Theriogenology 81 (2014) 966–973

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Influences of a diet supplemented with linseed oil and antioxidants on quality of equine semen after cooling and cryopreservation during winter

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ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 30 December 2013
Accepted 16 January 2014

Keywords:
Stallion
Seasonal reproduction
Semen quality
Linseed oil
Antioxidants
Polyunsaturated fatty acids

ABSTRACT

Seasonal changes in the reproductive physiology of stallions contribute to a decrease in the quality of frozen-thawed semen during late winter. Changes in the lipid composition of the sperm plasma membrane may contribute to this phenomenon. In the present study, we have, therefore, investigated the effects of adding linseed oil (LO) in combination with antioxidants to the diet of breeding stallions on the motility and membrane integrity of cooled–stored and cryopreserved semen. Starting in November, the diet of LO stallions (n = 6) but not control (C) stallions (n = 5) was supplemented with LO (100 mL once daily) plus an antioxidant (Myostem Protect; Audevard, Clichy, France) for a total of 84 days. Before (November) and at the end of this period (February), ejaculates were processed for cryopreservation (n = 3 ejaculates per stallion) and cooled shipping at 5 °C. Frozen-thawed and cooled–shipped semen was sent to the laboratory for computer-assisted semen analysis of total motility, progressive motility, and velocity parameters (average path velocity [VAP], curved line velocity [VCL], and straight-line velocity [VSL]) and evaluation of membrane integrity. The quality of frozen-thawed semen decreased ($P < 0.05$) from November (e.g., total motility LO 69 ± 3% and C 67 ± 3%) to February (total motility: LO 55 ± 4% and C 59 ± 3%) independent of treatment ($P > 0.05$). A decrease in the velocity parameters VAP, VCL, and VSL was more pronounced in LO stallions than in C stallions (e.g., VSL: November LO 67 ± 1 μm/s, C 64 ± 2 μm/s; February LO 59 ± 2 μm/s, C 63 ± 2 μm/s; interaction month by treatment, $P < 0.05$). In cooled–stored semen, total motility, progressive motility, and membrane integrity were lower in February than in November ($P < 0.001$ for all parameters). Supplementation of the diet with LO and antioxidants attenuated this decrease (e.g., Day 1 of cooled storage = 24 hours after semen collection: total motility in November LO 88 ± 1% and C 87 ± 3%; in February LO 83 ± 2% and C 73 ± 11%; interaction month by treatment: $P < 0.05$). Velocity parameters VAP, VCL, and VSL were significantly lower in February than in November ($P < 0.001$), but this decrease was not affected by treatment. In summary, dietary supplementation of stallions with LO plus antioxidants attenuated a decline in motility and membrane integrity of cooled–stored stallion semen during winter. This may improve the fertility of cooled–shipped semen. In contrast, the treatment did not counteract the decrease in quality of frozen-thawed semen that occurs in late winter.

1. Introduction

The high variability of equine semen for cryosurvival is considered a major constraint for a wider use of

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http://dx.doi.org/10.1016/j.theriogenology.2014.01.021

cryopreserved semen in horse breeding [1]. In addition, even in stallions considered acceptable freezers, a large number of frozen ejaculates do not meet requirements for post-thaw motility [2]. Semen cryopreservation is often performed early in the year, that is, at a time when the stallion has already undergone breeding soundness examination as required for semen export but when the number of mares for insemination is still low. At this time, seasonal changes in male reproductive physiology contribute to the low quality of frozen-thawed semen [3–5].

The phospholipid profile of the equine sperm plasma membrane is similar to that of the boar. It contains high levels of docosapentaenoic acid, an omega-6 polyunsaturated fatty acid (PUFA), and docosahexaenoic acid, an omega-3-PUFA [6]. Docosapentaenoic acid content varies among stallions potentially causing differences in sperm cryosurvival [7]. Feed supplementation with docosahexaenoic acid improved the motility of cooled–stored and frozen-thawed stallion semen [8]. In rabbits, nutritional modification of spermatozoal lipid composition by supplementation of linseed improved membrane integrity and viability of sperm [9]. Linseed oil (LO) provides members of the two families of essential fatty acids, α-linolenic acid, an omega-3-PUFA, and linoleic acid, an omega-6-PUFA. Addition of LO to the diet of breeding stallions may change the lipid composition of their spermatozoa and improve cryosurvival [1,8].

With the freezing rates currently used, intracellular ice crystal formation does not occur in stallion sperm [10]. Nevertheless, extracellular ice formation causes osmotic stress. Sperm membranes also undergo a phase transition from a liquid to a crystalline state during freezing [11]. The related physical stress is a major cause of damage to the sperm plasma membrane [1]. Fluidity of the membrane affects the degree of damage. Modulation of the cholesterol–phospholipid ratio changes the temperature at which phase transition occurs and maintains membrane fluidity at lower temperatures [12]. In pigs, modification of the sperm membrane lipid composition by feed supplementation of LO changed membrane fluidity [13]. On the other hand, PUFAs are substrates for reactive oxygen species (ROS). ROS induce alterations in membrane fluidity, permeability, and the function of ion channels and receptors [14,15]. In bulls, the proportion of omega-3-PUFAs in sperm and seminal fluid decreases with age. This contributes to rising susceptibility of spermatozoa to cryoinjury [16]. Supplementation of omega-3-PUFAs under conditions where either their supply is reduced or lipid peroxidation is increased may improve semen quality.

In the present investigation, we studied effects of LO supplementation to the diet of breeding stallions. In addition, antioxidants were supplemented because an increase in PUFAs rises the sensitivity to ROS [1]. We hypothesized that this supplementation will maintain the quality of cooled–stored and of cryopreserved semen during late winter.

2. Materials and methods

2.1. Animals

A total of 11 Warmblood stallions aged 3 to 17 (7.1 ± 1.3) years were included in this study. All horses were stabled in loose boxes at the semen collection center of the Sachsen-Anhalt State Stud in Prussendorf, Germany. They were ridden or lunged once daily, with the exception of Sunday being a day of rest. Stallions were fed thrice daily. The standard diet was composed of hay, oats (4 kg/animal and day), and a concentrate feed (4 kg/animal and day; High Energy Müsli, Eggersmann, Rinteln, Germany; nutrient composition: crude protein 11.7%, crude fat 5.2%, crude fiber 5.4%, crude ash 5.7%, main sources for crude fat are extraction meals from soybean, linseed, and sunflower seed). Water was freely available. All stallions were active breeding stallions of excellent to good fertility. They were located in a commercial AI center approved by the European Union and meeting requirements of European Council directive 92/65/EEC. The study was done in accordance with Germany legislation on animal experimentation.

2.2. Experimental design

The project lasted from November to February. Stallions were allocated to the LO (n = 6) or control group (C; n = 5), and groups were balanced for age and quality of raw semen (sperm concentration, total and progressive motility, morphology). At the beginning of the project, semen was collected once daily on five consecutive days in all stallions to deplete epididymal sperm reserves. After two days of sexual rest, semen was collected every other day for a total of three collections. All collections were processed for cryopreservation with only the first ejaculate processed for cooled storage at 5 °C and shipped to the laboratory for semen analysis within 24 hours. After collection of the third ejaculate, feed of stallions in the LO group was supplemented with 100 mL of LO once daily (Masterhorse, Schwieberdingen, Germany; containing 60% α-linolenic acid and 15% linoleic acid) plus 30 mL Myostem Protect (Audevard, Clichy, France; contents: magnesium HCL 114 g/L, Promutase 3.3 g/L, Sel-plex 3.4 g/L, vitamin E 83.7 g/L, lysin 83.3 g/L) for a total of 84 days, whereas C stallions received their standard diet, only. Promutase is a patented form of bioavailable superoxide dismutase [17]. Sel-plex is a selenomethionine compound providing selenium in a bioavailable form [18]. During the last 2 weeks of the supplementation phase (February of subsequent year), semen collections were performed as above (5 days of daily semen collection followed by 2 days of sexual rest and collection of three ejaculates every other day for processing of one sample of cooled–stored semen and 3 ejaculates of cryopreserved semen).

2.3. Semen collection and processing

Semen was collected with an artificial vagina (Hannover Model; Minitube, Tiefenbach, Germany). Immediately after collection, the gel fraction of the ejaculate was removed and semen was filtered through sterile gauze. Volume and color were determined and sperm concentration was measured photometrically (Spermacue; Minitube). For cooled transportation, raw semen was diluted with EquiPro extender (Minitube) to a final concentration of 50×10^6/mL. One syringe was filled with 12 mL of the diluted semen without air and closed with a plastic cap. The syringe was