

THE OUTPUT OF SPERMATOOZOA IN RAMS

I. RELATIONSHIP WITH TESTICULAR OUTPUT OF SPERMATOOZOA AND THE EFFECT OF EJACULATIONS

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[Manuscript received 26 July 1971]

Abstract

The mean daily output of spermatozoa was estimated for each of eight Merino rams from the numbers of spermatozoa eliminated from the urogenital tract both in the urine and in spontaneous discharges over 20 days (DSOU) and by doubling the number of spermatozoa collected daily from one testis by a rete testis catheter (DSOT). The two estimates (DSOU and DSOT) were highly correlated ($r = 0.999$), but the DSOU was usually a little lower than the DSOT. The mean difference was 3.4%.

The effect of 20 ejaculations collected over 2 days was studied in four rams. The DSOU was severely depressed for a period of 9–16 days after the ejaculations but when the number of spermatozoa ejaculated was added to the DSOU in this period the mean daily spermatozoan output was similar to that in the periods immediately preceeding and succeeding it.

It is concluded that the daily spermatozoan production of rams may be estimated accurately from the number of spermatozoa eliminated daily from the urogenital tract. It is unlikely that significant numbers of spermatozoa are resorbed or phagocytosed in the epididymides of normal rams.

I. INTRODUCTION

Attempts to measure the daily spermatozoan production of the testis have been made from:

- (1) quantitative studies of the testicular histology (Kennelly and Foote 1961, 1964; Amann and Almquist 1962*b*; Swiestra 1966, 1968*a*; Amann 1970);
- (2) counts of spermatid nuclei in testicular homogenates (Ortavant 1958; Almquist and Amann 1961; Amann and Almquist 1962*b*; Kirton, Desjardins, and Hafs 1967; Swiestra 1968*b*; Orgebin-Crist 1968; Amann and Lambiase 1969);
- (3) counts of the number of spermatozoa passed through a rete testis catheter (Voglmayr, Waites, and Setchell 1966; Voglmayr *et al.* 1967);
- (4) the number of spermatozoa in many ejaculates (Ortavant 1952, 1958; Almquist and Hale 1956; Vandemark 1956; Boyd and Vandemark 1957; Hale and Almquist 1960; Almquist and Amann 1961; Bielanski and Wierzbowski 1961*a*, 1961*b*; Kirton, Desjardins, and Hafs 1967; Lino, Braden, and Turnbull 1967; Lambiase and Amann 1969).

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All these methods have disadvantages since the first three involve surgical intervention and the fourth is tedious and of uncertain accuracy.

Resorption of spermatozoa was considered by Simeone and Young (1931) to be the main means of removing unejaculated spermatozoa from the genital tract. This concept, and that of the phagocytosis of spermatozoa in the epididymis, has had considerable support (Amann and Almquist 1962*a*; Koefoed-Johnsen 1964; Roussel, Stallcup, and Austin 1967; Orgebin-Crist 1968; Salamon 1968; Amann 1969; Lambiase and Amann 1969; Zankl 1969, 1970).

The recent histological studies of Bedford (1965), Martan (1969), and Fulka, Kopečný, and Koefoed-Johnsen (1971) indicate that it would be impossible for the large numbers of spermatozoa produced by the testis to be resorbed in the epididymis. Other studies have revealed the presence of spermatozoa in the urine of men (Oslund 1928; Wilhelm and Seligmann 1937), guinea pigs (Simeone and Young 1931), marsupial species (Bolliger and Carrodus 1938; Bolliger 1942), rams (Bielanski and Wierzbowski 1961*a*; Lino, Braden, and Turnbull 1967; Bielanski and Tischner 1968), and bulls (Koefoed-Johnsen 1964).

The number of spermatozoa eliminated from males at sexual rest should reflect accurately the number produced by the testes, if the proportion of spermatozoa resorbed or phagocytosed in the urogenital tract is low. Lino, Braden, and Turnbull (1967) concluded that resorption was not very important as a mechanism for disposing of the surplus spermatozoa in sexually inactive rams. Although the mean difference was only 12% between the daily spermatozoan output as measured by the number of spermatozoa voided with the urine and the daily spermatozoan output estimated by the exhaustion test, there was considerable variation between rams in the relative magnitude of the two estimates.

The present experiments compare the numbers of spermatozoa eliminated daily from the urogenital tract (DSOU) with the daily output of spermatozoa from a rete testis catheter (Voglmayr, Waites, and Setchell 1966; Voglmayr *et al.* 1967). The changes in DSOU induced by ejaculations and the evidence for resorption are also examined.

II. MATERIALS AND METHODS

(a) *Comparison of Methods of Estimating Daily Spermatozoan Production*

Eight 4- to 5-year-old Merino rams were maintained in an animal house on a constant daily ration for at least 60 days prior to the experiments. For urine collection, each ram was placed in a canvas sling so that he could stand but not lie down. A large cup-shaped silicone-rubber collecting apparatus (Dick and Mules 1954) was strapped under the prepuce. The urine, when voided, ran through a tube into a silicone-coated bottle containing 300 ml of 0.7% saponin made up in 0.4% aqueous formalin. Spermatozoa which were spontaneously discharged from the urogenital tract were caught on the inside of the collector and were rinsed into the urine-collecting bottle. The bottle was changed daily and the total number of spermatozoa in the urine (DSOU) estimated from haemocytometer counts. Four independent replicates were counted from each daily collection. The urine was collected for 20 days, after which a catheter was implanted in the rete testis of one testis of each ram by the technique of Voglmayr *et al.* (1967).

The catheters were placed alternately on the right and left sides in successive rams. The testicular fluid flowing from the catheter was usually collected for 24 hr in a small polythene bottle attached to the scrotum and the total number of spermatozoa estimated from haemocytometer counts. At least four replicates were counted for each collection. The first and last collections were discarded since they often varied widely from the mean stable flow. This was probably a result of the recent surgical intervention in the first collection and the failure of the

catheter in the last collection. The catheters gave usable collections over periods ranging from 40 to 212 hr.

No significant size or weight differences were noted between the testes of other Merino rams (Lino 1972), so bilateral symmetry was assumed where no difference in testis size was detectable by palpation. The mean daily output of spermatozoa from the rete testis catheter was doubled to give the estimate of the mean daily output of spermatozoa from both testes (DSOT).

(b) *Effect of 20 Ejaculations on Daily Spermatozoan Output*

Four 5-year-old Merino rams that had been maintained in an animal house on a constant ration for 18 months and trained for the exhaustive ejaculation technique were used. Ejaculates had not been collected from them for at least 5 weeks before urine collections were begun. Urine collections were made for 9 days, then 20 ejaculates were collected from each ram over the course of 2 days using an artificial vagina. The artificial vagina was rinsed with 0.9% saline between ejaculations and the washings were added to the combined ejaculates for the appropriate ram. Agglutination of the spermatozoa was prevented by the addition of the saponin-formalin solution used in the urine collections. The number of spermatozoa ejaculated was estimated from haemocytometer counts. Urine collections were continued between the ejaculations and for 18–29 days afterwards, and the DSOU estimated as previously stated.

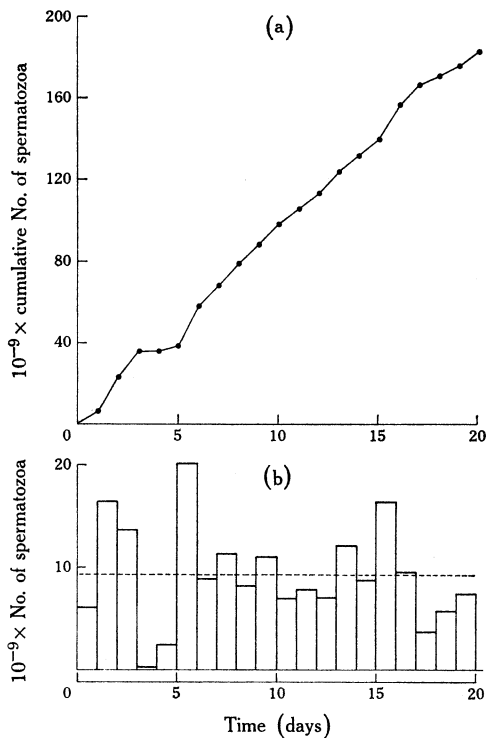


Fig. 1.—Number of spermatozoa eliminated from the urogenital tract of a ram (ram F) at sexual rest. (a) Cumulative total; (b) daily output and mean value.

III. RESULTS

(a) *Comparison of Methods of Estimating Daily Spermatozoan Output*

For each ram there was a large between-day variation in the number of spermatozoa eliminated from the urogenital tract so that the collections had to be made for a sufficient length of time that the estimates could not be influenced

significantly by any particular count. Twenty days was chosen arbitrarily as the length of the collections in the present experiments. The daily spermatozoan output from the urogenital tract (DSOU) of ram F is presented as an example of the variation between successive days (Fig. 1). It can be seen that the cumulative total of DSOU in this stabilized ram produces a nearly straight line despite the large day-to-day variation (Fig. 1) which is strong evidence that the total spermatozoan output over a period provides the best estimate of DSOU.

There was little variation in the rate of spermatozoan output from the rete testis catheter between successive collections except for the first and last collections which were excluded from consideration.

The mean daily output of spermatozoa both from the rete testis catheter and from the urogenital tract, together with their "standard errors", are presented in Table 1. It was found that the magnitude of the "standard deviation" was propor-

TABLE 1

COMPARISON OF THE MEAN DAILY SPERMATOZOAN OUTPUT FROM THE UROGENITAL TRACT (DSOU) AND FROM THE RETE TESTIS CATHETER (DSOT) IN EIGHT RAMS

Ram	DSOT (millions)	Hours of collection (No. of samples)	DSOU (millions)	Days of collection	Difference DSOU-DSOT (%)
A	3909 $\pm$ 105*	112.05 (5)	3576 $\pm$ 660†	20	-8.5
B	7118 $\pm$ 514	40.30 (2)	7135 $\pm$ 1248	20	+0.2
C	4081 $\pm$ 170	112.17 (9)	3823 $\pm$ 799	20	-6.3
D	2642 $\pm$ 249	139.33 (6)	2523 $\pm$ 386	20	-4.5
E	4076 $\pm$ 234	140.08 (6)	3971 $\pm$ 624	20	-2.6
F	9361 $\pm$ 291	48.50 (2)	9177 $\pm$ 1093	20	-2.0
G	8788 $\pm$ 256	88.25 (4)	8528 $\pm$ 1759	20	-3.0
H	7788 $\pm$ 579	161.00 (7)	7389 $\pm$ 525	20	-5.1
Mean	5970		5765		-3.4

* Calculated as normal standard error. The lack of independence of the daily counts and the probability of serial correlation means that the value only gives a measure of the dispersion of the daily estimates about the mean. It probably does not give a measure of the accuracy of the mean.

tional to the mean which would indicate that the square root transformation may be useful in normalizing the data when comparing between rams.

Since all the spermatozoa were collected from the rete testis catheter and from the urogenital tract, the daily counts of spermatozoa were probably not independent of each other. Each count can be affected by the magnitude of the count(s) immediately preceding and can itself influence the count(s) following. The lack of independence of the daily totals and the probable existence of serial correlation in this type of data implies that the standard errors calculated from the values of daily spermatozoan output may not reflect the accuracy of the means. In the case of the DSOU data, only a standard error based on successive 20-day tests from each ram should be used to determine the accuracy of the estimates. The standard error gives an idea of the dispersion about the mean of the daily totals of spermatozoan output. There was a very high correlation ($r = 0.999$) between the DSOU estimated

over the 20-day collection period and the DSOT for the eight rams (Table 1). In seven of the eight rams the DSOU was 2–9% lower than the DSOT, the overall means differing by 3·4% (Table 1).

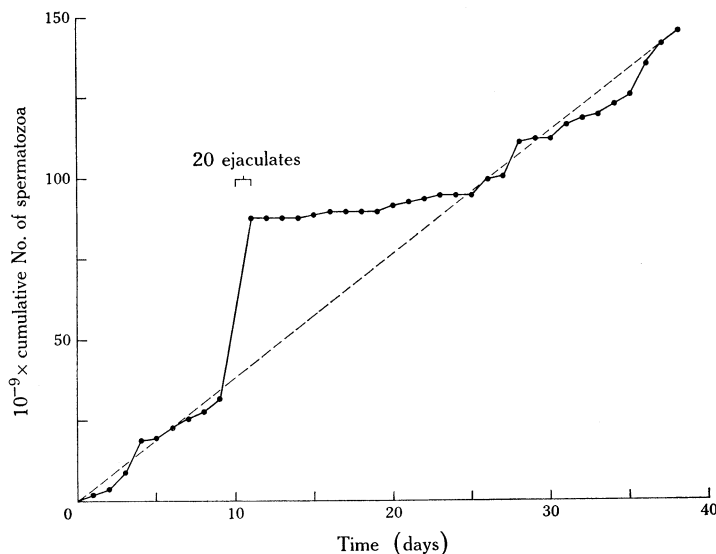


Fig. 2.—Cumulative number of spermatozoa eliminated from the urogenital tract and removed by multiple ejaculation of ram J for 9 days before ejaculation and during and 29 days after 20 ejaculations.

(b) *Effect of 20 Ejaculations on Daily Spermatozoan Output*

The number of spermatozoa eliminated from the urogenital tract was very low for a period of 9–16 days after the 20 ejaculations, before returning to the mean level of the spermatozoan output in period 1. The response of the DSOU following

TABLE 2

COMPARISON OF THE MEAN DAILY SPERMATOOAN OUTPUT OF FOUR RAMS DURING THE 9 DAYS BEFORE THE COLLECTION OF EJACULATES (PERIOD 1), DURING THE COLLECTION OF 20 EJACULATES AND FOR 9–16 DAYS THEREAFTER (PERIOD 2), AND DURING A FURTHER 9 DAYS (PERIOD 3)

Ram	Daily spermatozoan output (millions)			Overall mean
	Period 1	Period 2	Period 3	
J	3713	3855 (16)*	3817	3795
K	3693	3471 (9)	3147	3437
L	557	646 (16)	579	594
M	2817	2599 (9)	2826	2747

* Number of collection days in period 2.

the collection of 20 ejaculates in ram J (Fig. 2) is typical of the response of the DSOU in all the rams examined. For each ram the DSOU was measured during

three consecutive periods: (1) 9 days before the collection of ejaculates; (2) from the start of the collection of 20 ejaculates until the daily count of the spermatozoan output from the urogenital tract first equalled or exceeded the mean DSOU for period 1; and (3) for a further 9 days after this recovery point. The mean daily output of spermatozoa for each of these three consecutive periods for the four rams is shown in Table 2 (the output for period 2 includes the number of spermatozoa collected in the 20 ejaculates). The mean daily spermatozoan output in period 2 was very similar to that in periods 1 and 3.

IV. DISCUSSION

The finding that the daily spermatozoan output from the urogenital tract (DSOU) and the output of spermatozoa from a rete testis catheter (DSOT) are very highly correlated strengthens our earlier conclusion (Lino, Braden, and Turnbull 1967) that the DSOU is a simple means of reliably estimating the daily spermatozoan production of rams.

The large between-day variation in DSOU probably results from the capacity of the cauda epididymis, vas deferens, and ampulla, to retain large numbers of spermatozoa for an ejaculatory reserve. The occasional and random occurrence of spontaneous ejaculations and emissions of semen into the urine collector would contribute to this variation. On the other hand, the flow rate of the rete testis catheter was very stable.

The small mean difference (3.4%) between the DSOU and DSOT is probably attributable to some loss of cells during the collection of spermatozoa from the urogenital tract. Spontaneous ejaculation was one of the ways the ram eliminated spermatozoa from the urogenital tract and, although the semen was usually deposited in the urine-collecting apparatus, small amounts of semen occasionally were sprayed onto the harness and wool.

The close agreement between the two estimates of spermatozoan production shows that any resorption of spermatozoa in the genital tract of sexually inactive rams is negligible. Even if only half of the apparent 3.4% difference between the DSOU and DSOT were due to resorption, the number of spermatozoa destroyed would be about 50 million per epididymis per day. Histological examination of ram epididymides provided no evidence of resorption or phagocytosis of such a magnitude (unpublished observations). The studies of Bedford (1965), Martan (1969), and Fulka, Kopečný, and Koefoed-Johnsen (1971) indicate that resorption is also negligible in other species.

The DSOU fell to low levels after the 20 ejaculations, but soon return to pre-ejaculation levels. This suggests that the cauda epididymis, vas deferens, and ampulla function as a "reservoir", with spermatozoa continually entering from the testis, passing through, and being eliminated from the urogenital tract and, once this reservoir is depleted, few spermatozoa are eliminated until the reservoir is replenished.

Amann and Almquist (1962a) postulated that in bulls the rate of resorption of spermatozoa in the male genital tract was variable, being higher when the number of spermatozoa in the caudae epididymides was higher. Lambiase and Amann (1969) concluded that their results with rabbits supported this hypothesis. There were no data presented either on the number of spermatozoa voided in the urine

or lost by spontaneous ejaculations, nor was there histological evidence for the resorption of the large numbers of spermatozoa that were not accounted for. In the present study the mean daily output of spermatozoa was similar in the pre-ejaculation, recovery, and post-recovery periods, which indicates that the number of spermatozoa in the caudae epididymides could have little or no influence on the number of spermatozoa resorbed.

It is concluded that resorption or phagocytosis of spermatozoa in the excurrent ducts can be ignored and that the daily spermatozoan output from the urogenital tract provides a reliable estimate of testicular spermatozoan production in rams.

V. ACKNOWLEDGMENTS

We are grateful to Dr. T. W. Scott for advice and to Dr. J. K. Voglmayr for demonstrating the rete testis cannulation technique. Thanks are due also to Mr. K. E. Turnbull for technical assistance, and to Mrs. N. B. Carter, Division of Mathematical Statistics, CSIRO, for statistical advice.

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