THE OUTPUT OF SPERMATOZOA IN RAMS

II.* RELATIONSHIP TO SCROTAL CIRCUMFERENCE, TESTIS WEIGHT, AND THE NUMBER OF SPERMATOZOA IN DIFFERENT PARTS OF THE UROGENITAL TRACT

By B. F. Lino†

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Abstract

Daily spermatozoan output from the urogenital tract (DSOU), scrotal circumference, testis weight, and the numbers of spermatozoa in the epididymis and other parts of the urogenital tract were measured in 10 rams. Testis weight and the numbers of spermatozoa in the head, body, and tail of the epididymis and in the vas deferens and ampulla were found to be bilaterally symmetrical in each ram. The daily spermatozoan output from the urogenital tract was linearly correlated with testis weight ($r = 0 \cdot 83$), with epididymal spermatozoan reserves ($r = 0 \cdot 86$), with extragonadal spermatozoan reserves ($r = 0 \cdot 90$), and with the total number of spermatozoa in the urogenital tract ($r = 0 \cdot 84$). The significance levels of the regression coefficients indicated that these parameters would give good estimates of each other. The total number of spermatozoa in the urogenital tract of all rams at slaughter divided by the predicted time taken for spermatozoa to traverse the tract was found to be approximately 6% below the mean value for the daily spermatozoan output of all rams.

I. Introduction

Quantitative estimates of daily spermatozoan production by the testis are important in the analysis of many reproductive problems. Although it is difficult to make direct estimates of the spermatozoan production of the testis, much data can be obtained from the measurement of some parameters of the genital tract.

Daily spermatozoan output has been found to be correlated with testis weight in the rabbit (Edwards 1940) and in the bull (Vandemark 1956; Boyd and Vandemark 1957) and with the number of spermatozoa in the epididymides of bulls (Amann and Almquist 1961*b*; Almquist, Amann, and Hale 1961). Spermatozoan output is also correlated with both scrotal circumference and testis weight in the bull (Willett and Ohms 1955, 1957).

The estimates of daily spermatozoan output in those studies were made from either partial exhaustion tests or from relatively few ejaculates taken at a regular rate. Since estimates of daily spermatozoan output made in this way are affected by the number of ejaculates taken and by the neuromuscular efficiency of the

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† School of Veterinary Science, University of Melbourne, Werribee, Vic., and Department of Anatomy, School of Medicine, University of Hawaii, Honolulu, Hawaii 96822.

individual (Hale and Almquist 1960) they are subject to a large amount of variation which severely reduces their predictive value.

The mean daily output of spermatozoa from the urogenital tract (DSOU), which includes all spermatozoa eliminated in urinations, spontaneous emissions, and ejaculations, has been found to be within 4% of the mean number of spermatozoa passed daily from a catheter implanted in the rete testis (Lino and Braden 1972). The agreement between these two estimates of spermatozoan production was much closer than that found between DSOU and estimates of daily spermatozoan output obtained from an exhaustive ejaculation test (Lino, Braden, and Turnbull 1967) and indicated that DSOU gives a very good estimate of the daily spermatozoan production of the testis.

Since spermatozoa in rams at sexual rest are almost completely eliminated from the urogenital tract and the spermatozoan transit time is constant for rams ejaculated up to three times a week (Amir and Ortavant 1968), the total number of spermatozoa in the tract at slaughter should also give a good estimate of the spermatozoan production of the testis.

The relationships between DSOU, scrotal circumference, testis weight, and the numbers of spermatozoa in the epididymis and other parts of the urogenital tract were examined in the ram. Alternative methods for predicting the daily spermatozoan production of the testis using these data are presented.

II. Materials and Methods

Ten four-year-old Finewool Saxon Merino rams were housed and fed a weight-maintaining ration supplemented with vitamins and minerals for at least 4 months prior to the commencement of the experiments. The rams were placed in a harness and their urine collected for 28 days (Lino, Braden, and Turnbull 1967). The rams were electroejaculated once a week for routine semen examination. One drop of semen was taken for examination and the remainder of the ejaculate was washed into the urine collector. It has been demonstrated that the rate of spermatozoan output measured by DSOU is not affected by ejaculation provided that the spermatozoa removed by ejaculation are included with the number of spermatozoa voided (Lino and Braden 1972). The number of spermatozoa removed in a drop of semen once a week was found to be less than 0·5% of the total number of spermatozoa produced. The total number of spermatozoa were calculated in the daily urine samples from haemocytometer counts.

When the urine had been collected for 28 days, the scrotal circumference was measured by applying a tape measure at the point of maximum diameter while the skin of the scrotum was stretched taut around the base of the testes. The rams were killed by an intravenous overdose of barbiturate. The urethra was closed with a ligature and the genital tract and bladder were removed intact.

The epididymis was dissected from the testis and the testis was weighed after removing excess connective tissue and trimming the pampiniform plexus. The epididymis was cut into three sections corresponding to the head, body, and tail regions. After the tunica vaginalis and other connective tissue was removed, each section was cut into small pieces and homogenized in physiological saline, using the 50 ml chamber of a Sorvall omnimixer (Ivan Sorvall, Inc., Norwalk, U.S.A.). The homogenate was filtered through coarse gauze which was then rinsed with saline. Fifty ml of 0·17% saponin in 0·5% formalin was added to the homogenate as a preservative.

The vas deferens and ampulla were flushed with 50 ml physiological saline into 15 ml of the saponin–formalin preserving solution. The bladder was emptied and flushed with 100 ml saline into 50 ml of the preserving solution. The urethra was flushed with 50 ml saline and its flushings added to the bladder contents.

The volume of each of the homogenates and flushings from the sections of the tract was measured and after an appropriate dilution, the total number of spermatozoa in each was determined from haemocytometer counts.

III. Results

The scrotal circumference, testis weights, and the numbers of spermatozoa in the head, body, and tail regions of each epididymis, in each vas deferens and ampulla, and in the bladder and urethra were determined for each ram. Analysis of variance showed that there were highly significant ($P<0\cdot001$) differences between rams in all the measurements. However, there were no significant differences between the right and left sides within rams either in testis weight or in the numbers of spermatozoa in each of the three regions of the epididymis and in each vas deferens and ampulla, showing that the genital tract was bilaterally symmetrical.

The epididymal spermatozoan reserves included the total number of spermatozoa contained in the three regions of both epididymides. The extragonadal spermatozoan reserves included the spermatozoa contained in both vasa deferentia and ampullae and the epididymal spermatozoan reserves. The total number of spermatozoa in the urogenital tract included the spermatozoa contained in the bladder and urethra and in the extragonadal spermatozoan reserves. The distribution of the spermatozoa in the urogenital tract, the mean testis weight, and the scrotal circumferences for the 10 rams are shown in Table 1.

Table 1

NUMBERS OF SPERMATOZOA IN THE DIFFERENT REGIONS OF THE UROGENITAL TRACT

Mean single and paired testis weights were $268\pm8\cdot57$ (S.E.) and $536\pm24\cdot05$ g respectively, whilst mean scrotal circumference was $37\cdot88\pm0\cdot79$ cm

Part of tract	$10^{-9}\times$ mean No. of spermatozoa ($\pm$ S.E.)	Contribution (%) of region to: Epididymal sperm reserves	Extragonadal sperm reserves	Total sperm in tract
Epididymides				
Head	$17\cdot705\pm2\cdot469$	19·63	18·95	17·96
Body	$10\cdot330\pm0\cdot977$	11·45	11·05	10·48
Tail	$62\cdot172\pm5\cdot402$	68·92	66·53	63·07
Total epididymal sperm	$90\cdot206\pm7\cdot024$	100		
Vasa deferentia and ampullae	$3\cdot245\pm0\cdot820$		3·47	3·29
Total extragonadal sperm	$93\cdot451\pm7\cdot439$		100	
Bladder and urethra	$5\cdot122\pm1\cdot333$			5·20
Total sperm in tract	$98\cdot573\pm8\cdot012$			100

Linear correlation coefficients (r) were calculated between scrotal circumference, single and paired testis weight, the numbers of spermatozoa in the spermatozoan reserves, and the mean DSOU for both the 12 and 28 days prior to slaughter (Table 2).

Linear regression coefficients (b) were calculated for the significant correlations (Table 2). The significance levels of the correlation and regression coefficients were calculated.

The regression lines and equations useful for prediction (Fig. 1) were calculated using a least-squares procedure (Steel and Torrie 1960). The regressions of the

TABLE 2

LINEAR CORRELATION AND REGRESSION COEFFICIENTS BETWEEN REPRODUCTIVE CHARACTERISTICS OF NORMAL RAMS

Independent variables	Coefficient†	Dependent variables: Paired testis weight	Epididymal sperm reserves	Extragonadal sperm reserves	Total sperm in tract	Mean DSOU (12 days)‡	Mean DSOU (28 days)‡
Scrotal circumference	r	0·7408*	0·6439*	0·6968	0·6138	0·3950	0·4030
	b	22·5921*	5·7348*	6·1606*			
Single testis weight§	r		0·8887***	0·8820***			
	b		0·2541***	0·2672***			
Paired testis weight	r		0·9205***	0·9728***	0·8742***	0·7924**	0·8343**
	b		0·2688***	0·2825***	0·2912***	0·02163**	0·02037**
Epididymal sperm reserves	r					0·8815***	0·8553**
	b					0·08241***	0·07173**
Extragonadal sperm reserves	r					0·8471**	0·9010***
	b					0·07586**	0·07609***
	b'					0·09108***	0·09132***
Total sperm in tract	r					0·8337**	0·8448**
	b					0·06834**	0·06211**
	b'					0·08670***	0·08695***

* $P<0·05$. ** $P<0·01$. *** $P<0·001$.

† r = correlation coefficient; b = regression coefficient calculated by least-squares method; b' = regression coefficient calculated assuming line passes through origin (0,0).

‡ Indicates the length of the collection period (days) prior to slaughter.

§ Single testis weight coefficients were calculated with the corresponding single side reserves.

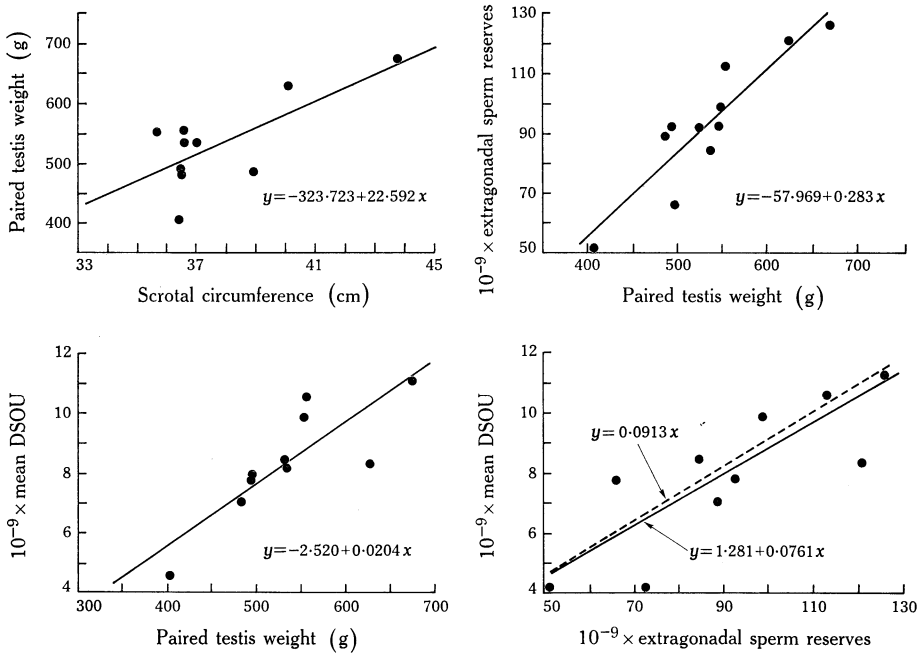

Fig. 1.—Regression lines and regression equations of paired testis weight against scrotal circumference, extragonadal spermatozoan reserves, and daily spermatozoan output from the urogenital tract (DSOU), and of extragonadal spermatozoan reserves against DSOU.

extragonadal spermatozoan reserves and the total number of spermatozoa in the urogenital tract on DSOU were also calculated as lines passing through the origin.

The total number of spermatozoa in the urogenital tract and the mean DSOU for both the 12 days and the 28 days prior to slaughter for each ram are shown in Table 3. The total spermatozoa in the urogenital tract divided by the mean DSOU for the 12 or 28 days gave mean values of $11 \cdot 85 \pm 0 \cdot 67$ and $11 \cdot 89 \pm 0 \cdot 57$ respectively.

TABLE 3

RELATIONSHIP BETWEEN THE TOTAL NUMBER OF SPERMATOZOA IN THE UROGENITAL TRACT (TSR) AND DAILY SPERMATOZOAN OUTPUT FROM THE UROGENITAL TRACT (DSOU) IN NORMAL RAMS

Ram	$10^{-9} \times$ TSR	$10^{-9} \times$ mean DSOU (12 days)*	$10^{-9} \times$ mean DSOU (28 days)†	$\frac{\text{TSR}}{\text{DSOU}}$ (12 days)*	$\frac{\text{TSR}}{\text{DSOU}}$ (28 days)†
1	103·780	8·653	7·824	11·99	14·00
2	117·697	11·613	10·563	10·13	11·32
3	108·862	9·077	9·819	11·99	11·49
4	134·642	10·809	11·127	12·46	12·10
5	101·042	7·902	8·391	12·79	12·04
6	122·897	10·158	8·374	12·10	14·68
7	93·428	5·668	7·091	16·48	13·18
8	66·146	7·474	7·779	8·85	8·51
9	84·538	6·973	8·419	12·12	10·05
10	52·702	5·494	4·597	9·59	11·48
Mean ± S.E.				$11 \cdot 85 \pm 0 \cdot 67$	$11 \cdot 89 \pm 0 \cdot 57$

* Collections made for 12 days prior to slaughter.

† Collections made for 28 days prior to slaughter.

These values should represent the number of days for the spermatozoa to traverse the genital tract and they were not significantly different ($\chi^2 = 3 \cdot 53$ and $2 \cdot 66$ respectively) from an estimate of spermatozoan transit time of $12 \cdot 5$ days [the midpoint of the range of spermatozoan transit times determined for rams ejaculated once per week (Amir and Ortavant 1968)].

IV. DISCUSSION

The testis weights in these rams were in the range described for normal rams by Ortavant (1958) and Dott and Skinner (1967). Although the total numbers of spermatozoa in the epididymis were generally lower than those reported by Polovceva (1938) and Ortavant (1958) they were much higher than those of Dott and Skinner (1967). The percentage distribution of spermatozoa in the tract was similar to that described by Polovceva (1938), Chang (1945), Ortavant (1952, 1958), and Dott and Skinner (1967). However, the percentages of spermatozoa were higher in both the head and body and lower in the tail of the epididymis than those reported by most of these workers. It is difficult to make meaningful comparisons with the reported results as the structure of the epididymis varies from animal to animal and its division is arbitrary.

Preliminary trials showed that there was no significant breakdown of spermatozoa due to the homogenization of the epididymis. These findings agreed with the

results of Amann and Almquist (1961*a*), Dott and Skinner (1967), and Orgebin–Crist (1968). Significant numbers of spermatozoa were frequently found in the bladder and urethra post mortem. These spermatozoa are thought to enter the bladder at death since such large numbers are seldom found in samples taken from the bladder of living rams by means of a catheter (Lino, unpublished data).

The scrotal circumference was significantly correlated with testicular weight although not as highly significantly as in bulls (Willett and Ohms 1955, 1957). The scrotal circumference was also significantly correlated with the epididymal spermatozoan reserves and the extragonadal spermatozoan reserves but not with daily spermatozoan output from the urogenital tract (DSOU). The correlation between scrotal circumference and DSOU was not improved by substituting the mean DSOU for the 12 days immediately prior to slaughter for the mean DSOU for the 28 days prior to slaughter. The low correlation ($r = 0 \cdot 40$) between scrotal circumference and spermatozoan production may reflect variations in scrotal wool cover, subcutaneous fat, and in the thickness of the scrotal skin. This is in contrast with correlations of $0 \cdot 68$ and $0 \cdot 92$ in bulls and young bulls respectively where the spermatozoan production was assessed by exhaustive ejaculation (Willett and Ohms 1955, 1957). However, in aged bulls, these workers obtained a negative correlation coefficient ($r = -0 \cdot 53$) which could have resulted from insufficient spermatozoa being removed by the exhaustive ejaculation.

Single and paired testis weight was highly correlated with the epididymal spermatozoan reserves and extragonadal spermatozoan reserves. In sexually rested bulls, the testis weight was also significantly correlated ($r = 0 \cdot 451$) with the number of spermatozoa in the epididymis (Amann and Almquist 1961*b*). Verma, Singh, and Sharma (1965) reported a non-significant correlation ($r = 0 \cdot 065$) between testis weight and the number of spermatozoa in the epididymides of buffalo bulls.

Paired testis weight was highly correlated with the mean DSOU estimated over the 12 and the 28 days prior to slaughter. Edwards (1940) obtained a highly significant regression of the rate of spermatozoan production on testis weight in rabbits. Testis weight and testis plus epididymal weight were also correlated ($r = 0 \cdot 80$ and $0 \cdot 74$) with the total number of spermatozoa produced in 90 min during a partial exhaustive ejaculation test in bulls (Vandemark 1956; Boyd and Vandemark 1957).

The epididymal spermatozoan reserves, the extragonadal spermatozoan reserves, and the total number of spermatozoa in the urogenital tract were highly correlated with DSOU. Almquist and Amann (1961) and Almquist, Amann and Hale (1961) also found a highly significant correlation ($r = 0 \cdot 87$) between the extragonadal spermatozoan reserves and the number of spermatozoa removed from bulls in six or seven ejaculates per week.

The highly significant linear regression coefficients for many of the relationships in the present study indicated that the deviations from regression were small and that the assumption of linearity conformed well to the data. For prediction purposes scrotal circumference could provide useful estimates of testis weight and epididymal spermatozoan reserves. Testis weight should give good estimates of the spermatozoan reserves and DSOU while the spermatozoan reserves should give good estimates of the DSOU. However, it should be remembered that each of these rams had a fairly stable DSOU and, presumably, stable spermatogenesis and that use of the regression equations would necessitate a similar stability.

Almquist and Amann (1961) found that the number of testicular spermatozoa was not significantly correlated with the epididymal spermatozoan reserves and stressed the independence of the testicular spermatozoan production and the storage of spermatozoa in the excurrent duct system. The highly significant correlation between DSOU and the epididymal spermatozoan reserves in the present study is contrary to this finding especially as, in the ram, most of the spermatozoa produced by the testis are eliminated from the urogenital tract (Lino and Braden 1972). Indeed, the interrelationship between spermatozoan reserves, DSOU, and spermatozoan transit time indicates that the total number of spermatozoa in the urogenital tract would provide a measure of the rate of spermatozoan production. The non-significant difference of approximately 6% between the ratio of the total number of spermatozoa in the urogenital tract to the DSOU and epididymal spermatozoan transit time shows that DSOU could be estimated from the total number of spermatozoa in the urogenital tract, and vice versa, since the transit time is known.

The duration of spermatozoan transit through the epididymis of unejaculated bulls (Koefoed–Johnsen 1960), rabbits (Orgebin–Crist 1965), and boars (Swiestra 1968) has been found to be reasonably consistent within species. This suggests that the relationship between spermatozoan production, spermatozoan reserves and spermatozoan transit time may hold for species other than ovine.

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VI. References

Almquist, J. O., and Amann, R. P. (1961).—Reproductive capacity of dairy bulls. II. Gonadal and extra-gonadal sperm reserves as determined by direct counts and depletion trials, dimensions and weight of genitalia. *J. Dairy Sci.* **44**, 1668–78.

Almquist, J. O., Amann, R. P., and Hale, E. B. (1961).—Measurement of sperm production and its relation to sperm output and reserves in dairy bulls. Proc. 4th Int. Congr. Anim. Reprod. The Hague, 1961. Vol. 2. pp. 270–3.

Amann, R. P., and Almquist, J. O. (1961*a*).—Reproductive capacity of dairy bulls. I. Technique for direct measurement of gonadal and extragonadal sperm reserves. *J. Dairy Sci.* **44**, 1537–43.

Amann, R. P., and Almquist, J. O. (1961*b*).—Reproductive capacity of dairy bulls. V. Detection of testicular deficiencies and requirements for experimentally evaluating testis function from semen characteristics. *J. Dairy Sci.* **44**, 2283–91.

Amir, D., and Ortavant, R. (1968).—Influence de la fréquence des collectes sur la durée du transit des spermatozoides dans le canal épididymaire du bélier. *Ann. Biol. anim., Biochem., Biophys.* **8**, 195–207.

Boyd, L. J., and Vandemark, N. L. (1957).—Spermatogenic capacity of the male bovine. I. A measurement technique. *J. Dairy Sci.* **40**, 689–97.

Chang, M. C. (1945).—The sperm production of adult rams in relation to frequency of semen collection. *J. agric. Sci., Camb.* **35**, 243–6.

DOTT, H. M., and SKINNER, J. D. (1967).—A reassessment of extragonadal sperm reserves in Suffolk rams. *J. agric. Sci., Camb.* **69**, 293–5.

EDWARDS, J. (1940).—The effect of unilateral castration on spermatogenesis. *Proc. R. Soc. Lond.* B. **128**, 407–21.

HALE, E. B., and ALMQUIST, J. O. (1960).—Relation of sexual behaviour to germ cell output in farm animals. *J. Dairy Sci.* **43** (suppl.), 145–69.

KOEFOED-JOHNSEN, H. H. (1960).—Influence of ejaculation frequency on the time required for sperm formation and epididymal passage in the bull. *Nature, Lond.* **185**, 49–50.

LINO, B. F., and BRADEN, A. W. H. (1972).—The output of spermatozoa in rams. I. Relationship with testicular output of spermatozoa and the effect of ejaculations. *Aust. J. biol. Sci.* **25**, 351–8.

LINO, B. F., BRADEN, A. W. H., and TURNBULL, K. E. (1967).—Fate of unejaculated spermatozoa. *Nature, Lond.* **213**, 594–5.

ORGEBIN-CRIST, M. C. (1965).—Passage of spermatozoa labelled with thymidine-^{3}H through the ductus epididymis of the rabbit. *J. Reprod. Fert.* **10**, 241–51.

ORGEBIN-CRIST, M. C. (1968).—Gonadal and epididymal sperm reserves in the rabbit: estimation of the daily sperm production. *J. Reprod. Fert.* **15**, 15–25.

ORTAVANT, R. (1952).—Recherches quantitatives sur la spermatogénése et les reserves spermatiques du bélier. Proc. 2nd Int. Congr. Physiol. Pathol. Anim. Reprod. Artif. Insem., Vol. 1. pp. 63–9.

ORTAVANT, R. (1958).—Le cycle spermatogénétique chez le bélier. D.Sc. Thesis, University of Paris.

POLOVCEVA, V. (1938).—The rate of movement and the time of maturation of spermatozoa in the epididymis of the ram. *Dokl. Adak. Seljskohos Nauk.* **15/16**, 43–52. [*Anim. Breed. Abstr.* **7**, 227 (1939).]

STEEL, R. G. D., and TORRIE, J. H. (1960).—"Principles and Procedures of Statistics." (McGraw-Hill Book Co. Inc.: New York.)

SWIESTRA, E. E. (1968).—Cytology and duration of the seminiferous epithelium of the boar; duration of spermatozoan transit through the epididymis. *Anat. Rec.* **161**, 171–86.

VANDEMARK, N. L. (1956).—Quantitative aspects of semen production in bulls. Proc. 3rd Int. Congr. Anim. Reprod. Cambridge, 1956. Plenary papers, pp. 80–9.

VERMA, M. C., SINGH, G., and SHARMA, V. D. (1965).—Studies on sperm production. II. Testicular and epididymal sperm reserves in Buffalo-bulls as determined by direct counts. *Ind. J. Vet. Sci. Anim. Husb.* **35**, 331–6.

WILLETT, E. L., and OHMS, J. I. (1955).—Testicular size and production of spermatozoa by bulls. *J. Anim. Sci.* **14**, 1261. [Abstract.]

WILLETT, E. L., and OHMS, J. I. (1957).—Measurement of testicular size and its relation to production of spermatozoa by bulls. *J. Dairy Sci.* **40**, 1559–69.