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Effect of vitamin E and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen

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ABSTRACT

This study was conducted to determine the combined effects of adding vitamin E (VitE) and selenium nanoparticle (Nano-Se) as antioxidant supplements to rooster semen extender for freezing. Semen samples were collected from 12 White Leghorn roosters and pooled. Subsequently, the samples were divided into nine equal groups using modified Beltsville extender. Extenders were supplemented with either two amounts of VitE (5 and 10 μg/mL) or two amounts of Nano-Se (1% and 2%) or a combination of both VitE and Nano-Se, and no antioxidants extender (control group). Using 5 μg/mL VitE and 1% of Nano-Se improved ($P < 0.05$) total sperm motility (79.28 ± 3.86%), progressive sperm motility (18.03 ± 1.02%), sperm viability (81.46 ± 2.16%) and integrity of the sperm membrane (77.21 ± 2.12%) after the freeze–thawing process compared with control group (54.08 ± 3.86%, 10.96 ± 1.02%, 60.53 ± 2.16%, and 54.47 ± 2.12%; respectively). Also, extenders supplemented with 5 μg/mL Vit E or 5 μg/mL VitE and 1% Nano-Se had a lesser malondialdehyde (MDA) concentration compared to control extender (1.15 ± 0.32, and 1.29 ± 0.32, respectively). Total abnormal morphology of sperm was decreased ($P < 0.05$) by adding 5 or 10 μg/mL VitE alone or in combined with 1% or 2% Nano-Se. Moreover, extenders containing Nano-Se demonstrated greater glutathione peroxidase (GPx) activity compared to other extenders. Catalase (CAT) activity was greater in extender supplemented with 10 μg/mL VitE and 2% Nano-Se. Moreover, higher TAC was observed in extenders supplemented with VitE and Nano-Se. It can be concluded that addition of 5 μg/mL vitamin E in combined with 1% Nano-Se improved the post-thawing quality and oxidative variables of rooster semen.

1. Introduction

Optimization of the management of rooster breeder birds is needed for efficient methods of semen storage such as semen cryopreservation (Blesbois, 2011). However, use of these processing technologies induce some irreversible damage to sperm. These damages can compromise anatomical, and structural integrity of sperm. Additionally, there is substantial evidence that cryopreservation results in increase DNA damage, aneuploidy, and chromosome fragmentation (Baumber et al., 2003; Li et al., 2007).

An important factor in the fertilizing capability of sperm is the lipid content of the plasma membranes (Cerolini et al., 1997). Avian sperm cell membranes have a much greater concentration of polyunsaturated fatty acids (PUFA) than mammalian sperm cells (Surai et al., 2001). This rela-

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tively greater PUFA content is essential for normal sperm function, as it is the foundation for the sperm plasma membrane's greater relative fluidity (Force et al., 2001; Gulaya et al., 2001). Therefore, avian sperm are more susceptible to lipid peroxidation (LPO) by reactive oxygen species (ROS) during *in vitro* handling and storage (Cerolini et al., 1997).

Sperm and seminal plasma have several mechanisms that generate ROS. Although the antioxidant capacity of sperm is low, enzymatic anti-oxidative compounds in seminal plasma comprising glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and also natural antioxidants such as vitamins A, E, C, uric acid, glutathione and carotenoids have been described as functioning as a defense mechanism against ROS and LPO in semen of numerous animal species (Bréque et al., 2003; Cerolini et al., 2006; Partyka et al., 2012b). The activity of the antioxidant system is, however, affected by cryopreservation, which increases the intensity of LPO (Partyka et al., 2012a).

Results of human studies suggested that amount of total antioxidant capacity (TAC) in the seminal plasma of infertile men was less than fertile men (Lewis et al., 1995). Because avian sperm cells have relatively greater amounts levels of PUFA than most sperm cells, these cells require additional antioxidants in the media to overcome the LPO during freezing-thawing process.

Vitamin E is generally considered the main component of the antioxidant system of sperm (Lenzi et al., 1996). Because vitamin E is located in the lipid part of membrane, where the process of lipoperoxidation occurs, this vitamin can function to prevent membrane LPO formation and, therefore, enhance sperm quality variables (Surai et al., 2001). A series of experiments have demonstrated that the vitamin E can improve post-thaw sperm qualities in bulls (Towhidi and Parks, 2012), rams (Silva et al., 2013) and roosters (Amini et al., 2015a; Moghbeli et al., 2016). Amini et al. (2015a, b) reported the supplementation of freezing diluents with 5 and 10 μg/mL vitamin E improved sperm motility and viability and decreased LPO in post-thaw rooster semen. In general, vitamin E has been observed to have synergistic effects with selenium as an antioxidant (Talib et al., 2009). Talib et al. (2009) observed that vitamin E combined with selenium had positive effects on reproductive performance of rams compared to vitamin E supplementation alone.

Addition of selenium not only improved male reproductive performance by potentiating semen quality butalso suppressed free radicals (Kleene, 1994). Numerous studies have been performed to evaluate the effect of *in vivo* selenium administration on semen characteristics in mice (Watanabe and Endo, 1991) rams (Marai et al., 2009), boars (Horky, 2012) and roosters (Edens and Sefton, 2009). The effect of selenium on semen quality during the *in vitro* storage and the post-thawing process is, however, not well documented. Moreover, bioavailability and toxicity are limiting factors for using selenium. For example, selenium can be metabolized into hydrogen selenide which is a highly toxic selenium compound (Tarze et al., 2007). In this regard, all of the comparative toxicity and efficacy studies on Nano-Se confirm its efficacy on inducing selenoproteins such as GPx with less toxicity and acceptable bioavailability (Peng et al., 2007; Wang et al., 2007)

Several clinical and experimental studies have examined the effect of antioxidants on rooster sperm variables after cryopreservation (Amini et al., 2015a, b; Moghbeli et al., 2016). Despite this large body of literature, the effect of these additives on sperm antioxidant capacity and activity has not been assessed. Therefore, the present study was conducted to evaluate the combined effects of vitamin E and selenium nanoparticles (Nano-Se) on post-thawed rooster semen quality variables and oxidative status.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Selenium nanoparticles were purchased from Nanoshel (Nanoshel, USA, cat no: NS6130-01-171).

2.2. Animals

This research was conducted at the Department of Animal Sciences, Faculty of Agriculture, University of Tabriz, Tabriz, Iran. Twelve White leghorn layer breeder roosters were selected at 30 weeks of age from a commercial flock and were housed individually in cages ($70 \times 70 \times 85$ cm) at 18 to 22 °C, in a 15 L: 09 D photoperiod system. Animals were fed a diet contained 10% crude protein, 3170 kcal ME/kg, 0.9% calcium and 0.45% available phosphate without additional antioxidant supplementation. The diets were fed twice daily, at the beginning of the photoperiod and in the evening. Roosters received an average of 90 g of feed during the experiment. Water was provided *ad libitum*. The males were habituated (for 2 weeks) to dorso-abdominal massage for semen collection (Burrows and Quinn, 1937).

2.3. Semen collection

Semen samples were collected twice a week by massage technique (Burrows and Quinn, 1937) during the morning hours of the day. The milky drops of the ejaculate were immediately aspirated by sterile tuberculin syringes and the semen samples were then transferred to sterile test tubes kept in a water bath at 37 °C. On each day of semen collection, seminal volume (mL) was measured in graduated collecting tubes. Also, sperm motility was evaluated using a phase-contrast microscope with 400× magnification, and sperm concentrations were calculated by determining the light absorption of the semen at a wavelength of 540 nm using a spectrophotometer (Spectronic 20, Bausch and Lomb Co., USA). Only ejaculates with volume of 0.2–0.6 mL, concentration of $\geq 3 \times 10^9$ sperm/mL, motility of ≥80%, and ≤10% of abnormal morphology were used in this study. To eliminate individual differences and obtain sufficient sperm for analysis, the semen samples were pooled and subsequently divided them into nine aliquots.