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Cryo-survival, cryo-capacitation and oxidative stress assessment of buffalo spermatozoa cryopreserved in new soya milk extender ☆

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ABSTRACT

Egg yolk is most commonly used in semen extenders for cryopreservation but, wide variability in composition and potential risk of xenobiotic contamination has raised questions upon the use of egg yolk. Therefore, the present study was designed to develop a soya milk based phytoextender for buffalo semen cryopreservation. Soya milk was prepared from fresh soya beans in laboratory. Soya milk (25% v/v) and glycerol (6.4% v/v) was added in Tris citrate buffer to prepare Soya Milk Tris (SMT) extender. To improve post-thaw sperm quality, cryo-protectants like trehalose (100 mM) or taurine (50 mM) was supplemented in SMT extender before semen cryopreservation. Post-thaw quality parameters, degree of cryocapacitation and oxidative stress (lipid peroxidation) of spermatozoa cryopreserved in SMT with or without additives were assessed and compared with Egg Yolk Tris (EYT) extender. SMT extender showed no significant ($P > 0.05$) differences in sperm motility, viability, membrane integrity and acrosome integrity as compared to EYT extender. The degree of cryocapacitation and oxidative damage were found significantly ($P < 0.05$) lower in spermatozoa cryopreserved in SMT extender as compared to EYT extender. Supplementation of additives (trehalose or taurine) in SMT extender significantly ($P < 0.05$) reduced oxidative damage. Thus, soya milk extender (25% soya milk v/v; 6.4% Glycerol in tris citrate buffer) with additives (trehalose or taurine) may substitute conventional egg yolk extender (20% egg yolk v/v; 7% Glycerol in tris citrate buffer) for better cryopreservation of buffalo semen.

1. Introduction

Artificial Insemination (AI) extensively depends on frozen semen. However, the process of cryopreservation damages plasma membrane, cytoskeleton, motility apparatus, and nucleus of spermatozoa (Ragoonanan et al., 2010). The process of cryopreservation also induces premature capacitation (Reddy et al., 2010), osmotic stress and oxidative damage (Thuwanut et al., 2008) that reduce functional life of spermatozoa. Therefore, quality evaluation of spermatozoa based on motility is not sufficient to judge true fertilizing capacity of spermatozoa.

Currently, egg yolk-based extenders are globally used for cryopreservation of semen from bull and many other species. However, wide variability, risk of xenobiotic contamination (Aires et al., 2003) and presence of steroid hormones (Lipar et al., 1999) have raised questions upon the use of egg yolk in freezing extender. Furthermore, egg yolk contains substances (e.g. high-density lipoproteins and minerals) that inhibit cellular respiration and affect

Abbreviations: EYT, egg yolk tris; SMT, soya milk tris; HOST, hypo-osmotic swelling test

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metabolic activities (Pace and Graham, 1974). Hence, to circumvent these problems a non animal origin based semen extender with comparable cryoprotective effect was desirable. Recently, many commercial extenders like AndroMed® (Aires et al., 2003) and Bioxcell® (Akhter et al., 2010) have been evaluated for preservation of bovine and buffalo semen and found to maintain better semen quality than egg yolk based extenders. But, high cost and limited availability of these commercial extenders make them unobtainable for emerging and small-scale semen banks especially in developing countries. We have developed an economic, pathogen free and non animal origin based soya milk semen extender to replace egg yolk based extender and reported successful liquid preservation of buffalo semen upto 72 h (A.K. Singh et al., 2012). A further study was required to establish this extender for cryopreservation of buffalo semen.

Recently, cryoprotectants like taurine or trehalose, have been supplemented in freezing extenders of bull (Sariozkan et al., 2009) and buffalo (Kumar and Atreja, 2012, Kumar et al., 2013) semen prior to cryopreservation, to improve the post thaw sperm quality. Hence, the present study was designed first; to cryopreserve buffalo semen in soya milk tris (SMT) extender, second; to evaluate cryo-survival, cryo-capacitation and oxidative stress of sperm cryopreserved in SMT extender in comparison to conventional EYT extender and third; to further improve the post thaw sperm quality by supplementing cryoprotectants like trehalose or taurine in SMT extender.

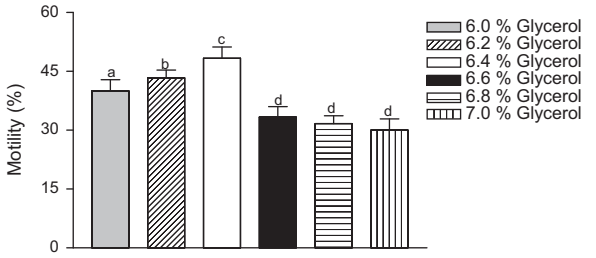

Fig. 1. Post thaw sperm motility of buffalo spermatozoa cryopreserved in soya milk extender with different glycerol concentration for optimization of glycerol percentage. Values are the mean ± S.E.M. of nine experiments. Means with different letters are significantly different ($P < 0.05$).

2. Results

2.1. Optimization of glycerol for cryopreservation

Post thaw motility of buffalo spermatozoa cryopreserved in SMT extender with gradient of glycerol ranging from 6.0–7.0% with a difference of 0.2% revealed that motility of spermatozoa cryopreserved in SMT extender with 6.4% of glycerol was significantly higher ($P < 0.05$) than other concentrations (Fig. 1).

2.2. Post-thaw motility, viability, membrane integrity and acrosome integrity

The standard semen quality parameters such as percent motility, viability, membrane integrity and acrosomal integrity in fresh semen ejaculates and post thawed semen are shown in Table 1. Cryopreservation of buffalo spermatozoa resulted in a significant ($P < 0.05$) decrease in all the standard semen quality parameters as compared to fresh. Buffalo spermatozoa cryopreserved in SMT extender did not show significant ($P > 0.05$) differences in any of the standard sperm quality parameters as compared to spermatozoa cryopreserved in EYT extender. Supplementation of trehalose (100 mM) or taurine (50 mM) to freezing extender showed a significantly ($P < 0.05$) higher post-thaw sperm motility, viability, percent membrane integrity and acrosome integrity as compared to sperm cryopreserved in their absence (Table 1).

2.3. Extent of cryocapacitation of spermatozoa

Sperm cryocapacitation was assessed by lysophosphatidyl choline (LPC)-induced acrosome reaction followed by dual staining of spermatozoa. Cryopreservation of buffalo spermatozoa in EYT extender resulted in a significant ($P < 0.05$) increase of capacitation as compared to freshly ejaculated buffalo semen. On the other hand, extent of cryocapacitation was found significantly ($P < 0.05$) lower in buffalo spermatozoa cryopreserved in SMT extender than EYT extender. Moreover, Supplementation of 100 mM trehalose or 50 mM taurine to the freezing medium prior to cryopreservation significantly ($P < 0.05$) decreased the extent of cryocapacitation in buffalo spermatozoa as compared to control (Table 1).

Table 1
Post thaw parameters of freshly ejaculated and cryopreserved Buffalo spermatozoa in extended in egg yolk tris extender (EYT), soya milk tris extender (SMT), soya milk tris extender supplemented trehalose (SMT+Trehalose) and soya milk tris extender supplimented with taurine (SMT+Taurine) respectively. Values are Mean ± Standard Error Mean (S.E.M.) of nine experiments. Mean ± S.E.M. with different letters (a,b,c,d) in row are significantly different ($P < 0.05$).

	Fresh	EYT	SMT	SMT+Trehalose (100 mM)	SMT+Taurine (50 mM)
Motility (%)	81.0 ± 1.87^{a}	47.0 ± 1.22^{b}	46.0 ± 1.87^{b}	54.0 ± 1.87^{c}	57.0 ± 1.22^{c}
Viability (%)	89.0 ± 1.83^{a}	61.4 ± 1.12^{b}	62.2 ± 1.58^{b}	69.2 ± 1.11^{c}	70.0 ± 1.26^{c}
Membrane integrity (%)	74.6 ± 3.04^{a}	38.2 ± 1.31^{b}	39.2 ± 1.52^{b}	42.4 ± 1.93^{b}	42.2 ± 2.03^{b}
Acrosome integrity (%)	96.2 ± 1.48^{a}	84.4 ± 3.40^{b}	86.4 ± 2.20^{bc}	90.6 ± 2.28^{cd}	91.4 ± 2.16^{d}
Sperm capacitation (%)	5.6 ± 0.67^{a}	17.2 ± 0.73^{b}	$12. \pm 0.83^{c}$	9.8 ± 0.58^{d}	9.0 ± 0.44^{d}
Lipid peroxidation (nmol MDA/10^{8}cells)	1.13 ± 0.31^{a}	3.26 ± 0.10^{b}	2.48 ± 0.10^{c}	1.48 ± 0.02^{d}	1.47 ± 0.16^{d}