PHOSPHOLIPID COMPOSITION OF BOVINE SPERM FRACTIONS, SEMINAL PLASMA AND CYTOPLASMIC DROPLETS

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Individual phospholipids have been quantified in bull semen (Scott & Dawson, 1968), whole spermatozoa and seminal plasma (Pursel & Graham, 1967). Masaki & Hartree (1962) analysed the total phospholipid content of sperm head and mid-piece–tail fractions from a bull with a congenital sperm defect resulting in separation of the head from the mid-piece before ejaculation. Little is known about the phospholipid composition of sperm fractions from normal bulls. The present study was conducted to determine the phospholipid content of sperm heads, mid-pieces, tails and cytoplasmic droplets to provide a basis for further work on the structure and function of these subcellular systems.

Two ejaculates from each of three normal bulls were combined to obtain a minimum pooled sample of 35×10^9 spermatozoa. This was replicated five times. Volumes of semen containing 10×10^9 and 20×10^9 spermatozoa were removed from the pooled semen sample for phospholipid analysis of the whole spermatozoa and the sperm fractions, respectively. The samples were centrifuged, washed twice with Ca-free Ringer solution containing 1 mM-sodium fluoride, and whole spermatozoa were removed for lipid extraction.

Spermatozoa were separated into head, mid-piece and tail fractions by the method of Mohri, Mohri & Ernster (1965), using sonication for 20 sec at 0° C, and 4-ml samples of seminal plasma were used for lipid extraction. Cytoplasmic droplets were removed from seminal plasma by dilution (1:1) with 0.25 M-sucrose containing 1 mM-EDTA, and were centrifuged at 10,000 g for 1 hr at ambient temperature. Droplets were resuspended and recentrifuged.

Lipids were extracted by the method of Folch, Lees & Sloane-Stanley (1957). Whole spermatozoa, head and mid-piece fractions were cooled to 0° C, sonicated for 5 min in 19 vols of 2:1 chloroform–methanol at 0 to 4° C, agitated for 30 min at room temperature and, after phase separation, solvents were evaporated at 50° C. The volume was adjusted to 10 ml with methanol, samples were flushed with nitrogen, and then sealed and stored at 5° C until chromatography.

Thin-layer chromatography (TLC) plates were prepared with silica gel H that had been washed by the procedure of Parker & Peterson (1965). Plates were 'pre-run' in chloroform–methanol–water (65:25:4) and activated for 1 hr

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at 110 to 120° C. Phospholipids were separated into major classes with chloroform-methanol-acetic acid-water (60:25:8:4; Skipski, Peterson & Barclay, 1962). Appropriate controls were included. Plasmalogens were separated from the corresponding diacyl compounds by a modification of the two-dimensional technique of Horrocks (1968), using HCl fumes to cleave the alk-1-enyl group of the plasmalogen.

After treatment with iodine vapour, phospholipid spots were scraped from the plates and eluted from the silica gel as described by de Bohner, Soto & de Cohan (1965), followed by successive rinses with 2 ml methanol and 2 ml chloroform. Elution solvents were evaporated.

Two phosphorus methods differing in sensitivity were used for lipid analysis. For samples containing 0.25 to 6 µg lipid phosphorus, the method of Chen, Toribara & Warner (1956) was used, except that the digestion was carried out with 0.5 ml of 70% perchloric acid. Parts of two procedures (Marinetti, 1962; Sumner, 1944) were modified and combined to derive another method for samples containing 2.5 to 50 µg phosphorus. The colour reaction was developed with an ammonium molybdate-sulphuric acid-ferrous sulphate solution and percentage transmittance was measured at 660 nm.

Statistically, phospholipids and sperm fractions were analysed as fixed variables, while replicates were considered as random variables. Least significant difference multiple comparisons were made among the various sources of phospholipid.

Sonication produced virtually complete disruption of the cells into head, mid-piece and tail fragments. Careful fractionation resulted in little visible contamination of each fraction with other components, as determined microscopically. The tail fragments sedimented slowly in the sucrose solution. Each fraction was recentrifuged after separation. The tail fraction was essentially free of heads and mid-pieces, but probably contained plasma-membrane and acrosomal material.

Nine phosphorus-containing classes were detected by TLC in all fractions (Table 1). Since the TLC technique did not always distinguish clearly between phosphatidylinositol and phosphatidylserine, these two compounds have been combined into one fraction designated phosphatidylinositol(-serine).

The results in Table 1 are presented as absolute values and as percentages of the recovered phospholipid. The total phospholipid phosphorus (P) content of whole spermatozoa before TLC was 54.0 µg/10⁹ cells. Of the total phospholipid, 40, 24 and 36% were contributed by the head, mid-piece and tail fractions, respectively. Choline phosphatide was the predominant sperm phospholipid, with the head fraction containing significantly more than the tail fraction. The ratio of phosphatidylcholine to phosphatidylcholine was approximately 1:1 in whole spermatozoa and in all sperm fractions.

The level of phosphatidylethanolamine was about double that of phosphatidylethanolamine in sperm heads and mid-pieces, and about equal in sperm tails. The mid-piece fraction was significantly higher ($P < 0.05$) in phosphatidylethanolamine and significantly lower ($P < 0.05$) in sphingomyelin than in the head or tail fraction. These trends are characteristic of mitochondria, as is the relatively high cardiolipin content (Green & Tzagoloff, 1966). Phosphatidyl-

Table 1. Phospholipid composition of whole bovine spermatozoa, sperm fractions, seminal plasma and cytoplasmic droplets

Component	$\mu\text{g P}/10^9$ spermatozoa in				$\mu\text{g P}$ in		Percentage composition†					
	Whole sperm.	Sperm heads	Sperm midpieces	Sperm tails	Seminal plasma (per ml)	Cyto-plasmic* droplets	Whole sperm.	Sperm heads	Sperm midpieces	Sperm tails	Seminal plasma	Cyto-plasmic droplets
Choline phosphatide	31.1	13.5	7.9	9.7	13.9	102.6	61.9 ^{ab}	65.8 ^a	60.6 ^{ab}	54.0 ^{bc}	43.9 ^{cd}	43.4 ^d
Phosphatidylcholine	15.2	6.9	3.7	4.7	8.2	52.8	30.1 ^a	34.0 ^a	28.6 ^a	25.9 ^a	26.3 ^a	22.5 ^a
Phosphatidylcholine	15.9	6.6	4.2	5.0	5.7	49.8	31.8 ^a	31.8 ^a	32.0 ^a	28.0 ^a	17.6 ^a	20.9 ^a
Ethanolamine phosphatide	7.0	2.1	2.6	2.0	6.2	41.5	13.8 ^b	9.8 ^c	19.6 ^a	10.5 ^c	19.5 ^a	17.2 ^{ab}
Phosphatidylethanolamine	5.0	1.4	1.7	1.0	4.2	22.8	9.7 ^{ab}	6.7 ^b	12.8 ^a	5.4 ^b	13.4 ^d	9.8 ^{ab}
Phosphatidylethanolamine	2.0	0.7	0.9	1.0	2.0	18.7	4.1 ^a	3.1 ^a	6.8 ^a	5.0 ^a	6.1 ^a	7.4 ^a
Sphingomyelin	5.7	2.7	0.8	2.8	4.2	37.0	11.5 ^{ab}	13.2 ^a	5.9 ^b	15.9 ^a	13.2 ^a	15.8 ^a
Cardiolipin	3.4	0.8	1.3	1.4	2.8	27.2	6.3 ^b	3.6 ^c	9.9 ^{ab}	6.5 ^{abc}	8.8 ^{ab}	11.5 ^a
Phosphatidylinositol(-serine)†	0.4	0.1	0.03	0.2	1.2	2.0	0.7 ^a	0.4 ^a	0.2 ^a	0.9 ^a	3.6 ^a	1.0 ^a
Lysophosphatidylcholine	0.8	0.7	0.2	0.5	0.7	8.4	1.7 ^a	3.8 ^a	1.8 ^a	2.8 ^a	2.2 ^a	3.8 ^a
Origin	2.0	0.7	0.2	1.9	2.8	16.1	4.0 ^{ab}	3.4 ^{ab}	2.0 ^b	9.5 ^a	8.8 ^{ab}	7.3 ^{ab}
Sum of components	50.5	20.5	13.0	18.5	31.7	234.9	100.0	100.0	100.0	100.0	100.0	100.0
Total phospholipid analysis	54.0	23.7	14.1	21.6	39.4	305.4	—	—	—	—	—	—

The individual values given are the mean of five replicates.

* Not quantitatively recovered from 15 to 17 ml seminal plasma.

† Phosphatidylinositol and phosphatidylserine.

‡ Values with different superscript letters, within phospholipid classes, differ significantly ($P < 0.05$).

inositol(-serine) and lysophosphatidylcholine were found in trace amounts in all sperm fractions and were quantified for the first time in bull semen.

The seminal plasma and cytoplasmic droplets were similar in composition. This may have been due in part to some cytoplasmic droplets remaining in the seminal plasma fraction. The droplets were relatively high in lysophosphatidylcholine. The percentage of choline phosphatide in seminal plasma was significantly lower ($P < 0.05$) than in the spermatozoa.

Most of the previous studies of sperm phospholipids have presented data calculated as μg of lipid phosphorus multiplied by 25, on the assumption that all phospholipids have a similar P content and an average molecular weight of 750. On this basis, the whole sperm total phospholipid content was $1350 \mu\text{g}/10^9$ spermatozoa, a value similar to that of $1377 \mu\text{g}$ obtained by Pursel & Graham (1967). Comparable values reported by other workers using a variety of analytical techniques are 810, 1790 and $2050 \mu\text{g}/10^9$ spermatozoa (Lardy, Winchester & Phillips, 1945; Masaki & Hartree, 1962; Masaki & Tomizuka, 1966).

The total phospholipid content in the seminal plasma was $960 \mu\text{g}/\text{ml}$. This value is similar to the total phospholipid level of $890 \mu\text{g}/\text{ml}$ seminal plasma calculated from the gravimetric data of Komarek, Pickett, Lanz & Jensen (1964) and is approximately three times the value obtained by Pursel & Graham (1967). The difference may be due to a substantial membrane contribution from cytoplasmic droplets (Dott & Dingle, 1968) which were probably present in the seminal plasma in the current study where centrifugation was at $1000 g$ for 15 min. If this assumption is valid, cytoplasmic droplets account for approximately two-thirds of the extracellular lipid phosphorus in bull semen.

In the present report, the distribution of phospholipid between the head, mid-piece and tail fractions was 40, 24 and 36%, respectively. Based on nitrogen content, Mohri *et al.* (1965) reported that the fractions obtained with a similar procedure contributed 52, 18 and 30%, respectively. These proportions differ from the 30% phospholipid in heads and 70% in mid-pieces and tails of congenitally defective spermatozoa with detached heads (Masaki & Hartree, 1962).

These studies have resulted in the quantification of various phospholipids in the different fractions of bull sperm cells and seminal plasma. Significant quantitative differences among fractions were found for several phospholipids. Phosphatidylinositol and/or phosphatidylserine and cardiolipin were identified and these, as well as lysophosphatidylcholine, were quantified for the first time in bull semen.

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