Theriogenology 87 (2017) 259–265

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Evaluation of quail and turkey egg yolk for cryopreservation of Nili-Ravi buffalo bull semen

S. Akhter [a,*], B.A. Rakha [b], M.S. Ansari [c], A.U. Husna [a], S. Iqbal [d], M. Khalid [e]

[a] Department of Zoology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[b] Department of Wildlife Management, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[c] Department of Zoology, University of Sargodha-Lyallpur Campus, Faisalabad, Pakistan
[d] Semen Production Unit, Qadirabad, Sahiwal, Pakistan
[e] Department of Production and Population Health, Royal Veterinary College, London, UK

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form 1 September 2016
Accepted 1 September 2016

Keywords:
Coturnix coturnix
Meleagris gallopavo
Chromatin damage
Extender
Sperm motility
Viability

ABSTRACT

Egg yolk is used as a cryoprotectant for semen in different mammalian species including buffalo. Egg yolk from different sources may affect freezability of buffalo bull semen. Quail egg yolk (QEY) and turkey egg yolk (TEY) in Tris-citric acid extender was evaluated for post-thaw quality and *in vivo* fertility rate of cryopreserved buffalo bull semen. Ejaculates were collected on weekly basis from six Nili-Ravi buffalo bulls (12 ejaculates/bull) for a period of 6 weeks and diluted at 37 °C with tris-citric egg yolk extender (50×10^6 motile spermatozoa mL^{-1}) containing different levels of QEY or TEY (5%, 10%, 15%, and 20%) or 20% chicken egg yolk (CEY; controls) and cryopreserved. Percent post-thaw sperm motility (48.3 ± 3.8), plasma membrane integrity (67.9 ± 5.3), live/dead ratio (68.2 ± 5.0), and viability (50.5 ± 3.7) were recorded higher ($P < 0.05$) in extender containing 5% QEY compared with control. However, TEY at 10% in extender improved ($P < 0.05$) the post-thaw sperm motility (57.5 ± 5.2), plasma membrane integrity (53.5 ± 4.5), livability (75.3 ± 6.0), and viability (73.5 ± 6.5) compared with higher concentrations of TEY and controls (20% CEY). The chromatin damage (2.0 ± 0.9) and intracellular enzymes, glutamic oxaloacetic transaminase (24.8 ± 3.5) and lactic dehydrogenase (77.7 ± 4.5), release were lower ($P < 0.05$) in extender containing 10% TEY compared with the controls. *In vivo* fertility was compared after artificial insemination with semen from two buffalo bulls that was cryopreserved in extenders containing 5% QEY, 10% TEY, or 20% CEY. A total of 600 inseminations (200 inseminations per extender) were recorded; the overall fertility rate was significantly higher ($P < 0.05$) with semen cryopreserved in extender containing 5% QEY (57.5 vs. 42%) and 10% TEY (57.5 vs. 42%). compared with 20% chiken egg yolk. In conclusion, QEY at 5% and TEY at 10% offers advantages over 20% CEY in terms of *in vitro* post-thaw semen quality and *in vivo* fertility of buffalo.

1. Introduction

Artificial insemination using cryopreserved semen is the optimal way of disseminating germplasm of the superior sires to a large number of females. It also facilitates sanitary, quarantine, and international exchange of germplasm [1,2]. However in buffalo, fertility rates following artificial insemination with cryopreserved semen are quite low and not commercially acceptable. These low fertility rates are attributed to the low quality of cryopreserved buffalo semen [3]. There are studies to show that buffalo spermatozoa are damaged heavily during freezing and thawing process [4,5]. The freezing–thawing process exerts physical and chemical stress to the sperm, which ultimately renders

* Corresponding author. Tel.: + 923335206056; fax: +92-51-9290160.
E-mail address: sashraf1993@gmail.com (S. Akhter).

0093-691X/$ – see front matter © 2016 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2016.09.002

the frozen-thawed semen to have reduced motility, viability, and fertilizing ability when compared with fresh semen [6–8]. This has led to a continuous effort to improve the post-thaw semen quality with the objective to achieve promising results after insemination with frozen-thawed semen [9].

The cholesterol/phospholipid ratio determines the sensitivity of the sperm to cold shock damage [7]. Therefore, sperm with high cholesterol to phospholipid ratio such as rabbit and human sperm [10] are more resistant to the "cold shock" damage than sperm having low cholesterol to phospholipid ratio like boar, ram, and bull sperm [11]. Buffalo bull sperms have comparatively lower cholesterol content in their membranes [12–14] that is further decreased during freeze–thaw process. Because egg yolk from different avian species has different ratios of fatty acids, phospholipids, and cholesterol, it could have different effects on freezability of the sperm [15–21].

Quail egg yolk (QEY) has higher amount of phosphatidylcholine, less phosphatidylethnaolamine, and a smaller ratio of polyunsaturated fatty acids to saturated fatty acids that could contribute additional protective effect to spermatozoa during cryopreservation than chicken egg yolk (CEY) [15]. It is relevant to mention that saturated fatty acids are less vulnerable to lipid peroxidation than unsaturated fatty acids, and this characteristic makes QEY a more suitable cryoprotectant than CEY as has been reported previously for jackass [15] and rooster [22] sperm. In the same context, turkey egg yolk (TEY) has a higher content of cholesterol compared with CEY and has been reported to result in a better post-thaw semen quality in boar and stallion [21,23,24].

Considering the role of cholesterol to phospholipids ratio in the freezability of semen, the present study was conducted to determine if the addition of QEY and TEY in extender improves the spermatozoa after cryopreservation. The objective of the study was to investigate if QEY or TEY in tris-citric acid extender improve the post-thaw quality and fertility of Nili-Ravi buffalo bull spermatozoa.

2. Materials and methods

All experimental procedures and animals used in this study were approved by the ethical committee of the Department of Zoology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

2.1. Animals and local

Nili-Ravi buffalo breeding bulls (n = 6) of known fertility and similar age (7–8 years) with clinically normal reproductive tracts, kept under uniform feeding and handling conditions at Semen Production Unit, Qadirabad, Sahiwal, Pakistan were used in this study.

2.2. Preparation of extenders

Tris-citric acid buffer was used for the semen extender. It was prepared by dissolving 1.56-g citric acid (Fisher Scientific, UK) and 3.0-g Tris-(hydroxymethyl)-aminomethane (Research Organics, USA) in 73-mL distilled water. The pH of buffer was 7.0, and the osmotic pressure was 320 mOsmol kg^{-1}. Apart from the buffer, the semen extender contained 0.2% (wt/v) fructose (Scharlau, Spain); 7% (v/v) glycerol (Riedel-deHaen, Germany), and a combination of antibiotics consisting of streptomycin sulfate (1 mg/mL), procaine penicillin (300 IU/mL), and benzyl penicillin (Sinbiotic, China) (100 IU/mL). The experimental egg yolks (QEY or TEY) were added at 5%, 10%, 15%, and 20%, whereas 20% CEY in extender was kept as control.

2.3. Semen collection and evaluation

Semen was collected with artificial vagina (42 °C) and transferred to the laboratory for initial evaluation (volume, sperm motility, and sperm concentration). Semen volume was measured using graduated glass collection tube.

Sperm progressive motility was assessed with phase contrast microscope at × 400 at 37 °C by placing a drop semen sample on a prewarmed glass slide and covered with a cover slip [25]. Sperm concentration was measured by taking 1 μL of semen and 200 μL of formal citrate solution (1 mL of 37% formaldehyde in 99 mL of 2.9% sodium citrate) with Neubauer hemocytometer (Marienfeld, Germnay). Only those ejaculates that qualified a minimum standard of 1 mL volume, 60% motility, and 0.5 billion spermatozoa mL^{-1} of semen were selected for further processing. The qualifying ejaculates (n = 36 per experiment; two ejaculates/bull/collection) were split into five aliquots for dilution in experimental extenders containing QEYs or TEYs (5%, 10%, 15%, and 20%) or 20% CEY (controls) and were cryopreserved.

2.4. Semen processing and cryopreservation protocol

Semen from experimental animals was collected during the peak breeding season (September–November) at weekly intervals for a period of 6 weeks (3 weeks [replicates] for each of the separate experiments on QEY and TEY) during early morning (before sunrise) with the help of an artificial vagina (IMV, France) connected with a rubber cone and graduated glass collection tube at a temperature of 42 °C, using an intact bull as a teaser. Semen aliquots were diluted in a single step at 37 °C with each of the experimental extenders at 50 × 10^6 motile spermatozoa mL^{-1}. Diluted semen was cooled to 4 °C for 2 hours and equilibrated during 4 hours at 4 °C before being filled in 0.5-mL French straws (IMV, France) with suction pump at 4 °C in a cold cabinet (Minitub, Germany). Then the straws were kept 5-cm over liquid nitrogen vapors for 10 minutes before being plunged into liquid nitrogen (−196 °C) and stored. The samples from each treatment were thawed at 37 °C for 30 seconds in water bath and assessed for post-thaw quality.

2.5. Post-thaw sperm assays

2.5.1. Sperm motility

Sperm progressive motility was assessed with phase contrast microscope at × 400 at 37 °C by placing a drop (10 μL) semen sample on a prewarmed glass slide and covered with a cover slip [25].