EFFECT OF SUPEROXIDE DISMUTASE AND CATALASE SUPPLEMENTATION IN SOYA MILK EXTENDER ON BUFFALO POST-THAW SEMEN QUALITY

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ABSTRACT

Using the split sample technique, the semen quality parameters of 36 ejaculates from 3 buffalo bulls were studied after extension in soya milk extender ± superoxide dismutase (SOD) and catalase (CAT). Among sperm defects, tail defects increased ($p<0.01$) at post-thaw stage as compared to post-equillibration stage in antioxidant group. The reduction in acrosomal integrity from post-equilibration to post-thaw stage was observed in both control and treatment groups, however, the reduction was prominent (25%, $p<0.01$) in control as compared to treatment (13%, $p<0.05$). The integrity of plasmalemma followed a similar trend as that of acrosomal integrity. Thus, antioxidant fortification prevented reduction in sperm motility and viability which was otherwise observed in non-supplemented group. It is concluded that SOD and CAT enzyme supplementation to soya milk extender can be used to improve post-thaw semen quality in buffalo.

Keywords: Buffalo, Catalase, Cryopreservation, Semen, Superoxide dismutase

INTRODUCTION

Artificial insemination with frozen thawed buffalo semen is less popular due to low conception rate (30%) under field conditions (Anzar *et al.,* 2003). The underlying reason is poor post-thaw performance of buffalo semen as the process of cryopreservation decreases viability, motility as well as plasma membrane, acrosome and DNA integrity of buffalo bull spermatozoa **(**Kadirvel *et al.,* 2009). The cryopreservation as a free radical generating process is well documented in buffalo semen, resulting in increased production of reactive oxygen species (ROS), membrane lipid peroxidation and apoptosis (Kadirvel *et al.,* 2009). Buffalo spermatozoa are more susceptible to peroxidative damage than that of cattle, since buffalo spermatozoa are rich in polyunsaturated fatty acids like arachidonic acid and decosahexaenoic acid (Nair *et al.,* 2006). Buffalo semen is equipped with indigenous antioxidant system that is insufficient to protect the spermatozoa from oxidative stress (Nair *et al.,* 2006). Therefore, the supplementation of antioxidants is recommended to protect buffalo bull spermatozoa integrity during freeze-thawing process (Andrabi *et al.,* 2008).

Buffalo semen is preserved in extenders that contain additives of animal origin (egg yolk and/or milk), which may pose a potential risk of microbial contamination. The presence of bacteria in the inseminate may cause reproductive problems in female and result in low fertility rate. Therefore, a well defined and pathogen-free substitute for egg yolk (preferably of non-animal origin) would be more suitable for extenders used for semen. In the last few years, new diluents with lecithin based cryo-protective components have been introduced into practice (Aires *et al.,* 2003). Soya bean, a plant based cryopreservative, contains kathin - a high molecular weight lipoprotein substitute for egg yolk, to prevent or repair damage to spermatozoa plasma membrane during cryopreservation (Aires *et al.,* 2003). The hypothesis of present study was to supplement superoxide dismutase (SOD) and

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catalase (CAT) to soya milk extender to protect buffalo spermatozoa from cryodamages.

MATERIALS AND METHODS

The present study was undertaken at Central Artificial Breeding station, Hakkal, Jammu (332 m above MSL, 74°50'E 32°40'North). The minimum and maximum temperature and humidity recorded during the study period were 5°C and 40°C and 60% and 95%, respectively. The daily ration of each bull consisted of 2-3 kg high protein feed supplemented with 30 gm mineral mixture, chopped barseem fodder *ad libitum* and 3 kg wheat bhoosa. Water was available *ad libitum*.

Soya milk was prepared by soaking the soya seed overnight in 0.3% sodium bicarbonate. Next morning, it was washed and boiled in fresh 0.3% sodium bicarbonate for 60 minutes. Slurry was then prepared with boiling water (1:8) and filtered through four folds of muslin cloth (Kapoor and Gupta, 1981). The composition (per 100 ml) of soya milk extender (SME) was Tris (hydroxy methyl amino methane) 2.42g, Citric acid 1.38g, Fructose 1.0g, Glycerol 6.4ml, Soya milk 25ml, Penicillin G 1000IU and Streptomycin 1000µg.

Using artificial vagina method, 36 ejaculates (12 ejaculate / buffalo bull; 2 ejaculate / bull / day about 15-20 minutes apart) from three buffalo bulls (age 3-5 yr, body weight ~450 kg) were utilized. Ejaculates having mass motility >3, progressive motility >70% and sperm concentration of >500 million/ml were selected for further processing. One fraction of a semen sample was diluted with SME without antioxidant additives (Control) and the other fraction was diluted with SME supplemented with antioxidant additives (Treatment) to final concentration 80 million sperms/ml. Antioxidants (SOD and CAT) were added to SME @100 units/ml each. The diluted semen was filled in appropriately marked 0.25ml straws and was kept for equilibration for 4h. At the end of equilibration period, the semen from three straws were pooled and subjected to evaluation tests. Immediately after equilibration, the straws were cryopreserved in liquid nitrogen using standard techniques for 10-15 days.

Post-thaw evaluation of semen samples for progressive motility, viability, abnormal sperm count, acrosomal integrity and hypo-osmotic swelling test was conducted immediately after thawing (0h) and after incubation for 30 min in water bath maintained at 37°C. Supra-vital staining technique (Eosin-Nigrosin) was used to determine the percentage of live spermatozoa and sperm abnormalities. Acrosomal integrity was assessed by Giemsa staining. Plasma membrane integrity was evaluated using Hypo-osmotic swelling test (HOST). Data were analysed by student's t-test to find difference between individual groups.

RESULTS AND DISCUSSION

A freeze-thaw cycle of buffalo semen resulted in 18% reduction each in sperm motility and viability ($p<0.05$), and 4% increase in sperm tail defects in non-supplemented semen (Table 1). However, similar reduction in sperm motility and viability was not observed ($p>0.05$) in antioxidant (SOD and CAT) fortified group. Among sperm defects, tail defects increased ($p<0.05$) at post-thaw stage as compared to post-equillibration stage in control but not in treatment group. Furthermore, tail defects were more ($p<0.05$) at post-thaw stage in control as compared to antioxidant fortified group (Table 1). It is pertinent to describe that cryopreservation of buffalo semen accelerated the production of ROS molecules through higher lipid peroxidation levels (Kadirvel *et al.,* 2009) beyond physiological levels. The levels of ROS are negatively correlated with sperm motility of buffalo semen (Kadirvel *et al.,* 2009) and a reduction in sperm motility along with total antioxidant potential was reported in cryopreserved buffalo semen (Kumar *et al.,* 2011). The addition of exogenous SOD to SME reduces available free radicals in sperm, decreases protein carbonylation (amount of damaged intracellular proteins) levels and helps protect the vitality of sperms (Stefanov *et al.,* 2011). A combination of SOD and CAT in tris-glucose-yolk diluents had an additive effect to improve the

Table 1: Effect of soya milk extender supplemented with antioxidants on sperm parameters at post-equillibration and post-thaw stage (n=36)

Parameter	Fresh semen	Gp	Post-equillibration	Post-thaw
Motility	72.6±3.5	**C**	69.3±4.3^{a}	51.2±4.3^{b,A}
		T	68.2±5.9	61.8±3.9^{B}
Viability	76.3±4.2	**C**	72.5±5.2^{a}	55.0±3.6^{b}
		T	68.3±5.2	55.3±4.2
Head defects	3.15±0.4	**C**	3.77±0.38	4.42±0.63
		T	3.62±0.42	5.49±0.24
Mid-piece defects	3.39±0.5	**C**	4.18±0.27	6.42±0.29
		T	3.78±0.44	5.76±0.35
Tail defects	3.8±0.5	**C**	4.35±0.21^{a}	8.31±0.46^{b,A}
		T	4.23±0.33	5.95±0.38^{B}
Intact acrosome	82.0±6.1	**C**	74.7±5.2^{a}	50.0±3.9^{b,A}
		T	72.6±4.5^{a}	60.0±4.3^{b,B}
Plasmalemma Integrity	69.4±4.7	**C**	52.5±3.2^{a,A}	41.3±4.1^{b,A}
		T	62.2±4.6^{a,B}	54.2±4.8^{b,B}

$^{a\ vs.\ b}p<0.05$, between column; $^{A\ vs.\ B}p<0.05$, between row of a parameter; C, Control; T, Treatment

survival of ram spermatozoa at 5°C (Maxwell and Stojanov, 1996) and post-thaw sperm survival in boar (Roca *et al.,* 2005).

The reduction in acrosomal integrity from post-equillibration to post-thaw stage was observed in both control and treatment groups, however, the reduction was more (25%; $p<0.05$) prominent in control as compared to treatment (13%; $p<0.05$; Table 1). Moreover, at post-thaw stage, treatment group had higher ($p<0.05$) acrosomal integrity than control. The integrity of plasmalemma followed a similar trend as that of acrosomal integrity (Table 1). Antioxidant fortification resulted in a preservation of progressive motility and integrity of plasmalemma which were reduced ($p<0.05$) in control after incubation of post-thawed semen at 37°C for 30 minutes (Figure 1). The beneficial effects of SOD (Perumal, 2014) and CAT (Peruma *et al.,* 2013) on keeping quality of mithun semen at 5°C was demonstrated recently. These studies showed that the semen quality can be preserved up to 30 h by addition of SOD or CAT. The supplementation of catalase alone or in combination with SOD improved physico-morphological attributes of buffalo spermatozoon including integrity of plasma membrane and acrosome (El-Sissy *et al.,* 2008). In a more recent study, supplementation of combination of SOD cell-permeable SOD mimetic [Manganese(III) meso-tetrakis(N-ethylpyridinium-2-yl)porphyrin chloride (MnTE)] and catalase improved motility, membrane integrity and viability of goat semen cryopreserved in Andromed extender (Shafiei *et al.,* 2015). In conclusion, SOD and CAT enzyme supplementation to soya milk extender in combination can be used to improve post-thaw quality of buffalo semen.

REFERENCES

Aires, V.A., Hinsch, K.D., Schloesser, F.M., Bognera, K. and Schloesser, S.M. (2003). In vitro and in vivo comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of bovine semen. *Theriogenology,* **60**: 269-279.

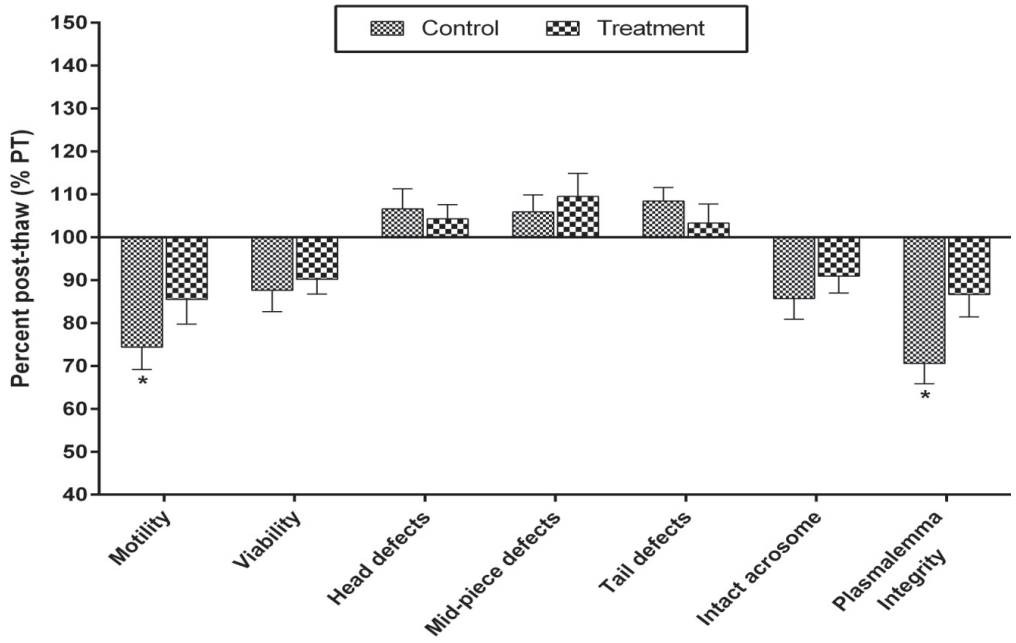

Figure 1: Effect of soya milk extender supplemented with antioxidants on sperm parameters after incubation at 37°C for 30 minutes after freeze-thaw (n=36). *p<0.05 *vs.* **post-thaw**

Andrabi, S.M.H., Ansari, M.S., Ullah, N. and Afzal, M. (2008). Effect of non-enzymatic antioxidants in extender on post-thaw quality of buffalo (*Bubalus bubalis*) bull spermatozoa. *Pak. Vet. J.,* **28**: 159-162.

Anzar, M., Farooq, U., Mirza, M.A., Shahab, M. and Ahmad, N. (2003). Factors affecting efficiency of artificial insemination in cattle and buffalo in Punjab, Pakistan. *Pak. Vet. J.,* **23**: 106-113.

El-Sisy, G.A., El-Nattat, W.S. and El-Sheshtawy, R.I. (2008). Effect of superoxide dismutase and catalase on viability of cryopreserved buffalo spermatozoa. *Global Veterinaria,* **2**: 65-61.

Kadirvel, G., Kumar, S. and Kumaresan, A. (2009). Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. *Anim. Reprod. Sci.,* **114**: 125-134.

Kapoor, C.M. and Gupta, S.K. (1981). Soy-whey weaning food. Method of manufacture. *J. Food Sci. Technol.,* **18**: 55-58.

Kumar, R., Mohanarao, G.J. and Atreja, S.K. (2011). Freeze–thaw induced genotoxicity in buffalo (*Bubalus bubalis*) spermatozoa in relation to total antioxidant status. *Mol. Biol. Reports,* **38**: 1499-1506.

Nair, S.J., Brar, A.S., Ahuja, C.S., Sangha, S.P.S. and Chaudhary, K.C. (2006). A comparative study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. *Anim. Reprod. Sci.,* **96**: 21-29.

Peruma, P., Chamuah, J.K. and Rajkhowa, C. (2013). Effect of catalase on liquid storage of mithun (*Bos frontalis*) semen. *Asian Pacific J. Reprod.,* **2**: 209-214.

Perumal, P. (2014). Effect of superoxide dismutase on semen parameters and antioxidant enzyme activities of liquid stored (5°C) Mithun (*Bos frontalis*) semen. *J. Anim.*, doi:10.1155/2014/821954.

Roca, J., Rodríguez, M.J., Gil, M.A., Carvajal, G., Garcia, E.M., Cuello, C., Vazquez, J.M. and Martinez, E.A. (2005). Survival and in vitro fertility of boar spermatozoa frozen in presence of superoxide dismutase and/or catalase. *J. Androl.* **26**: 15-24.

Shafiei, M., Forouzanfar, M., Hosseini, S.M. and Esfahani, M.H.N. (2015). The effect of superoxide dismutase mimetic and catalase on quality of postthawed goat semen. *Theriogenology,* **83**: 1321-1327.

Stefanov, R. Sabev, M. and Ivanova-Kicheva, M. (2011). Protective effect of antioxidant enzyme on sperm from Bubalus bubalis type after cryoconservation. *Biotechnol. Anim. Husb.,* **27**: 943-949.