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Effects of adding taurine and trehalose to a tris-based egg yolk extender on buffalo (*Bubalus bubalis*) sperm quality following cryopreservation

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ABSTRACT

Cryopreservation induces sublethal damage to the spermatozoa, which leads to their reduced fertile life. Under the present study, the cryoprotective effect of taurine and trehalose on buffalo sperm quality parameters after freeze–thaw process was studied. Buffalo semen was cryopreserved in tris-based egg yolk extender along with cryoprotectants like taurine (50 mM) or trehalose (100 mM) and used for the assessment of sperm quality parameters like motility, viability, plasma membrane integrity, total antioxidant status and the extent of cryocapacitation. The results were compared to semen cryopreserved in tris-based egg yolk extender only as control. Post-thaw semen evaluation clearly indicated that the addition of taurine or trehalose significantly improved ($P < 0.05$) the motility, viability and membrane integrity compared to control spermatozoa. The extent of sperm cells underwent cryocapacitation was significantly lowered ($P < 0.05$) in presence of taurine or trehalose. Moreover, the percentage of in vitro capacitated cells in the treated samples was comparable to the control spermatozoa along with maintaining other sperm quality parameters. Finally, compared to the control and trehalose treated sample, addition of taurine to the freezing extender showed more positive effect on the total antioxidant power of seminal plasma and spermatozoa. It is concluded that the addition of taurine or trehalose to the freezing extender led to the reduction of cryodamage to the buffalo spermatozoa.

1. Introduction

The foremost artificial breeding technology being widely applied to buffalo husbandry is the artificial insemination with cryopreserved semen. However, the low success rates of the cryopreserved semen, as compared to natural breeding, due to sublethal damage that is not completely understood, withhold its wider acceptability in the field. The basic studies on semen preservation and cryopreservation have not been done up to the desired extent to completely understand the male fertility factor for effective fertilization. Compared to fresh spermatozoa, eight times more cryopreserved bovine sperm were required to achieve equivalent fertilization rates in vivo (Shannon and Vishwanath, 1995). The post-thaw motility of the cryopreserved buffalo sperm is poor and the success rate of IVF with buffalo sperm is only 10–20% as compared to cattle which is 30–35% (Nandi et al., 2006; Totey et al., 1992).

The standard cryopreservation method includes temperature reduction, cellular dehydration, freezing and thawing (Medeiros et al., 2002). Procedures associated to cryopreservation increase premature capacitation of spermatozoa. These alterations may not affect motility

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but reduces lifespan, ability to interact with the female reproductive tract and sperm fertility (Medeiros et al., 2002). Freezing and thawing processes also lead to the generation of reactive oxygen species (ROS). Excess production of ROS during cryopreservation has been associated with reduced post-thaw motility, viability, membrane integrity, antioxidant status, fertility and sperm functions (Aitken et al., 1998; Bilodeau et al., 2000; White, 1993; Zhao and Buhr, 1995). Sperm cells contain high concentrations of polyunsaturated fatty acids (PUFA), and therefore are highly susceptible to lipid peroxidation (LPO) (Aitken and Fischer, 1994; Cassani et al., 2005; Storey, 1997). Tris-based extenders are frequently used for semen cryopreservation in domestic animals (Purdy, 2006) as also in buffaloes. In recent years, cryoprotectants like taurine and trehalose were supplemented to the freezing extender of bull (Chen et al., 1993; Sarıözkan et al., 2009; Uysal et al., 2007), boar (Funahashi and Sano, 2005; Gutiérrez-Pérez et al., 2009; Hu et al., 2009), ram (Bucak et al., 2007, 2008), goat (Ateşşahin et al., 2008), dog spermatozoa (Martins-Bessa et al., 2009; Michael et al., 2007) to improve the semen characteristics after cryopreservation. The sulfonic amino acid, taurine, acts as an antioxidant and can traverse the sperm plasma membrane and inhibit lipid peroxidation and protects the cells against the accumulation of ROS (Chen et al., 1993; Foote et al., 2002). Trehalose, a non-reducing disaccharide, has a protective role against osmotic effect and forms specific interactions with membrane phospholipids, rendering hypertonic media, causing cellular osmotic dehydration before freezing, and hence decreasing the amount of cell injury by ice crystallization (Anchordoguy et al., 1987; Liu et al., 1998; Molinia et al., 1994; Storey et al., 1998). So far, the effect of additives like taurine and trehalose on the cryopreserved buffalo sperm quality has not been reported. Therefore, the present study was conducted to determine the effect of these additives on sperm quality parameters like post-thaw motility, membrane integrity and total antioxidant status during cryopreservation of buffalo spermatozoa.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA, fraction V), heparin, lysophosphatidyl choline (LPC) were procured from Sigma Chemical Company (St. Louis, MO, USA). Taurine and Trehalose were purchased from Sisco Research Laboratories (Mumbai, India). Sodium citrate, citric acid, dextrose, and fructose were procured from Central Drug House private limited (New Delhi, India). Sodium pyruvate, sodium bicarbonate, eosin B and nigrosin were purchased from Sd Fine-chem limited (Mumbai, India).

2.2. Semen collection and cryopreservation

The Murrah buffalo bulls (3–5 years of age) were housed at Artificial Breeding Complex, National Dairy Research Institute, Karnal, India, under uniform nutritional conditions. A total number of 32 ejaculates from four buffalo bulls (eight ejaculates from one bull) were collected twice a week by artificial vagina (IMV, L'Aigle cedex, France). Immediately after collection the volume of the semen was measured in a conical tube graduated at 0.1 ml intervals, mass motility was assessed by light microscopy, and ejaculates were immersed in a warm water bath at 38.5 °C until semen was extended (Tomar, 1997). Standard semen characteristics like progressive motility, viability, membrane integrity and acrosomal abnormalities in the fresh semen ejaculates were also studied (Revell and Mrode, 1994; Therien and Manjunath, 2003; Tomar, 1997; Watson, 1975). The data of field pregnancy rates of the four buffalo bulls used in this study was collected from Artificial Insemination Laboratory, Cattle yard, National Dairy Research Institute, Karnal, India. The field pregnancy rate of a buffalo bull was calculated by the number of successful pregnancies diagnosed from the number of artificial inseminations performed. On an average 40 animals were inseminated with the semen of a single bull over a period of 10 months. Ejaculates containing spermatozoa with >80% forward progressive motility were used in this study. Tris-based egg yolk extender (Tris 33.2 g/l, citric acid 18.3 g/l, dextrose 7.8 g/l, egg yolk 20% (v/v), glycerol 6.4%, benzyl penicillin 10,00,000 IU/l, streptomycin 1 g/l) was used as the freezing extender. Each ejaculate was split into aliquots and diluted with the freezing extender containing taurine (50 mM), trehalose (100 mM), and without additives (control) to a final concentration of approximately 80×10^6 cells ml^{-1}. Diluted samples were aspirated into 0.25 ml (medium-sized) French straws, sealed with polyvinyl alcohol powder and equilibrated at 4 °C for 4 h. After equilibration, the straws were frozen in liquid nitrogen vapour, 5 cm above liquid nitrogen, for 10 min and then the straws were plunged into liquid nitrogen for storage. After storage for 6–8 weeks, frozen straws were thawed at 37 °C for 30 s in a water bath and used for the study of different semen parameters.

2.3. Sperm quality assessment

2.3.1. Post-thaw sperm motility and viability

For the motility analysis of the post-thaw semen samples, five straws per treatment were thawed by immersion in a water bath at 37 °C for 30 s. Immediately, 10 μl of thawed semen aliquots were transferred into glass slides and cover-slips were applied. Randomly a microscopic field was chosen in the slide and motile sperm showing any movement of the flagellum and non-motile sperm with no movement of flagellum were counted at 37 °C by light microscope at 400×. Another field was chosen after counting in the first field. The setting of the field has always been from left side of the slide to right side. Likewise, 300 cells were counted in at least 5 fields per slide. Minimum 3 slides were evaluated per ejaculate/bull. The mean of the three estimations was used as the final motility score (Tomar, 1997). The sperm viability was assessed according to Therien and Manjunath (2003) using eosin B and nigrosin stains. Briefly, 10 μl of each sperm suspension was applied on a microscope slide and mixed with 5 μl of 5% eosin B and 5 μl of 10% nigrosin. The stained sperm were spread on the slide. Randomly a microscopic field was chosen in the slide and viable sperm (white) and nonviable