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Lipidomics of equine sperm and seminal plasma: Identification of amphiphilic (O-acyl)-ω-hydroxy-fatty acids

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ABSTRACT

Using a nontargeted lipidomics analysis of equine sperm and seminal plasma, we were able to characterize a diverse array of individual lipids including ethanolamine and choline ether lipids and seminolipids essential to membrane raft function. We also detected, for the first time in sperm, the presence of (O-acyl)-ω-hydroxy-fatty acids (OAHFA) with up to 52 carbon chain lengths, which were localized to the head and not the tail of sperm. The only previous identification of OAHFAs has been in meibomian glands and their sebaceous secretions. The identities of these lipid amphiphiles were validated both by high-resolution mass spectrometry and by tandem mass spectrometry (<1 ppm mass error), which identified the fatty acid (FA) and hydroxy-FA components of individual OAHFAs. The amphiphilic and surfactant properties of these unique lipids could provide an interface between the complex lipid layers of the acrosome and the aqueous environment of the suspending seminal plasma. The potential roles of OAHFAs in orientation of critical proteins in the acrosomal membrane also remain to be explored with these new findings. Another unique finding of our lipidomics study was that phosphatidylethanolamines with mono- or di-unsaturated FA substitutions are present in seminal plasma but not in sperm suggesting a potential role of these glycerophospholipids in sperm capacitation and protecting sperm cells in the female reproductive tract. In summary, we have identified for the first time, the presence of OAHFAs in sperm and several phosphatidylethanolamines in seminal plasma, suggesting that these complex lipids may play critical roles in sperm function.

1. Introduction

There are currently limited lipidomics data for equine sperm [1–3]. A key observation from multiple mammalian species is that the lipid makeup of sperm is extremely complex, including high concentrations of ether glycerolipids, which contribute to the function of membrane lipid rafts that are essential for male fertility, sperm motility, and direct binding of sperm with the zona pellucida of the oocyte [4–6]. These ether glycerolipids include choline and ethanolamine plasmalogens, as well as seminolipids [7–9], which are only present in sperm and not in other tissues or body fluids. However, individual ether glycerophospholipids and seminolipids have never been characterized in equine sperm. To fully investigate the lipidomics profile of equine sperm and seminal plasma, we used a nontargeted high-resolution mass spectrometric approach. This experimental design minimized bias and generated highly accurate data sets including our identification, for the first time, of cholesterol sulfate and amphiphilic (O-acyl)-ω-hydroxy-fatty acids (OAHFAs) in equine sperm. Amphiphilic OAHFAs have been identified previously in meibomian glands and

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meibum of humans, dogs, rabbits, and mice but not in any other tissue or body fluid [10–16]. Our data suggest that the surfactant properties of OAHFAs may play an essential role in sperm function.

2. Materials and methods

2.1. Semen collection and processing

Stallions (n = 6) had semen collected with an artificial vagina. For separation of intact sperm from seminal plasma, semen was diluted (1:1) with PBS and centrifuged at 800 × *g* for 20 minutes. The supernatant was removed carefully leaving only the intact sperm pellet. The resulting pellet was resuspended in 10 mL of cold PBS and centrifuged a second time (800 × *g*; 20 minutes) before removal of the supernatant and resuspension of the pellet in 1.0 mL of PBS, which was snap frozen in liquid nitrogen for storage before analysis. This procedure does not remove all seminal plasma components from washed spermatozoa because some seminal plasma components are adherent to ejaculated sperm.

For collection of seminal plasma, semen samples were centrifuged at 10,000 × *g* for 10 minutes, and the supernatants were snap frozen after the addition of Halt Protease Inhibitor (ThermoFisher). This procedure ensured that no contaminating sperm were present in the recovered supernatant; however, the use of such a high centrifugal force for recovery of sperm has the potential to damage sperm and result in possible changes in the composition of the recovered plasma membrane components.

For the isolation of heads and tails, sperm were sonicated (Qsonica Sonicator, VWR) on ice with three pulses for 30 seconds. The sonicates were next layered on top of Androcoll (Minitube Int., Tiefenbach, Germany) and centrifuged at 200 × *g* for 10 minutes. The upper layer containing the tail fraction was isolated and snap frozen. Separated sperm heads were isolated at the bottom of the tube after Androcoll separation consistent with prior studies of bovine sperm [17]. The purity of separated heads and tails was estimated on the basis of phase-contrast, microscopic evaluation of the respective populations. Purity estimates for both populations were greater than 90% for both separated heads and tails.

2.2. Sperm lipidomics

After thawing, sperm suspension, sperm head fraction, sperm tail fraction, or seminal plasma were sonicated in 1 mL of water and 1 mL of methanol containing stable isotope internal standards [18–20]. Next, the tubes were vigorously shaken at room temperature for 30 minutes after the addition of 2 mL of methyl-tert-butyl ether. After centrifugation at 3000 × *g* for 10 minutes, 1 mL of the upper organic layer was dried by centrifugal vacuum evaporation and dissolved in 150 μL of isopropanol: methanol: chloroform (4:2:1) containing 15 mM ammonium acetate.

Shotgun lipidomics (5 μL/min) were performed using high-resolution data acquisition (140,000 at 200 amu; 0.3–3 ppm mass error; m/z 200–1400) with an orbitrap mass spectrometer (Thermo Q Exactive) [18–20]. In negative ion electrospray ionization (ESI), the anions of OHFAs were quantitated, and the identities of the fatty acid (FA) and hydroxy-FA composition of individual OHFAs were validated by MS^2 (Fig. 1). For MS/MS analyses, precursor ions were passed with unit mass resolution, whereas the product ions were scanned using high-resolution (140,000 at 200 amu; < 2 ppm mass error) data acquisition.

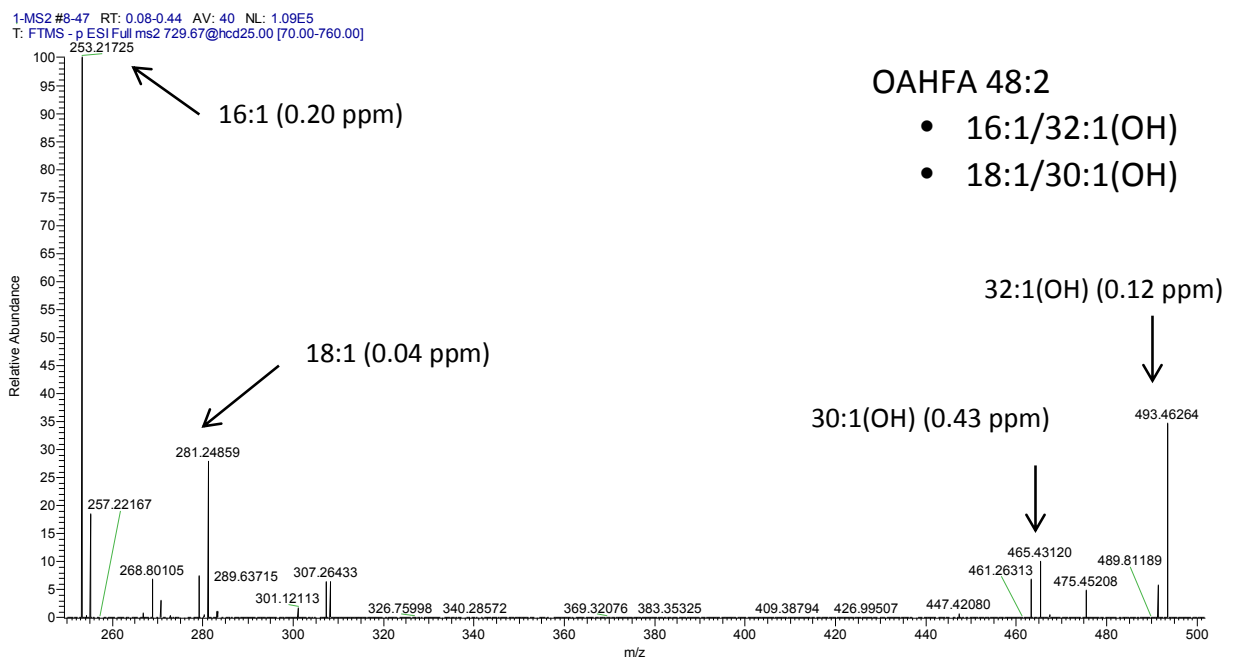

Fig. 1. MS^2 of OAHFA 48:2 validating that this mass included OAHFA 16:1/32:1(OH) and OAHFA 18:1/30:1(OH). OAHFA, (O-acyl)-ω-hydroxy-fatty acids.