



Role of reduced glutathione in improving post-thawed frozen seminal characters of poor freezable Jersey crossbred bull semen

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ABSTRACT

The role of reduced glutathione in improving the post thaw seminal characters in good and poor freezable Jersey crossbred bull semen was studied. And from each group 18 ejaculates were taken where tris extender supplementing with reduced glutathione @ 5mM was used for the experiment; thereafter, rapid freezing in liquid nitrogen was followed by thawing. The post-thawed seminal characters were studied to observe efficacy of addition of glutathione. The total motility showed significant difference between the 2 groups in both freezable bull semen, whereas livability, total sperm abnormality, loss of acrosomal integrity, percentage of hypo osmotic swelling positive sperm and vanguard distance travelled by sperm (mm/hour) in cervical mucus were found highly significant between the reduced glutathione treated and control group. However, the lipid peroxide levels as assessed by malondialdehyde formation were nonsignificant between the 2 groups, but the glutathione had reduced the production of more ROS as compared to the control group. The addition of reduced glutathione had maintained the functional state of good semen and enhances freezability of poor semen. Thus the reduced glutathione improves post thaw sperm functional parameters such as motility, viability, functional membrane and acrosomal integrity in poor freezable semen suggesting that addition of reduced glutathione in semen might have improved post-thawed seminal characters of crossbred bull frozen semen.

Key words: Bulls, Frozen thawed semen, Post-thawed seminal characters, Reduced glutathione, Semen

The conception rate in frozen thawed semen has been reported to be low (Waberski 2007). The process of cryopreservation exert physiological, osmotic and chemical stress on the sperm membrane and sperm structure, which may result into damage of spermatozoa and post-thawed quality of spermatozoa (Ozkavukcu *et al.* 2008, Muller *et al.* 2008). The good semen has the ability to tolerate various adverse effects during freezing process like osmotic stress, solution effect and large ice crystal formation (Morris *et al.* 2007, Ozkavukcu *et al.* 2008). The spermatozoa membrane has highly unsaturated fatty acids content (Sinha *et al.* 1996),

lipid peroxidation of spermatozoa membrane eventually leads to apoptotic cell changes and loss of sperm function (Aitken *et al.* 1989, Sikka 1996) in post-thaw semen samples.

Usually, the semen additives may maintain frozen-thawed seminal quality and improve the conception rate (Perumal *et al.* 2010). The cryopreservation reduces the content of reduced glutathione of spermatozoa and seminal plasma (Bilodeau *et al.* 2000, 2001, Gadea *et al.* 2005, 2008, Stradaoli *et al.* 2007) and leading to changes in membrane transportation (Alvarez and Storey 1982), affecting fertility of the spermatozoa. Perusal of literature revealed paucity of information on the ability of reduced glutathione in improving freezability of poor freezable bull by maintaining the sperm membrane integrity during cryopreservation and thawing. Hence, the objective of this study was to assess the role of reduced glutathione in improving semen quality in poor freezable Jersey crossbred bull semen.

MATERIALS AND METHODS

The experiment was conducted in 3 good freezable and 3 poor freezable Jersey crossbred bulls of 4 to 6 years of age belonging to Frozen Semen Bank, Cuttack, under Animal Husbandry and Veterinary Services, Government of Odisha,

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India. The experimental bulls of 4 to 6 years of age with good body condition (score 5–6) were selected and maintained under optimum managerial practices as per the standard criteria fixed for maintenance of breeding bulls in bull stations. The pre freezing seminal parameters of both groups of semen had >60% progressive forward motility, less than 20% total abnormality. The good and poor freezable bulls were classified based on post-thaw motility parameter. Those bulls provided consistently more than 40% post thaw motility were considered as good freezer bulls (n=3), whereas those bulls with less than 40% post-thaw motility were considered as poor freezer bulls (n=3).

Ejaculates (6) from each bull were collected twice in 1 week. Each ejaculate was split in to 2 equal parts to use 1 part as control without glutathione and another part with reduced glutathione @ 5mM for cryopreservation and subsequent evaluation. All experimental semen samples were extended with Tris extender and frozen by rapid freezing method in mini straws and cryopreserved using programmable freezer and stored in liquid nitrogen. Then frozen–thawed seminal characters like individual motility, percentage of live sperm, abnormal spermatozoa and acrosomal integrity were studied by staining with eosin-nigrosin and giemsa staining methods respectively. The *in vitro* fertility test like vanguard distance travelled by sperm in cervical mucus penetration test, percentage of hypo-osmotic swelling test (HOST) positive sperm (at 150 mOsm) were assessed as per Jeyendran *et al.* (1984). The lipid peroxide assay (MDA) was conducted as per Suleiman *et al.* (1996).

The results were analyzed statistically after arcsine transformation of percentage data by using SPSS 15.0 software.

RESULTS AND DISCUSSION

The post-thaw seminal parameters significantly ($P < 0.01$) differed between the glutathione treated and control group in both good and poor freezable bull semen (Tables 1, 2) except the lipid peroxide assay (Fig. 1). The glutathione treated group had produced nonsignificantly less malondialdehyde MDA as compared to untreated control group in both good and poor freezable semen. In good freezable semen the glutathione enhanced the post-thaw seminal parameters, whereas in poor freezable semen it helped to maintain the post-thaw seminal parameters.

The glutathione treated group had significantly ($P < 0.05$) higher post-thaw motility as compared to control group in both good freezable and poor freezable bull semen, which is very comparable to the earlier findings (Jain and Arora 1988, Uysal *et al.* 2007). However, slightly lower motility was reported in glutathione added semen in crossbred bulls (Zakariya *et al.* 2005, Munsu *et al.* 2007). The reduced glutathione treated sperm has more and fast forward movement as compared to control group because the reduced glutathione neutralizes and prevent the formation of free radical and maintains the membrane integrity and increases the efficiency of flagellar motion efficiently in fluid medium. Moreover, glutathione also improves the efficiency of mitochondria (Perumal *et al.* 2010) and also protect the other parts of sperm membrane to maintain the motility (Gonçalves *et al.* 2010). During freezing, the spermatozoa membrane is damaged resulting in leakage of enzymes. The present study revealed that addition of glutathione in freezing extender prevents leakage of enzymes through the action of membrane stabilizer as stated by Maxwell and Stojnov (1996).

The total live sperm percentage of glutathione treated

Table 1. Post-thaw sperm motility, livability and total abnormal sperm per cent of glutathione added semen (n=18) of good and poor freezable Jersey crossbred bulls

Groups	Motility (%)		Livability (%)		Total sperm abnormality (%)	
	Good freezer (18)	Poor freezer (18)	Good freezer (18)	Poor freezer (18)	Good freezer (18)	Poor freezer (18)
Control	62.08 ± 0.39 ^a	45.32 ± 0.24 ^a	63.09 ± 0.37 ^a	56.78 ± 0.56 ^a	13.18 ± 1.41 ^a	18.06 ± 0.18 ^a
Glutathione	74.12 ± 0.39 ^b	66.83 ± 0.42 ^b	76.28 ± 0.37 ^b	69.32 ± 0.35 ^b	9.45 ± 0.94 ^b	14.26 ± 0.18 ^b

Means with different superscripts in a column varied significantly ($P < 0.01$); figures in parenthesis indicate number of ejaculates.

Table 2. Post-thaw loss of acrosomal integrity, hypo-osmotic swollen positive sperms and cervical mucus penetration rate of glutathione added semen (n=18) of good and poor freezable Jersey crossbred bulls

Groups	Loss of acrosomal integrity(%)		Hypo-osmotic swelling test (HOST),(%)		Cervical mucus penetration Test (CMPT), (mm/h)	
	Good freezer (18)	Poor freezer (18)	Good freezer (18)	Poor freezer (18)	Good freezer (18)	Poor freezer (18)
Control	12.20 ± 0.71 ^a	15.97 ± 0.5 ^a	65.74 ± 0.27 ^a	57.03 ± 0.32 ^a	25.84 ± 1.35 ^a	20.24 ± 1.09 ^a
Glutathione	8.44 ± 0.63 ^b	13.01 ± 0.6 ^b	81.19 ± 0.30 ^b	71.29 ± 0.26 ^b	31.16 ± 1.09 ^b	25.44 ± 1.35 ^b

Means with different superscripts a, b in a column varied significantly ($P < 0.01$); figures in parenthesis indicate number of ejaculates.

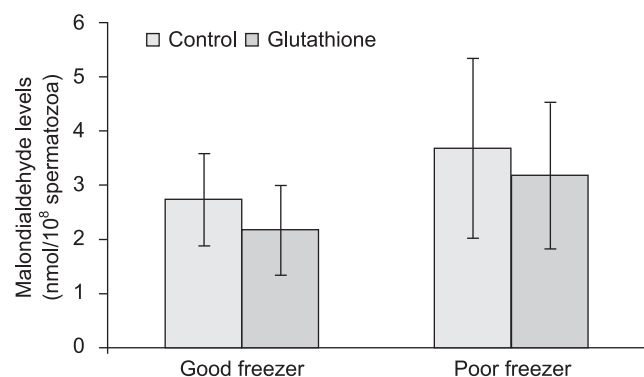


Fig. 1. Post-thawed lipid peroxidation levels ((MDA) malondialdehyde formation, $\times 10^8$ spermatozoa) of glutathione added semen of good and poor freezable Jersey crossbred bulls.

group was higher than control group of different freezable bulls. Addition of glutathione proportionally enhanced the viability in poor freezable semen and maintained the viability in good freezable bull sperm. The present observation in the loss of acrosomal integrity in post thaw semen was partially in accordance with the findings of Uysal *et al.* (2007). The frozen thawed value of HOST revealed a significant difference ($P < 0.01$) between experimental groups. These observations indicated that the glutathione acts as membrane stabilizer. Glutathione plays an important role in scavenging reactive oxygen species and their intermediates (Meister and Anderson 1983). The reduced lipid peroxidation potential of the glutathione treated semen samples, suggested that glutathione might protect the spermatozoa from membrane damage by inhibiting the lipid peroxidation process as suggested earlier (Irvine 1996, Kefer *et al.* 2009).

The present study revealed, that bull semen extended with glutathione has showed higher percentage of cervical mucus penetration than control group semen. The migration of the vanguard distance was higher due to enhanced straight forward motility, which was ascribed to normally produced adequate mucus penetration (Munsi *et al.* 2007). The glutathione incorporation to the extended semen might have potentiated the viability of the sperm with respect to its motility and morphological integrity, which was reflected by the highest rate of penetration through the cervical mucus in the present course of investigation.

The present study suggested that addition of glutathione in semen extender might improve post-thawed seminal characters of poor freezable Jersey crossbred bull semen.

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