SHORT COMMUNICATION

L-Cysteine Ameliorates Ultra-low Temperature Preservation Induced Cryodamage of Murrah Buffalo Spermatozoa

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Abstract

The present study was carried out with the aim to evaluate the effect of L-cysteine hydrochloride as an antioxidant on the *in-vitro* fertilizing potentials of buffalo spermatozoa through Hypo-osmotic swelling test (HOST) and sperm mucus penetration test (CMPT). Thirty-six ejaculates were collected from six Murrah bulls. The semen samples were divided into two groups, and were extended with Tris-citric-acid-fructose-egg-yolk-glycerol (TFYG) extender without additive, i.e., control (C) extender and with L-cysteine hydrochloride (1.0 mM) i.e. treatment (T) extender. The HOST and CMPT were evaluated at both post-dilution and post-thaw stages. The cysteine hydrochloride-fortified group (T) recorded a significant ($p<0.05$) improvement in HOS-positive spermatozoa and the distance traveled by vanguard spermatozoa as compared to control group. In conclusion, cysteine hydrochloride improves post-thawed sperm functional parameters in Murrah bull semen, indicating that incorporating cysteine hydrochloride into a semen extender may improve fertility.

Key words: Cryopreservation, Cysteine hydrochloride, Hypo-osmotic swelling test, Murrah bull, Sperm penetration distance.

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Introduction

Buffaloes have a lower reproductive potential and efficiency rate when it comes to artificial insemination (AI) than cattle, as it depends on the frozen-thawed quality of spermatozoa, and it has a significant impact on the success rate of AI (Duran *et al.,* 2005). The cryopreservation process causes physiological, osmotic, and chemical stress on the sperm membrane and structure, resulting in damage to the spermatozoa and poor post-thawing quality of the sperm (Ozkavukcu *et al.,* 2008). The buffalo sperm membrane is rich in unsaturated fatty acids and lipid peroxidation of the spermatozoa membrane leads to apoptotic cell changes and loss of sperm function in post-thawed semen samples (Sikka, 1996). Semen additives can usually maintain frozen-thawed seminal quality and increase conception rates (Perumal *et al.,* 2011). Cysteine, a sulfur-containing amino acid and precursor of glutathione, is considered an intracellular antioxidant that protects the cells from ROS-mediated damage under oxidative stress (Topraggaleh *et al*., 2014) and is used as one of the semen additives in freezing extenders to improve post-thaw sperm parameters of several species (Holt, 1997).

Plasma membrane integrity is critical for optimal sperm function because only sperm with an intact plasma membrane can undergo a series of complex changes in the female reproductive tract and acquire the ability to fertilize an oocyte (Khan and Ijaz, 2008). The hypo-osmotic swelling test determines the functional integrity of the sperm membrane (Mandal *et al.,* 2003; Iqbal *et al.,* 2009). According to Srivastava and Kumar (2006), the HOS test helps in measuring the various aspects of sperm membrane behavior separately, particularly biochemical activity and membrane integrity. Similarly, estimating the movement of spermatozoa in bovine cervical mucus is pivotal in the clinical management of bovine infertility. Sperm penetration provides reliable data for fertility assessment, allows for significant discrimination of sperm function, and can be used to diagnose and manage fertility (Oehninger and Ombelet, 2019). This test is based on a visual assessment of the linear distance covered by the first sperm cell (vanguard spermatozoa) in a capillary tube. For optimum fertility, a bull spermatozoon is expected to penetrate beyond 20 mm in cervical mucus from the capillary tube's base (Ivic *et al.,* 2002). This study was, therefore, aimed at evaluating the effect of cysteine hydrochloride as an antioxidant on functional aspects of spermatozoa through these *in-vitro* fertility tests.

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Materials and Methods

Collection, Dilution and Cryopreservation of Semen

This study was carried out at the Deep Frozen Semen Laboratory of the College of Veterinary Science & animal Husbandry, ANDUAT, Kumarganj, Ayodhya (UP, India) on semen of six Murrah bulls, aged between 4 and 8 years following approval from the institutional ethics committee. Thirty six ejaculates were collected from these bulls using artificial vagina (42°C) at weekly interval. The ejaculates having >75% visual motility were diluted in Tris-citric-acid-fructose-egg-yolk-glycerol (TFYG) extender. The ejaculates were split into two parts, part one was diluted with TFYG as such that served as control (C) and part two was diluted with TFYG containing 1.0 mM L-cysteine hydrochloride (Mercks, USA) referred to as treatment (T).

Extended semen was filled into 0.25 mL French mini straws and sealed with polyvinyl alcohol powder. These straws were equilibrated at 4°C in a cold handling cabinet for 4 h and then exposed to liquid nitrogen vapours for 10 min in a programmable bio-freezer and then stored in liquid nitrogen (-196°C) for 48 h before thawing at 37°C for 30 seconds. The post-diluted and post-thawed seminal attributes, *viz.,* HOST (Jeyendran *et al.,* 1984) and CMPT-SPD (Kremer, 1980, using the "Penetrak kit" (Serono Diagnostic, USA) were assessed adopting standard procedures as *in-vitro* fertility tests. The data were analyzed statistically using analysis of variance (ANOVA) using Graph Pad Prism 5 software.

Results and Discussion

The average percent HOS-reactive spermatozoa (Fig. 1) in post-diluted Murrah bull semen in the control and treatment groups were 49.11±0.23 and 51.97±0.18, respectively. The corresponding post-thaw values were 37.86±0.31 and 41.94±0.21 %, respectively. The post-thawed HOS-positive spermatozoa were significantly ($p<0.05$) higher in the cysteine hydrochloride-treated group as compared to the control group. Present findings are in accordance with Saurabh (2021), whereas lower and higher values were reported by Kumar (2015) and El-Sheshtawy and El-Nattat (2020). These findings suggested that cysteine hydrochloride acts as a membrane stabilizer (Szczesniak-Fabianczyk *et al.,* 2003). The reduced lipid peroxidation potential of cysteine hydrochloride-treated semen samples showed that cysteine hydrochloride might preserve spermatozoa from membrane damage by inhibiting the lipid peroxidation process (Mazur *et al.,* 2000). This is due to cysteine's ability to form a layer on the surface of spermatozoa, as these positively charged molecules can bind with the phosphate groups of sperm plasma membrane phospholipids (Kundu *et al.,* 2001). Moreover, Ansari *et al.* (2010) reported that cysteine supplementation in the extender protects the membrane integrity by scavenging the ROS molecules during the cryopreservation process.

The overall mean (±SE) distance travelled by vanguard spermatozoa in post-diluted semen of buffalo bulls in the control and cysteine-treated groups were 34.72±0.47 and 39.00±0.23 mm/h, and in post-thawed semen 26.97±0.29, 30.75±0.29 mm/h, respectively. The SPD value of frozen thawed semen was significantly ($p<0.05$) higher in cysteine-treated groups than the control. This was in tune with the observations recorded by Kumar (2015) and lower than Saurabh (2021). Indeed, cysteine is a low-molecular-weight amino acid that contains thiols. It is a precursor to intracellular glutathione biosynthesis and enhances GSH levels, which neutralize and prevent the formation of free radicals, maintain membrane integrity, and improve flagellar motion in the extender. Furthermore, cysteine hydrochloride also improves the efficiency of mitochondria (Perumal *et al.*, 2011). It acts as an antioxidant to lessen the formation of free radicals in semen and safeguards the other components of the sperm membrane to maintain motility (El-Sheshtawy *et al.,* 2008).

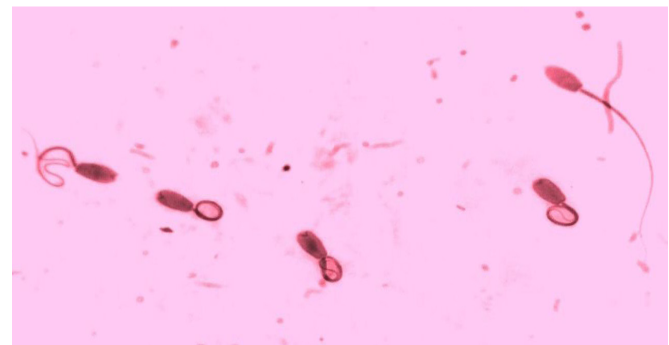

Fig. 1: Images of HOST reactive (coiled tail) and HOST non-reactive (normal tail) Murrah buffalo bulls spermatozoa (1000X).

The study concludes that the addition of L-cysteine hydrochloride in semen extender as an antioxidant improves the fertility of the Murrah bull semen through *in-vitro* fertility tests by maintaining the sperm membrane integrity during cryopreservation and thawing.

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