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Seasonal variation in the histology of the testis of the red deer, *Cervus elaphus*

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Summary. A histological study of the testes of stags shot in autumn (sexual season) and spring (quiescent period) indicated that the 3-fold increase in testicular size observed in the autumn was accompanied by increases in nearly all features studied (volumes of intertubular tissue, Leydig cells, blood vessels and peritubular cells; diameter and length of seminiferous tubules; the number of A_1 spermatogonia and products of spermatogonial divisions, meiosis and spermiogenesis). There were, however, fewer A_0 spermatogonia in the testes in autumn.

Introduction

Seasonal changes in the testis of the red deer, *Cervus elaphus*, have been reported previously (Frankenberger, 1953; Bruggerman, Adam & Karg, 1965; Lincoln, 1971a, b) but no precise analysis of the variations is available. The object of the present work was to describe in detail the changes which occur in the cellular components of the testes during the year, and to correlate these with the hormonal status of the animals.

Materials and Methods

Adult red deer stags were shot in the spring (February, March and April) during the non-sexual season (N = 5) or at the end of summer and beginning of autumn (August, September and October) during the sexual season (N = 6). Dissection of the animals and recovery of tissues was as described previously (Lincoln, 1971b). Frozen samples of seminal vesicles were assayed for fructose by a colorimetric method (Lindner & Mann, 1960). The samples of testicular tissue were assayed for testosterone by a specific gas chromatographic technique (Mann, Rowson, Short & Skinner, 1967), and the blood serum levels of LH were determined by radioimmunoassay (Scaramuzzi, Caldwell & Moor, 1970). The LH assay used ovine NIH-LH-S14 as reference standard, and the sensitivity was 0·5 ng/ml.

Fragments of testicular tissue were fixed in Bouin's solution, and embedded in paraffin wax and 10 μm sections were cut for histological study. The cycle of the seminiferous epithelium was classified in the eight stages described by Roosen-Runge & Giesel (1950) for the rat.

The type A spermatogonia and the Sertoli cells were counted in 10 cross-sections of seminiferous tubules at stages 7, 8 and 1, and leptotene primary spermatocytes, round spermatids and elongated spermatids were counted in 10 cross-sections at stages 1, 8 and 7 respectively. The total counts for each cell type were corrected for differences in nuclear volume by the formula of Abercrombie (1946) as modified by Ortavant (1958), although no correction was made for the Sertoli cells and elongating spermatids which were irregular in form. The nuclear and nucleolar diameters of A_0 and A_1 spermatogonia (Clermont, 1967) were measured with an ocular micrometer on 10 nuclei per cell type. The diameter of the tubules was measured on 20 cross-sections per testis. The relative volume of the intertubular tissue and seminiferous tubules was determined with a 25-point ocular integrator

(Hennig, 1957) on 20 microscope fields for each testis. The relative proportion of Leydig cells, blood vessels, peritubular cells and fibrolasts in the peritubular tissue was determined with the same method. The diameter of the Leydig cell cytoplasm was measured in 10 randomly selected cells per animal and the mean cell volume was determined.

The total length of the seminiferous tubules/testis was calculated from the above data by the formula of Attal & Courot (1963). The total number of type A_0 and A_1 spermatogonia per testis was calculated from the total length of the seminiferous tubules and the mean corrected number of cells per 10 μm tubular cross-section. The yield of spermatogonial divisions was determined from the ratio of primary spermatocytes at leptotene to A_1 spermatogonia, the yield of meiosis was determined from the ratio of round spermatids (stage 8) to primary spermatocytes at leptotene, and the yield of spermiogenesis from the ratio of elongated spermatids (stage 7) to round spermatids (stage 8). Statistical comparisons were made throughout by analysis of variance (Snedecor & Cochran, 1957).

Results

As shown in Table 1 all the reproductive characteristics that were measured were greater in the autumn-killed animals than in the spring-killed animals except for the testicular testosterone content which varied greatly between animals.

Table 1. Comparisons of various characteristics (mean ± s.e.m.) of red deer stags shot in spring and autumn

	Spring (N = 5)	Autumn (N = 6)	Significance
Age (years)	6·4 ± 0·4	7·5 ± 0·9	N.S.
Body weight (kg)	91·5 ± 1·9	104·6 ± 5·8	N.S.
Pituitary weight (g)	1·09 ± 0·04	1·41 ± 0·04	$P < 0·01$
Plasma LH (ng/ml)	4·5 ± 0·6	7·3 ± 0·5	$P < 0·05$
Testis (left only)			
Weight (g)	24·4 ± 2·7	70·7 ± 7·5	$P < 0·01$
Intertubular tissue vol. (cm^3)	5·4 ± 0·5	13·5 ± 1·6	$P < 0·01$
Seminiferous tubule length (m)	1273 ± 72	2487 ± 325	$P < 0·01$
Seminiferous tubule diam. (μm)	137 ± 5·3	180 ± 8·5	$P < 0·01$
Testosterone content (μg)	3·4 ± 2·8	265·6 ± 136·9	N.S.
Seminal vesicles			
Total weight (g)	19·9 ± 1·0	54·5 ± 11·7	$P < 0·05$
Fructose content (mg)	3·9 ± 0·5	168·8 ± 57·7	$P < 0·05$

The relative frequency of the seminiferous epithelium stages was similar for the animals shot in both seasons (Table 2). The total number of Sertoli cells/testis and the size of the Sertoli cell nucleus did not differ significantly in the two groups of animals (Tables 3 and 4). Two types of stem spermatogonia were recognized; Type A_0 spermatogonia had a round and pale nucleus with granular chromatin and 1 or 2 nucleoli (mean ± s.e.m. nuclear and nucleolar diameter = 7·5 ± 0·02 and 1·6 ± 0·01 μm respectively) and an eccentricity coefficient of the nucleus of 0·89 ± 0·02. Type A_1 spermatogonia had a pale and ovoid nucleus, fine granular chromatin and one central spherical nucleolus (nuclear and nucleolus diameter = 8·6 ± 0·04 and 2·5 ± 0·01 μm respectively, and an eccentricity coefficient of the nucleus of 0·73 ± 0·04). The nuclear and nucleolar size were larger and the eccentricity coefficient lower in A_1 compared to A_0 spermatogonia. The total number of A_0 spermatogonia/testis was significantly less in the testes from autumn-killed animals. The seasonal difference in the total number of A_1 spermatogonia/testis was less conspicuous, although A_1 stem cells tended to be more numerous in the autumn (Table 3).

Table 2. Relative frequencies of the stages of the seminiferous epithelium in red deer stags culled in spring (N = 5) and autumn (N = 6)

	Stage							
Season	1	2	3	4	5	6	7	8
Spring	22·7 ± 2·5	8·7 ± 1·4	23·7 ± 1·3	9·1 ± 1·4	4·5 ± 1·3	20·7 ± 1·2*		10·9 ± 1·1
Autumn	26·3 ± 1·9	10·0 ± 1·4	20·8 ± 1·6	9·9 ± 1·0	2·8 ± 0·8	10·9 ± 1·4	8·7 ± 0·7	10·3 ± 1·2

* During the non-sexual season stages 6 and 7 cannot be clearly defined and are counted together.

Table 3. Seasonal variations of total numbers of Sertoli cells and stem spermatogonia per testis and of yields of spermatogenesis in the red deer stag

	Season		
	Spring (N = 5)	Autumn (N = 6)	Significance
Sertoli cells/testis ($\times 10^6$)	340·2 ± 34·6	463·5 ± 41·4	N.S.
A_0 spermatogonia/testis ($\times 10^6$)	57·6 ± 4·8	31·8 ± 4·5	$P < 0.01$
A_1 spermatogonia/testis ($\times 10^6$)	37·0 ± 5·7	48·7 ± 6·6	N.S.
Leptotene I spermatocytes: A_1 spermatogonia	4·6 ± 1·9	14·2 ± 2·3	$P = 0.01$
Round spermatids (Stage 8): leptotene I spermatocytes	2·8 ± 0·3	3·3 ± 0·5	N.S.
Elongated spermatids (Stage 7): round spermatids (Stage 8)	0·43 ± 0·17	0·93 ± 0·15	$P = 0.05$

Table 4. Seasonal variation in the size of various structural components of the testis in the red deer stag

	Season		
	Spring (N = 5)	Autumn (N = 6)	Significance
Sertoli cells			
Nuclear area (μm^2)	54·3 ± 1·81	53·4 ± 2·11	N.S.
Leydig cells			
Nuclear area (μm^2)	20·7 ± 1·69	28·8 ± 1·01	$P < 0.01$
Cytoplasmic volume (μm^3)	7055 ± 403	13 544 ± 403	$P < 0.01$
Total volume of tissue (cm^3)	2·13 ± 0·15	6·06 ± 0·94	$P < 0.01$
Fibroblastic cells			
Total volume of tissue (cm^3)	1·66 ± 0·26	3·91 ± 0·55	$P = 0.01$
Blood vessels			
Total volume of tissue (cm^3)	0·45 ± 0·13	1·26 ± 0·22	$P < 0.05$
Peritubular cells			
Total volume of tissue (cm^3)	1·13 ± 0·11	2·24 ± 0·24	$P < 0.01$

The yield of spermatogonial divisions, i.e. the number of leptotene primary spermatocytes produced by an A_1 spermatogonia, was greater in the autumn than the spring (Table 3). Similarly, the yield of meiosis and early spermiogenesis, i.e. the number of round spermatids at stage 8 produced by a leptotene primary spermatocyte, was also slightly greater in the autumn although the difference was not significant (Table 3). The yield of spermiogenesis doubled between spring and autumn (Table 3).

The total volumes of the different components of the intertubular tissue were all significantly greater in the autumn-killed animals (Table 4) and there was an increase in the nuclear and cytoplasmic volume of the interstitial cells (Table 4).

Discussion

The histology of the testis of the stag is similar to that of the domestic ungulates. The two types of stem spermatogonia are nearly identical to those observed in the adult bull (Hochereau-de Reviers, 1970) and ram (Hochereau-de Reviers, Ortavant & Courot, 1976). The relative frequencies of the stages of the seminiferous epithelium cycle are also similar, the divisions of the B-type spermatogonia into primary spermatocytes occurring at stage 1 as in ram and bull testis. However, the seasonal changes in the various components of the stag testis are more conspicuous than are those in the other species.

Both the seminiferous tubules and the intertubular tissue change markedly with the seasonal sexual cycle of the stag. While the Sertoli cell numbers remain constant in the adult animal, the numbers of the various germ cell types involved in spermatogenesis change with season. Outside the sexual season there is a decrease in the yield of the spermatogonial divisions, meiosis and spermiogenesis, as occurs in both the ram (Ortavant, 1958; Hochereau-de Reviers, Loir & Pelletier, 1976) and vole (Grocock & Clarke, 1975). The seasonal changes in the stem spermatogonia are of special interest because they differ from those described for the ram in which the total number of both A_0 and A_1 spermatogonia per testis increase in the sexual season (Hochereau-de Reviers *et al.*, 1976).

The intertubular tissue of the testis is greatly expanded in the sexually active stags killed in the autumn. This is largely the result of changes in the Leydig cells which expand in both nuclear and cytoplamic mass; similar changes in the Leydig cells occur in sexually active hamsters (Vendrely Guerillot & Da Lage, 1972). This Leydig cell hypertrophy in the stag coincides with the time when testicular testosterone concentrations are maximal (Lincoln, 1971b) and the blood levels of testosterone are also high (Lincoln & Kay, 1979). In other seasonal breeders large increases in blood flow through the testis are known to occur during the sexual season (dormouse, ferret and fox: Joffre & Joffre, 1973; Joffre, 1977; ram: Courot & Joffre, 1977).

Seasonal changes in the pituitary content of LH (Bruggermann *et al.*, 1965) and in the blood levels of LH (Lincoln & Kay, 1979) occur in the stag, and it is possible that changes in gonadotrophin secretion dictate the seasonal cycle of testicular activity. It is not clear whether there is a direct effect of gonadotrophins on the efficiency of spermatogenesis or whether this effect is mediated via testosterone. The direct control of spermatogenesis by gonadotrophins is indicated by the observation in the ram that testosterone alone is unable to maintain spermatogonial divisions or fully support meiosis and spermiogenesis (Monet-Kuntz, Terqui, Locatelli, Hochereau-de Reviers & Courot, 1976).

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