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Hyaluronic acid improves frozen-thawed sperm quality and fertility potential in rooster

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ABSTRACT

Beneficial effects of Hyaluronic acid (HA) has not been yet assessed for cryopreservation of rooster sperm. This study was conducted to evaluate the effects of different concentrations of HA (0, 1, 2, 4 and 8 mM) in Beltsville extender on the cryopreservation of rooster sperm. Semen samples were collected from six Ross broiler breeders (24-week) using abdominal massage, then divided into five equal aliquots and cryopreserved in Beltsville extender that contained different concentrations of HA. Motion characteristics, morphology, membrane functionality, viability, acrosome integrity, lipid peroxidation and fertility potential of sperm were assessed after thawing. HA at concentration of 2 mM (HA2) resulted in the highest ($P < 0.05$) total motility (55.3 ± 1.1%) and progressive motility (25.2 ± 0.8%) compared to the other groups. HA8 produced the lowest significant ($P < 0.05$) percentage of total (38.6 ± 1.1%) and progressive (14.7 ± 0.8%) motility. High significant percentage of membrane functionality were observed in HA1 and HA2 (43.2 ± 1.0 and 46.1 ± 1.0%, respectively) compared to HA4 (40.1 ± 1.0%) and HA8 (32.5 ± 1.0%). Moreover, HA1 and HA2 produced the higher percentage of acrosome integrity (54.8 ± 1.2 and 57.5 ± 1.2, respectively) compared to other groups. HA1 and HA2 reduced ($P < 0.05$) malondialdehyde formation (3.66 ± 0.08 and 3.75 ± 0.08 nmol/ml) compared to other groups. Fertility rate and hatching rate obtained from artificial insemination were significantly higher in HA1 (63.7 and 54.7%) and HA2 (67.5 and 57.7%) compared to control group (40 and 37%). Our results showed that supplementation of Beltsville extender with 1 and 2 mM HA significantly improved the quality of rooster sperm after freeze thawing.

1. Introduction

Cryopreservation is a valuable technique for long-term storage of sperm in avian species. This process is accompanied by thermal shocks resulting in destruction of sperm plasma membrane which may decrease the viability and fertility potential of sperm, consequently reducing the efficiency of artificial insemination (AI) (Sar & özkan et al., 2015; Grossfeld et al., 2008). Reactive oxygen species (ROS), which are main products during cryopreservation can attack the bisallylic methylene group of plasma membrane phospholipids and lead to lipid peroxidation (LPO) (Agarwal et al., 2014; Aitken et al., 2014). Therefore, optimizing this technique is essential to improve the quality of rooster sperm after freeze-thawing.

Antioxidants are chemicals that have cryoprotective effects on sperm in mammalian and avian species (Amini et al., 2015; Sharafi et al., 2015a, 2015b; El-Sheshtawy et al., 2015; Bucak et al., 2009). Among the antioxidants known, hyaluronic acid (HA) has been

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considered a suitable additive for cryopreservation in Ram (Najafi et al., 2014; Bucak et al., 2007), bull (Eidan, 2016) and goat (Salmani et al., 2013). HA belongs to the glycosaminoglycan family and is produced naturally in the synovial fluid that surrounds the joints. The chemical structure of HA consists of disaccharide units composed of β-D-glucuronic acid and N-acetyl β-D-glucosamine (Simulescu et al., 2016). HA has many properties; it contributes to cellular cohesion and growth regulation (Mendoza et al., 2009). It has been reported that HA can increase the physiological functions of human sperm such as motility and capacitation (Bansal, 2015; Agarwal et al., 2014). Other studies have shown that HA preserves post-thawed sperm motility and membrane stability in human (Sbracia et al., 1997), stallion (Baumber et al., 2000) and boar (Qian et al., 2016; Pena et al., 2004). Nevertheless, to our knowledge, there have been no studies that have evaluated the effects of HA on cryopreservation of rooster semen. The purpose of this study was to survey the effects of different concentrations of HA in Beltsville extender on the post-thawed quality of rooster sperm. Several parameters such as motion characteristics, morphology, viability, membrane functionality, acrosome integrity, lipid peroxidation and fertility potential were assessed to find the optimum concentration of HA.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma (St. Louis, MO, USA), and Merck (Darmstadt, Germany). The Research Ethics Committees of Azad University approved the present study.

2.2. Farm management and semen collection

This experiment was conducted on Ross broiler breeders comprising 6 adult roosters which were housed individually in cages (70 × 70 × 85 cm) at 18–22 °C, under a 15 l: 09 D photoperiod. Animals were fed a diet that contained 10% crude protein, 3170 kcal ME/kg, 0.9% calcium and 0.45% available phosphate. Sperm collection was performed once a week during two months from roosters by a method described by Burrows and Quinn (1937). After collection, semen was placed in a water bath (37 °C), for primary evaluation in the laboratory. The criteria for normal quality of sperm which was previously described by Shahverdi et al. (2015) were consist of: volume: 0.3–0.6 ml; sperm concentration of $\geq 3 \times 10^9$ sperm/ml; motility ≥80% and abnormal morphology ≤ 15%. Then, to eliminate individual differences between roosters, semen samples in each day of sampling were pooled and divided into five aliquots according to the experimental design.

2.3. Extender preparation and cryopreservation

The components of Beltsville extender were dipotassium phosphate (7.59 g/l), sodium glutamate (8.67 g/l), fructose (5 g/l), sodium acetate (3.2 g/l), TES [*n*-tris (hydroxymethyl) methyl 1-2 amino ethane sulfonic acid] (3.2 g/l), potassium citrate (0.64 g/l), monopotassium phosphate (0.7 g/l), magnesium chloride (0.34 g/l). Glycerol was added to the basic medium at 3% (v/v) with pH of 7.1 and osmolarity of 310 mOsm/kg. Different concentrations of HA (sodium salt; Sigma 53747) were used as follows; Beltsville freezing medium without hyaluronic acid (Control, HA0), Beltsville freezing medium with 1 mM (HA1), 2 mM (HA2), 4 mM (HA4) and 8 mM (HA8) hyaluronic acid. Aliquoted sperm samples were diluted with Beltsville according to the above groups and then aspirated into 0.25 ml French straws (IMV, L'Aigle, France) to have a final concentration of 400×10^6 sperm/ml and sealed with polyvinyl alcohol powder and equilibrated at 5 °C for a period of 3 h. After equilibration, the straws were frozen in liquid nitrogen vapor, 4 cm above the liquid nitrogen for 7 min in a 40 × 20 × 20 cm cryobox containing 8000 cm^3 liquid nitrogen. Then, the straws were plunged into liquid nitrogen for storage.

2.4. Evaluation of post-thawed sperm

2.4.1. Motion characteristics

Motion characteristics of post-thawed sperm were analyzed using a computer assisted semen analysis (CASA) system fitted with the Sperm Class Analyzer (SCA) software (Version 5.1; Microptic, Barcelona, Spain) with settings adjusted to detecting avian sperm (Fattah et al., 2017a). A previously heated (37 °C) Makler chamber was placed under the phase-contrast microscope (Nikon, ECLIPSE E200, Japan) with magnification of 100X and the following motility values were recorded: total motility (TM, %), progressive motility (PM, %), straight linear velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), average path velocity (VAP, μm/s), Amplitude of lateral Head displacement (ALH, Mm) Beat-cross frequency (BCF, Hz) sperm track straightness (STR, %) and linearity (LIN, %).

2.4.2. Membrane functionality

Hypo osmotic swelling test (HOST) was applied to evaluate the integrity of sperm plasma membrane functionality after freeze-thawing (Masoudi et al., 2016a). This assay was carried out by mixing 5 μl of semen with a 50 μl hypoosmotic solution (100 mOsm/l, 57.6 mM fructose and 19.2 mM sodium citrate). After 30 min of incubation, the sperm were checked under a phase-contrast microscope (E200, Nikon, Tokyo, Japan) and 200 sperm with swollen and non-swollen tails were evaluated, and swollen tails were recorded as sperm with functional membrane.

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