LIPIDS OF PORCINE SPERMATOZOA, SEMINAL PLASMA AND GEL*

R. J. KOMAREK, B. W. PICKETT, E. W. GIBSON AND
R. G. JENSEN

*Department of Animal Industries, University of Connecticut, Storrs,
Connecticut, U.S.A.*

(Received 30th June 1964)

Summary. In forty-one ejaculates from six boars totalling 7.5 l. the spermatozoa, analysed on an individual ejaculate basis where possible, contained an average of 12.0% lipid which was composed of phospholipids (74.7%), cholesterol (12.6%), diglycerides (5.7%), triglycerides (4.5%) and wax esters (2.4%). Only a small portion of the dry matter of seminal plasma was lipid (0.23%), which was composed of phospholipids (64.7%), cholesterol (17.7%), diglycerides (5.8%), triglycerides (5.3%) and wax esters (6.5%). The gel fraction contained 0.32% lipid, composed of 51.6% phospholipid, 28.3% cholesterol, 6.3% diglycerides, 6.0% triglycerides and 7.8% wax esters.

INTRODUCTION

The importance of the lipid composition of cells has increased in view of the growing realization of the role of lipids in cellular structure and function (Locke, 1964). Lipids may also play a central role in the cells' ability to resist the effects of certain stress conditions (Martin, 1963). Limited data are available concerning the lipids of boar semen. For example: Mann (1954) reported that boar semen contained 6 mg of lipid phosphorus per 100 ml of semen, Dallam & Thomas (1952, 1953) isolated a lipoprotein fraction from spermatozoa and Hartree & Mann (1959) found plasmalogen and Dawson, Mann & White (1957) glycerylphosphorylcholine in seminal plasma. More comprehensive information on the lipid composition of semen from the ram and bull is available. Ram spermatozoa contained 7 to 9% lipid, which was primarily composed of phospholipid (plasmalogen 55 to 60%, sphingomyelin 5.5%), with some cholesterol (8%), hydrocarbons (2%) and perhaps small quantities of glycerides and wax esters (Lovern, Olley, Hartree & Mann, 1957). Bull spermatozoa contained 12% lipid, which was principally phospholipids (73.3%) and cholesterol (14.5%), with smaller quantities of diglycerides (6.5%), triglycerides (3.1%) and wax esters (2.6%) (Komarek, Pickett, Lanz & Jensen, 1964b). Bovine seminal plasma contained 1.35% lipid composed of the same lipid classes, in similar proportions, as found in bovine spermatozoa (Komarek *et al.*, 1964b).

* Scientific contribution No. 95, Agricultural Experiment Station, University of Connecticut, Storrs.

The purpose of this investigation was to obtain information on the lipids of the various fractions of boar semen as well as its variation in composition among boars and in some cases among ejaculates.

MATERIALS AND METHODS

Semen was collected from six boars of the Yorkshire breed with an artificial vagina and a female in oestrus as a teaser. The gel was separated from the fluid portions of the semen during collection, using a polyethylene bottle (500 ml capacity), from the top of which a small sac, composed of fine glass wool with a single layer of cheese cloth on either side, was suspended and held in place by a rubber stopper. The bottle was attached to the artificial vagina with a short glass tube which penetrated the rubber stopper. The fluid portion of the semen was consequently separated from the gel during ejaculation and resulted in little contamination of the gel by fluid portions of the ejaculate. The concentration of spermatozoa was estimated using a haemocytometer counting chamber. The gel-free semen was centrifuged at 25,000 *g* for 30 min, after which the supernatant seminal plasma was carefully drawn off, and the cells and plasma stored at -20°C . The seminal plasma was thawed, shell frozen, freeze-dried and stored under nitrogen at -20°C , which allowed, during extraction, the use of more manageable volumes of solvent.

In order to determine the dry matter content of seminal plasma and spermatozoa an aliquot of semen was centrifuged, the seminal plasma drawn off, dried in an oven at 115°C and weighed; the spermatozoa were washed with distilled water, dried and weighed (Komarek *et al.*, 1964b). The dry weights of aliquots of gel and semen were similarly determined. The cells used for dry weight determinations were washed because the residual seminal plasma left with the cells after centrifugation and separation would cause considerable error in dry weight if it were not removed. The cells used for lipid analysis were not washed because the residual seminal plasma contained an insignificant amount of lipid and washing would involve greater error due to loss of lipid from the cells (Hartree & Mann, 1959).

Prior to extraction of the lipids, the spermatozoa were insonated (treated with ultrasonic vibrations) in a mixture of chloroform-methanol (2:1, v/v) (Komarek *et al.*, 1964b) while the freeze-dried seminal plasma was reconstituted to 1/5 its original volume with distilled water before extraction. The frozen gel was crushed, mixed in a Waring blender with methanol until finely dispersed and then chloroform was added to make a solvent ratio of 2:1 (v/v) chloroform-methanol. To obtain the lipids, the spermatozoa, seminal plasma and gel were extracted with chloroform-methanol (2:1, v/v), followed by purification of the extracts by the procedure of Folch, Lees & Sloane-Stanley (1957) using 0.05 M-KCl as wash solution. The solvents were then removed in a rotary vacuum evaporator at 45°C and the residues were filtered through a sintered glass filter (fine grade) using chloroform-methanol (2:1, v/v). Separation of the extracts into major lipid classes was accomplished by thin-layer chromatography (TLC) and the quantities of each class estimated gravimetrically (Komarek, Jensen & Pickett, 1964a). Identification of each lipid

class was based on analytical methods outlined previously (Komarek *et al.*, 1964a).

RESULTS

Some of the characteristics of the semen, consisting of a total of forty-one ejaculates and amounting to more than 7.5 l. are presented in Table 1. While some difficulty was encountered in estimating the number of cells in a few ejaculates, due to microscopic gel particles, the incorporation of a filter in the collection vessel produced, in most cases, good separation of the gel from the fluid portions of the semen. In the few exceptions, the interference of the gel probably added to the variation found in cell numbers.

TABLE 1
SOME CHARACTERISTICS OF PORCINE SEMEN

Characteristics	No. ejaculates	Mean	Standard deviation	
			Boars*	Ejaculates within boars
Age (months)	—	23	24.6	—
Ejaculate vol. (ml)	41	186	219	58.4
Gel (% ejaculate)†	41	23	3.16	6.97
Cells/ml (10 ⁶) (gel-free semen)	39	212	123	137
Dry weight				
Semen (g/100 g)‡	41	4.9	3.00	0.96
Seminal plasma (g/100 g)	41	4.6	2.87	1.08
Gel (g/100 g)	40	10.8	2.08	1.08
Cells (mg/ml semen)	41	8.0	5.59	2.88
Cells (µg/10 ⁶)	39	50	40	36

* Six animals.

† Assuming a specific gravity of 1.

‡ Minus gel.

The lipid content as well as the distribution (%) of phospholipids, cholesterol, diglycerides, triglycerides and wax esters in the lipid extracted from spermatozoa, seminal plasma and gel of boar semen are presented in Tables 2, 3 and 4, respectively. The majority of the specific determinations of the various classes of sperm lipid represents duplicate analyses on individual ejaculates. These are summarized and statistically described in Table 2. Although all the major lipid classes present in spermatozoa were found in both seminal plasma and gel, spermatozoa contained a much higher percentage of lipid on a dry weight basis.

DISCUSSION

The boars used in this study represented two age groups. One of the oldest and the largest boar averaged 424 ml semen per ejaculate which is more than double the overall average. The gel fraction accounted for approximately one-fourth of the total ejaculate, agreeing with the data presented by Maule (1962). The average number of spermatozoa (212 million/ml) was double the average reported by Maule (1962) but within the range of values (25 to 1000 million/ml)

given by Anderson (1945). The gel-free semen contained on average 4.9% dry matter, which falls within the range of values given by Mann (1954). The least amount of variation was found in the dry matter content of the gel indicating

TABLE 2
LIPID COMPOSITION OF PORCINE SPERMATOZOA

Criteria	No. determinations*	Mean	Standard deviation	
			Boars†	Samples within boars
Total lipid (g/100 g)‡	21	12.0	3.58	1.77
Phospholipids (%)§	23	74.7	2.90	4.34
Cholesterol (%)§	23	12.6	1.33	1.38
Diglycerides (%)§	23	5.7	1.44	1.26
Triglycerides (%)§	23	4.5	2.08	2.74
Wax esters (%)§	23	2.4	1.21	1.46

* All determinations were an average of duplicates, except 6.

† Average of six boars.

‡ Lipid per 100 g of dry matter.

§ Per cent of total recovered (average recovery 97.3%, s.d. = 7.59).

TABLE 3
LIPID COMPOSITION OF PORCINE SEMINAL PLASMA

Boar	No. ejaculates	Total lipid (g/100 g*)	Percentage of total recovered				
			Phospholipid	Cholesterol	Diglycerides	Triglycerides	Wax esters
A	12	0.29	66.2	19.3	2.9	6.0	5.5
B	9	—	64.4	19.0	3.8	6.0	6.6
C	12	0.25	71.3	14.0	2.4	4.1	8.4
D and F	5	0.15	56.8	18.4	14.2	5.2	5.4
Mean		0.23	64.7	17.7	5.8	5.3	6.5

* Lipid per 100 g of dry matter.

TABLE 4
LIPID COMPOSITION OF PORCINE SEMINAL GEL

Boar	No. ejaculates	Total lipid (g/100 g*)	Percentage of total recovered				
			Phospholipid	Cholesterol	Diglycerides	Triglycerides	Wax esters
A	12	0.29	33.6	40.4	8.5	8.7	8.7
B	8	0.34	55.0	22.6	6.7	6.2	9.6
H†	13	0.34	66.3	21.8	3.8	3.0	5.1
Mean		0.32	51.6	28.3	6.3	6.0	7.8

* Lipid per 100 g of dry matter.

† Represents composite from six boars.

the constancy of the water content and indirectly indicates the effectiveness of the filtering device used to separate the gel during ejaculation.

The composition of the lipid did not seem to vary greatly between boars.

The average total lipid content of boar spermatozoa (12.0%) was the same as that found in bovine spermatozoa (Komarek *et al.*, 1964b). There were notable variations in the percentage of various lipid fractions of individual ejaculates. On several occasions diglycerides and triglycerides have displayed considerable increases in their relative percentages. Although we have collected no parallel data on fertility or stress-resistance, it is interesting to speculate on the significance of these variations relative to changes in the physiological capabilities of the cell. The average distribution of the major lipid classes in porcine spermatozoa lipid was similar to that in bovine spermatozoa but slightly higher phospholipid and lower cholesterol percentages were present (Komarek *et al.*, 1964b).

Seminal plasma did not contain sufficient lipid to analyse each ejaculate, therefore the seminal plasma lipid from each boar, where possible, was pooled and analysed (Table 3). Although the percentage of total lipid in seminal plasma from boars A and B differed markedly, the composition of the lipid was remarkably similar. Considerable variation was found in total lipid and lipid composition in the last two determinations (Table 3). Boar seminal plasma contained less than one-sixth the lipid as found in the bovine species. Thus it would appear in the case of the boar that the accessory sex gland secretions are almost devoid of lipid. The distribution of the major lipid classes, phospholipids and cholesterol, is close to that of bovine seminal plasma. However, in the boar, there were slightly larger quantities of diglycerides and triglycerides, with substantially greater quantities of wax esters.

On a dry weight basis gel contained slightly more lipid than seminal plasma. The gel lipid was also composed of a higher proportion of neutral lipids. Undoubtedly some of the lipid extracted from the gel samples originated from the other semen fractions which, because of their initial contact, could not be completely separated.

The lipids generally associated with cell membranes in animal cells are mostly phospholipids, with some cholesterol (Locke, 1964). Since these are the predominant lipids of porcine spermatozoa, it is therefore possible that the lipids extracted from the spermatozoa are a reflection of the lipids associated with the membranes of that cell. It is to be hoped that information concerning the composition of the membranes of a cell will give an indication of the factors important to the stability of the cell.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service Research Grant GM 08738 from the National Institutes of Health, Division of General Medical Sciences.

REFERENCES

- ANDERSON, J. (1945) *The semen of animals and its use for artificial insemination*, p. 20. I.A.B., Imperial Bureau of Animal Breeding and Genetics, Edinburgh.
- DALLAM, R. D. & THOMAS, L. E. (1952) Chemical composition of mammalian sperm. *Nature, Lond.* **170**, 377.
- DALLAM, R. D. & THOMAS, L. E. (1953) Chemical studies on mammalian sperm. *Biochim. biophys. Acta*, **11**, 79.

- DAWSON, R. M. C., MANN, T. & WHITE, I. G. (1957) Glycerylphosphorylcholine and phosphorylcholine in semen and their relation to choline. *Biochem. J.* **65**, 627.
- FOLCH, J., LEES, M. & SLOANE-STANLEY, G. H. (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J. biol. Chem.* **226**, 497.
- HARTREE, E. F. & MANN, T. (1959) Plasmalogen in ram semen and its role in sperm metabolism. *Biochem. J.* **71**, 423.
- KOMAREK, R. J., JENSEN, R. G. & PICKETT, B. W. (1964a) Quantitative gravimetric analysis of bovine semen lipids by thin-layer chromatography. *J. Lipid Res.* **5**, 268.
- KOMAREK, R. J., PICKETT, B. W., LANZ, R. N. & JENSEN, R. G. (1964b) Lipid composition of bovine spermatozoa and seminal plasma. *J. Dairy Sci.* **47**, 531.
- LOCKE, M. (1964) (ed.) *Cellular membranes in development*. The 22nd Symposium of the Society for the Study of Development and Growth. Academic Press, New York.
- LOVERN, J. A., OLLEY, J., HARTREE, E. F. & MANN, T. (1957) The lipids of ram spermatozoa. *Biochem. J.* **67**, 630.
- MANN, T. (1954) *The biochemistry of semen*, p. 32. Methuen, London.
- MARTIN, I. C. A. (1963) Effects of lecithin, egg-yolk, fructose and period of storage at 5° C on bull spermatozoa deep-frozen to -79° C. *J. Reprod. Fertil.* **6**, 441.
- MAULE, J. P. (1962) *The semen of animals and artificial insemination*, p. 264. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.