Theriogenology 116 (2018) 89–94

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of egg yolk plasma and soybean lecithin on rooster frozen-thawed sperm quality and fertility

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ARTICLE INFO

Article history:
Received 6 January 2018
Received in revised form
10 May 2018
Accepted 12 May 2018

Keywords:
Freezing
Rooster semen
Artificial insemination
Cryoprotectant

ABSTRACT

This experiment was conducted to study the effects of egg yolk plasma (10%, 15% and 20%), soybean lecithin (0.5%, 1% and 1.5%) and whole egg yolk (WEY) (control) on post-thawed sperm quality, hatchability and fertility outcomes. In experiment 1, sperm motility, abnormalities, membrane integrity, viability, apoptosis status, mitochondrial activity were studied following freeze-thawing. The best quality of frozen-thawed rooster sperm was chosen to be used for the assessment of the hatchability and fertility rate in experiment 2.

The significantly higher percentages of post-thawing sperm total and progressive sperm motilities, membrane integrity, viability were observed in 1% soybean lecithin and 20% egg yolk plasma in comparison with 0.5 and 1% soybean lecithin, 10% egg yolk plasma and control, except for 15% egg yolk plasma ($P < 0.05$). Using 20% egg yolk plasma in the extender improved mitochondrial activity. Supplementation of 1% soybean lecithin and 20% egg yolk plasma into the extender resulted in the least percentages of dead sperm ($P < 0.05$). Sperm abnormalities and early apoptosis did not differ in various extender supplementations. In experiment 2, higher percentages of hatchability and fertility rate were observed in semen containing 1% soybean lecithin and 20% egg yolk plasma compared with the WEY group. The results showed that supplementation of the rooster sperm extender with 1% soybean lecithin and 20% egg yolk plasma resulted in higher quality of frozen-thawed sperm.

1. Introduction

The most worthwhile technique for preservation and management of bird genetic resources is semen cryopreservation, which has been studied in domestic birds like chicken [1]. Despite years of intensive investigations, still more work should be done in order to perform successful cryopreservation of poultry sperm.

The lower quality of frozen-thawed poultry sperm and consequently the poor fertilization rates compared to mammalian species are due to the exceptional morphological properties of poultry sperm, which cause freeze damages because of their vulnerable structure to low temperatures [2]. Egg yolk was quickly adopted as anon-permeable cryoprotectant that decreases the freezing point of the medium and produces less ice crystals [3]. Although it has been documented that the cryoprotective effect of egg yolk is attributed to low density lipoproteins, phospholipids or triglycerides [4] but the mechanism by which it protects the sperm cell during cryopreservation, is not exactly recognized. Reasonable results achieved from different studies have made egg yolk extender as a protective agent to defend sperm against the harmful effects of low temperature. Despite its benefits, several arguments have been grown against the use of egg yolk, mostly due to its proneness to contamination with animal pathogens [5].

Egg yolk consists of two major parts including plasma and granules. Plasma contains a wide amount of valuable lipids named low-density lipoproteins (LDL), whereas granules are mainly constituted of high-density lipoproteins (HDL) which are known as unfavorable structures [6]. Different investigations have shown that HDL have the ability to disturb the function of sperm [7]. It has been revealed that the part responsible for the cryoprotective properties is LDL. Some properties of egg yolk plasma such as its easy extraction and comparable effect to LDL have made it a suitable

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https://doi.org/10.1016/j.theriogenology.2018.05.013
0093-691X/

alternative to whole egg yolk [8].

Moreover, soybean lecithin is suggested to replace WEY for sperm cryopreservation in most of the livestock species [9,10]. It has been proposed that the lecithin protection mechanism is due to the replacement of sperm membrane phospholipids, with minimizing the freezing point. Also, it may form a protective layer around the sperm, avoiding the formation of ice crystals, consequently preventing the physical damage to the sperm membranes [11]. Extenders including soybean lecithin are successfully used worldwide for sperm cryopreservation in variety of livestock species [12–14]. However, its effects on sperm functional properties yet need to be entirely studied.

Therefore, the aim of the present study was to analyze the use of egg yolk plasma or soybean lecithin instead of WEY in extenders for the cryopreservation of rooster semen. Our hypothesis was that soybean lecithin and egg yolk plasma extenders would be more effective than whole egg yolk-based extender at cryopreserving rooster sperm. This strategy might improve the protocol of rooster sperm freezing by developing a new extender before using in artificial insemination. Nevertheless, cellular mechanism of egg yolk plasma or soybean lecithin on in vivo fertility potential in rooster has not been completely clarified. Therefore, several parameters such as sperm motility, abnormalities, membrane integrity, viability, mitochondrial activity, apoptosis status, hatchability and fertility rate were assessed in this study to find the best cryoprotectant for cryopreservation of rooster sperm.

2. Materials and methods

2.1. Chemicals

The chemicals used in this experiment were obtained from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Egg yolk plasma

The hen egg yolk was separated into two fractions containing plasma and granules using the technique conducted by Pillet et al. [15]. Plasma fractionation was carried out by diluting an equal volume of yolk and 0.17-M NaCl solution and stirring with a magnetic stirrer for 1 h at 4 °C.Then centrifugation was carried out two times at 10,000 × g for 45 min at 4 °C.The supernatant containing egg yolk plasma was collected and maintained in glass containers of 2 mL at 4 °C for later use.

2.3. Animal ethics, farm management and semen collection

The study was carried out after approval of Animal Ethics Committee of the University of Tabriz (Tabriz, Iran). Ten Ross broiler breeders at 28 weeks of age were kept individually in cages (60 cm × 60 cm × 75 cm (at 20 °C and 14L:10D photo period at the Poultry Unit, Animal Production Centre, University of Tabriz (Tabriz, Iran). Birds were offered fresh water and a standard commercial chicken breeder diet (2750 kcal ME/kg, 11.99% CP).

They were first trained for semen collection for two consistent weeks. Then, all of the semen roosters were regularly collected twice a week. The semen was collected using the method conducted by Fattah et al. [16].

The collection was always carried out by the same person and under the same condition. The criteria in normal quality of sperm were as follows: the volume: 0.2–0.6 ml (semen volume was measured visually using a graduated collection tube); the concentration of sperm $\geq 3 \times 10^9$ sperm/ml (ejaculate concentration was evaluated by haemocytometer); total motility (CASA) ≥80% and abnormal morphology (Hancock method [17]) ≤ 10%. Then, to remove individual variations and obtain sufficient sperm for analysis, the semen samples were pooled and subsequently divided into seven aliquots in accordance with the experimental design.

2.4. Extender preparation

The components of Beltsville extender contained potassium citrate tribasic monohydrate (20.8 mM), sodium-*l*-glutamate (512.8 mM), magnesium chloride anhydrous (35 mM), D-(−)-fructose (277.5 mM), potassium phosphate dibasic trihydrate (435.7 mM), potassium phosphate monobasic (51.4 mM), n-[tris (hydroxymethyl) methyl]-2 (2.71), sodium acetate tri hydrate (139.5 mM) (pH (7.2), osmolarity (310 mOsm/kg), glycerol (8%) and purified water for a total volume of 1000 mL.

2.5. Experiment 1: in vitro assessment of frozen-thawed sperm quality

In experiment 1, the pooled semen samples were split into seven equal aliquots and diluted with the basic extender containing soybean lecithin (0.5%, 1% and 1.5%), egg yolk plasma (10%, 15% and 20%), and WEY (control).

Then, French 0.25 mL straws were filled with the diluted semen samples with the final concentration of 400×10^6 sperm/mL, sealed with PVA sealing powder and equilibrated for 3 h at 5 °C. Using a cryobox, the straws were arranged horizontally 4 cm above the surface of liquid nitrogen for 7 min and they were finally immersed in liquid nitrogen (−196 °C) for storage for further tests. For different evaluations, the straws were thawed individually at 37 °C for 30 s in a water bath.

2.5.1. Motion parameters

The motility parameters of sperm after thawing were evaluated using computer-assisted analyses (CASA; 12.3 CEROS, Hamilton-Thorne Biosciences, Beverly, MA, USA). At least 200 sperm were checked per sample using standard settings (a constant temperature of 37 °C). The proportions of total motile (MOT, %), and progressive sperm (PROG, %) were specified. The motility parameters including the straight-line velocity (VSL, μm/s), the curvilinear velocity (VCL, μm/s), the average path velocity (VAP, μm/s), the linearity (LIN, %; VSL/VCL), the beat cross frequency (BCF, Hz), the straightness (STR, %; VSL/VAP), and the amplitude of lateral head displacement (ALH, μm) were determined for each sample.

2.5.2. Abnormal forms

Morphology was analyzed in preparations comprising 15 μL sperm samples fixed in 300 μL Hancock solution under a phase-contrast microscope (Labomed LX400; Labomed Inc., Culver City, CA, USA) at magnification of 1000×. A total of 200 sperm in each sample were checked for the percentage of total sperm abnormalities (head abnormalities, detached heads, abnormal midpieces and tail defects).

2.5.3. Membrane integrity

Hypo-osmotic swelling (HOS) assay, a previously conducted method [13], was used to estimate the alterations in functional integrity based on swollen and curled tails. The test was performed by incubating a part of semen (10 μL; including 400×10^4 sperm) with 100 μL of hypo osmotic solution (100 mOsm/kg; sodium citrate 1.9 mM, fructose 5.0 mM) at 37 °C for 30 min. Afterward, a phase-contrast microscopy (Labomed LX400; Labomed Inc., Culver City, CA, USA) was used to count 200 sperm per slide and the percentage of sperm with swollen and curved tails was determined.