THE SPLIT EJACULATE OF THE BOAR: CONTRIBUTIONS OF THE EPIDIDYMIDES AND SEMINAL VESICLES

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Summary. The epididymal and seminal vesicular contributions to split-ejaculate fractions from boars were analysed for sperm concentration, glycerylphosphorylcholine (GPC), total-N, ethanol-soluble and insoluble N, citrate, zinc and haemagglutinin. The same components were also determined in epididymal plasma (EP), vesicular secretion (VS) and whole seminal plasma (SP). Isoelectric focusing of protein patterns was studied in the fractions.

With the exception of haemagglutinin, the components were present to a major extent in either VS or EP and in lower concentrations in the other secretion. The parameters in VS or EP were positively correlated among themselves and negatively correlated with most of the parameters of the other fluid. The correlation coefficients were not significant in all cases for individual animals, but the degree of significance was greater for the over-all correlations.

The EP components were mainly secreted in the first three or four fractions, but occasionally from fraction four onwards. Those of VS were emitted during the entire ejaculation, the maximum occurring in the sperm-rich fraction or the immediately succeeding fraction. The first fractions were devoid of VS components in only one case.

The majority of the EP proteins could be identified electrophoretically in the sperm-rich fractions, but the protein patterns in the other fractions were similar to those of VS. The results are discussed and compared with previous findings.

INTRODUCTION

The semen of the boar differs from that of the ram and bull in several ways; the volume is large, the sperm concentration very low and ejaculation occurs in several waves, the process occupying several minutes (McKenzie, Miller & Bauguess, 1938; Glover & Mann, 1954). In the boar ejaculate, it is possible to

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distinguish three major fractions, designated pre-sperm, sperm-rich and post-sperm according to the sperm distribution. Most of the physiological and biochemical studies on the characteristics of fractionated (split) ejaculates have been limited to those of the main fractions (McKenzie *et al.*, 1938; Glover, 1955; Archipovec, 1961; Johnson, Pursel & Gerrits, 1969a, b), but in several cases semen has been collected according either to volume or time sequence (Glover & Mann, 1954; Bialy & Self, 1959; Einarsson, Crabo & Ekman, 1970). The majority of the constituents which have been studied originate in the seminal vesicle secretion (VS), and have enabled an assessment to be made of the contribution of this gland to the various fractions (Glover & Mann, 1954) and to the final volume of the ejaculate (Mann & Glover, 1954).

Little attention has been given to the contribution of the epididymal plasma (EP) to the various fractions and in particular to the sperm-rich fraction which is so frequently used in insemination.

The chemical composition of EP, VS and seminal plasma (SP) has been studied by various investigators. The EP contains high levels of glyceryl-phosphorylcholine (GPC) (Dawson, Mann & White, 1957; Mann, 1959) and a considerable proportion of the nitrogenous material was soluble in ethanol (Mann, 1959). The major constituents of the VS are citrate (Humphrey & Mann, 1949), ergothioneine (Leone & Mann, 1951; Mann & Leone, 1953), inositol (Mann, 1951, 1954) and protein (Mann, 1959; Bournsnel, Baronos, Briggs & Butler, 1972). Bournsnel & Coombs (1966) reported the presence of a powerful haemagglutinin in boar VS, later identified as a mixture of proteins ('protein H') of high isoelectric point by Nelson & Bournsnel (1966), Bournsnel & Briggs (1969) and Lavon, Briggs & Bournsnel (1973). Bournsnel *et al.* (1972) confirmed a high level of zinc in boar VS (first observed by Bertrand & Vladesco, 1921), and also found a highly significant correlation between the zinc concentration and those of other substances characteristic of this fluid, particularly citrate and total N. The SP, a mixture of the secretions of various accessory sex glands, contained large quantities of all these components.

In addition, Lavon & Bournsnel (1971), Lavon, Bournsnel & Briggs (1972) and Lavon *et al.* (1973) have studied and characterized, by gel disc electrophoresis and isoelectric focusing on acrylamide gel, many discrete protein components of VS and EP. Striking differences were shown between the proteins arising from the seminal vesicles and the epididymides.

This work was undertaken to extend the results of the above studies and to investigate the contribution of many of the VS and EP components to the composition of the split ejaculate fractions, paying special attention to the sperm-rich fraction.

MATERIALS AND METHODS

Collection of semen and fluids

Split ejaculate fractions, collected at 30-sec intervals from four adult boars (A, B, F and W), housed at the Animal Research Station, were immediately strained through muslin to remove the gel. The volume of each fraction was measured and the spermatozoa were counted in a haemocytometer. Whole

semen was also collected from the same animals, at a different time, and the gel was separated.

The VS was obtained from seminal vesicle glands freshly excised from slaughterhouse material as described by Bournsnel, Hartree & Briggs (1970). Epididymal semen from freshly excised epididymides from the same carcasses was collected by the method of Lasley & Bogart (1944) as described by Walton (1957).

The spermatozoa were separated by centrifugation (10,000 *g* for 20 min at room temperature) and the supernatants were stored at -25°C until required. The VS was similarly stored, after centrifugation to remove cell debris.

Determination of total and ethanol-soluble nitrogen

Total N was determined by the micro-Kjeldahl procedure described by Bournsnel *et al.* (1970). Soluble N was similarly determined in supernatants of samples precipitated by addition of 2 vols redistilled ethanol (Bournsnel, Briggs, Lavon & Butler, 1973b). The ethanol-insoluble N was determined by difference.

Determination of glycerylphosphorylcholine

The supernatants of the ethanol-precipitated samples used for total N were taken to dryness and the residue was dissolved in water. Aliquots were hydrolysed in *N*-HCl at 100°C for 20 min (Brooks, 1970) and the liberated choline was precipitated by ammonium reineckate (B.D.H. Chemicals, Poole) according to the method of Glick (1944). The absorbance of the final acetone solution of the choline reineckate salt was measured at 345 nm in an SP 500 spectrophotometer (Pye Unicam, Cambridge).

Determination of citrate

This was determined by a modification (Lindner & Mann, 1960) of the method of Speck, Moulder & Evans (1946).

Determination of zinc

This was determined after suitable dilution as described by Bournsnel, Briggs, Lavon & Butler (1973a) in an SP 90 atomic absorption spectrophotometer (Pye Unicam, Cambridge).

Haemagglutinin titration

This was carried out by serial doubling dilution in buffered saline (0.15 *M*-NaCl) using a 1% (v/v) suspension of washed sheep red cells (Bournsnel, 1967).

Isoelectric focusing

This was performed on polyacrylamide-ampholine plates of pH 3 to 10 (L.K.B. Instruments, South Croydon) which were prepared and used as described by Lavon & Bournsnel (1971) and by Lavon (1972).

Statistical analysis of the results

The correlation coefficients between the various parameters were calculated according to the method described by Bliss (1967).

RESULTS

Concentration of components in boar vesicular secretion, epididymal plasma and seminal plasma

The mean contents of various components in VS and EP obtained from slaughterhouse material and in whole SP collected from the four boars providing the split ejaculates, together with the ratios between the concentrations of each of the components in the various secretions, are given in Table 1. The mean dilutions of the VS and EP in the SP during ejaculation were found to be $\times 6$ and $\times 30$, respectively.

Table 1. Mean concentrations (mg/100 ml) of GPC, total, ethanol-soluble and -insoluble nitrogen, citrate and zinc in samples of boar epididymal plasma, vesicular secretion and whole seminal plasma

<i>Sample</i>	<i>GPC</i>	<i>Total nitrogen</i>	<i>Soluble nitrogen</i>	<i>Insoluble nitrogen</i>	<i>Citric acid</i>	<i>Zinc</i>
Epididymal plasma						
Mean	2306	565	336	253	21.1	1.02
S.E.M.	236	18	16	25	1.2	0.58
Ratio*	13.10	(1.00)	8.62	(1.00)	(1.00)	(1.00)
Vesicular secretion						
Mean	176	1658	39	1619	756	21.5
S.E.M.	15	91	3	91	36	1.2
Ratio*	(1.00)	2.93	(1.00)	6.40	35.83	21.08
Seminal plasma						
Mean	114	222	27.1	195	153	2.00
S.E.M.	15	58	4.4	54	42	0.59
Ratio*	0.65	0.39	0.69	0.77	7.25	1.96

The seminal plasma samples were obtained from four boars. The epididymal plasma and vesicular secretion samples were obtained from slaughterhouse material.

* Ratios of these concentrations to the lower of the mean concentrations occurring in epididymal plasma or vesicular secretions.

Total quantities of the components in the split ejaculate fractions

The total number of spermatozoa and the total quantities of the components in the split ejaculate fractions are presented in Text-figs 1 to 4 which also give the volumes of the fractions. The concentrations of the components may be calculated from these results. The partial correlations between the various concentrations for the individual animals are given in Table 2 and the over-all correlations are given in Table 3.

Protein patterns in vesicular secretion, epididymal plasma and in the split ejaculate fractions

These isoelectric focusing patterns are shown in Pl. 1, Figs 1 to 14.

PLATE 1

Protein patterns (band numbers on left of Fig. 1) obtained by isoelectric focusing on ampholine-acrylamide plates (pH 3 to 10).

FIG. 1. Boar epididymal plasma.

FIGS 2 to 13. Fractions 1 to 12 of the split ejaculate of Boar A (13/7/71).

FIG. 14. Boar vesicular secretion.

(Facing p. 544)

TEXT-FIG. 1. Number of spermatozoa and total quantities of substances in fractions of a split ejaculate (Boar A, 13/7/71). The vols (ml) of the fractions in order were: 3.0, 47.5, 43.0, 17.0, 39.5, 23.5, 30.0, 20.0, 32.0, 9.0, 23.5 and 7.5.

TEXT-FIG. 2. As Text-fig. 1. Values for Boar B (24/6/71). The vols (ml) of the fractions in order were: 19.5, 16.5, 52.0, 36.0, 34.0, 14.0, 47.0, 26.0 and 15.0.

TEXT-FIG. 3. As Text-fig. 1. Values for Boar F (1/7/71). The vols (ml) of the fractions in order were: 15.5, 41.0, 82.5, 29.0, 22.5, 36.0 and 16.0.

TEXT-FIG. 4. As Text-fig. 1. Values for Boar W (8/7/71). The vols (ml) of the fractions in order were: 8.5, 10.0, 13.0, 31.0, 40.0, 19.0, 43.0, 42.0, 16.5 and 18.0.

Table 2. Partial correlation coefficients between the concentrations of various components determined in the split-ejaculate fractions from four boars

	1 Fraction vol.	2 Sperm concentration	3 GPC content	4 Total nitrogen	5 Soluble nitrogen	6 Insoluble nitrogen	7 Citric acid	8 Zinc	9 No. of tubes titrated
1 Fraction volume		A ± E ± F ± W	0.6439* 0.4968 0.5053 0.4002	-0.7073** -0.5468 -0.3595 0.2457	0.7838*** 0.5807 0.8074* 0.7500**	-0.7333*** -0.4987 0.2275 0.1764	-0.6160* -0.4356 -0.0396 0.1832	-0.6286* -0.3978 0.2004 0.2354	-0.5397 -0.6595 0.0153 0.4566
2 Sperm concentration	A ± E ± F ± W		0.9897*** 0.9493*** 0.9928*** 0.9128***	-0.5110 -0.3308 -0.4454 -0.0767	0.8220*** 0.8863*** 0.9047*** 0.6062	-0.5682 -0.4162 -0.5644 -0.1514	-0.6073* -0.2787 -0.7225 -0.1423	-0.7172*** -0.3404 -0.6033 -0.1497	-0.7640*** -0.0941 -0.6686 -0.1051
3 GPC	A ± E ± F ± W	+		-0.5386 -0.0666 -0.4865 0.2161	0.8312*** 0.9735*** 0.9055*** 0.6307	-0.5939* -0.1601 -0.6042 0.1584	-0.5826* -0.0391 -0.7570* 0.1750	-0.7078** -0.0721 -0.6432 0.1462	-0.7890*** -0.2791 -0.6959 0.2532
4 Total nitrogen	A ± E ± F ± W	±	-0.5386 -0.0666 -0.4865 0.2161	-0.7904*** -0.0603 -0.1571 0.6250	0.7904*** 0.0603 -0.1571 0.6250	0.9962*** 0.9954*** 0.9892*** 0.9960***	0.8746*** 0.8832*** 0.8795*** 0.9781***	0.8623*** 0.9460*** 0.9800*** 0.9921***	0.6617** 0.4204 0.7745* 0.8368***
5 Soluble nitrogen	A ± E ± F ± W	±	0.8312*** 0.9735*** 0.9055*** 0.6307	-0.7904*** -0.0603 -0.1571 0.6250	0.7904*** 0.0603 -0.1571 0.6250	-0.8405*** -0.0356 -0.3000 0.5526	-0.8260*** -0.0832 -0.5434 0.5187	-0.7533*** -0.0083 -0.3435 0.5722	-0.7047** -0.3127 -0.4957 0.7778***
Insoluble nitrogen	A ± E ± F ± W	±	-0.5939* -0.1601 -0.6042 0.1584	0.9962*** 0.9954*** 0.9892*** 0.9960***	-0.8405*** -0.0356 -0.3000 0.5526	0.8904*** 0.8763*** 0.9301*** 0.9848***	0.8695*** 0.9472*** 0.9975*** 0.9936***	0.6849** 0.3909 0.8217* 0.8042	0.6380* 0.9338 0.7743* 0.7604***
7 Citric acid	A ± E ± F ± W	±	-0.5826* -0.0391 -0.7570* 0.1750	0.8746*** 0.8832*** 0.8795*** 0.9781***	-0.8260*** -0.0832 -0.5434 0.5187	0.8904*** 0.8763*** 0.9301*** 0.9848***	0.8720*** 0.8507*** 0.9448*** 0.9699***	0.8720*** 0.8507*** 0.9448*** 0.9699***	0.6380* 0.9338 0.7743* 0.7604***
8 Zinc	A ± E ± F ± W	±	-0.7172*** -0.3404 -0.6033 -0.1497	0.8623*** 0.9460*** 0.9800*** 0.9921***	-0.7553*** 0.0083 -0.3435 0.5722	0.8695*** 0.9472*** 0.9975*** 0.9936***	0.8729*** 0.8507*** 0.9448*** 0.9699***	0.8729*** 0.8507*** 0.9448*** 0.9699***	0.7613*** 0.3953 0.8415* 0.8217***
9 No. of tubes titrated	A ± E ± F ± W	-	-0.7890*** -0.0941 -0.6686 0.1051	0.6617** 0.4204 0.7745* 0.8368***	-0.7047** 0.3127 -0.4957 0.7778***	0.6849** 0.3909 0.8217* 0.8042***	0.6380* 0.2338 0.7743* 0.7604**	0.6380* 0.2338 0.7743* 0.7604**	0.7613*** 0.3953 0.8415* 0.8217***

A, E, F, W = individual boars. +, Significant correlation (negative or positive); -, no trend; ±, high correlation not always significant.

Correlations (those in parentheses not significant for all boars).

Positive: (1,9); (2,3); (2,5); (3,5); (1,2); (1,3); (4,6); (4,7); (4,8); (4,9); (6,7); (6,8); (6,9); (7,8); (7,9); (8,9).

Negative: (2,4); (2,6); (2,7); (2,8).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Over-all correlation coefficients between the concentrations of various components determined in the split-ejaculate fractions from four boars

	Fraction vol.	Sperm concentration	GPC	Total nitrogen	Soluble nitrogen	Insoluble nitrogen	Citric acid	Zinc	No. of tubes titrated
1	0.5089***	0.5089***	0.5066***	0.0004	0.7005***	-0.0900	-0.1169	-0.0431	0.1585
2	0.5066***	0.9696***	0.9696***	-0.3764*	0.7986***	-0.4639***	-0.4993***	-0.5230***	-0.2232
3	0.0004	-0.3764*	-0.3174*	-0.3174*	0.8244***	-0.4107**	-0.4122**	-0.4383***	-0.1810
4	0.0004	-0.3764*	-0.3174*	-0.2613	-0.2613	0.9922***	0.8973***	0.9238***	0.6203***
5	0.7005***	0.7986***	0.8244**	-0.2613	-0.3795**	-0.3795**	-0.4047**	-0.2973	0.1017
6	-0.0900	-0.4639***	-0.4107**	0.9922***	-0.3795**	0.9122***	0.9122***	0.9238***	0.5814***
7	-0.1169	-0.4993***	-0.4122**	0.8973***	-0.4047**	0.9238***	0.8926***	0.8926***	0.5310
8	-0.0431	-0.5230***	-0.4383***	0.9238***	-0.2973	0.9238***	0.5310***	0.6065***	0.6065***
9	0.1585	-0.2232	-0.1810	0.6203***	0.1017	0.5814***	0.6065***	0.6065***	

Correlations (those in parentheses not significant for all boars).
Positive: 1, 2; 1, 3; 1, 5; 2, 3; 2, 5; 3, 5; 4, 6; 4, 7; 4, 8; 4, 9; 6, 7; 6, 8; 6, 9; 7, 8; 7, 9; 8, 9.
Negative: 2, 4; 2, 6; 2, 7; 2, 8; (3, 4); 3, 6; 3, 7; 3, 8; 5, 6; 5, 7; (2, 9); (5, 8).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

In the present study, many of the results include important constituents of boar seminal plasma other than those described by Glover & Mann (1954) and Bialy & Self (1959). Special attention was given to those constituents originating particularly in the EP, such as GPC and ethanol-soluble N or to those from the VS which had not been studied before: total N, ethanol-precipitable N, zinc and haemagglutinin. In addition, the protein patterns of the various fractions were studied in order to identify proteins particularly characteristic of the secretions of the epididymides or the seminal vesicles.

From all the results of the concentrations of the components in VS, EP and SP presented in Table 1, an attempt was made to calculate the dilutions of VS and EP in the SP, although the sources of the secretory glands were different from those of the SP samples. Moreover, none of the substances was unique to one gland, although the relative VS and EP concentrations of the components differed considerably. The SP contains considerable quantities of urethral gland secretion, the composition of which was not examined. The values obtained for the dilutions of VS and EP in SP are in reasonable agreement with those reported by McKenzie *et al.* (1938) and Mann & Glover (1954), but they are greater than those obtained by Einarsson (1971).

The constituents secreted by the two glands can be divided into two groups. In addition to the spermatozoa, the first group, from the epididymis, include GPC and ethanol-soluble N (mainly GPC and free amino acids). The second group, secreted by the seminal vesicles, include citrate, zinc, haemagglutinin, total N and ethanol-insoluble N. The parameters of each group are positively correlated between themselves and negatively correlated with most of those included in the other group. The correlation coefficients are not significant in all cases for individual animals, but the degree of significance is greater for the over-all correlations.

A study of the concentrations of the components found in the split-ejaculate fractions show that for three boars the GPC and ethanol-soluble N were secreted in the first three or four fractions with the spermatozoa. The VS components were emitted throughout the entire ejaculation and, in Boars F and W, the secretory peak was in the sperm-rich fractions. In the work of Glover & Mann (1954), the secretory peak of SV components followed shortly after the occurrence of the spermatozoa. In Boar W, the first fractions were almost devoid of any VS components but in the collections from the other boars their appearance as soon as ejaculation commenced was in agreement with the observations of Bialy & Self (1959). It should also be pointed out that, except for Boar W, some spermatozoa were present from the first fraction onwards and it was difficult to distinguish a pre-sperm fraction.

The time required for the collection of the two ejaculatory waves did not exceed 4 to 5 min. More fractions (twelve) were collected from Boar A than from the others, but the fractions represented four ejaculatory waves. Two of the split ejaculates showed patterns (Boar E and the first two waves from Boar A) similar to those reported by Glover & Mann (1954).

In view of the results obtained in this study, it seems reasonable to conclude

that various animals yield different fractional distribution patterns of the secretions. It seems possible that the results of Glover & Mann (1954), obtained with many collections from one boar, represented a distinctive distribution pattern characteristic of that particular animal.

The electrophoretic distribution of the VS and SP proteins in the fractions of the split ejaculate agreed with those reported earlier by Lavon & Bournnell (1971) and Lavon *et al.* (1972, 1973). The number of proteins in EP (Pl. 1, Fig. 1) was eighteen, and twelve or more of these could be detected in the sperm-rich fraction (Plate 1, Figs 3 and 4). This number was greater than the three proteins obtained by Einarsson *et al.* (1970) using paper electrophoresis at pH 8.6, a pH shown by Lavon & Bournnell (1971) to be unsuitable for boar SP, VS and EP protein differentiation.

From Fraction 4 onwards (Pl. 1, Fig. 5), the patterns become more similar to that of the VS (Pl. 1, Fig. 14). The haemagglutinin (Lavon *et al.*, 1972) could be identified as a scattered band at the basic end of Fraction 1 (Pl. 1, Fig. 2), Fractions 3 to 12 (Pl. 1, Figs 4 to 13) and the VS pattern, but was not identified in Fraction 2 (Pl. 1, Fig. 3). These observations are applicable also to some other VS proteins.

It is clear from these results that, while EP components are found in greater concentration in the sperm-rich fraction than in the other fractions or in SP, the sperm-rich fraction still contains a number of VS components. These observations may be relevant to the study of the protective action of phosphatidyl compounds in cold-shock damage to boar spermatozoa (Butler & Roberts, 1975). The action of phosphatidyl compounds on the zinc-precipitable protein (Bournnell & Roberts, 1974) in boar seminal plasma has been studied by Roberts & Bournnell (1974). The work reported in this paper was completed before the zinc-precipitable protein was discovered; it seems most likely that this protein, arising from the seminal vesicles, must also be present in the sperm-rich fraction.

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