RESEARCH ARTICLE

Effect of Different Concentrations of Trehalose on Post-Thaw Quality of Surti Buffalo (*Bubalus bubalis*) Bull Semen

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Abstract

The present study was conducted to investigate the effects of supplementation of trehalose as an additive in Tris-Fructose-Egg Yolk-Citrate extender on post-thaw semen quality in 24 ejaculates of six Surti buffalo bulls. Semen extended @ 80 million sperm /ml was divided into four equal parts and served as three treatment group as T1 (50 mM trehalose), T2 (100 mM trehalose) and T3 (150 mM trehalose) and one as control (T0). The percentage of post-thaw sperm progressive motility, sperm viability, HOS responsive sperm were higher, whereas sperm abnormalities were lower in treatment T1, T2 and T3 as compared to control. The treatment T2 (100 mM trehalose) had significantly higher post-thaw progressive motility, sperm viability, HOS response sperm percentage and reduced sperm abnormalities as compared to control and other levels of trehalose. In conclusion, supplementation of trehalose at 100 mM concentration significantly ($p<0.05$) improved freeze-thawed semen quality of buffalo bulls in comparison to control and trehalose concentration of 50 mM and 150 mM in Tris extender.

Key words: Cryopreservation, Extender, Semen, Surti bull, Trehalose.

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Introduction

The foremost artificial breeding technology being widely applied to buffalo husbandry is the artificial insemination (AI) with cryopreserved semen. Cryopreservation is a technique adopted for the propagation of superior germ plasm of dairy animals (Anzar and Graham, 1995). However, the biggest problem to exploiting cryopreserved buffalo semen is damage of sperm membrane structures during freezing and thawing, which leads to fewer viable and motile cells post-thawing (Holt, 2000). During processing procedures of semen for cryopreservation, sperm are exposed to various stresses including plasma membrane destabilization with impaired motility and functions. During cryopreservation, sperm damage occurs due to formation of intracellular ice crystals, phase transition induced membrane changes due to cooling (Watson, 2000; Medeiros *et al.,* 2002), and osmotic stresses related to change of cryoprotectants from the freezing media (Morris *et al.,* 2007). The composition of the extender in which semen is diluted before freezing is one of the most important factors affecting cryopreservation (El-Sheshtawy *et al.,* 2015). Trehalose (non-permeating disaccharide) acts as a hypertonic media causing cellular osmotic dehydration before freezing and decreasing the amount of cell injury by crystallization (Bucak *et al.,* 2007). Trehalose also acts as an enzymatic scavenger and through its osmotic effect trehalose induces its protective effects against oxidative damage rendering a role in the protection of spermatozoa against ROS. Therefore, this study was aimed to assess the effects of different concentrations of trehalose on post-thaw quality of Surti buffalo bull semen.

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Materials and Methods

The study was conducted on six Surti buffalo bulls of the age group 5-7 years, weighing 440-500 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 processed 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). Trehalose was added into aliquot 2, 3 and 4 at the rate of 50 mM, 100 mM, and 150 mM, respectively (treatment T1, T2, T3), while aliquot 1 served as untreated control (T0). These samples were then processed for cryopreservation, and immediately after thawing at 37°C for 30 seconds, samples were evaluated for percentage progressive sperm motility, live sperm, abnormal sperm and HOS response, following standard procedure. The data were statistically analyzed using one way ANOVA and Duncan's NMRT test, and compared as per the standard statistical procedures described by Snedecor and Cochran (1994) by using SPSS 20.0.0 version.

Results and Discussion

Individual Sperm Progressive Motility (%)

In the present study, significantly higher (P< 0.05) percentage of mean post-thaw sperm motility was observed with trehalose concentration of 50 mM (50.83±0.40), 100 mM (54.25±0.28) and 150 mM (52.75± 0.28) as compared to control (48.83±0.38). Furthermore, among all three treatment groups, T2 group had significantly higher mean post-thaw motility percentage in comparison to others (Table 1). Similarly, Badr *et al.* (2010) also reported a significantly (p<0.05) higher individual progressive motility of spermatozoa as 51.25±1.25, 61.25±1.25 and 56.25±2.39 % with 50 mM, 100 mM and 150 mM concentrations of trehalose, respectively, as compared to control (41.25±4.32 %) in buffalo semen. Our results are in agreement with Reddy *et al.* (2010) who also reported a significantly (p<0.05) higher individual progressive motility of spermatozoa with 100 mM (41.67±1.67%) concentration of trehalose as compared to control (31.67±1.67%) in Murrah buffalo bulls semen. Likewise, Hu *et al.* (2010) also recorded significantly (p<0.05) higher individual progressive motility of spermatozoa with 50 mM (44.36±1.83) and 100 mM (46.61±1.62) concentrations of trehalose as compared to control (36.88±1.53) in bulls.

Trehalose supplementation to cryopreservation medium significantly improves motility and viability, and has been reported in different species at a varying concentration ranging from 25 mM to 435 mM (Bucak *et al.,* 2007; Tuncer *et al.,* 2013; Ghallab *et al.*, 2017; Bittencourt *et al.,* 2018). The ameliorative effect of trehalose can be explained with its ability to replace water at the membrane-solution interface (Aisen *et al.,* 2002), which improves sperm function, such as motility (Bucak *et al.*, 2013). According to the water replacement hypothesis, trehalose replaces water through hydrogen bonding to polar residues and prevents the denaturation of proteins and the fusion of membranes and thereby exerting a cryoprotective effect.

Sperm Viability (%)

In the present study, a significant (p<0.05) improvement in the per cent live sperm count was observed with a trehalose concentration of 100 mM (80.58±0.93) and a non-significant elevation in the sperm viability was observed in 50 mM (78.08±0.94) and 150 mM (79.42±0.99) concentrations of trehalose as compared to control (76.17±1.09, Table 1). These results supported well the earlier observations of El-Sheshtawy *et al.* (2015) and Al-Badrany *et al.* (2017) in the bulls. Reddy *et al.* (2010) also observed a significant (p<0.05) improvement in per cent sperm viability upon the addition of 100 mM trehalose as compared to the control group in Murrah buffalo bulls.

The amelioration of cell viability by trehalose supplementation was interpreted by Chhillar *et al.* (2012). Trehalose acts like a non-permeating cryoprotectant which causes dehydration of spermatozoa due to the osmotically driven flow of water. Due to this mild dehydration, spermatozoa have less intracellular water which results in reduced intracellular ice crystal formation. This is beneficial for sperm because intracellular ice crystal formation results in cell death.

Sperm Abnormalities (%)

There was a significant (p<0.05) reduction in the sperm abnormalities with trehalose concentration of 100 mM (11.92±0.75%) and a non-significant decrease was observed in 50 mM (13.75±0.59%) and 150 mM (13.08±0.62%) concentrations of trehalose as compared to control (14.75±0.62%). Our results were in agreement with Al-Badrany *et al.,* (2017) in Holstein bulls. In an earlier study, El-Sheshtawy *et al.* (2015) found that the sperm abnormality was significantly (p<0.05) lower with the supplementation of 50 mM (6.20±0.58) and 100 mM (7.60±0.24) concentrations of

Table 1: The effect of trehalose on bull semen after freezed-thawing process

Post-thaw parameters	Control	Treatment 1	Treatment 2	Treatment 3
Progressive motility (%)	48.83^{a}±0.38	50.83^{b}±0.40	54.25^{d}±0.28	52.75^{c}± 0.28
Sperm viability (%)	76.17^{a}±1.09	78.08^{ab}±0.94	80.58^{b}±0.93	79.42^{ab}±0.99
Sperm abnormalities (%)	14.75^{b}±0.62	13.75^{ab}±0.59	11.92^{a}±0.75	13.08^{ab}±0.62
HOS response (%)	41.67^{a}±0.67	45.67^{b}±0.60	50.33^{c}±0.60	46.92^{b}±0.49

Mean values with different superscripts between treatment groups differ significantly (p<0.05). Control and treatment 1, 2 & 3 contained 0 mM, 50 mM, 100 mM and 150 mM trehalose, respectively.

trehalose as compared to control (16.40±0.51) in cattle bull. As per Jhamb (2021) the sperm abnormality was significantly (P<0.05) decreased upon the addition of 50 mM (14.72±0.12) trehalose as compared to the control (16.52±0.13) in Marwari stallion also. 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)

Sperm Plasma Membrane Integrity (HOST %)

In the present study, there was a significant (p<0.05) improvement in HOST (%) with the addition of trehalose at 50 mM (45.67±0.60), 100 mM (50.33±0.60) and 150 mM (46.92±0.49) concentrations as compared to control (41.67±0.67; Table 1). Reddy *et al*. (2010) also found a significantly (P<0.05) higher sperm plasma membrane integrity by using a concentration of 100 mM trehalose as compared to control in Murrah and Karan-fries bulls. In many earlier studies in various livestock species quoted above, the HOST (%) was found to be significantly (p<0.05) higher upon the addition of 50 mM and 100 mM concentrations of trehalose as compared to the control.

Aboagla and Terada (2003) emphasized that trehalose inserts itself into the membrane phospholipids bilayer, thus modulating membrane fluidity and therefore rendering the membrane more stable during freezing, providing cryoprotection. Further, the action of trehalose appears to be associated with its ability to replace water at the membrane-solution interface (Aisen *et al.,* 2002) which improves sperm functions like membrane integrity (Bucak *et al.,* 2013).

Conclusion

From the present study, it can be concluded that the addition of trehalose in controlled manner into Tris extender may be beneficiary to sperm cells during cryopreservation process. In our study, Trehalose at 100 mM concentration showed significant improvement in post-thawed semen quality in terms of progressive sperm motility, sperm viability, HOS response and reduced sperm abnormalities besides maintaining the morphological characteristics of Surti buffalo bull spermatozoa in a better manner, in comparison to 0 mM, 50 mM or 150 mM concentration of trehalose supplementation.

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