Effects of LH-RH infusion, castration and cryptorchidism on gonadotrophin and testosterone secretion in developing rams

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Summary. The relationship between the pituitary gland and testis in rams was studied from birth to sexual maturity. The concentrations of LH, FSH and testosterone increased between 5 and 7 weeks of age; the rise was not correlated with any specific cytological change in the testis. An augmented pituitary response to LH-RH was demonstrated as levels of gonadotrophin increased. It is unclear whether this change in sensitivity plays a role in initiation of the pubertal process because Sertoli cell maturation, the earliest detectable change in the seminiferous epithelium, occurs between 17 and 21 weeks of age. Spermatocytes were first seen in biopsies taken at 31–36 weeks and spermatogenesis was established fully by 45 weeks. This second phase of testicular development was characterized by increases in prolactin, testosterone and LH. Leydig cells previously difficult to identify became recognizable at the time of sexual maturation.

In newborn rams, castration produced significant increases in LH and FSH levels within 2–3 weeks, but higher basal FSH levels (2- to 3-fold) were observed at 5 than at 3 weeks of age. Cryptorchidism did not elevate LH or FSH significantly during the first year of life. FSH rose after this period to levels 2- to 3-fold higher than in normal rams, while LH and testosterone values remained in the normal range in spite of diminished spermatogenic activity; spermatids were absent and testis size was approximately 60% of that recorded in a normal ram.

These studies demonstrate a rise in gonadotrophin and testosterone secretion in rams during the first 5–7 weeks of life, followed by a quiescent period of 8–9 months before a secondary increase occurs coincident with the establishment of sexual maturity.

Introduction

The relationship between the pituitary gland and testis during sexual development has received close attention in recent years. Studies of the hypophysial–gonadal axis from the time of birth to sexual maturity have been performed to delineate the mechanisms of pubertal development. The rat has been studied most extensively although in this species spermatogenesis begins soon after birth (Lee, de Kretser, Hudson & Wang, 1975). Because rams have a relatively long and well-defined prepubertal period (Dun, 1955; Watson, Sapsford & McCance, 1956; Courot, 1967; Skinner, Booth, Rowson & Karg, 1968), this species is a suitable model for investigations concerning the control of spermatogenesis. We have studied rams from birth to sexual maturity

(i) to define the gonadotrophin (FSH, LH and prolactin) and testosterone concentration patterns, and to relate these hormone changes to testicular development (Lee *et al.*, 1976a); (ii) to determine whether changes occur in the sensitivity of the pituitary gland to LH-RH (Lee *et al.*, 1976b); and (iii) to examine the effect of neonatal castration or cryptorchidism on the patterns of gonadotrophin and testosterone secretion.

Pituitary–testicular relationships with age

Longitudinal hormone profiles

Follicle-stimulating hormone. At birth, plasma FSH levels are low and range between 11 and 22 ng/ml (Text-fig. 1). The levels progressively increase to reach a maximum level by the 5th postnatal week, followed by a decrease to 30–39 ng/ml between 11 and 45 weeks of age. Similar observations of raised FSH levels between 4 and 8 weeks of age in rams of other breeds have been reported (Blanc & Terqui, 1976; Walton, Evins & Waites, 1978), but the significance of this FSH rise remains·unknown.

Luteinizing hormone. Plasma LH levels are low at birth (<0·5 ng/ml) and remain at these levels for the first 4 weeks of postnatal life (Text-fig. 1). An abrupt increase in LH occurred during the next week to reach a peak level of 2·2 ng/ml. A large standard error was associated
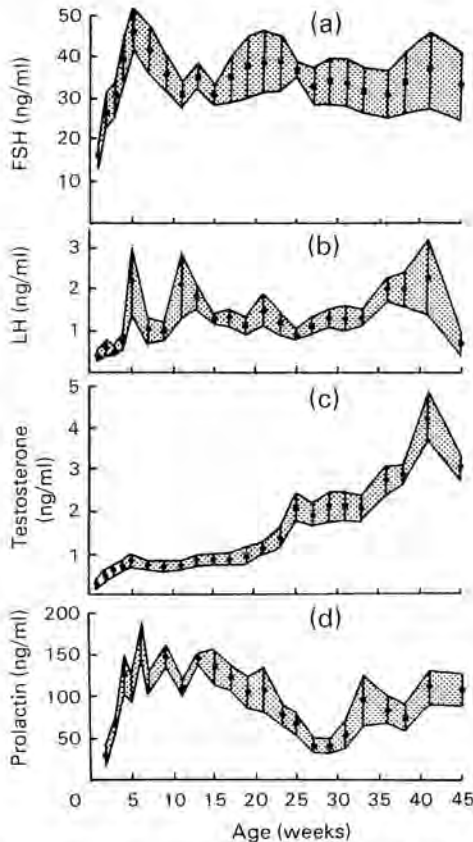
Text-fig. 1. Mean ± s.e.m. circulating concentrations of (a) FSH, (b) LH, (c) testosterone and (d) prolactin during sexual development of the Merino–Corriedale ram. Drawn from data published by Lee *et al.* (1976a). Reference standards are NIH-FSH-S6, NIH-LH-S8 and NIH-P-S6.

with this value, indicating that pulsatile secretion of LH is probably present in these young rams, an inference confirmed by more frequent sampling studies during the early neonatal period (Lee *et al.*, 1976a; Foster *et al.*, 1978). The frequency of LH pulses in Shropshire and Suffolk rams has been shown to increase 20-fold between 1 and 8 weeks of age (Foster *et al.*, 1978) and decrease 3-fold between Weeks 8 and 16. In our studies, mean LH levels remained in the range 0·9–1·3 ng/ml between Weeks 15 and 33 and this phase was followed by a secondary increase in LH. The secondary rise in LH coincided with the period when most rams showed sexual maturity, i.e. the first appearance of spermatozoa in the seminiferous tubules.

Testosterone. During the first week of neonatal life plasma testosterone levels are <0·3 ng/ml (Text-fig. 1). A gradual increase in testosterone occurs during the next 4 weeks to reach mean levels of 0·8 ng/ml by Week 5. The testosterone values remain fairly constant during the next 15 weeks and subsequently increase progressively to reach a mean value of 4·3 ng/ml by Week 41. The secondary rise in testosterone occurs before the secondary increase of LH, suggesting perhaps that the Leydig cells of the testis have acquired increased response to LH during this period. The rising levels of testosterone could also be associated with increases in Leydig cell numbers (Sapsford, 1962) or testicular testosterone content (Skinner *et al.*, 1968). The rise in testosterone occurred at a time when spermatids appear in the seminiferous tubules, i.e. between 30 and 40 weeks of age. This observation suggests that testosterone may be involved in the transition of spermatocytes to spermatids, in accord with the hypothesis proposed for the rat (Steinberger, 1971).

Prolactin. Plasma prolactin levels are <40 ng/ml during the first 2 weeks of postnatal life and rapidly increase to reach levels of >100 ng/ml by Week 4 (Text-fig. 1). The levels remain elevated during the next 15 weeks, then decline to a nadir between 27 and 31 weeks of age (coincident with the period of decreasing daylength). After the nadir a further rise in prolactin levels is evident and mean levels of 112 and 108 ng/ml are reached at 41 and 45 weeks of age respectively (i.e. at sexual maturity). A similar pattern of prolactin secretion in developing rams which were born in the spring has been reported by Ravault, Courot, Garnier, Pelletier & Terqui (1977) and Wilson & Lapwood (1979a). This pattern was not observed in rams born in autumn (Ravault & Courot, 1975; Ravault *et al.*, 1977). Bromocriptine treatment (which suppressed prolactin levels) in spring- and autumn-born rams did not affect FSH, LH and testosterone concentrations, testicular weight or the onset of spermatogenesis. These observations indicate that the change in prolactin levels of developing rams is more closely associated with seasonal and photoperiodic variations than with the control of pubertal development.

Pituitary and testicular responses to LH-RH

The pituitary–testicular axis in rams is responsive to LH-RH stimulation from the time of birth (Lee *et al.*, 1976b). Intracarotid infusion of LH-RH (5 µg for 60 min) significantly elevated LH within 10 min, the levels remaining elevated during the course of infusion, and declining once the infusion was stopped. In newborn ram lambs (Days 1–2), pituitary gonadotrophs are markedly responsive to LH-RH (Text-fig. 2). A decrease in the pituitary response occurs during the next week followed by a progressive increase with enhanced responses in FSH at 6–8 weeks and in LH at 2–3 months. Surprisingly, the maximum FSH response was at least 2–3-fold greater than the FSH response seen at other ages. A change of this magnitude in the response to LH-RH was not apparent for LH and clearly demonstrates a preferential release of FSH in rams between Weeks 6 and 8.

In all the animals infused with LH-RH (Lee *et al.*, 1976b), testosterone levels increased significantly within 60 min of the start of infusion and remained elevated for at least 60 min after the end of infusion. Similar observations of rapid increases in testosterone concentrations following LH-RH (Wilson & Lapwood, 1979b) or hCG (Foster *et al.*, 1978) injections have been observed in rams of different ages. The finding of rapid increases in testosterone secretion in
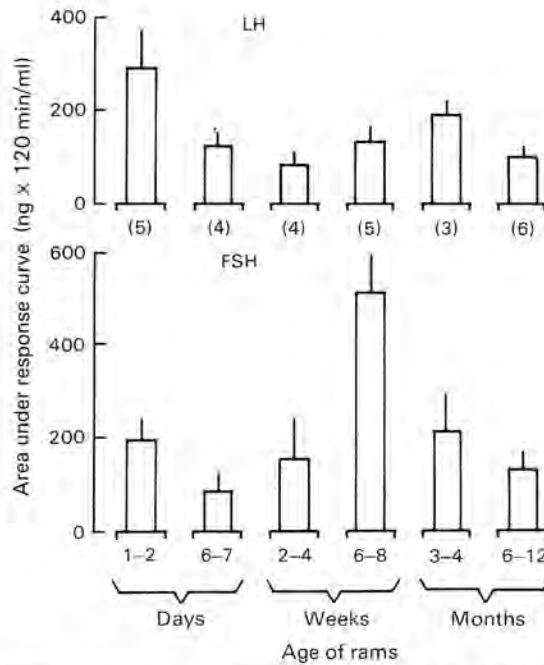
Text-fig. 2. Age-related changes in LH and FSH response to LH-RH infusions in Merino–Corriedale rams. Individual responses to LH-RH infusion (5 μg for 60 min) have been described previously (Lee *et al.*, 1976b); the areas under the LH and FSH response curves were estimated from planimeter measurements and are summarized in this text-figure. Numbers in parentheses indicate the number of animals.

newborn ram lambs (Lee *et al.*, 1976b), at a time when mature Leydig cells could not be identified in the testis (see below), suggests that the undifferentiated elongated, mesenchymal cells in the intertubular spaces are capable of steroid biosynthesis.

PLATE 1

Histological sections (7 μm) of testes of rams at various ages. Bouin fixation, haematoxylin and celestine blue staining. ×400

Fig. 1. At 5 weeks of age the gonocytes (G) are centrally positioned and supporting cells (sc) are peripherally localized.

Fig. 2. At 18 weeks of age the tubules have increased in diameter but only gonocytes (G) and Sertoli cells (Sc) are present.

Fig. 3. At 24 weeks of age there is an increase in the number of spermatogonia (sg) and the appearance of 'mature' Sertoli cells (Sc). IT = intertubular tissue which does not yet contain many Leydig cells.

Fig. 4. At 34 weeks of age the tubules increase in size and the spermatogonia (sg) have developed nuclei and the Sertoli cells (Sc) nucleoli. LC = Leydig cells.

Fig. 5. At 36 weeks of age the tubule size has increased further and primary spermatocytes (sp) and early spermatids are present.

Fig. 6. At 39 weeks of age the seminiferous tubule is fully mature with the appearance of late spermatids (sd).

PLATE 1
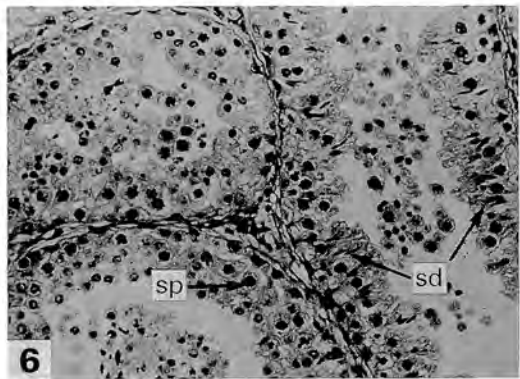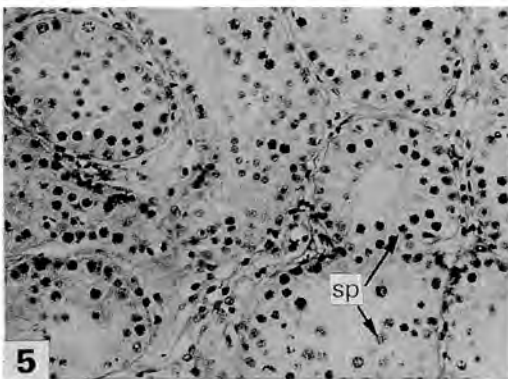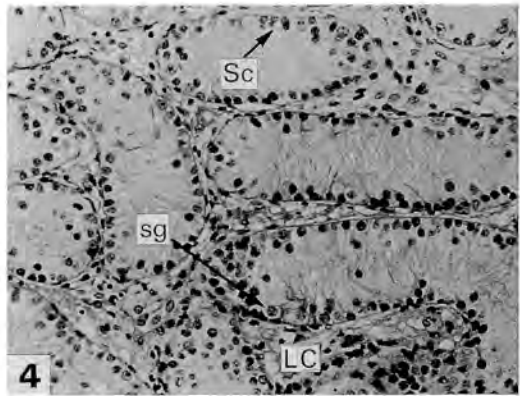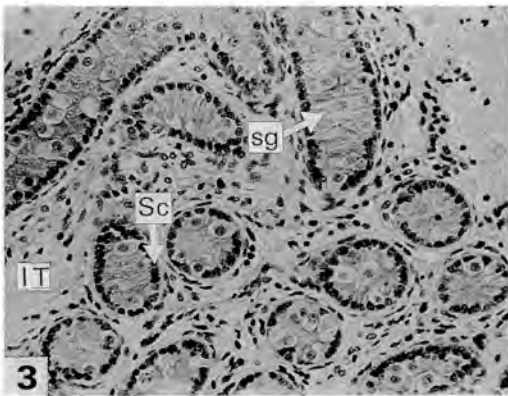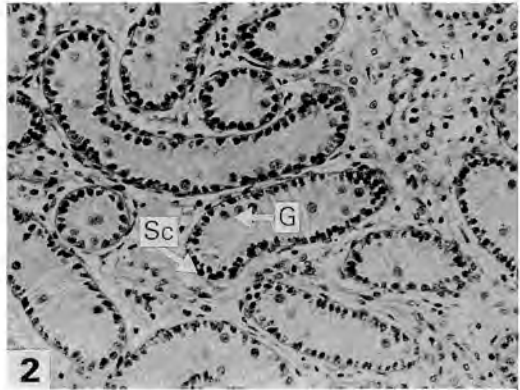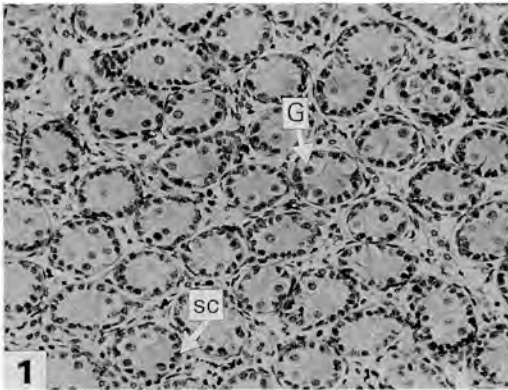
Histology of the testis

The seminiferous tubule diameter of the ram testis remains relatively uniform in size during the first 17 weeks of life and throughout this phase the tubules maintain a similar appearance, with supporting cells at the periphery and gonocytes centrally located (Table 1). The earliest cell changes are those of supporting cells which become differentiated into Sertoli cells between 17 and 21 weeks after birth. Spermatocytes are first seen in biopsies taken between 31 and 36 weeks and the full complement of spermatogenesis is established by 45 weeks, at a time much later than in rams studied by other investigators (Table 2). Despite differences in the time at which spermatozoa are first seen in the ram testis, the body weights of the different breeds of rams at the time of sexual maturity are comparable. This would suggest that the rate of sexual development is dependent on growth rate and is in accord with the observations of Watson *et al.* (1956) who found a close relationship between changes in testicular histological characteristics and testicular weight in Merino rams. The testicular weight is also related to body weight. It would therefore be interesting to determine the ratio of testicular size to body weight for the various breeds of rams and to determine whether a constant ratio exists betwen breeds at the time of sexual maturity.

Table 1. Summary of testicular histology and live weight of rams at various ages (see Plate 1)

| Age (weeks)	Mean body wt (kg)	Testicular histology		
		Mature Leydig cells	Sertoli cells	Germinal cells
5	8·8	Absent	Immature	Central gonocytes
9	11·8	Absent	Immature	Central gonocytes
13	15·6	Absent	Immature	Central gonocytes
17	16·6	Absent	Immature	Central gonocytes
21	—	Absent	Some mature	Central gonocytes
25	—	Absent	Some mature	Central gonocytes
31	—	Absent	Mature	Few spermatocytes
36	24·2	Absent	Mature	Few spermatocytes
45	33·2	Few	Mature	Full spermatogenesis

Table 2. Live weight and age of rams when spermatozoa appeared in the testes

Breed	No. of rams	Locality	Age when spermatozoa present (weeks)	Live wt at puberty (kg)	Reference
Ile-de-France	56	France	20–22	~35	Courot (1962, 1971)
Merino	110	Australia	18–32	~27	Watson *et al.* (1956)
Namaqua Africander	12	South Africa	28	~32	Skinner (1970)
Suffolk	54	England	16	~35	Skinner *et al.* (1968)
Merino–Corriedale	29	Australia	39–45	~30	Lee (1976)

Leydig cells are difficult to identify during the course of sexual development and become recognizable only about the time of sexual maturation (Table 1). This difficulty in identifying Leydig cells in rams has also been noted by previous investigators (Hay & Deane, 1966; Skinner *et al.*, 1968). The period when Leydig cells were first identified coincided with the peak of circulating testosterone levels (Text-fig. 1), i.e. between 36 and 45 weeks. 'Primitive' Leydig cells in the intertubular spaces, i.e. the undifferentiated, elongated mesenchymal cells, are presumably capable of secreting testosterone as evidenced by the rapid response to LH-RH in neonatal rams.

Effect of neonatal castration and cryptorchidism

Neonatal castration of ram lambs leads to a significant elevation of circulating levels of LH and FSH (Text-fig. 3). Similar rises in LH and FSH after castration of neonatal lambs have been described by Foster, Cook & Nalbandov (1972) and Walton *et al.* (1978). Together, these results indicate that the inhibitory feedback relationship of the testis and pituitary gland is established very early in neonatal life. In cryptorchid animals FSH levels during the first 12 months did not differ markedly from those seen in intact rams (Text-fig. 3). Mean plasma FSH levels ranged between 45 and 63 ng/ml in normal rams and between 39 and 69 ng/ml in cryptorchid rams. Surprisingly, at age 13 months and older the cryptorchid animals exhibit significantly higher FSH levels (80–108 ng/ml; $P < 0.01$) than normal rams (39–45 ng/ml). This increase in FSH secretion occurred at a time coincident with the period when sexual maturity was established in the first study (Text-fig. 1). Since LH and testosterone concentrations in cryptorchid animals did not differ significantly from those of normal rams the rise in FSH in cryptorchids at 13 months may reflect a testicular defect in the secretion of an FSH inhibitory factor(s). This speculation would require confirmation by direct measurement of
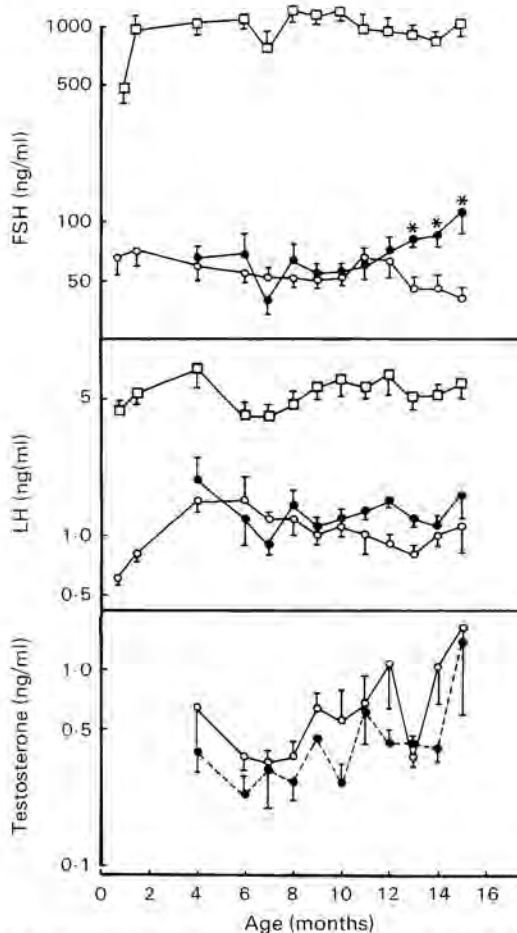
Text-fig. 3. Mean ± s.e.m. circulating concentrations of (a) FSH, (b) LH and (c) testosterone in intact (O, N = 4), castrated (□, N = 4) or cryptorchid (●, N = 4) rams. Differences in FSH levels between normal and cryptorchid animals were analysed by approximate F test (Gill & Hafs, 1971); *$P < 0.01$.

such inhibitory factor(s) in the testis or in testicular venous blood. The evidence that cryptorchid rams lack FSH inhibitory substances has been provided from studies demonstrating rapid increases in FSH following LH-RH infusion in these animals (unpublished observations).

Discussion and Conclusions

Our studies demonstrate that changes occur in the secretion of pituitary gonadotrophins and in the morphology of the testis in Merino–Corriedale rams during the course of sexual development. Increased pituitary responses to LH-RH occurred at about the same time as the gonadotrophin levels increased. Besides the rise in pituitary responsiveness to LH-RH, rapid increases in testosterone levels followed the LH-RH-stimulated rise in LH concentrations. The studies involving neonatal castration demonstrate the existence of a feedback relationship between the testis and pituitary gland in newborn rams. These changes in hormone levels and responses could not be correlated with any specific cytological changes in the testis. It is possible that the rise in FSH and LH at this time may reflect some alteration in the sensitivity of the hypothalamus or pituitary gland which facilitates the onset of puberty. The exact time of onset of puberty in the ram is difficult to define but changes in the seminiferous epithelium are evident as early as 17–21 weeks and consist of Sertoli cell maturation. The demonstration that the secretion of FSH and LH, in response to infusions of LH-RH, is augmented at about the same time as the levels of FSH and LH increase supports the concept of a change in hypothalamic–pituitary sensitivity at 5–7 weeks of age. Additional evidence of a change in the hypothalamic control of FSH secretion has been provided by the observation of differences in the basal secretion of this hormone in castrated ram lambs (Lee, Bremner & Burger, 1981). Ram lambs castrated within 24 h of birth have significantly higher FSH levels (2- to 3-fold) at 5 weeks than at 3 weeks of age (V. W. Lee, unpublished data). This increase in circulating levels of FSH occurs in spite of the absence of the testes, suggesting a change in the hypothalamic control of FSH which is independent of testicular secretion. These observations, together with the low circulating levels of testosterone in newborn rams, support the thesis that the feedback inhibition of FSH secretion is already established on the first neonatal day and that the inhibitory FSH factor(s) from the testis in these young rams may be due to substances other than testosterone. The source of the inhibitory factor from the testis of newborn lambs is unknown.

The significance of the rises in LH and FSH levels seen during the neonatal period (Weeks 4–8) is presently unknown. It is presumably important in triggering the initiation of spermatogenesis in the ram. The studies of Courot (1967), using hypophysectomized lambs treated with LH, FSH or a combination of both, have indicated an unequivocal need of gonadotrophins for the establishment of spermatogenesis and testicular growth during sexual development. Hypophysectomy led to inhibition of testicular growth and prevention of spermatogenic differentiation but treatment with LH or a combination of LH and FSH reversed the effects of hypophysectomy. The results of the latter treatment regimen also indicated a synergistic action of FSH with LH upon testicular development. These observations are in agreement with studies performed in hypophysectomized rats in which increased testicular sensitivity to LH occurred after pretreatment with FSH or prolactin (Odell, Swerdloff, Jacobs & Hescox, 1973; Bartke & Dalterio, 1976). The mechanisms for the initiation of spermatogenesis in the rat and the ram were probably similar but further comparative investigations on the role of the neonatal increases in LH, FSH and prolactin are required before any conclusions can be reached.

The physiological role of prolactin in the ram is presently unknown. It is clear that changes occur in the secretion of prolactin during the course of sexual development in the ram (Text-fig. 1), but there is evidence that prolactin does not play an important role (Ravault *et al.*, 1977). Bromocriptine, a prolactin inhibitor, did not prevent the onset of spermatogenesis in rams treated in the neonatal period.

These studies have increased our knowledge of changes in pituitary secretion of LH, FSH and prolactin during prepubertal development in the ram. The studies have also indicated that the hypothalamus and testes are contributing towards control of pituitary hormone secretion but the exact nature of this control remains to be elucidated.

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