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 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 seasonally 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 cell 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 germinai 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 its physiological analysis has been done. Tissue-type plasminogen activator 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 A₁ 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 [³H]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 I).

The origin of the cycling stem cells and the significance of A₀ 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 A₀ and A₁ (ovoid pale with a central dark nucleolus) spermatogonia differs markedly: for example, this ratio, A₀/(A₀ + A₁), 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 A₁ spermatogonia disappearing after hypophysectomy and being at least partly related to FSH secretion (Courot et al., 1979). A₀ spermatogonia could represent the first step of the cell cycle of A₁ spermatogonia (G₀ or beginning of G₁; Hochereau-de Reviers, 1981). The A₁ spermatogonia which are isolated or single (A₂) could represent the basic stem cell, the A paired (A₃) and aligned (A₄) spermatogonia being the multiplying cells which ensure the formation of new A₁ spermatogonia (Lok et al., 1982). In the ram the total numbers of A₂, A₃ and intermediate spermatogonia are observed (Hochereau-de Reviers et al., 1976b). At two
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Table 1. Comparison of duration of seminiferous epithelial cycle in sheep and cattle

| Species | Reference | Method | Duration (days) |
|---------|-----------|--------|----------------|
| Sheep   | Ortavant (1958) | $^{32}$P | 10.4 |
|         | Hochereau et al. (1964) | $[^3H]$thymidine | 10.4 |
| Cattle  | Orgehin (1961) | $^{32}$P | 13.4 |
|         | Hochereau et al. (1964) | $[^3H]$thymidine | 13.5 |
| (Bos indicus × Bos taurus) | Salim & Entwistle (1982) | $[^3H]$thymidine | 13.4 |
| (Bos indicus) | Cardoso & Godinho (1983) | $[^3H]$thymidine | 14.0 |
| (Bubalus bubalis) | Sharma & Gupta (1980) | $[^3H]$thymidine | 8.6 |
|          | Bilaspuri & Guraya (1980) | $[^3H]$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 ($r > +0.65$) in rams and bulls. This is not observed for the $A_0$ spermatogonia population (de Reviers & Courrot, 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 $1 \times 10^6$ per testis (Courrot, 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                                                   | 4.3 ± 0.4                                           | —                                                                   |
| 60-70 days of age   | 17.0 ± 6.5                                                      | —                                                      | 20.6 ± 1.4                                         | 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              |                                                                 |                                                        |                                                     |                                                                     |
| Birth               | —                                                               | —                                                      | —                                                   | —                                                                   |
| 120 days            | —                                                               | —                                                      | —                                                   | 9.3 ± 0.4                                                          |
| 240 days            | —                                                               | —                                                      | —                                                   | 4.6 ± 7.1                                                          |
| 18 months           | —                                                               | —                                                      | —                                                   | 4.4 ± 4.4                                                          |
| 3 years             | —                                                               | —                                                      | —                                                   | 54.6 ± 3.6                                                         |
| 6 years             | 36.6 ± 2.7                                                      | —                                                      | —                                                   | —                                                                   |

Values are mean ± s.e.m. x 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. x 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 x 10⁸ respectively) than those classified as ‘bad’ (22 ± 1 x 10⁸; 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 x 10⁸ 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

| 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                    | 22.7 + 1.8                          | 2.7 + 0.2                   |
| Lafortune (1983)  | Française Frisonne            | 23.0 + 2.4                          | 2.7 + 0.2                   |
|                  | Pie Noire                     | 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 bred, 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 multiplicactions 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 |                         |                         |
|-------------------------|--------------|-------------------------|-------------------------|
|                         | Total no.    | Nuclear area           | Seminiferous tubule mean diam. (µm) |
|                         | (x 10^8)     | (µm²)                  |                         |
| 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             | 65.5 ± 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     | 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 x 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 [3H]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 x Finn rams selected for their high (H) and low (L) rate of testicular growth (J. R. McNeilly, unpublished data)

| Age      | Testis weight (g) | Total no. of Sertoli cells/tetis (× 10^8)* | Sertoli cell nuclear area (µm²) | Seminiferous tubule diam. (µm) | Daily production of round spermatids (× 10^-9) |
|----------|-------------------|-------------------------------------------|--------------------------------|--------------------------------|---------------------------------------------|
| Birth    | H 0.7 ± 0.07^a     | 4.30 ± 0.4^a                             | 17.4 ± 0.4^a                   | 36.0 ± 0.4^a                   | —                                           |
|          | L 0.79 ± 0.16^a    | 4.4 ± 0.7^a                              | 17.7 ± 0.2^a                   | 34.0 ± 1.3^a                   | —                                           |
| 6 weeks  | H 1.97 ± 0.23^b    | 6.64 ± 0.3^b                             | 22.3 ± 0.8^b                   | 61.7 ± 2.8^b                   | —                                           |
|          | L 1.47 ± 0.18^b    | 5.82 ± 0.8^b                             | 20.4 ± 1.0^b                   | 51.0 ± 2.8^b                   | —                                           |
| 20 weeks | H 106.6 ± 4.00^d   | 19.3 ± 1.0^c                             | 45.1 ± 1.0^d                   | 203.3 ± 4.7^e                  | 1.57 ± 0.08^b                               |
|          | L 74.6 ± 11.10^e   | 21.4 ± 0.5^e                             | 39.2 ± 0.6^e                   | 172.4 ± 11.7^e                 | 0.97 ± 0.24^e                               |
| Adult    | H 218.7 ± 8.10^f   | 20.3 ± 1.1^e                             | 64.15 ± 1.6^e                  | 242.0 ± 7.0^f                  | 2.62 ± 0.16^e                               |
|          | L 222.8 ± 15.80^e  | 23.7 ± 2.8^e                             | 63.93 ± 1.6^e                  | 237.0 ± 4.0^e                  | 2.75 ± 0.28^e                               |

Values are mean ± s.e.m.

*Corrected number.

Within columns, values with different letters indicate significant differences (P < 0.05).

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

### Spermatogenesis and Sertoli cells in rams and bulls

Spermatogenesis and Sertoli cells in rams and bulls 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 µm²) 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.

| Parameter                                    | Cryptorchid | Cryptorchid + orchidopexy at 2 months of age |
|----------------------------------------------|-------------|----------------------------------------------|
| Testis weight (g)                            | 14          | 84                                           |
| % of empty tubules*                          | 100         | 16.5                                         |
| Sertoli cell total number/testis (× 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 (× 10^-6) | 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 [3H]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 [3H]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 x 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 (x 10^-9) | 1.89 ± 0.35    | 3.34 ± 0.48*          |
| DSP/Sertoli cell (x 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 ± 26a           | 219 ± 28b       | 275 ± 20a        | 369 ± 29c       |
| Total no. of Sertoli cells/testis (×10⁹) | 31.4 ± 2.9a        | 36.8 ± 3.2b     | 30.3 ± 4.5a      | 35.5 ± 2.4a     |
| FSH binding (pmol/testis) | 20.7 ± 3.7        | 25.9 ± 3.4      | 161 ± 12a        | 212 ± 18b       |
| Cytoplasmic androgen binding (pmol/testis) | 117 ± 25a       | 37.1 ± 30b      | 125 ± 12a        | 82 ± 18c        |
| ABP concentration in rete-testis fluid (10⁷ NO) | —                 | —               | 10.7 ± 1.4a      | 20.0 ± 6.4a     |
| Daily production of spermatids (×10⁻⁹) | 1.95 ± 0.14a       | 2.44 ± 0.26a    | 3.33 ± 0.55b     | 5.02 ± 0.38c    |

Values are mean ± s.e.m.
Within rows, values with different letters are significantly different. P < 0.05.

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 spermatiation, entrance of meiotic cells into the adluminal compartment and the division of type A₁ 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 A₀ stem cells and of the different ratio of A₀/(A₀ + A₁) 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 A₀ and A₁, spermatogonia vary inversely in the ovine testis and the A₀ population is negatively correlated to FSH plasma concentrations. Furthermore, A₁ 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 (Forest 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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