

Original article

## Ram lambs need FSH for normal testicular growth, Sertoli cell numbers and onset of spermatogenesis

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**Abstract** — The effect of FSH on the development of the testis in the ram lamb was examined in two experiments where lambs were passively immunized against ovine  $\beta$ -FSH from birth until 100 or 160 d. In both experiments, immunization resulted in a slower testicular growth relative to that of controls. This effect became apparent at around the start of the period of rapid testicular growth (60–70 d of age) and resulted in testicular weights at the end of treatment ranging from 37 to 51 % of those of control groups. Within the testis, this was reflected in shorter seminiferous tubules (48–64 % of controls) and in lower numbers of Sertoli cells per testis (57–82 %). In the rams immunized until 160 d of age, spermatogenesis had begun and immunization against FSH provoked a lower production of germinal cells which was not solely due to the lower number of Sertoli cells but also due to fewer germinal cells being supported by each Sertoli cell. However, the numbers of  $A_0$  spermatogonia per testis and the daily production of the  $A_1$  spermatogonia were unaffected by immunization, but the production of the  $B_2$  spermatogonia and, as a consequence, of leptotene and pachytene spermatocytes and of round spermatids were all markedly lower (43–47 % of controls). These effects were not due to any decreases in the secretion of LH or testosterone as seen in the blood levels of these two hormones. These results show that, in the ram lamb, FSH is essential for normal testicular development and for the establishment of a normal population of Sertoli cells. They also confirm that, once spermatogenesis is established, FSH is necessary for a normal production of germinal cells, with one or more of the divisions between the  $A_1$  and  $B_2$  spermatogonia being sensitive to suppression of FSH. © Inra/Elsevier, Paris.

**FSH / testis / spermatogenesis / Sertoli cells / ram lamb**

**Résumé** — FSH est nécessaire pour le développement normal des testicules, la mise en place de la population des cellules de Sertoli et l'établissement de la spermatogenèse chez l'agneau. L'effet de FSH sur le développement des testicules de l'agneau a été étudié par l'immunisation pas-

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sive d'agneaux mâles contre  $\beta$ -FSH ovine de leur naissance jusqu'à l'âge de 100 ou 160 j. L'immunisation a provoqué une croissance testiculaire plus faible que celle observée chez les témoins. Cet effet apparaît à partir du début de la période de croissance rapide des testicules (60 à 70 j) et aboutit, en fin d'expérience, à un poids testiculaire significativement inférieur à celui des témoins (37 à 51 %). Ceci résulte de tubes séminifères plus courts (48 à 64 % des témoins) et d'un moindre nombre de cellules de Sertoli par testicule (57 à 82 %). Chez les animaux immunisés jusqu'à 160 j, la spermatogenèse s'est tout de même établie mais avec une plus faible production de cellules germinales due à un plus petit nombre de cellules de Sertoli et à un plus faible nombre de cellules germinales soutenues par chaque cellule de Sertoli. Toutefois, le nombre de spermatogonies  $A_0$  par testicule et la production quotidienne de spermatogonies  $A_1$  ne sont pas affectés par l'immunisation contre FSH. Par contre, la production quotidienne des spermatogonies  $B_2$  et, par conséquent, celle des spermatocytes leptotène et pachytène et celles des spermatides rondes sont toutes significativement plus faibles que chez les témoins (43 à 47 %). Ces effets ne sont pas dus à des modifications des sécrétions de LH ou de testostérone qui ne sont pas diminuées. Ces résultats montrent que, chez l'agneau, FSH est indispensable au développement normal des testicules ainsi qu'à l'établissement d'une population suffisante de cellules de Sertoli. Ils confirment aussi que, lorsque la spermatogenèse est établie, FSH est nécessaire pour assurer une production quantitative normale de cellules germinales puisque son immunosuppression affecte fortement une ou plusieurs générations de spermatogonies situées entre les spermatogonies  $A_1$  et  $B_2$ . © Inra/Elsevier, Paris.

### FSH / testicule / spermatogenèse / cellules de Sertoli / agneau

## 1. INTRODUCTION

In the most detailed studies on the hormonal control of spermatogenesis in the developing ram, Courot [10, 12] showed that hypophysectomy of ram lambs soon after birth resulted in a decrease in testicular weight compared to intact initial controls. This was reflected in a decrease in the number of supporting cells and an arrest in the differentiation of the germinal cells. Treatment with FSH and LH partially overcame these effects; when the two hormones were given singly, LH was the more effective but when both hormones were given together, they acted in synergy.

Since hypophysectomy is an operation that disrupts the normal functioning of an animal, these effects may have simply reflected this disruption. However, there is other evidence supporting the hypothesis that at least FSH affects Sertoli cell numbers in the developing ram. First, supplementation of normal ram lambs with FSH increased Sertoli cell numbers after only 5 d of treatment [8, 9]. Second, hemicastration of ram lambs early in their life provoked an

immediate increase in blood FSH levels followed soon after by an increase in Sertoli cell divisions [42, 43] and, later, by an increase in spermatid production [21].

Evidence for the role of FSH is also available in other species (see review by Sharpe [35]). In the immature rat, hypophysectomy followed by supplementation with FSH [13] or suppression of FSH either by immunization [34] or with testosterone propionate [2, 3] or in vitro organ culture of the immature rat testis [6] indicated that the premeiotic steps of spermatogenesis are sensitive to FSH deprivation. The sensitivity of these steps to FSH has also been shown in mature rats treated with a GnRH antagonist [37] or immunized against GnRH [28] as well as in juvenile and adult monkeys [5, 27, 41] and hpg mice [36]. Finally, hemicastration of boars at a young age has also been shown to increase Sertoli cell numbers in the remaining testis [26, 33] accompanied by a transient increase in blood FSH levels [25].

However, most of this evidence is indirect. Direct evidence for a role of FSH in testicular function is seen in studies on the adult ram [23, 24], wherein passive immu-

nization against ovine  $\beta$ -FSH severely disrupted spermatogenesis at the level of the later spermatogonial divisions without interfering with Sertoli cell numbers. However, similar direct evidence is not available for young rams where the Sertoli cell population is still establishing and spermatogenesis has not yet reached adult levels. Therefore, the experiments reported here were designed to test the hypothesis that long-term immunosuppression of FSH in ram lambs would interfere with the establishment of the normal population of Sertoli cells and also disrupt the onset of spermatogenesis.

## 2. MATERIALS AND METHODS

### 2.1. Animals and experimental protocol

The three groups of ram lambs used in the study were all of the Ile-de-France breed born at the Inra Reproductive Physiology Research Station. Lambs in any one group were all born within a 5-d period and killed at the end of treatment.

The first group of five lambs (initial controls) was born in autumn and killed at birth. The second group of rams (experiment 1) was born in autumn and immunized with one of two batches of antiserum, Anti-FSH 1 ( $n = 7$ ) or Anti-FSH 2 ( $n = 6$ ) from birth until 100 days of age. Control animals ( $n = 6$ ) were treated with a similar volume of normal horse  $\gamma$ -globulins. Animals were housed in a light-proof room with a photoperiod similar to that of spring. The third group of rams (experiment 2) was born in spring and treated from birth with antiserum (Anti-FSH 3) or normal horse  $\gamma$ -globulins (controls). Some of these animals were treated for 100 d (six controls, five treated animals), while the rest were treated for 160 d (seven controls, six treated animals). These animals were also housed in a light-proof room with a photoperiod similar to that of the animals in experiment 1.

The rationale behind the treatments was as follows. The initial controls were used to measure testicular parameters at birth and serve as a baseline, particularly for Sertoli cells. Experiment 1 lasted until the commencement of the phase of rapid testicular growth, as determined by weekly measurements of testicular volume by compara-

tive palpation using an orchidometer [30]. Some of the animals of experiment 2 were also treated until this age, while the rest were allowed to proceed another 2 months to allow spermatogenesis to establish [12]. In order that the results of experiments 1 and 2 should be comparable, the animals were housed and subjected to artificial photoperiodic regimes similar to a natural springtime photoperiod. Animals were weighed monthly and their weekly weights, estimated from their growth curves, were used to calculate the volume of the testes relative to liveweight.

### 2.2. Preparation of antisera

Anti-FSH antisera were prepared in two mares by intradermic immunization at approximately monthly intervals with the  $\beta$  sub-unit of ovine FSH (provided by M.R. Sairam). Two weeks after immunization, a large volume of blood (at least 1 L) was taken from each mare and the serum collected. Control serum was obtained from the blood of non-immunized horses. The  $\gamma$ -globulin fractions of the antisera and the normal serum were prepared by precipitation with ammonium sulphate followed by dialysis against physiological saline; these fractions were then prepared for injection by sterile filtration.

Three batches of antiserum were used in the two experiments. Two of these batches (Anti-FSH 1, 3) came from one mare but on different dates separated by at least 4 weeks; the other batch (Anti-FSH 2) came from a second mare.

The titres of the antisera were measured by determining the capacity of serial dilutions to bind  $^{125}\text{I}$ -labelled FSH, while their cross-reactions with LH were defined as the ratio of FSH to LH which gave 50 % displacement of labelled FSH. The titres of the three antisera were 1/64 000, 1/8 000 and 1/32 000 for Anti-FSH 1, 2 and 3, respectively, while their respective cross-reactions with LH were 0.3, 0 and 0.3 %. The ability of the three antisera to neutralize FSH *in vivo* was assessed using the bioassay for FSH described by Steelman and Pohley [38]. A dose of 0.1 mL of each of the antisera negated the response in terms of rat ovarian weight augmentation when 20  $\mu\text{g}$  of FSH (equivalent to 10  $\times$  NIH S1) was injected in conjunction with 20 IU hCG.

### 2.3. Immunization

Ram lambs were injected subcutaneously twice weekly with a dose of antiserum that

increased with age. This dose started at 1.0 mL at birth and increased to 5.0 mL at a liveweight of approximately 35 kg; control rams received an equivalent dose of normal horse  $\gamma$ -globulins. At weekly intervals a blood sample was taken from the animals of experiment 1 and the plasma stored at  $-20^{\circ}\text{C}$ . In experiment 2, lambs underwent serial bleeding at ages of 59 and 99 d of age and, in the animals treated for 160 d, at 159 d of age. At these bleeds, a blood sample was taken every 20 min over an 8-h period and the plasma stored at  $-20^{\circ}\text{C}$ . Because of the apparent lack of efficacy of antiserum from the second mare in experiment 1, only antiserum from the first mare was used in experiment 2.

#### 2.4. Morphometry and germinal cell production

All animals were weighed immediately prior to death. The procedures relating to testicular morphology and germinal cell production have been described in detail by Kilgour et al. [23]. Briefly, immediately after death, the testes were removed and weighed and a small portion of each testis was processed for light microscopy.

The relative volume of the seminiferous tubular tissue and the diameter and length of the seminiferous tubules were measured. Since treatment with anti-FSH antisera has been previously shown to affect the nuclear size of Sertoli cells but not germinal cells [23], the perimeters of the nuclei of ten randomly selected Sertoli cells were measured for each ram using a semi-automatic image analyser (Mini-MOP, Carl Zeiss). The perimeter measurements were then transformed to diameters, assuming the nuclei to be spherical. These diameters were then used in the calculation of the number of Sertoli cells per testis by correcting the raw counts according to Abercrombie [1].

In the lambs of experiment 2 treated for 160 d, spermatogenesis had begun but had not reached adult levels as adjudged by the number of tubules with incomplete spermatogenesis. In order to account for this in the measurement of the germinal cellular component of the testis, 200 tubules of round cross-section were selected at random and their stage classified according to Ortavant's [31] classification of the stages of the cycle of the seminiferous epithelium.

The testicular content of Sertoli cells and of type  $A_0$  spermatogonia was measured as were the daily productions of  $A_1$  and  $B_2$  spermatogonia, of leptotene and pachytene spermatocytes and of round spermatids [4]. These measurements of daily production were then corrected

for the proportion of tubules with identifiable stages. In the case of the  $A_1$  and  $B_2$  spermatogonia, this was done by calculating for each ram the proportion of tubules at stage 8 and dividing this by the expected proportion of tubules at this stage in the mature ram. The calculated daily production of  $A_1$  and  $B_2$  spermatogonia was then multiplied by this proportion. Similar corrections were made for the daily production of leptotene (stage 2) and pachytene (stage 3) spermatocytes and of round spermatids (stage 6).

Finally, the daily production of the various germinal cells was expressed on a per-100-Sertoli-cell basis in order to assess whether any effects of immunization on daily production of germinal cells was simply a reflection of changes in Sertoli cell numbers.

#### 2.5. Hormonal measurements

The concentration of LH was measured in plasma using the double-antibody radioimmunoassay developed by M.R. Blanc and J.C. Poirier (unpublished) which was described in detail by Kilgour et al. [23]. Testosterone concentration was measured as described by Garnier et al. [19]. FSH was not measured owing to the presence of anti-FSH antibodies in the plasma of the treated animals.

All samples from the same animal were assayed together. The concentrations of LH and testosterone were estimated using the four-parameter procedure described by Dudley et al. [18]. In the animals of experiment 2, a pulse of LH was deemed to have occurred where there was a statistically significant rise (greater than three standard deviations of the lower concentration) in LH concentration between one sample and the next or between one sample and the next two followed by a consistent decrease over at least three consecutive samples. A peak of testosterone was deemed to have occurred where a statistically significant (five standard deviations) increase occurred within three or four samples. Four parameters relating to the secretion of LH and testosterone were then calculated for each animal. These were overall mean concentration, mean basal level, mean pulse height and pulse frequency.

#### 2.6. Statistical analyses

All data were analysed by least-square analysis of variance using raw data except in the case of number of pulses where data were transformed by  $\sqrt{(x + 0.5)}$ .

### 3. RESULTS

#### 3.1. Initial controls

The data pertaining to the ram lambs killed shortly after birth are presented in *table I*. As usually observed in newborn male lambs, the seminiferous epithelium was populated mainly by supporting cells, the precursors of the Sertoli cells, and occasional undifferentiated germinal cells or stem spermatogonia. There was no evidence of the evolution of the germinal cells into more highly differentiated cells such as spermatocytes or spermatids.

#### 3.2. Experiment 1

Immunization with Anti-FSH 1 retarded testicular development resulting in a 36 % lower ( $P < 0.05$ ) seminiferous tubular length and a 29 % lower ( $P < 0.05$ ) number of Sertoli cells per testis relative to control animals (*table II*). None of these effects was due to an effect on general body growth which was unaffected by treatment as shown by the lack of effect on live weight (*table II*). There were no other significant effects of Anti-FSH 1 on any of the other testicular parameters measured, although testicular weight, seminiferous tubular diameter and Sertoli cellular nuclear diameter were all lower in treated animals (*table II*). Immunization with Anti-FSH 2 had no significant effect on any of the testicular parameters measured, and the apparent increase in all of

these was due to three of the animals in this group entering the phase of rapid testicular growth well ahead of the controls.

#### 3.3. Experiment 2

Immunization with Anti-FSH 3 also significantly retarded testicular growth, wherein a marked slowing of testicular development occurred from around the time of the rapid phase of testicular development around 60 to 70 d of age seen in the controls (*figure 1*). This was also reflected in the following events within the testis.

##### 3.3.1. 100 days

In animals treated with Anti-FSH 3, testicular weight was only 37 % of that of the controls ( $P < 0.05$ ), while seminiferous tubular length was 62 % of that of the control animals ( $P < 0.05$ ; *table III*). Seminiferous tubular diameter, Sertoli cell nuclear size and the testicular number of Sertoli cells were also lower than in the controls, but none of these differences were statistically significant. Nor were any of these differences a result of an effect on liveweight which did not differ significantly between treated and control animals (*table III*).

At both 59 and 99 d of age, almost all rams had at least one pulse of testosterone during the sampling period, the number of which varied from 0 to 3. There were no significant differences between the control and treated rams in the number of LH pulses

**Table I.** Mean ( $\pm$  s.e.m.) testicular weight, diameter and length of the seminiferous tubules, and mean nuclear diameter and total numbers per testis of Sertoli cells of the five lambs killed at birth.

Testicular weight (g) <sup>1</sup>	Seminiferous tubular		Nuclear diameter of Sertoli cells ( $\mu$ )	No. of Sertoli cells per testis ( $\times 10^8$ )
	Diameter ( $\mu$ )	Length (m)		
0.68 $\pm 0.08$	37.4 $\pm 0.9$	138.0 $\pm 15.6$	5.44 $\pm 0.07$	2.5 $\pm 0.3$

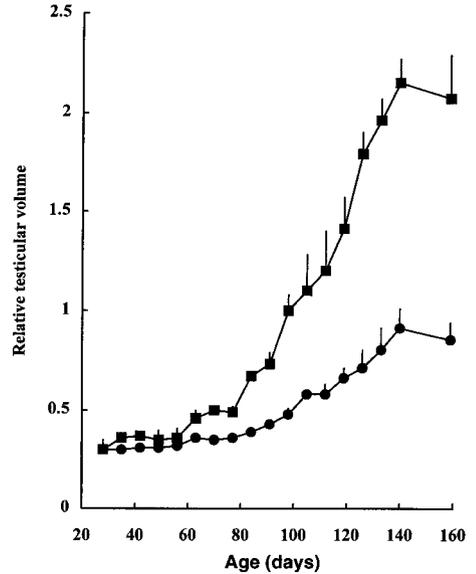
<sup>1</sup> Mean of left and right.

**Table II.** Mean ( $\pm$  s.e.m.) liveweight, testicular weight, diameter and length of the seminiferous tubules and nuclear diameter and total number per testis of Sertoli cells in the rams of experiment 1 treated from birth until 100 d of age with normal horse  $\gamma$ -globulins (controls), Anti-FSH 1 or Anti-FSH 2.

Group	Liveweight (kg)	Testicular weight (g) <sup>1</sup>	Seminiferous tubular		Nuclear diameter ( $\mu$ ) of Sertoli cells	Number of Sertoli cells per testis ( $\times 10^6$ )
			Diameter ( $\mu$ )	Length (m)		
Controls (n = 6)	30.3 $\pm$ 2.1	18.0 $\pm$ 4.4	92.2 $\pm$ 10.1	1 043 $\pm$ 49	6.8 $\pm$ 0.3	26.2 $\pm$ 2.4
Anti-FSH 1 (n = 7)	28.3 $\pm$ 2.0	9.1 $\pm$ 1.7	81.1 $\pm$ 6.0	655* $\pm$ 63	6.3 $\pm$ 0.2	18.5* $\pm$ 1.6
Anti-FSH 2 (n = 6)	31.0 $\pm$ 2.5	29.6 $\pm$ 7.2	104.9 $\pm$ 10.9	1 250 $\pm$ 152	7.1 $\pm$ 0.3	28.5 $\pm$ 2.3

<sup>1</sup> Mean of left and right.

\* Significantly different from controls,  $P < 0.05$ .



**Figure 1.** Mean ( $\pm$  s.e.m) relative testicular volume (mL testicular tissue/kg liveweight) of the ram lambs of experiment 2 passively immunized for 160 d with normal horse  $\gamma$ -globulins (squares) or with Anti-FSH 3 (circles). Where no error bar appears, error is smaller than the symbol.

at 59 and 99 d. However, at 59 d, pulse height was significantly higher in the treated rams, while at 99 d overall mean, pulse height and base level were all significantly higher in treated rams (figure 2). There were no significant differences between the two groups of rams in any of the measurements relating to the secretion of testosterone (figure 2).

### 3.3.2. 160 days

In the animals treated with Anti-FSH 3, testicular weight was only 42 % ( $P < 0.001$ ) of that of controls, seminiferous tubular length was 48 % ( $P < 0.001$ ), nuclear diameter of the Sertoli cells was 92 % ( $P < 0.05$ ) and the number of Sertoli cells per testis was 57 % ( $P < 0.01$ ) of that of controls (table III). Treatment with antiserum also had significant depressive effects on the

**Table III.** Mean ( $\pm$  s.e.m.) liveweight, testicular weight, diameter and length of the seminiferous tubules, nuclear diameter and total number per testis of Sertoli cells in the rams of experiment 2 treated from birth until either 100 or 160 d of age with normal horse  $\gamma$ -globulins (controls) or with Anti-FSH 3.

Group	Liveweight (kg)	Testicular weight (g) <sup>1</sup>	Seminiferous tubular		Nuclear diameter ( $\mu$ ) of Sertoli cells	Number of Sertoli cells per testis ( $\times 10^6$ )
			Diameter ( $\mu$ )	Length (m)		
<b>(a) 100 days</b>						
Controls (n = 6)	29.5 $\pm$ 2.0	37.1 $\pm$ 7.4	116.1 $\pm$ 10.3	1 210 $\pm$ 100	7.0 $\pm$ 0.2	26.0 $\pm$ 3.3
Anti-FSH 3 (n = 5)	28.6 $\pm$ 1.6	13.6* $\pm$ 3.4	85.3 $\pm$ 10.0	747* $\pm$ 27	6.4 $\pm$ 0.2	21.2 $\pm$ 1.0
<b>(b) 160 days</b>						
Controls (n = 7)	47.3 $\pm$ 1.6	85.3 $\pm$ 8.4	174.2 $\pm$ 8.6	1 751 $\pm$ 69	8.4 $\pm$ 0.2	30.4 $\pm$ 2.8
Anti-FSH 3 (n = 6)	46.5 $\pm$ 2.3	35.9*** $\pm$ 4.2	154.1 $\pm$ 5.5	844*** $\pm$ 58	7.8* $\pm$ 0.1	17.3** $\pm$ 1.4

<sup>1</sup> Mean of left and right.\* Significantly different from controls,  $P < 0.05$ ; \*\* significantly different from controls,  $P < 0.01$ ; \*\*\* significantly different from controls,  $P < 0.001$ .

**Table IV.** Mean ( $\pm$  s.e.m.) number of  $A_0$  spermatogonia per 100 Sertoli and daily production per 100 Sertoli cells of  $A_1$  and  $B_2$  spermatogonia, of leptotene and pachytene spermatocytes and of round spermatids in the rams of experiment 2 treated from birth until 160 d of age with normal horse  $\gamma$ -globulins (controls) or with Anti-FSH 3.

Group	Number per 100 Sertoli cells of $A_0$ spermatogonia	Daily production per 100 Sertoli cells of					
		Spermatogonia		Primary spermatocytes		Round spermatids	
		$A_1$	$B_2$	Leptotene	Pachytene		
Controls ( $n = 7$ )	6.4 $\pm 0.9$	9.2 $\pm 0.2$	17.7 $\pm 1.2$	15.6 $\pm 2.4$		50.5 $\pm 7.3$	
Anti-FSH 3 ( $n = 6$ )	5.5 $\pm 1.2$	4.1*** $\pm 0.9$	8.4* $\pm 1.1$	6.7* $\pm 1.2$		22.1* $\pm 2.7$	

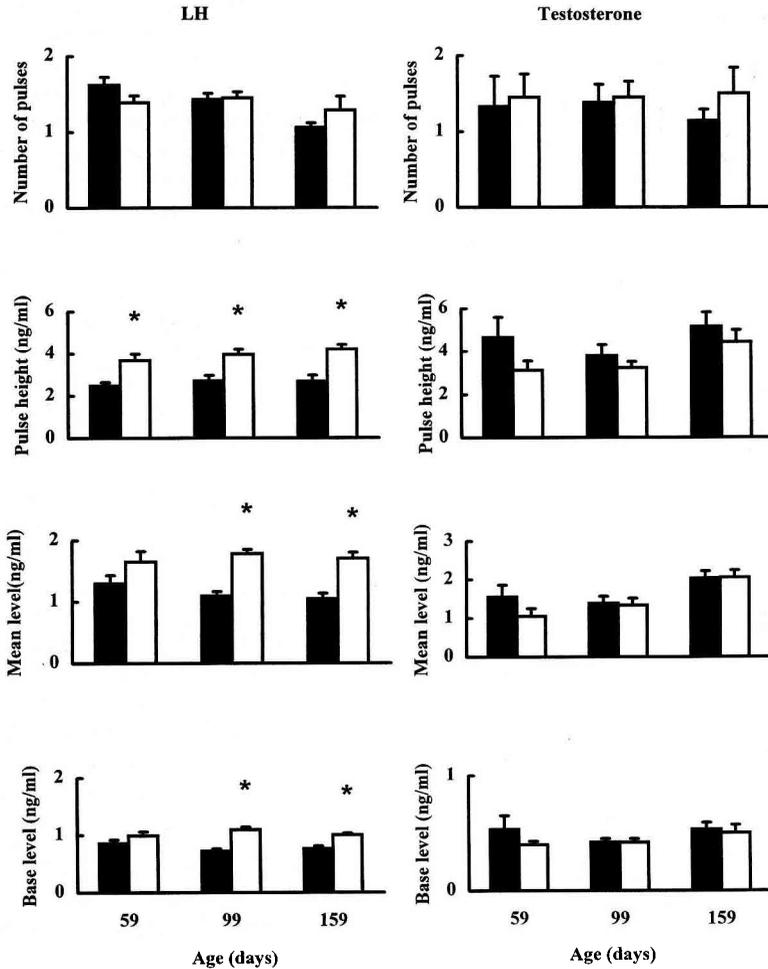
\* Significantly different from controls,  $P < 0.05$ ; \*\*\* significantly different from controls,  $P < 0.001$ .

total number of  $A_0$  spermatogonia per testis and on the total daily production of  $A_1$  and  $B_2$  spermatogonia, of leptotene and pachytene spermatocytes and of round spermatids (data not shown). However, when these data were adjusted for the size of the Sertoli cell population, treatment no longer had any significant effect on the number of  $A_0$  spermatogonia per 100 Sertoli cells nor on the daily production per 100 Sertoli cells of  $A_1$  spermatogonia (table IV). However, treatment did significantly impair the daily productions of all other types of germinal cells, which were all 43–47 % of their respective controls (table IV). The ratios of the daily production per 100 Sertoli cells of round spermatids to pachytene spermatocytes (3.2:1 and 3.3:1 in control and treated lambs), of pachytene spermatocytes to leptotene spermatocytes (0.9:1 and 0.8:1) and of leptotene spermatocytes to  $B_2$  spermatogonia (1.9:1 and 2.1:1) were similar in the control and treated groups. However, the ratio of  $B_2$  spermatogonia to  $A_1$  spermatogonia of treated animals was only 51 % of that of control animals, suggesting that only the spermatogonial divisions between the  $A_1$  and the  $B_2$  spermatogonia require FSH.

At the serial bleed at 159 d of age, there were no differences between the treated and control rams in the number of pulses of LH, but pulse height, base level and mean level were all significantly higher in the treated lambs (figure 2). There were no differences between the two groups of animals in any of the parameters relating to testosterone secretion (figure 2).

#### 4. DISCUSSION

These two experiments have shown that FSH is necessary for the normal testicular development and for the establishment of the Sertoli cell population in the ram lamb. Furthermore, these experiments have shown that, once spermatogenesis is established, FSH is essential for the differentiation of



**Figure 2.** Mean ( $\pm$  s.e.m.) pulse number, pulse height, overall mean and base level of LH (left panel) and testosterone (right panel) in the serial bleeds at 59, 99 and 159 d in the ram lambs of experiment 2 passively immunized for 100 or 160 d with normal horse  $\gamma$ -globulins (black columns) or with Anti-FSH 3 (white columns). An asterisk indicates that the treatment differs significantly from control ( $P < 0.05$ ).

A<sub>1</sub> spermatogonia into B<sub>2</sub> spermatogonia but that, from a quantitative point of view, earlier or later spermatogenic steps do not require FSH.

The experiments reported here, along with those reported by Kilgour et al. [23, 24] give new insight into the role played by FSH in the development of the testis, the

establishment of the Sertoli cell population and in the onset and maintenance of spermatogenesis in the ram.

As regards Sertoli cells, the ram lambs of the current study were born with a Sertoli cell population of around  $2.5 \times 10^8$  cells per testis. This population had increased approximately ten-fold by 100 d of age (controls,

experiments 1 and 2), after which time there was little increase up to 160 d (controls, experiment 2), even to adult ages [23, 24]. Evidence that FSH is necessary for this population to establish is seen in the present study where its withdrawal by passive immunization led to a lowering in Sertoli cell numbers relative to controls ranging from 18 to 43 %, but not complete arrest. Similar results were also seen in ram lambs passively immunized from 30 d of age against FSH with the same antisera [22]. However, in contrast to lambs, the adult rams of Kilgour et al. [23, 24], showed no effect of immunosuppression of FSH on the number of Sertoli cells per testis. Taken together, all of these results indicate that FSH is necessary only for the division of the precursors of the Sertoli cells but not for the quantitative maintenance of an already-established Sertoli cell population.

While FSH withdrawal only resulted in significant decreases in Sertoli cell numbers in two of the three cases reported here (experiment 1 and the 160-d treatment of experiment 2), anti-FSH treatment of the animals treated for 100 d in experiment 2 did result in an 18 % decrease in the size of the Sertoli cell population. One possible explanation for the lack of significance of this difference is the large coefficient of variation in the group treated with Anti-FSH 2 (greater than 30 %) compared to coefficients of close to 20 % in the other treated and control groups.

In those animals of the present study immunized up to 160 d of age, spermatogenesis had become established and, although FSH withdrawal did not completely inhibit spermatogenesis, it did result in large reductions in the production of several types of germinal cells. These reductions were not simply a reflection of the lowered number of Sertoli cells but also an effect on the numbers of germinal cells supported by each Sertoli cell. This evidence indicates that the differentiation of the  $A_0$  spermatogonia into  $A_1$  spermatogonia does

not require FSH, nor do any of the cellular types from the  $B_2$  spermatogonia onward. However, somewhere between the differentiation of the  $A_1$  into  $B_2$  exists one, or possibly more, steps that require FSH, a conclusion previously suggested by Courot et al. [14] that is corroborated by similar work in the adult mouse and hamster [17] ram [15, 23, 24], monkey [5, 27, 41] and rat [6, 28, 37].

From the experiment reported here, these above effects appear to be due to an effect on FSH alone, and not to any effect on the secretion of LH or testosterone. Evidence for this is seen in the lack of effect on the plasma levels of the two hormones which, if affected at all, were increased in the case of some LH parameters.

These results are consistent with other experiments on young animals which have shown an influence of FSH on the Sertoli cell population under a range of experimental conditions. These have included hypophysectomy or administration of antiserum to FSH to foetal rats [32], administration of FSH to neonatal rats [2, 40] and hpg mice [36], treatment of normal ram lambs with FSH for as little as 5 d [8, 9], hypophysectomy and hormonal replacement of young rams [10, 12] and rats [11, 29], suppression of FSH in immature and adult rats followed by treatment with hFSH ([2] see review by Sharpe [35]).

The results are also consistent with those concerning germinal cell production in the monkey [5, 27, 41] and young rat where the  $A_1$  spermatogonia appear not to be sensitive to hormones, while the more mature spermatogonia and, as a consequence, spermatocytes are under the influence of FSH [2, 6, 7, 11, 20, 28, 34, 37]. However, there is no evidence from the experiments reported here of a quantitative effect of FSH on spermiogenesis in the young ram as has been postulated in the rat [3, 28, 39].

In conclusion, FSH appears to be essential for the normal development of the testis and the establishment of the normal popu-

lation of Sertoli cells in at least two mammalian species because it is necessary for normal Sertoli cellular division. Once spermatogenesis has established, FSH also appears to be necessary for the late spermatogonial divisions, possibly by decreasing the number of spermatogonia that degenerate [16]. It is likely that this effect on the germinal cells operates through the Sertoli cells since immunosuppression of FSH decreased the size of their nuclei. However, the data reported here cannot be used to conclude that FSH is the sole hormone responsible for normal testicular development, since testicular growth and spermatogenesis were not completely arrested. While this may have been due to incomplete suppression of FSH, the evidence of the involvement of other hormones such as LH and GH [8, 12] cannot be discounted.

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