Effect of season of birth and of hemicastration on the histology of the testis of 6-month-old lambs

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Summary. Season but not hemicastration affected the cellular composition of the testis. Despite similar weight, the testicular histology differed markedly with season of birth. The number of Leydig cells and of Sertoli cells was greater in summer- than in winter-born lambs by factors of 2 and 1.5 respectively. Similarly the number of spermatogonia and their rate of production increased substantially in summer-born lambs. The rate of spermatid production was affected by both hemicastration and season. Season of birth exerted more modifications to testicular histology than did hemicastration.

Introduction

Season of birth affects testicular growth in ram lambs (Courot, de Reviers & Pelletier, 1975), the testes of lambs born in the autumn growing more rapidly than those of lambs born in the spring. This effect could be associated with variation in the sensitivity to the negative feedback effects of gonadal hormones on gonadotrophin release as demonstrated for females (Legan, Karsch & Foster, 1977). However, the assessment of the effects of variation in negative feedback by measurement of the response to hemicastration in two different seasons indicated that it was not a contributory factor for at least those seasons (Land, Drury & Fordyce, 1979). Alternatively, the relative proportion of particular cell types could be affected by the season of birth and growth. The aim of the present work was to study the populations of cells in the testis remaining after hemicastration and in the normal testis of lambs born in winter or summer.

Materials and Methods

Finn-Dorset lambs, 17 born in winter 1977 (31 December 1976 to 20 January 1977) and 21 born in summer 1977 (2 July to 12 August 1977) were studied. In both seasons, the lambs were born and reared indoors, and were weaned at 8 weeks. They were offered a creep feed (15% crude protein, 12.6 MJ/kg dry matter) *ad libitum* from 6 weeks, and hay *ad libitum* from 8 weeks, up to 19 weeks of age. Eight of the winter-born and 10 of the summer-born lambs served as controls; the remainder, chosen at random, were hemicastrated at 12 weeks $\pm$ 3 days of age. The animals were killed at 6 months of age and in each group one testis of each lamb was fixed in Bouin's solution.

Histological analysis of intertubular and tubular tissue was performed as previously described (Hochereau-de Reviers, Loir & Pelletier, 1976a; Hochereau-de Reviers *et al.*, 1979). The relative

volumes of intertubular tissue and seminiferous tubules were determined with a 25-point ocular integrator (Hennig, 1957) on 20 fields for each testis. The relative proportion of Leydig cells in the intertubular tissue was determined by the same method on 20 fields of intertubular tissue for each testis. The total volumes of intertubular tissue, Leydig cells and tubular tissue were then calculated from the testis volume and the relative volume of each element respectively. The diameter of the seminiferous tubules was measured with an ocular micrometer on 20 cross-sections of tubules per testis.

The cross-sectional areas of the cytoplasm and nuclei of Leydig cells and that of the nuclei of Sertoli cells were estimated with a microscopic planimeter (ASM Leitz) on 20 cells per animal. The total number of Leydig cells per testis was calculated from the estimation of Leydig cell volume.

The Sertoli cell and type A₀ and A₁ spermatogonia (Hochereau-de Reviers, Ortavant & Courot, 1976b) of each animal were counted in 10 cross-sections (10 µm thick) at stage 8 of the classification of Ortavant (1959). The true numbers of the spermatogenic cells per cross-section were calculated by the formulae of Abercrombie (1946) as modified by Ortavant (1959). The total length of the seminiferous tubules per testis was calculated from the testis weight, the relative volume of seminiferous tubules and the cross-sectional area of seminiferous tubules (Attal & Courot, 1963). The total area of the walls of the seminiferous tubules was calculated from the total length of seminiferous tubules and the mean cross-sectional tubular diameter. The total numbers of Sertoli cells and A₀ and A₁ spermatogonia per testis were determined as described by Attal & Courot (1963).

The daily productions of A₁ spermatogonia, leptotene primary spermatocytes and round spermatids were calculated by the method of Amann (1970). The yields of spermatogonial multiplication and of meiotic prophase were calculated from the ratio of the true number of leptotene primary spermatocytes to A₁ spermatogonia and that of round spermatids to leptotene primary spermatocytes respectively.

Least squares techniques were used to estimate the effects of season and hemicastration and of the interaction between them. The correlations amongst variables were calculated within hemicastration–season groups as well as independently of treatment.

Results

Testis weight. Testicular weight did not differ significantly between winter- and summer-born animals but was significantly higher in hemicastrated than in intact lambs (Table 1, $P < 0.001$). At 6 months of age, the weight of the testes was greater in summer- than in winter-born hemicastrated lambs, but the difference was not statistically significant.

Intertubular tissue. There was no significant effect of season of birth or of hemicastration on development of intertubular tissue (Table 1). The total volume of Leydig cells per testis was significantly higher in summer-born animals ($P < 0.001$) but hemicastration had no further effect.

The mean cross-sectional area of Leydig cells was also affected by season; it was significantly less in summer-born animals ($P < 0.05$). Variations in the mean cross-sectional area of Leydig cell nuclei paralleled those of the whole cell. The total number of Leydig cells per testis was significantly higher in summer-born than in winter-born animals (Table 1).

Seminiferous tubules and Sertoli cells. The mean seminiferous tubule diameter (Table 1) was significantly increased by hemicastration ($P < 0.05$) and was higher than in summer-born animals ($P < 0.001$).

The total length of seminiferous tubules was greater in summer- than in winter-born animals ($P < 0.001$). The surface area of tubular wall was increased by hemicastration ($P < 0.05$) and was greater in summer- than in winter-born animals ($P < 0.01$).

The total number of Sertoli cells per testis was greater in summer-than in winter-born animals ($P < 0.001$).

Table 1. Testicular weight and characteristics of 6-month-old winter- and summer-born hemicastrated and control lambs

Trait	Overall mean	Season of birth*			Treatment*		
		Summer	Winter	<i>P</i>	Hemi-castration	Control	<i>P</i>
Weight (g)	168	—	—	>0.05	186	147	<0.001
Intertubular volume (cm ³)	32.6	—	—	>0.05	—	—	>0.05
Total volume of Leydig cells (cm ³)	6.4	8.41	4.18	<0.001	—	—	>0.05
Cross-sectional area of Leydig cells (µm ²)	80.3	76.2	84.6	<0.05	—	—	>0.05
Cross-sectional area of nuclei of Leydig cells (µm ²)	26.0	25.0	27.2	<0.05	—	—	>0.05
Total no. Leydig cells/testis (× 10 ⁶)	1210	1663	731	<0.001	—	—	>0.05
Diameter of seminiferous tubule (µm)	235	218	253	<0.001	242	227	<0.05
Length of seminiferous tubules/testis (m)	2124	2496	1731	<0.001	—	—	>0.05
Area of tubule wall/testis (cm ²)	158	177	137	<0.01	171	142	<0.05
Sertoli cells/testis (× 10 ⁹)	4.02	4.81	3.19	<0.001	—	—	>0.05
Cross-sectional area of Sertoli cell nuclei (µm ²)	60.0	—	—	>0.05	—	—	>0.05
No. of A ₀ spermatogonia/testis × 10 ⁶	80.3	98	62	<0.07	—	—	>0.05
Daily production of A ₁ spermatogonia/testis × 10 ⁶	2.16	2.63	1.67	<0.01	—	—	>0.05
Daily production of leptotene 1° spermatocytes/testis × 10 ⁶	78.0	89.7	65.5	<0.01	86.1	68.2	<0.05
Daily production of round spermatids/testis × 10 ⁶	212	253	169	<0.001	235	186	<0.05
Yield of 1° spermatocytes/A ₁ spermatogonium (%)	41.3	—	—	>0.05	—	—	>0.05
Yield of meiotic prophase (spermatids/leptotene, %)	2.76	—	—	>0.05	—	—	>0.05

* Subclass means are not given for effects which are not statistically significant.

The area of the cross-section of Sertoli cell nuclei was not affected by season of birth or by hemicastration.

Spermatogenesis. The total number of A₀ reserve cells per testis was very variable so that even the 50% greater number present in summer- than in winter-born lambs was not statistically significant (Table 1). The daily production of A₁ spermatogonia was greater in summer- than winter-born animals ($P < 0.01$). The daily production of leptotene primary spermatocytes was increased by hemicastration ($P < 0.05$) and greater in summer-born animals ($P < 0.01$).

The daily production of round spermatids per testis was significantly affected by season ($P < 0.001$) and by hemicastration ($P < 0.05$).

The yield of spermatogonial multiplication varied considerably with season and hemicastration but, as with the number of reserve stem cells, the within-group variation was high and the effects not statistically significant. The size of the effects was such that the summer-born control lambs had only half the yield (27%) of the other three groups (45–47%). The yield of meiotic prophase did not vary significantly. With similar yields from meiosis, the low efficiency of spermatogonial multiplication was reflected in lower overall yields of round spermatids per A₁ spermatogonium in the summer-born control lambs than in the other three groups, but again this effect was not statistically significant.

Interrelationships. The within-group correlations showed relationships which were independent of the effects of treatment (Table 2).

Variations in testicular weight were equally associated with variation in the intertubular

Table 2. The correlations among testis weight, the characteristics of the interstitial tissue, seminiferous tubules and spermatogenesis of individuals within treatments

Interstitial tissue															Seminiferous tubules					Spermatogenesis				
 				
Interstitial tissue															Seminiferous tubules					Spermat				

$r > 0.48, P < 0.01$; $r > 0.37, P < 0.05$. With no significant correlations the area of cross-section of Leydig cells and the number of spermatogonia per testis were omitted as rows and the number of Sertoli cells per testis and the daily production of spermatids as columns.

volume and the length of the seminiferous tubules. The correlations between testicular weight and the production of spermatogonia, spermatocytes and spermatids are as high as those between the length or surface area of the tubular wall of the seminiferous tubule and these three components of spermatogenesis, indicating that testicular weight is an equally good predictor.

The only significant correlation between a component of the interstitial tissue and the characteristics of spermatogenesis was that of 0.42 between the total volume of Leydig cells and the daily production of spermatocytes.

The area of the nucleus of the Sertoli cells, but not the number of Sertoli cells, was related to the daily rate of production of spermatocytes and spermatids. Neither the size of the nucleus nor the number of Sertoli cells was related to either the size or number of Leydig cells.

Discussion

The weight of the testes of the lambs in this study was not affected by the season of birth, as reported earlier for testis diameter (Land *et al.*, 1979). With a constant age of sampling, however, season of birth is fully confounded with season of sampling so that the effects of birth on testis size reported by Skinner & Rowson (1968) for Suffolk $\times$ Welsh Mountain lambs could have been counterbalanced by effects of season of measurement.

Despite similar gross size, the testicular histology differed markedly in the lambs born in the two seasons. The number of Leydig cells per testis was approximately twice as great in the summer-born lambs, so that, even with a small cell volume difference in the opposite direction, the total volume of Leydig cells was approximately twice as great in the summer-born lambs. Equally, as reported by de Reviers *et al.* (1980), more Sertoli cells were observed in the testes of summer-born lambs although the difference was 1.5- rather than 2-fold. Of these differences, the greater number of Sertoli cells in summer-born lambs has been reported earlier by de Reviers *et al.* (1980).

Sertoli cell division ceases before puberty in the ram (Courot, 1971), at around 40 days of age in Romanov $\times$ Ile-de-France crossbred lambs (M. T. Hochereau-de Reviers, C. Monet-Kuntz, C. Perreau & M. Courot, unpublished data). Also, the total number of Sertoli cells per testis is similar at birth in lambs even in different seasons (M. T. Hochereau de Reviers & C. Perreau, unpublished observations). The present differences in Sertoli cell populations may be deduced to have arisen from an effect of season of rearing on the rate of Sertoli cell division during the first 2 months of the life of the ram.

In contrast to the Sertoli cells, it is known that the rate of division of Leydig cells is low until 2 months of age when it then increases dramatically while, as with the Sertoli cells, the number present at birth is not affected by season (M. T. Hochereau-de-Reviers & C. Perreau, unpublished observations). It may therefore be argued that the present difference in numbers arose during the phase of rapid testicular growth after 2 months of age. Again, the surface area of the basement membrane was greater in the summer-born lambs but this could have been an effect of the season of sampling.

The stock of reserve stem cells (A_0 spermatogonia) was not affected by the season of birth, confirming the conclusion of Hochereau-de Reviers (1981), from study of rams born in different seasons but sampled in the breeding season, that the number of A_0 stem cells is independent of time of birth. However, the rate of differentiation of these cells is affected by season; the greater rate of production of A_1 spermatogonia could be related to the greater number of Sertoli cells as postulated by de Reviers *et al.* (1980) for Ile-de-France rams. The possibility of such an association is supported by a correlation of 0.35 between the two characteristics within the present groups, even though this was not statistically significant ($P = 0.05$ for $r = 0.37$).

By contrast to these effects of season, none of the early stages of spermatogenesis was affected by hemicastration. Both hemicastration and season, however, affected the later stages of spermatogenesis. This differentiation of the effects of season and hemicastration could arise from

either of or a combination of two sources; the early stages of spermatogenesis could be determined before 12 weeks of age and hence be effects of birth, or the effects of season on the hormonal equilibria controlling spermatogenesis must differ from the perturbation due to hemicastration. The experiments presented above indicate that the number of stem cells at birth is a characteristic of the animal, independent of season and that the multiplication of these stem cells to A₁ spermatogonia is affected by the season through an effect on the number of Sertoli cells. Once the number of Sertoli cells is determined, any further modification of the rate of spermatogenesis can be influenced acutely by modification of the equilibria between pituitary trophic hormones and feedback hormones from the testis.

Courot (1970) showed in hypophysectomized lambs that mitosis of the precursors of Sertoli cells were under LH + FSH control, FSH alone being unable to maintain this cell population. Season is known to affect the frequency of LH pulses of young lambs (Lafortune *et al.*, 1982), and this led de Reviers *et al.* (1980) to argue that LH pulse frequency of the young lamb partly controls the number of Sertoli cells of the adult. In the present experiment, all the effects of season on the rate of production of spermatids (50%), as observed previously by Ortavant (1959), Hochereau-de Reviers *et al.* (1976b) and Schanbacher & Ford (1979), could have arisen from the difference in the daily rate of production of A₁ spermatogonia (57%), whereas the effect of hemicastration on the daily production of spermatids (26%) arose entirely from effects on the differentiation of similar numbers of A₁ spermatogonia.

Hemicastration showed that spermatogenesis can be increased in individual testes of summer-born lambs, thus indicating that the limit to spermatogenesis in the intact lamb is under systemic control. Changes in the concentration of FSH have been implicated in the increased rate of growth of the testis after hemicastration (Walton, Evins, Hillard & Waites, 1980; de Reviers *et al.*, 1980; Land, Baird & Carr, 1981) and passive immunization against oestrogens or oestrogen supplementation (Jenkins & Waites, 1983). Although the increase in the concentration of FSH was about 1.5 times in both studies, the variation amongst animals within groups was such that the effect was not statistically significant.

The season in which the lamb is reared has a marked effect on the establishment of interstitial cells and those of spermatogenesis. The histological components of the increased growth of the testis were therefore the same at both times of the year. This indicates that the systemic feedback equilibria controlling spermatogenesis were not affected by season.

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