Journal of Equine Veterinary Science 45 (2016) 73–77

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Quercetin Ameliorate Motility in Frozen-Thawed *Turkmen* Stallions Sperm

Afshin Seifi-Jamadi [a], Hamid Kohram [a,b,c,*], Ahmad Zare Shahneh [a], Mahdi Ansari [a], Beatriz Macías-García [d]

[a] *Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*
[b] *Razi Vaccine and Serum Research Institute (RVSRI), Karaj, Iran*
[c] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran*
[d] *Centre for Animal Science Studies (CECA/ICETA), Campus Agrario de Vairão, University of Porto, Vairão, Portugal*

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 17 June 2016
Accepted 17 June 2016
Available online 1 July 2016

Keywords:
Quercetin
Stallion
Sperm
Prooxidant
Reactive oxygen species

ABSTRACT

Overproduction of reactive oxygen species during sperm freeze-thawing cycles leads to membrane lipid peroxidation, DNA damage, motility loss, and subsequent death. This oxidative stress can be alleviated by the addition of some antioxidants to semen extenders prior freezing. The present study was performed to evaluate the in vitro effectiveness of quercetin on stallion sperm freezability. Ejaculates from healthy Turkmen stallions (n = 4), which exceeded minimum standards, were pooled, and aliquots of each pool were diluted in an egg yolk–based extender added with different concentrations of quercetin (0.1, 0.2, and 0.3 mM) and two control groups (positive: base extender + 0.5% ethanol and negative: base extender). The following parameters were determined: sperm motility and kinematics, viability, morphology, membrane integrity, and lipid peroxidation. Results showed that, except for motility and kinematics in which 0.1 mM quercetin exerted significant improving effects ($P < .05$), the other parameters investigated were not affected ($P > .05$). Additionally, higher concentrations of quercetin (0.2 and 0.3 mM) exerted partially prooxidant activity on sperm viability and membrane integrity. Therefore, 0.1 mM of quercetin seems to relatively protect sperm motility during cryopreservation.
© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Cryopreservation irreversibly damages spermatozoa resulting in their dysfunction. Some mechanisms such as thermal shock with intracellular and extracellular ice formation, cellular dehydration, and osmotic stress have been claimed to be the main insults occurring during cryopreservation [1,2]. Moreover, numerous studies suggest that sperm freeze-thaw cycles are associated with an increase in reactive oxygen species (ROS) production. Despite moderate levels of ROS are essential for successful capacitation, maturation, acrosome reaction, zona pellucida binding, and oocyte fusion [3], their overproduction during cryopreservation leads to sperm dysfunction [4].

Several protective antioxidants, such as the glutathione peroxidase/reductase system, superoxide dismutase, catalase and low-molecular weight antioxidants, vitamin E, vitamin C, and albumin, have been identified in sperm and seminal plasma presumably acting as ROS scavengers helping to prevent possible cellular damage [5]. Unfortunately, these protective systems are not sufficient to prevent the sperm damage exerted by higher ROS production during freeze/thaw cycles due to the high proportion of unsaturated fatty acids (~45% of total lipid fraction) present in the stallion spermatozoa and seminal plasma removal during the centrifugation and washing processes [6]. Consequently, to counteract ROS overproduction, the in vitro addition of

* Corresponding author at: Hamid Kohram, Department of Animal Science, Faculty College of Agriculture and Natural Resources, University of Tehran, P.O. Box 31587, Karaj 77871, Iran.
E-mail address: Hamid_Kohram@yahoo.com (H. Kohram).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.06.078

antioxidants has been tried in order to rise the scavenging capacity of the sperm extenders [7–11]. A large body of research has employed a wide variety of antioxidants such as catalase [12–15], butylated hydroxytoluene [1,12,13], vitamin E [8,11–14], Tempol [9,12,13], quercetin [16], vitamin C [12–14], N-acetyl-l-cysteine [9,14], EDTA [15], 5-(4-dimethylamino-phenyl)-2-phenyl-penta-2,4-dienoic acid [14], and rutin [9] in boar, ram, horse, human, bull, turkey, and dog frozen semen.

Quercetin belongs to the flavonoid family and is well known for its many biological activities including antioxidant [16–19], anti-inflammatory [19,20], and antimicrobial properties [21]. This dietary antioxidant is composes of two benzene rings connected by an oxygen containing a pyrene ring [22]. The presence and location of the hydroxyl (-OH) substitutions and the catechol-type B-ring make quercetin an effective antioxidant which possess more intensive ROS scavenger activity than vitamin E or C [23]. These properties, moreover, have been observed in both initial and propagation steps of the oxidative stress [24] through enzymatic and nonenzymatic systems, especially nicotinamide adenine dinucleotide phosphate oxidase [25] and nicotinamide adenine dinucleotide-dependent oxidoreductase [26] located in the mitochondria and sperm plasma membrane. Regulating calcium influx to sperm is another proposed mechanism which maintains internal adenosine triphosphate (ATP) concentration and prevents premature capacitation [27] and acrosome reaction [27,28] during sperm storage, so enhances sperm longevity by saving sperm limited energy content by delivering this crucial steps into female genital tract. It has been demonstrated that quercetin (0.15 mM) in comparison with catalase or cysteine improves post-thaw motility and zona binding ability of stallion spermatozoa in nonsorted, and DNA fragmentation in sex-sorted, stallion sperm [16]. However, other studies on human ram and mice species reported no effect or even a negative effect on sperm motility, progressive motility (PM), acrosome integrity or plasma membrane integrity, and sperm count [29–31]. So, in the present study, we evaluated a wider range of quercetin dosages compared to previous studies to further determine their effects on stallion sperm freezability.

2. Materials and Methods

2.1. Chemicals and Reagents

Eosin, nigrosin, and glycerol were obtained from Merck & Co Inc, penicillin from SHAFA-farmed industrial Co (Karaj, Iran), and all of other reagents used were obtained from Sigma-Aldrich Company (St Louis, MO).

2.2. Animal Husbandry, Semen Collection, and Primary Processing

Stallions were housed at the Razi Vaccine and Serum Research Institute, Karaj, Iran, and maintained according to institutional regulations. Semen was obtained from four healthy *Turkmen* stallions ranging from 8 to 10 years old, twice a week during July and August 2014. Ejaculates were collected using a Missouri model artificial vagina equipped with HAR-VET A/V filter to separate the gel fraction, lubricated and pre-warmed to 45°C to 50°C. The collected samples were immediately transported to the laboratory, and those showing over 80% total motility (TM) and less than 10% abnormal sperm were extended with INRA 82 (IMV, L'Aigle, France) and centrifuged (600 g for 10 minutes at room temperature). Finally, to eliminate individual stallion variability, the resulting sperm pellets were pooled [1]. The experiment was repeated six times.

2.3. Freezing Protocol

Sperm were reextended using an egg yolk–based extender composed of egg yolk (5% vol/vol), HEPES (10 g/L), citric acid (6.5 g/L), glucose monohydrate (50 g/L), penicillin (10 U/mL), and glycerol (4% vol/vol). Pooled semen was extended to a final concentration of 200×10^6 spermatozoa/mL, divided into five aliquots, and supplemented as follows: 0.1, 0.2, and 0.3 mM quercetin, negative control (no quercetin added), and positive control [0.5% (vol/vol) ethanol]. The treatment groups were slowly cooled to 4°C and equilibrated within 150 minutes, loaded into 0.5-mL plastic straws, layered horizontally in racks and placed 4 cm above the surface of liquid N_2 for 10 minutes, after which they were directly plunged in liquid N_2 and stored until evaluation [1].

2.4. Thawing and Post-Thaw Evaluation

Three frozen straws per each treatment were used to evaluate the post-thaw sperm quality. The thawing was carried out in water bath at 37°C for 30 seconds. The sperm quality was evaluated immediately after thawing.

2.5. Sperm Motility and Velocity Analysis

Motility and velocity parameters were evaluated by a phase contrast microscope (Labomed Lx 400, LA), equipped by a computer-assisted semen analyzer (CASA) software (VideoTesT, Sperm 3.1 St.-Petersburg, Russia). The assessed parameters included TM (%), PM (%), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s), curvilinear velocity (μm/s), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz), straightness (STR, %), and linearity (%). The system parameters for CASA were 50 frames per second; minimum contrast, 30; minimum cell size, 5 pixels; VAP cutoff 30 μm/s; VAP cutoff for progressive cells 70 μm/s; STR 80%; VSL cutoff, 0 μm/s; and ALH cutoff for progressive cells 0.3 μm.

2.6. Viability Test

Viability was assessed by eosin/nigrosin staining (eosin Y 0.67 g, nigrosin 10 g, NACl 0.9 g solved in 100 mL distilled water) [32]. Briefly, 10 μL of semen was mixed with 20 μL of stain and incubated for 30seconds at 37°C, smeared on a slide, and air dried. Sperm were classified as live if no stain was present and those which showed any pinkish coloration were considered as dead. At least 200 sperm per sample were assessed at a magnification of ×400 (Labomed).

Download English Version:

https://daneshyari.com/en/article/2394339

Download Persian Version:

https://daneshyari.com/article/2394339

Daneshyari.com