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MALDI-TOF mass spectrometry as a simple tool to determine the phospholipid/glycolipid composition of sperm: Pheasant spermatozoa as one selected example

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ABSTRACT

Cellular membranes are composed of highly variable lipid molecules, mainly cholesterol and phospholipids (PLs). The cholesterol moiety and the saturation degree of the fatty acyl residues of PL determine the fluidity of the membrane, which is particularly important for sperm because they have to undergo characteristic membrane-dependent processes (acrosomal exocytosis and fusion with the oocyte). Glycolipids are an essential part of the membrane surface acting as key mediators in the interactions of sperm with components of the female genital tract. Although the lipid composition of many mammalian spermatozoa has already been determined, the lipid composition of avian spermatozoa has scarcely been investigated.

Using spermatozoa extracts of the ring-necked pheasant (*Phasianus colchicus*) as a selected example, this work demonstrates that matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a simple and fast method to determine spermatozoal lipid compositions. The lipid compositions of pheasant spermatozoa have not yet been investigated. In addition to common membrane (primarily diacyl) PL (sphingomyelin, phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine), remarkable variation of different sulfoglycolipids (sulfogalactocerebrosides) was identified. This is in strong contrast to all other animal species investigated so far which nearly exclusively contain the sulfoglycolipid seminolipid (sulfogalactoalkylacylglycerol).

We emphasize that the MALDI MS approach allows the characterization of sulfoglycolipids of sperm within a few minutes without the necessity for previous chromatographic separation.

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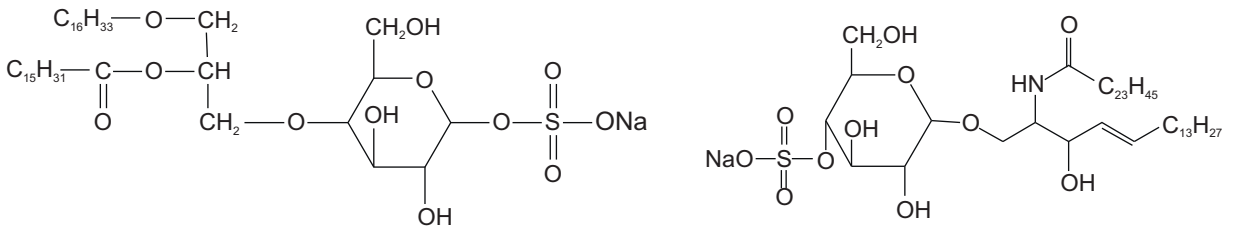

Fig. 1. Chemical structures of seminolipid (left) and sulfatide (right). The seminolipid possesses normally saturated fatty residues whereas the sulfatide is characterized by moderately unsaturated residues.

1. Introduction

Artificial insemination is a major technology for breeding domestic fowl such as the chicken and turkey. As a result, knowledge of the lipid composition of bird spermatozoa is largely restricted to these species as they provide sufficient amounts of semen for the respective analyses. Compared to mammalian spermatozoa that have extremely high proportions of docosahexaenoic (22:6) and docosapentaenoic acid (22:5), i.e. polyunsaturates of the *n*-3 series, the phospholipids (PLs) of avian spermatozoa mainly possess arachidonoyl (20:4) and docosatetraenoyl (22:4) acyl residues, i.e. polyunsaturates of the *n*-6 series (for review see Kelso et al., 1997; Surai et al., 1998). It has also been shown in previously investigated avian spermatozoa that they contain much smaller amounts of alkyl- or alkenyl-ether lipids (plasmalogens) than mammalian sperm—particularly from ruminants (Parks and Lynch, 1992; Schiller et al., 2003; Fuchs et al., 2007a, 2009a). In addition to common PL and cholesterol or cholesterol derivatives, sulfoglycolipids are also important constituents of membrane lipids. Mammalian sulfoglycolipids comprise two major members, sulfatide and seminolipid (Fig. 1). Sulfatide is a major lipid component of the myelin sheath, whilst seminolipid is synthesized in spermatocytes and maintained in the subsequent germ cell stages (Honke et al., 2004). Sulfatide and seminolipid play crucial roles in myelin function and spermatogenesis, respectively (Zhang et al., 2005). At present, nothing is known about the molecular structure of avian sperm glycolipids which are known to differ from mammalian sperm glycolipids in their physico-chemical properties, including their retardation factors (RF) in thin-layer chromatography (TLC) and their lipid phase behaviour observed using differential scanning calorimetry (Parks and Lynch, 1992).

Our current knowledge of avian sperm lipids is primarily based on classical chromatographic separation of the individual lipid species from spermatozoa and seminal fluid extracts, and the determination of their respective fatty acyl residues by gas chromatography/mass spectrometry (GC/MS) (Peterson and Cummings, 2006).

Matrix-assisted laser desorption and ionization mass spectrometry (MALDI MS) (often but not necessarily coupled with a time-of-flight (TOF) mass analyzer), particularly in combination with high-performance thin-layer chromatography (HPTLC), is a powerful tool that allows determining intact lipid molecules and provides important information, including the type of linkage between the apolar residues and the glycerol backbone of the PL (Leßig et al., 2004; Fuchs et al., 2009a). This information is lost if GC/MS is used because saponification of the samples is required in order to obtain the free fatty acids. Thus, MALDI MS permits the distinction between ester and alkyl/alkenyl ether lipids, the contribution of which may influence the physico-chemical properties of membranes. Furthermore, it is possible to analyze characteristic lipid oxidation products as detailed for spermatozoa of Ruminantia (Fuchs et al., 2007a).

To our knowledge, the lipid/phospholipid composition of semen has not been examined previously in pheasants. Therefore, we started analyzing pheasant sperm lipid compositions by HPTLC coupled to MALDI MS (Fuchs et al., 2007b), and this paper forms a short report of the first results. Our results emphasize that sulfoglycolipid composition can be determined directly from total extracts without the need for separation. Finally, we provide evidence that sulfatide but not seminolipid is present in pheasant spermatozoa.

2. Materials and methods

2.1. Animal husbandry

Ring neck pheasants (*Phasianus colchicus*) of mixed genetic stock (Holme Farm Hatcheries, Wokingham) were reared from one-day old chicks at the Game and Wildlife Conservation Trust HQ, Hampshire. For the first 8 weeks (commencing in early May), birds were housed in groups of 30 in indoor pens (1.8 m × 1.5 m) under dim light conditions within a semi-intensive brooder hut system. After 2 weeks of age, birds were provided daily access to outdoor pens with wire floors (3 m × 1.5 m) and fed following standard rearing practice. At 8 weeks of age, the birds were sexed and transferred to one of two outdoor single-sex pens (30 m × 27 m) with access to grass and provided with grit, feed and water *ad libitum*. At 47 weeks of age (i.e. at sexual maturity; Ohlsson et al., 2002), the males were euthanized and dissected as part of a series of experiments. To determine sperm lipid composition, which was only one experimental aspect, semen samples were removed from the distal end of the deferent duct (see Section 2.3).

2.2. Chemicals

All chemicals for sample preparation and all solvents (chloroform, methanol, isopropanol, ethanol, triethylamine and acetonitrile) were obtained in the highest commercially available purity from Fluka Feinchemikalien