

## Endocrine control of spermatogenesis in the ram

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**Summary.** In the ram, the size of the testes is related to the concentrations of FSH, LH and testosterone in the blood. A significant linear relationship is observed between testicular size and the level of FSH until a maximum after which no further increase in testis size is observed. Testicular size is also linearly related to the mean level (25 consecutive hours of sampling) of LH and testosterone in the blood, with apparently no upper limit, and to the frequency of peaks of these hormones in the sampling period.

Spermatogenesis in the ram is sensitive to variations in the levels of circulating hormones; there is a positive correlation between the number of renewing spermatogonia or the efficiency of spermatogonial multiplication and the mean LH value in the peripheral blood of the adult. Some of these relationships operate over long periods and involve the Sertoli cells. The level of circulating LH in the non-pubertal lamb is directly correlated with number of Sertoli cells per testis, and the latter is correlated with the number of renewing spermatogonia per testis in the adult ram.

Treatment of hypophysectomized rams with PMSG, hCG or testosterone shows that spermatogonial divisions are sensitive to the hormonal milieu with specific stages being controlled by the LH-like activity of hCG (A<sub>1</sub> spermatogonia), and the FSH-like activity of PMSG (transition from intermediate spermatogonia to leptotene spermatocytes). Testosterone has only a small effect at the beginning of the spermatogenic cycle (production of leptotene spermatocytes) and quantitatively maintains meiosis and spermiogenesis, but the differentiation of spermatids is dependent on information stored at the beginning of meiosis and requires the support of both testosterone and other factors.

### Introduction

In the sheep, as in other species, the success of fertilization depends on the number of spermatozoa deposited in the female genital tract (Salamon, 1962) as well as on their quality (Colas & Brice, 1976; Colas, 1979). Sperm production in the ram is known to vary quantitatively and qualitatively in northern latitudes according to season, mainly through photoperiodism. This is clearly shown in animals trained to be regularly ejaculated throughout the year (Colas, 1979; Barrell & Lapwood, 1979). This is also directly shown at the testicular level by the daily sperm production of rams in which the rete testis is cannulated (Dacheux, Pisselet, Blanc, Hochereau-de Reviers & Courot, 1981) and by data from a quantitative histological analysis of seminiferous tubules (Hochereau-de Reviers, Loir & Pelletier, 1976). Ram spermatogenesis therefore shows seasonal variation with a nadir in the non-breeding season and a maximum in the breeding season. In this report on the endocrine control of spermatogenesis in the ram we present the results of (1) a study of the relationships between circulating hormones and testicular size in normal animals, and (2) an examination of spermatogenesis of hypophysectomized rams treated with gonadotrophins or androgens.

### Testicular size and circulating hormones

In rams, the seasonal variation in testicular size has been well documented (Ortavant, 1959); it correlates with spermatogenic activity and testicular sperm production. How is it related to the secretion of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone? The variation of testicular size parallels the pattern of circulating LH and testosterone, but is not so clearly correlated with that of FSH (Sanford, Winter, Palmer & Howland, 1974; Sanford, Faïman, Howland & Palmer, 1976; Schanbacher & Ford, 1976; Schanbacher & Lunstra, 1976; Barrell & Lapwood, 1979). However, these results strongly suggest that testicular size and spermatogenesis are dependent on circulating pituitary hormones.

#### *Gonadotrophin-releasing hormone*

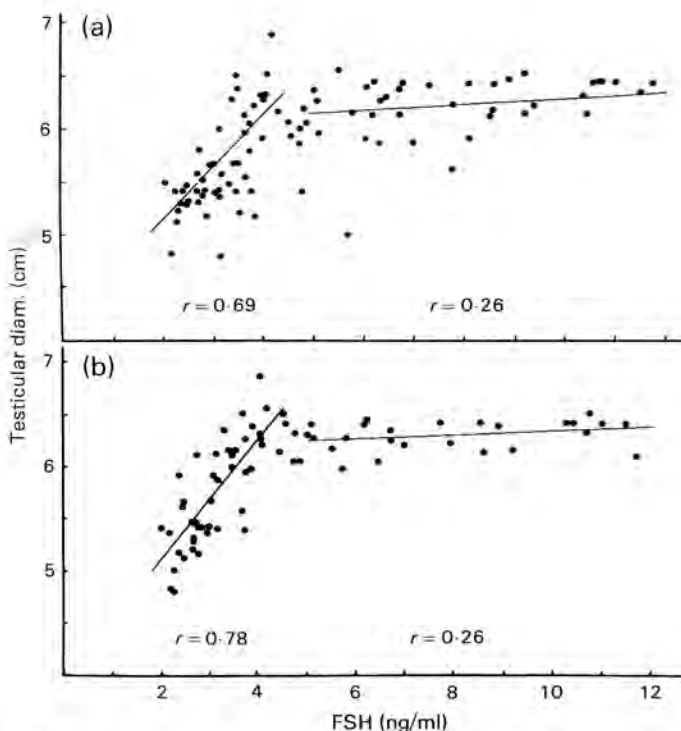
Lincoln (1979) has shown that prolonged treatment of adult Soay rams in the spring non-mating season, when the levels of LH and FSH in the peripheral blood were at minimum, with small pulses of 100 or 500 ng of synthetic gonadotrophin releasing hormone (Gn-RH) infused into the jugular vein for 60 sec every 2 h for 33–57 days resulted in enlargement of the testes. The first effects appeared 1–3 weeks after the start of the infusion and the testes continued to enlarge throughout the treatment. Growth ceased when the treatment was stopped. Each short infusion of Gn-RH resulted in a transitory increase in the levels of LH and testosterone while the concentration of FSH was affected only by the largest dose of Gn-RH used, 500 ng, and after a delay. Intramuscular injections of Gn-RH in the non-breeding season were previously shown to induce testicular enlargement in rams (Schanbacher & Lunstra, 1977) whereas active immunization of young rams induced a progressive reduction in testicular size related to a reduction of the releasable stores of LH in the pituitary gland (D. B. Crighton, B. Sweeten-Smith & I. A. Jeffcoate, unpublished). Collectively, these results clearly show the dependence of testicular enlargement on LH, testosterone and FSH, but they do not enable us to state which hormone is the most important.

#### *Circulating hormone concentrations*

In order to see how testicular enlargement depends on gonadotrophic hormones and on testosterone, two groups of 5 adult Ile-de-France rams were used to correlate testicular size with plasma levels of FSH, LH and testosterone. One group of rams (control) was subjected to natural photoperiod from January to September, i.e. from winter to autumn in the northern hemisphere. The other group (experimental) was kept in a controlled photoperiod of 8 h light and 16 h dark, in which the 8 h-period of light was split into a 7-h period and 1-h flash given in the dark period, 16 h after dawn. This treatment is known to induce enlargement of the testis in the non-breeding season (Ortavant, 1977). Testicular diameter was recorded every 2 weeks with callipers. Blood samples were collected, by acute puncture of the jugular vein, once a week for FSH measurements (Blanc & Poirier, 1979) and every hour during 25 consecutive hours at different times during the experiment for LH (Pelletier, Kann, Dolais & Rosselin, 1968) and testosterone (Garnier, Cotta & Terqui, 1978) measurements. FSH is relatively constant throughout a 24-h period (Ravault, Blanc, Ortavant, Pelletier & de Reviere, 1980), and so one sample per week was considered to give a representative value for the mean FSH value in each ram. LH is known to be secreted in pulses (Sanford *et al.*, 1974; Foster *et al.*, 1978; Lincoln, 1978; Terqui, Garnier, de Reviere, Huet & Pelletier, 1980; Ravault *et al.*, 1980). For this reason, the levels of LH have been estimated either by the mean level of the 25 values of a particular sampling period or by the number of peaks in the same period of time. Testosterone is secreted in response to LH (Sanford *et al.*, 1974; Schanbacher & Lunstra, 1976; Terqui *et al.*, 1980) and the testosterone values were therefore expressed in the same way as those of LH. In each group of

rams, testicular size was plotted against the level of FSH obtained on the same day or 2, 4 and 6 weeks before, to take into account the delay in the expression of hormone action. For LH and testosterone, the mean value per animal was compared with testicular size.

**FSH.** Low plasma concentrations of FSH were linearly related to testicular size (Text-fig. 1). Further increases in FSH ( $>4.5$ – $5$  ng FSH-HG-225/ml), were not related to any further increase in the size of the testis. The linear relationship between the two parameters was very clear when measurements of testicular size were plotted against those of FSH in blood taken 6 weeks earlier ( $r = 0.78$ ;  $P \leq 0.01$ ; Text-fig. 1b). The relationship seemed to be less strict between the two variables when concomitant measurements were plotted against each other ( $r = 0.69$ ;  $P \leq 0.01$ ; Text-fig. 1a). The results were quite similar in both groups of rams despite some differences in the response to the light regimen. These observations suggest that testicular enlargement is dependent on FSH levels in the peripheral blood. The improvement in the correlation between the level of plasma FSH and the diameter of the testis measured 6 weeks later compared to simultaneous measurements indicates that it may take some time for FSH to influence the testicular size. If FSH acts at the beginning of the spermatogenic cycle, some time would be needed to translate this stimulus into testicular enlargement due to increased numbers



**Text-fig. 1.** Relationship between testicular size and the mean level of FSH in the peripheral blood of adult Ile-de-France rams ( $N = 5$ ) kept under artificial lighting (8L : 16D with 1L given 16 h after dawn) from the winter solstice (December) to the autumn equinox (September). Testicular diameter was recorded every 2 weeks and blood samples collected once a week. Testicular measurements are plotted against plasma FSH levels observed (a) simultaneously or (b) 6 weeks previously. The coefficients of correlation noted on the graphs correspond to those observed between the testicular size and FSH levels from 0 to 4.5 ng/ml (highly significant) and from 5 to 12 ng/ml (non-significant). The corresponding regression equations are, from 0 to 4.5 ng/ml, (a)  $y = 4.1 + 0.49x$  and (b)  $y = 3.9 + 0.60x$  and, from 5 to 12 ng/ml, (a)  $y = 6.0 + 0.03x$  and (b)  $y = 6.2 + 0.17x$ . (By courtesy of M. R. Blanc.)

of cells in the seminiferous tubules. This may be linked to the negative correlation shown between the number of spermatogonia and the plasma level of FSH in the ram (Hochereau-de Reviers *et al.*, 1980).

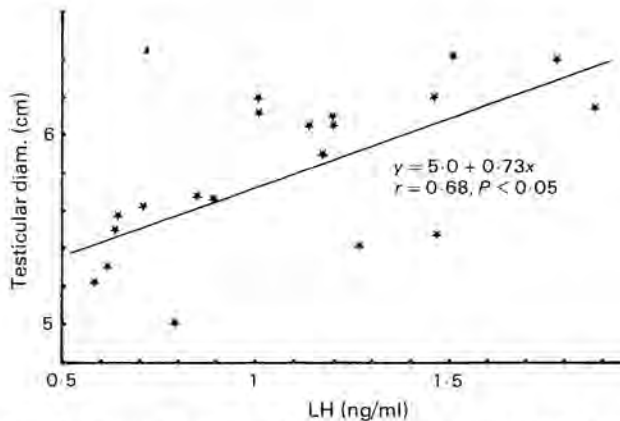
**LH.** The relationship between testicular size and the circulating level of LH differs from that observed with FSH. A positive linear relationship was observed between the diameter of the testis and the mean concentration of LH over its whole range of variation (Text-figs 2 and 3). There were some differences in the temporal dynamics of this relationship between groups (Table 1). In the experimental group, the coefficient of correlation between the two parameters increased with the delay between blood sampling and testicular measurement—non-significant for a delay of 0 or 2 weeks, highly significant for a delay of 6 and 8 weeks. In the control group the coefficient of correlation initially decreased and then increased when the delay between hormone measurement and the testicular measurement was increased from 2 to 6 and then to 8 weeks.

When the number of LH peaks per period of sampling was counted the results were similar to those obtained with the mean concentration of LH: the higher the number of peaks of LH, the larger the diameter of the testis (Text-fig. 3; Table 2).

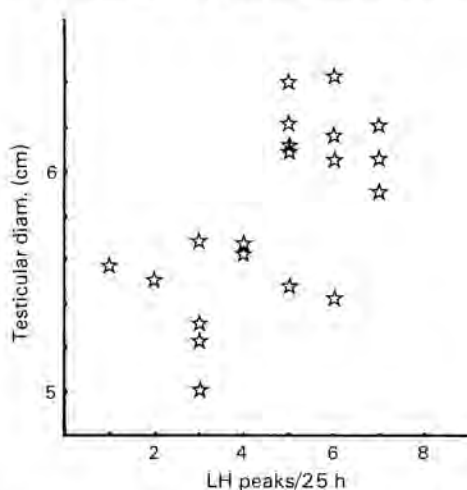
**Table 1.** Relationship between testicular size and the mean levels of LH and testosterone in the peripheral blood of adult Ile-de-France rams

Delay between testis and endocrine measurements (weeks)	Experimental rams		Control rams	
	LH	Testosterone	LH	Testosterone
0	0.14	0.64**	0.54*	0.62**
2	0.40	0.75**	0.66**	0.37
6	0.68**	0.84**	0.47*	0.45*
8	0.72**	0.88**	0.71**	0.54*

Results are given as the coefficients of correlation ( $r$ ) between the testicular diameter and the mean level of LH or testosterone in the peripheral blood plasma (mean of samples taken every hour during 25-h periods) either in the same sampling period or 2, 6 or 8 weeks previously. There were 5 rams per group and 20 pairs of experimental data: \*  $P < 0.05$ ; \*\*  $P < 0.01$ .



**Text-fig. 2.** Relationship between testicular size and the mean level of LH in the peripheral blood of adult Ile-de-France rams kept under artificial lighting (see Text-fig. 1). Testicular diameter was recorded 6 weeks after blood collection (every hour for 25 consecutive hours on 16 December, 11 February, 8 April and 9 June). LH is reported as the mean level of the 25 samples collected per ram per period, as ng LH per ml plasma (CNRS-LH-M3). (By courtesy of J. Pelletier.)



**Text-fig. 3.** Relationship between testicular size and the number of LH peaks in the peripheral blood of adult Ile-de-France rams kept under artificial lighting (same experimental conditions as in Text-fig. 2). The number of LH peaks per 25-h sampling period was determined 6 weeks previously as stated in Table 2. Spearman's rank correlation coefficient of 0.59 is significant ( $P < 0.001$ ). (By courtesy of J. Pelletier & M. Terqui.)

**Table 2.** Influence of the delay between endocrine measurements and recording the testicular diameter on Spearman's rank coefficient for the testicular size and the frequency of LH and testosterone peaks in adult Ile-de-France rams

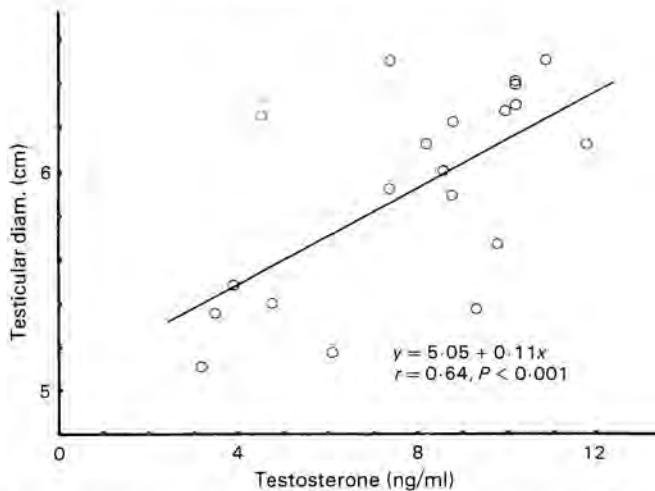
Delay (weeks)	Experimental rams		Control rams	
	LH	Testosterone	LH	Testosterone
0	0.32	0.37	0.25*	0.48*
2	0.43*	0.37	0.20	0.20
6	0.59**	0.60*	0.26	0.28
8	0.73**	0.71**	0.42*	0.44*

The frequency of peaks was calculated on the basis of hourly sampling during 25 consecutive hours. The existence of a peak of LH (or testosterone) was confirmed by relating it to the presence of a corresponding peak of testosterone (or LH). \*  $P < 0.005$ ; \*\*  $P < 0.01$ .

**Testosterone.** Testicular size appeared to be positively related to the mean level of circulating testosterone over its whole range of variation (Text-fig. 4), as it was with LH. Here again some differences in the temporal dynamics of the relationships were observed for the 2 groups. They were quite similar to those noticed for LH (Table 1).

These results suggest that testicular enlargement was related to the levels of LH and (secondarily?) of testosterone over the whole range of concentrations observed. However, the situation is more complicated than with FSH due to the pattern of secretion of LH and testosterone. From results obtained from continuous blood sampling of rams (Terqui *et al.*, 1980) as well as from serial bleeding every hour for 24 or 25 h at different periods of the year (Ortavant, 1977; Lincoln, 1978; Ravault *et al.*, 1980), it is now apparent that seasonal or light-controlled variations in LH and testosterone in the ram do not result from modification in the basal release of these hormones but to changes in the frequency of pulsatile discharge, and, to a certain extent, to the amplitude of the LH peaks. As already seen for FSH, the improvement in the correlation between the LH levels and the size of the testis measured several weeks later is





**Text-fig. 4.** Relationship between testicular size and the mean level of testosterone in the peripheral blood of adult Ile-de-France rams kept under artificial lighting (see Text-fig. 1). Testicular diameters are plotted against plasma testosterone levels observed simultaneously (the mean level of testosterone was calculated in the same way as for LH, see Text-fig. 2). (By courtesy of M. Terqui & D. H. Garnier.)

indicative of a positive effect of LH on the first steps of spermatogenesis. This is in agreement with the positive correlation already reported for plasma LH concentrations and yield of spermatogonial multiplication in the ram (Hochereau-de Reviers *et al.*, 1976, 1980; see also below).

**Prolactin.** A group of 3 adult Romanov rams kept in the natural light environment were treated twice daily (08:00 and 20:00 h) with 2 mg bromocriptine for 50 days at the beginning of the summer. This dose totally suppressed prolactin values in the peripheral blood. Compared with a control group, the treated rams showed a significant delay in testicular enlargement (Barenton & Pelletier, 1980). Changes of plasma prolactin levels by bromocriptine treatment (decreased) or by photostimulation (increased), did not affect the concentration of FSH receptors (Barenton & Hochereau-de Reviers, 1981), or LH receptors (Barenton & Pelletier, 1980) in the ram testis, whereas prolactin has been shown to increase the number of LH receptors in the hamster testis (Bex & Bartke, 1977). There is therefore no clear relationship between the seasonal variations in the pituitary secretion of prolactin and those in spermatogenesis in the ram.

#### *Long-term effect of gonadotrophins*

A positive correlation has been found between the number of renewing spermatogonia and the number of Sertoli cells per testis in the adult ram (de Reviers & Courot, 1976; Hochereau-de Reviers & Courot, 1978). Therefore, Sertoli cells may quantitatively regulate spermatogenesis in the adult. These cells originate from the supporting cells, a cellular population which is established by mitosis in the testis of the non-pubertal sheep before any spermatogenetic activity had commenced. Differentiation of supporting cells into Sertoli cells occurs concomitantly with the beginning of spermatogenesis and before the release of the first spermatozoa into the lumen of the seminiferous tubules. Thereafter, the Sertoli cells do not divide and represent a population of perennial cells (Courot, Hochereau-de Reviers & Ortavant, 1970). Thus the number of Sertoli cells is determined in infancy and factors like gonadotrophins which regulate cell number will consequently influence spermatogenesis in the adult. The dependence of supporting cells on gonadotrophins has been documented for the non-pubertal lamb: these cells regress after

hypophysectomy and they are quantitatively maintained by gonadotrophins with a major role for LH, which cannot be mimicked by testosterone. There is also a synergistic effect of FSH and LH when administered simultaneously (Courot, 1970). These experimental data are in accordance with the relationship observed between testicular size and the mean level of circulating LH in the blood of the lamb (Carr & Land, 1975). They also agree with the long-term correlation noticed between the level of circulating LH in non-pubertal lambs and the number of Sertoli cells per testis (Hochereau-de Reviers *et al.*, 1980).

### Regulation of spermatogenesis

The various relationships reported between the levels of circulating hormones and testicular size give an indication of the hormonal dependence of the testis. However, they do not explain the endocrine control of spermatogenesis. The latter was analysed in a quantitative and qualitative study of the cellular activity of the seminiferous tubules of normal and hypophysectomized rams.

With the seasonal variations of the northern hemisphere, spermatogenesis is more efficient in the autumn, when FSH, LH and testosterone concentrations are elevated in the peripheral blood. Quantitative analysis of the seminiferous epithelium shows that increased spermatogenesis in autumn results mainly from a higher yield of spermatogonial divisions ( $P \leq 0.001$ ) (Hochereau-de Reviers *et al.*, 1976) and to a lesser extent from improvements in the other steps of spermatogenesis (Ortavant, 1959). Thus, the higher sperm production in autumn is mainly due to an increased production of leptotene-phase primary spermatocytes. To answer the question of what hormones are concerned in this process, we undertook an experimental analysis of the endocrine control of spermatogenesis in adult Ile-de-France rams hypophysectomized during the autumn (Monet-Kuntz, Terqui, Locatelli, Hochereau-de Reviers & Courot, 1976; Courot *et al.*, 1979). They were immediately treated twice daily with testosterone, hCG or PMSG for 15, 20 or 40 days and compared to normal or hypophysectomized untreated rams. The doses of hormones were calibrated to maintain a concentration of testosterone within the seminiferous tubules similar to or higher than those of sexually active animals. At the end of the experiment, the testicular weights were recorded and quantitative histological analyses of the testes were performed. Results are given as total number of Sertoli cells and reserve stem spermatogonia per testis and as daily production of other germ cells (see Courot *et al.*, 1979). According to the fixed duration of the various spermatogenic stages in the ram, 15 days each for meiotic prophase and for spermiogenesis (Ortavant, 1959), it was possible to infer the stage at which germ cells required hormonal support.

#### *Testicular weight*

Testicular weight progressively diminished in hypophysectomized rams, to reach a value of 28% of that of controls by 40 days. None of the treatments fully maintained testicular weight (Table 3). The most efficient was PMSG for 15 days, after which the weight of the gonad decreased but at a slower rate than in non-treated animals (–35%, 40 days after hypophysectomy). Testosterone and hCG were less effective (Table 3).

#### *Diameter and length of seminiferous tubules*

A progressive reduction was observed in the diameter of the seminiferous tubules after hypophysectomy. This was partly prevented by treatment with exogenous hormones whose efficiency 40 days after the beginning of the experiment was ranked in the same order as for the maintenance of testicular weight: PMSG—testosterone—hCG (Table 3). The total length of

seminiferous tubules per testis appeared to be independent of treatment until 20 days after hypophysectomy. By Day 40, it was significantly diminished in hypophysectomized rams as well as in those treated with testosterone or hCG (Table 3).

**Table 3.** Endocrine control of the testis in hypophysectomized rams (adapted from Courot *et al.*, 1979)

Duration of treatment	No. of rams	Testis wt (g)	Seminiferous tubules		Sertoli cells	
			Diameter ( $\mu\text{m}$ )	Length (m)	Total no./testis ( $\times 10^8$ ) $\S$	Nuclear cross-sectional area ( $\mu\text{m}^2$ )
None (intact control)	3	230 $\pm$ 14	225 $\pm$ 6	2750 $\pm$ 280	48.4 $\pm$ 6.8	51.0 $\pm$ 2.3
15 days	5	184 $\pm$ 18	214 $\pm$ 5	2900 $\pm$ 415	47.1 $\pm$ 8.2	—
20 days	4	172 $\pm$ 7	162 $\pm$ 10	3000 $\pm$ 125	60.0 $\pm$ 6.0	47.8 $\pm$ 1.7
40 days	9	65 $\pm$ 4	136 $\pm$ 11	1950 $\pm$ 220	52.6 $\pm$ 7.2	45.0 $\pm$ 2.0
15 days + PMSG*	5	224 $\pm$ 16	227 $\pm$ 5	2940 $\pm$ 250	55.5 $\pm$ 4.9	—
20 days + PMSG*	4	197 $\pm$ 9	188 $\pm$ 4	3530 $\pm$ 245	61.4 $\pm$ 6.2	53.3 $\pm$ 3.8
40 days + PMSG*	5	150 $\pm$ 27	173 $\pm$ 13	2810 $\pm$ 230	56.1 $\pm$ 2.3	55.7 $\pm$ 0.7
15 days + hCG†	5	197 $\pm$ 8	224 $\pm$ 6	2620 $\pm$ 210	47.9 $\pm$ 5.0	—
20 days + hCG†	4	178 $\pm$ 19	185 $\pm$ 6	3260 $\pm$ 460	57.8 $\pm$ 6.3	53.7 $\pm$ 1.1
40 days + hCG†	5	83 $\pm$ 7	144 $\pm$ 8	1750 $\pm$ 150	47.4 $\pm$ 4.3	47.7 $\pm$ 1.6
20 days + testosterone‡	4	184 $\pm$ 9	204 $\pm$ 5	2980 $\pm$ 170	56.1 $\pm$ 4.2	45.4 $\pm$ 2.9
40 days + testosterone¶	5	94 $\pm$ 8	167 $\pm$ 12	1990 $\pm$ 60	42.8 $\pm$ 4.3	46.5 $\pm$ 1.7

The experiments were carried out in the autumn. Results are given as mean  $\pm$  s.e.m.

\* 300 i.u. twice daily.

† 250 i.u. twice daily.

‡ 0.25 g (N = 2) and 0.5 g (N = 2) twice daily.

§ Calculated as no. of Sertoli cells per cross section  $\times$  length of tubules/thickness of sections.

¶ 0.25 g twice daily.

### Sertoli cells

The total number of Sertoli cells per testis was not significantly altered by any treatment. However, their mean nuclear cross-sectional area, indicative of the metabolic activity, was slightly reduced after hypophysectomy and testosterone therapy. Treatment with PMSG for 20 or 40 days maintained the nuclear size of Sertoli cells at values similar to those of controls, but hCG maintained nuclear size for only 20 days (Table 3).

### Germ cells

Hypophysectomy induced a severe regression in spermatogenesis (Table 4). Quantitatively, the earlier in the spermatogenic cycle that the cells were affected, the more dramatic was the effect of hypophysectomy on the subsequent production of spermatozoa.

*Spermatogonia.* The undifferentiated spermatogonia ( $A_0$ ) appeared to require no support from pituitary hormones since their total number per testis was not significantly altered by hypophysectomy (Table 4). This is in accordance with the failure of hypophysectomy to affect the number of primordial germ cells in the lamb testis a long time before the onset of spermatogenic activity (Courot, 1970). However, as seen in lambs, they are qualitatively under pituitary control since their capacity to differentiate into 'renewing' stem spermatogonia ( $A_1$ ) was depressed after hypophysectomy. Indeed, the daily production of  $A_1$  spermatogonia was markedly diminished 20 and 40 days after hypophysectomy. Up to 20 days, PMSG and hCG fully maintained the daily production of  $A_1$  spermatogonia whereas testosterone did not. However, the effect of hCG was lost after 40 days of treatment.

The more differentiated spermatogonia,  $A_3$ , intermediate and B spermatogonia, which are



involved in an active process of multiplication and differentiation before entering meiosis, were highly sensitive to deprivation of pituitary hormones. Many of them rapidly degenerated and/or ceased to divide, thus inducing a depletion in the subsequent population of germ cells. The daily production of intermediate spermatogonia was drastically reduced after hypophysectomy (–80% at 20 and 40 days) and testosterone therapy (–40 and –70%). It was maintained by PMSG and hCG but only for 20 days (not significantly different from controls); after 40 days of such therapy the production of intermediate spermatogonia was reduced, possibly as a consequence of a depressed production of A<sub>1</sub> spermatogonia (Table 4).

**Table 4.** Endocrine control of spermatogenesis in hypophysectomized rams (adapted from Courot *et al.*, 1979)

Duration of treatment*	No. of rams	A <sub>0</sub> spermatogonia/testis (× 10 <sup>6</sup> )†	Daily production (× 10 <sup>7</sup> )‡					
			Spermatogonia		Spermatocytes		Spermatids	
			A <sub>1</sub>	Intermediate	Leptotene	Pachytene	Round	Elongated§
None (intact control)	3	206 ± 42	2.6 ± 0.1	11.8 ± 1.8	95 ± 9	94 ± 9	379 ± 26	459 ± 39
20 days	4	279 ± 46	0.9 ± 0.3	2.2 ± 0.8	3 ± 2	30 ± 15	144 ± 77	126 ± 73
40 days	9	163 ± 38	0.8 ± 0.2	2.2 ± 0.6	3 ± 2	2 ± 2	4 ± 4	8 ± 8
20 days + PMSG	4	258 ± 36	3.9 ± 0.8	11.5 ± 2.6	104 ± 16	94 ± 7	346 ± 18	614 ± 24
40 days + PMSG	5	218 ± 46	2.0 ± 0.4	3.5 ± 1.0	43 ± 20	44 ± 23	237 ± 91	293 ± 130
20 days + hCG	4	257 ± 71	3.9 ± 0.8	10.8 ± 2.4	26 ± 5	76 ± 16	395 ± 89	638 ± 129
40 days + hCG	5	92 ± 19	1.1 ± 0.1	2.1 ± 0.4	1 ± 1	4 ± 2	19 ± 10	112 ± 36
20 days + testosterone	4	221 ± 53	2.1 ± 0.3	7.0 ± 1.8	34 ± 4	37 ± 3	353 ± 22	594 ± 42
40 days + testosterone	5	140 ± 30	1.2 ± 0.3	3.3 ± 0.6	16 ± 3	17 ± 5	98 ± 49	166 ± 61

\* See Table 3.

† Calculated as corrected no. of cells per cross-section × length of tubules/thickness of sections.

‡ Calculated as no. of cells per testis/duration of the cycle of seminiferous epithelium (Amann, 1970). The number was corrected by Abercrombie's formula for spermatogonia to round spermatids, but was the crude number for elongated spermatids.

§ The larger production of elongated spermatids by treated rams as compared to controls may be due to the experimental conditions: the control animals had been used at the beginning of the sexual season whereas the others were treated at the height of season when the production in control rams was estimated to be around 600 × 10<sup>7</sup> cells/day.

**Spermatocytes.** Following from the observations described above, it was not surprising to find that the daily production of leptotene-stage primary spermatocytes fell to nearly zero 20 and 40 days after hypophysectomy. Testosterone therapy maintained only a low production of cells, 36% and 17% of the controls after 20 and 40 days, respectively. Most interesting was the effect of PMSG: this maintained a quantitatively normal production of leptotene spermatocytes for 20 days whereas hCG did not; however, the stimulatory effect of PMSG decreased thereafter. At 40 days, the production of leptotene spermatocytes was only 55% of that of controls (Table 4).

The decrease in daily production of 'old' pachytene primary spermatocytes appeared to result from a previous decrease of leptotene spermatocytes and not from the direct sensitivity of cells in meiotic prophase to pituitary removal. Indeed, in the testes of rams castrated 15 days after hypophysectomy, old pachytene spermatocytes were as numerous as in controls (daily production of 97 ± 20 × 10<sup>7</sup> and 94 ± 9 × 10<sup>7</sup> respectively). It was only from 20 days after hypophysectomy that the daily production of old primary spermatocytes was significantly diminished (Table 4). In PMSG-, hCG- or testosterone-treated rams, the daily production of old primary spermatocytes varied mainly as a consequence of the effect of treatment on the yield of spermatogonial divisions.

The maturation divisions, giving rise to young spermatids, appeared to be quantitatively insensitive to the deprivation of pituitary gonadotrophins as shown by the daily production of round spermatids in rams hypophysectomized 15 days previously—387 ± 76 × 10<sup>7</sup> compared with 379 ± 26 × 10<sup>7</sup> in controls.

**Spermatids.** More highly differentiated germ cells, the spermatids, were affected by the removal of the pituitary gland. However, since they were not engaged in an active process of multiplication and their life-span was longer (15 days) than that of spermatogonia (around 40 h),

their degeneration did not occur so rapidly as for spermatogonia and was delayed to more sensitive stages in their development, such as condensation of chromatin and elongation of the nucleus.

By 15 days after hypophysectomy, the daily production of round and elongated spermatids was still similar to that of control animals ( $387 \pm 76 \times 10^7$  and  $379 \pm 26 \times 10^7$  for round spermatids and  $553 \pm 105 \times 10^7$  and  $459 \pm 39 \times 10^7$  for elongated spermatids), but after 20 days it was significantly reduced ( $-62$  and  $-72\%$ ). This strongly suggests that information controlling the evolution of spermatids is stored by spermatocytes in meiotic prophase between 15 and 20 days earlier. This information is under endocrine control and PMSG, hCG and testosterone were equally effective in quantitatively maintaining spermiogenesis for 20 days (Table 4). At 40 days after hypophysectomy, the production of elongated spermatids was markedly depressed, possibly as a consequence of a reduced production of primary spermatocytes.

Besides the quantitative control of spermiogenesis, it must be noted that hormones also controlled the morphological differentiation of spermatids. Most of the spermatids from 15- or 20-day testosterone-treated animals displayed a normal nuclear appearance but possessed acrosomes with morphological abnormalities: the process of acrosome formation, as well as its binding to the nucleus, was severely impaired in young spermatids. Only morphological changes in the acrosome were seen in old spermatids (Courtens & Courot, 1980). Again, in accordance with the duration of spermatogenesis in the ram, it is suggested that acrosome development is under the control of endocrine-dependent events occurring before the beginning of spermiogenesis. After treatment with PMSG or hCG, abnormalities were also observed in the morphogenesis of the nucleus of round and elongated spermatids (J. L. Courtens & M. Courot, unpublished data).

### Discussion

These results establish a relationship between testicular size, spermatogenesis and the blood concentrations of gonadotrophins and testosterone in the ram. The coefficients of correlation for each hormone and diameter of the testis suggest that no hormone is preferentially active, but that the various hormones are equally important for the male gonad, with the possible exception of prolactin. The various hormones (FSH, LH and testosterone) appear to act simultaneously on the testis, apparently on the beginning of the spermatogenetic process as shown by the increased correlation coefficients with increasing delay between endocrine and testicular size measurements.

The importance of FSH, LH and testosterone in the control of ram spermatogenesis is confirmed. These results differ from those obtained for rats in which spermatogenesis can be maintained by testosterone without gonadotrophins (Clermont & Harvey, 1967; Ahmad, Haltmeyer & Eik-Nes, 1975; Chemes, Podesta & Rivarola, 1976; Harris, Bartke, Weisz & Watson, 1977; Rivarola, Podesta, Chemes & Calandra, 1977; Chowdhury & Tcholakian, 1979), although the state of sperm production is below normal (Chowdhury, 1979). In the ram, testosterone is relatively inactive in supporting spermatogonial divisions and, consequently, sperm production. This would not appear to be due to inappropriate doses of testosterone because the concentration of this hormone in the testes of hypophysectomized testosterone-treated rams (Courot *et al.*, 1979), or in the seminiferous tubules as seen by testosterone concentration in rete testis fluid (Monet-Kuntz *et al.*, 1976), was not different from normal.

The experiments indicate that some stages of ram spermatogenesis are more sensitive to hormones than others (Table 4). The reserve stem cells, A<sub>0</sub> spermatogonia, are not hormone dependent since their number did not vary with treatment, with the possible exception of the 40-day treatment with hCG which is not yet explained. The reserve stem spermatogonia have

already been observed to be quantitatively independent of hormones in non-pubertal and prepubertal ram lambs (Hochereau-de Reviere & Courot, 1978). The differentiation from  $A_0$  to  $A_1$  spermatogonia is gonadotrophin-dependent, probably most LH-dependent since hCG and PMSG are similarly effective in maintaining it, at least for 20 days of treatment. This agrees with the observation of Courot (1970) who showed that in the hypophysectomized lamb, LH, FSH and both hormones acting synergistically induce spermatogenic activity. Conversely, in the rat, testosterone has been claimed to control this stage of spermatogonial differentiation (Steinberger, 1971; Chowdhury, 1979) which can be partly blocked by antiandrogens (Viguier-Martinez & Hochereau-de Reviere, 1977). The differentiation from  $A_1$  to intermediate spermatogonia is equally maintained (see ratio Intermediate/ $A_1$ ) by PMSG, hCG and testosterone during 20 days of treatment. This suggests that the gonadotrophins are acting through induced testosterone secretion. The final spermatogonial divisions from intermediate spermatogonia to leptotene primary spermatocytes, including the  $B_1$  and  $B_2$  spermatogonia, are maintained only by PMSG. This gonadotrophin-like hormone contains both FSH and LH activities in the molecule (Stewart, Allen & Moor, 1976). hCG is unable to maintain this stage of spermatogenesis. Therefore the transition from intermediate spermatogonia to primary spermatocytes is likely to be dependent on FSH, or the FSH-like activity of PMSG. FSH has been shown to stimulate spermatogonial divisions in the rat (Courot, Ortavant & de Reviere, 1971; Cunningham & Huckins, 1979).

Once the leptotene stage has been reached, meiotic prophase and spermiogenesis in the ram are quantitatively maintained by PMSG, hCG or testosterone (Table 4). This suggests that the effect of gonadotrophin-like hormones is mediated through testosterone secretion. A similar observation has been made for the hypophysectomized rat in which testosterone maintains meiosis and spermiogenesis (Chowdhury & Tcholakian, 1979). However, electron microscopic studies show that there are some morphological abnormalities in ram spermatids after treatment with PMSG, hCG or testosterone. Thus hormonal therapy which is able to maintain spermatogenesis quantitatively may not necessarily do so qualitatively, and both parameters must be considered when looking for semen with high fertilizing ability (Colas, 1979).

All spermatogonia and the leptotene spermatocytes are located in the basal compartment of the seminiferous tubules (Dym & Fawcett, 1970) where they can be reached directly by the hormones supplied by the blood stream or present in the peritubular fluid (Comhaire & Vermeulen, 1978). However, this does not rule out a possible control of spermatogonia through Sertoli cells. Indeed, positive correlations have been observed between the total number of Sertoli cells and stem spermatogonia in the adult rat, ram and bull (Hochereau-de Reviere & Courot, 1978). Our results clearly show that the cells of the basal compartment are more rapidly affected than others by any change of the hormonal balance and suggest that they represent a direct target to gonadotrophins and testosterone. The presence of FSH receptors has been shown in Sertoli cells (Castro, Alonso & Mancini, 1971; Means, 1977; Steinberger & Steinberger, 1977) and spermatogonia of the rat (Orth & Christensen, 1978). LH receptors have not been demonstrated in Sertoli cells in spite of alteration of these cells after treatment with antibody to LH in the rat (Chemes, Dym & Raj, 1979) and increased number of pinocytotic vesicles on their membrane after treatment with hCG in the dog (Connell, 1977). The presence of LH receptors on spermatogonia has been reported in only one paper (Fabrini, Santiemma, Fraioli & Spera, 1975) and a specific role for LH (not operating through testosterone) on seminiferous tubules has been documented (Courot *et al.*, 1970). Testosterone receptors have also been demonstrated in the rat Sertoli cells (Grootegeod, Peters, Mulder, Rommerts & van der Molen, 1977; Steinberger & Steinberger, 1977).

At the present time one cannot decide whether FSH and/or LH act directly on ram spermatogonia or only indirectly by causing Sertoli cells to secrete a substance that promotes or enhances spermatogenesis. The Sertoli cells are actually considered as the main target cells to FSH in the testis. Their response to this hormone is now well documented for the rat (Means, 1977) and this response ends in a 'message' translated to the germinal cells, especially those of

the adluminal compartment. Androgen binding protein (ABP) is an end product of FSH-stimulated Sertoli cells (Hansson, Ritzén, French & Nayfeh, 1975; Means, 1977; Steinberger & Steinberger, 1977) and is present in the ram testis cytosol (Carreau, Drosdowsky & Courot, 1979). Does ABP represent the message which supports meiosis and spermiogenesis? This is likely because receptors to androgen have been shown in spermatids of the rat (Wright & Frankel, 1980), but several other polypeptides are secreted by Sertoli cells after hormonal stimulation (Wilson & Griswold, 1979) and it cannot be ruled out that one of them might control spermatogenesis.

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