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# Effect of glutathione on the quality of frozen buck semen

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ABSTRACT

Ejaculates (30) collected twice weekly from five Beetal bucks were used to study the effect of addition of 0 (control), 4 mM, 6 mM and 8 mM glutathione on the quality of frozen Beetal buck semen by split sample technique. After removal of seminal plasma, the semen was primarily extended with Tris extender (1: 5) considering the volume of semen prior to removal of seminal plasma and then split into 4 parts and finally extended with equal volume of Tris extender that rose the extension rate to 1: 10. The mean percentage of sperm motility, live sperm, live intact acrosome, HOST-reacted sperm was significantly higher in Tris extender containing 4 mM glutathione than that containing 6 mM, 8 mM glutathione and control. The release of ALT and AST from post-thaw spermatozoa was also the lowest in semen containing 4mM concentration of glutathione. Based on post-thaw sperm motility, live sperm, live intact acrosome, HOST-reacted sperm, and ALT and AST release, addition of 4 mM glutathione in Tris extender was superior to 6 mM, 8 mM and 0 mM (control). It was concluded that glutathione at 4 mM could be used as an antioxidant in Tris for cryopreservation of Beetal buck semen which could provide a better environment in protecting the functional capacity of spermatozoa.

**Key words**: Beetal buck, Freezing, Glutathione, Semen quality

Cryopreservation is one of the best methods for long term preservation of germplasm. The process of cryopreservation causes damage to sperm organelles and membrane in particular and induce changes in capacitation and acrosome reaction. Freezing and thawing have been reported to cause comparatively more damage to spermatozoa as compared to other stages (dilution, cooling and equilibration). During cryopreservation one of the main problems is the production of reactive oxygen species (ROS) which leads to lipid peroxidation of sperm membrane due to oxidative stress resulting in loss of sperm motility, viability and fertility (Bucak *et al.* 2007). Glutathione (GSH) is a tripeptide, ubiquitously distributed in living cells, and plays an important role in intracellular defence mechanism against oxidative stress (Irvine 1996). The process of freezing causes a significant reduction in GSH content in bovine (Bilodeau *et al.* 2000) and porcine (Gadea *et al.* 2004) semen. Addition of GSH in the extender during freezing improved the quality and fertilizing ability of frozen thawed spermatozoa (El-Kishk 2010). Therefore the present study was taken up to find the effect of incorporation of GSH in the semen extender on the quality of frozen semen in goats.

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## MATERIALS AND METHODS

Ejaculates (30) collected using artificial vagina from five adult healthy Beetal bucks maintained at Goat Research Station, AAU, Burnihat were used in the present study. Ejaculates having ≥ 0.8 ml volume, ≥3+ mass activity and ≥70 per cent initial sperm motility were used in the present study. Each ejaculate was diluted (1: 5) with warm (35°C) Tris buffer, centrifuged at room temperature for 5 min at 3,000 rpm and the supernatant was discarded. The sperm pellet was primarily extended (1: 5) using Tris extender taking into account the volume of fresh semen prior to removal of seminal plasma. The primarily extended semen was then split into 4 aliquots and finally extended with equal volume of Tris extenders containing 8 mM, 12 mM, 16 mM and 0 mM glutathione making final concentration of 4 mM, 6 mM, 8 mM and 0 mM of glutathione in 4 different fractions respectively. The extended semen was cooled gradually to 5°C @ 1ºC per 3 min and equilibrated in cold

handling cabinet for 4 h at 5°C. The extended semen was frozen in French mini straws as per standard technique and making use of different colour combinations of straws and polyvinyl alcohol powder for a particular buck and concentration of the antioxidant. The semen was evaluated for sperm motility, live sperm, live intact acrosome (Tamuli and Watson 1994) and hypo osmotic swelling test (HOST)-reacted sperm (Revel and Mrode 1994) after equilibration and after freezing and the frozen semen was thawed in warm water (37°C) for 30 sec. Further, frozen semen was estimated for extracellular release of alanine transaminase (ALT) and aspartate transaminase (AST). Statistical analysis of the obtained data was done by 2-way ANOVA using SAS Enterprise Guide 4.2 version.

## RESULTS AND DISCUSSION

The mean sperm motility, live sperm, live intact acrosome and HOST-reacted sperm recorded both after equilibration and after freezing and extracellular release of ALT and AST recorded after freezing of Beetal buck semen in Tris extender containing different concentrations of Glutathione are presented in the Table 1. The mean sperm motility, live sperm, live intact acrosome, and HOST-reacted sperm after equilibration and after freezing was highest when semen was extended with Tris extender with 4 mM followed by 6 mM and 8 mM Glutathione. The present highest value of sperm motility obtained after equilibration was close to that reported by Saraswat *et al.* (2012) in Sirohi goat semen extended with Tris extender containing 7 mM glutathione. However, it was higher than that reported by Bucak and Tekin (2007) after equilibration in ram semen extended with 5 mM glutathione. The obtained post-thaw sperm motility was in agreement with that reported in ram by Uysal and Bucak (2007) and in goat by Sinha *et al.* (1996) with Glutathione supplementation in the extender. It was much higher than that reported in goat by Saraswat *et al.* (2012). Percentage of live spermatozoa after equilibration was comparable with that recorded by Bucak and Tekin (2007) in ram semen with addition of 5 mM glutathione but it was lower than that noted by Saraswat *et al.* (2012) in Sirohi goat semen with Tris extender containing 7 mM glutathione. The live sperm percentage obtained with different concentrations of glutathione was higher than the corresponding value after equilibration (79.10 ± 2.40%) but lower than that after freezing in ram semen with supplementation of 5 mM glutathione as reported by Bucak and Tekin (2007) and Uysal and Bucak (2007) respectively. The incidence of live intact acrosome (LIA) after equilibration and after freezing could not be compared due to lack of data in the available literature, however, the percentage of post-thaw LIA obtained with 4 mM glutathione compared favourably with the occurrence of post-thaw intact acrosome in Sirohi goat recorded by Saraswat *et al.* (2012). The percentage of HOST-reacted sperm post thaw obtained in the present study was lower than that reported in ram semen with addition of 5 mM glutathione in the extender (Uysal and Bucak 2007). On the other hand, the present figure for post-thaw HOST-reacted sperm was much higher than that found by Saraswat *et al.* (2012) in Sirohi goat. The variations in the values for different sperm parameters after equilibration and after freezing from the present study could be due to difference in species and age of the animal, concentration of antioxidants and other components in the extender, processing and freezing techniques, season and method of estimation.

In the present investigation, the percentage of motile sperm, live sperm, live intact acrosome and HOST-reacted sperm in extended semen after equilibration and after freezing differed significantly (P<0.01) between different concentrations of glutathione used, the values for 4 mM glutathione being significantly (P<0.05) higher than that for 6 mM,8 mM and 0 mM. Sinha *et al.* (1996) also found significant differences in buck sperm motility after freezing with different concentrations of glutathione. Saraswat *et al.* (2012) in goat and Uysal and Bucak (2007) in ram made similar observations for live sperm. Sinha *et al.* (1996) in buck and Uysal and Bucak (2007) in ram semen also observed significant difference in percentage of intact acrosome after thawing between different concentrations of Glutathione. Uysal and Bucak (2007) recorded higher percentage of HOST-reacted ram spermatozoa with different concentrations of glutathione in the extender as compared to control. Saraswat *et al.* (2012) could not find significant

Table 1. Percentage of sperm characteristics (mean*± SE) after equilibration (AE) and after freezing (AF) and post-thaw extracellular release of ALT and AST in Tris extender containing different concentrations of glutathione

| Glutathione | Sperm Motility (%) | | Live sperm (%) | | LIA (%) | | HOST (%) | | ALT (U/L) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Concentration | AE | AF | AE | AF | AE | AF | AE | AF | AF | AF |
| 0 (Control) | $74.67^{d}$ | $60.17^{f}$ | 82.17 | 63.52 | $74.18^{d}$ | $42.07^{h}$ | 67.88 | 51.73 | $197.65^{d}$ | $253.72^{d}$ |
| | ±0.23 | ±0.17 | ±0.28 | ±0.35 | ±0.42 | ±0.28 | ±0.43 | ±0.39 | ±0.18 | ±0.27 |
| 4 mM | $84.50^{a}$ | $64.67^{e}$ | 87.73 | 70.07 | $82.37^{a}$ | $48.53^{e}$ | 72.07 | 54.75 | $176.46^{a}$ | $221.80^{a}$ |
| | ±0.28 | ±0.33 | ±0.15 | ±0.26 | ±0.23 | ±0.35 | ±0.20 | ±0.28 | ±0.32 | ±0.29 |
| 6 mM | $79.76^{b}$ | $60.17^{f}$ | 83.67 | 64.30 | $75.87^{b}$ | $43.57^{f}$ | 66.52 | 51.32 | $190.02^{b}$ | $243.66^{b}$ |
| | ±0.23 | ±0.29 | ±0.21 | ±0.27 | ±0.27 | ±0.28 | ±0.31 | ±0.30 | ±0.26 | ±0.33 |
| 8 mM | $71.17^{c}$ | $55.17^{g}$ | 77.82 | 58.82 | $66.17^{c}$ | $36.92^{g}$ | 63.10 | 46.70 | $210.12^{c}$ | $265.19^{c}$ |
| | ±0.45 | ±0.17 | ±0.38 | ±0.35 | ±0.37 | ±0.46 | ±0.29 | ±0.45 | ±0.29 | ±0.28 |

*30 Observations, means bearing different superscripts within a parameter differ significantly (P<0.05).

differences in sperm motility after equilibration and after freezing in goat among reduced glutathione (7 mM), ascorbic acid (4 mM) and vitamin E (4.5 mM) when added in Tris-based extender. Bucak and Tekin (2007) also did not record significant difference in live spermatozoa in ram between different concentrations of Glutathione during storage at liquid state. Sperm motility, live sperm, live intact acrosome and HOST-reacted sperm were found to decrease significantly (P<0.01) after freezing as compared to their levels at equilibration. Significant drop in sperm motility due to freezing was also reported in goat by Deka *et al.* (2012) and Saraswat *et al.* (2012) and in ram by Bucak and Tekin (2007) and Uysal and Bucak (2007). Significant decline in live sperm percentage following freezing was also recorded in goat by Saraswat *et al.* (2012) and in ram by Uysal and Bucak (2007). In the present study sperm motility and live intact acrosome varied significantly (P<0.01) due to glutathione concentration and stage interaction, which indicated that the main effects were not independent.

The extracellular ALT and AST activities in frozen Beetal buck semen differed significantly (P<0.01) between different concentrations of glutathione used (Table 1). ALT and AST are intracellular enzymes and their release into the extracellular fluids indicated sperm cell damage. Since assay of ALT and AST was reported to evaluate semen quality (Veerabramhaiah *et al.* 2011, Sharma *et al.* 2013), significantly (P<0.05) lower post-thaw ALT and AST levels obtained with 4 mM glutathione as compared to other concentrations could indicate the superiority of 4 mM glutathione in providing protection to sperm membrane thus producing better quality of frozen semen. Slaweta and Laskowska (1987) also reported that addition of glutathione (5 mM) decreased the release of AST in thawed spermatozoa.

During cryopreservation of semen, reactive oxygen species (ROS) or free radicals are produced which are associated with oxidative stress (Bilodeau *et al.* 2000). The free radicals such as hydrogen per oxide, superoxide anion and dihydral radical act on the phospholipid of the sperm cell membrane leading to lipid peroxidation (LPO) and inflict damage to the spermatozoa (Kim and Parthasarathi 1998) in the form of loss of membrane integrity, increased cell permeability, enzyme inactivation, structural damage to DNA and cell death. Susceptibility of the sperm cell to LPO is induced by its high content of unsaturated fatty acids in membrane and low cytoplasmic component. Antioxidants partially ameliorate the negative effect of ROS produced during cryopreservation by acting against the free radicals and scavenging them to protect the cells from sub lethal damage. In the present study glutathione used at a concentration of 4 mM in Tris extender yielded significantly better quality of frozen Beetal buck semen as compared to other concentrations and control. Better efficacy of 4 mM glutathione in comparison with 6 mM, 8 mM or without glutathione could be due to better maintenance of sperm plasma membrane and internal structures of sperm by reducing lipid peroxidation over other levels of glutathione. Slaweta and Laskowska (1987) suggested that glutathione played an active role in fructolysis, improved the metabolic activity and motility of spermatozoa and reported increase in motility of thawed spermatozoa with addition of 5 mM glutathione in frozen semen. Higher concentration of exogenous GSH was reported to cause lower membrane stability when endogenous GSH levels were within normal limits (Foote *et al.* 2002). The extent of dilution of semen adopted in the present study might not have highly altered the content of glutathione within the spermatozoa. This might explain the conferment of better protective effect on spermatozoa with 4 mM glutathione as compared to higher concentration, viz. 6 mM and 8 mM glutathione used in the extender. Present findings gain corroboration from the observations that incorporation of glutathione in the extender resulted in higher post thaw sperm parameters as reported by Sinha *et al.* (1996), Uysal and Bucak (2007) and Saraswat *et al.* (2012) in buck and ram.

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