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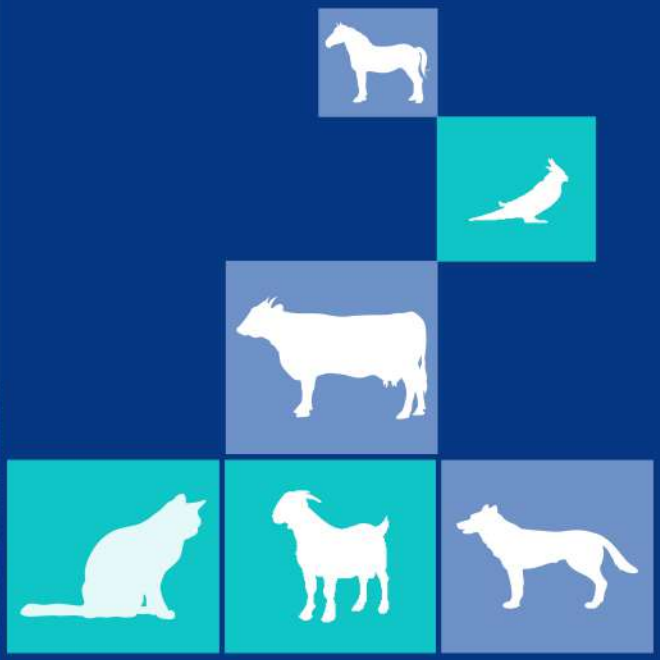

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Full Length Article

EFFECT OF GLUTATHIONE ON SEMINAL QUALITY PARAMETERS AT EQUILIBRATION IN SURTI BUFFALO BULL

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ABSTRACT

The main aim of this study was to investigate the effect of different concentrations of glutathione supplementation on liquid storage of Surti buffalo bull semen. This study was performed on adult Surti buffalo bull (n = 6), and seminal ejaculates (24) were collected and evaluated for various microscopic seminal quality parameters for further processing. After preliminary evaluation, ejaculates of each collection session were mixed and divided into four equal aliquots. All the aliquots were diluted (1:10) with Tris fructose egg yolk citrate extender contained glutathione served as control (T0), whereas the other three aliquots were supplemented with 0.5, 2.0 and 5.0 mM glutathione which were grouped as Treatment-1 (T1), Treatment-2 (T2) and Treatment-3 (T3), respectively. Thereafter, the samples were stored at 4 ºC for 4 h, and various seminal parameters (individual sperm progressive motility, viability, abnormalities, plasma membrane functionality) were evaluated at equilibration. The results indicated that the mean percent values for pre-freeze sperm progressive motility, live sperm percentage and HOS responsive spermatozoa were found to be significantly higher (P<0.05) (except individual progressive motility which was non-significantly higher in Treatment-3); whereas sperm abnormalities were significantly lower (P<0.05) in semen samples treated with glutathione (Treatment-1, Treatment-2 and Treatment-3) in comparison to control. The Treatment-2 (2.0 mM glutathione), had the highest pre-freeze sperm progressive motility percentage, live sperm percentage, HOS response sperm percentage and reduced sperm abnormalities percentage compared to other three groups.

Keywords: Glutathione, Pre-freeze quality, Surti buffalo, *Bubalus bubalis*, Semen additive.

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INTRODUCTION

The process of artificial insemination (AI) is employed in livestock by using semen either in its liquid or frozen state. The utilization

of liquid semen in artificial insemination has led to increased rates of fertility (Anzar *et al.,* 2003) with lower numbers of spermatozoa (Vishwanath *et al.*, 1996). Tris, citrate, and milk-based buffers are commonly employed to preserve buffalo semen at cooling temperatures, effectively maintaining the quality and fertility of stored semen for a period up to three days (Sansone *et al.*, 2000). Glutathione naturally present in buffalo semen has been recognized as an essential intracellular antioxidant (Andrabi, 2009). In mammalian semen, glutathione (GSH) predominantly represents the non-enzymatic antioxidant defense system. Glutathione, a tripeptide thiol (γ glutamylcysteinylglycine), is the primary non-protein sulphydryl compound in mammalian cells, recognized for its numerous biological functions. The fundamental role of GSH in mammalian semen is associated with its interactions with other systems, serving as a preventive mechanism against reactive oxygen species (ROS). This scavenging function of GSH helps to counteract the effects of oxidative stress in sperm cells, which could result in lipid peroxidation of plasmalemma, irreversible loss of motility, leakage of intracellular enzymes and damage of the chromatin. In bovine semen, both enzymatic (catalase, superoxide, dismutase, glutathione peroxidase/reductase) and non-enzymatic (vitamin C and E, glutathione, cysteine) antioxidants are present to protect the spermatozoa from reactive oxygen species (ROS) molecules (Nichi *et al.,* 2006). Nevertheless, the natural levels of antioxidants are insufficient to provide complete protection for sperm integrity against oxidative stress (Sreejith *et al.,* 2006). The processes of cooling and freeze-thawing exert physical and chemical stresses on the sperm membrane, leading to a decrease in sperm viability and fertilizing ability (Stradaioli *et al.,* 2007). The fertilizing capacity of chilled semen decreases with prolonged storage time (Shamsuddin *et al.,* 2000). Oxidative stress during liquid storage is a significant factor that diminishes sperm quality (El-sissy *et al.*, 2007) and fertility (Vishwanath and Shannon, 1997) by producing reactive oxygen species molecules (ROS; superoxide, hydroxyl, hydrogen peroxide, nitric oxide, peroxynitrile) (Baumber *et al.*, 2000). These substances elevate the levels of lipid peroxidation (LPO) in unsaturated fatty acids within the plasma membrane (Kadirvel *et al.*, 2009; El-sissy *et al.*, 2007). Reactive oxygen species (ROS) at low concentrations play a crucial role in various sperm physiological processes, including capacitation, hyperactivation, acrosome reactions, and signaling processes essential for fertilization. It is well documented that during the liquid storage of buffalo semen, oxidative stress triggers an excessive production of ROS, leading to elevated levels of LPO in the cell membrane (El-sissy *et al.*, 2007; Kadirvel *et al.*, 2009). The resulting oxidative stress may induce mitochondrial dysfunction and deterioration of sperm motility, viability, plasmalemma integrity and sperm morphology

(Kadirvel *et al.*, 2009). This study aimed to assess the comparative impact of adding varying concentrations of glutathione to a semen extender on pre-freeze semen quality parameters in Surti buffalo bulls.

MATERIALS AND METHODS

The study was conducted on six Surti buffalo bulls at the age group of 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. Totally 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 it's 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, respectively), while aliquot 1 served as untreated control (T0).

The extended semen of each treatment was then filled and sealed in French mini straws (0.25 ml, 135 mm length and 2 mm diameter) at room temperature by a manual method. They were kept for 4 hours of equilibration at 4°C, 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).

RESULTS AND DISCUSSION

Pre-freezing Individual Progressive Motility (%)

In the present study, the effect of various concentrations of glutathione (0.5 mM, 2.0 mM and 5.0 mM) on the pre-freezing semen attributes in Surti buffalo bull were studied after the equilibration period of 4°C for 4 hours.

The mean progressive motility percent was significantly ($p<0.05$) higher in T2 (72.80±0.27 %) compared to control (70.13±0.34 %) however non-significant improvement was observed in T2 (72.80±0.27 %) compared to T1 (72.13±0.31 %). Also, T3 (70.82±0.32) was significantly lower than T1 (72.13±0.31 %) and T2 (72.80±0.27 %) but non-significantly higher than control (70.13±0.34 %).

In an earlier study, Gangwar *et al.* (2018) reported progressive motility (%) of spermatozoa at pre-freeze stage. Progressive motility of sperms in semen samples of the two groups was examined at the end of the equilibration period

and there was no significant difference observed between the control (68.00±1.11) and the treatment group (68.25±1.21) with 0.5 mM glutathione at post equilibration stage in Murrah buffalo bulls.

Ansari *et al.* (2011) reported that in Nili-Ravi buffalo bulls, sperm motility did not differ in all experimental extenders on 1st day of storage. Higher ($p<0.05$) sperm motility (%) was observed at 3rd and 5th day of storage in extender containing glutathione 0.5 mM (56.7±2.9, 46.7±2.9) and 1.0 mM (55.0±0.0, 46.7±2.9) as compared to extender containing glutathione 3.0 mM (48.3±2.9, 33.3±7.6) and control (48.3±2.9, 35.0±5.0).

Ismail and Darwish (2011) reported individual motility percentages of buffalo spermatozoa preserved in tris egg yolk extender supplemented with 0.0, 0.5 and 1.0 mM glutathione at pre-freeze (74.67±0.69, 79.00±0.67 and 80.00±1.02, respectively).

The improvement in the progressive motility (%) observed in the T2 (2.0 mM glutathione supplementation) over control and T3 (5.0 mM glutathione supplementation) might be due to the protection of the sperm during the chilling and extension against oxidative stress and ROS deleterious effects. A positive relationship has been reported between level of glutathione and sperm motility (Gadea *et al*., 2004; Stradaioli *et al*., 2007) in bovine (Munsi *et al*., 2007; Foote *et al.,* 2002), swine (Funahashi and Sano, 2005) and ovine semen (Bucak and Tekin, 2007); these results substantiates improvement in sperm motility after the addition of 2.0 mM glutathione in the present study.

Live Sperm (%)

Livability is one of the major factors in the assessment of semen quality. During cryopreservation, the spermatozoa are exposed to a foreign diluting media and very low temperature. Death of sperm might occur due to the release of toxic substances, ultra-low temperature exposure, enzymatic leakage, medium of preservation, degree of sperm permeability, aging effect of sperm and individual variation (Watson, 2000).

In the current study, there was a significant ($p<0.05$) increase in live sperm count in T1 (79.03±0.28 %), T2 (80.61±0.29 %) and T3 (77.21±0.30 %) during pre-freeze equilibration of semen in comparison to control (75.21±0.32 %).

Gangawar *et al.* (2018) reported a similar effect in the treatment group with 0.5 mM glutathione supplementation. The treatment group (82.35±0.71) had significantly ($p<0.05$) higher percent of live spermatozoa than the control group (81.50±0.73) at post equilibration stage in Murrah buffalo bull.

Ansari *et al.* (2011) reported that in all five experimental extenders, the viability of buffalo bull spermatozoa was similar at 1st day of storage. Percentage of

viable sperm was higher ($p<0.05$) at 3rd and 5th day of storage in extender containing glutathione 0.5 mM (76.7±2.9, 66.7±2.1), 1.0mM (75.0±0.0, 66.7±2.1) and 3.0mM (68.3±3.1, 55.0±6.6) as compared to control (68.3±2.1, 58.3±1.5).

Ismail and Darwish (2011) reported the effect of different concentrations of glutathione on the percentage viability in Egyptian buffalo bulls. There was higher viability at 0.50 mM (83.30±0.75) and 1.00 mM (82.60±0.58) than control (75.60±0.53) semen samples at pre freeze stage.

Also, Dushyant *et al.* (2019) reported a significantly higher ($p<0.05$) mean percentage of live bovine spermatozoa with intact acrosome at the pre-freezing stage in treatment T1 and T2 groups (0.5 mM - 79.58±0.52 and 1.0 mM - 74.31±0.48) than in the control group (68.87±0.52).

Behnsawy *et al.* (2017) reported the effect of the different concentrations of glutathione; control (58.0±2.52), 2 mM (58.8±3.29), 4 mM (66.3±1.17) and 6 mM (66.6±2.15) on live sperm percentage. The addition of 4 or 6 mM concentration of glutathione significantly ($p<0.05$) improved the percentage of live spermatozoa (post-equilibration) of goat semen extender. While the lowest value of live spermatozoa percentage was recorded in the control group.

Live sperm percentage has been reported to vary due to methodological errors, feeding variation, breeds and their adaptability in varying agro-climatic conditions of the places of investigation, season and frequency of semen collection etc. (Mittal and Pandey, 1972; Pandey *et al.,* 1985). The lower viability of buffalo semen has been reported due to poor antioxidant activity and increased ROS production during liquid storage at 4°C (El-Sissy *et al.,* 2007). Therefore, the increased live sperm count observed in the present study could be due to the antioxidant nature of the supplemented glutathione.

Sperm Abnormalities (%)

In this study, as compared to control (10.13±0.23 %), there was significant ($p<0.05$) reduction in sperm abnormalities in T1 (8.50±0.19 %), T2 (7.86±0.19 %) and T3 (9.34±0.23 %) groups during pre-freeze equilibration of semen in Surti buffalo bulls.

Munsi *et al.* (2007) reported significantly ($p<0.01$) lower acrosomal abnormality in 0.5 mM glutathione-treated pre-freeze equilibration to the bull semen on Day 5, in comparison to control, 1.0 mM, 2.0 mM, 3.0 mM treatments.

Similarly, Slaweta and Laskowska (1987) reported significantly ($p<0.01$) lower acrosomal abnormality in 5.0 mM glutathione-treated pre-freeze equilibration bull semen in comparison to

control.

Behnsawy *et al.* (2017) reported that the lower percentage of sperm abnormality in buck semen supplemented with different concentrations of glutathione at 2 mM (8.0±0.365), 4 mM (8.2±0.166), 6 mM (8.0±0.365) than the control (8.2±0.365) group. The experimental results in the current study show that the sperm abnormality was not significantly ($p<0.05$) affected by different levels of glutathione during post-equilibration. Sperm abnormality decreased progressively with the addition of glutathione concentrations and the control group recorded the highest level of sperm abnormality.

The lowest sperm abnormalities in the current study were observed in 2.0 mM glutathione (T2); this variation from other studies could be due to many factors including the species variation, methodological errors, feeding variation, season and frequency of semen collection, place of investigation and their adaptability in that agro-climatic condition etc.

Hypo-Osmotic Swelling Test (%)

Routine semen evaluation has certain limitations for comprehensive prediction of fertility of bull semen. The HOS response of the sperm highlights the permeability of the sperm membrane to hypo-osmotic solution and the projection of a higher value is a valid indication of an intact membrane and a sample with a higher value is regarded as potent for establishing pregnancy.

In the current study, the HOS response during pre-freeze equilibration of semen, in T2 (70.55±0.55 %) group was significantly ($p<0.05$) higher than T3 (67.61±0.67 %) and control (65.37 ±0.45) group but non-significantly higher than T1 (69.00 ±0.52 %) group.

Similar results were observed by Gangawar *et al.* (2018) who reported significantly ($p<0.05$) higher HOS response at post equilibration stage in the 0.5 mM glutathione-supplemented group (73.55±0.67) than the control group (71.40±0.69) in Murrah buffaloes.

Ismail and Darwish (2011) reported the effect of different concentrations of glutathione on the percentage of intact plasma membranes in Egyptian buffaloes. The intact plasma membrane integrity percentage at 0.5 mM (68.10±0.71) and 1.00 mM (69.50±0.58) was higher than the control (63.70±0.70) groups of the pre-freezing semen samples.

Ansari *et al.* (2011) reported that glutathione addition (0.5-1.0 mM) in extender improved the plasma membrane integrity of cooled buffalo (*Bubalus bubalis*) bull semen. Plasma membrane integrity of buffalo spermatozoa did not differ due to glutathione in extenders at 1st day of storage; whereas, sperm with intact plasma membrane was higher ($p<0.05$) at 3rd and 5th days of storage in extender containing glutathione 0.5 mM (71.7±2.9,

Table 1: Pre-Freeze semen traits in Surti Buffalo bull after glutathione supplementation in extender (Mean ±SE, n=24)

Pre-Freeze semen trait (%)	Control	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)
Progressive motility (%)	70.13±0.34^{a}	72.13±0.31^{b}	72.80±0.27^{b}	70.82±0.32^{a}
Live sperm (%)	75.21±0.32^{a}	79.03±0.28^{c}	80.61±0.29^{d}	77.21±0.30^{b}
Sperm abnormalities (%)	10.13±0.23^{d}	8.50±0.19^{b}	7.86±0.19^{a}	9.34±0.23^{c}
HOS Response (%)	65.37 ±0.45^{a}	69.00±0.52^{c}	70.55±0.55^{c}	67.61±0.67^{b}

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

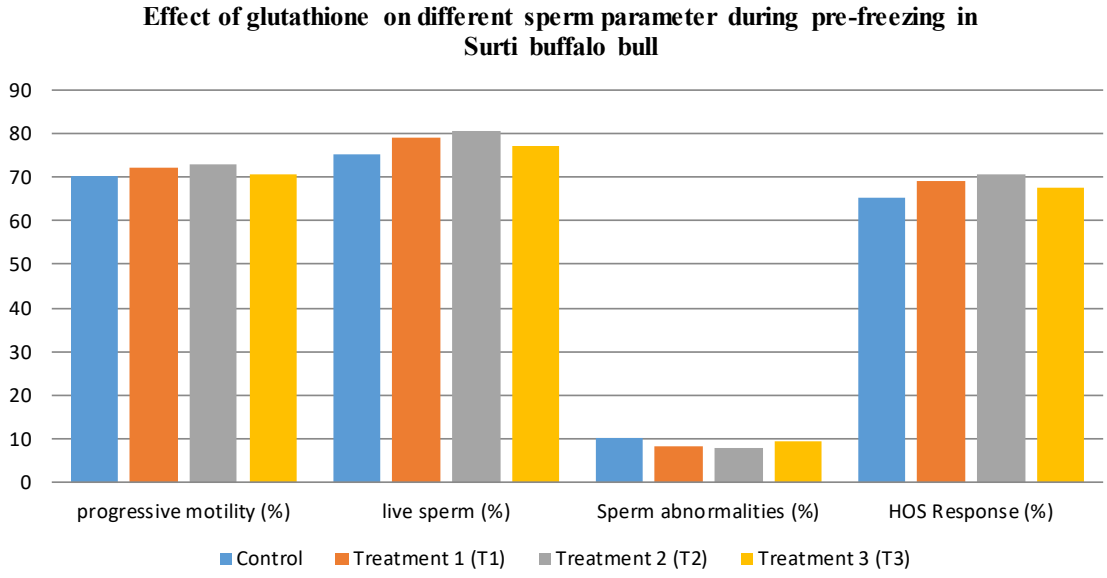

Fig. 1. Effect of different concentrations of glutathione on percent motility, sperm viability, abnormality and HOS responsive sperm during cooled storage of buffalo bull semen.

61.7±2.9) and 1.0 mM (70.0±0.0, 61.7±2.9) as compared to extender containing glutathione 3.0 mM (63.3±2.9, 48.3±7.6) and control (63.3±2.9, 50.0±5.0).

The glutathione supplementation helps maintain the integrity of normal acrosome and stabilises the plasma lemma of spermatozoa (Sinha *et al.,* 1996); this explains the increased HOS response in glutathione-supplemented pre-freeze semen samples, in the present study.

It could be concluded that glutathione supplementation in the TFYC extender at 2.0 mM concentration showed significant improvement in pre-freeze 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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