SHORT COMMUNICATION

Glutathione as a Semen Additive to Improve Surti Buffalo (*Bubalus bubalis*) Bull Semen Cryopreservation

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Abstract

The present study evaluated the effects of glutathione as an additive in Tris fructose egg yolk citrate extender on Surti buffalo bull semen. Extended semen @ 80 million sperm/mL was divided into 4 equal aliquots and glutathione was added at 0.5 mM (T1), 2.0 mM (T2), and 5.0 mM (T3) with one control (T0) group. The percentages of post-thaw sperm progressive motility, live sperm, and HOS responsive sperm were significantly higher ($p < 0.05$), whereas sperm abnormalities were significantly lower ($p < 0.05$) in treatment T1, T2 and T3 compared to T0 control. The treatment T2 (2.0 mM glutathione) had the highest post-thaw sperm progressive motility, live sperm, HOS response sperm percentage and reduced sperm abnormalities compared to other three groups. It was concluded that glutathione at 2.0 mM concentration significantly ($p < 0.05$) improved post-thaw semen quality of buffalo bulls in comparison to control and glutathione concentrations of 0.5 mM and 5.0 mM in Tris extender.

Keywords: Cryopreservation, Glutathione, Post-thaw quality, Surti buffalo, Semen additive.

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Introduction

Sperm cryopreservation, as a common and important procedure in ART labs, provides a valuable option in the field of assisted reproduction (Oehninger *et al.,* 2000 and O'Connell *et al.,* 2002). The causes deteriorating the qualitative and functional characteristics of semen after freezing and thawing are those linked to oxidative stress and decreased detoxification of antioxidants present in the cells and seminal plasma (Stradaioli *et al*., 2007). Glutathione naturally present in buffalo semen has been recognized as an essential intracellular antioxidant (Andrabi, 2009). Extender supplementation with glutathione (GSH) resulted in a better post-thaw semen quality (Bilodeau *et al.,* 2001; Tuncer *et al.,* 2010). It was demonstrated that GSH added to bovine semen extender resulted in a decreased LPO level during freezing, and ultimately improved fertility rates (Perumal *et al.,* 2011). The purpose of this study was to evaluate the comparative effect of addition of different concentration of glutathione to semen extender on post-thaw semen quality parameters of Surti buffalo bulls.

Materials and Methods

The study was conducted on six Surti buffalo bulls of the age group 6.5–7.5 years, weighing 445–520 kg, reared at Network Project on Buffalo Improvement at College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur, (Rajasthan, India). Semen samples were collected from each bull twice a week in the morning hours by Artificial Vagina method. Total twenty-four ejaculates (4 x 6) were collected from these bulls. The ejaculated semen samples were evaluated for their quality by routine tests to confirm suitability for further processing and only those with more than 70% initial motility were utilized for this study.

After evaluation, the fresh semen samples were diluted with Tris-fructose-egg yolk-citrate extender @ 80 million spermatozoa/mL and were divided into four equal aliquots (1-4). Glutathione was added into aliquot 2, 3 and 4 at the rate of 0.5 mM, 2.0 mM, and 5.0 mM, respectively (treatment T1, T2, T3), while aliquot 1 served as untreated control (T0). These samples were then processed for cryopreservation and after thawing immediately at 37°C for 30 seconds, samples were evaluated for progressive sperm motility (%), live sperm (%), abnormal sperm (%), HOS response (%) using standard procedure. The data were analysed statistically using CRD and one way ANOVA (Sendecor and Cochran, 1994).

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Results and Discussion

Post-Thaw Motility (%)

In the present study, significantly higher ($p < 0.05$) mean post-thaw motility was observed with glutathione concentration of 0.5 mM (67.46 ± 0.24 %), 2.0 mM (68.57 ± 0.36 %) and 5.0 mM (65.65 ± 0.32 %) in comparison to control (64.78 ± 0.25 %). These observations concurred well with the reports of Ansari *et al.* (2012) and Ismail and Darwish (2011) on buffalo semen cryopreserved with varying levels of glutathione. In Nili-Ravi buffalo bull semen, Ansari *et al.* (2010) observed significantly ($p \leq 0.05$) increased post-thaw progressive sperm motility at 0, 3, 6 hours of post-thaw incubation in glutathione supplemented semen extender in a dose-dependent manner and 2.0 mM glutathione was found to be the best (56.7 ± 2.9, 41.7 ± 2.9, 28.3 ± 2.9) compared to lower or higher levels. The increase in sperm motility with glutathione supplementation might be due to a decrease in oxidative stress and ROS production that is associated with lipid peroxidation of the sperm plasma membrane causing poor sperm motility. However, Perumal *et al.* (2011) and Tuncer *et al.* (2010) did not find any significant effect on the progressive motility of sperm in frozen thawed bull semen cryopreservation in extender containing varying levels of antioxidants at immediate post-thaw.

Physico-chemical properties of a diluent can affect the pattern of sperm motility. Results of the present study have shown that post-thaw sperm progressive motility was significantly increased with glutathione supplementation in the extenders with 2.0 mM concentration compared to the control or 0.5 mM and 5 mM glutathione concentration.

Live Sperm (%)

Significantly higher ($p < 0.05$) mean post-thaw live sperm (%) was observed with glutathione concentration of 0.5 mM (74.40 ± 0.38%), 2.0 mM (75.90 ± 0.38%) and 5.0 mM (72.30 ± 0.40%) in comparison to control (70.11 ± 0.44%). These findings supported well the earlier observations of Ansari *et al.* (2010) in Nili-Ravi buffalo semen. Ismail and Darwish (2011) and Gangawar *et al.* (2018) also reported significantly ($p < 0.05$) higher percentage of live spermatozoa in Murrah buffalo bull semen at post-thaw stage in 0.5 or 1.0 mM glutathione group than the control group.

According to Kothari *et al.* (2010) continuous peroxidation process results in high membrane damage, decreased or increased fluidity on certain ions. Holt (2000) reported that glutathione prevents lipid peroxidation of sperm cell membrane during the freezing process of semen, thereby increasing the motility and integrity of the acrosome after thawing. Glutathione has a positive effect on maintaining motility and sperm viability. An improvement in sperm viability has been reported to be highly correlated with glutathione content of the bull spermatozoa (Stradaioli *et al.,* 2007). Our findings corroborate with the above reports.

Sperm Abnormalities (%)

A significant ($p < 0.05$) reduction in the post-thaw abnormal sperm percentage was observed with glutathione concentration of 0.5 mM (11.42 ± 0.20%), 2.0 mM (10.44 ± 0.23%) and 5.0 mM (12.25 ± 0.17%) in comparison to control (12.88 ± 0.15%) in post-thaw Surti buffalo bull semen samples. Uysal and Bucak (2007) found that addition of glutathione at 5 mM had the lowest percentage of sperm abnormalities in ram frozen semen followed by 10 mM, 20 mM and control group, respectively. The reduction in sperm abnormalities in general can be explained by the fact that the cold shock of sperm cells during the freezing-thawing process is associated with oxidative stress induced by free radicals and the free radicals are eliminated by antioxidant systems (Sanocka and Kurpisz, 2004).

Hypo-Osmotic Swelling Test (%)

In the present study, a significant ($p < 0.05$) improvement in the mean post-thaw HOS response was observed with glutathione concentration of 0.5 mM (65.32 ± 0.39%), 2.0 mM (66.90 ± 0.42%) and 5.0 mM (63.38 ± 0.30%) in comparison to control (60.53 ± 0.23%). These findings supported the observations of Ansari *et al.* (2010, 2012) and Ismail and Darwish (2011) that fortification of semen extender with 2.0 mM glutathione significantly ($p \leq 0.05$) increased the Nili-Ravi buffalo bull sperm plasma membrane integrity while 3.0 mM did not show any improvement in the percentage of spermatozoa with intact plasmalemma. Gangawar *et al.* (2018) also reported significantly ($p < 0.05$) higher HOS response at post-thaw stage in the treatment (0.5 mM) group (60.30 ± 0.89) than the control group (54.30 ± 1.03). Previously, high post-thaw plasma membrane integrity along

Table 1: Post-Thaw semen traits in Surti Buffalo bull after glutathion supplementation in extender (Mean ± SE, n=24)

Post-thaw semen trait	*Control*	*Treatment 1 (T1)*	*Treatment 2 (T2)*	*Treatment 3 (T3)*
Progressive motility (%)	64.78 ± 0.25^{a}	67.46 ± 0.24^{c}	68.57 ± 0.36^{d}	65.65 ± 0.32^{b}
Live sperm (%)	70.11 ± 0.44^{a}	74.40 ± 0.38^{c}	75.90 ± 0.38^{d}	72.30 ± 0.40^{b}
Sperm abnormalities (%)	12.88 ± 0.15^{d}	11.42 ± 0.20^{b}	10.44 ± 0.23^{a}	12.25 ± 0.17^{c}
HOS Response (%)	60.53 ± 0.23^{a}	65.32 ± 0.39^{c}	66.90 ± 0.42^{d}	63.38 ± 0.30^{b}

Values are presented as mean ± SE of mean of twenty four replicates. Different superscripts within a row indicates significant difference ($P<0.05$). Control, T1, T2 and T3 contained 0.0, 0.5, 2.0 and 5.0 mM concentration of glutathione.

with a reduction in left posterior oblique (LPO) level was observed in bovine semen after extender supplementation with GSH (Perumal *et al.,* 2011). Plasma membrane of buffalo bull spermatozoa is high in polyunsaturated fatty acids, so it is more prone to oxidative stress during cryopreservation (Andrabi, 2009; Kadirvel *et al.,* 2009; Garg *et al.,* 2009).

The processes of capacitation, acrosome reaction and the oocyte penetration require a biochemically active plasma membrane. Since glutathione plays an important role in scavenging reactive oxygen intermediates and other radicals with the help of glutathione reductase (Meister and Andersson, 1983), it is a possibility that glutathione might protect the spermatozoa from membrane damage by inhibiting the lipid peroxidation process (Sinha *et al.,* 1996).

Conclusion

It can be concluded that glutathione supplementation in the TFYC extender at 2.0 mM concentration shows significant improvement in post-thawed semen quality in terms of progressive motility, live sperm percentage, HOS response, and reduced sperm abnormalities, in comparison to 0.5 mM or 5.0 mM concentration of glutathione supplementation.

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