SPERM PRODUCTION OF BOARS AS MEASURED FROM EPIDIDYMAL SPERM RESERVES AND QUANTITATIVE TESTICULAR HISTOLOGY

E. E. SWIERSTRA

*Research Station, Canada Department of Agriculture,
Brandon, Manitoba, Canada*

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Summary. Four methods of measuring daily sperm production (DSP) were evaluated using seventy-two boars. The DSP was determined; I, by unilaterally castrating boars 0 and 48 hr after depletion of the epididymal sperm reserves and then measuring changes in the reserves (fourteen boars); II, by measuring changes in the epididymal sperm reserves of four groups of boars killed 5, 24, 52 and 72 hr following depletion of the reserves (forty-six boars); III, by dividing the total number of spermatozoa in the right and left epididymis of sexually active boars by the epididymal transit time (twelve boars); IV, by quantitative testicular histology (same twelve boars as for Method III). Method I was unsuitable for estimating the DSP of individual boars because of normal variation in sperm numbers between epididymides within boars. Method II revealed that there was too much variation in epididymal sperm reserves among boars to obtain a reliable mean estimate of DSP for the forty-six boars. Method III did not give reliable results for individual boars because epididymal transit time varies among boars. It was concluded that the DSP of individual boars could best be measured by Method IV though Methods III and IV gave similar estimates for the mean DSP of the twelve boars (15.0×10^9 versus 16.2×10^9). The sperm output of these boars averaged 15.5×10^9 /day when ejaculates were collected at 72-hr intervals, suggesting little or no absorption of spermatozoa from the epididymides or excretion of spermatozoa in the urine of sexually active boars. The mean DSP/g of net testis was 24.5×10^6 (S.E. ± 0.3) as determined by quantitative histology. Only spermatozoa from the cauda epididymidis were ejaculated during depletion.

INTRODUCTION

Quantitative testicular histology has been used to determine daily sperm production (DSP) in the boar (Kennelly & Foote, 1964; Swierstra, 1968a) and the bull (Amann & Almquist, 1962b; Swierstra, 1966). In the bull, DSP has also been determined by counting the number of spermatids in suspensions of homogenized testes and dividing the total number counted by their lifespan (Amann & Almquist, 1962b). Singh (1962) determined the DSP of

boars by dividing the total epididymal sperm reserves by the time interval in days required for spermatozoa to pass through the epididymis. Recently, the DSP of the ram and bull has been measured by collecting spermatozoa and fluid directly from the rete testis of the living animal (Voglmayr, Waites & Setchell, 1966; Voglmayr, Larsen & White, 1970). Procedures for measuring DSP have been reviewed by Foote (1969) and Amann (1970).

The objectives of this research were (1) to evaluate three methods for measuring DSP from epididymal sperm reserves, (2) to compare DSP as measured from epididymal sperm reserves with DSP as measured by quantitative testicular histology, and (3) to compare sperm production with sperm output when semen samples were collected at 48- and 72-hr intervals.

MATERIALS AND METHODS

Three experiments were conducted to measure DSP from epididymal sperm reserves in the boar. All boars were kept in individual pens in a windowless piggery with a mean temperature of $17^{\circ}\text{C} \pm$ only a few degrees and with light supplied from 08.00 to 17.00 hours. The boars had free access to water and were hand-fed 1.36 kg of pelleted ration (16% protein) twice daily. Using a dummy sow as teaser, semen was collected by artificial vagina (Swierstra & Rahnefeld, 1967), and the sperm concentration was determined in the gel-free fraction of the ejaculate by optical density (Young, Foote, Turkheimer & Hafs, 1960). The total number of spermatozoa per ejaculate was calculated, taking into account sperm losses in the collection equipment and gelatinous fraction of the ejaculate. Swierstra & Rahnefeld (1967) determined that these losses equalled 3.28% of the number of spermatozoa present in the collection bottle.

Epididymal sperm reserves

Each epididymis was divided into the caput, corpus and cauda, and each part was processed separately. The tissue was minced with a pair of scissors and transferred to a Waring blender using 150 ml of homogenizing solution. This solution consisted of physiological saline with 0.05% Triton X-100 added (Amann & Lambiase, 1969). The suspension was homogenized for 1 min and transferred to a glass jar. The homogenates were diluted further with homogenizing solution to obtain sperm concentrations that were convenient for accurate haemocytometer counting (400 to 600 spermatozoa per chamber). Sperm concentrations were determined by counting spermatozoa present in ten large squares of each of eight haemocytometer chambers. Each chamber was filled with a different pipette and the homogenates were mixed for 2 min with a magnetic stirrer just before filling the pipettes.

Daily sperm production by the testes

The daily sperm production was determined from quantitative testicular histology (DSP-T) using a modification by Swierstra (1968a) of a method developed by Kennelly & Foote (1964):

$$\text{DSP-T per testis} = \frac{\text{Corrected testis volume} \times \text{Volume \% round spermatid nuclei in the testis}}{\text{Av. volume/round spermatid nucleus} \times \text{Life-span of round spermatids in days}}$$

The corrected testis volume was obtained by the following formula:

Corrected testis volume =

$$\left[\left(\frac{\text{Gross testis weight} - \text{Tunica albuginea weight}}{\text{Testis density}} \right) - \text{Volume of mediastinum testis} \right] \times \text{Shrinkage correction}$$

Tissue samples were taken from three locations in the testes: near the caput epididymidis, midway between the poles of the testis and near the cauda epididymidis. The samples were fixed in Allen's fixative (Gray, 1964) for 3 days and processed routinely for embedding in paraffin wax. The tissue was serially sectioned (8 μm) and stained with a Feulgen technique using fast green as a counterstain. The percentage volume of round spermatid nuclei in the testes was determined by the method of Chalkley (1943).

Experiment 1

Ejaculates were collected at 48-hr intervals from seven Yorkshire and seven Lacombe boars for 8 weeks. The last semen collection on this schedule was made in the morning. Epididymal sperm reserves were further depleted by collecting a second semen sample from each boar on the afternoon of the same day and another the following morning. One randomly selected testis of each boar was removed after the last semen collection and the other 48 hr later. At castration, the Yorkshire boars were 316 days (S.E. ± 4) old and weighed 140 kg (S.E. ± 5). Corresponding figures for the Lacombe boars were 332 days (S.E. ± 5) and 157 kg (S.E. ± 4). Daily sperm production (DSP-E₁) was determined from the epididymal sperm reserves by subtracting the sperm reserves present at 0 hr from the sperm reserves present at 48 hr. The value thus obtained can be taken directly as a measure of DSP-E₁ per boar, because it represented repletion of the sperm reserves of only one testis over a 2-day period (Foote, Kennelly & Swierstra, 1965). The DSP-T was determined by classifying 700 'random hits' (Chalkley, 1943) per location or 2100 hits per testis.

Sperm production was compared with the average sperm output in the last ten ejaculates collected on the regular 48-hr collection schedule taking into account sperm losses in the collection equipment and gelatinous fraction of the ejaculate. Detailed information on the semen characteristics of these boars have been presented elsewhere (Swierstra & Rahnefeld, 1967).

Experiment 2

Twenty-four Yorkshire and twenty-two Lacombe boars were used in this experiment. Semen was collected from these boars every 72 hr for 5, 10 or 15 weeks, after which the epididymal sperm reserves were depleted as outlined under Exp. 1. The boars were assigned to four similar groups on the basis of body weight and mean number of spermatozoa in the last ten ejaculates collected on the regular 72-hr schedule. Group I consisted of seven Yorkshire and five Lacombe boars; Group II of seven Yorkshire and seven Lacombe boars; and Groups III and IV both included five boars from each breed. Groups I, II, III and IV were killed 5, 24, 52 and 72 hr after depletion. At killing, the Yorkshire boars were 320 (S.E. ± 3) days old and weighed 132 kg (S.E. ± 2). The age and weight of the Lacombe boars were 321 days (S.E. ± 2) and 147 kg

(S.E. $\pm$ 2), respectively. The mean daily sperm production (DSP-E₂) was determined from the repletion rate of the epididymal sperm reserves.

The DSP-T was measured for Group II by classifying 1400 'random hits' per location or 4200 hits per testis. Sperm production was compared with the average sperm output in the last ten ejaculates collected on the regular 72-hr collection schedule.

Experiment 3

Ejaculates were collected at 72-hr intervals from six Yorkshire and six Lacombe boars for an 11-week period, and killed 24 hr after the last semen collection. At slaughter, the Yorkshire boars were 334 days (S.E. $\pm$ 6) old and weighed 166 kg (S.E. $\pm$ 3). Corresponding figures for the Lacombe boars were 337 days (S.E. $\pm$ 3) and 178 kg (S.E. $\pm$ 2).

Daily sperm production (DSP-E₃) was determined from the epididymal sperm reserves by dividing the total sperm reserves in the right and left epididymides by the number of days required for spermatozoa to pass through the epididymis. Studies with tritiated thymidine, in which semen was collected from eight boars at 24-hr intervals, have indicated a mean minimum transit time through the epididymis of 10.2 days (S.E. $\pm$ 0.5). The percentage of labelled spermatozoa increased from 18% on the 1st day to 58% on the 2nd day after labelled spermatozoa appeared in the ejaculate (Swierstra, 1968b, unpublished data). In this study, therefore, 10.7 days were taken as the average transit time through the epididymis for calculating DSP-E₃. The DSP-T was determined by classifying 1400 'random hits' per location or 4200 hits per testis. Sperm production was compared with the average sperm output in the last ten ejaculates after allowing for sperm losses as before.

RESULTS

Experiment 1

The mean DSO of the fourteen boars was 13.1×10^9 (Table 1), and there was no significant difference between breeds with respect to sperm output. The mean estimate for daily sperm production was 16.6×10^9 when based on quantitative testicular histology and 11.5×10^9 when based on sperm repletion rate in the epididymides (DSP-E₁). The DSP-E₁ estimates for individual boars ranged from $+40.9 \times 10^9$ to -10.1×10^9 with a coefficient of variation of 142%. Sperm output was correlated with DSP-T ($r = 0.61$, $P < 0.05$) and with testis weight ($r = 0.57$, $P < 0.05$). Correlations between DSO and DSP-E₁ ($r = 0.19$) and between DSP-T and DSP-E₁ ($r = 0.02$) were not significant.

The epididymal sperm reserves in the caput and corpus were similar for the depleted and repleted epididymides, indicating that only spermatozoa from the cauda were ejaculated (Table 1).

Experiment 2

The mean epididymal sperm reserves per boar for Groups I, II, III and IV were 128.2, 150.4, 142.4 and 165.8×10^9 , respectively (Table 2). The linear regression equation including all data was $Y = 131.37 (10^9) + 0.42X (10^9)$,

TABLE 1

EPIDIDYMAL SPERM RESERVES OF SEVEN YORKSHIRE AND SEVEN LACOMBE BOARS UNILATERALLY CASTRATED AT 0 AND 48 HR AFTER DEPLETION OF THE EPIDIDYMAL SPERM RESERVES* (EXP. 1)

Characteristic	Time after depletion	
	0 hr	48 hr
Testis weight (g)	339±15	349±15
Epididymal weight (g)	99±4	108±5
Epididymal sperm reserves (10 ⁹)		
Caput	25.1±3.2	23.2±3.2
Corpus	18.5±2.5	18.1±2.0
Cauda	30.8±3.3	44.6±3.7
Per side	74.4±6.0	85.9±6.5
DSP-E ₁ † (10 ⁹)		11.5±4.3
DSP-T ₁ ‡ (10 ⁹)		16.6±0.8
DSO§ (10 ⁹)		13.1±0.8

* Values in the body of the table are means±S.E.

† Daily sperm production obtained by subtracting the epididymal sperm reserves present after depletion from the epididymal sperm reserves after 48 hr of repletion.

‡ Daily sperm production calculated from quantitative testicular histology.

§ Mean number of spermatozoa in the last ten ejaculates collected at 48-hr intervals divided by the collection interval in days.

TABLE 2

EPIDIDYMAL SPERM RESERVES OF TWENTY-FOUR YORKSHIRE AND TWENTY-TWO LACOMBE BOARS KILLED AT DIFFERENT INTERVALS AFTER DEPLETION OF THE EPIDIDYMAL SPERM RESERVES* (EXP. 2)

Characteristic	Time after depletion			
	5 hr	24 hr	52 hr	72 hr
No. of boars				
Yorkshire	7	7	5	5
Lacombe	5	7	5	5
Testis weight (g)	293±9	313±9	289±8	310±11
Epididymal weight (g)	60±3	66±2	62±2	66±3
Epididymal sperm reserves (10 ⁹)				
Caput	21.4±1.2	23.7±1.5	20.6±1.2	23.9±1.4
Corpus	15.2±1.8	16.2±1.4	16.1±1.3	16.4±1.5
Cauda	27.5±2.7	35.3±2.9	34.5±3.1	42.6±2.5
Per side	64.1±4.5	75.2±4.7	71.2±4.4	82.9±4.3
Per boar	128.2±12.8	150.4±13.1	142.4±11.9	165.8±12.0
DSO† (10 ⁹)	10.1±0.5	10.9±0.8	10.6±0.5	10.9±0.7

* Values in the body of the table are means±S.E.

† Mean number of spermatozoa in the last ten ejaculates collected at 72-hr intervals divided by the collection interval in days.

where Y is the predicted epididymal sperm reserve for the right and left testes, and X is the time interval after depletion in hours (Text-fig. 1). The sample standard deviation of the regression coefficient (S_b) was 0.25 ($P=0.10$). Groups I and II each contained two boars with large epididymal sperm reserves. By excluding the two values outside the 95% confidence limits from the calculation, the linear regression coefficient was significantly different from zero ($S_b = 0.22$).

The DSP-T for Group II was 13.8×10^9 (S.E. ± 0.6), and the DSP-T/g of net testis weight (gross testis weight minus the weight of the tunica albuginea) was 23.5×10^6 . Daily sperm production calculated from the repletion rate of

TEXT-FIG. 1. Epididymal sperm reserves of four groups of boars killed 5, 24, 52 and 72 hr after depletion. Sample regression including all data (—); sample regression excluding two boars outside the 95% confidence limits (---).

the epididymal sperm reserves (DSP-E₂) was 10.08×10^9 when using the equation $Y = 131.37 (10^9) + 0.42X (10^9)$, and 12.24×10^9 when using the equation $Y = 123.38 (10^9) + 0.51X (10^9)$. These estimates are 73 and 89% of the value 13.8×10^9 for DSP-T. The DSO was 10.9×10^9 (S.E. ± 0.8) or 79% of the DSP-T.

Experiment 3

There was no significant difference between breeds with respect to sperm output and the DSP, as determined by quantitative histology, was similar for the two breeds (Table 3). The epididymal sperm reserves varied considerably among boars and between testes within boars (Table 4). The overall DSP-T, DSP-E₃ and DSO for the twelve boars were 16.3×10^9 (S.E. ± 0.5), 15.0×10^9 (S.E. $\pm$

TABLE 3

EPIDIDYMAL SPERM RESERVES OF SIX YORKSHIRE AND SIX LACOMBE BOARS HAVING BEEN ON A 72-HR SEMEN COLLECTION SCHEDULE AND KILLED 24 HR AFTER THE LAST SEMEN COLLECTION (EXP. 3)

Characteristic	Mean $\pm$ S.E.	
	Yorkshire	Lacombe
Testis weight (g)	357 $\pm$ 6	360 $\pm$ 13
Epididymal weight (g)	69 $\pm$ 3	70 $\pm$ 3
Epididymal sperm reserves (10^9)		
Caput	22.3 $\pm$ 2.3	34.1 $\pm$ 2.5
Corpus	19.2 $\pm$ 2.4	16.0 $\pm$ 1.6
Cauda	38.7 $\pm$ 3.6	30.8 $\pm$ 2.5
Per side	80.2 $\pm$ 4.8	80.9 $\pm$ 5.5
Per boar	160.4 $\pm$ 13.8	161.8 $\pm$ 14.0
DSP-E ₃ * (10^9)	15.0 $\pm$ 1.3	15.1 $\pm$ 1.3
DSP-T† (10^9)	16.5 $\pm$ 0.5	16.0 $\pm$ 0.9
DSO‡ (10^9)	15.3 $\pm$ 0.2	15.8 $\pm$ 0.8

* Daily sperm production calculated from epididymal sperm reserves.

† Daily sperm production calculated from quantitative testicular histology.

‡ Mean number of spermatozoa in the last ten ejaculates divided by the collection interval in days.

0.9) and 15.5×10^9 (S.E. ± 0.4), respectively. The DSP-T/g of net testis weight was similar to that found in Exp. 2 (24.2×10^6 versus 23.5×10^6). Sperm output was significantly correlated with DSP-T ($r = 0.65$, $P < 0.05$) and with paired testis weight ($r = 0.70$, $P < 0.05$). There was no significant correlation between DSP-T and DSP-E₃ ($r = 0.25$). Sperm output equalled 95% of the DSP-T and 103% of the DSP-E₃.

Considering all three experiments, the overall mean DSP-T/g of testis

TABLE 4

VARIATION IN EPIDIDYMAL SPERM RESERVES BETWEEN RIGHT AND LEFT TESTES, AND AMONG BOARS HAVING BEEN ON A 72-HR SEMEN COLLECTION SCHEDULE AND KILLED 24 HR AFTER THE LAST SEMEN COLLECTION

Yorkshire boars			Lacombe boars		
Boar	Sperm numbers (10^9)		Boar	Sperm numbers (10^9)	
	Right epididymis	Left epididymis		Right epididymis	Left epididymis
1	95.5	97.3	7	99.3	108.9
2	91.3	104.2	8	77.8	73.2
3	52.1	65.4	9	60.3	62.2
4	88.1	94.5	10	64.2	67.7
5	66.3	71.9	11	73.0	88.5
6	64.7	71.6	12	119.0	76.8
Mean	76.3	84.2		82.3	79.6

was 24.2×10^6 (S.E. ± 0.4) for the Yorkshire boars and 24.8×10^6 (S.E. ± 0.4) for the Lacombe boars.

DISCUSSION

Evidence from Exps. 1 and 2 suggests that only sperm numbers in the cauda epididymidis of the boar are reduced by repeated ejaculation (Tables 1 and 2). This agrees with results obtained in bulls and rabbits (Amann & Almquist, 1962a; Kirton, Desjardins & Hafs, 1967). In Exp. 1, DSP estimated from replenishment of epididymal sperm reserves after unilateral castration was about 31% lower than using quantitative testicular histology (Table 1). The sperm output also exceeded the estimate for DSP-E₁. Thus, unilateral castration appears to alter epididymal function or interfere with the release of spermatozoa from the remaining testis. Furthermore, because of the variation in sperm reserves between epididymides within boars (Table 4), it is concluded that unilateral castration or any technique involving ligation of the epididymides (Foote *et al.*, 1965) will not be likely to give reliable results for measuring DSP in individual boars.

In Exp. 2, it was postulated that the mean DSP of the forty-six boars could be determined from the rate of sperm accumulation in the epididymides following depletion. However, in each of Groups I and II (5 and 24 hr after depletion) there were two boars with large epididymal sperm reserves (Text-fig. 1). In fact, three of these four boars had epididymal sperm reserves exceeding those of the boars in Group IV (72 hr after depletion). These four boars affected the slope of the regression line to a considerable extent. Because of this variation, forty-six boars were insufficient to provide an accurate mean value for DSP.

Sperm production and sperm output were of similar magnitude in Exp. 3 (Table 3) suggesting that, in sexually active boars, few if any spermatozoa are absorbed from the epididymis or excreted in the urine. This was further verified by collecting urine from two boars that had been on a regular semen collection schedule. For the first 3 days after the last semen collection, the urine of these boars was devoid of spermatozoa. In sexually inactive rams, it has been shown that there is little resorption of spermatozoa in the epididymis, vas deferens or ampulla. These rams eliminated 88% of the DSP in the urine. However, sperm output in the urine was 'severely depressed for several days' after ejaculates had been collected from the rams (Lino, Braden & Turnbull, 1967).

As transit time of spermatozoa through the epididymis varies among boars, DSP for individual boars can be determined more accurately by quantitative testicular histology than by dividing the epididymal sperm reserves by the mean transit time. Swierstra (1968b, unpublished data) found that the minimum transit time through the epididymides of eight Yorkshire boars ranged from 9 to 13 days. This variation means that the DSP-E₃ of some boars will be underestimated and of others overestimated by using a mean of 10.7 days and this probably accounts for the low correlation between DSP-E₃ and DSP-T. However, a mean DSP for a group of boars can be determined from the epididymal sperm reserves fairly accurately. Only sexually active animals should be

considered, because sperm production and output have to be stabilized. In sexually inactive males, the epididymal sperm reserve increases considerably and this would result in erroneous values for DSP (Singh, 1962). It is postulated that reliable DSP estimates for individual animals can only be obtained by measuring epididymal transit time and epididymal sperm reserves in the same animals. Estimates for DSP so obtained should be comparable to those obtained by quantitative testicular histology.

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REFERENCES

- AMANN, R. P. (1970) *Sperm production rates*. In: The Testis, Vol. I, pp. 433–482. Eds. A. J. Johnson, W. R. Gomes and N. L. VanDemark. Academic Press, New York and London.
- AMANN, R. P. & ALMQUIST, J. O. (1962a) Reproductive capacity of dairy bulls. VI. Effect of unilateral vasectomy and ejaculation frequency on sperm reserves; aspects of epididymal physiology. *J. Reprod. Fert.* **3**, 260.
- AMANN, R. P. & ALMQUIST, J. O. (1962b) Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. *J. Dairy Sci.* **45**, 774.
- AMANN, R. P. & LAMBIASE, J. T. (1969) The male rabbit. III. Determination of daily sperm production by means of testicular homogenates. *J. Anim. Sci.* **28**, 369.
- CHALKLEY, H. W. (1943) Method for quantitative morphologic analysis of tissues. *J. natn. Cancer Inst.* **4**, 47.
- FOOTE, R. H. (1969) *Research techniques to study reproductive physiology in the male*. In: Techniques and Procedures in Animal Science Research. American Society of Animal Science, c/o Q Corporation, Albany, New York.
- FOOTE, R. H., KENNELLY, J. J. & SWIERSTRA, E. E. (1965) Predicting maximum daily sperm production by the testis. *Cornell Univ. Lab. Proc.* **14**, 1.
- GRAY, P. (1964) *Handbook of basic microtechnique*, 3rd edn, p. 91. McGraw-Hill, New York.
- KENNELLY, J. J. & FOOTE, R. H. (1964) Sampling boar testes to study spermatogenesis quantitatively and to predict sperm production. *J. Anim. Sci.* **23**, 160.
- KIRTON, K. T., DESJARDINS, C. & HAFS, H. D. (1967) Distribution of sperm in male rabbits after various ejaculation frequencies. *Anat. Rec.* **158**, 287.
- LINO, B. F., BRADEN, A. W. H. & TURNBULL, K. E. (1967) Fate of unejaculated spermatozoa. *Nature, Lond.* **213**, 594.
- SINGH, G. (1962) Réserves spermatiques épидидymaires chez le verrat. *Annls Biol. anim. Biochim. Biophys.* **2**, 47.
- SWIERSTRA, E. E. (1966) Structural composition of Shorthorn bull testes and daily spermatozoa production as determined by quantitative testicular histology. *Can. J. Anim. Sci.* **46**, 107.
- SWIERSTRA, E. E. (1968a) A comparison of spermatozoa production and spermatozoa output of Yorkshire and Lacombe boars. *J. Reprod. Fert.* **17**, 459.
- SWIERSTRA, E. E. (1968b) Cytology and duration of the cycle of the seminiferous epithelium of the boar; duration of spermatozoan transit through the epididymis. *Anat. Rec.* **161**, 171.
- SWIERSTRA, E. E. & RAHNEFELD, G. W. (1967) Semen and testis characteristics in young Yorkshire and Lacombe boars. *J. Anim. Sci.* **26**, 149.
- VOGLMAYR, J. K., LARSEN, L. H. & WHITE, I. G. (1970) Metabolism of spermatozoa and composition of fluid collected from the rete testis of living bulls. *J. Reprod. Fert.* **21**, 449.
- VOGLMAYR, J. K., WAITES, G. M. H. & SETCHELL, B. P. (1966) Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. *Nature, Lond.* **210**, 861.
- YOUNG, D. C., FOOTE, R. H., TURKHEIMER, A. R. & HAFS, H. D. (1960) A photoelectric method for estimating the concentration of sperm in boar semen. *J. Anim. Sci.* **19**, 20.