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Effect of sperm concentration on characteristics and fertilization capacity of rooster sperm frozen in the presence of the antioxidants catalase and vitamin E

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ABSTRACT

The objective of this study conducted was to determine the influence of different levels of sperm concentration, including catalase (CAT) and vitamin E (VitE) in rooster semen extender on postthawed quality and fertility of rooster semen. Semen was collected twice a week from six roosters (Arian) and diluted according to experimental treatments consisting of sperm suspensions containing different sperm concentrations (200, 400, and 600 × 106 sperm/mL) without antioxidant supplementation as control (Con) groups (Con200, Con400, and Con600, respectively), sperm suspensions containing different sperm concentrations (200, 400, and 600 × 106 sperm/mL) supplemented with 5-μg/mL VitE (VitE200, VitE400, and VitE600, respectively) and different sperm concentrations (200, 400, and 600 × 106 sperm/mL) supplementation with 100 IU/mL CAT (CAT200, CAT400, and CAT600, respectively). After thawing; sperm motility, membrane integrity, and mitochondrial function were assessed. Fertility and hatchability rates were determined by using 100 artificially inseminated hens. The percentage of total motility (TM) and activity of mitochondria decreased ($P < 0.05$) as the sperm concentration increased in control groups. So, the lowest percentage of the TM and activity of mitochondria were observed in the Con600 as compared with other treatment groups. Extenders containing 100 IU/mL CAT and 5-μg/mL VitE resulted in higher ($P < 0.05$) TM, progressive motility, membrane integrity, and activity of mitochondria compared with control groups. Adding VitE and CAT in different sperm concentrations, the percentage of TM, membrane integrity, and activity of mitochondria decreased ($P < 0.05$) as the sperm concentration decreased. The highest ($P < 0.05$) membrane integrity, TM, and progressive motility were recorded at VitE400 and CAT400. Including VitE and CAT in rooster extender with different level sperm concentrations had no effect ($P > 0.05$) on fertility and hatchability rates. In conclusion, although adding VitE and CAT in extender with different levels of sperm concentration improved postthawed quality of rooster semen, but adding VitE and CAT in the extender have no effect on fertility rate.

1. Introduction

Semen cryopreservation is a key tool for long-term storage of genetic material in avian species [1]. The cryopreservation of sperm requires the use of cryoprotectants that minimize cell damage during freezing and thawing.

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Glycerol is the least toxic cryoprotectant for rooster semen and most effective at protecting fowl spermatozoa against cold shock [2]. However, semen cryopreservation induces a series of biochemical and physical alterations in spermatozoa such as reducing the activity of antioxidant [3], and increasing reactive oxygen species (ROS) production [4]. When the generation of ROS surpasses the sperm capacity to eliminate the free radicals and reactive metabolites that are generated, oxidative stress occurs [5]. Oxidative stress may lead to a decrease in sperm motility as a result of reduced ATP production [6] and also lead to a loss of membrane integrity as well as DNA damage and apoptosis [5]. Avian semen normally contains antioxidant system such as glutathione peroxidase, catalase (CAT), superoxide dismutase, and also vitamin E (VitE) and vitamin C to counter ROS, but the activity is affected by cryopreservation [3].

The primary function of CAT in the cells is to convert hydrogen peroxide radicals into O_2 and H_2O. This enzyme is effective in the cells, protecting them against hydrogen peroxide toxicity [3]. Vitamin E is considered to be the main component of the antioxidant system of spermatozoa [7]. This lipid-soluble vitamin directly blocks lipid peroxidation (LPO) in the sperm membrane by neutralizing ROS–generated free radicals [8]. Recent studies found that supplementation of freezing diluents with VitE (5 μg/mL) and CAT (100 IU/mL) has been improved the postthawed sperm functions of rooster [8,9]. Also, in studies performed on cryopreservation of cat and boar sperm, they were suggested that the supplementation of freezing diluents with VitE resulted in a positive effect on mitochondrial membrane potential and integrity of the sperm membrane and DNA [10,11].

The production of ROS may be infused by sperm concentration [12]. Recent studies on donkey [13], horse [14], dog [15], and ram [12] reported that greater concentrations of sperm in the straw having detrimental effect on postthawed sperm quality. On the other hand, the interaction of sperm concentration and adding the antioxidant to semen extender of the rooster has not yet been investigated. Therefore, the present study was conducted to determine the influence of different level sperm concentrations, including CAT and VitE in a modified Beltsville extender (in a lecithin-based medium) on sperm motility, membrane integrity, mitochondrial function, fertility, and hatchability rate after freezing and thawing.

2. Materials and methods

All experimental procedures used were approved by the Animal Welfare Committee of the Department of Animal Science, University of Tehran.

2.1. Chemicals

All chemicals used in this study were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Birds and semen collection

This research was conducted at the Department of Animal Sciences, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. Six mature Arian breeder roosters were selected at 35 weeks of age from a commercial flock and were caged individually (60 × 50 × 75 cm) at 22 ± 2 °C under a 15L:09D photoperiod. Roosters were fed a common breeder diet with 2740 kcal of MEn/kg and 12.5% CP and 0.9% calcium. Water was provided *ad libitum*. Semen was collected twice a week from the roosters according to the method described by Burrows and Quinn [16]. Immediately after collection, semen transferred to the water bath (37 °C) and then evaluated for primary criteria such as volume, color, morphology, and concentration. To determine sperm concentration, semen was collected 10 times from six roosters, pooled, and measured by using FACSCCalibur (Becton Dickinson San Khosoz, CA, USA) flow cytometer. The average concentration of sperm was 3.510 ± 0.008 × 109 sperm/mL.

2.3. Extender preparation, sperm dilution, and freezing/thawing protocols

The composition of the diluent used in this study (modified Beltsville extender) has been shown in Table 1 [17]. The osmotic pressure and pH were set at 310 mOsm/kg and 7.2, respectively. Immediately after primary evaluation, the sperm samples were pooled and diluted into three dilutions (200, 400, and 600 × 106 sperm/mL) at 37 °C. The three dilutions were split into nine equal aliquots (Fig. 1). The sperm suspensions containing different sperm concentrations were supplemented with 5-μg/mL VitE (VitE200, VitE400, and VitE600, respectively), 100-IU/mL CAT (CAT200, CAT400, CAT600, respectively), or without antioxidant supplementation as control (Con) groups (Con200, Con400, Con600, respectively). The nine treatments were cooled slowly at 5 °C. After 2 hours (incubation time), the semen was frozen in liquid nitrogen vapor by aspirating into 0.25-mL French straws (and sealed with polyethylene glycol). The straws were placed 5 cm above the surface of the liquid nitrogen for 7 minutes in a 40 × 20 × 20 cm cryobox that

Table 1
Composition of the diluents used.

Ingredient	Modified Beltsville extender	Medium A amount	Medium B
Potassium citrate tribasic monohydrate (g)	0.64	0.93	1.25
Sodium-L-glutamate (g)	8.67	8.67	12.27
Magnesium chloride anhydrous (g)	0.34	0.75	0.34
D-(-)-fructose (g)	5	5	10
Potassium phosphate dibasic trihydrate (g)	7.59	7.59	8.35
Potassium phosphate monobasic (g)	0.7	—	0.7
N-[Tris (hydroxymethyl) methyl]-2 (g)	2.7	3.0	3.5
Sodium acetate trihydrate (g)	3.1	3.0	4.8
Myo-inositol (g)	—	6.0	—
Purified water (mL)	1000	1000	1000
pH	7.2	7.1–7.3	7.2–7.4
Osmolarity (mOsm/kg)	310	330–340	390–400
Soybean lecithin (%)	1	—	—
Glycerol (%)	11	—	—