

EFFECTS OF ADDITIN OF TAURINE AND MANGANESE IN EXTENDER ON POST THAW SEMEN QUALITY OF SAHIWAL BULLS

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ABSTRACT: Effect of two antioxidants (Taurine and Manganese) in extender on different sperm parameters (sperm motility, live sperm percentage, membrane and acrosome integrity) of Sahiwal bulls was evaluated. Semen was collected twice a week for 8 weeks from six adult, regular donor and clinically healthy Sahiwal bulls. A total of 96 ejaculates were collected and pooled after initial evaluation. The pooled semen samples were diluted at with tris-citric acid, fructose, egg yolk and glycerol extender either containing different level of taurine (20.0, 40.0 and 60.0 mM), manganese (100.0, 150.0 and 200.0 μ M), or combinations of taurine and manganese (20.0 mM + 100.0 μ M, 40.0 mM + 150.0 μ M and 60 mM + 200 μ M) at a temperature of 37°C and without antioxidants as control. Semen samples were loaded in 0.5 mL French straws and cooled to 4°C. Samples were frozen and stored in liquid nitrogen. These samples were thawed and analyzed for different parameters. The post-thaw sperm motility (%) was significantly ($P<0.01$) higher in treatment groups containing 20.0 mM of taurine, 100.0 and 150.0 μ M of manganese and 20.0 mM + 100.0 μ M of taurine and manganese respectively. Likewise, the live sperm percentage was higher ($P<0.01$) in 20.0 mM of taurine and 100.0, 150.0 μ M of manganese supplemented groups. The maximal sperm acrosome integrity of 20.0 mm was observed in taurine group. A higher proportion ($P<0.01$) of sperm with intact membrane was observed in 20.0 mM of taurine and 150.0 μ M in manganese containing groups. It was concluded that addition of taurine and manganese in cryodiluents with a concentration of 20 mM and 150 μ M respectively could improve the post-thaw sperm quality in Sahiwal bulls.

Key words: Taurine, Manganese, Tris Citric Acid extender, Semen quality.

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INTRODUCTION

Cryopreservation of semen is helpful in preserving the superior germplasm for years. During cryopreservation, several factors can deteriorate the semen quality (Curry, 2007). The sperm cell damaged during cryopreservation and thawing is mostly due to free radicals or excessive reactive oxygen species (ROS) production (Tatoneet *et al.*, 2010). The major ROS produced in semen are hydroxyl radical (OH^-), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), alkoxy (RO $^-$) and peroxy (ROO $^-$) radicals (Tariq *et al.*, 2015).

Sperm membrane mainly comprises of polyunsaturated fatty acids. Reaction of free radicals with poly unsaturated fatty acids leads to lipid peroxidation (Choudhary *et al.*, 2010) and results in the production of low quality semen (Bucak *et al.*, 2010).

Antioxidants are the molecules which show inhibitory activity against reactive oxygen species and free radicals. These antioxidants like catalase, superoxide dismutase and glutathione reductase (Hamid *et al.*, 2010) are also present in the body. Taurine performs many biological and physiological functions. It shows non-enzymatic antioxidant activity by protecting the sperm

from ROS especially during cooling and aerobic conditions (Beheshtiet *et al.*, 2013). In recent studies, there is special focus on taurine addition as a cryoprotectant in Mithun (*Bos Frontalis*) bull semen (Perumale *et al.*, 2013). Manganese acts as a co-factor of mitochondrial superoxide dismutase enzyme (Macmillan and Cruthirds, 2001). It has chain breaking non-enzymatic antioxidant activity in the semen (Cossinet *et al.*, 1992).

Manganese and taurine individually have been used for cryopreservation of semen in bulls (Bilaspuri and Bansal, 2008) and rams (Bucak and Tekin 2007) respectively. Information regarding the combined addition of taurine and manganese in extender to cryopreserve the Sahiwal cattle semen is lacking. Keeping in view the individual and combined effects of these antioxidants on cryopreservation, various concentrations of taurine and manganese on post-thaw semen quality of Sahiwal bull were used.

MATERIALS AND METHODS

Experimental animals: This study was conducted at the Semen Production Unit (SPU), Qadirabad, District Sahiwal, Pakistan. Six adult and healthy Sahiwal bulls were selected for a period of 8 weeks. Bulls were fed

seasonal green fodder and concentrates. Clean water was provided ad-libitum.

Semen collection and evaluation: Semen was collected using an artificial vagina early in the morning. Immediately after collection, semen ejaculates were transferred to the laboratory and evaluated for initial gross physical and microscopic evaluation. Semen ejaculates (n=96) having acceptable color (creamy white/yellow), volume >2.0 ml, mass activity >3+, sperm motility percentage >60% and sperm concentration >500 $\times 10^6$ /ml of semen were selected. After initial evaluation, semen samples were pooled to eliminate the individual bull effect.

Semen processing: Initially, the semen was diluted with Tris citric acid, egg yolk and glycerol extender at 37°C to keep the spermatozoa at the rate of 20×10^6 /0.5 mL per straw. The diluted semen was divided in aliquots (n=10) and different doses of taurine (20.0, 40.0, 60.0 mM), manganese (100.0, 150.0, 200.0 μ M) and their combinations (20.0 mM+100.0 μ M, 40.0 mM+150.0 μ M, 60 mM+200 μ M respectively) were mixed. One aliquote was kept as control containing no antioxidant. Semen straws of 0.5 mL capacity were filled, cooled from 37°C to 4°C in a cold cabinet and equilibrated for further 4 hours. Later, the straws were placed horizontally on freezing grills 5cm above the liquid nitrogen for 8 minutes and immersed into liquid nitrogen. The straws were stored in liquid nitrogen until further evaluation, (Chaudhari and Mshelia, 2002).

Post thaw semen evaluation: A total of 30 cryopreserved straws per replicate were thawed at 37°C for 30 sec and evaluated for sperm motility, viability, plasma membrane, and acrosomal integrity.

Concentration of spermatozoa was determined by Accucell Bovine Photometer. The sperm motility (%) was assessed by placing a drop of semen on glass slide and examined under microscope as described by Asret *et al.* (2011).

Spermatozoa viability: Eosin-Nigrosin stain was used to determine the sperm viability. Equal drops of semen and stain were placed on prewarmed slide and smear was prepared. The prepared smear was air dried and evaluated under phase contrast microscope. A total of 100 sperm were counted in each slide. The sperms having unstained head were considered alive, whereas stained or partially stained head were counted as dead.

Spermatozoa acrosomal integrity: For determination of sperm acrosomal integrity, 1% formaldehyde citrate solution was prepared by adding 2.9 g of sodium citrate and 1 ml of 37% formaldehyde into 100 ml of distilled water. Acrosomal integrity was measured by adding 500 μ l of semen and mixing with 50 μ l of 1% formaldehyde citrate. The samples were evaluated according to criteria

mentioned by Asret *et al.* (2011). Two hundred heads of spermatozoa per slide were counted for acrosome abnormalities such as swollen, ruffled or absent acrosomes.

Spermatozoa plasma membrane integrity: The hypoosmotic swelling test (HOST) solution (150 mOsm/L) was prepared by dissolving 7.35g sodium citrate and 13.5g fructose in 1000 ml of distilled water as described by Adeeleet *et al.* (2009). For examination 1.0 mL of hypoosmotic solution was mixed with 0.1 mL of post thawed semen and incubated at 37°C for 1 hour. Spermatozoa with coiled/swollen tails were considered to have normal plasma membrane (Andrabiet *et al.*, 2008).

Data analysis: The data obtained was analyzed using one-way analysis of variance (ANOVA). The differences in groups were compared by Duncan's Multiple Range Test (DMRT) (Fay and Proschan, 2010).

RESULTS AND DISCUSSION

It was observed that the addition of taurine (20 mM) and manganese (150 μ M) individually improved the post-thaw sperm motility, viability, membrane integrity, and acrosome integrity (Table1). However, no improvement was seen in any of combination group. Reactive oxygen species were excessively produced during cryopreservation, subsequently decreasing motility of sperms through less production of ATP (Dandekaret *et al.*, 2002). The presence of polyunsaturated fatty acids in mammalian sperm plasma membrane made it more susceptible to the damage of the free radicals (Choudhary *et al.*, 2010).

Results of the study showed that addition of antioxidants and their combination in tris citric acid extender at the level of 20 mM taurine, 150 μ M manganese and 20 mM taurine+100 μ M manganese increased the post thaw motility. These results were similar to the previous findings in bovine bull reported by Bansal and Bilaspuri 2008a and buffalo bull by (Mughal *et al.*, 2013). This increase in the motility may be due to detoxification of reactive oxygen species (ROS) by either taurine or manganese which led to reduce the oxidative stress and less cell damages (Bilodeau *et al.*, 2001).

The higher live spermatozoa were observed in 100 μ M and 150 μ M of manganese added group. These observations are inline with the results reported in cattle bull by (Cheema *et al.*, 2009). Significant increase in post-thaw viability in 150 μ M manganese supplemented extender may be due to the decline in lipid peroxidation by antioxidant ability of manganese as been reported by (Cavallini *et al.*, 1984).

It was also noted that supplementation of 20 mM of taurine significantly increased live sperm percentages compared to control group. The current results were alike to the previous findings in buffalo bull reported by

(Mughal *et al.*, 2013). Oxidants and free radicals were required in a limited amount for certain normal physiological functions of sperm. Taurine at higher concentration severely reduce the level of oxidants i.e. below the normal physiological level that was required for normal membrane fluidity in spermatozoa. In contrast, the high doses of taurine produced cryo-injury as has been reported by (Roca *et al.*, 2004) that in turn lowered the functionality of ATPases/ionic channels in membrane and further reduced the nutrients entry in the sperm (Shoe and Zamiri, 2008).

Fertilizing ability was related to the intactness of plasma membrane as it prevented spermatozoa from harmful effects of external damages (Perumalet *et al.*, 2013). The present study showed significantly higher membrane integrity in treatment group containing 20 mM of taurine. These findings were in accordance with the observations in ram reported by (Bucaket *et al.*, 2007). Taurine conserved the concentration of superoxide dismutase and catalase during cryopreservation. These enzymes scavenge the intracellular and extracellular superoxide anion and reduced the lipid peroxidation in plasma membrane (Perumalet *et al.*, 2013). This protective mechanism might be responsible for the maintenance of higher membrane integrity during the freezing and thawing procedure. In addition, there was significant increase in membrane intactness of sperm supplemented with 150 μ M of manganese. Similar findings were reported by Cheema *et al.*, (2009) in crossbreed cattle bull semen. The manganese has the capability to increasing the intracellular (Ca_i^{++}) uptake by increasing the cAMP activity which in turn increasing sperm membrane integrity (Bansal and Bilaspuri, 2008b).

The present study exhibited that supplementation of 20 mM of taurine significantly improved acrosome integrity in post-thawed semen. These results are similar to the findings of Mughal *et al.*, (2013) in frozen-thawed semen of Nilli-Ravi buffalo bull.

Higher post thaw acrosomal integrity in 20 mM of taurine added group might be due to the beneficial effects of taurine as it helped to maintain the normal acrosome integrity (Perumalet *et al.*, 2013). Similarly, the acrosome integrity intact percentage was higher in 150 μ M of manganese group. This increase in sperm acrosome integrity might be associated with the supplementation of Mn, as it enhanced Ca^{++} entrance into the cell without decreasing its viability and it maintained the sperm survival and acrosome (Bansal and Bilaspuri, 2008b).

The supplementation of 40 mM or 60 mM of taurine resulted in decrease of sperm quality (motility, viability, membrane and acrosome integrity). Similar results have been documented by Beheshtiet *et al.*, (2013) in buffalo bulls when higher concentration of taurine was supplemented in semen extender. The reactive oxygen species interacted with the sperm plasma membrane for normal hyper-activation and capacitation of sperm; however, the higher concentration of antioxidants in extender blocked the normal physiological function of free radicals (Roca *et al.*, 2004). The lower values of semen quality parameters may be due to higher doses supplementation of antioxidants in freezing dilutants.

The current results showed that supplementation of 20 mM taurine and 100 of μ M manganese in combination provided beneficial impact on sperm cryosurvival. On the other hand, combination of higher doses of antioxidants badly influenced the semen parameters. This negative effect could be due to the reduction in the normal concentration of oxidants that were usually required for normal physiological functions like sperm capacitation and membrane fluidity. This imbalance in oxidant and antioxidants concentration led to the death of sperm as has been reported by (Roca *et al.*, 2004). Additionally, functionality of ATPases/ionic channels in membrane was also deteriorated which lowered the nutrients entry in the sperm and subsequently sperm survival was questionable (Shoe and Zamiri, 2008)

Table 1: Effects of antioxidants on post thaw semen parameters.

Treatment	Motility %	Live Sperm %	HOST %	Acrosomal Integrity %
20.0 mM taurine	50.50 \pm 0.563 ^C	62.75 \pm 1.230 ^A	63.75 \pm 0.559 ^A	65.00 \pm 0.796 ^A
40.0 mM taurine	45.25 \pm 0.761 ^{DE}	56.75 \pm 1.230 ^B	55.50 \pm 0.592 ^D	61.00 \pm 0.796 ^{CDE}
60.0 mM taurine	42.50 \pm 0.742 ^{EF}	57.25 \pm 0.938 ^B	51.50 \pm 0.428 ^E	58.25 \pm 0.739 ^{DEF}
100.0 μ M manganese	53.63 \pm 0.821 ^B	63.25 \pm 0.844 ^A	59.75 \pm 0.528 ^B	63.00 \pm 0.548 ^{ABC}
150.0 μ M manganese	59.75 \pm 0.642 ^A	64.25 \pm 0.844 ^A	64.75 \pm 0.528 ^A	64.75 \pm 0.496 ^{AB}
200.0 μ M manganese	54.19 \pm 0.726 ^B	60.75 \pm 0.883 ^{AB}	55.75 \pm 0.528 ^{CD}	58.00 \pm 0.816 ^{EF}
20.0 mM taurine+100.0 μ M manganese	50.00 \pm 0.730 ^C	60.25 \pm 0.938 ^{AB}	54.75 \pm 0.559 ^D	62.25 \pm 0.739 ^{ABC}
40.0 mM taurine+150.0 μ M manganese	46.25 \pm 0.588 ^D	56.75 \pm 1.055 ^B	49.00 \pm 0.632 ^F	58.00 \pm 0.796 ^{EF}
60.0 mM taurine+200.0 μ M manganese	41.50 \pm 0.428 ^F	45.50 \pm 0.224 ^C	44.50 \pm 0.428 ^G	56.00 \pm 0.796 ^F
Control (no antioxidant)	45.50 \pm 0.742 ^{DE}	58.00 \pm 1.211 ^B	58.00 \pm 0.632 ^{BC}	61.50 \pm 0.940 ^{BCD}

Means sharing similar superscripts (A,B,C,D,E and F) are showing statistically non-significant ($P>0.05$) difference among different groups.

Conclusion: It was concluded that taurine and manganese supplementation in freezing extender with a concentration of 20 mM and 150 μ M or improved the post-thaw sperm quality in Sahiwal bulls.

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