

A COMPARISON OF SPERMATOOZA PRODUCTION AND SPERMATOOZA OUTPUT OF YORKSHIRE AND LACOMBE BOARS

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Summary. A procedure is described for measuring daily spermatozoa production (DSP) from quantitative testicular histology. The mean DSP, as determined by this procedure, was 16.5×10^9 for ten Yorkshire boars (av. age 11.2 months) and 17.8×10^9 for ten Lacombe boars (av. age 11.3 months). The spermatozoa output was 88% of the DSP for the Yorkshire boars and 83% of the DSP for the Lacombe boars when semen samples were collected at 48-hr intervals. Spermatozoa output was significantly correlated (+0.54) with spermatozoa production. The two breeds did not differ significantly with respect to DSP, but within breeds certain boars produced more spermatozoa than others ($P < 0.05$). The DSP per gram of testis was 25.1×10^6 for Yorkshire boars and 24.3×10^6 for Lacombe boars ($P > 0.05$). The relative volume of the testes occupied by spermatids with round nuclei did not differ significantly between breeds, among boars within breeds nor between right and left testes. This suggests that for the boar, spermatozoa production is mainly a function of testis size. Results obtained by the procedure for measuring DSP were not significantly influenced by different staining techniques.

INTRODUCTION

Daily spermatozoa production (DSP) and daily spermatozoa output (DSO) have been measured for several species of animals. Daily spermatozoa production is the number of spermatozoa passing from the testis into the caput epididymidis daily. Spermatozoa output generally refers to the number of spermatozoa collected by means of an artificial vagina or by electro-ejaculation. There are many reports in the literature on spermatozoa output but results vary greatly among experiments. Differences in age of the animals, in semen collection techniques, in extra-gonadal spermatozoa reserves at the start of the semen collection period, in degree of sexual preparation before ejaculation and in ejaculation frequencies account for much of the variability of DSO (Hale & Almquist, 1960).

Procedures reputed to measure DSP can be grouped into three categories;

methods employing frequent semen collections, methods employing direct counts of spermatids in homogenized testes, and methods based on quantitative testicular histology. As discussed by Hale & Almquist (1960) and Amann & Almquist (1962) the first method does not measure DSP but rather DSO. Amann & Almquist (1962) first suggested that DSP could be calculated from the number of spermatids present in suspensions of homogenized testes. The total number of spermatids counted was divided by the life-span of the counted spermatids in order to derive an estimate of DSP. Using this technique, the DSP for twenty-five mature dairy bulls was found to average 13.1×10^9 (Almquist & Amann, 1961). Two approaches have been used to measure DSP from quantitative testicular histology. Amann & Almquist (1962) counted the number of spermatids in a series of seminiferous tubules and used these data combined with information on testis composition and specific knowledge of the duration of spermatogenesis as the basis for calculating DSP. The DSP of twelve dairy bulls averaged 12.76×10^9 . Kennelly & Foote (1964) used the total volume of primary spermatocytes in the testes and the life-span of primary spermatocytes as the basis for calculating DSP of boars. Swierstra (1966) modified this method by using spermatids instead of primary spermatocytes and by correcting for shrinkage due to histological processing.

The objectives of this research were (1) to compare daily spermatozoa production of Yorkshire and Lacombe boars, (2) to compare their daily spermatozoa production with the actual number of spermatozoa obtained when semen samples were collected at 48-hr intervals, (3) to relate testis characteristics to spermatozoa production and output, and (4) to evaluate certain aspects of the methodology for determining daily spermatozoa production.

MATERIALS AND METHODS

Animals and semen collection procedure

Spermatozoa production and output were studied in ten Yorkshire and ten Lacombe boars. The boars were housed in individual pens in a temperature-controlled barn (January to March). They had free access to water and were hand-fed twice a day. Semen samples were collected by means of a 'dummy' and an artificial vagina (Swierstra & Rahnefeld, 1967). One semen sample was collected every 48 hr for 8 weeks from each of the boars. Immediately after ejaculation, the percentage of motile spermatozoa, spermatozoa concentration, total volume and strained volume of each semen sample were determined. Spermatozoa concentration was determined by optical density (Young, Foote, Turkheimer & Hafs, 1960). The total number of spermatozoa per ejaculate was calculated, taking into account spermatozoa losses in the collection equipment and gelatinous fraction of the ejaculate. Swierstra & Rahnefeld (1967) calculated that these losses equalled 3.28% of the number of spermatozoa present in the collection bottle.

After the last ejaculation all boars were castrated and the weight of each testis, tunica albuginea and epididymis recorded. At castration, the Yorkshire boars were 11.2 months (10.5 to 11.7 months) and the Lacombe boars 11.3 months

(10.3 to 11.7 months) old. The Yorkshires averaged 150 kg and the Lacombe 165 kg in body weight.

Histological procedures

The testes were cut mid-sagittally and pieces of tissue removed from three specified loci. Locus A was chosen near the caput epididymidis, locus B midway between the poles of the testis and locus C near the cauda epididymidis. Tissue samples were fixed in Allen's fixative (Gray, 1958) for 3 days. After fixation the samples were left overnight in 70% ethyl alcohol. The next morning they were dehydrated in a graded series of alcohols and cleared in xylene. Paraffin impregnation (Tissuemat, m.p. 55° C) was carried out under vacuum (50 cm of Hg). Three changes of paraffin were used and the samples were left in each change for 30 min. Histological sections were prepared from tissue representing each locus. Some of the histological slides from each locus were stained by the periodic acid-Schiff-haematoxylin (PAS-haem) technique and some by the Feulgen technique using fast green as a counterstain.

Predicting daily spermatozoa production from quantitative histology

The daily spermatozoa production of the testes (DSP) was determined by the method of Swierstra (1966) using the following formula:

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

The corrected testis volume was obtained by the following formula:

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

The volume % of round spermatid nuclei in the testes was determined by Chalkley's procedure (Chalkley, 1943). One Feulgen stained and one PAS-haem stained section (4 μ thick) were randomly selected from each location for analysis. For each section, two technicians observed 130 randomly chosen microscope fields and recorded the structures at the end of the five pointers in the ocular. This resulted in 7800 hits or recorded structures per testis. The structures were classed as (1) spermatids with round nuclei, (2) other testicular structures, and (3) artifacts. Before analysis of variance all percentages were transformed to arcsin (Snedecor, 1957).

The average volume per round spermatid nucleus was obtained by calculating a weighted mean diameter and substituting this value in the formula for a sphere. This weighted mean diameter was obtained from spermatid diameters in stages 4, 5, 6, 7, 8 and 1 of the cycle of the seminiferous epithelium, and the duration of the respective stages (Swierstra, 1968). In each stage, twelve whole nuclei were measured in each of five boars. The nuclei were measured at right angles and the two measurements averaged. All measurements were

made on Feulgen stained sections cut at 12 μ . The weighted mean diameter of a round spermatid nucleus was 5.07 μ and the mean volume 68.20 μ^3 .

Studies with [^3H]methylthymidine have shown that the life-span of boar spermatids with round nuclei is 6.34 days (Swierstra, 1968).

It was assumed that the volume of the mediastinum equalled 1% of the testis volume. This value was reported by Amann (1962a) for the volume of the mediastinum of bull testes.

Shrinkage due to histological processing

Shrinkage of testicular tissue due to histological processing was determined in a separate experiment involving an additional five Yorkshire and five Lacombe boars. These boars were of a similar age (10.6 to 11.6 months) to the boars used for studying spermatozoa production and output. Eight pieces of tissue were removed from each of the twenty testes. The tissue samples, similar in size to the pieces ultimately used for histological analyses averaged 0.81 ± 0.01 g. The eight tissue samples from each testis were randomly assigned to four groups and fixed in Allen's fixative (Gray, 1958). Groups I, II, III and IV were fixed for 3, 6, 6 and 9 days, respectively. Groups I, II and IV were dehydrated and embedded as outlined under *Histological procedure*. Group III tissue samples were processed as above except that they were left for 60 min in each of the three changes of Tissuemat (50 cm of Hg vacuum). Before fixation, the volume and density of each tissue sample was determined by Archimedes' principle. After paraffin impregnation, the volume of each sample was again determined by Archimedes' principle but absolute alcohol was substituted for water as the displacement fluid.

RESULTS

Semen characteristics

The mean values for certain semen characteristics are presented in Table 1. More detailed information on the semen characteristics of the boars has been presented elsewhere (Swierstra & Rahnefeld, 1967). For each semen component the difference between breeds was not significant. However, boars within breeds differed ($P < 0.01$) with respect to total volumes, strained volumes, gel volumes, spermatozoa concentrations and total spermatozoa per ejaculate. The total number of spermatozoa per ejaculate increased gradually during the first 5 weeks of the collection period and then remained relatively constant (Text-fig. 1). This increase in spermatozoa output was associated with an increase in spermatozoa concentrations and a slight decrease in ejaculate volumes. Only the last ten ejaculates were used to calculate spermatozoa output for comparison with DSP. The spermatozoa output/48 hr was $28.9 \pm 1.1 \times 10^9$ for the Yorkshire boars and $29.7 \pm 1.1 \times 10^9$ for the Lacombe boars. These values include a 3.28% correction for spermatozoa losses in the collection equipment and gelatinous fractions of the ejaculates (Swierstra & Rahnefeld, 1967).

Testis density and shrinkage due to histological processing

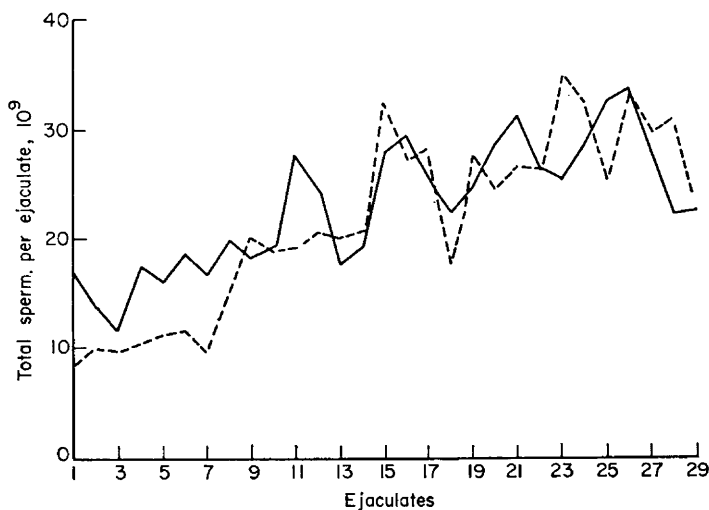
The mean testis density was 1.041 for Yorkshires and 1.038 for Lacombes.

TABLE 1
SEMEN CHARACTERISTICS OF YORKSHIRE AND LACOMBE BOARS EJACULATED
AT 48-HOUR INTERVALS

Characteristic	Yorkshire boars		Lacombe boars	
	Mean	Range*	Mean	Range*
No. of boars	10		10	
No. of ejaculates	290		290	
Age at start of collection (months)	9.4	8.6 to 9.8	9.4	8.4 to 9.8
Total volume (ml)	235	150 to 299	244	166 to 339
Strained volume (ml)	186	118 to 254	192	125 to 282
Gel volume (ml)	49	31 to 73	51	41 to 71
Sperm concentration (10^6 /ml)	147	78 to 219	123	68 to 178
Motile sperm. (%)	74	68 to 80	62	43 to 72
Total sperm./ejaculate† (10^9)	23.8	20.7 to 28.3	22.3	18.8 to 33.7

* Figures are on a per boar basis (av. for twenty-nine ejaculates/boar).

† Corrected for spermatozoa losses in collection equipment and gel.



TEXT-FIG. 1. Mean spermatozoa output for ten Yorkshire boars (—) and ten Lacombe boars (---), ejaculated at 48-hr intervals for an 8-week period.

Differences in testis density between breeds and between right and left testes were not significant. However, among animals within breeds testis density differed ($P < 0.01$).

Shrinkage of testis tissue was affected by the duration of fixation and paraffin impregnation (Table 2). Less shrinkage occurred when tissue was fixed for 9 days rather than for 3 or 6 days. Leaving testis tissue in paraffin for a total of 180 min rather than 90 min resulted in more shrinkage ($P < 0.01$). Although the extent of shrinkage did not differ between the two breeds, the differences among animals within breeds were highly significant.

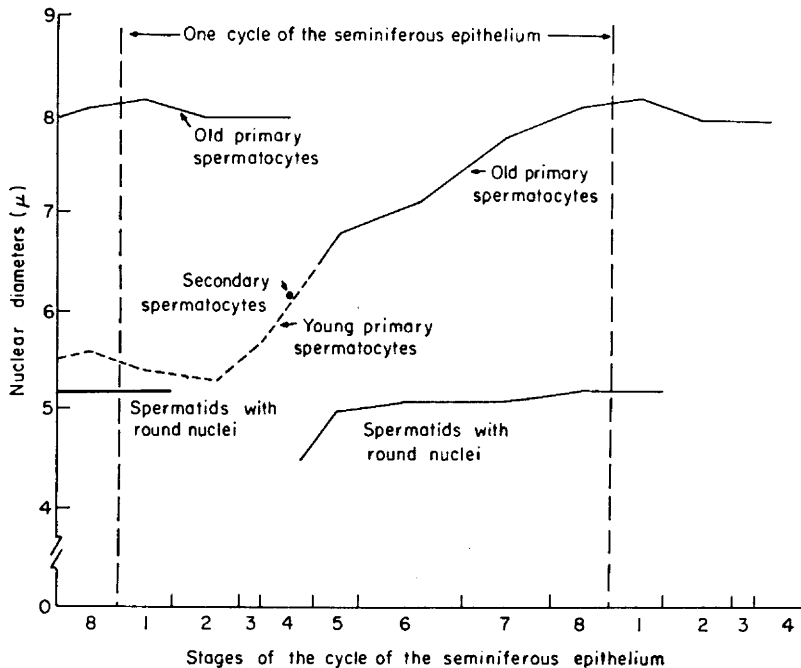
TABLE 2
SHRINKAGE OF TESTICULAR TISSUE DUE TO DIFFERENT METHODS OF HISTOLOGICAL PROCESSING

Method of processing	% of original vol.† (mean ± S.E.)	Sources of variation‡					
		B	A:B	T	BT	TA:B	O: TAB
I	47.6 ± 0.6	24	49**	6*	(0)	8	13
II	47.7 ± 0.6	33	42**	(0)	(0)	(0)	25
III	46.3 ± 0.6	16	44**	(0)	(0)	6	34
IV	49.2 ± 0.7	21	14	1	(0)	(0)	65

* $P < 0.05$; ** $P < 0.01$.

† % of original volume remaining after fixation, dehydration and paraffin impregnation.

‡ Breeds (B) = 2 (fixed), Animals within breeds (A:B) = 5 (random), Testes (T) = 2 (fixed), Observations (O) = 2 (random). Figures are percentages of total variance. Negative variance components are denoted by (0) and are not included in the computation of the percentages.



TEXT-FIG. 2. Nuclear diameters of certain porcine germ cells during the eight stages of the cycle of the seminiferous epithelium. Sixty nuclei of each cell type were measured per stage.

A schedule of 3 days fixation and 90 min of paraffin impregnation (three changes of 30 min each) was adopted for processing the testis tissue from the boars used for studying DSP. Thus, the value 0.476 was used in all computations to correct for shrinkage due to histological processing.

Testis composition and staining techniques

The testes of the Yorkshires and Lacombe contained 2.38 ± 0.15 and $2.31 \pm$

0.12% of round spermatid nuclei, respectively. This difference and differences among animals within breeds (Tables 3 and 4) were not significant. The respective values for the percentage of round spermatid nuclei in right and left testes were 2.33 ± 0.04 and 2.36 ± 0.03 , and in locations A, B and C 2.32 ± 0.06 , 2.41 ± 0.05 and 2.30 ± 0.05 . Differences between right and left testes and among the three locations were not significant.

TABLE 3

SPERMATOZOA PRODUCTION AND SPERMATOZOA OUTPUT OF YORKSHIRE BOARS

Boar	Testis	Testis wt. (g)	Tunica albuginea wt. (g)	Corrected testis volume* (ml)	Round spermatid nuclei in testis (%)	DSP		Sperm. output per 48 hr† (10 ⁹)	Sperm. output as % DSP
						per testis (10 ⁹)	per boar (10 ⁹)		
84	R	348.1	21.2	147.9	2.32	8.0	17.0	36.7	108
	L	407.2	20.8	174.8	2.22	9.0			
173	R	360.4	19.6	154.2	2.40	8.6	17.8	33.1	93
	L	388.5	20.5	166.5	2.39	9.2			
352	R	339.5	24.3	142.6	2.22	7.4	15.8	27.0	85
	L	342.2	22.9	144.4	2.50	8.4			
402	R	275.7	15.8	117.6	2.33	6.4	13.7	24.1	88
	L	288.5	15.7	123.4	2.56	7.3			
441	R	342.0	18.8	146.2	2.54	8.6	17.2	31.1	90
	L	359.5	19.2	154.0	2.41	8.6			
635	R	300.7	18.8	127.5	2.23	6.6	13.4	25.5	95
	L	311.1	19.1	132.1	2.22	6.8			
714	R	313.5	17.5	133.9	2.14	6.7	13.3	19.0	71
	L	270.5	18.9	113.8	2.50	6.6			
742	R	442.5	26.8	188.1	2.08	9.1	18.1	32.9	91
	L	409.0	28.9	171.9	2.24	9.0			
921	R	375.1	19.4	160.9	2.35	8.8	19.5	29.8	76
	L	402.4	23.2	171.6	2.68	10.7			
941	R	343.1	19.5	146.4	2.69	9.2	18.8	30.1	80
	L	362.0	20.9	154.3	2.67	9.6			
Mean		349.1	20.6	148.6	2.38	8.2	16.5	28.9	88

* Values obtained by the formula given in Materials and Methods section.

† Based on the number of spermatozoa in the last ten ejaculates, and corrected for losses in the collection equipment and in the gelatinous fraction of the ejaculate.

The analysis of variance indicated that there was no significant difference between the techniques of the technicians. The percentage of round spermatid nuclei in Feulgen-stained sections was 2.31 ± 0.04 and in PAS-haem-stained sections 2.38 ± 0.03 ($P > 0.10$).

Nuclear diameters of spermatids showed little variation from the time spermatids were formed until the elongation of the nuclei (Text-fig. 2).

Testis size, spermatozoa production and spermatozoa output

The mean testis weight of the Yorkshire boars was 349.1 g and that of the Lacombe boars 389.1 g (Tables 3 and 4). This difference between breeds was not significant. However, animals within breeds differed with respect to paired

TABLE 4
SPERMATOZOA PRODUCTION AND SPERMATOZOA OUTPUT OF LACOMBE BOARS

Boar	Testis	Testis wt. (g)	Tunica albuginea wt. (g)	Corrected testis volume* (ml)	Round spermatid nuclei in testis (%)	DSP		Sperm. output per 48 hr† (10 ⁹)	Sperm. output as % DSP
						per testis (10 ⁹)	per boar (10 ⁹)		
143	R	404.0	31.4	169.0	2.20	8.6	17.5	29.3	84
	L	429.1	33.2	179.6	2.14	8.9			
382	R	331.6	22.8	140.1	2.25	7.3	14.7	24.7	84
	L	350.0	24.2	147.8	2.14	7.4			
392	R	544.2	30.2	233.2	2.32	12.6	22.8	29.2	64
	L	466.2	31.0	197.4	2.22	10.2			
421	R	304.5	21.3	128.5	2.46	7.3	14.8	39.6	100
	L	320.5	23.7	134.6	2.38	7.5			
452	R	420.0	23.9	179.7	2.25	9.4	20.2	39.6	98
	L	470.1	27.0	201.1	2.30	10.8			
471	R	391.8	23.2	167.2	2.14	8.3	16.7	32.2	96
	L	398.8	26.4	169.0	2.13	8.4			
591	R	343.9	19.2	147.3	2.42	8.3	16.4	32.7	100
	L	347.5	19.8	148.7	2.33	8.1			
676	R	346.6	22.6	147.0	2.53	8.6	18.4	24.3	66
	L	399.3	24.5	170.1	2.48	9.8			
679	R	342.5	19.0	146.8	2.58	8.8	16.9	23.8	70
	L	357.0	18.9	153.4	2.26	8.1			
1012	R	409.8	20.5	176.6	2.17	8.9	19.2	32.0	83
	L	420.0	18.8	182.1	2.43	10.3			
Mean		389.9	24.1	165.9	2.31	8.9	17.8	29.7	83

* Values obtained by the formula given in the Materials and Methods section.

† Based on the number of spermatozoa in the last ten ejaculates, and corrected for losses in the collection equipment and in the gelatinous fraction of the ejaculate.

TABLE 5
ANALYSIS OF VARIANCE OF PREDICTED DAILY SPERMATOZOA PRODUCTION

Source of variation*	d.f.	Mean square	Expectation of mean square	F Ratio	Level of probability
B	1	4.23	$\sigma_w^2 + 2\sigma_{a:b}^2 + 20\sigma_b^2$	1.47	$P > 0.10$
T	1	1.30	$\sigma_w^2 + \sigma_{a:t:b}^2 + 20\sigma_t^2$	3.10	$P > 0.05$
BT	1	0.48	$\sigma_w^2 + \sigma_{a:t:b}^2 + 10\sigma_{b:t}^2$	1.14	$P > 0.25$
A:B†	18	2.87	$\sigma_w^2 + 2\sigma_{a:b}^2$	6.83	$P < 0.01$
AT:B	18	0.42	$\sigma_w^2 + \sigma_{a:t:b}^2$		

* Breeds (B) = 2 (fixed); Testes (T) = 2 (fixed); Animals (A) = 10 (random); Colon denotes a within classification.

† F ratio was calculated by using AT:B as denominator mean square.

testis weight ($P < 0.01$). The tunica albuginea accounted for 6.1% of the testis weight.

The DSP of the Yorkshires was $16.5 \pm 0.7 \times 10^9$ and that of the Lacombe was $17.8 \pm 0.8 \times 10^9$. Although this difference between the breeds was not significant, DSP differed significantly among boars within breeds (Table 5).

Paired testes weight was correlated ($r = +0.90$, $P < 0.01$) with DSP. The DSP can be estimated from paired testes weight by the following regression equation in which testes weight is expressed in grams and DSP in thousand millions:

$$\text{DSP} = 2.3 + 0.02 (\text{paired testes weight})$$

Based on the last ten ejaculates for each boar, spermatozoa output/48 hr was $28.9 \pm 1.1 \times 10^9$ for the Yorkshires and $29.7 \pm 1.1 \times 10^9$ for the Lacombe. Spermatozoa output equalled 88% of the DSP for the Yorkshires and 83% of the DSP for the Lacombe (Tables 3 and 4). Spermatozoa output was significantly correlated ($r = +0.54$, $P < 0.05$) with spermatozoa production.

The DSP/g net testis weight (gross testis weight minus the weight of the tunica albuginea) for the Yorkshire and Lacombe boars was $25.1 \pm 0.4 \times 10^6$ and $24.3 \pm 0.3 \times 10^6$, respectively. This difference between breeds was not significant.

DISCUSSION

Few reports have been published in which spermatozoa production was compared with spermatozoa output. In the present study the DSP of 11-month-old Yorkshire and Lacombe boars was 16.5×10^9 and 17.8×10^9 , respectively. Spermatozoa output was 88% of the spermatozoa production in the Yorkshire boars and 83% of the spermatozoa production in the Lacombe boars when semen samples were collected at 48-hr intervals. Kennelly & Foote (1964) measured DSP and DSO in six 2-year-old Yorkshire boars. The DSO of these boars was 19.7×10^9 and this equalled 63% of the DSP as measured by quantitative testicular histology. However, these researchers did not consider shrinkage due to histological processing and correction for this would undoubtedly lower the estimate of DSP.

Amann & Almquist (1962) compared DSO and DSP of twelve dairy bulls. The DSO averaged 4.81×10^9 when these bulls were ejaculated six times per week with intensive sexual preparation. This represented only 42% of the mean DSP of 11.49×10^9 . Losses of spermatozoa in the artificial vagina were not accounted for. Swierstra (1966) measured spermatozoa production and output of seven 18-month-old Shorthorn bulls. The DSP as measured by quantitative testicular histology, was 5.3×10^9 and 25% of these spermatozoa were ejaculated when semen samples were collected at 48-hr intervals, using electro-stimulation. Thus, in boars a much larger percentage of the spermatozoa produced by the testes are ejaculated as compared to bulls.

In the present study, testis weight and DSP were highly correlated ($r = +0.90$, $P < 0.01$). One reason for this high correlation is that testis weight is one of the two main variables used when calculating DSP.

The DSP/g gross testis weight averaged 23.1×10^6 for the twenty boars. Values for Shorthorn bulls of 14.8×10^6 (Swierstra, 1966), for dairy bulls of 17.7×10^6 (Amann & Almquist, 1962) and for rams of 12.2×10^6 (Ortavant, 1959) have been reported. There are many reasons for these differences in DSP/g of testis among species. The duration of the cycle of the seminiferous

epithelium is 8.6 days for boars (Swierstra, 1968), 13.5 days for bulls (Hochcreau, Courot & Ortavant, 1964) and 10.4 days for rams (Ortavant, 1956). Furthermore, one stem spermatogonium probably produces ninety-six spermatozoa in boars as contrasted to sixty-four spermatozoa in rams and bulls (Ortavant, 1959; Amann, 1962b).

When DSP, as determined by the procedure outlined in this paper, is compared with DSO then it is imperative that the time interval between the two measurements be taken into account. In the boar it takes about 11 days for spermatids with round nuclei to pass into the caput epididymidis as spermatozoa. An additional 10.2 days is needed for these spermatozoa to traverse the epididymis (Swierstra, 1968). Thus, for the boar the two measurements are separated by a 21.5-day interval. In animals with rapidly growing testes this results in a low estimate for DSO as a percentage of DSP. Theoretically, in normal animals the DSO should not be larger than the DSP. For one boar (No. 84) spermatozoa output exceeded the estimated production. The testes of this boar appeared to be normal. Possibly this aberrant value was caused by sampling errors and does not reflect testicular degeneration.

The relative volume of the testes occupied by spermatids with round nuclei did not differ significantly between testes, among boars within breeds, or between breeds. Similarly, Swierstra (1968) found that for boars there generally was a constancy of the relative frequencies of the stages of the seminiferous epithelium. Thus, since the duration of spermatogenesis is constant within a species, DSP is primarily a function of testis size in normal boars.

The procedure outlined for measuring DSP can be used for species other than the boar. However, the constants in the formulae vary among species and they would have to be determined for each species separately.

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