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Original Research

Quality of Fresh, Cooled, and Frozen Semen From Stallions Supplemented with Antioxidants and Fatty Acids

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ABSTRACT

This study assessed the effect of oral supplementation with the primary antioxidants and fatty acids involved in spermatogenesis (L-carnitine, selenium, vitamin E, omega-3, and omega-6) on the seminal quality in fresh, cooled, and frozen semen of stallions (n = 8), using a randomized design. The animals were divided into Group I (n = 4) and Group II (n = 4) for a 30-week experiment. The two groups alternated between nutraceutical supplementation and a placebo over the course of the experiment. Semen collections were performed in two sets: once in the middle of the experiment, before the two groups switched treatments, and once at the end. The volume, appearance, sperm concentration, spermatozoa kinetics, and membrane integrity of fresh semen were evaluated. The spermatozoa kinetics and membrane integrity of cooled (for 24, 36, and 48 hours) and frozen semen were also evaluated. No differences were observed in volume, appearance, and sperm concentration between treatment and control. However, compared with placebo, nutraceutical supplementation increased ($P < .05$) total motility, trajectory speed, as well as plasma and acrosomal membrane integrity in spermatozoa from fresh semen. In cooled semen, nutraceutical treatment also increased ($P < .05$) total motility, speed, and membrane integrity of spermatozoa compared with the control. In frozen semen, supplementation increased ($P < .05$) spermatozoa progressive motility and plasma membrane integrity. Our results suggest a positive, synergistic effect of the antioxidant L-carnitine and selenium on spermatozoa kinetics. Similarly, the increase in plasma and acrosomal membrane integrity could be attributed to higher concentrations of polyunsaturated fatty acids (a key cell-membrane component), combined with the prevention of excess lipid peroxidation by antioxidants. In conclusion, supplementation with nutraceuticals containing fatty acids and antioxidants improved the quality of fresh, cooled, and frozen stallion semen. Therefore, nutraceutical use should increase the success of artificial insemination with cooled and cryopreserved semen.

1. Introduction

The most commonly used technique in the global equine industry for assisted reproduction is artificial insemination with fresh, cooled, or frozen semen. Thus, semen quality is a major determinant of success in horse-breeding programs [1–3].

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High-quality semen is dependent on several major husbandry factors, including proper management, good health care, and sufficient nutrients to meet the needs of reproductive stallions. Unfavorable environmental conditions may affect testicular hormonal secretion and cell differentiation, as well as spermatozoa maturation and transport in the epididymis. Particularly adverse environments negatively influence fertility via causing temporary or permanent testicular degeneration, as well as other disturbances of varying intensities in reproductive tissue [4].

Supplementing the equine diet is a popular management method to improve stallion health and thereby, semen quality. Accordingly, the equine breeding industry has produced commercially available nutraceuticals aimed at optimizing nutrient use in stallions [4]. Some of these supplements are considered essential in equine husbandry; for example, polyunsaturated fatty acids (PUFAs), such as omega-3 and -6, are required to maintain the structure, function, and integrity of the spermatozoa plasma membrane. Because horses cannot synthesize PUFAs from saturated or monounsaturated fatty acids [5,6], these compounds must be added to their diets.

The high PUFA concentration in spermatozoa plasma membranes indicates their high susceptibility to oxidative stress, specifically lipid peroxidation by reactive oxygen species (ROS) [7]. Damage caused to spermatozoa structure and DNA decreases sperm motility and survival [8]. Thus, other major nutraceuticals are antioxidants such as L-carnitine, a compound that is vital in energy metabolism, which acts as a carrier of fatty acids into the internal mitochondrial membrane and facilitating β-oxidation [9,10]. Research on rats has shown that L-carnitine supplementation aid spermatozoa motility and maturation through increasing metabolic function [11].

Also present in semen are the synergistically acting vitamin E and selenium [12]. The fat-soluble vitamin E is considered a potent antioxidant, acting at the cell-membrane level to remove peroxyl radicals [13]. Next, selenium plays a major role in male fertility; in addition to protecting sperm cells through antioxidant action and membrane stabilization, selenium is also indispensable in testosterone synthesis [14].

Several studies have examined how nutraceuticals in equine diets affect sperm parameters and whether they interfere with the quality of stored (cooled or frozen) semen [3,6,15–18]. However, we currently do not know how a variety of nutraceuticals may interact to influence semen quality. Moreover, few studies have compared how fresh and stored semen may differ in their response to nutraceuticals. Thus, we aimed to evaluate how the quality of fresh, cooled, and frozen semen is affected by the combined effects of major antioxidants (L-carnitine, selenium, and vitamin E) and fatty acids (omega-3, omega-6) involved in spermatogenesis.

2. Materials and Methods

2.1. Animals

Eight Mangalarga Marchador stallions were used in a randomized design following the methods described by Brinsko et al. [6]. The animals ranged from 3 to 12 years old, and weighed between 345 and 430 kg. Their semen quality had been previously assessed and found to conform with the Brazilian College of Animal Breeding (CBRA) [19] recommended parameters for fresh semen in terms of total motility (≥60%), strength (≥3), and normal spermatozoa morphology (≥60%). Sperm strength was expressed as the movement speed of spermatozoa in the field, on a scale of 0 (no motion) to 5 (intense motion) [19].

The animals were housed in 20-m^2 stables on the same property, located in Brasília, DF (central Brazil: 15° 45′42.5″ S, 47° 52′ 10.5″ W). Food and water were provided *ad libitum*. The diet consisted of elephant grass (*Pennisetum purpureum*, Schumach, "Napier"), mineral supplementation for equines (Bellforte, Bellman Nutrição Animal, Cuiabá, MT), and 4 kg/day of concentrated ration (Nutrina Equinos Premium 150, Nutrina, Brasília, DF; nutritional composition: 15% crude protein, 2% ether extract, 10% fibrous matter, 13% mineral matter, 1.5% calcium, 0.5% phosphorus). The experiment was conducted during the 2014 and 2015 breeding season (October 2014 to April 2015 in the southern hemisphere). All procedures were approved by the Ethics Committee in Animal Experimentation (CEUA) of the Institute of Biological Sciences at the University of Brasília, under Protocol UnBDoc No. 12,701/2014.

2.2. Experimental Design

The experiment was conducted over 30 weeks, with two phases. Prior to the study, the animals were randomly assigned to Group I (n = 4) and Group II (n = 4). In the first phase (Weeks 1 to 9), along with their standard diet, Group I received 50 mL/d of commercial nutraceuticals (Table 1) administered through an oral drench syringe, whereas Group II were orally administered a placebo (50 mL of saline solution). Semen was collected once a week to control

Table 1
Guaranteed levels (minimum per kg) of Reproductive Garanhões JCR nutraceuticals (VETNIL, Louveira, SP).

Vitamin A	800,850 UI
Vitamin B12	17,292 μg
Vitamin B6	720 mg
Vitamin E	22,000 UI
Folic acid	1,326 mg
Beta-carotene	500 mg
L-carnitine	330.005 g
Glutamine	1,500 mg
Aspartic acid	280 mg
Glutamic acid	2,800 mg
Arginine	28.41 g
Phenylalanine	370 mg
Glycine	4,600 mg
Lysine	740 mg
Omega-3	110 g
Omega-6	55 g
Oleic acid	57.072 g
Proline	2,330 mg
Taurine	1,500 mg
Valine	460 mg
Selenium	150 mg
Zinc	3,303 mg
Copper	574 mg
Chromium	221 mg