Theriogenology 85 (2016) 954–959

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Trehalose improves semen antioxidant enzymes activity, post-thaw quality, and fertility in Nili Ravi buffaloes (*Bubalus bubalis*)

Sajid Iqbal [a], Syed Murtaza Hassan Andrabi [b], Amjad Riaz [a,*], Aneela Zameer Durrani [c], Nasim Ahmad [a]

[a] Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[b] Animal Reproduction Laboratory, Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan
[c] Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 5 November 2015
Accepted 6 November 2015

Keywords:
Buffalo semen
Trehalose
Cryopreservation
Antioxidant enzyme activity
Semen quality
Fertility

ABSTRACT

Our objectives were to study the effect of trehalose in extender on (1) antioxidant enzymes profile during cryopreservation (after dilution, before freezing, and after thawing), (2) *in vitro* quality (after thawing), and (3) *in vivo* fertility of Nili Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. Semen samples (n = 20) from four buffalo bulls were diluted in Tris-citric acid–based extender having different concentrations of trehalose (0.0, 15, 30, 45, and 60 mM) and frozen in French straws. At post dilution, profile of sperm catalase (U/mL) was higher ($P < 0.05$) in extenders containing 15, 30, and 45 mM of trehalose as compared to control. Although profiles of superoxide dismutase (U/mL) and total glutathione (μM) were higher ($P < 0.05$) in extenders containing 15 and 30 mM of trehalose as compared to control. At prefreezing, sperm catalase, superoxide dismutase, and total glutathione profiles were higher ($P < 0.05$) in all the treatment groups as compared to control. At post thawing, the profiles of catalase and total glutathione were higher ($P < 0.05$) in extender containing 30-mM trehalose as compared to other treatment groups and control. Whereas, profile of superoxide dismutase was higher ($P < 0.05$) in extenders containing 30, 45, and 60 mM of trehalose as compared to control and 15mM group. Post thaw total sperm motility (%) was higher ($P < 0.05$) in extender containing 30-mM trehalose as compared to control and 15 and 60-mM groups. Although sperm progressive motility (%), rapid velocity (%), average path velocity (μm/s), straight line velocity (μm/s), curvilinear velocity (μm/s), plasma membrane (structural and functional, %), acrosome (%), and DNA (%) integrity were higher ($P < 0.05$) in extender containing 30 mM trehalose as compared to other treatment groups and control. The fertility rates (61% vs. 43%) were higher ($P < 0.05$) in buffaloes inseminated with semen doses cryopreserved in extender containing 30 mM of trehalose than the control. It is concluded that addition of 30-mM trehalose in extender improves the semen antioxidant enzymes activity, post thaw quality, and fertility in Nili Ravi buffaloes.

1. Introduction

Cryopreservation is a technique adopted for the propagation of superior germplasm of dairy animals [1]. The success of this procedure primarily depends on the number of structurally and functionally viable spermatozoa [2].

* Corresponding author. Tel.: +92 333 5262325; fax: +92 42 99211461.
E-mail address: dramjadriaz@uvas.edu.pk (A. Riaz).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.11.004

Cryopreservation reduces semen antioxidant enzymes profile and fertility through generation of reactive oxygen species (ROS) [3]. High contents of saturated and polyunsaturated fatty acids particularly in buffalo sperm predominantly make them vulnerable to the injuries tempted by excessive ROS release [4–6]. The presence of higher profile of polyunsaturated fatty acids in sperm needs an effective antioxidant system to counter the per oxidative activity [7,8]. Nature has gifted spermatozoa with an intracellular and extracellular antioxidant defense mechanism, which includes enzymatic and nonenzymatic systems [9,10]. The enzymatic antioxidant defense system mostly comprises of superoxide dismutase, catalase, and glutathione peroxidase and reductase.

Catalase catalyzes the dissociation of H_2O_2 into H_2O and O_2 [11], reduces the oxidative stress, and finally enhances sperm motility [12,13]. The physiological role of superoxide dismutase is to prevent the lethal effect of ROS by dismutation of O_2 to H_2O_2, ultimately exert positive effect on membrane integrity during the cryopreservation process [14]. Glutathione belongs to the tripeptide thiols group and physiologically helps in protection of cell by reducing oxidative stress [15] through elimination of H_2O_2 [16].

The antioxidant defense systems of mammalian spermatozoa are predominantly of cytoplasmic in nature. Spermatozoa dispose of their cytoplasm during maturation stage of spermatogenesis. Consequently, mammalian spermatozoa are deficient of adequate reserves of natural antioxidants against the detrimental effects of ROS and lipid peroxidation (LPO) during cryopreservation [7,8,17]. Therefore, the consequences of oxidative stress can be reduced by adding antioxidants in semen extender. It has been documented that addition of antioxidants in extender enhanced the post-thaw sperm parameters (viability, motility, and fertility) in bovine [18], ovine [19], and caprine [20].

Trehalose is a nonreducing disaccharide sugar, and when used at higher doses in extender, it reduces intracellular ice crystal formation during cryopreservation [21–23]. Moreover, trehalose has the antioxidative property at lower doses and protects the spermatozoa by reducing LPO [24,25]. Literature regarding sperm antioxidant enzymes profile and use of trehalose as an antioxidant in water buffalo bulls is scarce. Therefore, the objectives of present study were to study the effect of trehalose in extender on (1) antioxidant enzymes profile during cryopreservation (after dilution, before freezing, and after thawing), (3) *in vitro* quality (after thawing), and (3) *in vivo* fertility of buffalo bull spermatozoa.

2. Materials and methods

2.1. Chemicals

The chemicals used in the preparation of extender including trehalose (T0167) were procured from Sigma–Aldrich Chemical Co., USA.

2.2. Selection of animals

Present study was conducted at Semen Production Unit, Qadirabad, Sahiwal, Pakistan (173 M, 73 74 E, 30 31.15 N). Four mature donor Nili Ravi buffalo bulls of similar age (6 years) were selected. Bulls were individually housed and maintained under uniform feeding and managemental conditions. Good quality seasonal green fodder was provided with 10% body weight of bulls. Fixed amount of concentrate (3 kg/day) was offered to each bull and water *ad libitum*.

2.3. Semen collection and initial evaluation

Artificial vagina maintained at 42 °C was used to collect the semen. Two consecutive ejaculates were collected once in a week from each animal for 5 weeks (replicate). Each ejaculate was shifted to water bath maintained at 37 °C in the laboratory within a minute. After collection percentage, motility was evaluated under phase-contrast microscope (Olympus, Japan) at magnification × 200. Each semen sample was evaluated for sperm concentration spectrophotometrically (IMV, France) [26]. Ejaculates having greater than 65% visual motility and greater than 500×10^6 sperm/mL were selected for further processing.

2.4. Semen extension and freezing

Tris-citric acid–based extender (Tris 199.80 mM, citric acid 69.75 mM, fructose 55.56 mM, egg yolk 20% [vol:vol], glycerol 7% [vol:vol], benzyl penicillin 1000 [IU/mL], and streptomycin sulfate 1000 [μg/mL]; pH 6.9) was used in the present study. Semen samples of individual animal were diluted with the extender containing different concentrations of trehalose (0, 15, 30, 45, and 60 mM; osmotic pressure 264, 273, 293, 307, and 322 mOsmol/kg, respectively) to a final concentration of 50×10^6 spermatozoa/mL at 37 °C. Cooling of extended semen samples to 4 °C was done in 2 hours and equilibration at 4 °C for 4 hours. Semen samples were packed and sealed in 0.54 mL French straws at 4 °C. After equilibration, semen-filled straws were frozen in liquid nitrogen vapors for 10 minutes. Finally, straws were dipped and stored in liquid nitrogen (−196 °C). To evaluate post-thaw sperm quality parameters, two straws per treatment were thawed at 37 °C in water bath for 30 seconds. Post-thaw semen quality parameters were evaluated after at least 24 hours of storage.

2.5. Sperm antioxidant enzymes profile

Antioxidant enzymes profile (catalase, superoxide dismutase, and total glutathione) was determined during different stages of cryopreservation (after dilution, before freezing, and after thawing). Pooled ejaculates of each bull were analyzed for antioxidant enzymes profile. Thawed semen sample (120 μL) were centrifuged at 1600 × *g* for 5 minutes to remove the seminal plasma. After discarding the supernatant, 360 μL of 1% Triton X-100 solution was added into the precipitate. The suspension was incubated for 20 minutes and was subsequently centrifuged at 25 °C for 30 minutes at 4000 × *g*. The precipitate was resuspended, and finally, the supernatant was collected containing crude extract of enzymes in the sperm [27]. The antioxidant enzymes (catalase, superoxide dismutase, and total glutathione) profile was analyzed through ELISA kit. Catalase enzyme profile was analyzed through the