Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 69 (2008) 940–945
Theriogenology
www.theriojournal.com

Effect of a dietary antioxidant supplementation on semen quality in pony stallions

K. Deichsel [a,1], F. Palm [b], P. Koblischke [a], S. Budik [a], C. Aurich [a,*]

[a] *Centre for Artificial Insemination and Embryo Transfer, Department for Animal Breeding and Reproduction, University for Veterinary Sciences, Veterinärplatz 1, A-1210 Vienna, Austria*
[b] *Clinic for Obstetrics, Gynecology and Andrology, Department for Animal Breeding and Reproduction, University for Veterinary Sciences, Vienna, Austria*

Received 31 May 2007; received in revised form 16 January 2008; accepted 29 January 2008

Abstract

Lipid peroxidation contributes to the damage of the sperm plasma membrane. In different species, dietary supplementation with antioxidants has been shown to improve semen quality. Therefore, we tested effects of dietary supplementation with antioxidants and L-carnitin on semen quality in Shetland pony stallions ($n = 6$). Semen was collected twice a week over a time period of 16 weeks. From weeks 5 to 12, a special diet for stallions containing a variety of antioxidants (STALLION®, Pavo Pferdenahrung GmbH, Goch, Germany; tocopherol 300 mg/day; ascorbic acid 300 mg/day; L-carnitin 4000 mg/day; folic acid 12 mg/day) was added to the basal diet (hay, mineral supplements, water). Ejaculates were evaluated for total sperm count, semen motility (percentage of totally and progressively motile spermatozoa, longevity for 24 h at 5 °C) and membrane integrity (SYBR-14/PI staining): All values given are means ± S.E.M. No changes in motility, progressive motility and membrane integrity or semen longevity for 24 h were detected. A slight but significant reduction of morphologically abnormal spermatozoa was found (weeks 1–4: 43.7 ± 7.1%; weeks 13–16: 39.4 ± 7.2%, $p < 0.05$). Results show that a supplementary diet with antioxidants in the given concentration and duration does not result in pronounced effects on semen quality of stallions. It is therefore questionable to support stallions with dietary antioxidants as long as they receive an adequately balanced basal diet.

Keywords: Stallion; Semen; Dietary supplementation; Vitamin E; Vitamin C

1. Introduction

Mammalian sperm plasma membranes contain very high concentration of long-chain (C_{22}) polyunsaturated fatty acids. Therefore and because of their inadequate defensive mechanisms they are highly susceptible to lipid peroxidation [1,2]. Spermatozoa have the ability to produce reactive oxygen species (ROS) which have physiological functions in signalling events controlling sperm capacitation, acrosome reaction and sperm-oocyte fusion as long as they are produced in a controlled manner [3]. An imbalance in the production or degradation of ROS may have serious adverse effects on sperm function [4], for example on sperm motility which declines prior to detectable changes in membrane integrity [5]. Oxidative stress appears as a consequence of the extreme ROS production and results in a decrease of intracellular ATP levels which initiates lipid peroxidation in the sperm plasma membrane [6]. To

* Corresponding author. Tel.: +43 1 25077 6400; fax: +43 1 25077 5491.
E-mail address: christine.aurich@vu-wien.ac.at (C. Aurich).
[1] Present address: Dr. Katharina Deichsel, Tierärztliche Klinik für Pferde Mühlen, Münsterlandstraße 42, D-49439 Mühlen, Germany.

0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.01.007

avoid such a ROS overproduction, organisms have developed several defence mechanisms that include an enzymatic defence system (superoxide dismutase, catalase, glutathione transferase, and glutathione peroxidase) and antioxidants (ascorbate, reduced glutathione, urate, vitamin E and β-carotene) [7]. Vitamin E (α-tocopherol) is believed to be the primary component of the antioxidant system of spermatozoa [8] and is one of the major membrane protectants against ROS and lipid peroxidation [9]. It is not synthesized by mammalian cells and once membrane tocopherol is consumed during periods of oxidative stress, cellular lipids are subject to peroxidation which can result in toxic damages [10]. Vitamin C is most effective as an antioxidant in the aqueous phase, and it seems to scavenge free radicals and positive interactions with vitamin E have been suggested [11].

Dietary supplementation with vitamin E, vitamin C, selenium and other antioxidative substances is claimed to be associated with improved antioxidant defense mechanism and prevention of free radical-associated damages in testes and epididymides. Recently an improvement in semen quality (semen motility, longevity, morphology as well as total sperm count) after dietary intake of antioxidants alone or in combination with polyunsaturated fatty acids has been reported in a variety of species [12–18]. It has therefore become popular to add antioxidants to the diet of breeding animals of various species. For the stallion, so far no controlled studies on the effects of dietary supplementation of antioxidants on semen quality have been performed. The objective of this study was thus to evaluate the effects of dietary supplementation with antioxidants on quality of equine semen immediately after collection and on longevity of semen cooled-stored for 24 h.

2. Materials and methods

2.1. Animals

A total of six fertile Shetland pony stallions were used in this study. These animals belonged to the Centre for Artificial Insemination and Embryo Transfer for several years (two animals were born from mares of our experimental herd, four were bought as 3-year-old stallions). At the start of the experiment, the animals were aged between 8 and 16 years (11.0 ± 1.3) and weighed between 150 and 190 kg. From these stallions, ejaculates are collected and examined on a regular basis for breeding or teaching purposes throughout the year (e.g. two to three times per week). The experimental stallions were kept as a group in a spacious stable with access to an outdoor paddock from 7:00 a.m. to 6:00 p.m. As all ponies from our experimental herd, they were kept on a diet of hay (fed twice daily), water and mineral supplements were freely available. Experiments were carried out from September to the end of December.

2.2. Experimental design

Semen was collected twice a week from each stallion over 16 weeks in total. For the first 4 weeks of the experiment feeding of the basal diet (hay, mineral supplements, water) was continued without any supplementation (weeks 0–4). From weeks 5 to 12 a special diet for stallions containing a variety of antioxidants and L-carnitin (STALLION®, Pavo Pferdenahrung GmbH, Goch, Germany) was added to the standard diet. The animals received 15 g daily. The composition of the supplemented diet is shown in Table 1. The last 4 weeks of the experiment, the stallions were again fed their basal diet without any supplementation (weeks 13–16). Ejaculates were evaluated for total sperm count, semen motility (percentage of totally and progressively motile spermatozoa, longevity for 24 h at 5 °C with a CASA system), morphological aberrations of spermatozoa and membrane integrity (SYBR-14/PI staining).

2.3. Semen collection and semen analysis

Semen was collected with an artificial vagina (Hannover model, Minitüb, Tiefenbach, Germany) after exposure of the stallion to a stimulus mare until erection and readiness to mount, followed by mounting of a dummy. Immediately after collection, the gel fraction of the ejaculate was removed and semen was filtered through sterile gauze and volume and colour were determined. The sperm concentration was measured photometrically (SpermaCue, Minitüb). Total sperm count per ejaculate was calculated from volume and

Table 1
Composition of supplementary diet for stallions (STALLION®, Pavo Pferdenahrung GmbH, Goch, Germany) in mg/(stallion day)

Vitamin E	300 mg
Vitamin C	300 mg
L-Carnitin	4000 mg
Folic acid	12 mg
Copper	–
Zinc	–
Manganese	–
Selenium	–