Animal Reproduction Science 160 (2015) 97–104

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effects of feeding omega-3-fatty acids on fatty acid composition and quality of bovine sperm and on antioxidative capacity of bovine seminal plasma

Hakan Gürler[a,*], Oguz Calisici[a,b], Duygu Calisici[a,b], Heinrich Bollwein[c]

[a] Clinic for Cattle, University of Veterinary Medicine Hannover, Germany
[b] Süt Kardesler A.S. Animal Breeding Center, Izmir, Turkey
[c] Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Switzerland

ARTICLE INFO

Article history:
Received 7 June 2015
Received in revised form 29 July 2015
Accepted 30 July 2015
Available online 5 August 2015

Keywords:
Bulls
Fatty acids
Seminal plasma
Total antioxidant capacity
Lipid peroxidation

ABSTRACT

The aim of the present study was to examine the effects of feeding alpha-linolenic (ALA) acid on fatty acid composition and quality of bovine sperm and on antioxidative capacity of seminal plasma. Nine bulls (ALA bulls) were fed with 800 g rumen-resistant linseed oil with a content of 50% linolenic acid and eight bulls with 400 g palmitic acid (PA bulls). Sperm quality was evaluated for plasma membrane and acrosome intact sperm (PMAI), the amount of membrane lipid peroxidation (LPO), and the percentage of sperm with a high DNA fragmentation index (DFI). Fatty acid content of sperm was determined using gas chromatography. Total antioxidant capacity, glutathione peroxidase, and superoxide dismutase activity were determined in seminal plasma. Feeding ALA increased ($P < 0.05$) the docosahexaenoic acid (DHA) content in bulls whereas in PA bulls did not change. PMAI increased after cryopreservation in ALA bulls as well as in PA bulls during the experiment period ($P < 0.005$). LPO of sperm directly after thawing did not change during the study period in ALA group, but decreased in PA group ($P < 0.006$). After 3 h of incubation LPO increased in the ALA group ($P < 0.02$), while LPO did not differ between phases within groups. In conclusion, feeding of neither saturated nor polyunsaturated fatty acids affect the antioxidant levels in seminal plasma. Both saturated as well as polyunsaturated fatty acids had positive effects on quality of cryopreserved bovine sperm, although the content of docosahexaenoic acid in sperm membranes increased only in ALA bulls.

1. Introduction

In mammalian spermatozoa, long-chain polyunsaturated fatty acids of the n-3 family, in particular docosahexaenoic acid (DHA; C22:6n-3), are predominant (Petit et al., 2007; Zachut et al., 2011). These fatty acids are important for sperm membrane integrity, sperm motility and viability. The proportion of omega-3-PUFAs in sperm and seminal fluid decreases with age in bulls. This contributes to rising susceptibility of spermatozoa to cryoinjury (Argov-Argaman et al., 2013). The lipid composition of the plasma membrane is also a major determinant of motility and freezability of sperm among species (Hammerstedt et al., 1990; Parks and Lynch, 1992; Hammerstedt, 1993). Furthermore, it was noticed that lower contents of C20 and C22 PUFAs in the sperm of older bulls (Kelso et al., 1997b) was related to a decrease in the ability to fertilize oocytes. It is suggested by

* Corresponding author at: Institute of Reproductive Biology, University of Veterinary Medicine Hannover, Bünteweg 2, D-30559 Hannover, Germany. Tel.: +49 511 856 7184; fax: +49 511 953 8504.
E-mail address: gurlerhakan@hotmail.com (H. Gürler).

http://dx.doi.org/10.1016/j.anireprosci.2015.07.010
0378-4320/

Argov-Argaman et al. (2013) that such alterations might affect the semen's capacity to successfully undergo the cryopreservation procedures, which are widely used in intensive reproduction management. Animals cannot synthesize n-6 or n-3 fatty acids de novo as they lack the appropriate fatty acid desaturase enzymes and should obtain them from dietary sources (Wathes et al., 2007). Furthermore, fatty acids in the diets are known to affect fatty acid composition of sperm in human (Conquer et al., 2000) and a variety of farm animals (Kelso et al., 1997a,b; Zaniboni et al., 2006; Brinsko et al., 2005; Harrison and Abhyankar, 2005).

The decrease in PUFA content was related to decreased antioxidant levels (GPx and SOD) in seminal plasma of aging bulls during reproductive period (Kelso et al., 1997b). Somatic cells contain several antioxidants, GPx, SOD, and catalase in their cytoplasm. As sperm are devoid of most of this cytoplasm, the main antioxidant source in semen is the seminal plasma (Bilodeau et al., 2000). For example, it was shown that GPx and SOD in seminal plasma play important roles in the inhibition of LPO (Kelso et al., 1996). Previous reports have shown that enzymatic and non-enzymatic antioxidants had positive effects on sperm freezability (Bilodeau et al., 2001; Fernandez-Santos et al., 2007; Bansal and Bilaspuri, 2008). An important positive effect of PUFA in sperm function has been attributed to its effect on fluidity of the plasma membrane (Gholami et al., 2010). In the last fifteen years, in various feeding experiments DHA or its precursors have been supplied to change the fatty acid composition of sperm membrane in order to improve sperm quality and fertility. Several different studies revealed that the fatty acid profile of sperm membrane can be modified with diet and, thus, improvement in sperm quality was demonstrated in a variety of livestock species including chicken (Zaniboni et al., 2006), turkey (Blesbois et al., 2004), boar (Maldjian et al., 2005); buffalo (Adeel et al., 2009) and stallion (Brinsko et al., 2005). However, diets that contain more PUFAs are associated with impaired antioxidant capacity of animal tissues, blood, and semen (Surai et al., 2000a; Castellini et al., 2003). Feeding bulls with a commercial nutriceutical product that contained 25% n-3 (eicosapentaenoic acid [EPA] and DHA) for 12 weeks increased the percentage of live sperm during the supplementation period and improved the motility and progressive motility in fresh but not in frozen sperm (Gholami et al., 2010). After feeding of more PUFA, elevated LPO of sperm and blood were noticed (Surai et al., 2000a; Castellini et al., 2003). Therefore, it has been recommended that the dietary supplementation of PUFAs has to comprise additional antioxidants (Surai et al., 2000a; Zanini et al., 2003; Cerolini et al., 2005). A recent study by Adeel et al. (2009) showed that the feeding of sunflower oil and sunflower seed (rich in PUFA) improves motility and plasma membrane integrity of post-thawed buffalo sperm. However, until now there is no evidence for the transport of PUFAs from the diet to spermatozoa and its effect on seminal levels of antioxidants in Holstein Friesian bulls. Thus, the aim of the present study was to characterize the effects of feeding alpha-linolenic (ALA) acid on fatty acid composition, sperm quality and antioxidative capacity of seminal plasma in Holstein Friesian bulls.

2. Material and methods

2.1. Bulls

Two consecutive experimental periods (P1 and P2) were carried out with the same fat supplementations. There were twenty bulls in both experimental periods (18 Holstein Friesian bulls and 2 Red Holstein bulls). The first experimental period (P1; $n = 10$) was conducted between August and October 2007 and the second period (P2; $n = 10$) was carried out between December 2007 and March 2008. The bulls were housed in an AI station (Masterrind GmbH; Verden, Germany). Two bulls from the first treatment period and one bull from the second treatment period had to be excluded due to general illness not related to fat supplementation. Nine bulls in the ALA group were 3.2 ± 1.0 years and bulls in the PA group were 3.7 ± 0.8 years old.

2.2. Dietary supplementation of bulls

The bulls in both groups were fed with a basal diet consisting of hay-silage (15 kg/day), concentrate and 300 g/day of a mineral feed. The basal ration of the ALA group was supplemented with 800 g coated alpha-linolenic acid per day (net alpha-linolenic acid = 400 g). The fat supplement contained at least 70% PUFA (oleic acid: 10–22%, linoleic acid: 12–18%, alpha linolenic acid: 56–71%). The ration of the PA group was supplemented with 400 g palmitic acid to achieve the same energy density for both groups. This supplement was a fractioned palm fat with a high percentage of palmitic acid (palmitic acid: minimum 75%, stearic acid: minimum 10%, myristic acid: ca. 1.5%, oleic acid: ca. 10%, linoleic acid: ca. 2%, eicosanoic acid (C20:0): ca. 0.5%). Both fat supplements were fed in two equal portions twice a day.

2.3. Semen collection, dilution and freezing

Semen was collected twice a week for 16 weeks (4 weeks before and 12 weeks after the start of the dietary treatment) using an artificial vagina. Concentration (Z2™ COULTER COUNTER® Cell and Particle Counter, Beckman Coulter GmbH, Krefeld, Germany), volume and total sperm number of each ejaculate were determined directly after collection. Progressive motility was estimated using microscopy at 37 °C at 200× magnification. A portion of each ejaculate with a total sperm number of 450×10^6 was diluted to a final concentration of 60×10^6 cells/mL using a Tris-egg yolk based extender. Five milliliters of each ejaculate were kept undiluted for the separation of seminal plasma. After the dilution of ejaculates at 20 °C, they were packaged in 30 French straws. Ten straws were used for sperm analyses before cooling and after cryopreservation. Twenty straws were cooled slowly to 5 °C over a period of 180 min and frozen to −110 °C within 390 s on racks in a freezer (Model K, Hede Nielsen, Horsens, Denmark). Frozen samples were placed directly into liquid nitrogen and stored at least 24 h until analysis.