Seasonal variations in rete testis fluid secretion and sperm production in different breeds of ram

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Summary. Production of spermatozoa and secretion of rete testis fluid (RTF) in rams was assessed by a rete testis cannulating technique. Four breeds (Ile-de-France, Romanov, Préalpes du Sud and cross-breed Romanov) were studied throughout the year. Inhibitory effects of the cannulation process on spermatogenesis were observed for some animals. Between-breed differences were found in sperm concentration and flow rate of the RTF. The seasonal variations in the daily sperm production of the testis were more pronounced for Ile-de-France rams than for the other breeds. There was a seasonal variation in the flow rate of RTF in Ile-de-France rams, the minimum flow being in February (winter) and the maximum in August–September (autumn).

Introduction

Spermatozoa formed in the seminiferous tubules are carried from the testis to the epididymis by the flow of a fluid actively secreted by the seminiferous epithelium (see review by Setchell, 1978). It is well established that the production of testicular spermatozoa is modified by seasonal factors (Ortavant, 1958, 1977; Lincoln & Short, 1980) but little is known about possible seasonal variations in testicular fluid secretion. By using a rete testis cannulation technique, the present study was undertaken to estimate sperm production and the fluid secretion of the testis in rams with respect to breed and sexual season.

Materials and Methods

Collection of rete testis fluid (RTF)

RTF was collected from rams throughout the year. Four breeds were studied (Ile-de-France, Préalpes du Sud, Romanov and cross-breed Romanov: Romanov × Ile-de-France or Romanov × Préalpes du Sud). The technique of Voglmayr, Waites & Setchell (1966) as modified by Edwards, Dacheux & Waites (1976) was employed. A 'T'-piece polyvinylchloride cannula (Dural plastic, Australia; i.d. 0.5 mm, o.d. 1.00 mm) was inserted into the rete testis of one or both testes of rams anaesthetized with fluothane in oxygen. Tetramycin chlorohydrate was sprayed into the wound before suture of the scrotum. After surgery, conscious animals were retained in a harness. RTF flowing from the cannula was withdrawn by a sterile air-circulating system into a stocking cylinder and kept at 4–7°C. The cylinder was changed every 24 h, when the volume of fluid collected was measured and the concentration of the spermatozoa was estimated using a haemocytometer. Before cannulation the animals were maintained under natural daylength and were fed according to current practice for animals kept indoors.

Fluid analysis. Spermatozoa were separated by centrifugation (20 min at 1200 g at 4°C) from an aliquot of fluid obtained each day and stored at -20°C until required for assays. Ionic analysis (K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Zn^{2+}) was performed with an atomic absorption spectrometer (Safas, Monaco), while chloride was estimated by titration (Radiometer CMT 10) and the protein content by the technique of Lowry, Rosebrough, Farr & Randall (1951).

Statistical analysis. Because of the decrease in the concentration of spermatozoa during the collection period (see 'Results'), the sperm concentration values in the RTF ($10^6/ml$) refer to the mean value during the first 4 days of cannulation, or less if the duration was shorter. As the RTF flowed steadily during the collection period, the mean value during the whole period of cannulation except the last day was taken as the 'flow rate' (ml/h) only the results of cannulations which were effective for more than 4 days were taken into account. The mean 'daily production of spermatozoa' per testis was taken as the product of 'sperm concentration' and 'flow rate' for a 24-h period for animals in which the cannulation was successful for 4 days or more. For the studies of seasonal and breed variations, the results of collections made from January to June were compared to those of collections during July to December, this division corresponding roughly to the non-breeding and the breeding seasons respectively. The differences between seasons and between breeds were assessed by Student's *t* test. For Ile-de-France rams, the maxima and minima of the fluid flow and production of spermatozoa were estimated from the mean curve using all the individual results adjusted by a significant polynomial regression curve tested by an analysis of variance (Snedecor, 1961). The same technique was applied to the calculation of variations in RTF flow rate, sperm concentration and daily sperm production during the collecting period for the Ile-de-France and cross-breed Romanov rams.

Results

Effects of the cannulation process on the characteristics of RTF secretion

Success of the procedure. The success of the cannulation procedure (see Table 1) was breed dependent. In Ile-de-France rams fluid flowed for at least 24 h in about 95% of cannulated animals. The success rate was lower in the other breeds: 48% in Romanov, 74% in Préalpes and 65% in cross-breed Romanov. Difficulty in cannulating Romanov rams increased during the non-breeding season.

Table 1. Mean ($\pm$ s.e.m.) duration of RTF collection, flow rate and total volume of fluid collected per testis according to breed of ram

Breed	No. of testes cannulated†	Duration of collection (days)	Flow rate (ml/h)‡	Total fluid collected per testis (ml)
Ile-de-France	129	$10.4 \pm 0.5^{**}$ (max: 36)	$1.12 \pm 0.03^{***}$ (100)	$248 \pm 17^{***}$
Préalpes du Sud	40	6.6 ± 1.2 (max: 45)	0.83 ± 0.06 (19)	119 ± 20
Romanov	22	6.2 ± 0.9 (max: 15)	0.75 ± 0.06 (12)	106 ± 17
Cross-breed Romanov	51	7.9 ± 0.6 (max: 18)	0.74 ± 0.05 (37)	143 ± 15

† Cannula remaining patent for more than 1 day.

‡ Includes animals with cannula remaining patent for more than 4 days: no. in parentheses. Values significantly different from those of other breeds: $^{**}P < 0.01$; $^{***}P < 0.001$.

Duration of cannulation. The average duration of RTF collection varied amongst the breeds; patency of the catheter was maintained for a longer period in Ile-de-France rams (Table 1).

Flow rate of RTF. For Ile-de-France and cross-breed Romanov rams, the flow rate of RTF during the cannulation period was little affected (Text-fig. 1a). However, the values obtained during the first 2 days, particularly in Ile-de-France rams, were significantly higher than those obtained thereafter ($P < 0.001$).

Text-fig. 1. Variations in (a) flow rate, (b) concentration of spermatozoa in rete testis fluid and (c) daily production of spermatozoa per testis during the period of collection in Ile-de-France (—) and cross-breed $\times$ Romanov rams (---). A regression curve (fifth degree, $P < 0.001$) was calculated using individual daily values. The daily values are the mean $\pm$ s.e.m. for the numbers of animals indicated along the axis of graph b for (1) Ile-de-France and (2) cross-breed Romanov rams.

Sperm concentration in RTF. For all 4 breeds the concentration of spermatozoa increased from the beginning of the collection period until the 4th day ($P < 0.001$) and then decreased slowly. In cross-breed Romanov rams, this decrease occurred later than in the Ile-de-France rams (Text-fig. 1b). This reduction was significant only after 15–16 days of cannulation. In Ile-de-France rams a progressive reduction in sperm concentration occurred after the 5th or 6th day, the value on the 17th day of cannulation being about 50% of the value on Day 4. However,

the extent of this reduction varied between animals. In Ile-de-France rams on Day 13 of collection, about 40% of animals yielded 20% or less of the average amount collected on the first 3 days, but 20% of animals showed no apparent changes. There was no difference between breeds in the pattern of sperm concentration decrease between the breeding and the non-breeding season.

Daily sperm production. In Ile-de-France rams the daily production of spermatozoa was stable during the 4 first days of collection, and then there was a steady decrease (Text-fig. 1c). In cross-breed Romanov rams, the variation in sperm production was not statistically significant, and the daily value was reduced only after 15–16 days of collection.

Breed and seasonal influences on the flow rate of RTF in the ram

Influence of breed on the flow rate. Values of the flow rate of RTF varied with the breed of ram, being greater in the Ile-de-France rams (Table 1, Text-fig. 1a).

Seasonal variations (Table 2). The mean flow rate of RTF collected during the period July–December was not different from that measured during January–June for the Romanov and cross-breed Romanov rams. Conversely, in Ile-de-France and Préalpes rams the flow rate of RTF increased significantly (by about 30 and 45% respectively) during the breeding season ($P < 0.01$) (Table 2). The minimum flow rate (Text-fig. 2) in Ile-de-France rams was observed in February–March (0.85 ml/h) and the maximum in September (1.45 ml/h). The amplitude of the seasonal variation in flow rate was about 70% in Ile-de-France rams.

Table 2. Variations in RTF characteristics (mean $\pm$ s.e.m. for no. of animals in parentheses) according to the breed and breeding season (January–June, non-breeding (NB) season; July–December, breeding season (B)) in rams

Breed	Season	Sperm conc. (10 ⁶ /ml)	Flow rate (ml/h)	Sperm production (10 ⁹ /day/testis)
Ile-de-France	NB	79.59 $\pm$ 5.66† (70)	0.98 $\pm$ 0.04 (53)	1.88 $\pm$ 0.15 (53)
	B	131.69 $\pm$ 4.64*** (59)	1.27 $\pm$ 0.04*** (47)	4.03 $\pm$ 0.19*** (47)
Préalpes du Sud	NB	116.16 $\pm$ 10.17 (30)	0.77 $\pm$ 0.04 (16)	2.37 $\pm$ 0.30 (16)
	B	131.37 $\pm$ 21.30 (10)	1.12 $\pm$ 0.19** (3)	4.62 $\pm$ 0.46*** (3)
Romanov	NB	118.15 $\pm$ 20.18 (10)	0.73 $\pm$ 0.11 (6)	2.33 $\pm$ 0.58 (6)
	B	114.23 $\pm$ 23.27 (12)	0.77 $\pm$ 0.07 (6)	3.27 $\pm$ 0.35*** (6)
Cross-breed Romanov	NB	115.56 $\pm$ 12.32 (11)	0.64 $\pm$ 0.10 (8)	1.76 $\pm$ 0.43 (8)
	B	161.11 $\pm$ 5.74***‡ (40)	0.77 $\pm$ 0.06 (29)	2.95 $\pm$ 0.24***‡ (29)

Values significantly different from those for other breeds in the NB season: † $P < 0.05$.

Values significantly different from those for that breed in the NB season: ** $P < 0.01$, *** $P < 0.001$.

Values significantly different from those for Ile-de-France rams in the B season: ‡ $P < 0.001$.

Breed and seasonal influence on the protein and ionic composition of ram RTF

The concentrations of Na⁺, K⁺, Cl⁻, Zn²⁺ (Table 3) were not significantly different between the breeding and non-breeding seasons for the Ile-de-France ram or between breeds (Ile-de-France and cross-breed Romanov) in the breeding season. During the breeding season, the

concentration of Ca^{2+} increased in Ile-de-France rams, but protein, Ca^{2+} and Mg^{2+} were more concentrated in the RTF of cross-breed Romanov than in Ile-de-France rams.

The concentration of spermatozoa in RTF was positively correlated with protein concentration ($r = 0.38$; $P < 0.001$), flow rate ($r = 0.19$; $P < 0.01$) and Ca^{2+} concentration ($r = 0.20$; $P < 0.01$).

Table 3. Composition of rete testis fluid in Ile-de-France and Romanov cross-breed rams in the non-breeding season (NB: January–June) and breeding season (B: July–December)

Breed	Season	Protein	Na^+	K^+	Ca^{2+}	Zn^{2+}	Mg^{2+}	Cl^-
Ile-de-France	NB	1.21	112.3	10.68	1.32	1.20	5.83	114.7
	(N = 26)	± 0.07	± 4.39	± 0.44	± 0.03	± 0.13	± 0.21	± 3.04
	B	1.22	106.3	11.26	1.44	0.93	6.02	112.5
Cross breed Romanov	(N = 24)	± 0.07	± 3.60	± 0.63	± 0.06	± 0.14	± 0.15	± 4.01
	B	1.67	101.2	10.50	1.57	1.41	6.89	114
	(N = 9)	$\pm 0.22^{**}$	± 4.80	± 5.6	$\pm 0.10^*$	± 0.42	$\pm 0.32^{**}$	± 5.76

The results are given as mean $\pm$ s.e.m. and expressed in mg/ml for protein, and mequiv./l for ionic values. Values significantly different from those of Ile-de-France rams in the NB season: * $P < 0.05$; ** $P < 0.01$.

Text-fig. 2. Seasonal variations in Ile-de-France rams of (a) the flow rate of the RTF (—) and the sperm concentration in RTF (----), and (b) the daily production of spermatozoa per testis. Monthly mean $\pm$ s.e.m. values are given for 129 observations and individual values are also shown in (b). Significant regression curves (fourth degree, $P < 0.001$) were calculated using individual values from each testis (see statistical analysis). Minimum and maximum values are indicated.

Breed and seasonal influence on sperm concentration in RTF and daily sperm production

Variations in sperm concentration in RTF. The concentration of spermatozoa during the breeding and the non-breeding season in cross-breed Romanov was significantly higher than that in Ile-de-France rams for the corresponding period ($P < 0.05$) (Table 2). For Ile-de-France and Romanov cross-breed rams (Table 2), there was a significant increase in sperm concentration between the two seasons (about 65 and 40% respectively; $P < 0.001$). For the other breeds, the increases were only small (13% in Préalpes, 22% in Romanov). For Ile-de-France rams, the minimum value of sperm concentration in RTF ($60 \times 10^6/\text{ml}$) occurred during March–April and the maximum ($150 \times 10^6/\text{ml}$) during October and November (Text-fig. 2a), so the maximal variation in the concentration of spermatozoa in RTF of Ile-de-France rams was about 150%.

Variations in daily sperm production per testis. There were few differences between breeds of ram during the non-breeding season. In all breeds (Table 2) the production values increased ($P < 0.001$) during the breeding season by 114% in Ile-de-France rams, 95% in Préalpes, 40% in Romanov and 68% in cross-breed Romanov. Ile-de-France rams had a greater daily production than did cross-breed Romanov during the breeding season ($P < 0.001$). In Ile-de-France rams (Text-fig. 2b), the daily sperm production increased by 370% from March ($1.02 \times 10^9/\text{day}$) to October ($4.82 \times 10^9/\text{day}$).

Discussion

Cannulation technique

The cannulation of the rete testis as described by Voglmayr *et al.* (1966) and Setchell, Voglmayr & Hinks (1971) is an efficient way of collecting testicular fluid secretions. However, in our hands, the success of the cannulation process depended on the breed of the ram. This was related to the different anatomical conformations of the rete testis and its relationships with the blood vessels which caused difficulties when inserting the PVC cannula, especially during the non-breeding season. Ile-de-France rams were the easiest to cannulate and a longer flow was obtained than in the other breeds used in this study. Any interruption of the flow was generally linked to the occlusion of the cannula by connective tissue proliferation around and into the cannula, by inspection at autopsy, or an accumulation of spermatozoa in the lumen of the cannula, especially when the sperm concentration in the fluid was high (cross-breed Romanov or Romanov).

The flow rate of RTF was very stable during the collection period. This confirms previous publications (Waites, 1977; see Setchell, 1978) but was not in agreement with the rapid decline in flow during cannulation claimed by Baker *et al.* (1977).

The only variation in flow rate from the stable mean levels observed thereafter was in Ile-de-France rams during the first 3 days of cannulation. This was probably a result of the surgical intervention as suggested by Lino & Braden (1972). Since the daily production of spermatozoa remained stable at this time, the increase in flow rate may explain the low sperm concentration observed during this period.

The ionic composition of the fluid was in agreement with other results (see reviews by Waites, 1977; Setchell, 1978) and did not change significantly during the cannulation period. The small variation in Ca^{2+} (correlated with spermatozoa and protein) might be related to the presence of specific proteins and spermatozoa bound with Ca^{2+} (Waites, 1977).

The concentration of spermatozoa seemed to be more affected by the cannulation process than was the flow rate. There was a breed difference in the degree of change of spermatogenesis, which appeared earlier in Ile-de-France (4–5 days) than in cross-breed Romanov (15–16 days) rams. This effect has been already described by Baker *et al.* (1977) and by

Cahoreau, Blanc, Dacheux, Pisselet & Courot (1979) but was not reported previously (see review by Setchell, 1978). Although the origin of this effect was unknown, the relatively early action on the testis may be linked to an alteration in sperm release and/or the late stages of spermatogenesis. A blockage of release of spermatozoa from the epithelium has been observed after antibiotic treatments in rabbits (Swierstra, Whitefield & Foote, 1964) and rats (Timmermans, 1974), and in the monkey immobilized with a harness for a long time (Gondos, Zemjanis & Cockett, 1970). But the fact that only a proportion of the animals and often only one testis was affected may mean that these disturbances are linked to an individual local reaction to the surgical procedure or antibiotic treatment. An oedematous reaction of the scrotum was more often seen in Ile-de-France rams than in other breeds.

Physiological results

Using this cannulation method, it has been possible to demonstrate marked breed differences in RTF flow rate in the ram, which was higher from the Ile-de-France testis (1.12 ml/h) than in other breeds, especially the Romanov (0.75 ml/h). This difference (50%) may be related to the large difference observed in the total number of Sertoli cells per testis: 50% more in the Ile-de-France as compared to the Romanov (de Reviers *et al.*, 1980).

Significant seasonal variations in the flow rate per testis were observed only in Ile-de-France and Préalpes rams. If the values of flow rates for Ile-de-France rams were expressed per gram of testis (182 ± 24 g in spring; 247 ± 12 g in autumn; Hochereau-de-Reviers, Loir & Pelletier, 1976), there was no between-season difference: $5.38 \mu\text{l/h/g}$ testis during the non-breeding season and $5.14 \mu\text{l/h/g}$ testis in the breeding season. However, in Ile-de-France rams the total length, and the mean diameter of the seminiferous tubules increases between spring and autumn by 30–45 and 15% respectively, and the total area of the basal membrane by 70% (M. T. Hochereau-de-Reviers, unpublished data). Although the total number of Sertoli cells per testis did not vary in the adult animal, the cross-sectional area of the nuclei of these cells nonetheless increased by 20% during the breeding season in Ile-de-France rams (Hochereau-de-Reviers *et al.*, 1976). This could reflect a seasonal modification of the activity of these cells and so in the flow rate of RTF. The lack of any significant difference for variations in the flow rate of RTF between non-breeding and breeding seasons for the other breeds of ram investigated in this study, especially the Romanov, may be related to a smaller seasonal difference in the variation of the total length of the seminiferous tubules and the total area of the basal membrane between breeds during the breeding season: 60 and 45% more respectively in Ile-de-France than in Romanov rams.

In spite of the effect of cannulation on spermatogenesis, the caution taken in collecting the data meant that it was possible to use this technique for evaluating the daily sperm production. Lino & Braden (1972) have previously shown that the estimate of testicular daily sperm output obtained by cannulation was similar to the daily production value. Blanc *et al.* (1979) also obtained similar results for the daily sperm production as judged by the sperm output from the rete during the 4 first days of collection and that calculated by quantitative histology.

As shown by Hochereau-de-Reviers *et al.* (1976) and de Reviers *et al.* (1980) the estimation of the daily sperm production by cannulation confirms that the Ile-de-France breed ram was the breed most susceptible to seasonal effects when compared to Préalpes, Romanov or cross-breed Romanov rams. The daily sperm production values (spermatozoa/day/testis) of Ile-de-France rams found in this study ($1.88 \pm 0.1 \times 10^9$ from January to June and $4.03 \pm 0.2 \times 10^9$ from July to December) were very similar to those previously published ($1.9 \pm 0.3 \times 10^9$ in spring and $3.9 \pm 0.3 \times 10^9$ in autumn; Hochereau-de-Reviers *et al.*, 1976). This 2-fold difference in total sperm production between seasons is similar to that reported by Schanbacher & Ford (1979).

The variations in the flow rate and concentration of spermatozoa in the fluid, the two components of the daily sperm production, were displaced from each other by about 1.5 months. The minimum and maximum were in February and August–September respectively for the flow rate, and March–April and November for the sperm concentration. This apparent delay may be compared to the duration of the spermatogenic cycle in the ram (40.5 days; Ortavant, 1958). The hormonal signal from the anterior pituitary, which varies according to the season, may be translated as a modification of the seminiferous membrane and epithelium modifying the flow rate of RTF relatively quickly, but a lag of one spermatogenic cycle is necessary to modify the concentration of spermatozoa leaving the testis.

It is well known that spermatogenesis is under gonadotrophic control (Courrot & Ortavant, 1981), but the flow of the rete testis fluid in the ram does not seem susceptible to direct gonadotrophic regulation or to be linked to spermatogenesis (Setchell & Linzell, 1968; Waites, 1977; Setchell, 1978). However, the possibility that gonadotrophins may act on the flow rate of RTF indirectly by modification of the seminiferous tubule cannot be excluded, and the effect may only be seen when between-season variations are marked.

This work was supported by grants from Délégation Générale à la Recherche Scientifique et Technique (DGRST-76-7-0047), Institut National de la Santé et de la Recherche Médicale (INSERM 25-75-48) and World Health Organisation (W.H.O. 76071). We thank Professor G. M. H. Waites for demonstrating the rete testis cannulation technique to J.L.D. who was the recipient of a British Council Bursary; C. Cornu for the care of rams; A. Lacroix, Ch. Perreau, J. Cl. Poirier, Ghislaine Garreau and Huguette Corbé for technical assistance; the staff of the hospital; and Dr John Williams for help with the manuscript.

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Received 8 May 1980