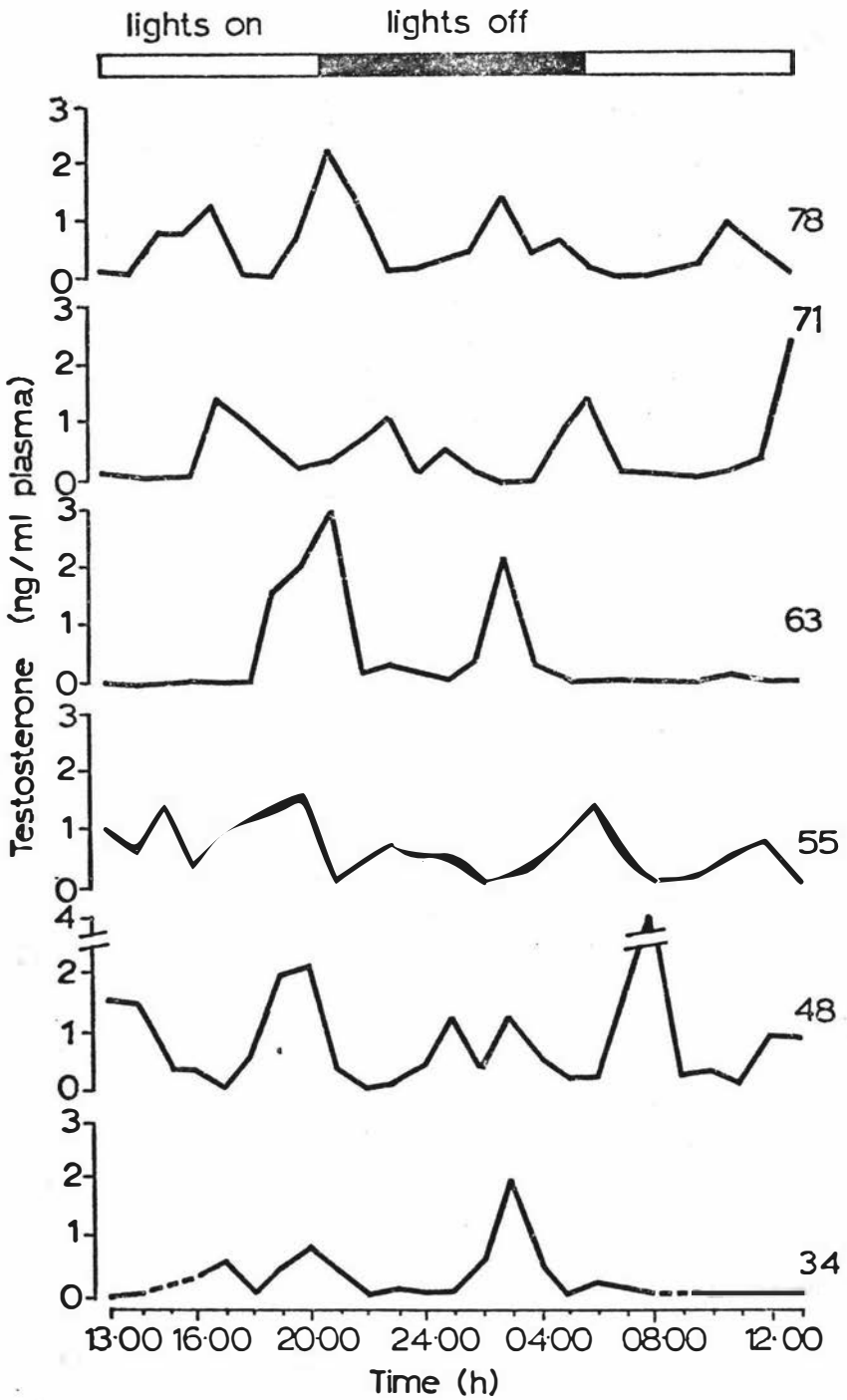

Figure 4.11 : Individual 24 hour profiles of plasma testosterone levels recorded from 6 non-ganglionectomized entire lambs at 100 days of age.

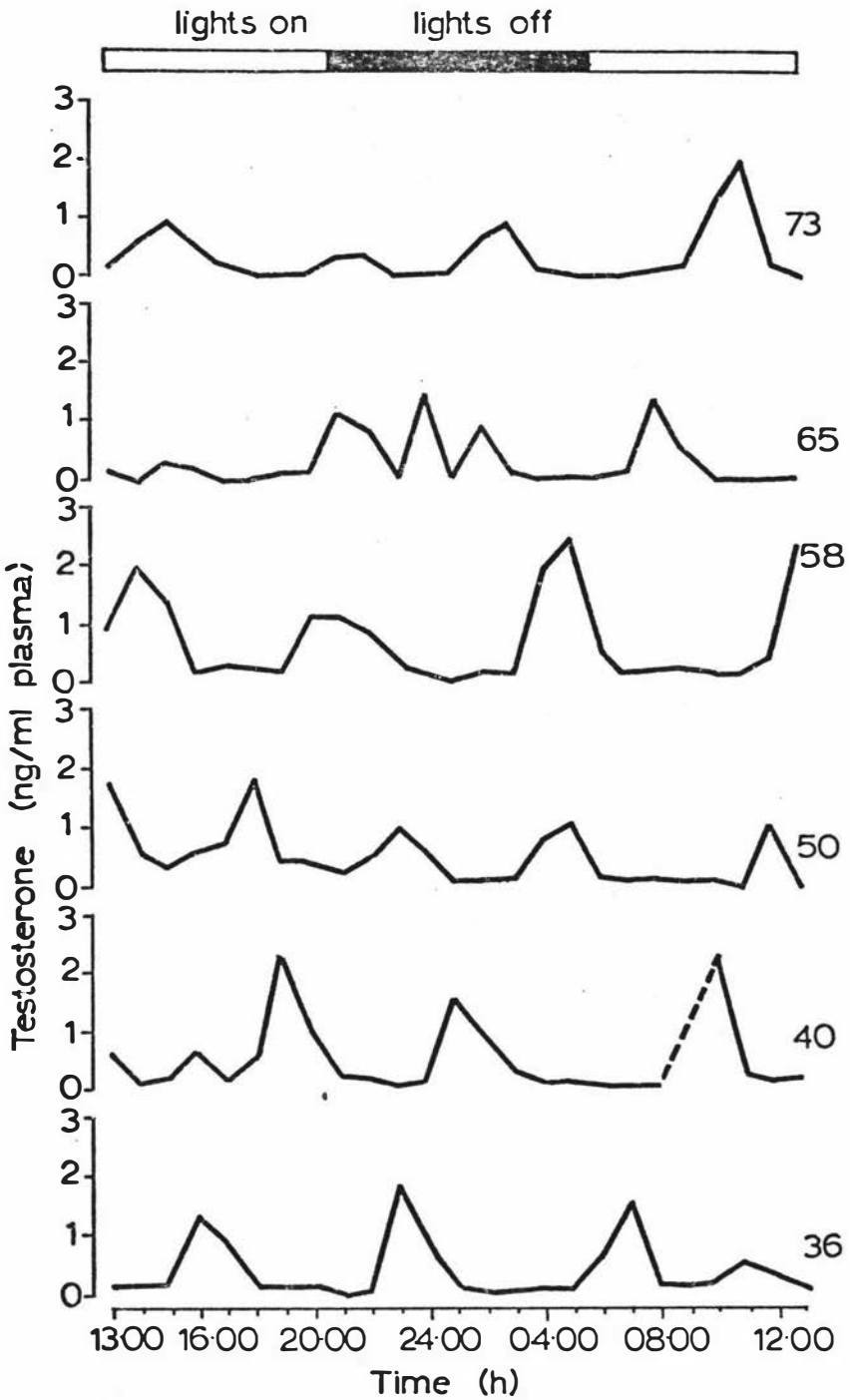

Figure 4.12 : Individual 24 hour profiles of plasma testosterone levels recorded from 6 ganglionectomized entire lambs at 100 days of age.

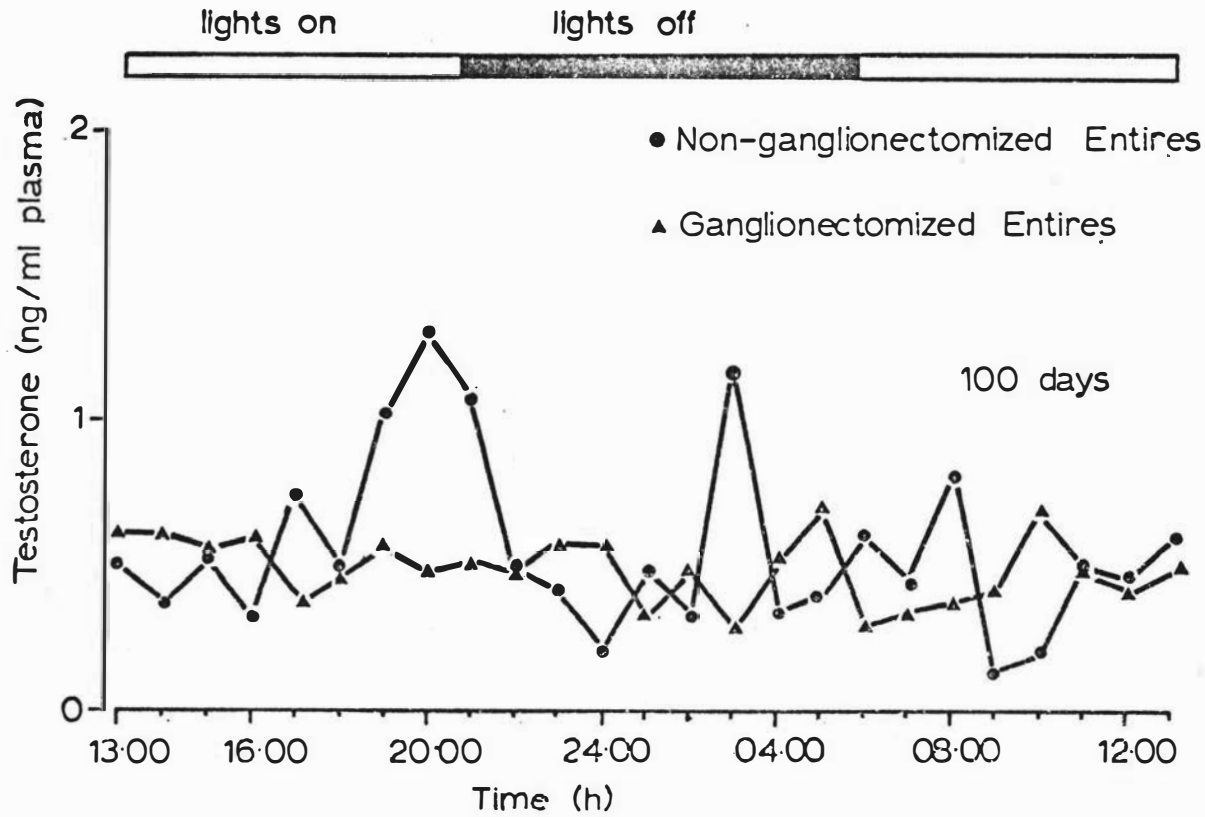

Figure 4.13 : Mean 24 hour plasma testosterone profiles recorded from lambs at 100 days of age.

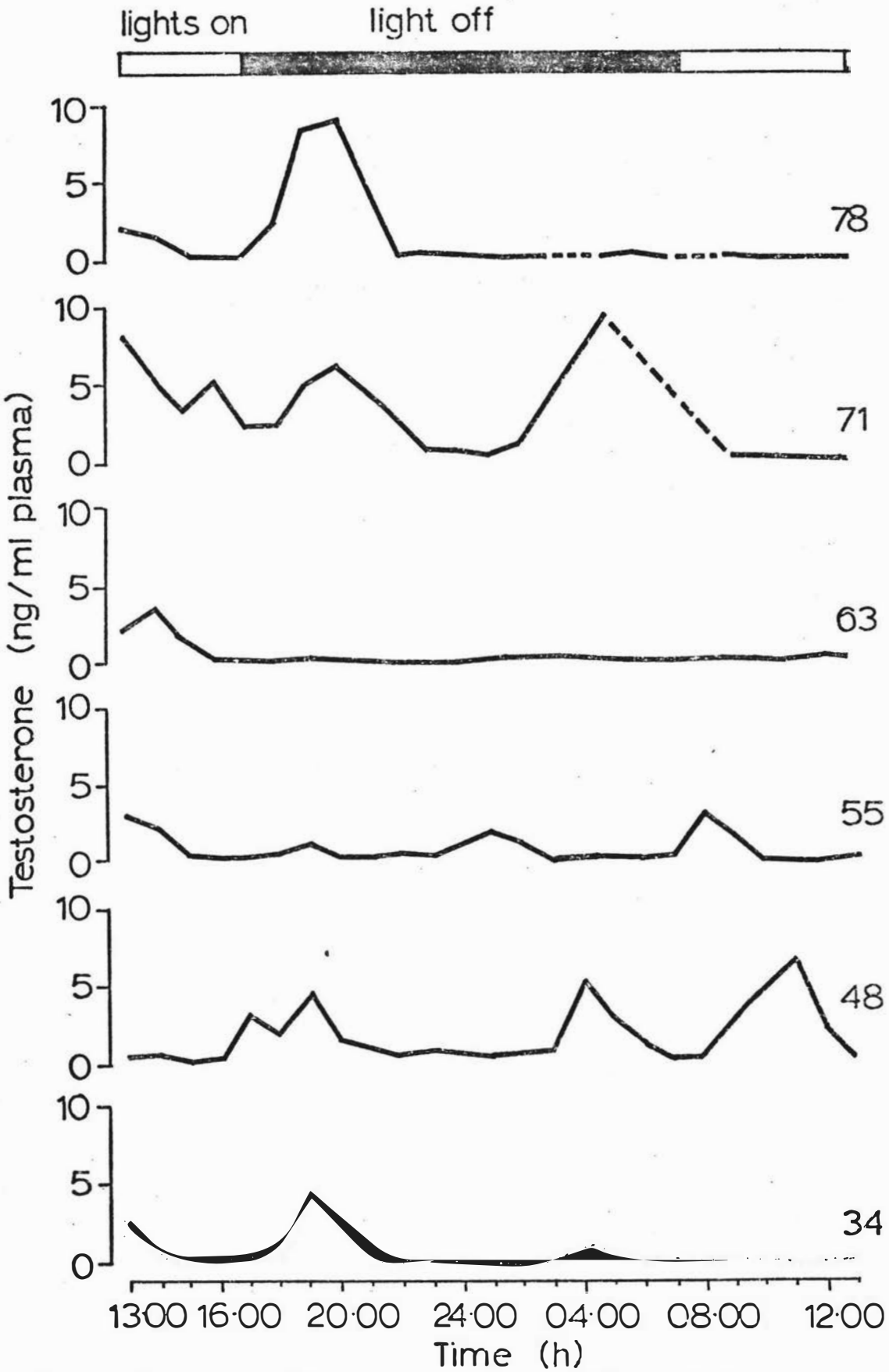

Figure 4.14 : Individual 24 hour profiles of plasma testosterone levels recorded from 6 non-gangl-ionectomized entire lambs at 300 days of age.

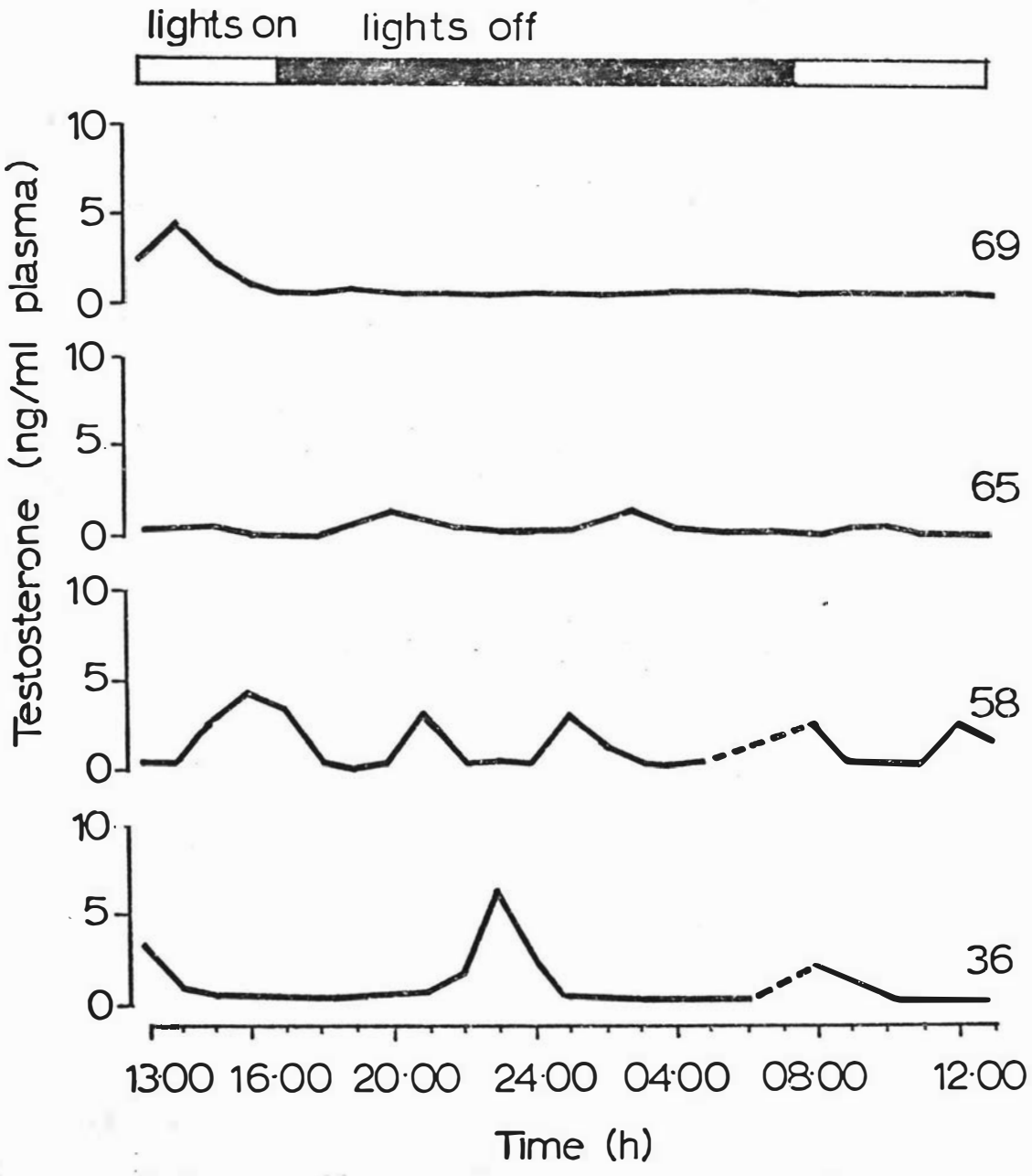

Figure 4.15 : Individual 24 hour profiles of plasma testosterone levels recorded from 4 gangl-ionectomized entire lambs at 300 days of age.

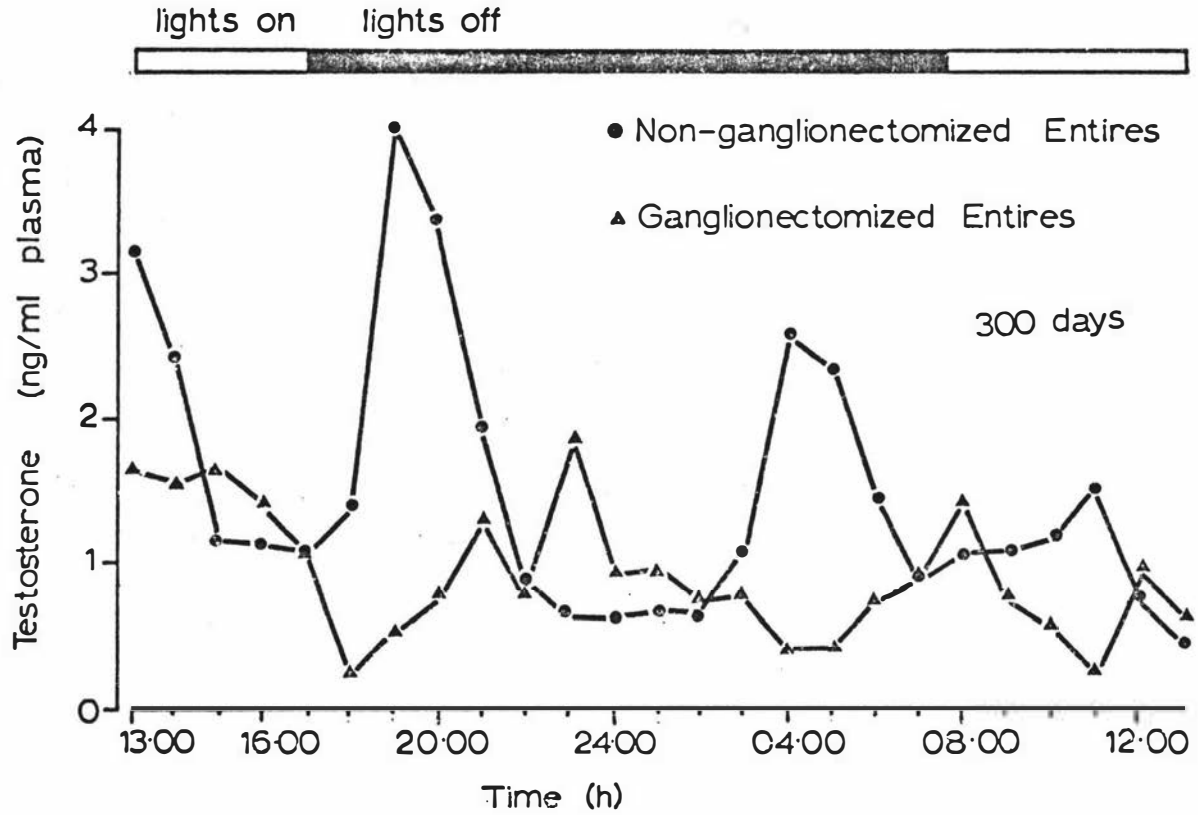

Figure 4.16 : Mean 24 hour plasma testosterone profiles recorded from lambs at 300 days of age.

3. Prolactin

Prolactin data is given in Figures 4.17 to 4.21 (pubertal), Figures 4.22 to 4.26 (post-pubertal) and Tables 4.1 and 4.2.

Circadian variations in plasma prolactin levels were not apparent in individual animals, nor in the mean profiles for any group of lambs at either age.

A normal seasonal variation in prolactin levels, with high levels during the summer months (100 day sampling) and low during winter (300 day sampling), was recorded from non-ganglionectomized entires (mean 24 hour levels were 100.6 ± 1.7 ng/ml at 100 days vs 9.0 ± 0.8 ng/ml at 300 days, $P < 0.001$) and non-ganglionectomized castrates (102.7 ± 1.5 ng/ml at 100 days vs 8.5 ± 0.5 ng/ml at 300 days, $P < 0.001$). Ganglionectomy resulted in a marked disruption of this pattern with no significant effects of age (season) on prolactin concentrations being recorded from ganglionectomized entires (35.1 ± 2.9 ng/ml at 100 days vs 50.7 ± 4.2 ng/ml at 300 days) or ganglionectomized castrates (51.8 ± 3.0 ng/ml at 100 days vs 50.7 ± 3.0 ng/ml at 300 days). Thus at 100 days of age ganglionectomy significantly reduced prolactin levels from a mean of 101.6 ± 1.1 ng/ml plasma in non-ganglionectomized lambs to 43.5 ± 2.1 ng/ml ($P < 0.01$). In comparison, at 300 days of age prolactin levels in ganglionectomized animals (mean 50.7 ± 2.5 ng/ml plasma) were significantly higher ($P < 0.001$) than those recorded from non-ganglionectomized lambs (mean 8.8 ± 0.5 ng/ml plasma); in the latter group

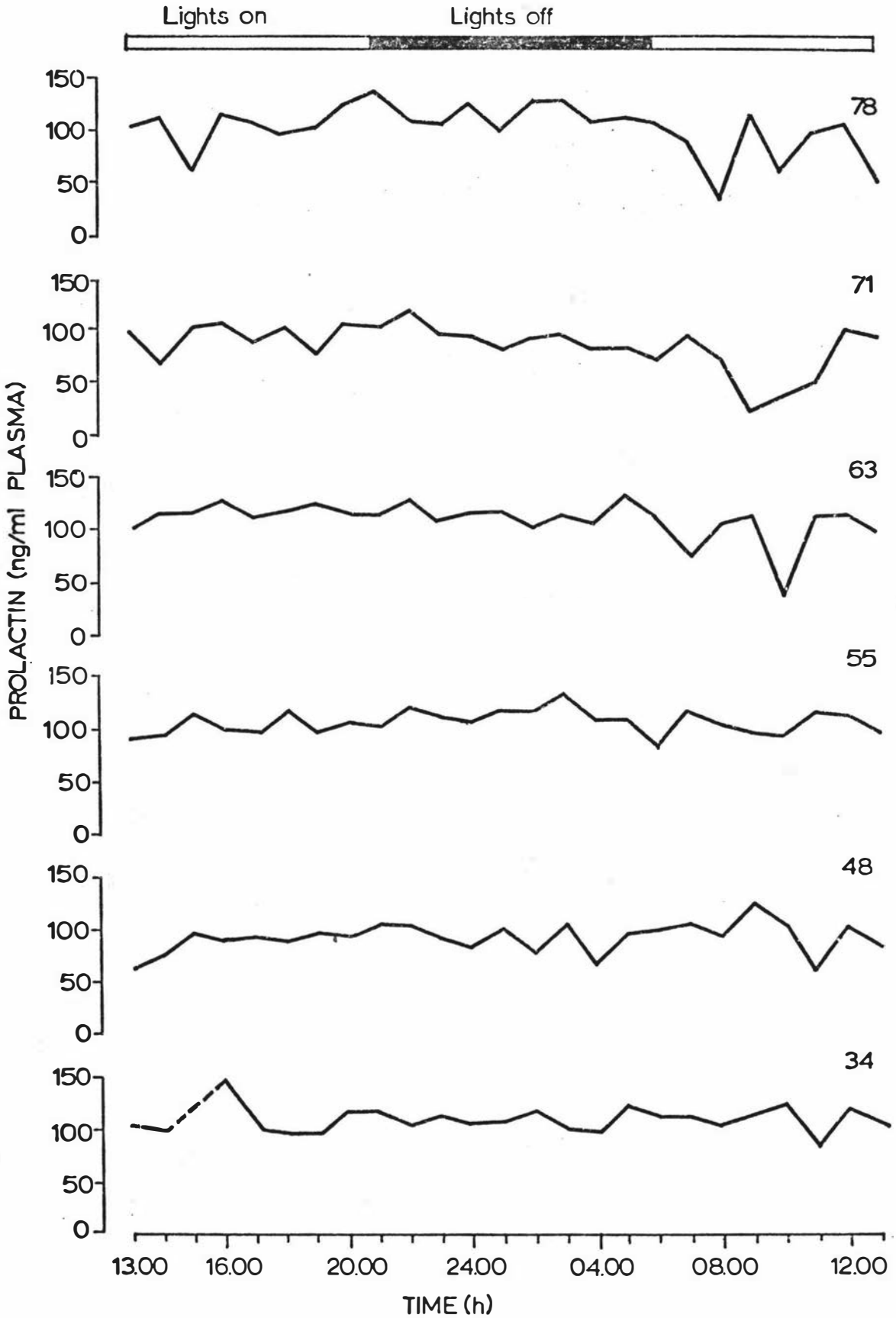

Figure 4.17 : Individual 24 hour profiles of plasma prolactin levels recorded from 6 non-ganglionectomized entire lambs at 100 days of age.

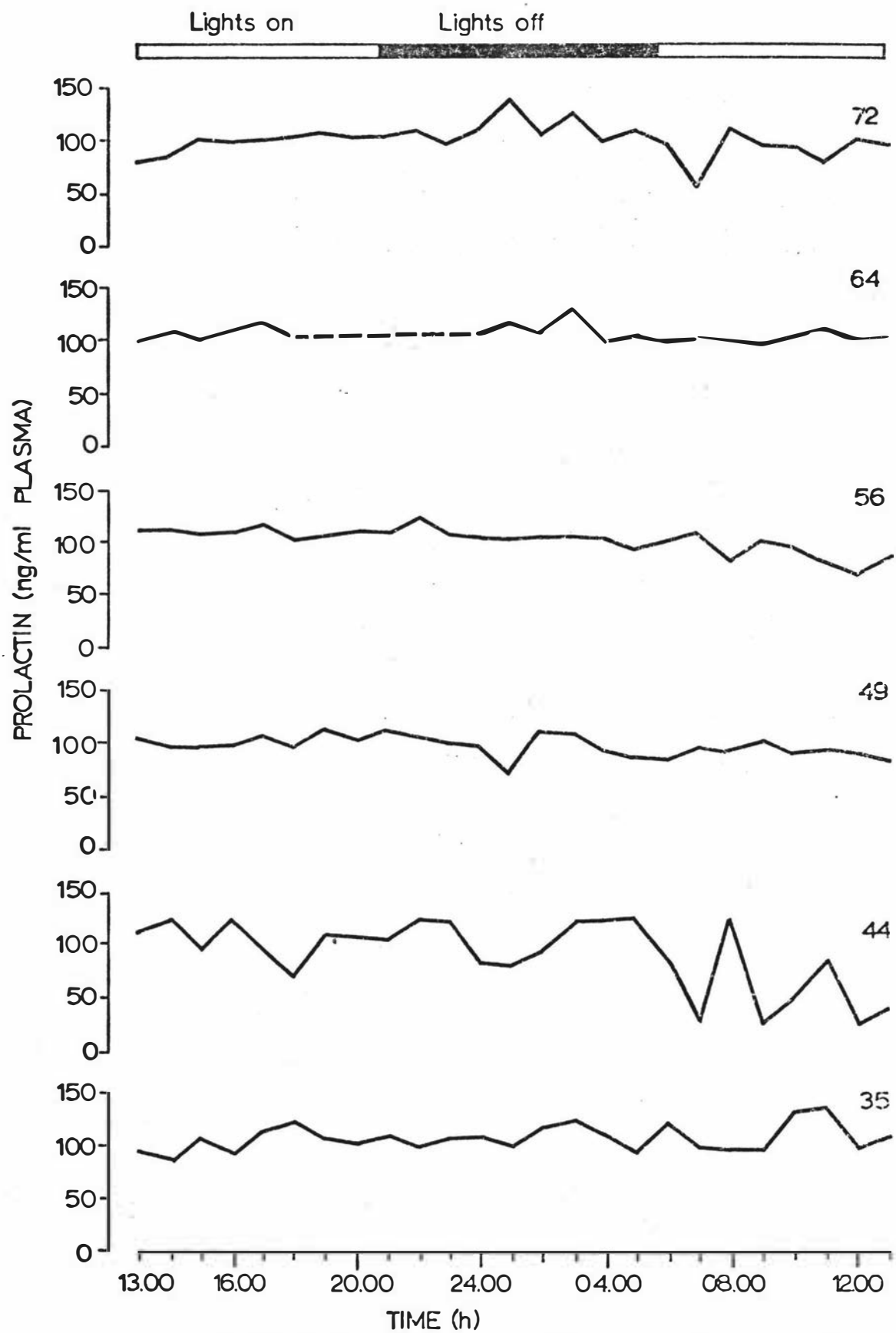

Figure 4.18 : Individual 24 hour profiles of plasma prolactin levels recorded from 6 non-ganglionectomized castrate lambs at 100 days of age.

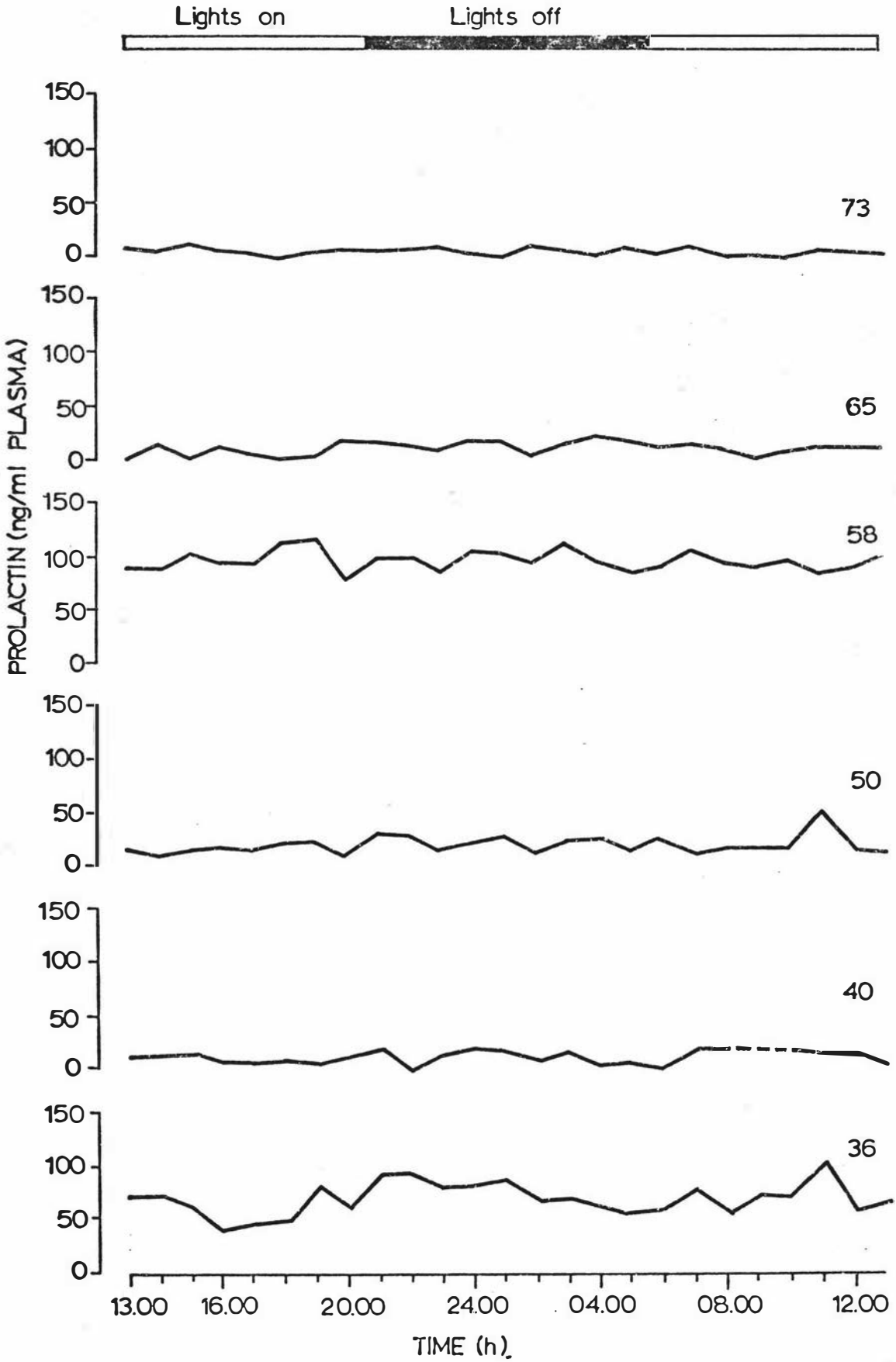

Figure 4.19 : Individual 24 hour profiles of plasma prolactin levels recorded from 6 ganglionectomized entire lambs at 100 days of age.

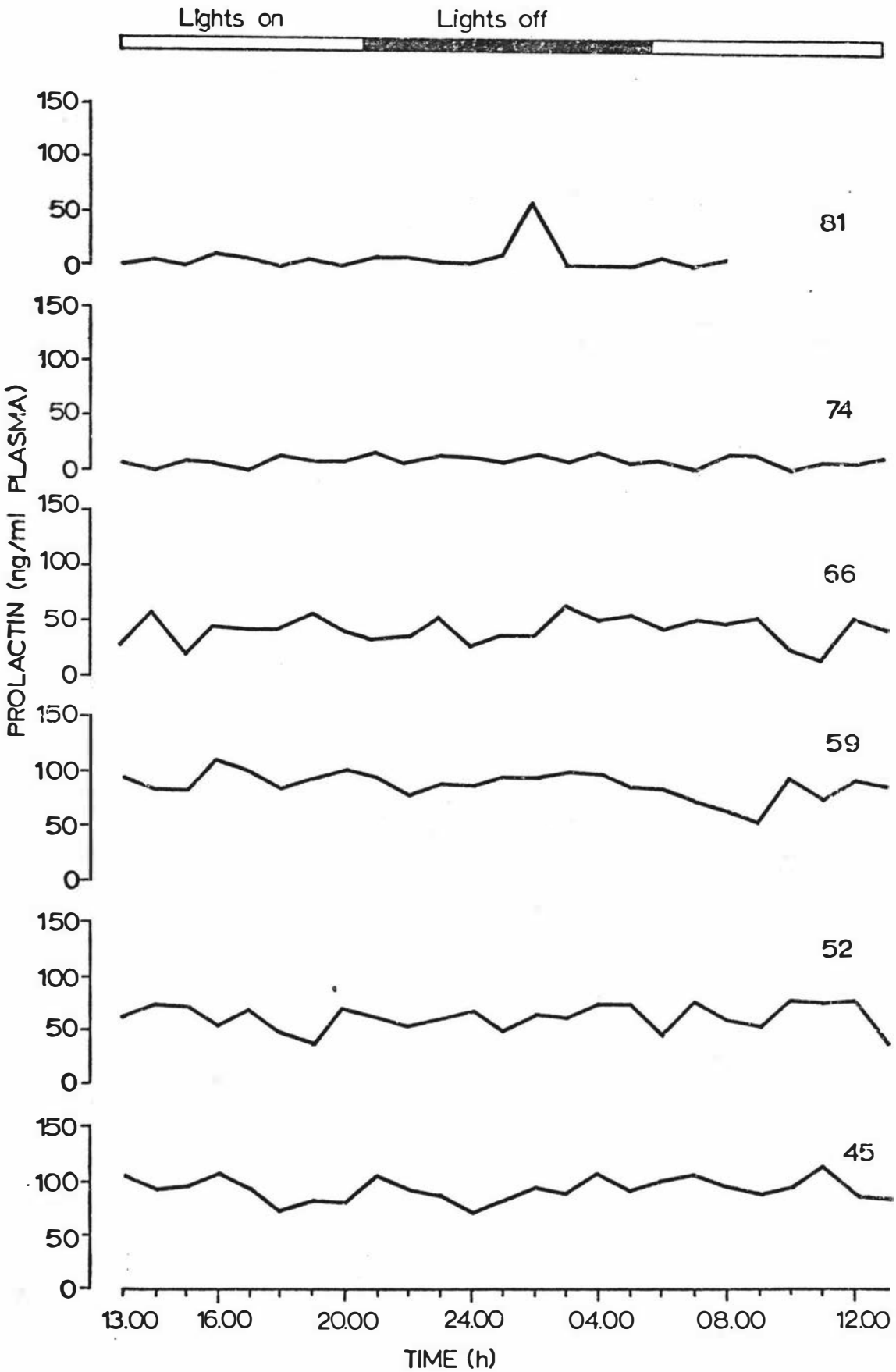

Figure 4.20 : Individual 24 hour profiles of plasma prolactin levels recorded from 6 ganglionectomized castrate lambs at 100 days of age.

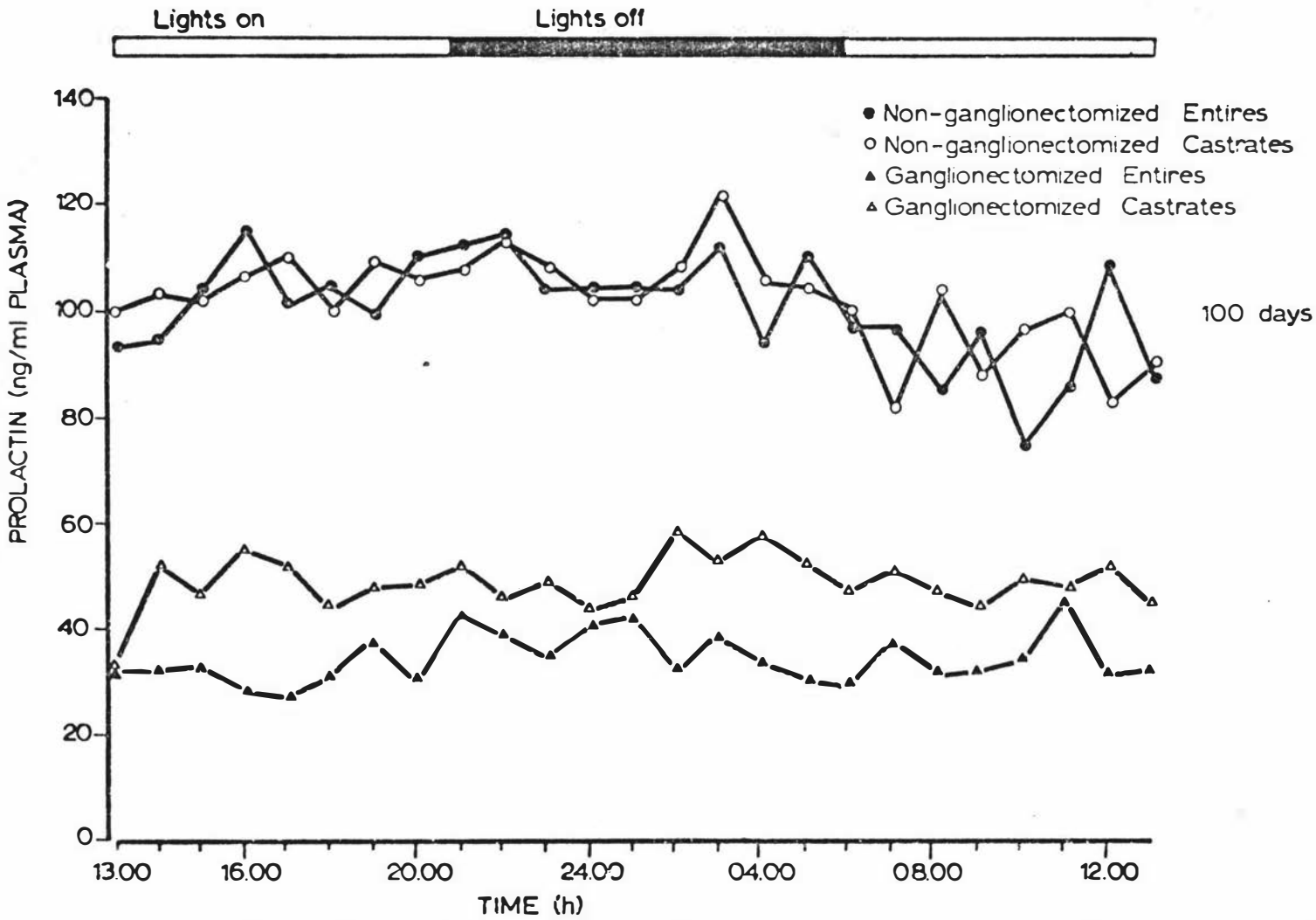

Figure 4.21 : Mean 24 hour plasma prolactin profiles recorded from lambs at 100 days of age.

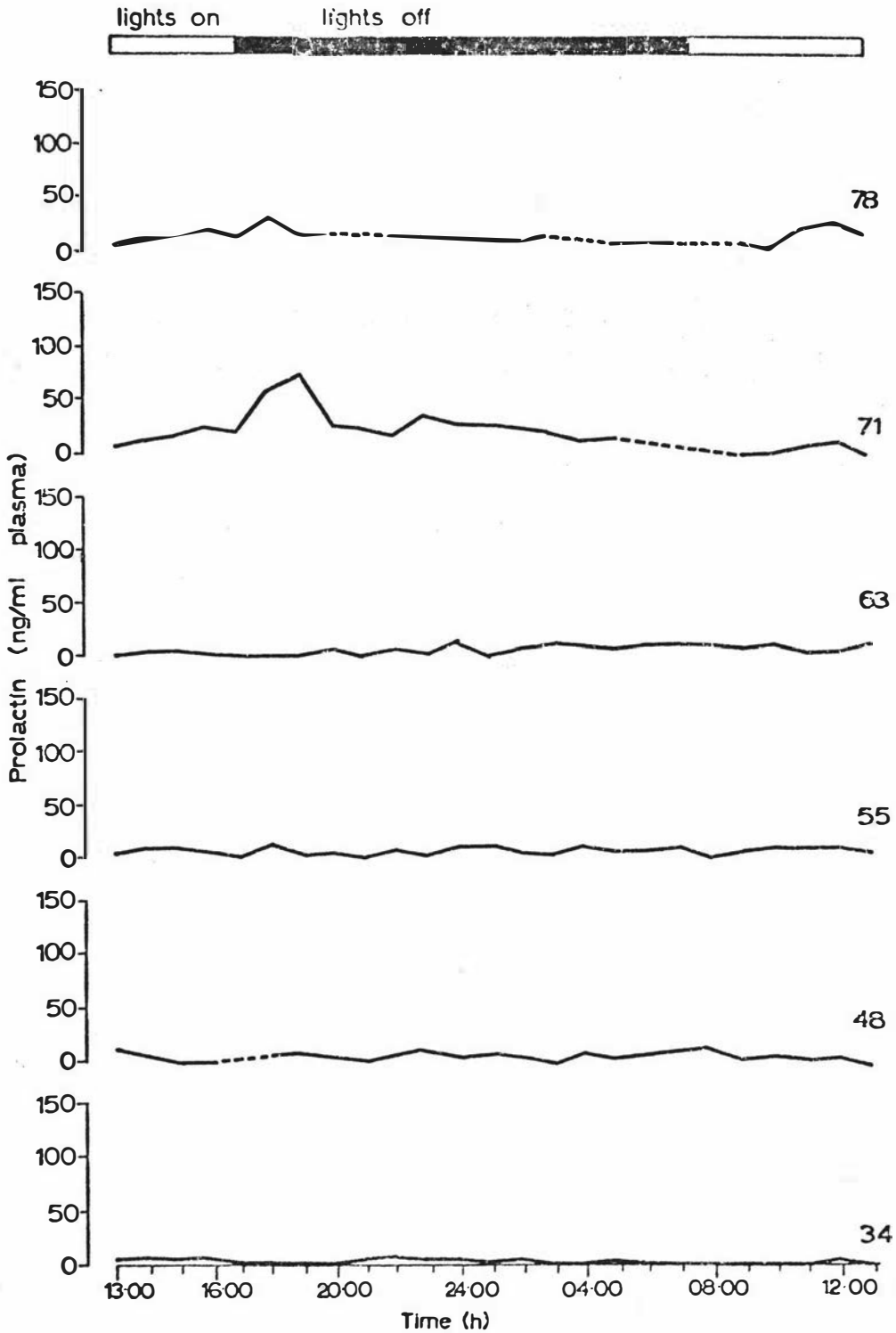

Figure 4.22 : Individual 24 hour profiles of plasma prolactin levels recorded from 6 non-gang-lionectomized entire lambs at 300 days of age.

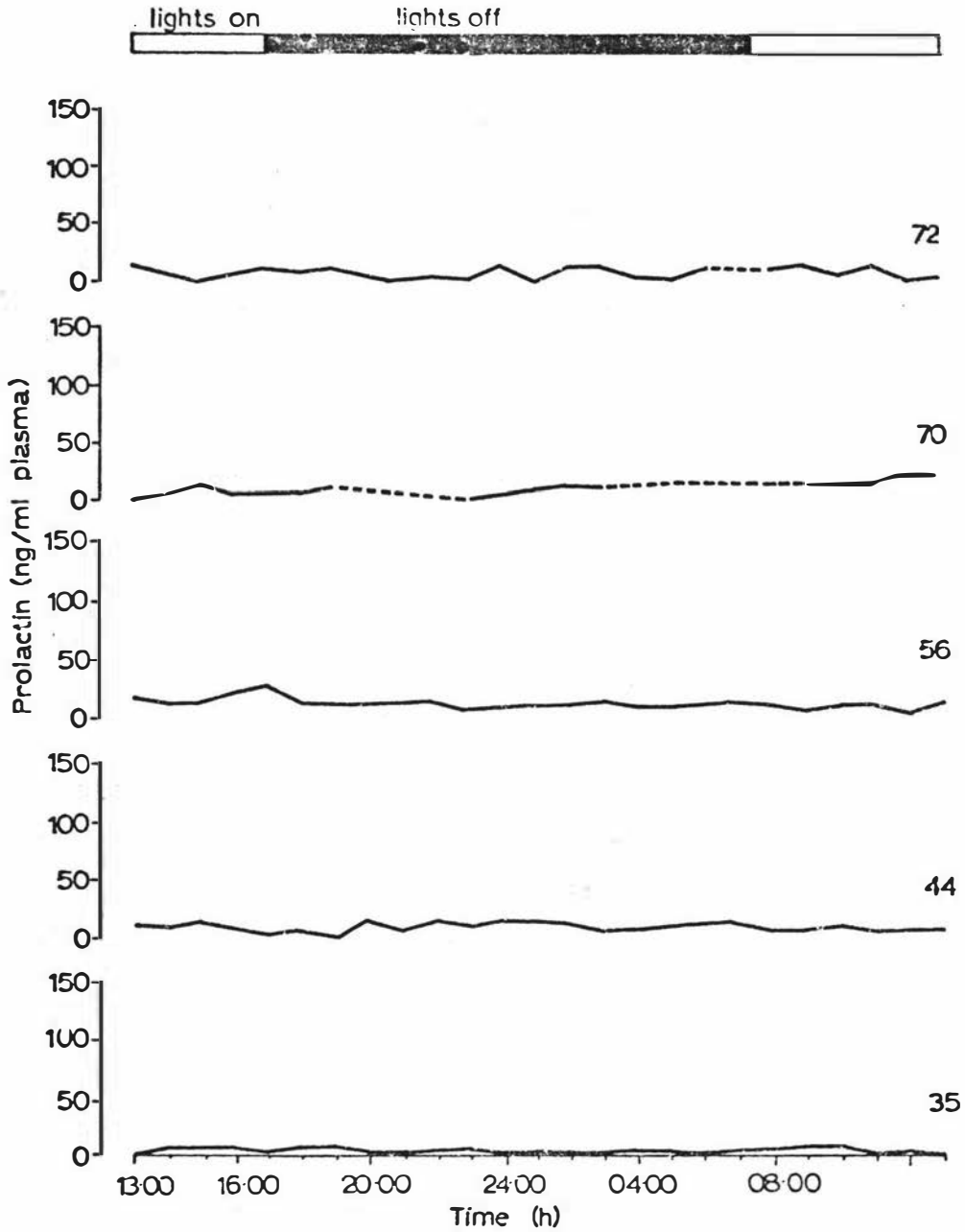

Figure 4.23 : Individual 24 hour profiles of plasma prolactin levels recorded from 5 non-ganglionectomized castrate lambs at 300 days of age.

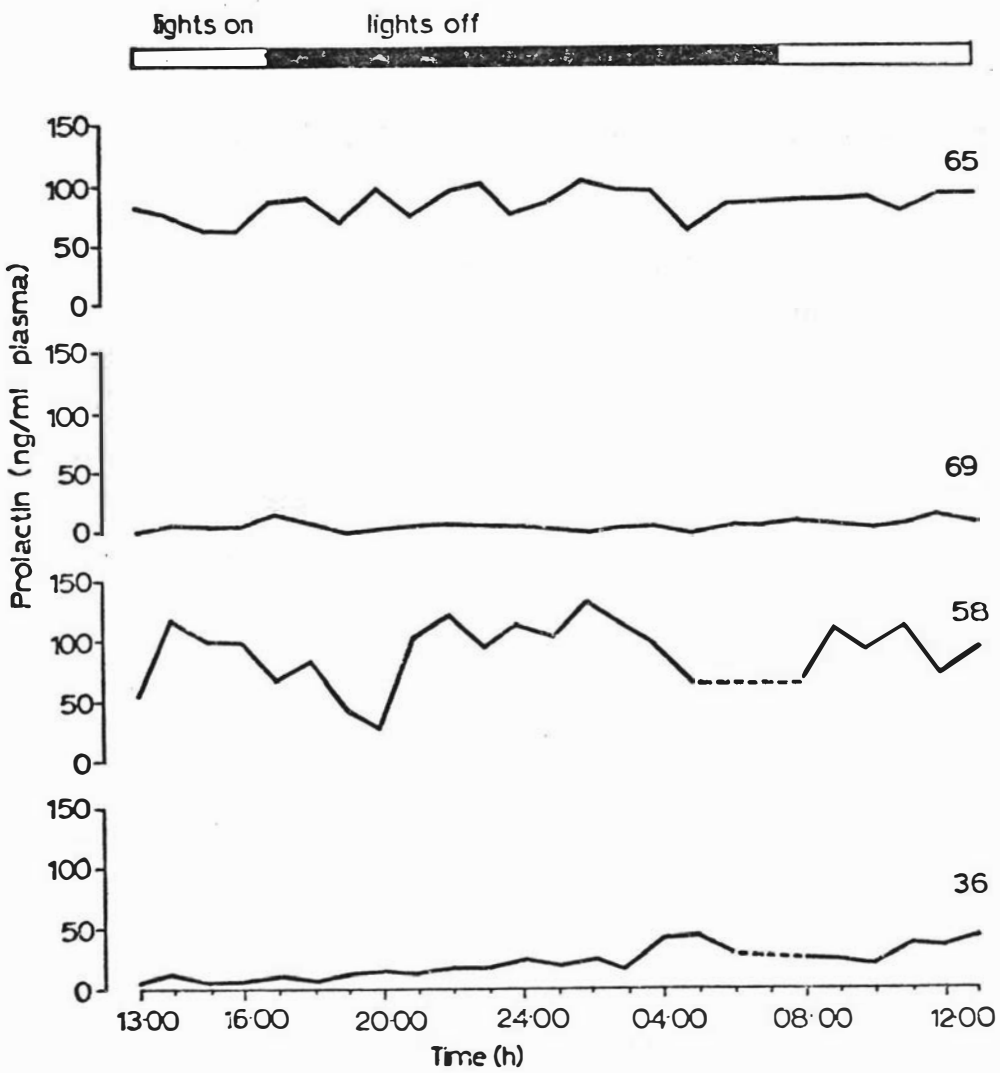

Figure 4.24: Individual 24 hour profiles of plasma prolactin levels recorded from 4 ganglionectomized entire lambs at 300 days of age.

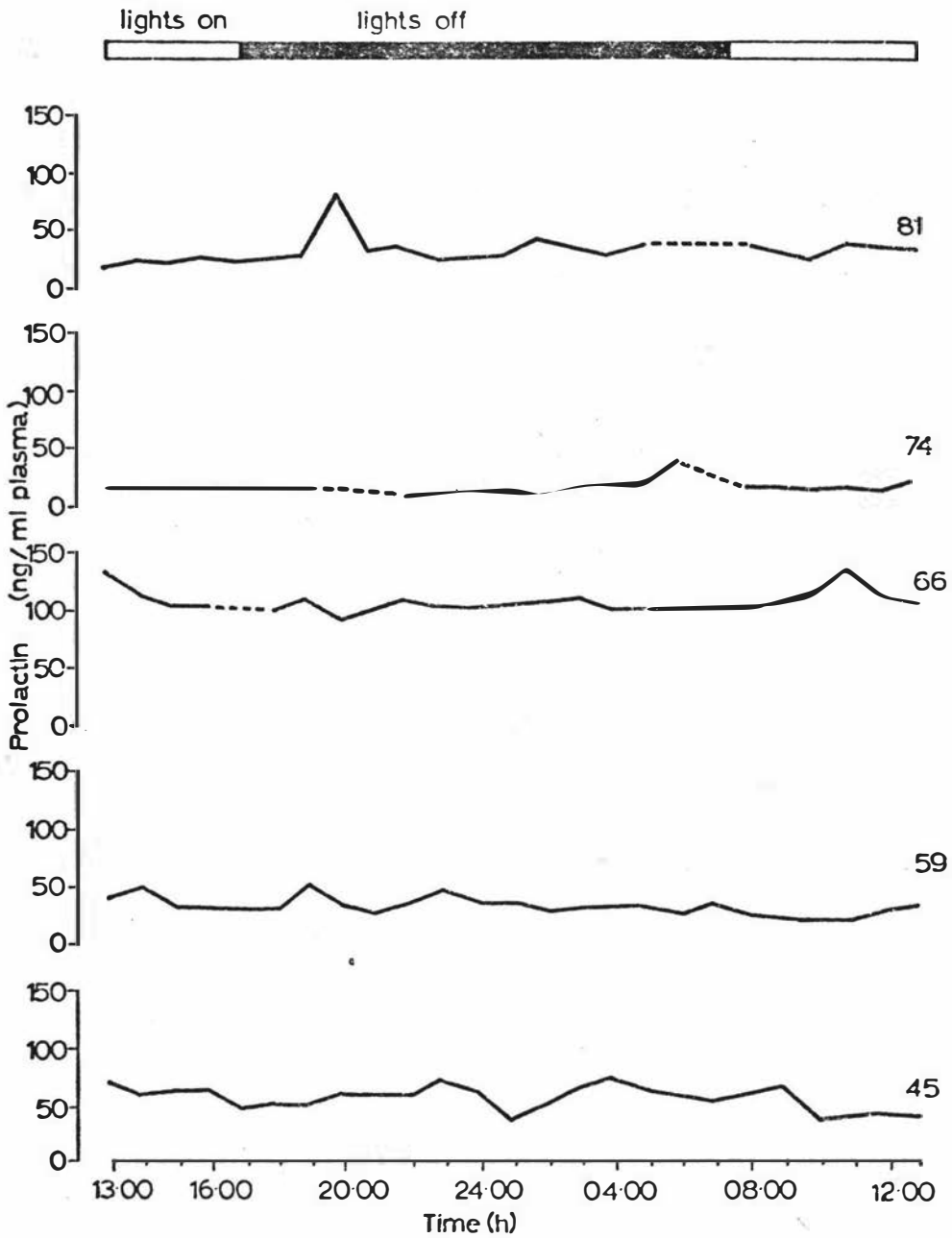

Figure 4.25 : Individual 24 hour profiles of plasma prolactin levels recorded from 5 ganglionectomized castrate lambs at 300 days of age.

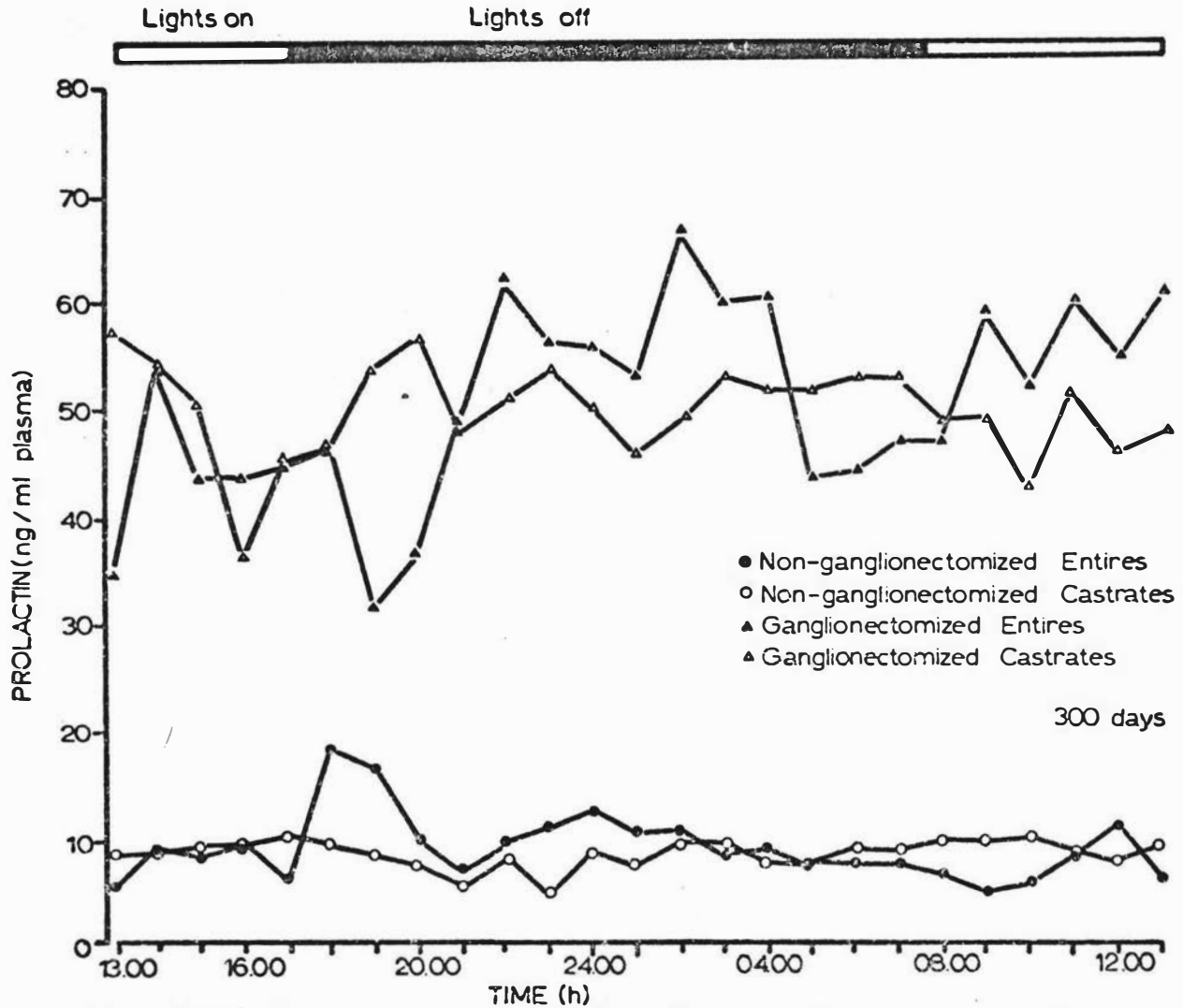

Figure 4.26 : Mean 24 hour plasma prolactin profiles recorded from lambs at 300 days of age.

prolactin concentrations often were below the limit of assay sensitivity.

Castration had no significant influence of prolactin secretion at either age, and there were no trends suggesting any influence. Similarly, the interaction of castration and ganglionectomy was not significant at either age, although at 100 days ganglionectomized entires had lower mean prolactin levels than ganglionectomized castrates (35.1 $\pm$ 2.9 ng/ml *vs* 51.8 $\pm$ 3.0 ng/ml plasma, respectively).

D. DISCUSSION

1. Pulsatile Hormone Secretion

All animals in this experiment showed evidence of episodic or pulsatile patterns of LH and testosterone secretion over the 24 hour sampling periods. However, with the hourly sampling regime utilized pulses of hormone release could not be delineated accurately, thus no attempts have been made to assess the effects of experimental treatments on such parameters of hormone secretion as pulse frequency and pulse height. A more frequent sampling regime was not practicable because of the number of animals utilized in the experiment. Episodic secretion of both these hormones is now clearly established in rams (Katongole *et al.*, 1974; Purvis *et al.*, 1974; Sanford *et al.*, 1974c, 1977; Schanbacher and Ford, 1976; Lincoln, 1976a; Barrell and Lapwood, 1978d; Wilson and Lapwood, 1978a), ram lambs (Foster, 1974; Carr and Land, 1975; Foster *et al.*, 1978; Wilson and Lapwood, 1978b), and wethers (Riggs and Malven, 1974a).

Although episodic LH release probably is determined by a pulsatile pattern of GnRH release from the hypothalamus, research so far has failed to show a direct relationship between peripheral GnRH levels and LH release (Crighton et al., 1973; Nett et al., 1974; Carmel et al., 1976).

In evaluation of the present results distinction could not be made between the effects of seasons and stages of sexual maturity on hormone secretion profiles, particularly because daily photoperiod was not held constant during the course of the experiment. In fact some parameters of pulsatile LH and testosterone secretion (e.g. frequency, amplitude and duration of pulses) have been shown to change with the stage of the photoperiod-induced seasonal cycle (Sanford et al., 1974c; Lincoln, 1976a; Schanbacher and Ford, 1976; Wilson and Lapwood, 1978d), probably resulting in greater basal hormone levels being associated with the autumn sexual season.

In developing ram lambs, Foster et al. (1978) reported that the onset of pulsatile LH secretion occurred between 3 and 7 weeks of age, and depended more on body-weight than chronological age. That study also described an increase in circulating testosterone levels after the first observed LH pulse. On the other hand, Wilson and Lapwood (1978a) found that testosterone secretory pulses did not follow pulsatile LH releases consistently in 6 week old rams, but did so in 14 and 22 week old animals.

Individual prolactin secretion profiles in non-ganglionectomized lambs were consistently high during the

pubertal sampling period (which took place at about the time of the summer solstice) and very low during the post-pubertal sampling period (which occurred at about the time of the winter solstice). This result was consistent with the photoperiod-induced seasonal cycle in prolactin secretion reported by Pelletier (1973), Ravault (1976), Lincoln et al. (1978) and Barrell and Lapwood (1978a,c), and with the results of the previous experiment (Chapter 3). In addition to recording high summer and low winter prolactin levels, Wilson and Lapwood (1978d) reported a relatively stable secretory pattern of prolactin release in adult rams in summer, but a pulsatile prolactin release pattern during winter. Similarly, Davis et al. (1978) found episodic prolactin release in rams in autumn. The hourly blood sampling regime utilized in the present experiment probably was too infrequent to allow accurate detection of prolactin secretory pulses.

2. Circadian Periodicity

A feature of this study was the absence of any clear evidence for circadian variations in the plasma levels of LH, testosterone or prolactin in individual animal secretion profiles, at either the pubertal or post-pubertal samplings. Any important circadian rhythms of hormone secretion should have been most obvious in graphs of mean secretory profiles in which influences of individual-animal secretory pulses were minimized; no such rhythms were recorded. Most previous reports of peripheral plasma hormone concentrations in rams have also failed to show any circadian rhythmicity in secretion

of LH, FSH, testosterone or prolactin (Katongole et al., 1974; Sanford et al., 1974a,c; 1977; Schanbacher and Ford, 1976; Wilson and Lapwood 1978d). On the other hand there are some reports of elevated nocturnal plasma levels of prolactin in sheep (Davis and Borger, 1973; Forbes et al., 1975; Ravault and Ortavant, 1977; Barrell and Lapwood, 1978e; Lincoln et al., 1978), but only a few reports of similar cyclicity in blood concentrations of gonadotrophins or testosterone (Wettemann and Desjardins, 1973; Lincoln et al., 1977).

It has been suggested that the circadian rhythms are determined by daily cycles in lighting and animal activity (Lincoln et al., 1977, 1978; Ravault and Ortavant, 1977; Barrell and Lapwood, 1978d), but the multitude of variations in environmental conditions, breeds, reproductive states and the methods and frequencies of blood collection utilized, makes it difficult to interpret the status of any rhythmicity in reproductive hormone secretion in the ram.

In the present study at least, the absence of any circadian trends in hormone secretion may have been due to the fact that the animals were removed from pasture and housed indoors in individual metabolism crates only 2 to 3 days prior to sampling. The stress of unfamiliarity with the environment may have abolished any usual circadian rhythm. Holley et al. (1975) reported that adult rams took 6 days of cage restraint before elevated and erratic daily plasma cortisol levels were replaced by the normal daily rhythm. A second, but less likely explanation is that as

LH, prolactin and testosterone have half-life's of 30 minutes or less (Geschwind and Dewey, 1968; Foster et al., 1972, 1975; Akbar et al., 1974; Haynes et al., 1976) the frequency of sampling may have precluded any rhythm from being detected.

The significance of any rhythmicity in hormone secretion is unclear, but it may be related to changes in reproductive states such as puberty or seasonality. MacKinnon et al. (1978) described changes in the phase and amplitude of circadian rhythms in FSH, prolactin and corticosterone secretion associated with the onset of puberty in female rats. Also Weitzman et al. (1975) reported that a sleep-related increase in episodic LH secretion first occurred in pubertal children.

The existence of a photosensitive phase in the 24 hour period in rams (Ravault et al., 1976; Garnier et al., 1977a; Ravault and Ortavant, 1977), during which exposure to light stimulates testosterone and diminishes prolactin secretion, suggests that the incidence of circadian hormone secretion may reflect the stage of the photoperiod-induced seasonal sexual cycle.

3. Castration

As expected, castration significantly elevated LH levels throughout the 24 hour sampling period, both in non-ganglionectomized and ganglionectomized animals at both ages. This result concurs with those of Pelletier (1968) and Foster (1974) who showed a rise in LH levels in adult rams and newborn ram lambs respectively, following castration. Normal testicular inhibition of LH secretion

has been attributed to two different actions of testosterone, one being an inhibition of pituitary responsiveness to GnRH and the second an inhibition of hypothalamic GnRH synthesis (Pelletier, 1970, 1974; Garnier et al., 1977b).

The increasing intensity of testicular steroid regulation of LH secretion in developing ram lambs is shown by the significant lowering of LH levels in entires but not castrate lambs at 300 days compared with 100 days of age, while at the same time plasma testosterone levels in entires had increased, but not significantly. This result also tends to confirm the elevated LH levels seen in non-ganglionectomized entires between 8 and 13 weeks of age described in Chapter 3, although the profile study was conducted at over 14 weeks of age when LH levels were falling.

Plasma prolactin profiles were not significantly influenced by castration at either age and this result, similar to that recorded in the longitudinal study (Chapter 3), is consistent with reports that prolactin secretion is not influenced by either endogenous or exogenous androgens in rams, or by exogenous androgens in wethers (Pelletier, 1973; Ravault et al., 1977a). However, Davis et al. (1978) did report that large doses of androgens or oestrogens could increase prolactin secretion in wethers.

4. Cranial Cervical Ganglionectomy

Cranial cervical ganglionectomy elevated LH levels at the post-pubertal sampling. This effect was due entirely

to the castrates, the two groups of entire animals having identical mean LH concentrations (0.41 ng/ml).

A previous study at this laboratory showed that pinealectomy of adult rams significantly elevated mean LH levels in acute secretion profile studies (Barrell and Lapwood, 1978e) and induced similar trends in testosterone levels.

Since ganglionectomy had no effect on LH secretion at the pubertal sampling it is possible that the pineal gland of young male lambs does not have a significant influence on LH secretion at that age, in contrast to the situation in post-pubertal and adult animals. Alternatively, during summer when the long daily photoperiod is thought to suppress pineal antigonadotrophic function (Reiter, 1977), any effect of ganglionectomy on LH secretion may be expected to be minimal.

The lack of effect of ganglionectomy on testosterone secretion was consistent with previous reports of altered LH, but not testosterone secretion, both in ganglionectomized and pinealectomized rams (Barrell and Lapwood, 1978b,e). These differential effects of the pineal on LH and testosterone secretion probably reflect the fact that the testes are more remote from the pineal, both anatomically and physiologically, than is the pituitary.

In comparison, ganglionectomy had a most marked influence on the 24 hour plasma profiles of prolactin, disrupting the normal seasonal variation of high levels during the summer months and low concentrations during

winter. This result was more clear cut than that recorded in the longitudinal study described in Chapter 3.

Length of the daily photoperiod is the most important regulator of prolactin secretion in rams (Pelletier, 1973; Lincoln et al., 1978; Barrell and Lapwood, 1978c,d). Pinealectomy abolished seasonal and circadian rhythms in plasma prolactin levels in adult rams (Barrell and Lapwood, 1978c,e) and similarly removed the effects of daylength on prolactin secretion in castrated lambs (Forbes, 1975; Brown et al., 1977). The present experiment provides further evidence that the pineal gland modulates the seasonality of prolactin secretion in rams.

There are no reports in the literature which have compared the effects of pineal function on entire and castrate domestic animals. However, in male rats, pinealectomy lowered serum prolactin levels both in castrate and intact animals, indicating that the stimulatory effect of the pineal gland on prolactin secretion was not dependent on the presence of the testes (Rønnekleiv and McCann, 1975). Similarly, plasma FSH and LH levels were not significantly influenced by pinealectomy in castrated rats (Talbot and Reiter, 1973/74; Slama-Scemama, 1976). However, Cardinali and his colleagues (see review by Cardinali and Vacas, 1978) have reported that pituitary and gonadal hormones may be involved in the regulation of pineal function by the operation of a feedback mechanism in rats.

CHAPTER V

THE EFFECTS OF CRANIAL CERVICAL GANGLIONECTOMY ON PITUITARY LH AND GONADAL TESTOSTERONE RESPONSES, AND OF CASTRATION ON PITUITARY LH RESPONSES, TO EXOGENOUS GnRH IN PUBERTAL AND POST-PUBERTAL MALE LAMBS

A. INTRODUCTION

Administration of synthetic GnRH induces release of LH in adult rams and wethers (Reeves et al., 1970; Hopkinson et al., 1974; Galloway and Pelletier, 1975; Pelletier, 1976; Stelmasiak and Galloway, 1977; Wilson and Lapwood, 1978e), ram lambs (Galloway and Pelltier, 1974; Lee et al., 1976b; Wilson and Lapwood, 1978b), and neonatal and foetal lambs (Foster et al., 1972; Foster, 1974). Changes in sensitivity of the pituitary to hypothalamic releasing hormones, as well as increasing steroidogenic capacity of the testes, may be part of the sexual maturational process (Odell and Swerdloff, 1976). In ram lambs maximal pituitary LH responses to exogenous GnRH occur between 2 and 3 months after birth (Lee et al., 1976b; Wilson and Lapwood, 1978b), while the testosterone response increases progressively with age, at least until 32 weeks (Wilson and Lapwood, 1978b).

This experiment was designed to supplement the study described in the previous chapter by investigating the influence of cranial cervical ganglionectomy on pituitary LH and gonadal testosterone responses, and of castration on pituitary LH responses, following synthetic GnRH administration to pubertal and post-pubertal male lambs.

B. MATERIALS AND METHODS

One hour after completion of the 24 hour profile sample collections described in Chapter 4, 10 µg of synthetic GnRH was administered via the venous cannulae. Blood samples were taken via the cannulae immediately prior to GnRH administration (time 0) then 30, 60, 90, 120, 180, 240, 360 and 480 minutes later. Constant artificial lighting was provided throughout the period of sample collection.

The total output of hormone secreted was calculated as the area under hormone response curves and expressed as ng/ml.hours (ng/ml.h). Statistical analyses of basal and peak hormone levels, and of total hormone responses, consisted of Student's t-test evaluations of the effects of castration, ganglionectomy, and their interaction, on logarithm transformed data. In addition, for lambs in each treatment group, t-tests were utilized to assess the effects of age on the above parameters.

C. RESULTS

See Tables 5.1 and 5.2

1. LH

Individual LH responses of pubertal lambs to exogenous GnRH are shown in Figures 5.1 to 5.4 with the mean responses for each treatment group shown in Figure 5.5. Similarly, the post-pubertal responses are shown in Figures 5.6 to 5.10.

Pre-injection or basal LH levels in castrate lambs were greater than in entires at both 100 days

Table 5.1 Mean (± S.E.M.) basal hormone levels, and peak and total hormonal responses to GnRH injection.

Treatment	Age (days)	Basal Levels (ng/ml)		Peak Levels (ng/ml)		Total Responses (ng/ml.h)	
		100	300	100	300	100	300
				Luteinizing Hormone			
Entire							
Non-ganglionectomized		6.85 ± 2.22	1.50 ± 0.53	27.64 ± 5.80	17.65 ± 2.63	43.53 ± 10.85	36.66 ± 5.93
Ganglionectomized		1.23 ± 0.67	0.95 ± 0.11	28.69 ± 4.61	21.56 ± 4.59	54.10 ± 11.05	52.31 ± 10.99
Castrate							
Non-ganglionectomized		11.14 ± 2.49	10.90 ± 1.49	57.74 ± 6.48	69.84 ± 7.91	125.09 ± 17.69	158.17 ± 19.51
Ganglionectomized		8.29 ± 2.0	18.88 ± 1.71	62.76 ± 0.14	80.67 ± 1.64	147.32 ± 22.94	213.38 ± 7.20
				Testosterone			
Entire							
Non-ganglionectomized		0.34 ± 0.10	0.63 ± 0.17	2.76 ± 0.14	7.70 ± 1.19	6.16 ± 0.68	22.92 ± 3.74
Ganglionectomized		0.40 ± 0.15	0.67 ± 0.18	2.81 ± 0.36	5.62 ± 0.91	7.30 ± 1.34	18.59 ± 3.80

Table 5.2 Summary of t-test analyses of LH and testosterone responses to GnRH.

	Basal Levels		Peak Levels		Total Output	
Age (days)	100	300	100	300	100	300
Treatment			**Luteinizing Hormone**			
A. Entire vs Castrate	3.605** (22)	7.718*** (20)	3.413*** (22)	8.557*** (20)	6.800*** (22)	8.980*** (20)
B. Non-gangX vs GangX	0.788 (22)	1.197 (20)	0.065 (22)	0.906 (20)	0.765 (22)	0.799 (20)
C. Interaction AxB	1.697 (20)	5.226*** (18)	0.114 (20)	0.297 (18)	1.125 (20)	0.407 (18)
D. Age (100 vs 300 days)						
(i) Non-gangX Entires	2.973* (11)		1.120 (11)		0.012 (11)	
(ii) Non-gangX Castrates	0.286 (10)		0.967 (10)		1.352 (10)	
(iii) GangX Entires	0.412 (10)		0.870 (10)		0.093 (10)	
(iv) GangX Castrates	2.609* (9)		1.287 (9)		2.304* (9)	
			Testosterone			
A. Non-gangX Entires vs GangX Entires	0.359 (10)	0.147 (11)	0.761 (10)	1.337 (11)	0.688 (10)	0.653 (11)
B. Age (100 vs 300 days)						
(i) Non-gangX Entires	1.380 (11)		5.383*** (11)		5.895*** (11)	
(ii) GangX Entires	1.101 (10)		2.560* (10)		2.700* (10)	

***P<0.001, **P<0.01, *P<0.05

Note : Figures in brackets are the numbers of degrees of freedom for each contrast.

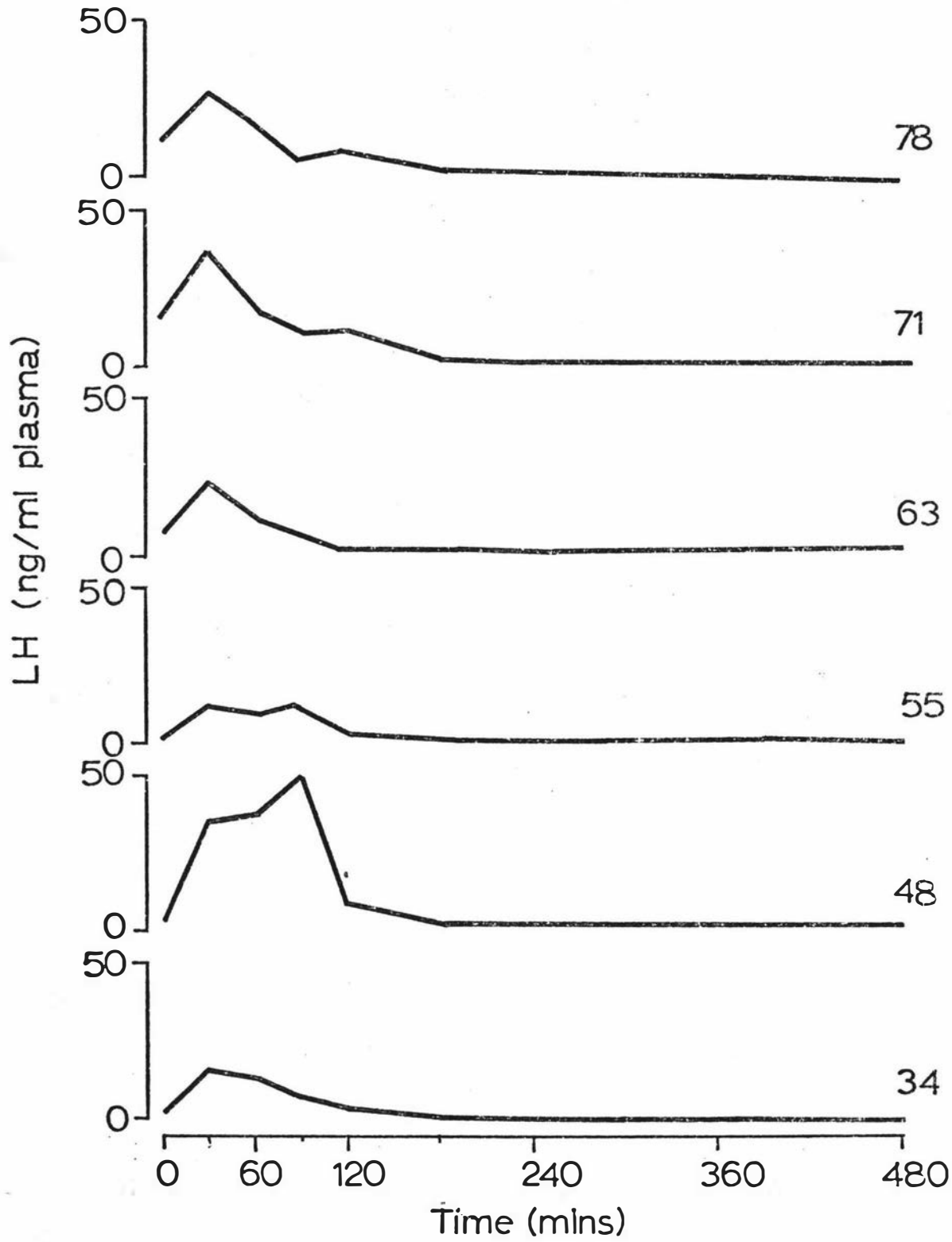

Figure 5.1 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 non-ganglionectomized entire lambs at 100 days of age.

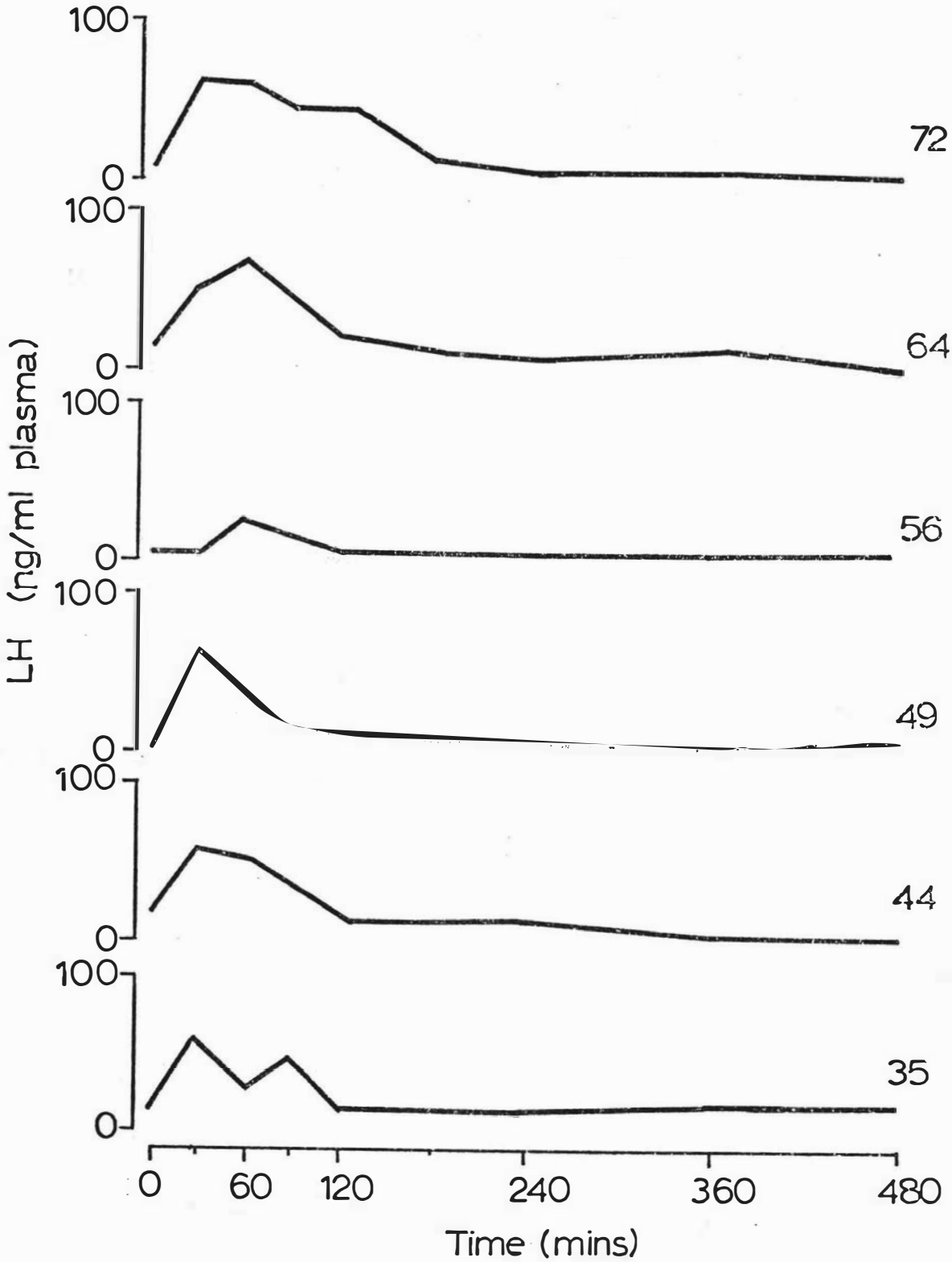

Figure 5.2 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 non-ganglionectomized castrate lambs at 100 days of age.

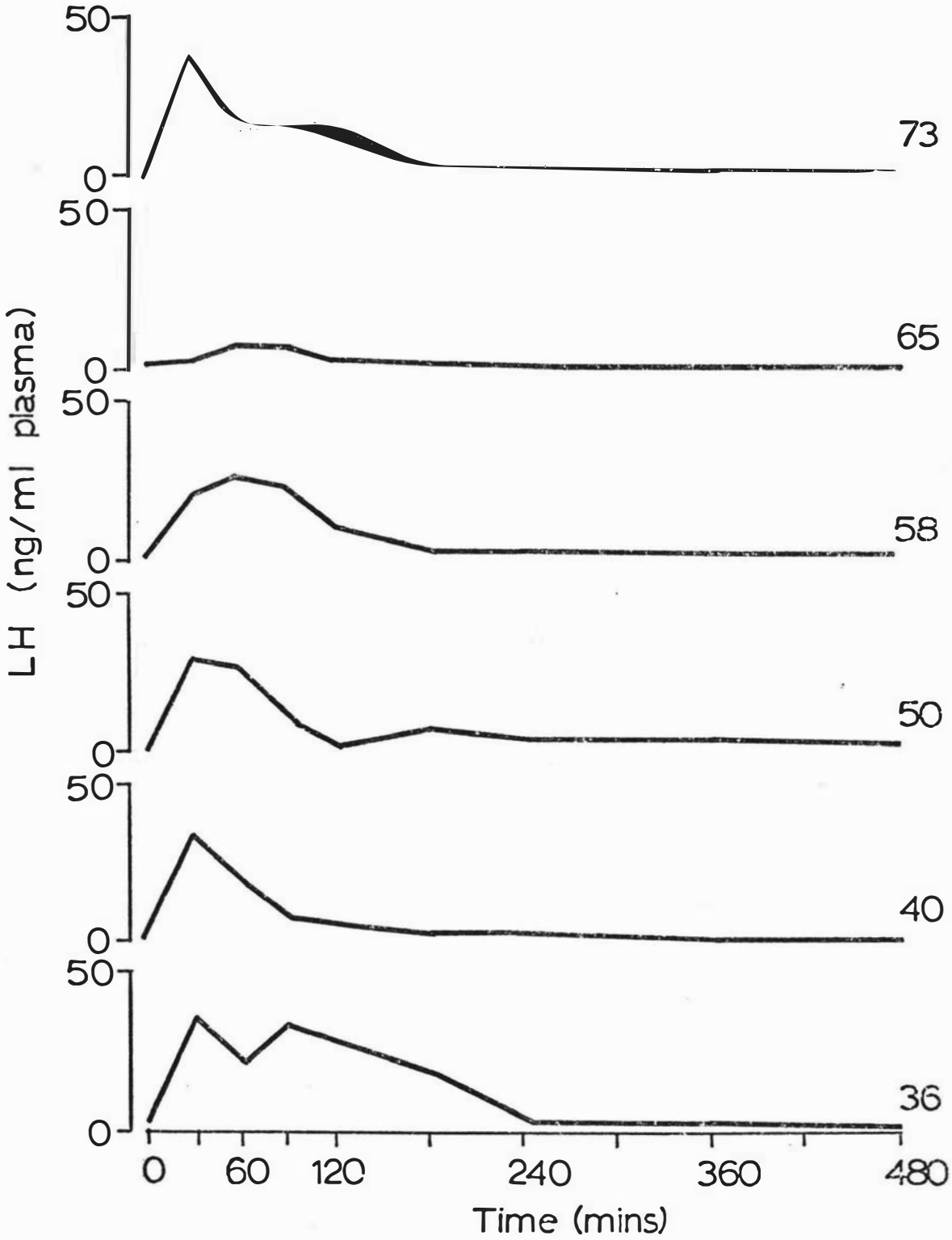

Figure 5.3 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 ganglionectomized entire lambs at 100 days of age.

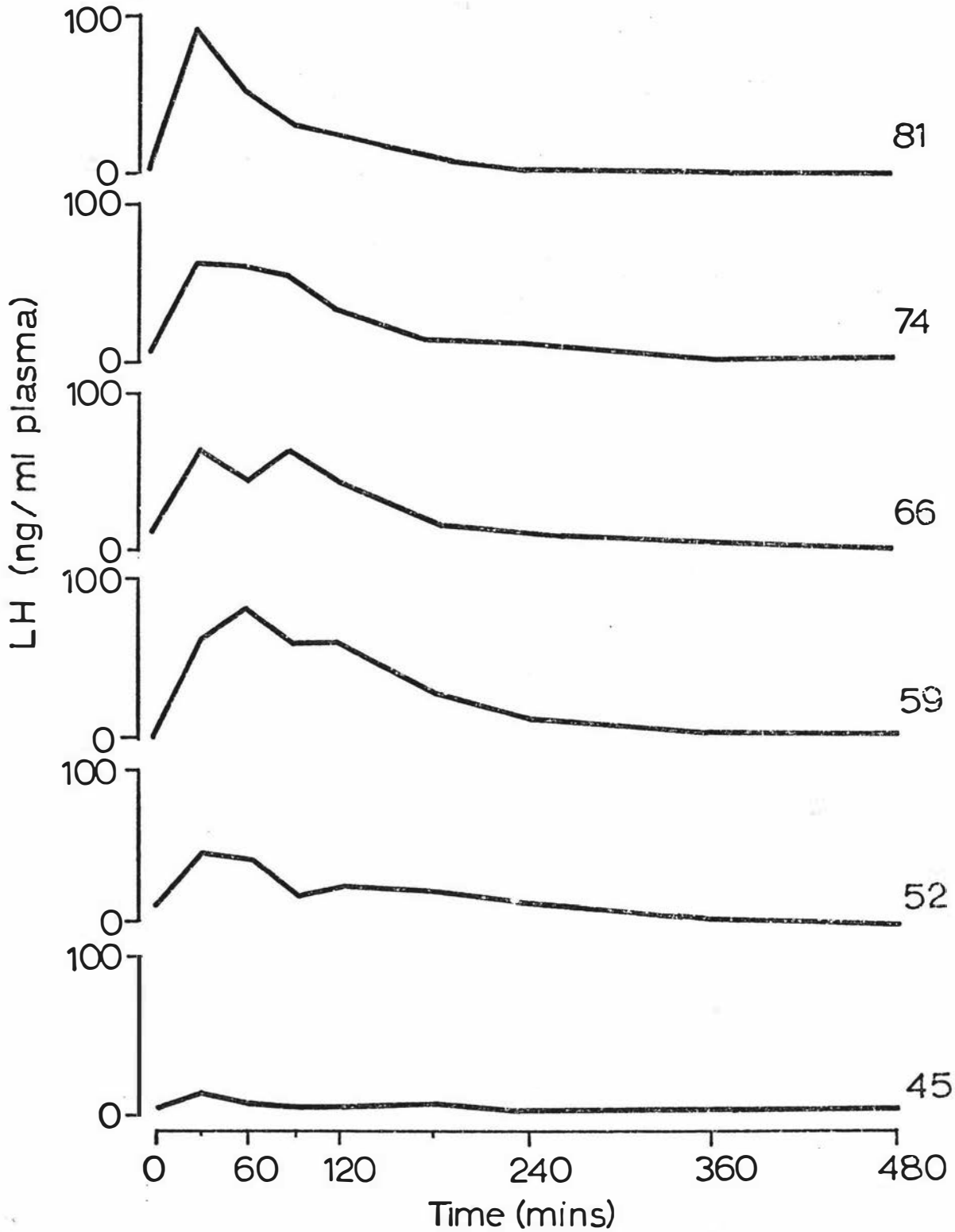

Figure 5.4 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 ganglionectomized castrate lambs at 100 days of age.

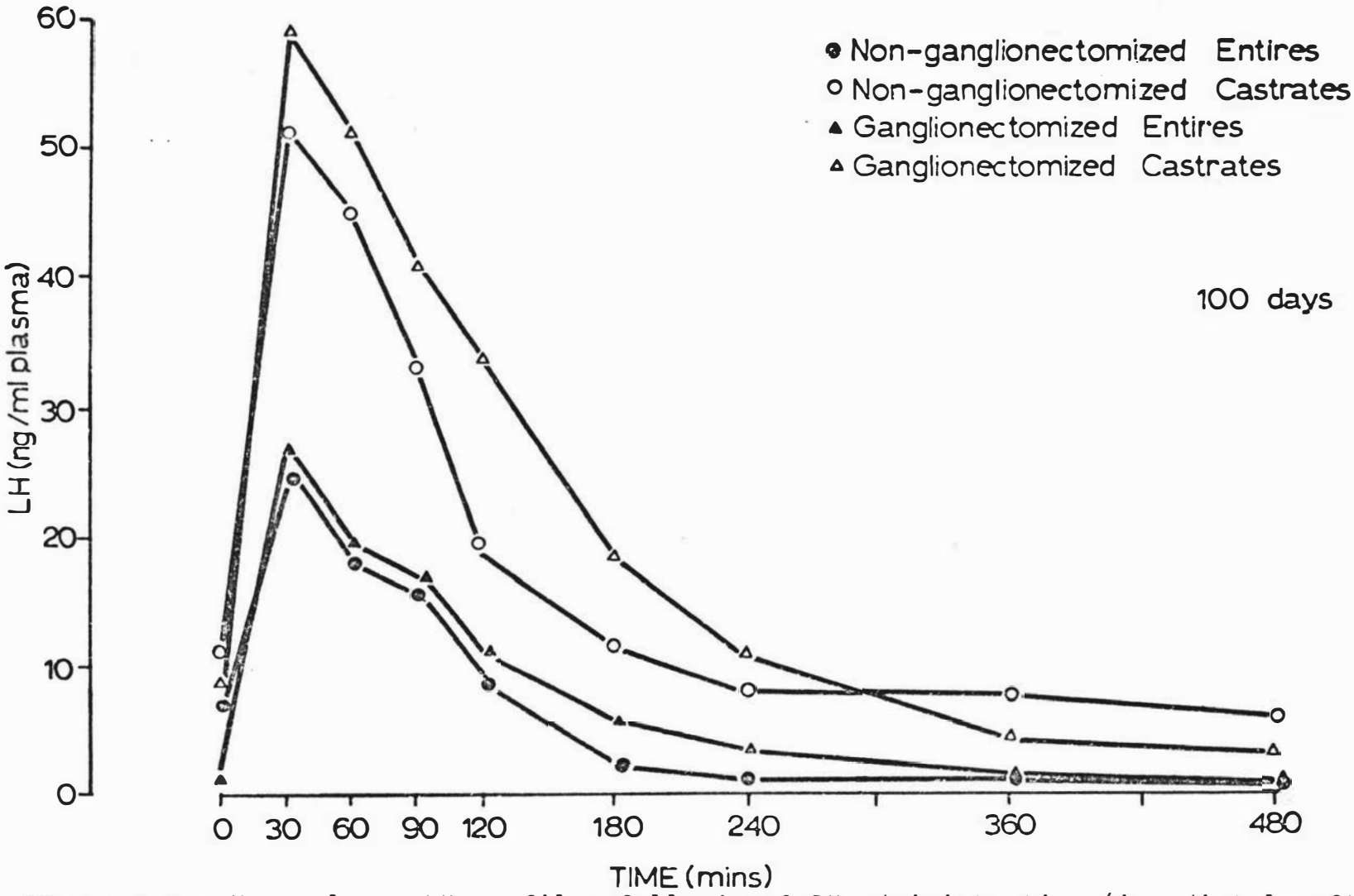

Figure 5.5 : Mean plasma LH profiles following GnRH administration (immediately after the zero min sample) to lambs at 100 days of age.

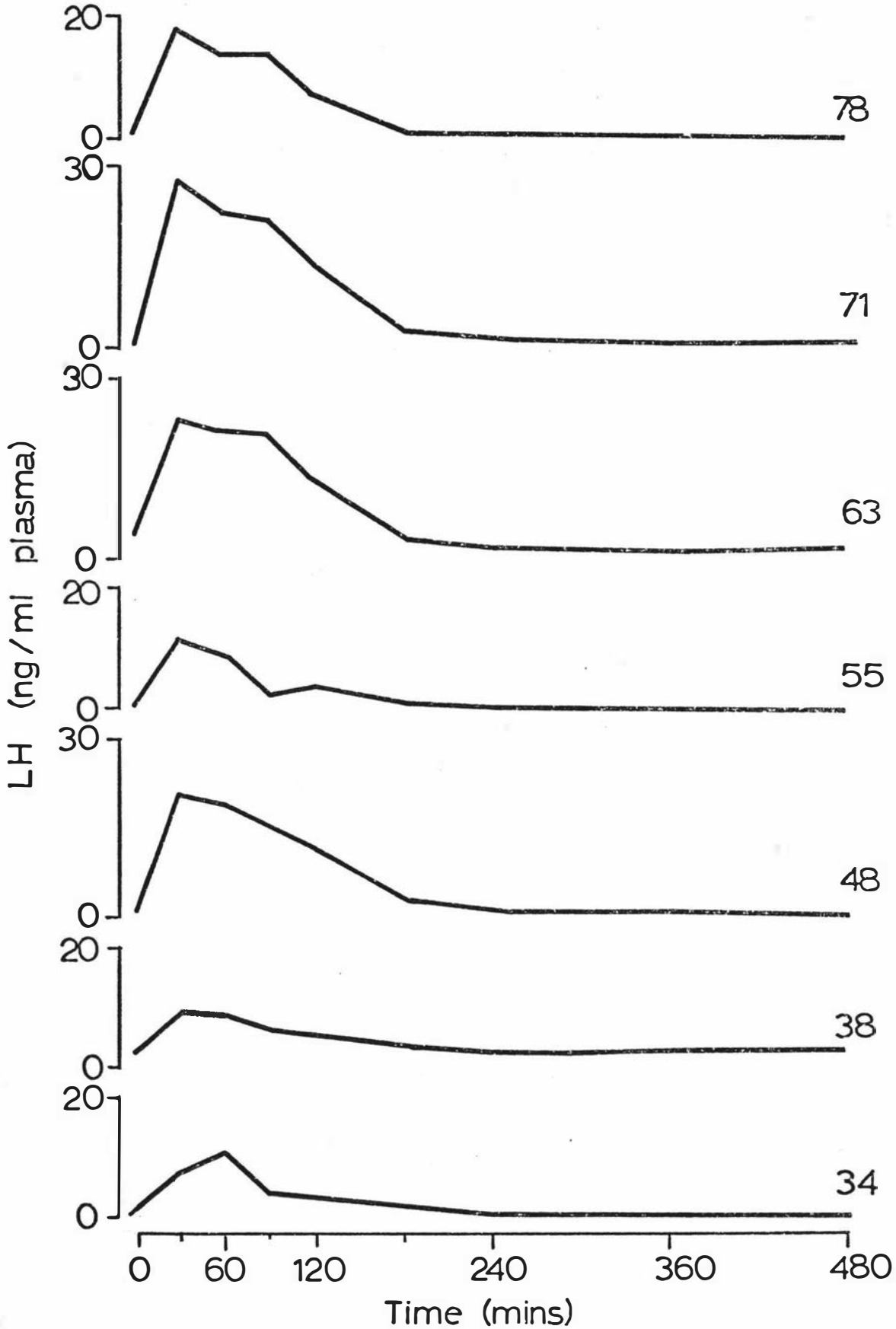

Figure 5.6 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 7 non-ganglionectomized entire lambs at 300 days of age.

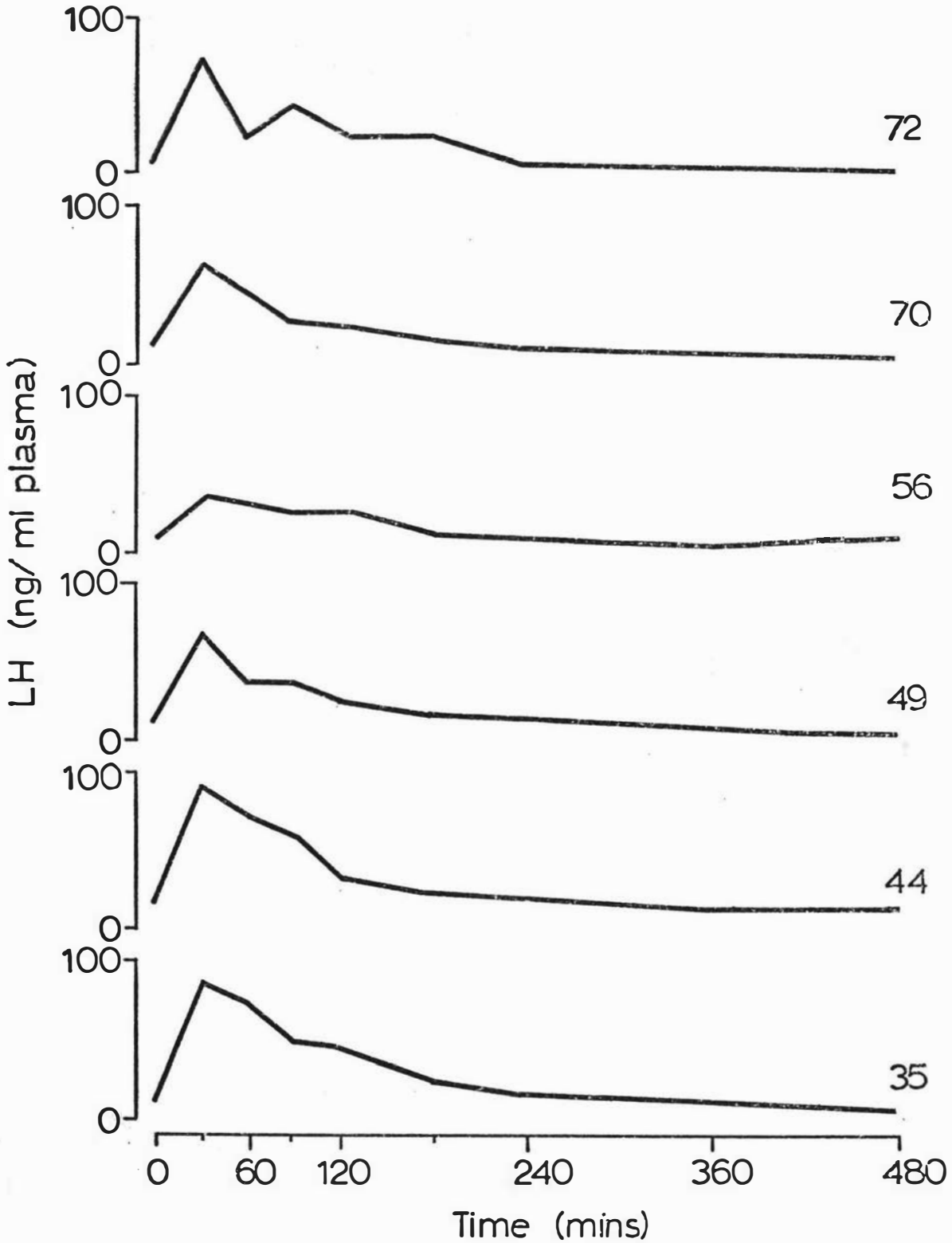

Figure 5.7 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 non-ganglionectomized castrate lambs at 300 days of age.

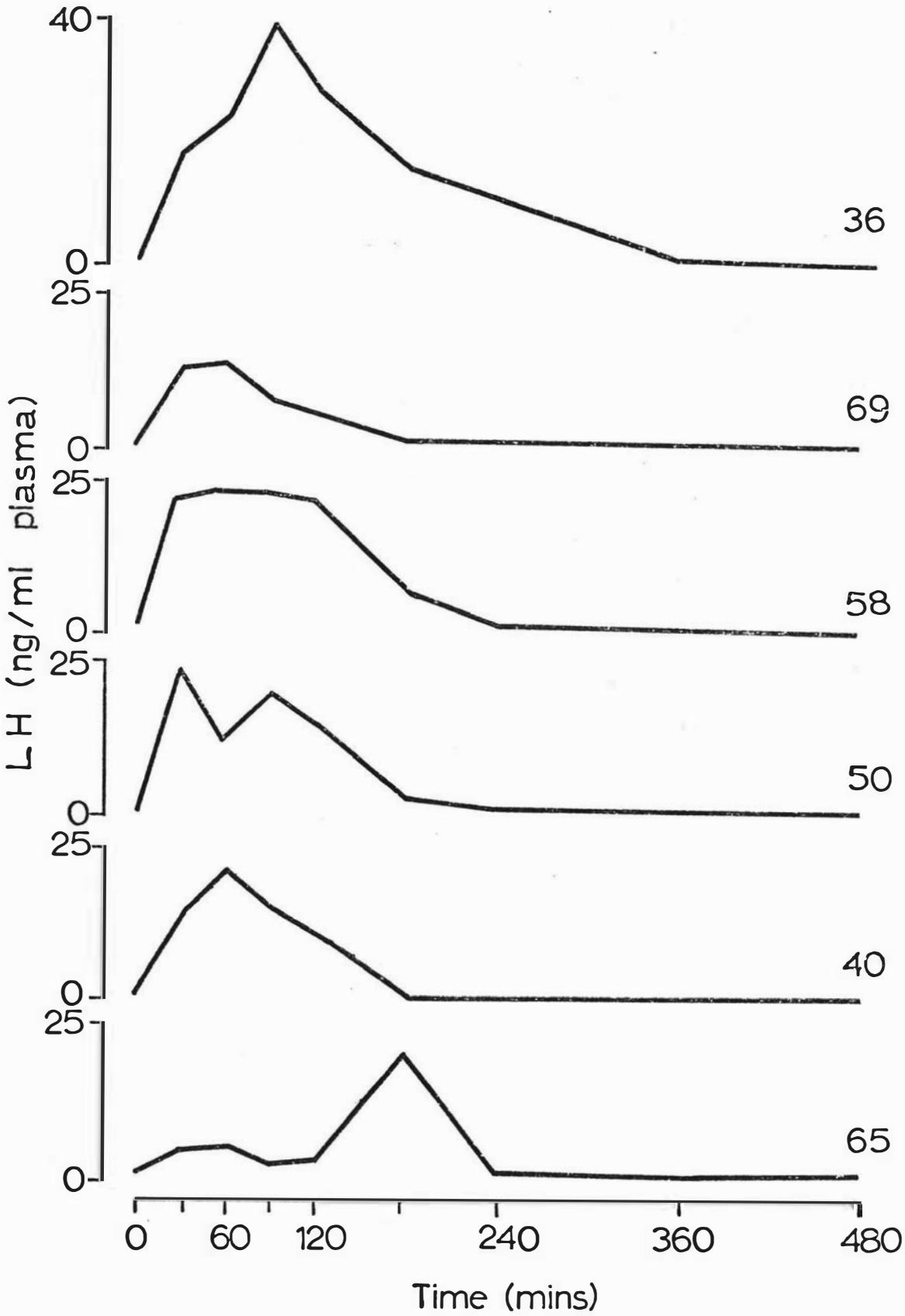

Figure 5.8 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 ganglionectomized entire lambs at 300 days of age.

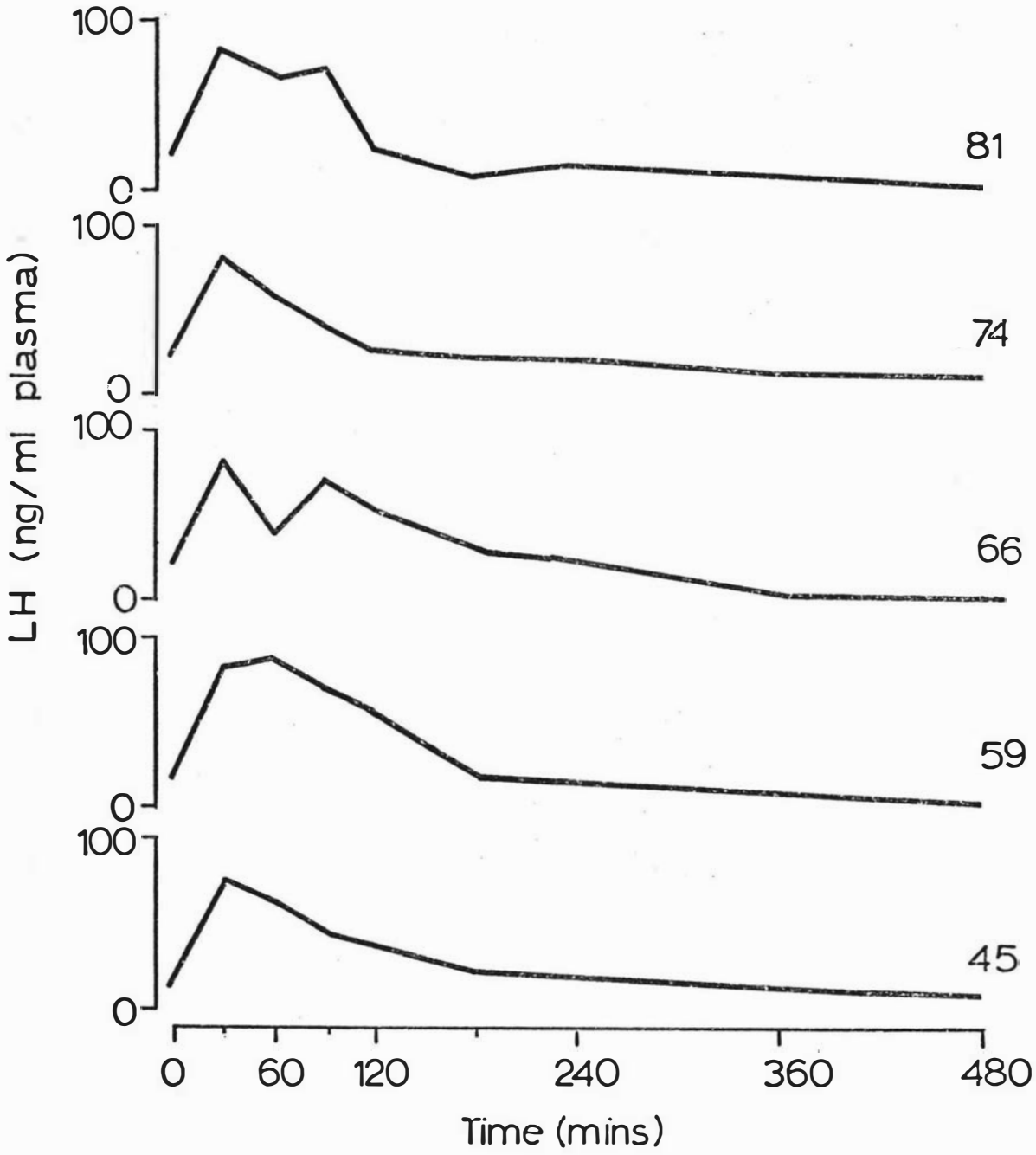

Figure 5.9 : Plasma LH profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 5 ganglionectomized castrate lambs at 300 days of age.

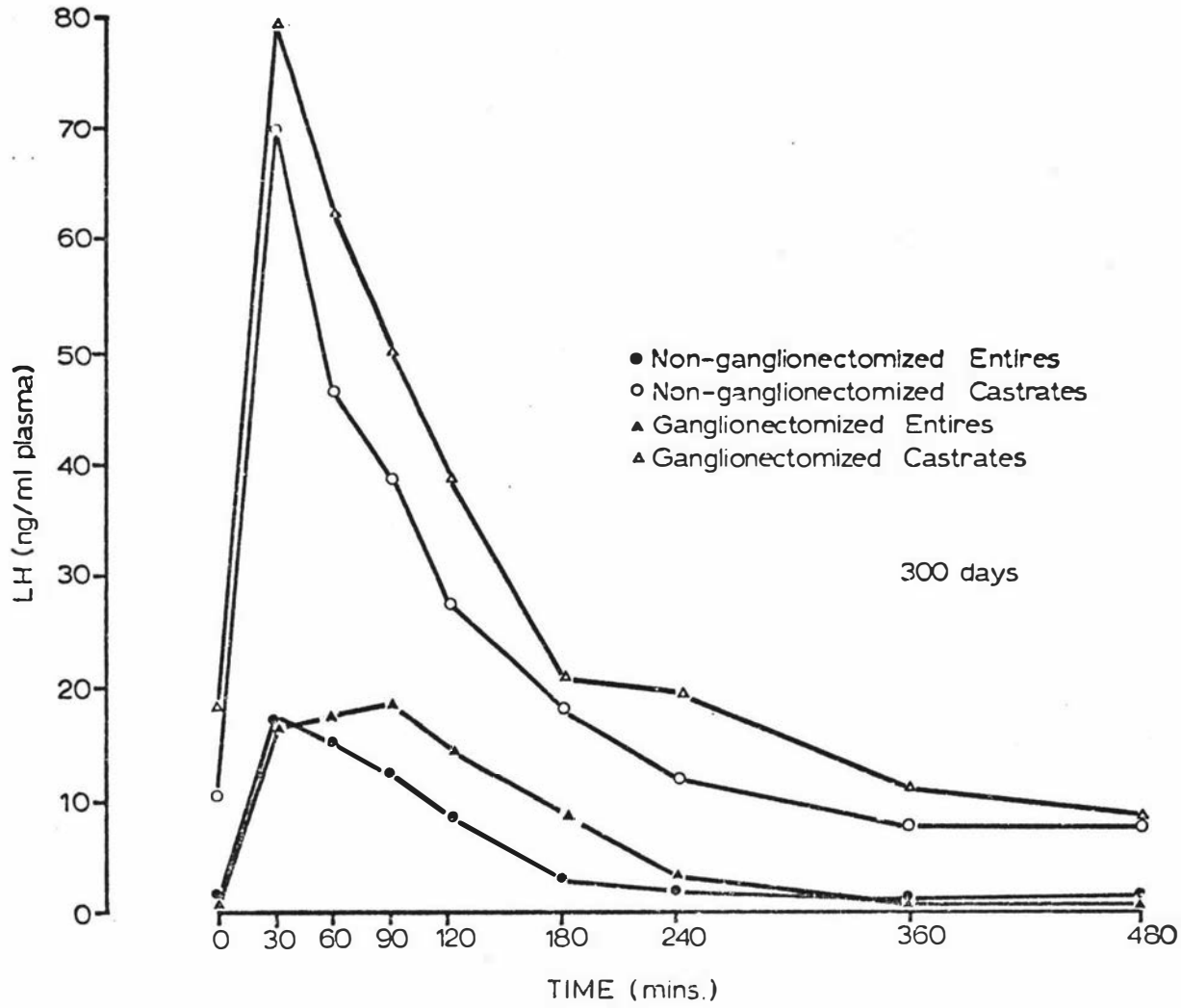

Figure 5.10 : Mean plasma LH profiles following GnRH administration (immediately after the zero min sample) to lambs at 300 days of age.

(mean 9.71 ± 1.58 ng/ml in castrates vs 4.04 ± 1.39 ng/ml in entires; $P < 0.01$) and 300 days (mean 14.89 ± 1.65 ng/ml in castrates vs 1.23 ± 0.29 ng/ml in entires; $P < 0.001$). The interaction of cranial cervical ganglionectomy with castration was significant for basal LH levels at 300 days of age ($P < 0.001$) because of the greatly elevated levels in the ganglionectomized castrates. This interaction was not significant at 100 days of age.

Following GnRH administration, LH concentrations rose quickly with peak levels being attained in nearly all animals within 30 - 60 minutes. Subsequently there was a return to basal levels, usually within 2 - 4 hours. However, the post-pubertal ganglionectomized entires did differ in that peak levels in some animals were not recorded until up to 180 minutes, with the return to basal levels taking 3 - 6 hours.

Castrates had greater peak LH concentrations than entires at both 100 days (mean 60.00 ± 6.34 ng/ml in castrates vs 28.16 ± 3.54 ng/ml in entires; $P < 0.001$) and 300 days (mean 75.25 ± 4.43 ng/ml in castrates vs 19.61 ± 2.50 ng/ml in entires; $P < 0.001$). Also the total output of LH secreted in response to GnRH was significantly higher in castrates than entires: mean total responses were 136.21 ± 14.22 ng/ml.h in castrates vs 48.81 ± 7.56 ng/ml.h in entires ($P < 0.001$) at the pubertal sampling; and 183.26 ± 13.74 ng/ml.h in castrates vs 43.88 ± 6.15 ng/ml.h in entires ($P < 0.001$) at the post-pubertal sampling.

Although mean LH responses tended to be slightly higher in ganglionectomized animals at both ages, there was

no significant effect of ganglionectomy. Likewise for peak LH levels and total LH responses the interactions of ganglionectomy and castration were not significant.

Comparison of pubertal and post-pubertal LH data showed that basal levels in non-ganglionectomized entires were higher at the pubertal rather than the post-pubertal sampling (mean levels were 6.85 ± 2.22 ng/ml at 100 days *vs* 1.50 ± 0.53 ng/ml at 300 days; $P < 0.05$). On the other hand in ganglionectomized castrates basal levels were lower in pubertal than post-pubertal animals (mean levels were 8.29 ± 2.00 ng/ml at 100 days *vs* 18.88 ± 1.71 ng/ml at 300 days; $P < 0.05$). There was no effect of age on the basal levels in the ganglionectomized entires or non-ganglionectomized castrates.

Peak levels and total LH responses to GnRH tended to be greater in entires at 100 days than at 300 days, whilst in castrates these values were greater at 300 days. However, for peak levels none of these differences were significant, while the only group of animals for which age had a significant effect on total LH output were the ganglionectomized castrates (mean totals were 147.32 ± 22.94 ng/ml.h at 100 days *vs* 213.38 ± 7.20 ng/ml.h at 300 days; $P < 0.05$).

2. Testosterone

See Figures 5.11 to 5.13 (pubertal) and 5.14 to 5.16 (post-pubertal) and Tables 5.1 and 5.2 for testosterone results.

Representative plasma samples collected from castrate animals at both ages showed there was no testosterone

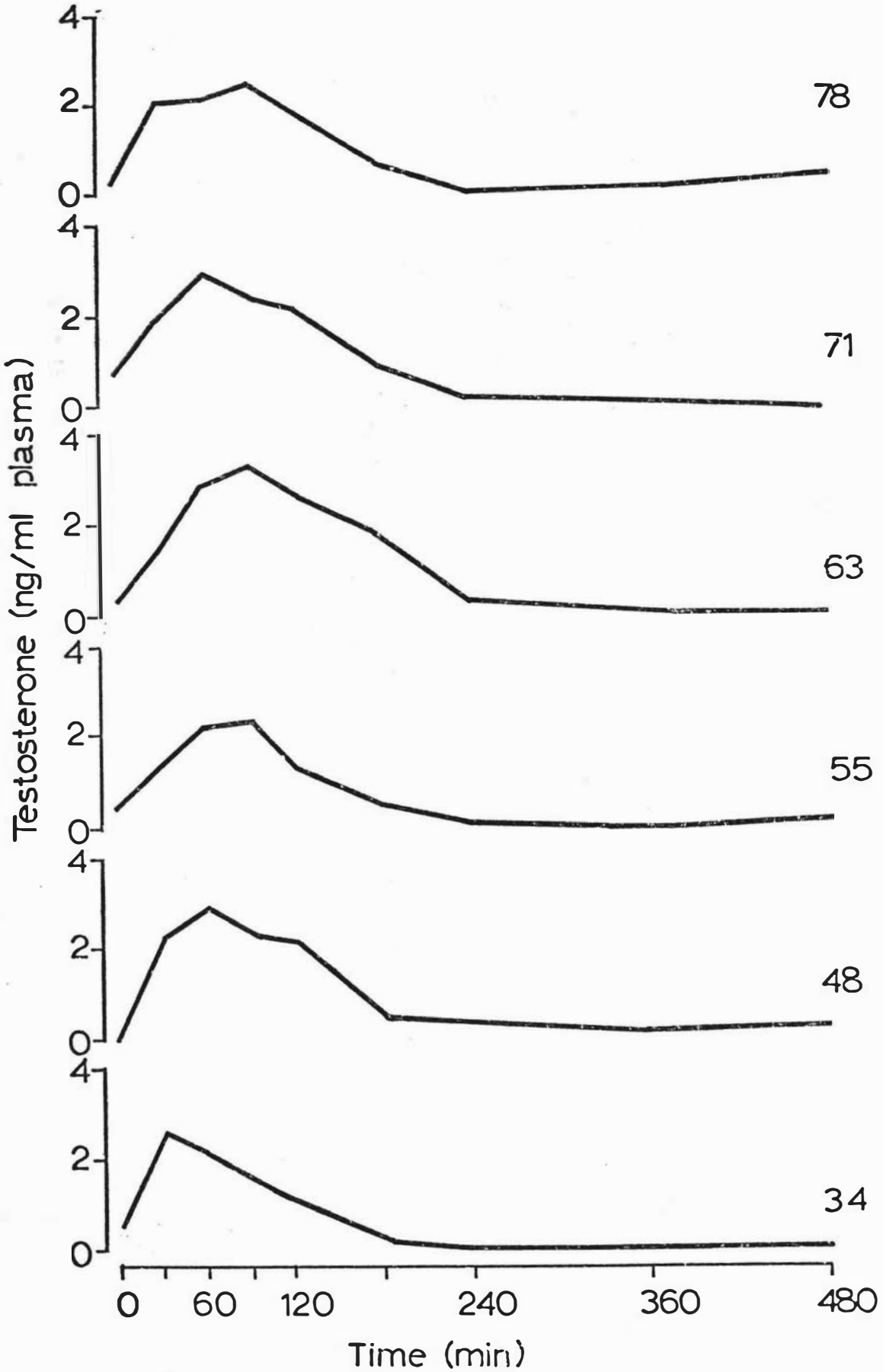

Figure 5.11 : Plasma testosterone profiles following administration of 10 µg GnRH (immediately after the zero min sample) to 6 non-ganglionectomized entire lambs at 100 days of age.

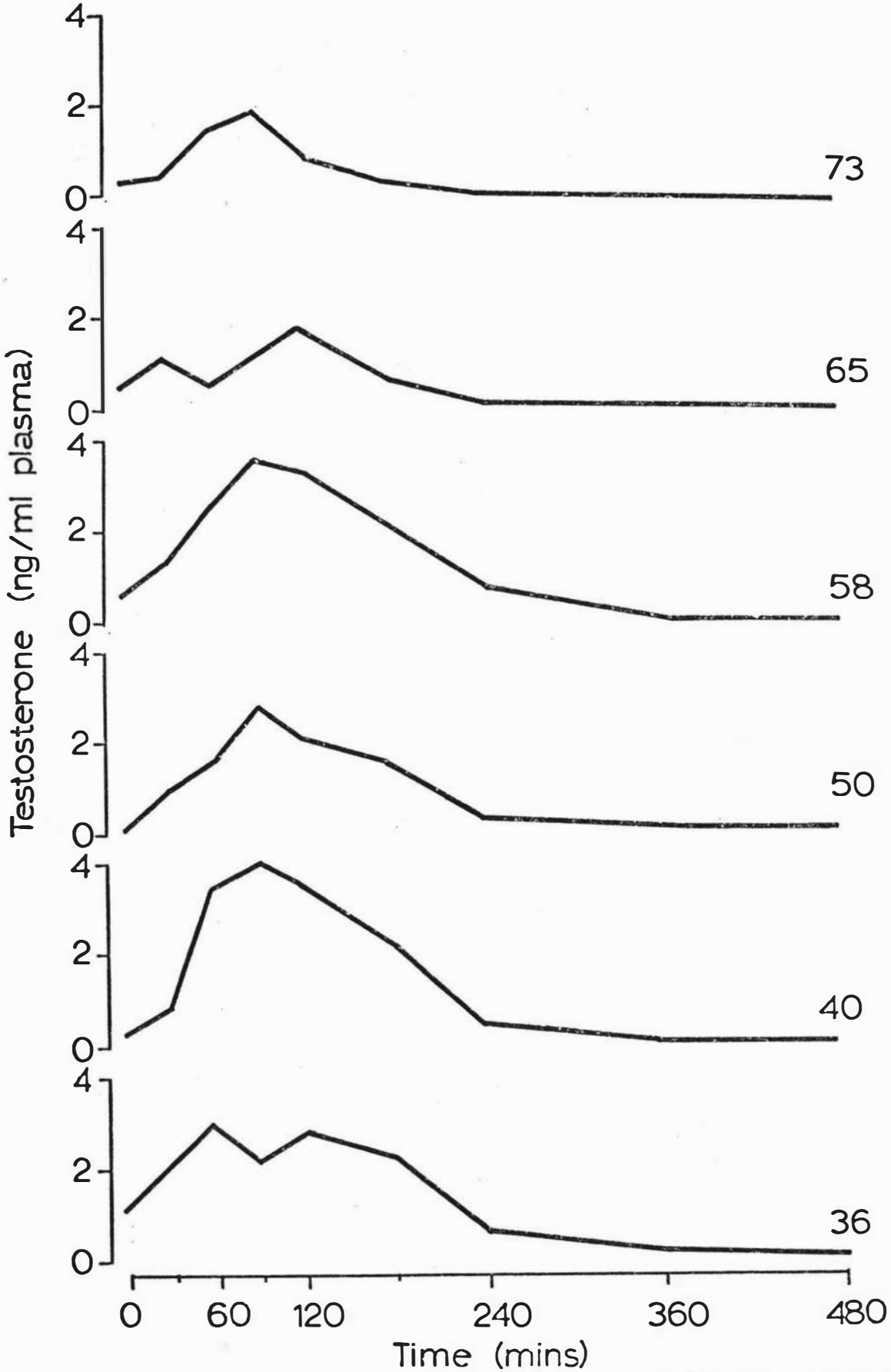

Figure 5.12 : Plasma testosterone profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 ganglionectomized entire lambs at 100 days of age.

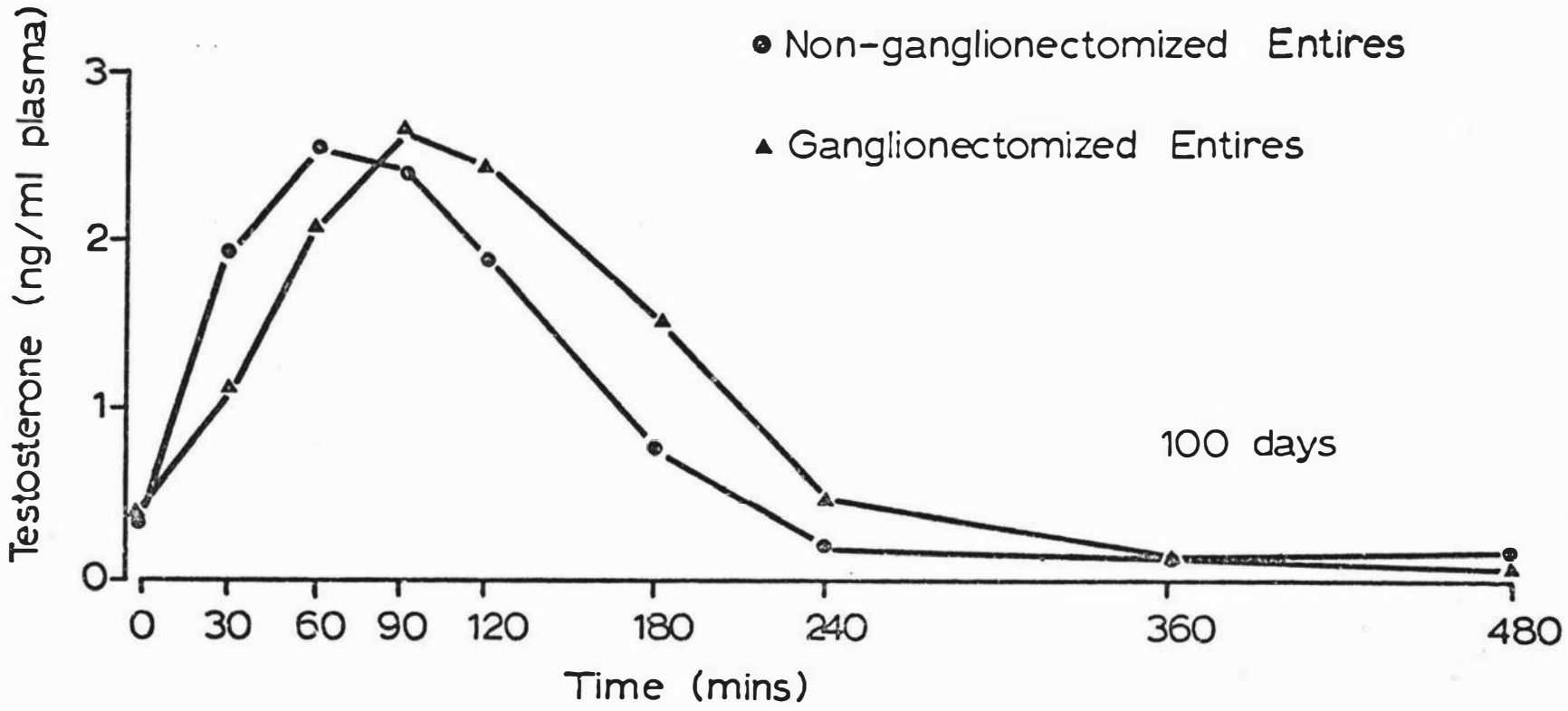

Figure 5.13 : Plasma testosterone profiles following GnRH administration (immediately after the zero min sample) to lambs at 100 days of age.

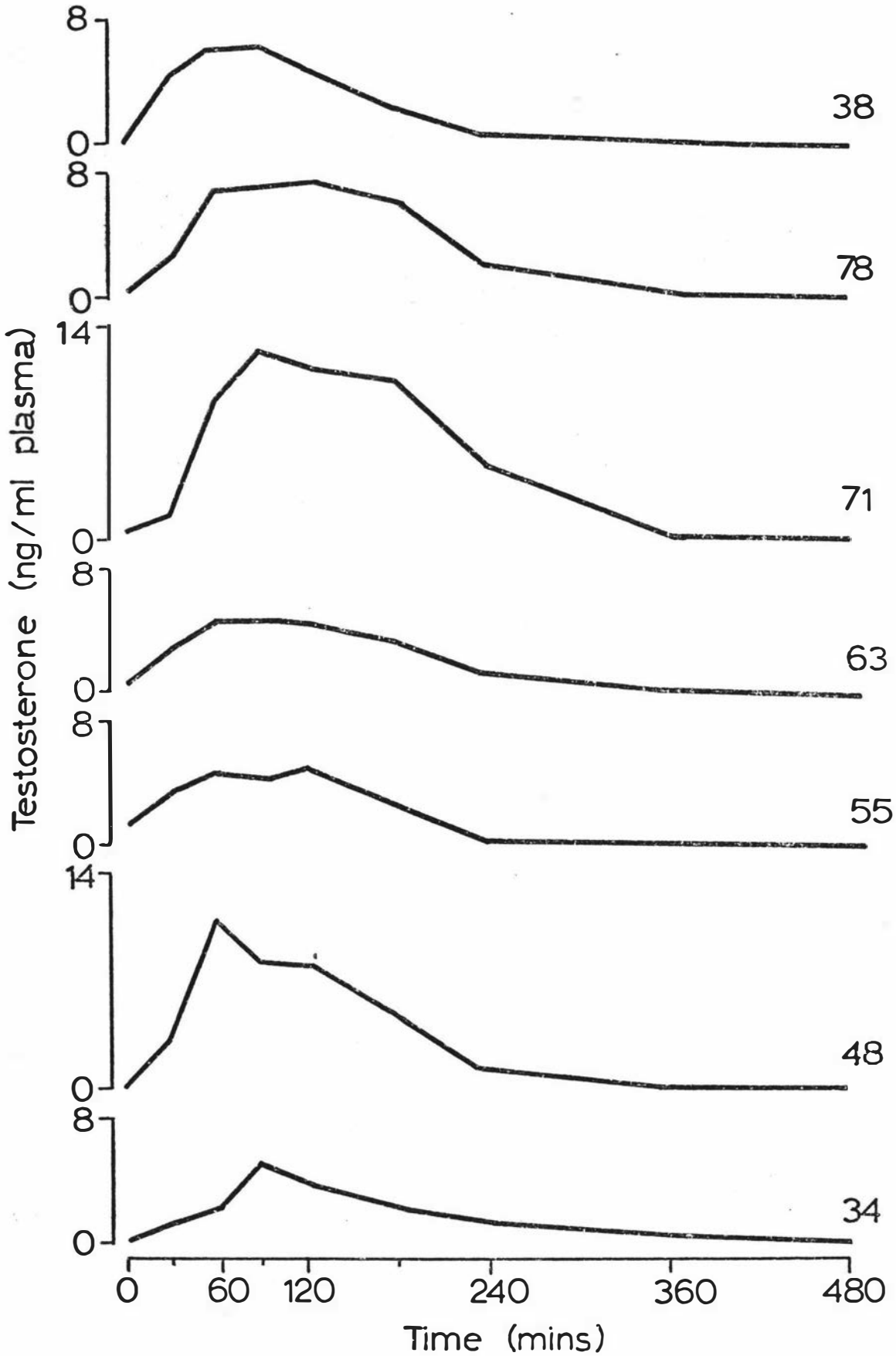

Figure 5.14 : Plasma testosterone profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 7 non-ganglionectomized entire lambs at 300 days of age.

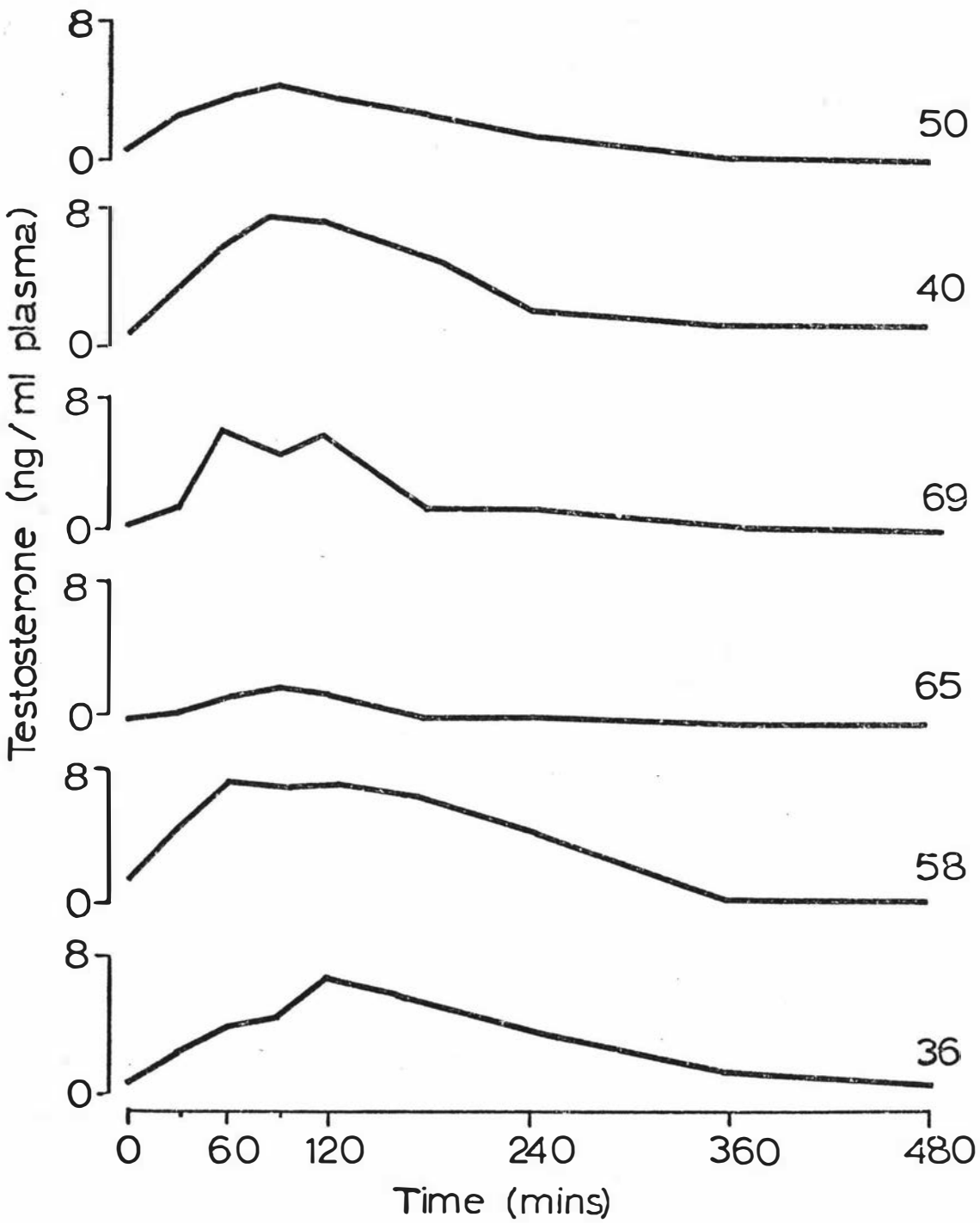

Figure 5.15 : Plasma testosterone profiles following administration of 10 μg GnRH (immediately after the zero min sample) to 6 ganglionectomized entire lambs at 300 days of age.

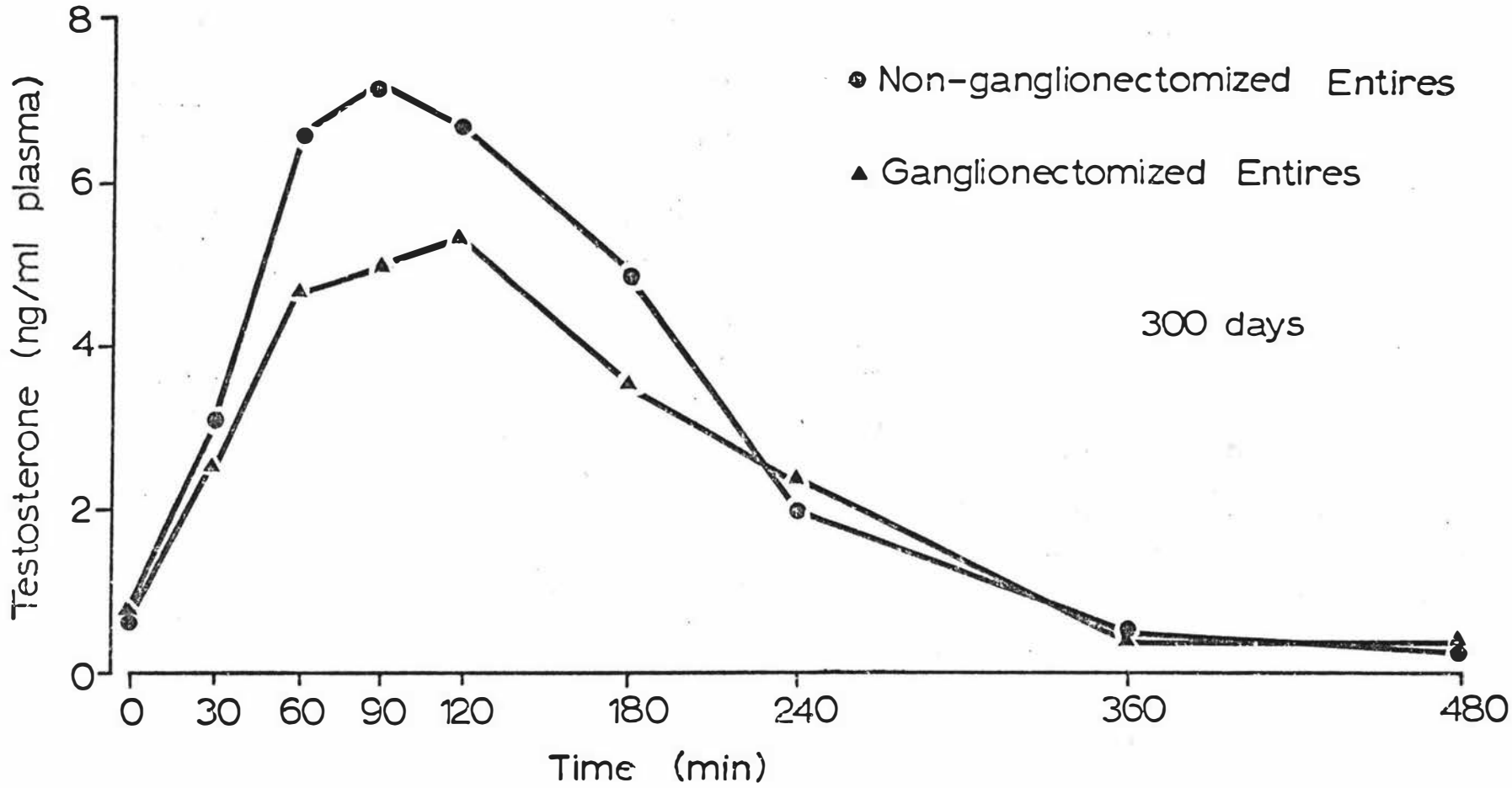

Figure 5.16 : Mean plasma testosterone profiles following GnRH administration (immediately after the zero min sample) to lambs at 300 days of age.

response to GnRH, the levels being below the limit of assay sensitivity.

Following GnRH administration testosterone levels in entire lambs rose from mean basal levels of 0.37 ± 0.09 ng/ml at 100 days and 0.65 ± 0.12 ng/ml at 300 days, to mean peak levels of 2.79 ± 0.19 ng/ml and 6.74 ± 0.80 ng/ml, respectively. Pubertal non-ganglionectomized entires reached this peak within 30 - 90 minutes and the return to basal levels occurred 3 - 4 hours post-injection. In contrast, in pubertal ganglionectomized entires and both groups at the post-pubertal sampling, testosterone concentrations took 60 - 120 minutes to reach peak values and about 4 - 6 hours to return to basal levels.

At both sampling ages cranial cervical ganglionectomy had no significant effect on basal or peak testosterone levels, nor on total testosterone responses.

Between-age comparisons of testosterone values (shown in Table 5.1) showed that: (i) mean basal levels were higher at 300 days but this difference was not significant; (ii) peak levels were greater at 300 days in both the non-ganglionectomized ($P < 0.001$) and ganglionectomized ($P < 0.05$) entires; and (iii) total testosterone responses were greater at 300 days in both non-ganglionectomized ($P < 0.001$) and ganglionectomized ($P < 0.05$) entires.

D. DISCUSSION

Administration of GnRH in this experiment resulted in the release of LH in all lambs, and in entire animals this in turn induced increases in plasma testosterone concentrations. These results are comparable with those of other studies of GnRH administration to rams, wethers and ram lambs (Reeves et al., 1970; Hopkinson et al., 1974; Foster, 1974; Galloway and Pelletier, 1974, 1975; Pelletier, 1976; Lee et al., 1976b; Wilson and Lapwood, 1978b,e).

Previous research has shown that pituitary responsiveness to synthetic GnRH can be influenced by daily photoperiod in rams (Lincoln, 1977), pregnancy, parturition, lactation and seasonality in ewes (Jenkin et al., 1977), and the stage of reproductive development in ram lambs (Galloway and Pelletier, 1974; Lee et al., 1976b; Wilson and Lapwood, 1978b).

Pituitary sensitivity to exogenous GnRH is maximal at about 6 - 8 weeks of age in rams, after which there is a progressive decrease in the time taken to reach peak concentrations (Lee et al., 1976b; Wilson and Lapwood, 1978b). The prepubertal peak of pituitary sensitivity to GnRH possibly is involved in initiation of pubertal processes (Odell and Swerdloff, 1976).

Wilson and Lapwood (1978b) also reported that with advancing age there was an increase in peak and total testosterone responses to single GnRH injections.

Although accurate analysis of the time taken to reach peak LH and testosterone levels could not be made in

this experiment, due to the infrequency of blood sampling, the data obtained suggested that the LH peak was progressively delayed with advancing age. Results of this experiment also showed that there was a decrease in the LH response and increase in testosterone secretion with increasing age, supporting the concept that reproductive development is associated with increasing steroidogenic capacity of the testes, and thus a greater intensity of feedback inhibition of LH secretion.

The greater and more rapid LH secretory response to synthetic GnRH in castrate animals, rather than entires, has been recorded in other studies (Reeves et al., 1970; Galloway and Pelletier, 1975; Pelletier, 1976). Undoubtedly, testicular inhibition of pituitary LH secretion in entire animals was induced by testosterone (Galloway and Pelletier, 1975; Pelletier, 1976). Pelletier (1976) suggested that "testosterone could act in two different, relatively independent, ways at pituitary level:

a) by inhibiting the magnitude of the pituitary response to LH-RF,

b) by delaying the LH response to LH-RF".

A significant interaction of cranial cervical ganglionectomy with castration, affecting basal LH levels at the post-pubertal sampling, as well as an effect of age on basal LH levels in the non-ganglionectomized entires and the ganglionectomized castrates, all were in accordance with results recorded in Experiment 2 (Chapter 4). Effects of ganglionectomy, and of its interaction with castration, on

the pituitary and gonadal responses to exogenous GnRH may have been anticipated if the pineal influences the pituitary-testicular axis directly. However, only an effect of age on the total LH output in the ganglionectomized castrates, compared with the non-ganglionectomized castrates, suggested that a direct effect on the pituitary may exist. Ellis (1976) has reviewed evidence showing that melatonin can inhibit testicular steroidogenesis and pituitary gonadotrophin synthesis by altering enzyme activity at both the gonadal and pituitary levels, as well as at the hypothalamus. Arginine vasotocin also appears to influence enzyme activities at the gonadal and pituitary levels, as well as at the hypothalamus, but its exact action on reproductive function is unclear (Ellis, 1976; Vaughan et al., 1976). However, the physiological significance of any direct effects of pineal secretory products on the pituitary and/or gonads compared with an influence within the brain, is unknown. Kamberi et al., (1970) showed that the indoleamines serotonin and melatonin, did not alter LH release in male rats when infused through the pituitary portal vessels, but they reduced LH levels significantly, when injected into the third ventricle. Thus, the indoleamines appear to act like neurotransmitters in influencing the synthesis and/or the release of GnRH in the hypothalamus, rather than influencing the secretion of LH from the pituitary.

Ganglionectomy might also have been anticipated to have effects on responses to GnRH if basal testosterone levels had been affected by ganglionectomy (see Chapter 4),

resulting in different levels of testicular steroid feedback influencing pituitary sensitivity. However, as yet the physiological significance of a direct influence of the pineal gland on the pituitary-testicular axis remains to be established.

CHAPTER VI

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 and becomes capable of gamete production. Induction of the pubertal process is initiated by the brain, but can be influenced by a number of genetic and environmental factors. The experiments described in this thesis have attempted to examine the influence of the pineal gland on pubertal development in ram lambs; this was assessed subsequent to pre-pubertal denervation of the gland.

1. Sexual Maturation in Rams

Results presented in this thesis and in other studies have shown that endocrine characteristics of sexual maturation in ram lambs include:

(i) Plasma LH levels are elevated in the first few months after birth (Crim and Geschwind, 1972b; Courot, 1974; Courot et al., 1975; Cotta et al., 1975; Lee et al., 1976a; Wilson and Lapwood, 1978a). Unfortunately, an FSH assay was not available during the present study, but Lee et al. (1976a) reported that FSH levels increased along with LH levels, early in life. Similar increases in pituitary weights and LH and FSH contents have been observed (Skinner et al., 1968; Courot et al., 1975) with values increasing gradually from birth to maximal levels within the first six months of life.

(ii) LH release is pulsatile in both pubertal and post-pubertal rams and in wether lambs. These results were consistent with other reports of LH release in lambs (Foster, 1974; Bindon and Turner, 1974; Carr and Land, 1975; Foster et al., 1978; Wilson and Lapwood, 1978a) and wethers (Riggs and Malven, 1974a).

(iii) Negative feedback regulation of LH secretion by the testes is present shortly after birth (Foster, 1974). An inhibin-FSH feedback system probably is not established until around 2 - 3 months of age (Blanc and Terqui, 1976).

(iv) A gradual increase in plasma testosterone levels occurs with advancing age, as has been reported previously (Crim and Geschwind, 1972a; Courot, 1974; Courot et al., 1975; Cotta et al., 1975; Lee et al., 1976a; Illius et al., 1976a; Wilson and Lapwood, 1978a). There is a corresponding increase in testicular androgen content (Skinner et al., 1968; Attal et al., 1972).

(v) Exogenous GnRH induces LH release, and this in turn resulted in elevated testosterone levels, at both the pubertal and post-pubertal ages. Lee et al. (1976b) and Wilson and Lapwood (1978b) reported that the pituitary was most sensitive to synthetic GnRH at between 6 and 8 weeks of age and suggested that this may have been an important facet of sexual maturation.

Concomitant with these endocrine changes there was an increase in testicular and accessory gland weights, while the establishment of spermatogenesis occurs in N.Z. Romney rams by 22 weeks of age (Steffert, 1971; Wilson and Lapwood, 1978c).

The above results are compatible with the model of sexual maturation proposed by Odell and Swerdloff (1976), based on research with sexually maturing rats. This model proposed:

1. Gonads of newborn and neonatal rats are poorly responsive to LH, but responsive to FSH.

2. The hypothalamic-hypophyseal unit is active in the neonate.

3. FSH induces development of testicular LH receptors, possibly in synergism with prolactin and/or growth hormone.

4. FSH secretion, which initially is high and relatively unrestrained, is gradually suppressed as gonadal responsiveness to LH increases, and gonadal steroid and possibly inhibin secretion increases.

5. Although hypothalamic-hypophyseal sensitivity to the feedback suppression of LH may decrease with sexual maturation, it probably is not an important part of maturation.

To date, this remains the most attractive model of sexual maturation but it does not elucidate the mechanism or mechanisms which begin or initiate the onset of puberty. Is it, as Levasseur (1977) suggested, caused by a dampening of the central nervous system inhibition of the hypothalamic-pituitary-gonadal axis? Lesions of the hypothalamus and limbic system of rats advance the time of pubertal onset, suggesting that the prepubertal brain exerts an inhibitory influence on reproductive development (Critchlow and Bar-Sela, 1967; Davidson, 1974).

Reproductive development is a sequential process

which probably is controlled by an inherent mechanism within the brain removing the reproductive axis from an inhibitory influence characteristic of juvenility. The timing of this event is most likely influenced by afferent inputs to the hypothalamus or other brain areas such as the limbic system, from the external and internal environments. This idea is suggested by the fact that temperature, stress, photoperiod, altitude, nutrition and growth rate, various social and economic factors which have been mainly studied in humans, as well as hormonal and pharmacologic substances, all can influence pubertal onset (Donovan and van der Werff ten Bosch, 1965; Critchlow and Bar-Sela, 1967; Tanner, 1967; Frisch, 1974).

2. Cranial Cervical Ganglionectomy

The effectiveness of cranial cervical ganglionectomy in modifying pineal function has been shown by many investigators. In laboratory animals removal of the ganglia produces the following effects: histological changes indicative of reduced protein synthesis and secretory activity (Romijn, 1975; Reiter et al., 1976); abolition of significant circadian changes in noradrenalin content (Morgan et al., 1976), NAT activity (Klein et al., 1971), and HIOMT activity (Wurtman et al., 1964; Eichler and Moore, 1971; Cardinali et al., 1976b); reduction in size of the pineal parenchymal serotonin pool (Bertler et al., 1964); and alterations in pineal steroid-receptor content and activity (see review by Cardinali and Vacas, 1978). Buttle (1977) reported that ganglionectomy reduced the number of nerve fibres in goat pineals, while in rams

Barrell and Lapwood (1978b) found significantly lower HIOMT activity and higher nuclear cell densities (indicative of less active pineals) following removal of the ganglia. Collectively these results indicate that the changes in endocrine and reproductive function which follow cranial cervical ganglionectomy probably can be attributed to modified pineal function.

However, the possibility that denervation affects the hypothalamo-hypophyseal-gonadal axis independently of the pineal should not be overlooked. Sympathetic neurons arising in the cranial cervical ganglia supply the heart, eye, salivary glands and the blood vessels of the head, as well as the pineal, so it may be possible to attribute the effects of ganglionectomy to factors such as alterations in cranial blood flow or optic function. Edvinnson et al. (1971) reported that cranial cervical ganglionectomy markedly changed the cerebral blood volume of mice with an increase in the first few days after the operation, and a slight reduction recorded at 14 days post-operation. Similarly, since blinding and eye removal influence pineal function (Reiter and Hester, 1966; Moore et al., 1967; Rønnekleiv and McCann, 1975) it could be argued that any effect of ganglionectomy on optic function, perhaps altering the perception of light, may account for any altered reproductive function. However, cranial cervical ganglionectomized goats showed no changes in pupillary diameter, iris dilatability, or tone of nictitating membranes, nor was there any degree of ptosis compared with sham-operated controls (Buttle, 1977). In the present

study, no ptosis was evident in ganglionectomized lambs.

As stress is thought to influence the secretion of LH, testosterone and prolactin (Raud et al., 1971; Davis, 1972; Sitarz et al., 1977; Wilson and Lapwood, 1978a), it is possible that any effects of ganglionectomy on hormone levels may have been due to alterations in stress susceptibility of treated animals. The response of the rat pineal to stress is thought to involve an increase in NAT and HIOMT activities and melatonin synthesis (Lynch et al., 1975; Urry et al., 1976). Parfitt and Klein (1976) found that superior cervical ganglionectomized rats responded to stress with an increase in pineal NAT activity whereas intact controls did not. This may have been related to the phenomenon of supersensitivity of the denervated pineal to stress and autonomic drugs (Axelrod, 1974, 1975). Super- (and sub-) sensitivity is thought to be related to the amount of catecholeamine transmitter impinging on the pineal β-receptors (Axelrod, 1974, 1975), but its significance with respect to interpreting the results of pineal denervation experiments in unknown.

While it must be emphasized that results of cranial cervical ganglionectomy experiments should be interpreted with caution, Barrell and Lapwood (1978b,c) found that ganglionectomy and pinealectomy had similar effects on some aspects of reproductive seasonality in rams. Thus ganglionectomy apparently is an acceptable way of studying the influence of the pineal on reproductive maturation in ram lambs, particularly in view of technical difficulties associated with complete removal of the pineal in young

sheep (J.M. Forbes, personal communication; Brown et al., 1977). Unfortunately, methods for measuring the function of the pineal, for example radioimmunoassays for plasma and cerebrospinal pineal products such as melatonin and arginine vasotocin, are still being developed in this laboratory.

Finally, since the pineal may influence the thyroid and the adrenal glands (De Fronzo and Roth, 1972; Vermes et al., 1974; Vriend et al., 1977) possibly some of the effects of ganglionectomy or pinealectomy are mediated via other organs, although Meijs-Roelofs and Moll (1978) concluded that the adrenals were not essential to sexual maturation in rats.

3. Bodyweight

Dýrmundsson (1973) stated "... the development of the ram lamb, as reflected in growth and subsequent bodyweight, is a better guide to puberty than chronological age". In Romney rams, testis growth increased sharply from 70 days of age when bodyweight exceeded 20 kg (Steffert, 1971). This change in testis growth is associated with the commencement of spermatogenesis and subsequently there is a close relationship between the development of spermatogenesis and testis weight (Courot, 1962). These observations, along with the view that the attainment of puberty is dependent on reaching a critical bodyweight, and thus a certain level of basal metabolism (Frisch, 1974), emphasize the need to consider growth and bodyweight when interpreting the results of studies of pubertal development in animals with different bodyweights. In particular, in

this experiment both groups of ganglionectomized animals grew more slowly than did the non-ganglionectomized lambs, so any influence of ganglionectomy on pubertal development may have been secondary to effects on growth rate. Also, since sham-operated control animals were not included in this study, a non-specific influence of the operation on growth rate cannot be discounted.

4. Prolactin

The reproductive functions of prolactin in rams have not been fully elucidated. Autumn born lambs show a distinct peak in plasma prolactin levels at 12 weeks of age, coincident with the onset of the rapid increase in testicular weight and spermatogenic activity (Ravault and Courot, 1975). Subsequently it has been demonstrated that inhibition of prolactin secretion during pubertal development depressed plasma testosterone levels and also resulted in significant decreases in seminal vesicle weights and fructose concentrations (Ravault et al., 1977b). These results raise the possibility that in rams prolactin may synergize with LH and testosterone in regulating testicular steroidogenesis and the functions of some accessory reproductive structures. Research with rats and hamsters has shown that prolactin does have such functions, especially during reproductive development (Bartke et al., 1978).

Spring-born lambs, such as used in the present study and in that of Wilson and Lapwood (1978a) show high plasma prolactin levels during the first few months of life; the long photoperiods recorded at that time of the year

undoubtedly were the major reasons for those high rates of prolactin secretion (Pelletier, 1973; Ravault, 1976; Barrell and Lapwood, 1978a,c). Thus, it is difficult to elucidate a function of prolactin in reproductive development when utilizing spring-born lambs. This is because the "prolactin-stimulatory" photoperiod to which these animals are exposed probably exerts an over-riding influence which would at least mask changes associated with reproductive development. In order to study the influence of prolactin on reproductive development then, it would be necessary to utilize autumn-born lambs or inhibit prolactin secretion as Ravault and colleagues have done (Ravault and Courot, 1975; Ravault, 1976; Ravault et al., 1977a,b), or to rear lambs under even or reversed artificial photoperiods. However, altering photoperiod may also influence the secretion of other reproductive hormones. Experiments with lambs born out of season also must be interpreted carefully, particularly since factors such as nutrition and environmental temperature may influence prolactin secretion and reproductive development.

The effects of cranial cervical ganglionectomy in this study, and of pinealectomy or ganglionectomy in those of Forbes (1975), Brown et al. (1977) and Barrell and Lapwood (1978b,c,d), provide strong evidence for the concept that the pineal gland is involved in mediating the effects of photoperiod on prolactin secretion in rams and wethers. In the present study, normal seasonal patterns of prolactin secretion, with high levels during the summer months and low during winter, were disrupted by

ganglionectomy to the extent that treated animals had lower summer levels and higher winter levels than untreated animals. If the pineal stimulates release of prolactin inhibiting factor (PIF), then in winter an active pineal would reduce prolactin secretion, whilst in summer a less active pineal would result in higher prolactin levels. If this was the case, ganglionectomy would be expected to elevate prolactin concentrations by removing the pineal stimulus to PIF secretion. Thus it was surprising to find that ganglionectomy diminished prolactin secretion in summer (Experiment 2), suggesting that the pineal stimulated prolactin release at that time. This would occur if more prolactin stimulating factor (PSF) than PIF regulated prolactin secretion. Further experiments, involving the effects of lighting on intact and pinealectomized or ganglionectomized rams, are required for elucidation of the mechanism by which photoperiod and/or the pineal modulates PIF and/or PSF release.

5. The Pineal and Puberty

The present study has shown that the pineal gland is involved in regulating prolactin, and possibly also LH and testosterone, secretion during the course of pubertal development in rams. Although a role of prolactin in reproductive maturation remains to be firmly established, it is feasible to suggest that the pineal gland is capable of influencing puberty.

If it is assumed that the pineal alters the timing of the onset of puberty, since pinealectomy in rats advances the age of puberty by up to 10 days (Kincl and Benagiano,

1967; Relkin, 1970a,b, 1971), then a more thorough examination of the age of pubertal onset in the ram (as judged by such parameters as testis histology, spermatozoal production, and accessory gland function) must be undertaken. Such an investigation should involve serial castrations of normal and ganglionectomized or pinealectomized ram lambs. Experiments should also include sham-operated controls, rather than non-operated controls such as were used in the present study.

Ideally, the lambs in these experiments should have been ganglionectomized at birth or even *in utero* since endocrine maturational changes related to puberty could have been initiated in the early post-natal period. For example Wilson and Lapwood (1978a) reported that plasma LH levels rose to a peak at 6 weeks of age in Romney ram lambs and a similar increase in Merino/Corriedale lambs at 5 weeks was reported by Lee *et al*. (1976a).

Since Odell and Swerdloff (1976) suggested that puberty is initiated by an FSH-induced increase in the number of testicular LH receptors, and FSH levels increase rapidly from birth to a peak at 5 weeks in the ram (Lee *et al*., 1976a), it would be necessary to measure plasma FSH before any definitive statements regarding the function of the pineal on the onset of puberty can be made. The non-availability of an FSH assay in this laboratory has already been mentioned.

Lincoln (1971) described the exaggerated reproductive seasonality in red deer stags as being similar to a yearly puberty; this type of observation emphasizes

the importance of considering seasonality and puberty together when working with seasonal breeders. Indeed, in animals born during the normal season of birth, seasonal influences may provide the impetus for reproductive development. Several studies have reported an influence of season of birth on the age and bodyweight at which puberty, and in turn sexual maturity, is attained, even in sheep (Ortavant et al., 1964; Dýrmundsson and Lees, 1972a, b,c; Land 1978). Although there are breed differences in the degree to which pubertal development is influenced by season, generally lambs born later in the spring lambing season reach puberty, and in turn sexual maturity, at a younger age and lighter bodyweight. For example, as time of birth advanced from March to May (in the northern hemisphere), testis diameter measured at 24 weeks of age increased in Finnish Landrace (Finn) rams but decreased in Merino/Finn crossbreds (Land, 1978). Land (1978) stated that "as the time of birth changes from spring to autumn, puberty would be expected to accelerate, and then suddenly recede as birth becomes too late to allow sexual maturation within the 'first' breeding season." Lincoln and MacKinnon (1976) reported that this is exactly what occurs in the male hare and apparently is related to the seasonal photoperiod, the effects of which possibly are mediated by the pineal (Lincoln, 1976c). Also, Hoffmann (1978) reported that raising hamsters exposed to short photoperiods (8L : 16D) delayed puberty for about 5 months compared with animals exposed to long daily photoperiods (16L : 8D).

In adult rams the pineal is involved in mediating the effects of photoperiod on reproduction (Barrell and Lapwood, 1978b,c,d,e) so an influence on pubertal development can reasonably be anticipated. It is envisaged that the more important role of the pineal is to entrain reproductive development with seasons rather than to initiate the onset of puberty. Future studies will have to be carried out on the influence of the pineal on development in spring and autumn born lambs and on those exposed to normal, even or reversed lighting cycles and possibly to constant light and darkness.

6. Possible Applications of the Present Study

An understanding of the basic physiological processes of reproductive development is a necessary prelude to the manipulation of pubertal processes in medical and animal sciences. For example, the treatment of precocious and delayed puberty, which has been well documented in humans (Kitay, 1954; Wurtman, 1968), may follow the identification and synthesis of the definitive pineal principles or its/their analogues. This may also lead to the treatment of other reproductive disorders and potentially the most useful application, the development of a contraceptive agent for use in humans.

Further research of the type described in this thesis may provide the basis of advancing the age of pubertal onset and sexual maturity. Advancing breeding age would permit more rapid genetic turnover, particularly in slowly maturing breeds such as the Merino (Land, 1978) and would be of practical benefit to animal breeding and selection

programmes. Ram lambs have already been successfully utilized as sires (Dýrmundsson, 1973). However, the possible methods for manipulating reproductive development, such as pineal denervation or removal, administration of principles or substances which interfere with their synthesis, secretion or actions, regulation of nutrition or growth, and the utilization of appropriate photoperiods probably are impractiable on a commercial scale within the New Zealand sheep industry, although they may be useful on a smaller scale in nuclear stud flocks and in other domestic animals. The degree to which puberty may be hastened probably is limited genetically and it will be necessary also to determine whether manipulation of reproductive development is detrimental or beneficial to further somatic growth and reproductive performance.

REFERENCES

Ahmed, N., Tiwari, S.B., and Sahni, K.L. (1978). Effect of castration on live weight growth and meat production in cross-bred lambs. Anim. Breed. Abstr. 46, 1310. Abstr.

Akbar, A.M., Nett, T.M., and Niswender, G.D. (1974). Metabolic clearance and secretion rates of gonadotropins at different stages of the estrous cycle in ewes. Endocrinology 94, 1318-1324.

Alberio, R., and Colas, G. (1976). Influence of photoperiodism on the sexual development of the young Ile-de-France ram. Proc. VIIIth Intern. Congr. Anim. Reprod. & A.I. Cracow 1976 p26-29.

Alifakiotis, T.A., Call, J.W., and Foote, W.C. (1976). Androgen induction of intersexuality in sheep. Theriogenology 6, 21-28.

Anderson, E. (1965). The anatomy of bovine and ovine pineals. J. Ultrastruct. Res. Suppl. 8, 1-80.

Appleton, A.B., and Waites, G.M.H. (1957). A surgical approach to the superior cervical ganglion and related structures in the sheep. J. Physiol., Lond. 135, 52-57.

Aragona, C., and Friesen, H.G. (1975). Specific prolactin binding sites in the prostate and testis of rats. Endocrinology 97, 677-684.

Arimura, A., Debeljuk, L., Matsuo, H., and Schally, A.V. (1972). Release of luteinizing hormone by synthetic LH-releasing hormone in the ewe and ram. Proc. Soc. Exp. Biol. Med. 139, 851-854.

Attal, J. (1969). Levels of testosterone, androstenedione, estrone and estradiol-17β in the testes of fetal sheep. Endocrinology 85, 280-289.

Attal, J., Andre, D., and Engels, J.A. (1972). Testicular androgen and oestrogen levels during the postnatal period in rams. J. Reprod. Fert. 28, 207-212.

Auty, R., Branch, R.A., Cole, E.N., Levine, D., and Ramsey, L. (1976). Prolactin, diuretics and urinary electrolytes in normal subjects. J. Endocr. 70, 173-181.

Axelrod, J. (1974). The pineal gland: a neurochemical transducer. Science, N.Y. 184, 1314-1348.

Axelrod, J. (1975). Relationship between catecholamines and other hormones. Rec. Prog. Horm. Res. 31, 1-35.

Barraclough, C.A. (1973). Sex steroid regulation of reproductive neuroendocrine processes. In Handbook of Physiology. Section 7: Endocrinology, Part 1. Eds. R.D. Greep and E.B. Astwood. American Physiological Society, Washington. p29-56.

Barrell, G.K., Lapwood, K.R. (1978a). Seasonality of semen production and plasma hormone levels in Romney, Merino and Polled Dorset rams. Anim. Reprod. Sci. (In press).

Barrell, G.K., and Lapwood, K.R. (1978b). Effects of modifying olfactory and pineal gland function on the seasonality of semen production and plasma luteinizing hormone, testosterone and prolactin levels in rams. Anim. Reprod. Sci. (In press).

Barrell, G.K., Lapwood, K.R. (1978c). Effects of pinealectomy on the secretion of luteinizing hormone, testosterone and prolactin in rams exposed to different lighting regimes. J. Endocr. (In press).

Barrell, G.K., Lapwood, K.R. (1978d). Effects of varying lighting regimes and pinealectomy on semen production in Romney rams. J. Reprod. Fert. (In press).

Barrell, G.K., and Lapwood, K.R. (1978e). Effects of pinealectomy of rams on secretory profiles of luteinizing hormone, testosterone, prolactin and cortisol. Neuroendocrinology. (In press).

Bartke, A., and Dalterio, S. (1976). Effects of prolactin on the sensitivity of the testis to LH. Biol. Reprod. 15, 90-93.

Bartke, A., Hafiez, A.A., Bex, F.J., and Dalterio, S. (1978). Hormonal interactions in regulation of androgen secretion. Biol. Reprod. 18, 44-54.

Ben-Jonathan, N., Mical, R.S., and Porter, J.C. (1974). Transport of LRF from CSF to hypophysial portal and systematic blood and the release of LH. Endocrinology 95, 18-25.

Benson, B. (1977). Current status of pineal peptides. Neuroendocrinology 24, 241-258.

Bertler, A., Falck, B., and Owman, C. (1964). Studies on the 5-hydroxytryptamine stores in the pineal gland of rat. Acta Physiol., Scand. 63, Suppl. 239, 1-18.

Bindon, B.M., and Turner, H.N. (1974). Plasma LH of the prepubertal lamb: a possible early indicator of fercundity. J. Reprod. Fert. 39, 85-88.

Blackwell, R.E., and Guillemin, R. (1973). Hypothalamic control of adenohypophysial secretions. Ann. Rev. Physiol. 35, 357-390.

Blanc, M.R., and Dacheux, J.L. (1976). Existence of inhibin activity in the ram rete testes fluid: effect of physiological doses on plasma LH and FSH. ICRS Medical Science 4, 460.

Blanc, M.R., and Terqui, M. (1976). Determination of the age of establishment of the inhibin-follicle stimulating hormone feed-back mechanism in the ram lamb. ICRS Medical Science 4, 17.

Blask, D.E., Vaughan, M.K., Reiter, R.J., Johnson, L.Y., and Vaughan, G.M. (1976). Prolactin-releasing and release-inhibiting factor activities in the bovine, rat, and human pineal gland: in vitro and in vivo studies. Endocrinology 99, 152-162.

Bolt, D.J. (1971). Changes in the concentration of luteinizing hormone in plasma of rams following administration of oestradiol, progesterone or testosterone. J. Reprod. Fert. 24, 435-438.

Bowers, C.Y., Currie, B.L., Johansson, K.N., and Folkers, K. (1973). Biological evidence that separate hypothalamic hormones release the follicle stimulating and luteinizing hormones. Biochem. Biophys. Res. Commun. 50, 20-26.

Bowie, E.P., and Herbert, D.C. (1976). Immunocytochemical evidence for the presence of arginine vasotocin in the rat pineal gland. Nature 261, 66.

Bremner, W.J., Findlay, J.K., de Kretser, D.M., Cumming, I.A., and Hudson, B. (1976). Pituitary-testicular responses in rams to prolonged infusions of LH-RH. J. Reprod. Fert. 46, 496-497. Abstr.

Brown, W., Jones, R., and Forbes, J.M., (1977). Effect of pinealectomy on prolactin, growth hormone and growth, in lambs. J. Endocr. 77, 35P Abstr.

Bubenik, G.A., Brown, G.M., and Grota, L.G. (1976). Differential localization of N-acetylated indolealkylamines in CNS and the Harderian gland using immunohistology. Brain Research 118, 417-427.

Burgus, R., Butcher, M., Amoss, M., Ling, N., Monahan, M., Rivier, J., Fellows, R., Blackwell, R., Vale, W., and Guillemin, R. (1972). Primary structure of ovine hypothalamic luteinizing hormone-releasing factor (LRF). Proc. Nat. Acad. Sci. USA 69, 278-282.

Burstyn, P.G., Horrobin, D.F., and Manku, M.S. (1972). Saluretic action of aldosterone in the presence of increased salt intake and restoration of normal action by prolactin or by oxytocin. J. Endocr. 55, 369-376.

Buttle, H.L. (1977). The effect of anterior cervical ganglionectomy on the seasonal variation in prolactin concentration in goats. Neuroendocrinology 23, 121-128.

Cardinali, D.P. (1974). Melatonin and the endocrine role of the pineal organ. Current Topics in Experimental Endocrinology. 2, 107-128.

Cardinali, D.P., and Wurtman, R.J. (1972). Hydroxyindole-O-methyl transferases in rat pineal, retina and Harderian gland. Endocrinology 91, 247-252.

Cardinali, D.P., Nagle C.A., and Rosner, J.M. (1974a). Metabolic fate of androgens in the pineal organ: uptake, binding to cytoplasmic proteins and conversion of testosterone in 5α-reduced metabolites. Endocrinology 95, 179-187.

Cardinali, D.P., Nagle, C.A., and Rosner, J.M. (1974b). Aromatization of androgens to oestrogens by the rat pineal gland. Experientia 30, 1222-1223.

Cardinali, D.P., Nagle, C.A., and Rosner, J.M. (1974c). Changes in pineal indole metabolism and plasma progesterone levels during the estrous cycle in ewes. Steroids Lipids Research 5, 308-315.

Cardinali, D.P., Nagle, C.A., and Rosner, J.M. (1975a). Control of estrogen and androgen receptors in the rat pineal gland by catecholamine transmitter. Life Sciences 16, 93-106.

Cardinali, D.P., Nagle, C.A., Gómez, E., and Rosner, J.M. (1975b). Norepinephrine turnover in the rat pineal gland. Acceleration by estradiol and testosterone. Life Sciences 16, 1717-1724.

Cardinali, D.P., Gómez, E., and Rosner, J.M. (1976a). Changes in [^{3}H] leucine incorporation into pineal proteins following estradiol or testosterone administration: involvement of the sympathetic superior cervical ganglion. Endocrinology 98, 849-858.

Cardinali, D.P., Nagle, C.A., and Rosner, J.M. (1976b). Gonadotrophin- and prolactin-induced increase in rat pineal hydroxyinole-O-methyl transferase: involvement of the sympathetic nervous system. J. Endocr. 68, 341-342.

Cardinali, D.P., and Vacas, M.I. (1978). Mechanisms underlying hormone effects on pineal function: a model for the study of integrative neuroendocrine processes. J. Endocr. Invest. 1, 89-96.

Carlson, J.C., Barcikowski, B., and McCracken, J.A. (1973). Prostaglandin F2∝ and the release of LH in sheep. J. Reprod. Fert. 34, 357-361.

Carmel, P.W., Araki, S., and Ferin, M. (1976). Pituitary stalk portal blood collection in Rhesus monkeys: evidence for pulsatile release of gonadotropin-releasing hormone (GnRH). Endocrinology 99, 243-248.

Carr, W.R., and Land, R.B. (1975). Plasma luteinizing hormone levels and testis diameters of ram lambs of different breeds. J. Reprod. Fert. 42, 325-333.

Carson, R.S., Matthews, C.D., Findlay, J.K., Symons, R.G., and Burger, H.G. (1977). Biological and immunological luteinizing hormone-releasing hormone (LH-RH) activity of the ovine pineal. Neuroendocrinology 24, 221-225.

Chamley, W.A., Fell, L.R., Alford, F.P., and Goding, J.R. (1974). Twenty-four hour secretory profiles of ovine prolactin and growth hormone. J. Endocr. 61, 165-166.

Chari, S., Duraiswami, S., and Franchimont, P. (1978). Isolation and characterisation of inhibin from bull seminal plasma. Acta Endocr., Copenh. 87, 434-448.

Christensen, A.K. (1975). Leydig cells. In Handbook of Physiology Section 7: Endocrinology Vol. V Male Reproductive System. Eds. D.W. Hamilton and R.O. Greep. American Physiological Society, Washington. p57-94.

Clegg, M.T., and Ganong, W.F. (1969). Environmental factors affecting reproduction. In Reproduction in Domestic Animals. Eds H.H. Cole and P.T. Cupps. Academic Press, New York. p473-488.

Convey, E.M., and Reece, R.P. (1969). Restoration of pituitary lactogen released in response to suckling. Proc. Soc. Expt. Biol. Med. 131, 543-546.

Cotta, Y., Terqui, M., Pelletier, J., and Courot, M. (1975). Testostérone et LH plasmatiques chez l'agneau de la naissance à la puberté. C.R. Acad. Sc. Paris. Série D. 280, 1473-1476.

Courot, M. (1962). Développement du testicule chez l'agneau. Établissement de la spermatogenèse. Ann. Biol. anim. Bioch. Biophys. 2, 25-41.

Courot, M. (1967). Endocrine control of the supporting and germ cells of the impuberal testis. J. Reprod. Fert., Suppl. 2, 89-101.

Courot, M. (1974). LH, testostérone et prolactine sanguines chez L'agneau male. I.N.S.E.R.M. 32, 157-160.

Courot, M. (1976). Hormonal regulation of male reproduction (with reference to infertility in man). Andrologia 8, 187-193.

Courot, M., de Reviers, M., and Pelletier, J. (1975). Variations in pituitary and blood LH during puberty in the male lamb. Relation to time of birth. Ann. Biol. anim. Bioch. Biophys. 15, 509-516.

Courot, M., and Joffre, M. (1977). Testicular capillary blood flow in the impubertal lamb and the ram during the breeding and non-breeding seasons. Andrologia 9, 332-336.

Crighton, D.B., Schneider, H.P.G., and McCann, S.M. (1970). Localization of LH-releasing factor in the hypothalamus and neurohypophysis as determined by an in vitro method. Endocrinology 87, 323-329.

Crighton, D.B., Foster, J.P., Holland, D.T., and Jeffcoate, S.L. (1973). Simultaneous determination of luteinizing hormone and luteinizing hormone releasing hormone in the jugular venous blood of the sheep at oestrus. J. Endocr. 59, 373-374.

Crim, L.W., and Geschwind, I.I. (1972a). Testosterone concentration in spermatic vein plasma of the developing ram. Bio. Reprod. 7, 42-46.

Crim, L.W. and Geschwind, I.I. (1972b). Patterns of FSH and LH secretion in the developing ram: the influence of castration and replacement therapy with testosterone propionate. Biol. Reprod. 7, 47-54.

Critchlow, V., and Bar-Sela, M.E. (1967). Control of the onset of puberty. In Neuroendocrinology Eds. L. Martini and W.F. Ganong. Academic Press, New York. 2, 101-162.

Davidson, J.M. (1967). Neuroendocrine mechanisms in the control of spermatogenesis. J. Reprod. Fert., Suppl. 2, 103-116.

Davidson, J.M. (1974). Hypothalamic-pituitary regulation of puberty, evidence from animal experimentation. In Control of the Onset of Puberty. Eds. M.M. Grumbach, G.D. Grave and F.E. Mayer. John Wiley and Sons, New York. p79-103.

Davies, A.G., Duncan, I.F., and Lynch, S.S. (1975). Autoradiographic localization of 125 I-labelled follicle-stimulating hormone in the rat hypothalamus. J. Endocr. 66, 301-302.

Davies, I.J., Naftolin, F., Ryan, K.J., and Siu, J. (1976). Specific binding of steroids by neuroendocrine tissues. In Subcellular Mechanisms in Reproductive Neuroendocrinology. Eds. F. Naftolin, K.J. Ryan and J. Davies. Elsevier, Amsterdam. p263-275.

Davies, R.V., Main, S.J., and Setchell, B.P. (1977). Seasonal changes in plasma follicle-stimulating hormone, luteinizing hormone and testosterone in rams. J. Endocr. 72, 12 P Abstr.

Davis, S.L. (1972). Plasma levels of prolactin, growth hormone, and insulin in sheep following the infusion of arginine, leucine and phenylalanine. Endocrinology 91, 549-555.

Davis, S.L., and Borger, M.L. (1973). Metabolic clearance rates and secretion rates of prolactin in sheep. Endocrinology 92, 1414-1418.

Davis, S.L., Ohlson, D.L., Klindt, J., and Anfinson, M.S. (1978). Episodic patterns of prolactin and thyrotropin secretion in rams and wethers: influence of testosterone and diethylstilbestrol. J. Anim. Sci. 46, 1724-1729.

DeFronzo, R.A., and Roth, W.D. (1972). Evidence for the existence of a pineal-adrenal and a pineal-thyroid axis. Acta Endocr., Copenh. 70, 35-42.

Domański, E. (1976). The role of the limbic formations of the central nervous system in the control of gonadotrophic hormones secretion and ovulation. Proc. VIIIth Intern. Congress Anim. Reprod. & A.I. Krakow. p1-7.

Domański, E., Przekop, B., Skubiszewski, B., Wolińska, E. (1975). The effect and site of action of indoleamines on the hypothalamic centres involved in the control of LH release and ovulation in sheep. Neuroendocrinology 17, 265-273.

Donovan, B.T. (1970). Mammalian Neuroendocrinology. McGraw-Hill, London. 208p.

Donovan, B.T. (1974). The role of the hypothalamus in puberty. Prog. Brain Res. 41, 239-253.

Donovan, B.T., and van der Werff ten Bosch, J.J. (1965). Physiology of puberty. Edward Arnold Ltd, London. 216p.

Dorrington, J.H., Fritz, I.B., and Armstrong, D.B. (1978). Control of testicular estrogen synthesis. Bio. Reprod. 18, 55-64.

Dutt, R.H., and Hamm, P.T. (1957). Effect of exposure to high environmental temperature and shearing on semen production of rams in winter. J. Anim. Sci. 16, 328-334.

Dyer, R.G., and Dyball, R.E.J. (1974). Evidence for a direct effect of LRF and TRF on single unit activity in the rostral hypothalamus. Nature 252, 486-488.

Dýrmundsson, Ó.R. (1973). Puberty and early reproductive performance in sheep. II. Ram lambs. Anim. Breed. Abstr. 41, 419-430.

Dýrmundsson, Ó.R., and Lees, J.L. (1972a). Puberal development of Clun Forest ram lambs in relation to time of birth. J. Agric. Sci., Camb. 79, 83-89.

Dýrmundsson, Ó.R., and Lees, J.L. (1972b). A note on mating ability in Clun Forest ram lambs. Anim. Prod. 14, 259-262.

Dýrmundsson, Ó.R., Lees, J. L. (1972c). A note on factors affecting puberty in Clun Forest female lambs. Anim. Prod. 15, 311-314.

Ebels, I., and Horwitz-Bresser, A.E.M. (1976). Separation of pineal extracts by gelfiltration. IV. Isolation, location and identification from sheep pineals of three indoles, identical with 5-hydroxytryptophol, 5-methoxytryptophol and melatonin. J. Neural Transm. 38, 31-41.

Edvinnson, L., Owman, Ch., and West, K.A. (1971). Changes in cerebral blood volume of mice at various time-periods after superior cervical sympathectomy. Acta Physiol., Scand. 82, 521-526.

Eichler, V.B., and Moore, R.Y. (1971). Pineal hydroxyindole-O-methyl transferase and gonadal responses to blinding or continuous darkness blocked by pineal denervation in the male hamster. Neuroendocrinology 8, 81-85.

Eik-Nes, K.B. (1975). Biosynthesis and secretion of testicular steroids. In Handbook of Physiology Section 7: Endocrinology Vol. V Male Reproductive System. Eds. D.W. Hamilton and R.O. Greep. American Physiological Society, Washington. p 95-115.

Ellendorff, F. (1976). Evaluation of extrahypothalamic control of reproductive physiology. Rev. Physiol. Biochem. Pharmacol. 76, 103-127.

Ellis, L.C. (1976). Peripheral and CNS effects of the pineal gland: target enzymes common to tissues and species. Am. Zoologist 16, 67-78.

Fawcett, D.W. (1975). Ultrastructure and function of the Sertoli cell. In Handbook of Physiology Section 7: Endocrinology Vol. V Male Reproductive System. Eds. D.W. Hamilton and R.O. Greep. American Physiological Society, Washington. p21-55.

Fell, L.R., Beck, C., Brown, J.M., Catt, K.J., Cumming, I.A., and Goding, J.R. (1972). Solid-phase radioimmunoassay of ovine prolactin in antibody-coated tubes. Prolactin secretion during estradiol treatment, at parturition, and during milking. Endocrinology 91, 1329-1336.

Fell, L.R., Findlay, J.K., Cumming, I.A., and Goding, J.R. (1973). Effect of synthetic TRF on prolactin release in the sheep. Endocrinology 93, 487-491.

Forbes, J.M. (1975). Effect of pinealectomy on growth and plasma prolactin in sheep at two daylengths. Acta Endocr., Copenh. Suppl. 199, 121. Abstr.

Forbes, J.M., Driver, P.M., El Shahat, A.A., Boaz, T.G., and Scanes, C.G. (1975). The effect of daylength and level of feeding on serum prolactin in growing lambs. J. Endocr. 64, 549-554.

Foster, D.L. (1974). Regulation of gonadotropins during fetal and early postnatal development in the sheep. I.N.S.E.R.M. 32, 143-156.

Foster, D.L., Cruz, T.A.C., Jackson, G.L., Cook, B., and Nalbandov, A.V. (1972). Regulation of luteinizing hormone in the fetal and neonatal lamb. III. Release of LH by the pituitary in vivo in response to crude ovine hypothalamic extract or purified porcine gonadotrophin releasing factor. Endocrinology 90, 673-683.

Foster, D.L., Jaffe, R.B., and Niswender, G.D. (1975). Sequential patterns of circulating LH and FSH in female sheep during the early postnatal period: effect of gonadectomy. Endocrinology 96, 15-22.

Foster, D.L., Mickelson, I.H., Ryan, K.D., Coon, G.A., Drongowski, R.A., and Holt, J.A. (1978). Ontogeny of pulsatile luteinizing hormone and testosterone secretion in male lambs. Endocrinology 102, 1137-1146.

Franchimont, P., Chari, S., Hagelstein, M., Debruche, M.L., Duraiswami, S., Walton, J., and Waites, G.M.H. (1976). Existence of a follicle-stimulating hormone-inhibiting factor in ram rete testis fluid. Andrologia 8, Suppl. 1, 127-128. Abstr.

Frisch, R.E., (1974). Critical weight at menarche, initiation of the adolescent growth spurt, and control of puberty. In Control of the Onset of Puberty. Eds. M.M. Grumbach, G.D. Grave, and F.E. Mayer. John Wiley and Sons, New York. p403-423.

Galloway, D.G., and Pelletier, J. (1974). Influence of age on the pituitary response of male lambs to synthetic LH-RH injection. Horm. Metab. Res. 6, 240-241.

Galloway, D.B., and Pelletier, J. (1975). Luteinizing hormone release in entire and castrated rams following injection of synthetic luteinizing hormone releasing hormone and effect of testosterone propionate pre-treatment. J. Endocr. 64, 7-17.

Ganong, W.F., Shepherd, M.D., Wall, J.R., Van Brunt, E.E., and Clegg, M.T. (1963). Penetration of light into the brain of mammals. Endocrinology 72, 962-963.

Garnier, D., Ortavant, R., Mansard, F., and Terqui, M. (1977a). Influence de la lumiére sur les variations de la testostéronémie chez le Bêlier: mise en évidence d'une phase photosensible au cours du rythme diurne. C.R. Acad. Sc. Paris. Série D. 284, 61-64.

Garnier, D.H., Terqui, M., and Pelletier, J. (1977b). Plasma concentrations of LH and testosterone in castrated rams treated with testosterone or testosterone propionate. J. Reprod. Fert. 49, 359-361.

Geschwind, I.I., and Dewey, R. (1968). Dynamics of luteinizing hormone (LH) secretion in the cycling ewe: a radioimmunoassay study. Proc. Soc. Exp. Biol. Med. 129, 451-455.

Gradwell, P.B., Millar, R.B., and Symington, R.B. (1976). Failure to demonstrate high concentrations of luteinizing hormone-releasing hormone in the bovine pineal body. S.A. Med. J. 50, 217-219.

Greep, R.O. (1973). Nature, rôle and control of the gonadotrophins. J. Reprod. Fert., Suppl. 18, 1-13.

Griffiths, E.C., Hooper., K.C., Jeffcoate, S.L., and Holland, D.T. (1974). The presence of peptidases in the rat hypothalamus inactivating luteinizing hormone-releasing hormone (LH-RH). Acta Endocr., Copenh. 77, 435-442.

Griffiths, E.C., Hooper, K.C., Jeffcoate, S.L., and Holland, D.T. (1975). The effects of gonadectomy and gonadal steroids on the activity of hypothalamic peptidases inactivating luteinizing hormone-releasing hormone (LH-RH). Brain Research 88, 348-388.

Hafiez, A.A., Lloyd, C.W., and Bartke, A. (1972a). The role of prolactin in the regulation of testis function: the effects of prolactin and luteinizing hormone on the plasma levels of testosterone and androstenedione in hypophysectomized rats. J. Endocr. 52, 327-332.

Hafiez, A.A., Bartke, A., and Lloyd, C.W. (1972b). The role of prolactin in the regulation of testis function: the synergistic effects of prolactin and luteinizing hormone on the incorporation of [1-^{14}C]acetate into testosterone and cholesterol by testes from hypophysectomized rats in vitro. J. Endocr. 53, 223-230.

Hall, P.F. (1970). Endocrinology of the testis. In The Testis. Eds. A.D. Johnson, W.R. Gomes, and N.L. Vandemark. Academic Press, New York. 2, 1-71.

Hall, P.F., and Young, D.G. (1968). Site of action of trophic hormones upon the biosynthetic pathways to steroid hormones. Endocrinology 82, 559-568.

Hansson, V., Ritzén, E.M., French, F.S., and Nayfeh, S.N. (1975). Androgen transport and receptor mechanisms in testis and epididymis. In Handbook of Physiology Section 7: Endocrinology Vol. V Male Reproductive System. Eds. D.W. Hamilton and R.O. Greep. American Physiological Society, Washington. p173-201.

Hansson, V., Djoseland, O., Torgersen, O., Ritzen, E.M., French, F.S., and Nayfeh, S.N. (1976a). Hormones and hormonal target cells in the testis. Andrologia 8, 195-202.

Hansson, V., Calandra, R., Purvis, K., Ritzen, M., and French, F.S. (1976b). Hormonal regulation of spermatogenesis. Vitamins and Hormones 35, 187-214.

Harris, G.W., and Jacobsohn, D. (1952). Functional grafts of the anterior pituitary gland. Proc. Roy. Soc., Lond. B. 139, 263-276.

Hart, I.C., and Buttle, H.L. (1975). Growth hormone levels in the plasma of male goats throughout the year. J. Endocr. 67, 137-138.

Haynes, N.B., Hafs, H.D., Purvis, K., and Manns, J.G. (1976). Studies on the effect of unilateral and bilateral castration on plasma testosterone and LH levels in the bull. J. Reprod. Fert. 46, 471-473.

Hedlund, L., Lischko, M.M., Rollag, M.D., and Niswender, G.D. (1977). Melatonin: daily cycle in plasma and cerebrospinal fluid of calves. Science, New York. 195, 686-687.

Herbert, J. (1972). Initial observations on pinealectomized ferrets kept for long periods in either daylight or artificial illumination. J. Endocr. 55, 591-597.

Herbert, D.C. (1976). Immunocytochemical evidence that luteinizing hormone (LH) and follicle stimulating hormone (FSH) are present in the same cell type in the Rhesus monkey pituitary gland. Endocrinology 98, 1554-1557.

Hoffmann, K. (1978). Effects of short photoperids on puberty, growth and moult in the Djungarian hamster (Phodopus sungorus). J. Reprod. Fert. 54, 29-35.

Holley, D.C., Beckman, D.A., and Evans, J.W. (1975). Effect of confinement on the circadian rhythm of ovine cortisol. J. Endocr. 65, 147-148.

Hopkinson, C.R.N., Pant, H.C., and Fitzpatrick, R.J. (1974). Release of LH and FSH in the normal intact ram by synthetic LH-RF and the effect of pretreatment with testosterone propionate. J. Reprod. Fert. 39, 135-139.

Horrobin, D.F. (1973). Prolactin: Physiological and Clinical Significance. Medical and Technical Publishing, Lancaster. 239p.

Horrobin, D.F. (1974). Prolactin 1974. Medical and Technical Publishing, Lancaster. 156p.

Horrobin, D.F. (1975). Prolactin 1975. Eden Press, Montreal.

Horrobin, D.F., Manku, M.S., and Burnstyn, P.G. (1973). Saluretic action of aldosterone in the presence of excess cortisol: restoration of salt-retaining action by prolactin. J. Endocr. 56, 343-344.

Hostetter, M.W., and Piacsek, B.E. (1977). The effect of prolacton deficiency during sexual maturation in the male rat. Bio. Reprod. 17, 574-577.

Hyyppä, M.T. (1974). Neuroendocrine control of puberty: role of perinatal neuroamines. I.N.S.E.R.M. 32, 395-406.

Illius, A.W., Haynes, N.B., Purvis, K., and Lamming, G.E. (1976a). Plasma concentrations of testosterone in the developing ram in different social environments. J. Reprod. Fert. 48, 17-24.

Illius, A.W., Haynes, N.B., and Lamming, G.E. (1976b). Effects of ewe proximity on peripheral plasma testosterone levels and behaviour in the ram. J. Reprod. Fert. 48, 25-32.

Jeffcoate, S.L. (1975). The control of testicular function in the adult. Clinics Endocr. Metab. 4, 521-543.

Jegou, B., Dacheux, J.L., Terqui, M., and Garnier, D.H. (1976). Étude de la protéine de liaison de la 5∝ dihydrotestosterone (5∝ DHT) et de la testostérone dans le tractus génital mâle (bélier). J. Physiol., Paris 72, 44A. Abstr.

Jegou, B., Dacheux, J., and Terqui, M. (1978a). Mise en évidence d'une protéine de liaison spécifique des androgènes de type ABP. C.R. Acad. Sci. Paris, Série D. 286, 347-350.

Jegou, B., Dacheux, J.L., Terqui, M., Garnier, D.H., and Courot, M. (1978b). Studies on the androgen binding protein in the rete testis fluid of the ram and its relation to sexual season. Molec. Cell. Endocr. 9, 335-346.

Jenkin, G., Heap, R.B., and Symons, D.B.A. (1977). Pituitary responsiveness to synthetic LH-RH and pituitary LH content at various reproductive stages in the sheep. J. Reprod. Fert. 49, 207-214.

Jewell, P.A., Milner, C., and Morton Boyd, J. (1974). Island Survivors: The Ecology of the Soay Sheep of St. Kilda. Athlone Press, London. 386p.

Johnson, B.H., Desjardins, C., and Ewing, L.L. (1973). Seasonal effects in testis function in rams. J. Anim. Sci. 37, 247. Abstr.

Jost, A. (1976). Hormonal and genetic factors affecting the development of the male genital system. Andrologia 8, Suppl. 1, 17-33.

Jost, A., Vigier, B., Prépin, J., and Perchellet, J.P. (1973). Studies on sex differentiation in mammals. Rec. Prog. Horm. Res. 29, 1-41.

Jutisz, M., Bérault, A., and Theoleyre, M. (1976). Cyclic AMP and the mechanism of action of gonadotropin releasing hormone (LH-RH). Ann. Biol. anim. Bioch. Biophys. 16, 205-211.

Kamberi, I.A. (1973). Biogenic amines and neurohumoral control of gonadotropin and prolactin secretion. Proc. 4th Intern. Congr. Endocr. Washington, D.C., 1972. Ed. R.O. Seaús. Elsevier, New York. p112-119.

Kamberi, I.A., Mical, R.S., and Porter, J.C. (1970). Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. Endocrinology 87, 1-12.

Kamberi, I.A., Mical, R.S., and Porter, J.C. (1971a). Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 88, 1288-1293.

Kamberi, I.A., Mical, R.S., and Porter, J.C. (1971b). Effect of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. Endocrinology 88, 1012-1020.

Kappers, J.A. (1965). Survey of the innervation of the epiphysis cerebri and the accessory pineal organs of vertebrates. Prog. Brain Research 10, 87-153.

Kappers, J.A. (1971). Innervation of the pineal organ: phylogenetic aspects and comparison of the neural control of the mammalian pineal with that of other neuroendocrine systems. Mem. Soc. Endocr. 19, 27-48.

Karsch, F.J., and Foster, D.L. (1975). Sexual differentiation of the mechanism controlling the preovulatory discharge of luteinizing hormone in sheep. Endocrinology 97, 373-379.

Kato, J. (1976). Ontogeny of 5∝-dihydrotestosterone receptors in the hypothalamus of the rat. Ann. Biol. anim. Bioch. Biophys. 16, 467-469.

Katongole, C.B., Naftolin, F., and Short, R.V. (1974). Seasonal variations in blood luteinizing hormone and testosterone levels in rams. J. Endocr. 60, 101-106.

Kennaway, D.J., Frith, R.G., Phillipou, G., Matthews, C.D., and Seamark, R.F. (1977). A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography-mass spectrometry. Endocrinology 101, 119-127.

Kennaway, D.J., Porter, K.J., and Seamark, R.F. (1978). Changes in plasma tryptophan and melatonin content in penned sheep. Aust. J. Biol. Sci. 31, 49-52.

Keogh, E.J., Lee, V.W.K., Rennie, G.C., Burger, H.G., Hudson, B., and de Kretser, D.M. (1976). Selective supression of FSH by testicular extracts. Endocrinology 98, 997-1004.

Kincl, F.A., and Benagiano, G. (1967). The failure of the pineal gland removal in neonatal animals to influence reproduction. Acta Endocr., Copenh. 54, 189-192.

Kitay, J.I. (1954). Pineal lesions and precocious puberty: a review. J. Clin. Endocr. Metab. 14, 622-625.

Kledzik, G.S., Marshall, S., Campbell, G.A., Gelato, M., and Meites, J. (1976). Effects of castration, testosterone, estradiol, and prolactin on specific prolactin-binding activity in ventral prostate of male rats. Endocrinology 98, 373-379.

Klein, D.C., Weller, J.L., and Moore, R.Y. (1971). Melatonin metabolism: neural regulation of pineal serotonin: acetyl coenzyme A N-acetyltransferase activity. Proc. Natl. Acad. Sci. USA 68, 3107-3110.

Koenig, M.P., Zuppinger, K., and Liechti, B. (1977). Hyperprolactinemia as a cause of delayed puberty: Successful treatment with bromocriptine. J. Clin. Endocr. Metab. 45, 825-828.

Kordon, C., Epelbaum, J., Enjalbert, A., and McKelvy, J. (1976). Neurotransmitter interactions with neuroendocrine tissue. In Subcellular Mechanisms in Reproductive Neuroendocrinology. Eds. F. Naftolin, K.J. Ryan and I. J. Davies. Elsevier, Amsterdam. p167-184.

Kordon, C., Enjalbert, A., and Carbonell, L. (1977). Central neurotransmitters and control of prolactin secretion. Ann. Biol. anim. Bioch. Biophys. 17, 425-439.

de Kretser, D.M., Atkins, R.C., and Paulsen, C.A. (1973). Role of the kidney in the metabolism of luteinizing hormone. J. Endocr. 58, 425-434.

Kuhl, H., and Taubert, H-D. (1975). Short-loop feedback mechanism of luteinizing hormone: LH stimulates hypothalamic L-cystine arylamidase to inactivate LH-RH in the rat hypothalamus. Acta Endocr., Copenh. 78, 649-663.

Labhsetwar, A.P., Joshi, H.S., and Zolovick, A. (1974). Possible role of prostaglandins in the secretion of gonadotropins. In Gonadotropins and Gonadal Function. Ed. N.R. Moudgal. Academic Press, New York. p357-363.

Labrie, F., Pelletier, G., Borgeat, P., Drouin, J., Ferland, L., and Belanger, A. (1976). Mode of action of hypothalamic regulatory hormones in the adenohypophysis. In Frontiers in Neuroendocrinology, Vol. 4. Eds. L. Martini and W.F. Ganong. Raven Press, New York. p63-93.

Land, R.B. (1978). Reproduction in young sheep: some genetic and environmental sources of variation. J. Reprod. Fert. 52, 427-436.

Lee, V.W.K., Cumming, I.A., de Kretser, D.M., Findlay, J.K., Hudson, B., and Keogh, E.J. (1976a). Regulation of gonadotrophin secretion in rams from birth to sexual maturity. I. Plasma LH, FSH and testosterone levels. J. Reprod. Fert. 46, 1-6.

Lee, V.W.K., Cumming, I.A., de Kretser, D.M., Findlay, J.K., Hudson, B., and Keogh, E.J. (1976b). Regulation of gonadotrophin secretion in rams from birth to sexual maturity. II. Response of the pituitary-testicular axis to LH-RH infusion. J. Reprod. Fert. 46, 7-11.

Lee, V.W.K., Keogh, E.J., Burger, H.G., Hudson, B., and de Kretser, D.M. (1976c). Studies on the relationship between FSH and germ cells: evidence for selective suppression of FSH by testicular extracts. J. Reprod. Fert., Suppl. 24, 1-15.

Lemay, J.P., and Corrivault, G.W. (1973). Variations saisonnières de l'activité sexuelle chez le bélier. Le Naturaliste Canadien 100, 19-24.

Lerner, A.B., Case, J.D., Takahashi, Y., Lee, T.H., and Mori, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes. J. Amer. Chem. Soc. 80, 2587.

Levasseur, M.-C. (1977). Thoughts on puberty. Initiation of gonadotropic function. Ann. Biol. anim. Bioch. Biophys. 17, 345-361.

Liao, S., and Fang, S. (1969). Receptor-proteins for androgens and the mode of action of androgens on gene transcription in ventral prostate. Vitamins and Hormones 27, 17-90.

Lincoln, G.A. (1971). The seasonal reproductive changes in the Red deer stag (Cervus elaphus). J. Zool., Lond. 163, 105-123.

Lincoln, G.A. (1976a). Seasonal variation in the episodic secretion of luteinizing hormone and testosterone in the ram. J. Endocr. 69, 213-226.

Lincoln, G.A. (1976b). Secretion of LH in rams exposed to two different photoperiods. J. Reprod. Fert. 47, 351-353.

Lincoln, G.A. (1976c). Seasonal changes in the pineal gland related to the reproductive cycle in the male hare, Lepus europaeus. J. Reprod. Fert. 46, 489-491.

Lincoln, G.A. (1977). Changes in pituitary responsiveness to luteinizing hormone releasing hormone in rams exposed to artificial photoperiods. J. Endocr. 73, 519-527.

Lincoln, G.A. (1978a). Induction of testicular growth and sexual activity in rams by a 'skeleton' short-day photoperiod. J. Reprod. Fert. 52, 179-181.

Lincoln, G.A. (1978b). The temporal relationship between plasma levels of FSH and LH in the ram. J. Reprod. Fert. 53, 31-37.

Lincoln, G.A., and Davidson, W. (1977). The relationship between sexual and aggressive behaviour, and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. J. Reprod. Fert. 49, 267-276.

Lincoln, G.A., and MacKinnon, P.C.B. (1976). A study of seasonally delayed puberty in the male hare, Lepus europaeus. J. Reprod. Fert. 46, 123-128.

Lincoln, G.A., and Peet, M.J. (1977). Photoperiodic control of gonadotrophin secretion in the ram: a detailed study of the temporal changes in plasma levels of follicle-stimulating hormone, luteinizing hormone and testosterone following an abrupt switch from long to short days. J. Endocr. 74, 355-367.

Lincoln, G.A., Peet, M.J., and Cunningham, R.A. (1977). Seasonal and circadian changes in the episodic release of follicle-stimulating hormone, luteinizing hormone and testosterone in rams exposed to artificial photoperiods. J. Endocr. 72, 337-349.

Lincoln, G.A., McNeilly, A.S., and Cameron, C.L. (1978). The effects of a sudden decrease or increase in daylength on prolactin secretion in the ram. J. Reprod. Fert. 52, 305-311.

Lino, B.F. (1972). The output of spermatozoa in rams. II. Relationship to scrotal circumference, testis weight, and the number of spermatozoa in different parts of the urogenital tract. Aust. J. Biol. Sci. 25, 359-366.

Lodge, J.R., and Salisbury, G.W. (1970). Seasonal variation and male reproductive efficiency. In The Testis. Eds. A.D. Johnstone, W.R. Gomes and N.L. Vandemark. Academic Press, New York. 3, 139-167.

Lostroh, A.J. (1969). Regulation by FSH and ICSH (LH) of reproductive function in the immature male rat. Endocrinology 85, 438-445.

Lynch, H.J., Hsuan, M., and Wurtman, R.J. (1975). Sympathetic neural control of indoleamine metabolism in the rat pineal gland. Adv. Exp. Med. Biol. 54, 93-114.

McCann, S.M. (1971). Mechanism of action of hypothalamic-hypophyseal stimulating and inhibiting hormones. In Frontiers in Neuroendocrinology, 1971. Eds. L. Martini and W.F. Ganong. Oxford University Press, London. p209-235.

McCann, S.M. (1974). Regulation of secretion of follicle-stimulating hormone and luteinizing hormone. In Handbook of Physiology Section 7: Endocrinology Vol. IV The pituitary gland and its neuroendocrine control, Part 2. Eds. E. Knobil and W.H. Sawyer. American Physiological Society, Washington. p489-517.

McCann, S.M. (1976). Development and maturation of the hypothalamo-hypophyseal control of the reproductive system. Ann. Biol. anim. Bioch. Biophys. 16, 279-289.

McCann, S.M., and Porter, J.C. (1969). Hypothalamic pituitary stimulating and inhibiting hormones. Physiol. Rev. 49, 240-284.

McCann, S.M., Krulich, L., Cooper, K.J., Kalra, P.S., Kalra, S.P., Libertun, C., Negro-Vilar, A., Orias, R., Rønnekleiv, O., and Fawcett, C.P. (1973). Hypothalamic control of gonadotrophin and prolactin secretion, implications for fertility control. J. Reprod. Fert., Suppl. 20, 43-59.

McCann, S.M., Ojeda, S.R., Harms, P.G., Wheaton, J.E., Sundberg, D.K., and Fawcett, C.P. (1976). Control of adenohypophyseal hormone secretion by prostaglandins. In Subcellular Mechanisms in Reproductive Neuroendocrinology. Eds. F. Naftolin, K.J. Ryan and J. Davies. Elsevier, Amsterdam. p407-422.

McKenna, T.J., Glick, A.D., Cobb, C.A. (Jnr.), and Jacobs, L.S. (1978). Galactorrhea and hypogonadism associated with radiologically-inapparent prolactin-secreting pituitary tumour. Acta Endocr., Copenh. 87, 225-233.

MacKinnon, P.C.B., Puig-Duran, E., and Laynes, R. (1978). Reflections on the attainment of puberty in the rat: have circadian signals a role to play in its onset? J. Reprod. Fert. 52, 401-412.

Marshall, F.H.A. (1937). On the change over in the oestrous cycle in animals after transference across the equator, with further observations on the incidence of the breeding seasons and the factors controlling sexual periodicity. Proc. Royal Soc. Lond. Ser. B 122, 413-428.

Martin, J.E., and Klein, D.C. (1976). Melatonin inhibition of the neonatal pituitary response to luteinizing hormone-releasing factor. Science, New York. 191, 301-302.

Martini, L. (1976). Androgen reduction by neuroendocrine tissues: physiological significance. In Subcellular Mechanisms in Reproductive Neuroendocrinology. Eds. F. Naftolin, K.J. Ryan and J. Davies. Elsevier, Amsterdam. p327-345.

Matsuo, H., Baba, Y., Nair, R.M.G., Arimura, A., and Schally, A.V. (1971a). Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 43, 1334-1339.

Matsuo, H., Arimura, A., Nair, R.M.G., and Schally, A.V. (1971b). Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. Biochem. Biophys. Res. Commun. 45, 822-827.

Means, A.R. (1974). Early sequence of biochemical events in the action of follicle stimulating hormone on the testis. Life Sciences 15, 371-389.

Meijs-Roelofs, H.M.A., and Moll, J. (1978). Sexual maturation and the adrenal glands. J. Reprod. Fert. 52, 413-418.

Meites, J., and Nicoll, C.S. (1966). Adenohypophysis: prolactin. Ann. Rev. Physiol. 28, 57-88.

Menaker, M. (1971). Rhythms, reproduction and photoreception. Bio. Reprod. 4, 295-308.

Menaker, M., Takahashi, J.S., and Eskin, A. (1978). The physiology of circadian pacemakers. Ann. Rev. Physiol. 40, 501-526.

Mess, B., Zanisi, M., and Tima, L. (1970). Site of production of releasing and inhibiting factors. In The Hypothalamus. Eds. L. Martini, M. Motta and F. Fraschini. Academic Press, New York. p259-276.

Minguell, J.J., and Sierralta, W.D. (1975). Molecular mechanism of action of the male sex hormones. J. Endocr. 65, 287-315.

Moore, R.Y. (1973). Retinohypothalamic projection in mammals: a comparative study. Brain Research 49, 403-409.

Moore, R.Y. (1978). Central neural control of circadian rhythms. In Frontiers in Neuroendocrinology. Eds. W.F. Ganong and L. Martini. Raven Press, New York. 5, 185-206.

Moore, R.Y., Heller, A., Bhatnager, R.K., Wurtman, R.J., and Axelrod, J. (1968). Central control of the pineal gland: visual pathways. Arch. Neurol. 18, 208-218.

Moore, R.Y., and Klein, D.C. (1974). Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Research 71, 17-33.

Moore, R.W., Whyman, D., and Wilson, P.R. (1978). Effects of sexual stimulation on plasma levels of LH and testosterone in rams from high- and low-fertility flocks. J. Reprod. Fert. 53, 67-70.

Morgan, W.W., Reiter, R.J., and Pfeil, K.A. (1976). Hamster pineal noradrenaline: levels over a regulated lighting period and the influence of superior cervical ganglionectomy. Life Sciences 19, 437-440.

Motta, M., Piva, F., and Martini, L. (1973). New findings on the central control of gonadotrophin secretion. J. Reprod. Fert., Suppl. 20, 27-42.

Nagle, C.A., Cardinali, D.P., and Rosner, J.M. (1975). Testosterone effects on protein synthesis in the rat pineal gland. Modulation by the sympathetic nervous system. Life Sciences 16, 81-91.

Nakane, P.K. (1970). Classifications of anterior pituitary cell types with immunoenzyme histochemistry. J. Histochem. Cytochem. 18, 9-20.

Neill, J.D. (1974). Prolactin: its secretion and control. In Handbook of Physiology Section 7: Endocrinology Vol. IV The Pituitary Gland and its Neuroendocrine Control, Part 2. American Physiological Society, Washington. p469-488.

Nett, T.M., Akbar, A.M., and Niswender, G.D. (1974). Serum levels of luteinizing hormone and gonadotropin-releasing hormone in cycling, castrated and anestrous ewes. Endocrinology 94, 713-718.

Nicoll, C.S., and Bern, H.A. (1972). On the actions of prolactin among the vertebrates: is there a common denominator? In Lactogenic Hormones. Eds. G.E.W. Wolstenholme and J. Knight. Churchill Livingstone, Edinburgh. p299-324.

Niswender, G.D., Reichert, L.E. (Jnr.)., Midgley, A.R. (Jnr.), and Nalbandov, A.V. (1969). Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84, 1166-1173.

Nunez, E.A., Benassaayag, C., Savu, L., Vallette, G., and Jayle, M.F. (1976). Serum binding of some steroid hormones during development in different animal species. Discussion on the biological significance of this binding. Ann. Biol. anim. Bioch. Biophys. 16, 491-501.

Odell, W.D., and Swerdloff, R.S. (1976). Etiologies of sexual maturation: a model system based on the sexually maturing rat. Rec. Prog. Horm. Res. 32, 245-288.

Ojeda, S.R., and Ramirez, V.D. (1973/74). Short-term steroid treatment on plasma LH and FSH in castrated rats from birth to puberty. Neuroendocrinology 13, 100-114.

O'Malley, B.W., and Schrader, W.T. (1976). The receptors of steroid hormones. Scientific American 234, 32-43.

Ortavant, R., Mauleon, P., and Thibault, C. (1964). Photoperiodic control of gonadal and hypophyseal activity in domestic mammals. Ann. N.Y. Acad. Sci. 117, 157-193.

Ortavant, R., Courot, M., and Hochereau, M.T. (1969). Spermatogenesis and morphology of the spermatozoon. In Reproduction in Domestic Animals. Eds. H.H. Cole and P.T. Cupps. Academic Press, New York. p251-276.

Ozaki, Y., and Lynch, H.J. (1976). Presence of melatonin in plasma and urine of pinealectomized rats. Endocrinology 99, 641-644.

Palkovits, M., Arimura, A., Brownstein, M., Schally, A.V., and Saavedra, J.M. (1974). Luteinizing hormone-releasing hormone (LH-RH) content of the hypothalamic nuclei in rat. Endocrinology 95, 554-558.

Pang, S.F., Brown, G.M., Grota, L.J., and Rodman, R.L. (1976). Radioimmunoassay of melatonin in pineal glands, harderian glands, retinas and sera of rats or chickens. Fed. Proc. 35, 691. Abstr.

Parfitt, A.G., and Klein, D.C. (1976). Sympathetic nerve endings in the pineal gland protect against acute stress-induced increase in N-acetyltransferase (EC 2.3.1.5.) activity. Endocrinology 99, 840-851.

Parker, D.C., Rossman, L.G., and Vanderlaan, E.F. (1973). Sleep-related, nyctohemeral and briefly episodic variation in human plasma prolactin concentrations. J. Clin. Endocr. Metab. 36, 1119-1124.

Pavel, S. (1973). Arginine vasotocin release into cerebrospinal fluid of cats induced by melatonin. Nature New Biology 246, 183-184.

Pelletier, J. (1968). Élévation du taux de LH dans le plasma sanguin du bêlier après castration. Ann. Biol. anim. Bioch. Biophys. 8, 313-315.

Pelletier, J. (1970). Mode of action of testosterone propionate on the secretion and release of luteinizing hormone (LH) in the castrated ram. Acta Endocr., Copenh. 63, 290-298.

Pelletier, J. (1973). Evidence for photoperiodic control of prolactin release in rams. J. Reprod. Fert. 35, 143-147.

Pelletier, J. (1974). Decrease in the pituitary response to synthetic LH-RF in castrated rams following testosterone propionate treatment. J. Reprod. Fert. 41, 397-402.

Pelletier, J. (1976). Influence of LH-RF on LH and FSH releases in domestic mammals. Ann. Biol. anim. Bioch. Biophys. 16, 213-234.

Pelletier, J., and Ortavant, R. (1975a). Photoperiodic control of LH release in the ram. I. Influence of increasing and decreasing light photoperiods. Acta Endocr., Copenh. 78, 435-441.

Pelletier, J., and Ortavant, R. (1975b). Photoperiodic control of LH release in the ram. II. Light-androgens interaction. Acta Endocr., Copenh. 78, 442-450.

Phifer, R.F., Midgley, A.R., and Spicer, S.S. (1973). Immunohistologic and histologic evidence that follicle-stimulating hormone and luteinizing hormone are present in the same cell type in the human pars distalis. J. Clin. Endocr. Metab. 36, 125-141.

Plapinger, L., and McEwen, B.S. (1973). Ontogeny of oestradiol-binding sites in rat brain. I. Appearance of presumptive adult receptors in cytosol and nuclei. Endocrinology 93, 1119-1128.

Pomerantz, D.K., and Nalbandov, A.V. (1975). Androgen level in the sheep fetus during gestation. Proc. Soc. Exp. Bio. Med. 149, 413-416.

Price, M.A. (1975). The effects of added dietary lipid on the body composition of rams and wethers. J. Agric. Sci., U.K. 84, 201-208.

Przekop, F., and Domański, E. (1970). Induction of ovulation in sheep by electrical stimulation of hypothalamic regions. J. Endocr. 46, 305-311.

Purvis, K., Illius, A.W., and Haynes, N.B. (1974). Plasma testosterone concentrations in the ram. J. Endocr. 61, 241-253.

Quay, W.B. (1974). Pineal Chemistry. C.C. Thomas, Springfield.

Quijada, M., Krulich, L., Fawcett, C.P., Sundberg, D.K., and McCann, S.M. (1971). Localization of TSH-releasing factor (TRF), LH-RF and FSH-RF in rat hypothalamus. Fed. Proc. 30, 197. Abstr.

Racey, P.A. (1978). Seasonal changes in testosterone levels and androgen-dependent organs in male moles (Talpa europaea). J. Reprod. Fert. 52, 195-200.

Radford, H.M. (1967). Electrical stimulation of ovulation in the ewe. J. Endocr. 38, 477-478.

Raikhlin, N.T., Kvetnoy, I.M., and Tolkachev, V.N. (1975). Melatonin may be synthesized in enterochromaffin cells. Nature 255, 344-345.

Raisman, G. (1970). Some aspects of the neural connections of the hypothalamus. In The Hypothalamus. Eds. L. Martini, M. Motta and F. Fraschini. Academic Press, London. p1-15.

Raisman, G., and Field, P.M. (1971). Anatomical considerations relevant to the interpretation of neuroendocrine experiments. In Frontiers in Neuroendocrinology, 1971. Eds. L. Martini and W.F. Ganong. Oxford University Press, London. p3-44.

Rajaniemi, H., and Vanha-Perttula, T. (1973). Evidence for LH and FSH binding proteins(s) in human and rat serum. Horm. Metab. Res. 5, 261-266.

Ramirez, D.V., and McCann, S.M. (1963). Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology 72, 452-464.

Raud, H.R., Kiddy, C.A., and Odell, W.D. (1971). The effect of stress upon the determination of serum prolactin by radioimmunoassay. Proc. Soc. Exp. Bio. Med. 136, 689-693.

Ravault, J.P. (1976). Prolactin in the ram: seasonal variations in the concentration of blood plasma from birth until three years old. Acta Endocr., Copenh. 83, 720-725.

Ravault, J.P., and Courot, M. (1975). Blood prolactin in the male lamb from birth to puberty. J. Reprod. Fert. 42, 563-566.

Ravault, J.P., and Ortavant, R. (1977). Light control of prolactin secretion in sheep. Evidence for a photoinducible phase during a diurnal rhythm. Ann. Biol. anim. Bioch. Biophys. 17, 459-473.

Ravault, J.P., Daveau, A., Garnier, D., Pelletier, J., de Reviers, M.M., Terqui, M., and Ortavant, R. (1976). Influence of some light pulses on the daily variations of prolactin secretion in the ram. Proc. VIIIth Intern. Congr. Anim. Reprod. and A.I. p81-84.

Ravault, J.P., Bartke, A., and Caraty, A. (1977a). Plasma prolactin levels in castrated lambs with and without testosterone treatment. J. Endocr. 72, 235-236.

Ravault, J.P., Courot, M., Garnier, D., Pelletier, J., and Terqui, M. (1977b). Effect of 2-bromo-α-ergocryptine (CB 154) on plasma prolactin, LH and testosterone levels, accessory reproductive glands and spermatogenesis in lambs during puberty. Bio. Reprod. 17, 192-197.

Reeves, J.J., Arimura, A., and Schally, A.V. (1970). Studies on dose response relationship of luteinizing hormone-releasing hormone (LH-RH) in sheep. J. Anim. Sci. 31, 933-936.

Relkin, R. (1970a). Influence of prepuberal and post-puberal pinealectomy on maze performance. Amer. J. Physiol. 218, 328-331.

Relkin, R. (1970b). The influence of prepuberal and post-puberal pinealectomy on treadwheel activity. Physiol. Behav. 5, 341-343.

Relkin, R. (1971). Relative efficacy of pinealectomy, hypothalamic and amygdaloid lesions in advancing puberty. Endocrinology 88, 415-418.

Relkin, R. (1976). The Pineal. Eden Press, Montreal. 187p.

Reiter, R.J. (1968). The pineal gland and gonadal development in male rats and hamsters. Fert. Steril. 19, 1009-1017.

Reiter, R.J. (1973). Comparative physiology: pineal gland. Ann. Rev. Physiol. 35, 305-328.

Reiter, R.J. (1974a). Pineal regulation of hypothalamicopituitary axis: gonadotrophins. In Handbook of Physiology Section 7: Endocrinology Vol. IV The Pituitary Gland and its Neuroendocrine Control, Part 2. Eds. E. Knobil and W.H. Sawyer. American Physiology Society, Washington. p519-550.

Reiter, R.J. (1974b). Circannual reproductive rhythms in mammals related to photoperiod and pineal function: a review. Chronobiologia 1, 365-395.

Reiter, R.J. (1977). The Pineal-1977. Annual Research Reviews. Eden Press, Montreal. 184p.

Reiter, R.J., and Hester, R.J. (1966). Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod in the regulation of the endocrine systems of hamsters. Endocrinology 79, 1168-1170.

Reiter, R.J., and Vaughan, M.K. (1977). Pineal antigonadotrophic substances: polypeptides and indoles. Life Sciences 21, 159-172.

Reiter, R.J., Welsh, M.G., and Vaughan, M.K. (1976). Age-related changes in the intact and sympathetically denervated Gerbil pineal gland. Amer. J. Anat. 146, 427-432.

Riggs, B.L., and Malven, P.V. (1974a). Spontaneous patterns of LH release in castrate male sheep and the effects of exogenous estradiol. J. Anim. Sci. 38, 1239-1244.

Riggs, B.L., and Malven, P.V. (1974b). Effects of intraventricular infusion of serotonin, norepinephrine, and dopamine on spontaneous LH release in castrate male sheep. Bio. Reprod. 11, 587-592.

Robyn, C., Leleux, P., Vanhaelst, L., Golstein, J., Herlant, M., and Pasteels, J.L. (1973). Immunohistochemical study of the human pituitary with anti-luteinizing hormone, anti-follicle stimulating hormone and anti-thyrotrophin sera. Acta Endocr., Copenh. 72, 625-642.

Robyn, C., Delvoye, P., Van Exter, C., Vekemans, M., Caufriez, A., de Nayer, P., Delogne-Desnoeck, J., and L'Hermite, M. (1977). Physiological and pharmacological factors influencing prolactin secretion and their relation to human reproduction. In Prolactin and Human Reproduction. Eds. P.G. Crosignani and C. Robyn. Academic Press, London. p71-96.

Roche, J.F., Foster, D.L., Karsch, F.J., Cook, B., and Dziuk, P.J. (1970a). Levels of luteinizing hormone in the sera and pituitaries of ewes during the estrous cycle and anestrus. Endocrinology 86, 568-572.

Roche, J.F., Foster, D.L., Karsch, F.J., and Dziuk, P.J. (1970b). Effect of castration and infusion of melatonin on levels of luteinizing hormone in sera and pituitaries of ewes. Endocrinology 87, 1205-1210.

Roche, J.F., Karsch, F.J., Foster, D.L., Takagi, S., and Dziuk, P.J. (1970c). Effect of pinealectomy on estrus, ovulation and luteinizing hormone in ewes. Bio. Reprod. 2, 251-254.

Rodríguez, E.M. (1976). The cerebrospinal fluid as a pathway in neuroendocrine integration. J. Endocr. 71, 407-443.

Rollag, M.D., and Niswender, G.D. (1976). Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology 98, 482-489.

Rollag, M.D., O'Callaghan, P.L., and Niswender, G.D. (1978a). Serum melatonin concentrations during different stages of the annual reproductive cycle in ewes. Bio. Reprod. 18, 279-285.

Rollag, M.D., O'Callaghan, P.L., and Niswender, G.D. (1978b). Dynamics of photo-induced alterations in pineal blood flow. J. Endocr. 76, 547-548.

Rollag, M.D., Morgan, R.J., and Niswender, G.D. (1978c). Route of melatonin secretion in sheep. Endocrinology 102, 1-8.

Romijn, H.J. (1975). The ultrastructure of the rabbit pineal gland after sympathectomy, parasympathectomy, continuous illumination, and continuous darkness. J. Neural Transm. 36, 183-194.

Rønnekleiv, O.K., and McCann, S.M. (1975). Effects of pinealectomy, anosmia and blinding on serum and pituitary prolactin in intact and castrated male rats. Neuroendocrinology 17, 340-353.

Rønnekleiv, O.K., and McCann, S.M. (1978). Growth hormone release in conscious pinealectomized and sham-operated male rats. Endocrinology 102, 1694-1701.

Rubin, R.T., Poland, R.E., Rubin, L.E., and Gouin, P.R. (1974). The neuroendocrinology of human sleep. Life Sciences 14, 1041-1052.

Rubin, R.T., Poland, R.E., and Tower, B.B. (1976). Prolactin-related testosterone secretion in normal adult men. J. Clin. Endocr. Metab. 42, 112-116.

Rusak, B., and Morin, L.P. (1976). Testicular responses to photoperiod are blocked by lesions of the suprachiasmatic nuclei in golden hamsters. Bio. Reprod. 15, 366-374.

Sanford, L.M., Palmer, W.M., and Howland, B.E. (1974a). Seasonal variation of serum levels of LH and testosterone in the ram. Can. J. Anim. Sci. 54, 247-249.

Sanford, L.M., Palmer, W.M., and Howland, B.E. (1974b). Influence of sexual activity on serum levels of LH and testosterone in the ram. Can. J. Anim. Sci. 54, 579-585.

Sanford, L.M., Winter, J.S.D., Palmer, W.M., and Howland, B.E. (1974c). The profile of LH and testosterone secretion in the ram. Endocrinology 95, 627-631.

Sanford, L.M., Faiman, C., Howland, B.E., and Palmer, W.M. (1976). The profile of follicle-stimulating hormone secretion in the ram. Can. J. Anim. Sci. 56, 497-504.

Sanford, L.M., Palmer, W.M., and Howland, B.E. (1977). Changes in the profiles of serum LH, FSH and testosterone, and in mating performance and ejaculate volume in the ram during the ovine breeding season. J. Anim. Sci. 45, 1382-1391.

Sanford, L.M., Beaton, D.B., Howland, B.E., and Palmer, W.M. (1978). Photoperiod-induced changes in LH, FSH, prolactin and testosterone secretion in the ram. Can. J. Anim. Sci. 58, 123-128.

Sapsford, C.S. (1962). The development of the testis of the Merino ram, with special reference to the origin of the adult stem cells. Aust. J. Agric. Res. 13, 487-502.

Schally, A.V., Kastin, A.J., and Arimura, A. (1971). Hypothalamic follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-regulating hormone: structure, physiology, and clinical studies. Fert. Steril. 22, 703-721.

Schally, A.V., Kastin, A.J., and Arimura, A. (1972). FSH-releasing hormone and LH-releasing hormone. Vitamins and Hormones 30, 83-164.

Schally, A.V., Arimura, A., Redding, T.W., Debeljuk, L., Carter, W., Dupont, A., and Vilchez-Martinez, J.A. 1976a). Re-examination of porcine and bovine hypothalamic fractions for additional luteinizing hormone and follicle stimulating hormone-releasing activities. Endocrinology 98, 380-391.

Schally, A.V., Dupont, A., Arimura, A., Takahara, J., Redding, T.W., Clemens, J., and Shaar, C. (1976b). Purification of a catecholamine-rich fraction with prolactin release-inhibiting factor (PIF) activity from porcine hypothalami. Acta Endocr., Copenh. 82, 1-14.

Schams, D., and Karg, H. (1972). The immediate response of the plasma prolactin level to oestrogen infusions in dairy cows. Acta Endocr., Copenh. 69, 47-52.

Schams, D., and Reinhardt, V. (1973). Immediate response of the plasma prolactin level to steroid hormone infusion in male and female cattle. Acta Endocr., Copenh. Suppl. 173, 38. Abstr.

Schanbacher, B.D., and Ford, J.J. (1976). Seasonal profiles of plasma luteinizing hormone, testosterone, and estradiol in the ram. Endocrinology 99, 752-757.

Schanbacher, B.D., and Lunstra, D.D. (1976). Seasonal changes in sexual activity and serum levels of LH and testosterone in Finnish Landrace and Suffolk rams. J. Anim. Sci. 43, 644-650.

Setchell, B.P., and Jacks, F. (1974). Inhibin-like activity in rete testes fluid. J. Endocr. 62, 675-676.

Setchell, B.P., and Main, S.J. (1974). Inhibin. Bibliography of Reproduction. 24, 245-252 and 361-367.

Shackell, G.H., Kelly, R.W., and Allison, A.J. (1977). Seasonal variation in libido of rams. N.Z. J. Exp. Agric. 5, 121-122.

Short, R.V. (1974). Sexual differentiation of the brain of the sheep. I.N.S.E.R.M. 32, 121-142.

Sitarz, N.E., Erb, R.E., Martin, T.G., and Malven, P.V. (1977). Effect of repeated sampling on concentrations of testosterone, LH and prolactin in blood of yearling Angus bulls. Theriogenology 7, 151-158.

Skinner, J.D., Booth, W.D., Rowson, L.E.A., and Karg, H. (1968). The post-natal development of the reproductive tract of the Suffolk ram, and changes in the gonadotrophin content of the pituitary. J. Reprod. Fert. 16, 463-477.

Skinner, J.D., and Rowson, L.E.A. (1968). Puberty in Suffolk and crossbred rams. J. Reprod. Fert. 16, 479-488.

Slama-Scemama, A. (1976). Épiphysectomie et castration précoces chez la Ratte Wistar. C.R. Acad. Sci. Paris, Serie D 282, 1741-1744.

Smith, I., Mullen, P., Silman, R., Snedden, W., and Wilson, B. (1976). Absolute identification of melatonin in human plasma and cerebrospinal fluid. Nature 260, 718-719.

Smith, V.G., Hacker, R.R., and Brown, R.G. (1977). Effect of alterations in ambient temperature on serum prolactin concentration in steers. J. Anim. Sci. 44, 645-649.

Sorrentino, S. (Jnr.) (1975). Ovulation in PMS-treated rats treated with gonadotropin releasing hormone after pentobarbital and melatonin block. Neuroendocrinology 19, 170-176.

Spona, J. (1973). LH-RH stimulated gonadotropin release mediated by two distinct pituitary receptors. FEBS Letters 35, 59-62.

Steffert, I.J. (1971). Studies on the testicular development of the New Zealand Romney ram. M.V.Sc. Thesis, Massey University.

Steinberger, E. (1971). Hormonal control of mammalian spermatogenesis. Physiol. Rev. 51, 1-22.

Steinberger, E. (1974). Maturation of male germinal epithelium. In Control of the Onset of Puberty. Eds. M.M.Grumbach, G.D. Grave and F.E. Mayer. John Wiley and Sons, N.Y. p386-402.

Stelmasiak, T., and Galloway, D.B. (1977). A priming effect of LH-RH on the pituitary in rams. J. Reprod. Fert. 51, 491-493.

Stetson, M.H., and Watson-Whitmyre, M. (1976). Nucleus Suprachiasmaticus: the biological clock in the hamster? Science, N.Y. 191, 197-199.

Talbot, J.A., and Reiter, R.J. (1973/74). Influence of melatonin, 5-methoxytryptophol and pinealectomy on pituitary and plasma gonadotropin and prolactin levels in castrated adult male rats. Neuroendocrinology 13, 164-172.

Tanner, J.M. (1967). Puberty. Adv. Reprod. Physiol. 2, 311-347.

Tindal, J.S. (1974). Hypothalamic control of secretion and release of prolactin. J. Reprod. Fert. 39, 437-461.

Urry, R.L., Dougherty, K.A., Frehn, J.L., and Ellis, L.C. (1976). Factors other than light affecting the pineal gland: hypophysectomy, testosterone, dihydrotestosterone, estradiol, cryptorchidism and stress. Am. Zoologist 16, 79-91.

Vaitukaitis, J.L., Ross, G.T., Braunstein, G.D., and Rayford, P.L. (1976). Gonadotropins and their subunits: basic and clinical studies. Rec. Prog. Horm. Res. 32, 289-331.

Vale, W., Rivier, C., and Brown, M. (1977). Regulatory peptides of the hypothalamus. Ann. Rev. Physiol. 39, 473-527.

Vaughan, M.K., Vaughan, G.M., Blask, D.E., Barnett, M.P., and Reiter, R.J. (1976). Arginine vasotocin: structure-activity relationships and influence on gonadal growth and function. Am. Zoologist 16, 25-34.

van de Veerdonk, F.C.G. (1965). Separation method for melatonin in pineal extracts. Nature 208, 1324-1325.

Vermes, I., Molnár, D., Dull, G., and Telegdy, G. (1974). Functional interrelationship between cervical superior ganglia, pineal gland and hypothalamo-pituitary-adrenal system in rats. Acta Physiol. Acad. Scient. Hung., Tomus 45, 53-62.

Vriend, J., Sackman, J.W., and Reiter, R.J. (1977). Effects of blinding, pinealectomy and superior cervical ganglionectomy on free thyroxine index of male golden hamsters. Acta Endocr., Copenh. 86, 758-762.

Warberg, J., Eskay, R.L., and Porter, J.C. (1976). Prostaglandin-induced release of anterior pituitary hormones: structure-activity relationships. Endocrinology 98, 1135-1141.

Wartman, S.A., Branch, B.J., George, R., and Newman Taylor, A. (1969). Evidence for a cholinergic influence on pineal hydroxyindole O-methyltransferase activity with changes in environmental lighting. Life Sciences 8, 1263-1270.

Weitzman, E.D., Boyar, R.M., Kapen, S., and Hellman, L. 1975). The relationship of sleep and sleep stages to neuroendocrine secretion and biological rhythms in man. Rec. Prog. Horm. Res. 31, 399-446.

Wettemann, R.P., and Desjardins, C. (1973). Relationship of LH to testosterone in rams. J. Anim. Sci. 36, 212. Abstr.

Wettemann, R.P., and Tucker, H.A. (1974). Relationship of ambient temperature to serum prolactin in heifers. Proc. Soc. Exp. Bio. Med. 146, 908-991.

Wetterberg, L., Geller, E., and Yuwiler, A. (1970). Harderian gland: an extraretinal photoreceptor influencing the pineal gland in neonatal rats? Science, N.Y. 167, 884-885.

Wheaton, J.E., Martin, S.K., Swanson, L.V., and Stormshak, F. (1972). Changes in hypothalamic biogenic amines and serum LH in the ewe during the estrous cycle. J. Anim. Sci. 35, 801-804.

White, W.F., Hedlund, M.T., Weber, G.F., Rippel, R.H., Johnson, E.S., and Wilber, J.F. (1974). The pineal gland: a supplemental source of hypothalamic-releasing hormones. Endocrinology 94, 1422-1426.

Williams-Ashman, H.G. (1975). Metabolic effects of testicular androgens. In Handbook of Physiology Section 7: Endocrinology Vol. V Male Reproductive System. Eds. D.W. Hamilton and R.O. Greep. American Physiology Society, Washington. p473-490.

Williams, G.L., Ruttle, J.L., and Ezaz, Z. (1976). Plasma androgen levels in yearling and mature rams. J. Anim. Sci. 43, 310. Abstr.

Wilson, J.D. (1975). Metabolism of testicular androgens. In Handbook of Physiology Section 7: Endocrinology Vol. V Male Reproductive System. Eds. D.W. Hamilton and R.O. Greep American Physiology Society, Washington. p491-508.

Wilson, P.R. (1977). Studies of testicular and reproductive endocrine parameters in the ram with particular reference to sexual maturation. Ph.D. Thesis. Massey University. 234p.

Wilson P.R., and Lapwood, K.R. (1978a). Studies of reproductive development in Romney rams: I. Basal levels and plasma profiles of LH, testosterone and prolactin. Bio. Reprod. (In press).

Wilson, P.R., and Lapwood, K.R. (1978b). Studies of reproductive development in Romney rams: II. LH and testosterone secretion following single or repeated doses of GnRH. Bio. Reprod. (In press).

Wilson, P.R., and Lapwood, K.R. (1978c). Studies of reproductive development in Romney rams: III Relationships between testicular development, spermatogenesis and endocrine parameters. Bio. Reprod. (In press).

Wilson, P.R., and Lapwood, K.R. (1978d). Studies of hormone secretion in Romney rams: luteinizing hormone, testosterone and prolactin plasma profiles, LH/testosterone interrelationships and the influence of seasons. Theriogenology 9, 279-294.

Wilson, P.R., and Lapwood, K.R. (1978e). Pituitary and gonadal secretory responses of rams following intravenous infusion or injection of graded doses of GnRH. Theriogenology 9, 417-428.

Wilson, P.R., and Tarttelin, M.F. (1978). Studies of sexual differentiation in sheep. I. Foetal and maternal modifications and post-natal plasma LH and testosterone content following androgenisation early in gestation. Acta Endocr., Copenh. 89, 182-189.

Wolińska, E., Polkowska, J., and Domański, E. (1977). The hypothalamic centres involved in the control of production and release of prolactin in sheep. J. Endocr. 73, 21-29.

Wolstenholme, G.E.W., and Knight, J. (1971). The Pineal Gland. Churchill Livingstone, Edinburgh. 401p.

Wurtman, R.J. (1967). Effects of light and visual stimuli on endocrine function. In Neuroendocrinology. Eds. L. Martini and W.F. Ganong. Academic Press, N.Y. Vol. II, 19-59.

Wurtman, R.J. (1968). The Pineal Gland. In Endocrine Pathology. Ed. J.M.B. Bloodworth. Williams and Wilkins, Baltimore. p117-132.

Wurtman, R.J., Axelrod, J., and Fischer, J.E. (1964). Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system. Science, N.Y. 143, 1328-1330.

Wurtman, R.J., Axelrod, J., and Kelly, D.E. (1968). The Pineal. Academic Press, N.Y. 199p.

Yeates, N.T.M. (1949). The breeding season of the sheep with particular reference to its modification by artificial means using light. J. Agric. Sci., Camb. 39, 1-43.

Zimmerman, E.A., Hsu, K.C., Ferin, M., and Kozlowski, G.P. (1974). Localization of gonadotropin-releasing hormone (GnRH) in the hypothalamus of the mouse by immunoperoxidase technique. Endocrinology 95, 1-8.