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Spermatogenesis and Sertoli cell numbers and function in rams and bulls

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Summary. The two main types of cellular associations (type I, 2 generations of spermatocytes + 1 of spermatids; type II, 1 of spermatocytes and 2 of spermatids) occupy, respectively, more than half and about a third of the seminiferous epithelium cycle in rams and bulls. However, the duration of the cycle of the seminiferous epithelium and that of spermatogenesis differ between the species. A_1 spermatogonia and Sertoli cell total numbers are highly correlated in adult rams and bulls. Mitosis in Sertoli cells occurs mostly *in utero* but may still occur for a short period after birth. Between birth and puberty there is about a 5-fold increase in the number of Sertoli cells. After that there are no seasonal- or age-related increases in the number of adult Sertoli cells. Some factors (season of birth; nutrition; genetics; hormones) affect mitosis of Sertoli cells in prepubertal animals. Sertoli cells differentiate after cessation of mitosis. Their differentiation is affected by cryptorchidism, nutrition, genetics and hormones. Their adult function is only poorly known. ABP and rete testis fluid secretions and nuclear Sertoli volume fluctuate under the influence of the same factors, but they are not always linked together. This reinforces the need for more knowledge of Sertoli cell secretions and function.

Introduction

Development and function of the germinal epithelium are linked to the development of the somatic elements of the testis. The Sertoli cells are permanent elements of the seminiferous epithelium which originate from the mesonephros (Zamboni & Upadhyay, 1982). In the male fetus primordial germ cells divide but do not differentiate in the male gonad, although when they occasionally enter the fetal adrenal, they initiate spermatogonial multiplications and undergo meiosis (Upadhyay & Zamboni, 1982). However, after birth and differentiation of Sertoli cells, germ cells enter spermatogenesis.

The Sertoli cells could play different roles in inhibiting germinal differentiation in the fetal testis, inducing germ cell multiplication and differentiation during and after puberty and probably influencing sperm quality (Hochereau-de Reviers & Courot, 1978). The different factors involved in such complex phenomena are not yet known. More than 80 proteins are secreted by rat Sertoli cells in culture (Wright *et al.*, 1981). Very few of these proteins have been identified in rams and bulls. Androgen-binding protein (ABP) is one of them (Jegou *et al.*, 1979). Clusterin, which is a cell aggregating factor, has been identified in testes of sheep (Blaschuk *et al.*, 1983) but no physiological analysis has been done. Tissue-type plasminogen activor is secreted by bovine Sertoli cells in culture (Jenkins & Ellison, 1986). The existence of bovine anti-Müllerian hormone (Josso, 1973) and its structure (Cate *et al.*, 1986) and its homology with inhibin (Mason *et al.*, 1985) have been reported. The object of this review is (1) to summarize the similarities and dissimilarities of the seminiferous epithelium in rams and bulls and (2) to analyse the control of Sertoli cell multiplication and function in both species.

Spermatogenesis in rams and bulls

Two main types of cellular associations have been distinguished: type I with two generations of primary spermatocytes and a single generation of spermatids and type II with only one generation of primary spermatocytes and two of spermatids. Types I and II represent 50–60% and 30–40% respectively in the seminiferous epithelial cycle of bulls and rams, and in both species, spermatozoa are released before the new generation of preleptotene primary spermatocytes is initiated. The two types of cellular associations can be subdivided further according to the arrangement and shape of the germ cells (Ortavant, 1958; Cupps & Laben, 1960; Amann, 1962; Hochereau, 1967; Guraya & Bilaspuri, 1976; Bilaspuri & Guraya, 1986) or the stages of development of the acrosome (Clermont & Leblond, 1955; Kramer, 1960; Berndtson & Desjardins, 1974). The relative frequencies of cellular association vary according to the classification method. However, equivalence can be drawn and comparisons can be made (Courot *et al.*, 1970).

There are no significant variations in frequencies between regions in the same testis, between testes or between individuals provided a sufficient number of cross-sections of tubules are analysed (Amann, 1962; Hochereau, 1963). If not enough tubules are counted, local variations in grouping of tubules at the same stage are observed, indicating a local control of onset of spermatogenesis as in the mouse (Redi, 1986). This local assembly of tubules at the same stages could result in apparent variation of relative frequencies of the cellular association (Kramer, 1960). This phenomenon has to be taken into account if analyses are performed on small biopsy specimens.

Spermatogonial divisions and stem cell renewal

The number of generations between A_1 and preleptotene spermatocytes has been analysed by different complementary methods: (1) incorporation of radiolabelled precursors of DNA (Hochereau *et al.*, 1964; Hochereau, 1967; Hochereau-de Reviers, 1970; Hochereau-de Reviers *et al.*, 1976b); (2) the morphological appearance of nuclei in the spermatogonia including their nuclear volume (Ortavant, 1958; Kramer, 1960; Amann, 1962; Bilaspuri & Guraya, 1986); and (3) the evolution of their number per cross-section of tubules during the seminiferous epithelial cycle (Ortavant, 1958; Amann, 1962).

Six spermatogonial divisions have been observed after labelling with [^{3}H]thymidine in the ram and the bull. They occur at the same stages in the seminiferous epithelial cycle. Three type A, one intermediate and two type B spermatogonial generations have been observed in rams and bulls (Hochereau, 1967; Berndtson & Desjardins, 1974; Hochereau-de Reviers *et al.*, 1976b; Bilaspuri & Guraya, 1986). However, the duration of the seminiferous epithelial cycle differs in the two species (Table 1).

The origin of the cycling stem cells and the significance of A_0 spermatogonia (round and pale type A) are still disputed (Hochereau-de Reviers, 1981; Lok *et al.*, 1982). In adult rams and bulls, the ratio between A_0 and A_1 (ovoid pale with a central dark nucleolus) spermatogonia differs markedly: for example, this ratio, $A_0/(A_0 + A_1)$, is about 15% in adult bulls (Hochereau-de Reviers, 1970) and about 50% in adult rams in the breeding season (Hochereau-de Reviers *et al.*, 1976b). In the sheep testis it varies with photoperiod (Hochereau-de Reviers *et al.*, 1985) and endocrinological status, the A_1 spermatogonia disappearing after hypophysectomy and being at least partly related to FSH secretion (Courot *et al.*, 1979). A_0 spermatogonia could represent the first step of the cell cycle of A_1 spermatogonia (G_0 or beginning of G_1: Hochereau-de Reviers, 1981). The A_0 spermatogonia which are isolated or single (A_S) could represent the basic stem cell, the A paired (A_p) and aligned (A_{a1}) spermatogonia being the multiplicating cells which ensure the formation of new A_1 spermatogonia (Lok *et al.*, 1982). In the ram the total numbers of $A_s + A_p + A_{a1}$ (Lok *et al.*, 1982) nearly double between stages 2 and 5 of the classification of Roosen-Runge & Giesel (1950) in which three peaks of [^{3}H]thymidine labelling corresponding to A_2, A_3 and intermediate spermatogonia are observed (Hochereau-de Reviers *et al.*, 1976b). At two

Table 1. Comparison of duration of seminiferous epithelial cycle in sheep and cattle

Species		Reference	Method	Duration (days)
Sheep	(*Ovis aries*)	Ortavant (1958)	^{32}P	10·4
		Hochereau *et al.* (1964)	[^{3}H]thymidine	10·4
Cattle	(*Bos taurus*)	Orgebin (1961)	^{32}P	13·4
	(*Bos taurus*)	Hochereau *et al.* (1964)	[^{3}H]thymidine	13·5
	(*Bos indicus* × *Bos taurus*)	Salim & Entwistle (1982)	[^{3}H]thymidine	13·4
	(*Bos indicus*)	Cardoso & Godinho (1983)	[^{3}H]thymidine	14·0
	(*Bubalus bubalis*)	Sharma & Gupta (1980)	[^{3}H]thymidine	8·6
		Bilaspuri & Guraya (1980)	[^{3}H]thymidine	8·5

divisions after labelling, most of the new A_1 labelled spermatogonia in rams and bulls arise from precursor mother cells labelled as A_1 and A_2 spermatogonia (Hochereau-de Reviers, 1970; Hochereau-de Reviers *et al.*, 1976b). In bulls, A_1 spermatogonia at stages 7 and 8 are present as single (25%) or grouped (75%) cells (Hochereau-de Reviers, 1970). This suggests segregation of the precursor cells of A_1 spermatogonia earlier than the A_2 divisions (Hochereau-de Reviers, 1971).

Nevertheless, A_1 spermatogonia and Sertoli cell total numbers per testis are highly and positively correlated ($r > +0{\cdot}65$) in rams and bulls. This is not observed for the A_0 spermatogonia population (de Reviers & Courot, 1976) and we conclude that A_1 spermatogonia are the first step of the spermatogenic cycle and are clearly dependent on Sertoli cell function.

Relations between Sertoli and germ cell populations

The existence of a correlation between Sertoli and A_1 spermatogonia indicates a control of spermatogenesis by the Sertoli population very early in the spermatogenic cycle, the end point of which is control of daily sperm production (de Reviers *et al.*, 1980). The establishment of a Sertoli cell population is therefore a primordial factor controlling sperm production.

Sertoli cell multiplications

Sertoli cell multiplications occur mostly during fetal life. In the Ile-de-France lamb shortly after sexual differentiation (40 days of fetal life) the total number of future Sertoli cells (supporting cells) is about 1×10^6 per testis (Courot, 1971). It increases 300- to 400-fold until birth (Table 2). Between birth and the post-pubertal phase of testicular growth, total number of Sertoli cells still increases 5- to 10-fold in rams according to breed and by a factor of 5 in Normand bulls (Table 2). The age at which mitosis of Sertoli cells is arrested varies from 40 to 80 days in different breeds of sheep. No further increase is observed after the prepubertal period in sheep and cattle (Table 2). No variation in total numbers of Sertoli cell is observed between breeding and non-breeding season in adult rams (Table 3).

After puberty the Sertoli cell population, estimated by the same technique, does not vary in numbers and so there is a quantitatively stable population of Sertoli cells in adult rams and bulls. However, the following factors can influence the Sertoli cell multiplications.

Genetic factors

Between breeds, variation in Sertoli cell populations has been reported (Hochereau-de Reviers *et al.*, 1984a) for rams and bulls (Table 4). These variations affect at least partly those of daily

Table 2. Comparative development of total numbers of Sertoli cells (corrected number for nuclear size) according to age in rams and bulls of different breeds

Sheep	Ile-de-France (adapted from Courot, 1971; Kilgour *et al.*, 1985)	Romanov (Lafortune *et al.*, 1984; and unpublished data)	Dorset Horn × Finn (J. R. McNeilly, unpublished data)	Romanov × Prealpes × Ile-de-France (Monet-Kuntz *et al.*, 1984, 1987)
Birth	3·1	3·1	4·30 ± 0·4	—
60–70 days of age	17·0 ± 6·5	—	—	22·5 ± 3·0
100–200 days of age	36·2 ± 3·0	20·6 ± 1·4	20·5 ± 1·1	25·6 ± 2·0
18 months	28·0 ± 3·3	19·8 ± 1·1	20·3 ± 1·1	—
5 years	33·7 ± 2·5	—	—	—

Cattle	Française Frisonne Pie noire (M.T. Hochereau-de Reviers, unpublished data)	Normand (adapted from Attal & Courot, 1963)
Birth	—	9·3 ± 0·4
120 days	—	46·8 ± 7·1
240 days	—	44·2 ± 4·4
18 months	35·9 ± 1·2	—
3 years	—	54·6 ± 3·6
6 years	36·6 ± 2·7	—

Values are mean ± s.e.m. × 10^{-8}.

Table 3. Comparison of total numbers (corrected for nuclear size) of Sertoli cells per testis in different breeds of sheep according to season

Breed of sheep	Reference	Rams/ group	Non-breeding season	Breeding season
Île-de-France	Hochereau-de Reviers *et al.* (1984a)	8	27·9 ± 7·7	33·2 ± 9·2
	B. D. Schanbacher (unpublished)	5	31·4 ± 6·7	31·2 ± 2·7
Soay	Hochereau-de Reviers *et al.* (1985)	5	15·4 ± 0·6	12·9 ± 0·6

Values are mean ± s.e.m. × 10^{-8}.

production of spermatozoa. In Montbeliard bulls there is a tendency for the animals classified as 'good' or 'intermediate' for their sperm characteristics to have a higher number of Sertoli cells per testis (30 ± 7 and 29 ± 4 × 10^8 respectively) than those classified as 'bad' (22 ± 1 × 10^8; Abdel Malak, 1983).

Environmental factors

Nutrition. Severe restriction of food, inducing a reduction of mean daily weight increase (136 versus 280 g/day), from the first week of age results in a decrease of the Sertoli cell population at 100 days of age (21 *vs* 39 × 10^8 Sertoli cells/testis) in cross-bred Romanov × Limousin lambs (Brongniart *et al.*, 1985). However, the Sertoli cell multiplications can be maintained for a longer period as rapid testis growth is delayed and starts just before 100 days of age in underfed lambs. Therefore, no conclusion can be reached on the presence or absence of a decrease in the adult Sertoli cell population.

Table 4. Breed differences in Sertoli cell populations (corrected numbers) and daily sperm production in sheep and cattle

	Reference	Breed	Sertoli cells (total no./testis $\times 10^{-8}$)	Daily sperm production ($\times 10^{-9}$)
Sheep	Hochereau-de Reviers *et al.* (1984a)	Soay	13·9 ± 1·4	2·1 ± 0·2
		Romanov	19·8 ± 1·3	2·5 ± 0·2
		Prealpes-du-Sud	24·9 ± 1·4	4·1 ± 0·2
		Ile-de-France	36·2 ± 3·1	4·1 ± 0·3
	J. R. McNeilly (unpublished)	Dorset Horn × Finn	20·3 ± 1·1	2·6 ± 0·2
	M. Seck (unpublished)	Merinos d'Arles	24·7 ± 1·4	2·8 ± 0·2
Cattle	Abdel Malak (1983)	Montbeliard	27·2 ± 1·8	2·7 ± 0·2
	Lafortune (1983)	Française Frisonne Pie Noire	23·0 ± 2·4	2·7 ± 0·2
		Zebu	21·0 ± 1·5	2·7 ± 0·1
	M. T. Hochereau-de Reviers (unpublished)	Holstein × FFPN	36·6 ± 2·7	—
	Attal & Courot (1963)	Normand	54·6 ± 3·6	3·4 ± 0·2

Values are mean ± s.e.m.

Season of birth. A higher number of Sertoli cells after puberty is observed in the testes of rams born in the summer: +25% in Ile-de-France adult rams (de Reviers *et al.*, 1980) and +50% in Finn × Dorset Horn cross breds, 6 months old (Hochereau-de Reviers *et al.*, 1984b). The photoperiod modifies the gonadotrophin secretion (Courot *et al.*, 1975; Lafortune *et al.*, 1984) during the prepubertal period in lambs. Gonadotrophin binding per testis (Barenton & Pelletier, 1983) and increased secretions of testosterone and ABP (Jegou *et al.*, 1979) are evidence of seasonal variations with a maximum during the summer months and a minimum during the winter ones.

Experimental situations

Unilateral castration. Unilateral castration of prepubertal lambs or calves resulted in a hyperplasia of the Sertoli cell population (Hochereau-de Reviers, 1976; de Reviers *et al.*, 1980; Waites *et al.*, 1985). Such a numerical increase is not obtained after unilateral castration of pubertal or adult animals (Hochereau-de Reviers *et al.*, 1984b).

Cryptorchidism. In rams and bulls testicular descent into the scrotum occurs early in fetal life (Hullinger & Wensing, 1985), around the end of the second third of gestation. Lambs have been rendered experimentally cryptorchid at birth and orchidopexy occurs at onset of prepubertal rapid testicular growth. After 2 months of cryptorchidism, a significant increase in the Sertoli cell population is observed compared to normal lambs (Monet-Kuntz *et al.*, 1987).

Hypophysectomy. Hypophysectomy of 50-day-old Ile-de-France lambs results in a decrease in Sertoli cell numbers (Courot, 1971; Table 5): 15 days after hypophysectomy total numbers are reduced by 40% compared to values of controls and by 60% compared to that of postpubertal animals of the same breed. Treatment with LH or FSH alone prevents Sertoli cell numbers from decreasing. However, treatment with both FSH and LH results in a synergistic action on Sertoli cell multiplications such that the normal adult number in that breed is restored (Table 5).

Table 5. Hormonal control of Sertoli cell population and of seminiferous tubule mean diameter in prepubertal Ile-de-France lambs (corrected number) (adapted from Courot (1971) and Kilgour *et al.* (1984))

	Sertoli cell		Seminiferous tubule mean diam. (μm)
	Total no. ($\times 10^{-8}$)	Nuclear area (μm^2)	
Control, 50 days	14·8 ± 2·3	22·5 ± 0·7	55·1 ± 1·7
Hypox. + 15 days	10·3 ± 3·1	17·5 ± 0·4	36·2 ± 1·2
Hypox. + FSH	14·9 ± 2·2	18·5 ± 0·3	39·4 ± 2·5
Hypox. + LH	17·3 ± 2·2	24·5 ± 0·4	58·0 ± 3·9
Hypox. + LH + FSH	30·6 ± 3·8	27·2 ± 0·5	63·5 ± 3·1
Control, 100 days	38·2 ± 5·5	33·1 ± 1·3	104·0 ± 13·2
Passively immunized against LH	20·7 ± 2·8	30·3 ± 1·3	104·9 ± 10·7
Control, 100 days	32·8 ± 0·3	30·4 ± 2·2	125·2 ± 5·7
Passively immunized against FSH	20·1 ± 2·7	28·9 ± 1·3	85·2 ± 9·9

Values are mean ± s.e.m.

Immunization against gonadotrophins. Continuous passive immunizations since birth until 100 days of age with antibodies against either FSH or LH decrease the total number of Sertoli cells per testis at 100 days of age in lambs; the value observed at birth (3×10^8) has not been obtained (Table 5; Kilgour *et al.*, 1984). Multiplications of Sertoli cells are not completely arrested.

Culture in vitro. Ovine Sertoli cells cultured *in vitro* incorporate [^{3}H]thymidine into DNA whatever the age, between 2 and 12 weeks of age. This incorporation is not stimulated but is decreased by FSH treatment, probably by an increase of their maturation (A. S. Speight, J. M. Clifford & G. M. H. Waites, unpublished data). A seminiferous growth factor has been isolated from calf and mouse testes; it stimulates Sertoli cell proliferation *in vitro* (Bellvé & Feig, 1984).

Functional differentiation of Sertoli cells

At the end of the prepubertal period, Sertoli cells progressively differentiate. One of the first morphological signs is an increase in cytoplasmic and nuclear cross-sectional area (Monet-Kuntz *et al.*, 1984). In the lamb, mean cellular and nuclear volumes increase 3- and 1·5-fold respectively, between 25 and 100 days of age. Between 6 weeks and 18 months of age Sertoli cell nuclear volume increases about 5-fold. In the Normand bull, the mean Sertoli nuclear volume increases by a factor of 3·5 between 4 months and 3 years of age.

In the lamb, the total number of FSH binding sites per testis increases about 150-fold between 10 and 120 days (Barenton *et al.*, 1983b), and those of androgen cytoplasmic and nuclear binding sites increase 16- and 12-fold between 25 and 100 days of age respectively (Monet-Kuntz *et al.*, 1984). In fact, between cessation of mitosis of Sertoli cells and puberty the total numbers of FSH and androgen-binding sites increase about 10-fold. ABP testicular content increases by a factor of 3·5 from 50 to 120 days of age (Carreau *et al.*, 1979). Similarly, an increase in FSH binding sites of 13-fold has been reported for calves between 100 days and 2·5 years of age (Dias & Reeves, 1982).

During pubertal testicular growth, inhibin secretion increases with Sertoli differentiation in the ram (Blanc *et al.*, 1981).

The factors which can alter Sertoli cell differentiation are as follows.

Genetic factors

In lambs from two established lines selected for high (H) and low (L) testicular growth between 6 and 20 weeks of age (McNeilly *et al.*, 1986; unpublished data), the total numbers of Sertoli cells

Table 6. Changes of testicular values in two fixed lines of Dorset Horn × Finn rams selected for their high (H) and low (L) rate of testicular growth (J. R. McNeilly, unpublished data)

		Testis weight (g)	Total no. of Sertoli cells/testis ($\times 10^{-8}$)*	Sertoli cell nuclear area (μm^2)	Seminiferous tubule diam. (μm)	Daily production of round spermatids ($\times 10^{-9}$)
Birth	H	$0{\cdot}7 \pm 0{\cdot}07^a$	$4{\cdot}30 \pm 0{\cdot}4^a$	$17{\cdot}4 \pm 0{\cdot}4^a$	$36{\cdot}0 \pm 0{\cdot}4^a$	—
	L	$0{\cdot}79 \pm 0{\cdot}16^a$	$4{\cdot}4 \pm 0{\cdot}7^a$	$17{\cdot}7 \pm 0{\cdot}2^d$	$34{\cdot}0 \pm 1{\cdot}3^a$	—
6 weeks	H	$1{\cdot}97 \pm 0{\cdot}23^b$	$6{\cdot}64 \pm 0{\cdot}3^b$	$22{\cdot}3 \pm 0{\cdot}8^b$	$61{\cdot}7 \pm 2{\cdot}8^b$	—
	L	$1{\cdot}47 \pm 0{\cdot}18^b$	$5{\cdot}82 \pm 0{\cdot}8^b$	$20{\cdot}4 \pm 1{\cdot}0^b$	$51{\cdot}0 \pm 2{\cdot}8^c$	—
20 weeks	H	$106{\cdot}6 \pm 4{\cdot}00^d$	$19{\cdot}3 \pm 1{\cdot}0^c$	$45{\cdot}1 \pm 1{\cdot}0^d$	$203{\cdot}3 \pm 4{\cdot}7^d$	$1{\cdot}57 \pm 0{\cdot}08^b$
	L	$74{\cdot}6 \pm 11{\cdot}10^e$	$21{\cdot}4 \pm 0{\cdot}5^c$	$39{\cdot}2 \pm 0{\cdot}6^e$	$172{\cdot}4 \pm 11{\cdot}7^e$	$0{\cdot}97 \pm 0{\cdot}24^a$
Adult	H	$218{\cdot}7 \pm 8{\cdot}10^f$	$20{\cdot}3 \pm 1{\cdot}1^c$	$64{\cdot}15 \pm 1{\cdot}6^e$	$242{\cdot}0 \pm 7{\cdot}0^f$	$2{\cdot}62 \pm 0{\cdot}16^c$
	L	$222{\cdot}8 \pm 15{\cdot}80^f$	$23{\cdot}7 \pm 2{\cdot}8^c$	$63{\cdot}93 \pm 1{\cdot}6^e$	$237{\cdot}0 \pm 4{\cdot}0^f$	$2{\cdot}75 \pm 0{\cdot}28^c$

Values are mean ± s.e.m.
*Corrected number.
Within columns, values with different letters indicate significant differences ($P < 0{\cdot}05$).

did not differ at any age between lines. However, their function could be different. The mean diameter of the seminiferous tubules, which reflects Sertoli cellular volume variation, and the nuclear area of Sertoli cells are greater at 6 and 20 weeks of age respectively in the H line than in the L line sheep. Seminiferous tubule diameter and daily production of round spermatids are also increased in the H line at 20 weeks of age (Table 6), but in adult rams no differences are observed. The two lines of sheep are therefore distinguished by a transitory difference in Sertoli and germ cell characteristics. Plasma FSH concentrations are significantly lowered in the H line sheep at 16 and 20 weeks of age (McNeilly *et al.*, 1986), and this could reflect a more precocious increase in FSH binding sites in the H than in the L line animals.

In Ile-de-France, Prealpes-du-Sud and Romanov lambs, at 8 months of age and during the non-breeding season, variations in FSH binding sites per testis or per Sertoli cell reflect variations in precocity and/or seasonality (Barenton *et al.*, 1983a).

Sertoli cell function during pubertal testis growth could therefore result in variations of testicular characteristics and possibly in earlier puberty.

Nutritional factors

In sheep that are underfed during the prepubertal period, there is reduced Sertoli cell nuclear size (mean nuclear area: 17·1 versus 28·4 μm^2) resulting in a decrease of 63% in volume (I. Brongniart, unpublished data).

Experimental situations

Cryptorchidism. In sheep experimental cryptorchidism at birth inhibits the normal differentiation of the Sertoli cells during the pubertal process. Nuclear volume of Sertoli cells is reduced by 30% by cryptorchidism. Total ABP (Table 7) content per testis, total numbers of FSH and cytoplasmic androgen binding sites per testis are greatly reduced (Monet-Kuntz *et al.*, 1987). After orchidopexy at 2 months of age the daily production of round spermatids, 5 months later, is only partly restored, due to the presence of seminiferous tubules empty of germ cells. However, the ABP content of testis and the total numbers of FSH and cytoplasmic androgen binding sites are restored (Monet-Kuntz *et al.*, 1987; Table 7). This indicates that restoration of Sertoli cell function in terms of ABP secretion

Table 7. Effect of experimental cryptorchidism on lamb testicular parameters (from Monet-Kuntz *et al.*, 1987), expressed as % of the normal values at 7 months of age

	Cryptorchid	Cryptorchid + orchidopexy at 2 months of age
Testis weight (g)	14	84
% of empty tubules*	100	16·5
Sertoli cell total number/testis ($\times 10^{-8}$)	88	149
FSH binding (pmol/testis)	3·6	90
Cytoplasmic androgen binding (pmol/testis)	14·3	96
ABP (pmol/testis)	19·2	100
Daily production of round spermatids ($\times 10^{-9}$)	0	45

*Empty tubules in normal lambs equals 1·9%.

or of FSH and androgen binding sites does not depend on that of spermatogenesis in the whole testis.

Hypophysectomy. Hypophysectomy of 50-day-old lambs provokes a decrease in cellular (as indicated by the variation in seminiferous tubule diameter) and nuclear size of Sertoli cells (Courot, 1971; Table 5). FSH treatment does not support these measures. LH supplementation maintains the initial cytoplasmic and nuclear size and LH + FSH treatment promotes the Sertoli cell development. ABP production is restored after hypophysectomy, mainly by testosterone treatment (Carreau *et al.*, 1980).

Immunization against gonadotrophins. Passive immunizations of sheep from birth until 100 days of age with antibodies against either LH or FSH do not modify Sertoli cell nuclear development significantly (Table 5).

Culture in vitro. Sertoli cells of prepubertal sheep and cattle have been cultured to assess their ability to be stimulated by FSH and/or androgen. In calves, 5–11 months old, FSH or testosterone treatments change the overall rate of protein synthesis and secretion without the detection of specific qualitative changes and age effects (Hayes & Brooks, 1985). In cultures of Sertoli cells from 6–8-month-old calves, FSH but not LH induces a synthesis of cAMP and [^{3}H]leucine-labelled proteins (Smith & Griswold, 1981). FSH stimulates the synthesis of tissue-type plasminogen activator of Sertoli cells of prepubertal calves but this response is abolished by dexamethasone, which induces a specific protease inhibitor (Jenkins & Ellison, 1986). In the lamb, from 2 to 12 weeks of age, Sertoli cells cultured *in vitro* demonstrate a significant age-dependent increase in the proportion of [^{3}H]leucine incorporation into Sertoli cell secreted proteins (Waites *et al.*, 1985).

Adult Sertoli cell functions

Cyclic variations of Sertoli cell nuclei according to the seminiferous epithelium stages have been observed in rams, with maximum development during the stages when elongation of spermatids takes place (Hochereau-de Reviers *et al.*, 1985). However, the separation of functional seminiferous tubules in bull and ram testis is not possible, due to the importance of connective fibres in intertubular tissue. A stage-dependent analysis of seminiferous epithelium function to compare with that of the rat (Parvinen, 1982) has not yet been possible.

Sertoli cell functions of adults may vary according to the factors below.

Table 8. Comparisons of testicular values and hormonal binding in 18-month-old adult Romanov and Ile-de-France rams during the breeding season (C. Monet-Kuntz, unpublished data)

	Romanov (N = 6)	Ile-de-France (N = 5)
Testis weight (g)	172·8 ± 26·9	255·2 ± 45·8*
Sertoli cell total no./testis $\times 10^{-8}$	13·6 ± 2·7	28·0 ± 2·3*
FSH receptors/Sertoli cell (pmol)	13·90	12·25
Sertoli nuclear cross-sectional area (μm^2)	70·4 ± 1·3	65·9 ± 1·1*
FSH binding (pmol/testis)	189·0 ± 38	343·0 ± 66·0*
Cytoplasmic androgen binding (pmol/testis)	52·7 ± 19	74·5 ± 22·0
Daily production of round spermatid ($\times 10^{-9}$)	1·89 ± 0·35	3·34 ± 0·48*
DSP/Sertoli cell ($\times 10^{-7}$)	13·9	11·9
Cross-sectional area of spermatid cell (μm^2)	53·3 ± 0·9	58·7 ± 1·7*
Rete testis fluid secretion (ml/h)	1·03 ± 0·14	1·35 ± 0·35

Values are mean ± s.e.m.
*Significantly different from value for Romanov rams, $P < 0·05$.

Genetic factors

In sheep, breed differences have been demonstrated in rete-testis fluid flow and compared to that of Sertoli cell numbers per testis (Dacheux *et al.*, 1981). Romanov as compared to Ile-de-France rams have 50% less of the number of Sertoli cells, of FSH binding sites and of daily spermatid production per testis, 30% less of testis weight, of androgen binding sites and of rete testis fluid flow rate (Table 8). However, the mean Sertoli cell nuclear cross-sectional area, the daily production of spermatids and the FSH binding sites are slightly greater in Romanov than in Ile-de-France rams, while cross-sectional area of spermatid cell is smaller.

Seasonal factors

In adult rams, seasonal variations in nuclear cross-sectional area of Sertoli cells have been observed with a maximum during the breeding season (Hochereau-de Reviers *et al.*, 1976a, 1985). Total numbers of FSH binding sites per testis (Barenton & Pelletier, 1983), ABP concentration in the rete testis fluid (Jegou *et al.*, 1979) and rete testis fluid flow rate (Dacheux *et al.*, 1981) exhibit seasonal variations. The maximum of rete testis fluid flow precedes by 1·5 months that of sperm production, and this delay corresponds approximately to the duration of one spermatogenic cycle and suggests that the increase of Sertoli cell function, indicated by rete testis flow rate, induces and/or is correlated with, that of spermatogenic functions.

Experimental situations

In adult Ile-de-France rams, hypophysectomy induces a decrease in Sertoli cell nuclear volume which is restored nearly completely by PMSG or hCG treatments but not by testosterone (Courot *et al.*, 1979).

In sheep, active immunization against oestradiol-17β provokes an increase in testicular volume (Schanbacher, 1984) which is accompanied by an increase in LH, FSH and testosterone plasma concentrations (Schanbacher *et al.*, 1986). During the breeding season, this testicular increase is related to an increase in sperm production and of ABP concentration in the rete testis fluid which is

Table 9. Effect of active immunization against oestradiol-17β on testicular values in Ile-de-France adult rams (B. D. Schanbacher, unpublished data)

	Non-breeding season		Breeding season	
	Control	Immunized	Control	Immunized
Testis weight (g)	173 ± 26[a]	219 ± 28[b]	275 ± 20[c]	369 ± 29[d]
Total no. of Sertoli cells/testis ($\times 10^{-8}$)	31·4 ± 2·9[a]	36·8 ± 3·2[a]	30·3 ± 4·5[a]	35·5 ± 2·4[a]
FSH binding (pmol/testis)	20·7 ± 3·7	25·9 ± 3·4	161 ± 12[a]	212 ± 18[b]
Cytoplasmic androgen binding (pmol/testis)	117 ± 25[a]	37·1 ± 30[b]	125 ± 12[a]	82 ± 18[c]
ABP concentration in rete-testis fluid (10^{-9} M)	—	—	10·7 ± 1·4[a]	20·0 ± 6·4[a]
Daily production of spermatids ($\times 10^{-9}$)	1·95 ± 0·14[a]	2·44 ± 0·26[a]	3·33 ± 0·55[b]	5·02 ± 0·38[c]

Values are mean ± s.e.m.
Within rows, values with different letters are significantly different, $P < 0{\cdot}05$.

nearly doubled. In parallel, the total number of FSH binding sites per testis is increased while that of androgen binding sites and androgen concentrations in the rete testis, are decreased (unpublished data; Table 9).

Passive immunization against FSH depresses Sertoli nuclear cross-area (by 14%) and sperm production (Courot *et al.*, 1984; unpublished data).

Discussion

The major events of spermatogenesis in ram and bull testes are now relatively well known. However, the determination of daily sperm production requires a precise knowledge of duration of the seminiferous epithelial processes in all domestic ruminants.

The relative proportion of associations of Types I and II is quite different from that observed in rodents in which Types I and II represent about 25% and 70% respectively of the seminiferous epithelial cycle, and in human male in which the two types are nearly equal. This reinforces the need for a comparative study between species in which spermiation, entrance of meiotic cells into the adluminal compartment and the division of type A_1 spermatogonia are concomitant or not in the seminiferous tubules. Compared to rodents or monkeys (*Cercopithecus aethiops*), the number of spermatogonial generations is similar but the ratio of type A and B spermatogonia differs (see review by Courot *et al.*, 1970).

As in rats, the mode of stem cell renewal is still disputed for sheep. However, the main problem is to understand the physiological significance of A_0 stem cells and of the different ratio of $A_0/(A_0 + A_1)$ in rams and bulls. These differences could be related to the presence of seasonal variation in sheep, but this needs to be tested. Total numbers of A_0 and A_1, spermatogonia vary inversely in the ovine testis and the A_0 population is negatively correlated to FSH plasma concentrations. Furthermore, A_1 spermatogonia and Sertoli cell population per testis are highly correlated in ram and bull testes. Firstly, this indicates that Sertoli cells control the very early step of spermatogenesis (inhibiting and/or permissive action) and not only the adluminal compartment. Secondly, the establishment of the Sertoli cell population before puberty could have specific consequences on sperm production in the adult. This reinforces the need for a better knowledge of the control of Sertoli cell multiplications in the fetus and neonate.

In sheep and cattle, most Sertoli cell multiplications occur during fetal life as observed in the rat (Orth, 1982) but multiplication does continue until the onset of prepubertal testicular growth. In rams and bulls between birth and adulthood the Sertoli cell population increases by a factor of 5–10

before the onset of spermatogenesis, while in rodents the two phenomena are concomitant. In these adult animals no numerical variations with age, after puberty or with season are observed. This is different from the conclusion of Johnson & Thompson (1983) for the stallion. However, these authors observe modifications in total volume of Sertoli nuclei according to age or season without variation in individual Sertoli nuclear volume and draw conclusions on variations in number. This is not confirmed by Jones & Berndtson (1986) who report a decline with age of the Sertoli cell population. Results from sheep and cattle testes support variations in nuclear volume of Sertoli cells with age, hormonal status, season and genetics without variations in numbers and correspond to the observation of Lino (1971).

In rats, Sertoli cell multiplications are under FSH control (Orth, 1984), but FSH alone appears to be unable to promote Sertoli cell multiplications in hypophysectomized lambs or *in vitro*. A synergistic effect of LH and FSH *in vivo* is necessary to ensure normal multiplications (Courot, 1971) and, as far as morphology of the cell is concerned, LH could be necessary to maintain normal Sertoli cells, possibly allowing further response to FSH. No such study has been done for the calf.

Before puberty, environmental factors, via variation in hormonal secretion, hormone binding to target cells and specific secretions induced by hormones, affect Sertoli cell multiplications and differentiation. Variation in precocity could be partly explained by variations in Sertoli function, possibly by those of FSH binding and further stimulation, as induced in Sertoli cells cultured *in vitro*. Hormonal control of Sertoli secretion in prepubertal or adult bull and rams is poorly understood as the only proteinaceous productions to be analysed are ABP and plasminogen activator. ABP is mainly testosterone dependent in the lamb (Carreau *et al.*, 1980) and fluctuates with season (Jegou *et al.*, 1979). Plasminogen activator secretion appears to be FSH dependent (Jenkins & Ellison, 1986). Inhibin variations had been suspected in normal and cryptorchid rams (Blanc *et al.*, 1978, 1981), but the relationship between anti-Müllerian hormone (Josso *et al.*, 1980) and inhibin production, their hormonal control and their respective role on germ cell multiplications or differentiation have to be investigated. Moreover, the respective roles of the EGF domain of tissue-type plasminogen activator (Patthy, 1985) and of the transforming growth factor β domain of inhibin (Mason *et al.*, 1985) and of anti-Müllerian hormone (Cate *et al.*, 1986) are probably of prime importance for the regulation of spermatogonial multiplications as for other cell types (Roberts *et al.*, 1985).

Furthermore, the relation between quality of spermatozoa and Sertoli cell secretion has been poorly investigated in cattle and sheep. The role of factors such as transferrin (Foresta *et al.*, 1986) or clusterin (Blaschuk *et al.*, 1983) has to be investigated.

In conclusion, the numerical variation of Sertoli cells and their relation with sperm production are now relatively well understood, but their secretions and hormonal control are still poorly understood in sheep and cattle, despite the economic importance of these species.

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