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Variation in lipid profiles within semen compartments—the bovine model of aging

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ABSTRACT

Semen lipid composition was examined in young and mature bulls. Given the specific roles of various semen compartments (i.e., seminal fluid, sperm head, and sperm tail) during fertilization, we hypothesized that altered fatty acid and cholesterol composition of a specific compartment might impair semen quality and sperm function. Semen samples were collected from five mature and five young Holstein Friesian bulls during the winter (December–January). Semen was evaluated by computerized sperm-quality analyzer for bulls and was centrifuged to separate the sperm from the seminal fluid. The sperm fraction was sonicated to separate its head and tail compartments. Cold extraction of lipids was performed, and fatty acids and cholesterol were identified and quantified by gas chromatography. Semen physiological features (concentration, motility, and progressive motility) did not differ between mature and young bulls. However, lipid composition within fractions varied between groups, with prominent impairments in the head compartment. In particular, the proportions of polyunsaturated fatty acids, omega-3 fatty acids, and docosahexaenoic acid in the intact sperm; seminal fluid; and sperm head were lower in semen collected from mature bulls than in that from young bulls. The finding suggests an age-differential absorption and/or metabolism through spermatogenesis. Reduced proportions of major fatty acids in mature bulls might reduce membrane fluidity, which in turn might affect the ability to undergo cryopreservation and/or oocyte-sperm fusion through fertilization.

1. Introduction

In modern dairy herds, where reproductive management is on the basis of artificial insemination, the bull is considered a risk factor for fertility [1]. In that respect, the association between bull age and ejaculate characteristics has become highly relevant for the study of reproduction. Ejaculate volume and total sperm number increase with age and have been found to vary among bovine breeds [2–4]. On the other hand, advancing bull age is associated with a decrease in sperm motility and an increase in sperm defects [5] and sperm concentration [6]. In contrast, Hallap et al. [7] reported an increase in sperm motility and membrane integrity and a higher proportion of sperm with normal tail and acrosome morphology as bull age increases. Nevertheless, the mechanism underlying these alterations through aging is not clear.

Lipid and fatty acid composition is associated with semen physiological characteristics, which is considered as important reproductive predictor. For instance, Cerolini et al. [8] found that increased concentrations of free cholesterol, free fatty acids, triacylglycerol, and cholesterol ester are associated with decreased sperm motility and fertility. Similarly, in chickens, reduced male fertility was associated with low lipid content in the seminal fluid and reduced content of polyunsaturated fatty acids (PUFAs) in the sperm membrane, most notably arachidonic acid and docosahexaenoic acid (DHA) [6]. The high concentration of PUFA in sperm makes it extremely vulnerable to

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nonenzymatic oxidation processes. The extent of PUFA (i.e., antioxidation capacity), determined by the presence of antioxidant agents, is reduced through aging in both seminal fluid [6] and epididymal sperm [9] in association with reduced activity of the antioxidant enzyme glutathione peroxidase 1 (GPX1). Cholesterol, the most abundant lipid molecule in the sperm membrane, is also sensitive to peroxidation. Reduced cholesterol concentration in the membrane impairs membrane stability, resulting in premature deterioration of the sperm cells [10]. Taken together, it is suggested that an adequate lipid and fatty acid composition with appropriate antioxidant activity plays a role in protecting the sperm and maximizing its fertilization potential.

Given the specific role and physical properties of each of the semen compartments required for successful fertilization, and due to the uneven distribution of fatty acids between sperm heads and tails [11,12], we hypothesized that altered fatty acid and cholesterol composition of a specific compartment might impair semen features and sperm function. The aim of the present study was to provide a wide lipid profile to determine whether age-related alterations in bull semen are associated with sperm characteristics. For this purpose, lipid composition was examined in different semen fractions: whole sperm, sperm head, sperm tail, and seminal fluid.

2. Materials and methods

2.1. Animals

Experiments were performed at the Israeli Artificial Insemination Center ("Sion," Hafetz-Haim, Israel), in accordance with the Israeli guidelines for animal welfare and experimentation (1994). Semen samples were collected from five mature (7.3 ± 0.6 years) and five young (1.8 ± 0.1 years) Holstein Friesian bulls during the winter to avoid any deleterious effects of summer heat stress. All animals were fertile bulls from the "Sion inseminating bulls" list. Bulls were routinely ejaculated at the same interval, and samples were taken only from the first ejaculate of the collection day. Bulls were fed the same total mixed ration throughout the experiment, containing 68.4% (wt/wt) dry matter (DM), 7.2% (wt/wt) protein, 36.2% (wt/wt) neutral detergent fiber, 20.0% (wt/wt) acidic detergent fiber, 1.45 net energy Mcal/kg, and 3.5 g minerals/kg (NaCl, Ca, and P) on a DM basis.

2.2. Semen collection and initial evaluation of physiological characteristics

Semen was collected routinely once a week ($n = 5$ samples per bull). To eliminate any potential differences in sperm quality due to serial ejaculates, the samples were obtained only once a day. Bulls were mounted on a live teaser and semen was collected into a disposable tube using a heated (38 °C), sterile artificial vagina. The ejaculate was immediately transferred to a nearby laboratory, and the semen was evaluated by computerized sperm-quality analyzer for bulls (SQA-Vb, Medical Electronic Systems, Caesarea, Israel). The physiological characteristics included semen volume (mL), concentration (million sperm/mL), motility (%), progressive motility (%), morphologically normal sperm (%), motile sperm concentration (MSC, million/mL), progressive motile sperm concentration (PMSC, million/mL), and velocity, that is, the average velocity of the progressively motile sperm (μm/s). According to routine procedures at "Sion," samples with a concentration higher than 650×10^6 cell/mL, motility higher than 70%, and progressive motility higher than 60% were defined as being of good quality.

2.3. Sperm handling

At each collection, 2 mL of the total volume of collected semen was centrifuged ($800 \times g$) for 10 minutes at room temperature to separate the sperm from the seminal fluid. The supernatant (i.e., seminal fluid) was collected and kept at −20 °C until further analysis. The pellet was resuspended and washed twice in 1 mL physiological solution (saline; 0.9% NaCl in double-distilled water, wt/wt) and centrifuged again to remove any remaining seminal fluid. The pellet from one tube was suspended in 200 μL saline and used for lipid profile analysis, whereas the pellet from the second tube was suspended in 2 mL saline and subjected to separation into sperm head and tail fractions.

2.4. Separation of spermatozoa into head and tail compartments

Spermatozoa were separated into head and tail compartments as previously described by Zalata et al. [13] with minor modifications. Briefly, samples were sonicated for 2 minutes at maximal power (Misonix Microson XL2000

Table 1
Fatty acid composition of bulls' rations.

Fatty acid	Mol%
c8:0	0.06
c12:0	0.21
c14:0	0.66
c15:0	0.23
c16:0	20.7
c16:1	0.85
c17:0	0.22
c18:0	4.04
c18:1	17.7
c18:2n6	32.5
c18:3n3	6.3
c20:0	0.63
c20:1	0.35
c20:2	0.3
c20:4n6	0.29
c20:5n3	0.4
c21:0	0.2
c22:0	0.8
c22:1	1.3
c22:2	0.2
c22:3	0.3
c22:5n3	0.1
c22:6n3	0.3
c23:0	0.3
c24:0	0.8
c24:1	0.3

Values are presented as mol% of each fatty acid from the total identified fatty acids in the sample.