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Role of Catalase as Semen Additive in Cryopreservation of Cattle Bull Semen

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A B S T R A C T

The present study was done at the Frozen Semen Laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu and Division of V.G.O., F.V.Sc & A.H., SKUAST- J, R.S.Pura, during the period between December 2017 and May 2018. This investigation was carried out with the objective to study the effect of antioxidant semen additive catalase on cryopreservation of semen. Semen samples (n=10) from mature cattle bull stationed at Frozen semen laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu, were used to evaluate the effect of catalase additive at post-dilution and at post thaw stage. The semen sample was extended with Tris-Egg-Yolk-Citric-acid-Fructose-Glycerol (TEYCAFG) extender and were split into two groups: Group 1: TEYCAFG without any additive/ control and group 2: TEYCAFG + Catalase (100 IU/ml). Progressive motility, live spermatozoa, acrosomal integrity, sperm abnormality, hypo-osmotic swelling test (HOST) was evaluated at both post-dilution and post-thaw stage. Whereas, oxidative stress tests *viz*. malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were evaluated at only post-thawed stage. Group 2 i.e. catalase group, showed significant ($p<0.05$) increased progressive motility, live spermatozoa, acrosomal integrity and HOST positive spermatozoa, while significant ($p<0.05$) decreased sperm abnormalities in post-thawed semen. In oxidative stress evaluation the MDA level was significantly ($p<0.05$) decreased, whereas, CAT and SOD levels significantly ($p<0.05$) increased in group 2 in comparison to control group. It was concluded that addition of catalase (100 IU/ml) as semen additive improves semen quality and minimize oxidative stress to the spermatozoa during cryopreservation of semen.

Introduction

Artificial insemination (AI) is the most important single technique devised for the genetic improvement of animals. This improvement has been exponential in dairy cattle, in which use of frozen semen is most common. A prerequisite for the best use of this genetic material is to obtain acceptable fertility after AI apart from achieving a high

milk production (Karunakaran *et al.,* 2012). But the freezing and thawing process leads to the generation of reactive oxygen species (ROS) that impairs post-thaw motility, viability, intracellular enzyme activity, fertility and sperm functions (Aitken *et al.,* 1998; Zhao and Buhr, 1995; White, 1993).

The damage during cryopreservation is mainly due to cold shock, ice crystal formation, oxidative stress, osmotic changes and lipid-protein reorganization within the cell membranes (Bailey *et al.,* 2000; Watson, 1995). The sperm cells contain high contents of polyunsaturated fatty acids (PUFA) which makes the membranes more susceptible to peroxidative damage. The protective antioxidant systems in spermatozoa are primarily of cytoplasmic origin which is generally lost during terminal stages of differentiation, thus lack antioxidants that counteract the damaging effects of ROS (Bucak *et al.,* 2010). Spermatozoa and seminal plasma possess an antioxidant system comprising of taurine, reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) to prevent oxidative damage. However, this antioxidant capacity in sperm cells, due to small cytoplasmic component containing antioxidants to scavenge oxidants, is limited. Thus, mammalian spermatozoa are unable to prevent formation of lipid peroxidation (LPO) during freeze –thawing process (Bucak *et al.,* 2010; Aurich *et al.,* 1997; Storey, 1997).

In recent years, the addition of antioxidants such as reduced glutathione (GSH), ascorbic acid, taurine, cysteine to bovine semen has shown to protect effects of ROS and improve post-thaw sperm motility, viability and fertility.

Catalase is frequently used additives to sperm suspensions in order to improve sperm characteristics. Catalase is a common enzyme found in all living organisms exposed to oxygen. Catalase specifically removes hydrogen peroxide by converting this oxidant to water and oxygen (Baker *et al.,* 1996). It is an enzymatic antioxidant exists in peroxisomes, which can convert hydrogen peroxide metabolism to water and oxygen and neutralize the toxic effects of free radicals (Teke, 2014). It is the first intracellular defense system against ROS and free radicals (Caballero, 2007). Catalase also improves oocyte penetration of bull spermatozoa (Rossi *et al.,* 2001).

To best of our knowledge, the studies are deficient in investigation of the roles of addition of catalase in semen of cross-bred bull of Jammu and Kashmir region. In continuation to our previous reported findings in cryopreservation of cross-bred cattle bull semen (Singh *et al.,* 2020) the present study was designed with the objective to evaluate the oxidative stress to the spermatozoa during cryopreservation of semen and the effects of catalase as semen additive.

Materials and Methods

The present study was undertaken at the Division of V.G.O., F.V.Sc & A.H., SKUAST- J, R.S. Pura and Frozen semen laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu during the period between December 2017 and May 2018. Semen samples (n=10) were collected randomly from mature cattle bull stationed at Frozen semen laboratory, U.T. of Jammu & Kashmir, Hukkal, Jammu, India (32.73º N, 74.87º E, altitude 300 m). Semen was collected twice a week from each bull by artificial vagina method. The semen ejaculates were initially evaluated for volume, mass activity, spermatozoa concentration and progressive motility. The ejaculates qualifying the minimum initial standards were further

processed. After initial assessment of semen, the selected samples were extended with pre-warmed (37°C) Tris egg yolk citric acid fructose glycerol (TEYCAFG) extender so that each ml of extended semen contained at least 40 x 10^6 spermatozoa (Anel *et al.,* 2003). After extension, the semen samples were divided into two parts. The first part was considered as group 1: TEYCAFG without any additive/ control and group 2: TEYCAFG + Catalase (100 IU/ml). The pH was adjusted within the range 7.2 to 7.4. The semen samples were filled in 0.5 ml capacity, polyvinyl straw (IMV, France). Equilibration was done in the cold handling unit maintained at 4° C for 4 hours. Freezing of the straws was done by programmable biofreezer (Digit Cool-530, IMV Technologies, France) with rate freezing from 4°C to -10°C @ 5°C, -10°C to -100°C @ 40°C, -100°C to -140°C @ 20°C, after attainment of -140°C temperature the straws were removed from the racks shifted to the pre-cooled goblets and plunged into liquid nitrogen and finally stored in liquid nitrogen tank where they were stored for definite period of time for future evaluation.

Total ejaculates (n=10) were evaluated at two stages of the semen processing viz. post-dilution and post thaw stage, for per cent progressive motility, live spermatozoa, acrosomal integrity, sperm abnormalities and hypo-osmotic swelling test. Whereas, only at post-thaw stage for oxidative stress test viz. MDA, SOD and Catalase. Thawing of frozen semen was done at 37° C for 30 seconds.

Volume was measured by graduated collecting tube, mass activity was graded as described by Tomar *et al.,* (1966). The concentration of the spermatozoa (millions/ml) was determined by Accucell photometer (IMV Technologies, France). Progressive motility, live spermatozoa and acrosome integrity were assessed as per Salisbury *et al.,* (1978); HOST test was performed as described by Jeyendran *et al.,* (1984); sperm abnormalities were assessed by Eosin-nigrosin stain with slight modification as described by Kumar (1993). Oxidative stress test viz. Malondialdehyde (MDA) was determined as described by Shafiq-ur-Rehman (1984); Catalase as per Aebi (1983) and Superoxide dismutase (SOD) as described by Marklund and Marklund (1974). The results were analysed statistically using Analysis of Variance (ANOVA) (Snedecor and Cochran, 1989).

Results and Discussion

In the present investigation EY-tris (egg yolk-tris-citric acid-fructose-glycerol) was used as control dilutor, EY-tris plus Catalase (100 IU/ml) were used as experimental groups.

Progressive motility

The per cent progressive motility (Mean ± SE) post-dilution and post-thawing in control group were 67.0 ± 1.52 and 48.0 ± 2.49; whereas in catalase added group were 68.0 ± 1.33 and 58.0 ± 1.33, respectively (Table 1). Perusal of the table 1 it was observed that the progressive motility (%) differed non significantly between post-diluted control and Catalase added group, whereas, in post-thaw a significant ($p<0.05$) difference in progressive motility was observed between control and catalase group.

In the present study in control group the per cent progressive motility of diluted semen (Table 1) was 67.0 ±1.52 which was in agreement with the finding of Pathak *et al.,* (1990) who reported per cent progressive motility as 65.30 ± 1.20 per cent in diluted semen of cattle bull. The per cent progressive motility of post thaw semen is reported as higher as 80.74 (Kishore, 2009), 61.7 ± 2.6 (Pawshe *et al.,* 2016), 55.5 ± 2.5 (Uysal *et al.,* 2007), 55.34 ± 1.02 (Ulfina and Raina, 2003),

36.88 ± 1.53 (Hu *et al.*, 2010) and as low as 14.7 ± 1.4 (Li *et al.*, 2016).

In the present study per cent post thaw motility in control group (Table 1) was 48.0 ± 2.49 which was less than reported by Kishore (2009), Pawshe *et al.*, (2016), Uysal *et al.*, (2007) and Ulfina and Raina (2003), while it was higher than reported by Hu *et al.*, (2010) and Li *et al.*, (2016). The main reason for the decrease in the per cent post thaw progressive motility might be due to cryopreservation damage, ROS production and damage caused due to formation of ice crystal formation in mitochondria and Axomemes during cryopreservation that impairs sperm motility.

In the present study in catalase added group the values of per cent progressive motility of diluted semen (Table 1) was 68.0 ± 1.33, The per cent progressive motility in diluted semen was observed by various scientist as 80 ± 7.58, 80 ± 1.54, 80 ± 2.16 in three different bulls with 100 IU of catalase and 85 ± 1.63, 85 ± 2.86, 80 ± 4.71 in three different cattle with 200 IU of catalase (Bansal and Cheema, 2016), 64.0 ± 18.2 in cattle bull with 200 IU of catalase (Fernandez-Santos *et al.*, 2009).

In the present study the per cent post thaw progressive motility in catalase added group was recorded as 58.0 ± 1.33. It has been previously reported as 65 ± 0.94, 50 ± 4.02, 57.5 ± 0.62 in post thaw semen of three different bulls with 100 IU of catalase and 70 ± 4.71, 57.5 ± 6.24, 63.75 ± 2.33 in post thaw semen of three different bulls with 200 IU of catalase (Bansal and Cheema, 2016), 46.70 ± 2.89 in post thaw semen of cattle bull with 100 IU of catalase and 43.30 in post thaw semen of cattle bull with 200 IU of catalase (Asadpour *et al.*, 2011), 41.0 ± 11.4 in post thaw semen of cattle bull with 200 IU of catalase (Fernandez-Santos *et al.*, 2009).

In present study the value of per cent progressive motility in diluted semen of catalase added group (Table 1) was 68.0 ± 1.33, which was in agreement with the Fernandez-Santos *et al.*, (2009), whereas lesser than the finding of Bansal and Cheema (2016).

The value of per cent progressive motility in post thaw semen of catalase added group (Table 1) in present study was 58.0 ± 1.33 which was in agreement with Bansal and Cheema (2016) with 200 IU of catalase, while it was higher than finding of Asadpour *et al.*, (2011), Fernandez-Santos *et al.*, (2009) and lesser than finding of Bansal and Cheema (2016) with 100 IU of catalase.

Live spermatozoa

The per cent live spermatozoa (Mean ± SE) post-dilution and post-thawing in control group were 74.4 ± 1.82 and 50.8 ± 1.17; whereas in catalase group were 77.7 ±1.25 and 60.8 ± 1.14, respectively (Table 1). Perusal of the table 1 it was observed that the live spermatozoa (%) differed non significantly between post-diluted control and catalase group, whereas, in post-thaw a significant ($p<0.05$) difference in live spermatozoa was observed between control and catalase group.

In the present study in the control group values of per cent live spermatozoa in diluted semen (Table 1) was 74.4 ± 1.82. in the previous study the per cent live spermatozoa in diluted semen was observed as 74.28 (Keshava, 1996), 73.0 ± 1.3 (Abdel-khalek *et al.*, 2008) and 72.44 ± 0.51 (Ulfina and Raina, 2003). In the present study the values of per cent live spermatozoa in diluted semen of control group (Table 1) was 74.4 ± 1.82, which was in agreement with the findings of Keshava (1996) and Abdel-khalek *et al.*, (2008). The per cent spermatozoa in post thaw semen was reported as 60.76 ± 0.68, 57.64 ± 0.78 (Bhalde *et al.*, 1991), 56.24 ± 0.01 (Rao *et al.*, 2017) and 66-73 per cent

(Abdel-khalek *et al.,* 2008). In the present study the values of per cent live spermatozoa of post thaw semen of control group (Table 1) was 50.8 ± 1.17 which was less than reported by Bhalde *et al.,* (1991), Rao *et al.,* (2017) and Abdel-khalek *et al.,* (2008). Decreasing proportion of live spermatozoa in post thaw semen may be due to cryo damage caused to formation of ice crystals in extra and intracellular environment, increasing solute concentration (Mazur, 1984) and sperm susceptibility for freezing and thawing temperature, ROS production and lipid peroxidation (Bucak *et al.,* 2008)

In the present study in catalase added group the value of per cent live spermatozoa of diluted semen was 77.7 ± 1.25. The per cent live spermatozoa in diluted semen was observed by various scientist as 97.29 ± 4.85, 88.42 ± 1.73, 91.02 ± 3.29 in three different bulls with 100 IU of catalase and 92.45 ± 5.91, 86.3 ± 3.44, 93.2 ± 1.79 in three different bulls with 200 IU of catalase (Bansal and Cheema, 2016). In the present study in catalase added group the value of per cent live spermatozoa of post thaw semen was 60.8 ± 1.14. In the previous study the per cent live spermatozoa in post thaw semen was observed as 59 ± 2.82, 64.39 ± 3.11, 61.69 ± 8.16 in post thaw semen of three different bulls with 100 IU of catalase and 59 ± 2.82, 64.39 ± 3.11, 61.69 ± 8.16 in post thaw semen of three different bulls with 200 IU of catalase (Bansal and Cheema, 2016), 59.0 ± 23.9 in post thaw semen of cattle bull with 200 IU of catalase (Fernandez-Santos *et al.,* 2009), 51.70 ± 2.90 in post thaw semen of cattle bull with 100 IU of catalase and 200 IU of catalase (Asadpour *et al.,* 2011).

In our study the value of per cent live spermatozoa in diluted semen of catalase added group (Table 1) was 77.7 ± 1.25, which was lesser than the finding of Bansal and Cheema (2016).

The value of per cent live spermatozoa in post thaw semen of catalase added group (Table 1) in present study was 60.8 ± 1.14 which was in agreement with finding of Fernandez-Santos *et al.,* (2009) and Bansal and Cheema (2016), whereas lower values were observed in the finding of Asadpour *et al.,* (2011).

Acrosomal integrity

The per cent acrosomal integrity (Mean ± SE) post-dilution and post-thawing in control group were 79.7 ± 1.52 and 53.9 ± 0.94; whereas in catalase group were 81.4 ± 1.32 and 66.3 ± 1.31, respectively (Table 1). Perusal of the table 1 it was observed that the acrosomal integrity (%) differed non significantly between post-diluted control and catalase group, whereas, in post-thaw a significant ($p<0.05$) difference in acrosomal integrity was observed between control and catalase group.

The evaluation of acrosomal integrity is important for determination of semen quality as enzyme necessary for fertilization present in the acrosomal cap. The per cent acrosomal integrity in diluted semen was reported as 85.77 ± 0.01 in crossbred bull (Rao *et al.,* 2017) and 80.17 ± 3.26 in buffalo bull (Lone *et al.,* 2017). In the present study the values of per cent acrosomal integrity of diluted semen of control group (Table 1) was 79.7 ± 1.52, which was in agreement with the finding of Lone *et al.,* (2017), it was less than the finding of Rao *et al.,* (2017).

The per cent acrosomal integrity in post thaw semen was reported as 56.24 ± 0.01 in crossbred bull (Rao *et al.,* 2017), 36.25 ± 0.91 in murrah buffalo bull (Sandeep *et al.,* 2015), 31.5 ± 1.3 in crossbred bull (Paudel *et al.,* 2010). In the present study the values of per cent acrosomal integrity of post thaw semen of control group (Table 1) was 53.9 ± 0.94 which was in agreement with the finding of

Rao *et al.*, (2017), however it was higher than the finding of Sandeep *et al.*, (2015) and Paudel *et al.*, (2010).

Studies have shown, Cryopreservation induces various degrees of acrosomal damage that may be primary, secondary or teriary damage. There is reduction of 20-30 % in acrosomal intactness during time of post thaw evaluation of spermatozoa. The decrease in acrosomal integrity at various stages may be due to loss of component of plasma membrane and loss of plasmalemma over entire acrosome during freeze- thawing.

In the present study in catalase added group the values of per cent acrosomal integrity of diluted semen (Table 1) was 81.4 ± 1.32, whereas, the values of per cent acrosomal integrity of post thaw semen (Table 1) was 66.3 ± 1.31. In previous study the value per cent acrosomal integrity in post thaw semen was recorded as 35.9 ± 1.4 (Paudel *et al.*, 2010) which was lower than the present study finding *i.e.* 66.3 ± 1.31.

Sperm abnormalities

The per cent sperm abnormalities (Mean ± SE) post-dilution and post-thawing in control group were 7.2 ± 0.63 and 16.8 ± 0.61; whereas in catalase group were 7.1 ± 0.69 and 12.7 ± 0.42, respectively (Table 1). Perusal of the table 1 it was observed that the sperm abnormalities (%) differed non significantly between post-diluted control and catalase group, whereas, in post-thaw a significant ($p<0.05$) difference in sperm abnormalities was observed between control and catalase group.

The per cent sperm abnormalities in diluted semen was observed as 4.91 ± 0.14 in bhadawari bull (Mittal *et al.*, 2014) 7.28 ± 0.02 in crossbred bull (Rao *et al.*, 2017) and 9.14 ± 0.09 in cattle bull (Gupta *et al.*, 1990). In the present study the values of per cent sperm abnormalities in diluted semen of control group (Table 1) was 7.2 ± 0.63, which was in agreement with the finding of Rao *et al.*, (2017), however it was higher than as reported by Mittal *et al.*, (2014) while it was lower than as reported by Gupta *et al.*, (1990).

The per cent sperm abnormalities in post thaw semen is reported by various scientists as 7.75 ± 0.17 in Bhadawari bull (Mittal *et al.*, 2014), 14.93 ± 1.07 in cattle bull (Sariozkan *et al.*, 2009a), 15.0 ± 1.1 in cattle bull (Sariozkan *et al.*, 2009b), in the range of 15.66 ± 0.39 to 16.75 ± 0.37 in Jersey bulls (Rao *et al.*, 1999), 17.63 ± 1.99 in cattle bull (Gupta *et al.*, 1990) and 18.36 ± 0.04 in crossbred bull (Rao *et al.*, 2017).

In the present study per cent post thaw sperm abnormalities in control group (Table 1) was 16.8 ± 0.61 which was in agreement with the finding of Rao *et al.*, (1999) while it was higher than as reported by Mittal *et al.*, (2014), Sariozkan *et al.*, (2009a) and Sariozkan *et al.*, (2009b) whereas lower than the finding of Gupta *et al.*, (1990) and Rao *et al.*, (2017).

In the present study in catalase added group the value of per cent sperm abnormalities of diluted semen (Table 1) was 7.1 ± 0.69. In previous work the value of per cent of sperm abnormalities in diluted and post thaw semen observed by Fernandez-Santos *et al.*, (2009) as 10.0 ± 2.0 and 14.0 ± 6.6, respectively, which is higher than the value in our present study in diluted semen and post thaw semen of catalase added group (Table 1) *i.e.* 7.1 ± 0.69 12.7 ± 0.42, respectively.

Table.1 The effect of catalase on different physio-morphological characteristics at post-dilution and post-thaw semen of cross-bred bulls

Parameter (%)	Stage	Control	Catalase (100 IU/ml)
Progressive motility	Post-dilution	67.0 ± 1.52[a]	68.0 ± 1.33[a]
	Post-thaw	48.0 ± 2.49[a]	58.0 ± 1.33[b]
Live spermatozoa	Post-dilution	74.4 ± 1.82[a]	77.7 ±1.25[a]
	Post-thaw	50.8 ± 1.17[a]	60.8 ± 1.14[b]
Acrosomal integrity	Post-dilution	79.7 ± 1.52[a]	81.4 ± 1.32[a]
	Post-thaw	53.9 ± 0.94[a]	66.3 ± 1.31[b]
Sperm abnormalities	Post-dilution	7.2 ± 0.63[a]	7.1 ± 0.69[a]
	Post-thaw	16.8 ± 0.61[a]	12.7 ± 0.42[b]
HOST	Post-dilution	71.0 ± 1.10[a]	73.1 ± 1.30[a]
	Post-thaw	45.4 ± 0.93[a]	53.8 ± 0.87[b]

Values are given as mean ± SE of 10 animals unless and otherwise stated
Values bearing different superscripts in a row differ significantly ($p<0.05$)

Table.2 The effect of Catalase on malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) activity in post-thawed semen of cross-bred bulls

Oxidative stress test	Control	Catalase (100 IU/ml)
MDA (nmol/10^8 Spermatozoa)	3.49 ± 0.19[a]	1.62 ± 0.04[b]
CAT (µmol/10^8 Spermatozoa)	1.18 ± 0.36[a]	1.46 ± 0.40[b]
SOD (U/ 10^8 Spermatozoa)	0.95 ± 0.06[a]	1.42 ± 0.05[b]

Values are given as mean ± SE of 10 animals unless and otherwise stated
Values bearing different superscripts in a row differ significantly ($p<0.05$)

Hypo-osmotic swelling test (HOST)

The hypo-osmotic swelling test positive spermatozoa per cent (Mean ± SE) post-dilution and post-thawing in control group were 71.0 ± 1.10 and 45.4 ± 0.93; whereas in catalase group were 73.1 ± 1.30 and 53.8 ± 0.87, respectively (Table 1). Perusal of the table 1 it was observed that the hypo-osmotic swelling test positive spermatozoa (%) differed non significantly between post-diluted control and catalase group, whereas, in post-thaw a significant ($p<0.05$) difference in hypo-osmotic swelling test positive spermatozoa was observed between control and catalase group.

The per cent hypo-osmotic swelling test (HOST) positive spermatozoa in diluted semen is reported as 72.54 ± 0.01 in crossbred bull (Rao *et al.*, 2017), 70.91 ± 5.92 in buffalo bull (Lone *et al.*, 2017) and 62.05 ± 0.80 in pure Jersey bulls and 62.11 ± 0.89 in crossbred Jersey bulls (Kumar *et al.*, 2018).

In the present study the per cent hypo-osmotic swelling test (HOST) positive spermatozoa of diluted semen in control group (Table 1) was 71.0 ± 1.10 which was in agreement with the findings of Rao *et al.*, (2017) and Lone *et al.*, (2017), however lower values of per cent hypo-osmotic swelling test (HOST) of diluted semen was observed by Kumar *et al.*, (2018).

The per cent hypo-osmotic swelling test (HOST) positive in post thaw semen is reported as 51.30 ± 4.43 in buffalo bull (Lone *et al.*, 2017), 49.97 ± 3.62 in sahiwal cattle

bull (Sohail *et al.,* 2015), 47.27 ± 1.05 in pure Jersey bulls and 45.94 ± 1.33 crossbred Jersey bulls (Kumar *et al.,* 2018), 43.70 ± 1.96 in cattle bull (Sariozkan *et al.,* 2009a), 39.6 ± 1.3 in crossbred bulls (Paudel *et al.,* 2010), 38.73 ± 0.01 in crossbred bull (Rao *et al.,* 2017) and 36.52 in cattle bull (Taraphdar, 1999). In the present study the values of per cent hypo-osmotic swelling test (HOST) of post thaw semen of control group (Table 1) was 45.4 ± 0.93, which was agreement with the finding of Kumar *et al.,* (2018) and Sariozkan *et al.,* (2009a), while lower values were observed by Paudel *et al.,* (2010), Rao *et al.,* (2017) and Taraphdar (1999) whereas higher finding was observed by Lone *et al.,* (2017) and Sohail *et al.,* (2015). The structural changes produced in the post thaw sperm cells membrane are primarily linked to altered abilities for energy sourcing which later on influence both cellular metabolism and other sperm functions (Dziekonska *et al.,* 2009; Gillan *et al.,* 2004).

In the present study in catalase added group the values of per cent HOST positive of diluted semen has been reported as 73.1 ± 1.30. The value per cent of hypo-osmotic swelling test (HOST) in diluted semen was reported as 93.63 ± 2.93, 55.78 ± 10.76, 74.7 ± 4.85 in three different bulls with 100 IU of catalase and 99 ± 13.81, 61 ± 5.65, 68 ± 4.24 in three different cattle with 200 IU of catalase (Bansal and Cheema, 2016) and the value per cent of hypo-osmotic swelling test (HOST) in post thaw semen reported as 41.6 ± 1.3 in cross bred bull with 200 IU of catalase (Paudel *et al.,* 2010), 52.34 ± 1.77, 36 ± 5.65, 44.17 ± 6.51 in three different bulls with 100 IU of catalase & 54.39 ± 1.31, 36 ± 1.69, 45.19 ± 3.21 in three different cattle with 200 IU of catalase (Bansal and Cheema, 2016). In the present study the per cent hypo-osmotic swelling test (HOST) of diluted semen with catalase added group (Table 1) was 73.1 ± 1.30 which is in agreement with diluted semen with 100 IU of catalase by Bansal and Cheema (2016).

The value for per cent hypo-osmotic swelling test (HOST) of post thaw semen with catalase added group was 53.8 ± 0.87 which is in agreement with post thaw semen with 100 IU and 200 IU of catalase by Bansal and Cheema (2016), however lower value was observed by Paudel *et al.,* (2010).

Malondialdehyde (MDA)

The MDA levels (Mean ± SE) post-thaw semen samples in control group and catalase group were 3.49 ± 0.19 and 1.62 ± 0.04, respectively (Table 2). Perusal of the table 2 it was observed that MDA levels in post-thaw semen samples differed significantly ($p<0.05$) between control and catalase group.

The MDA level (nmol H_2O_2 produced/ 10^8 spermatozoa) of post thaw semen in 712.1 ± 49.1 nmol MDA level/10^9 spermatozoa in crossbred bull (Paudel *et al.,* 2010), 496.02 ± 39.28 nmol MDA level/10^9 spermatozoa in buffalo bull (Lone *et al.,* 2017). In the present study MDA level (nmol H_2O_2 produced/ 10^8 spermatozoa) in post thaw semen of control group (Table 2) was 3.49 ± 0.19 which was lower than the value observed by Paudel *et al.,* (2010) and Lone *et al.,* (2017). The finding of other scientists could not be compared with our finding due to disparity in method of evaluation and units of measurement.

There was an increase in MDA production over a period of 72 hour storage at refrigeration temperature in both cattle and buffalo bull spermatozoa, it indicated that spermatozoa were under increased oxidative stress during storage that resulted in higher LPO (Nair *et al.,* 2006). Since the determination of LPO was based on reaction of thiobarbituric acid (TBA) with the MDA

produced during the process of oxidative destruction of membranes (Buege and Aust, 1978), it was, therefore, an indirect measure of the peroxidation of polyunsaturated fatty acyl moieties of spermatozoa membranes. The mammalian sperm membrane has high polyunsatured fatty acids (PUFA), it renders the sperm very susceptible to LPO, which occurs as a result of the oxidation of the membrane lipids by partially reduced oxygen molecules, such as superoxide, hydrogen peroxide, and hydroxyl radicals. Jones and Mann (1976) also reported that phospholipids in spermatozoa were the prime targets for peroxidation, of which certain polyunsaturated acyl moieties were particularly vulnerable.

In the present study, the MDA level (nmol H_2O_2 produced/ 10^8 spermatozoa) post thaw semen of catalase added group (Table 2) was 1.62 ± 0.04. The MDA level (nmol H_2O_2 produced/ 10^8 spermatozoa) of post thaw semen was 483.6 ± 45.0 (MDA level nmol/10^9 spermatozoa) in 200 IU/ml of catalase in crossbred bulls (Paudel *et al.*, 2010) and 58.56 ± 6.63, 539.97 ± 10.78, 129.26 ± 40.37 (nmoles of MDA/µg protein/ml/10^6 spermatozoa) in three different bulls with 100 IU of catalase and 277.28 ± 38.58, 73.83 ± 6.85, 75.55 ± 2.77 (nmoles of MDA/µg protein/ml/10^6 spermatozoa) in three different cattle with 200 IU of catalase (Bansal and Cheema, 2016) and 5.32 ± 1.32 (nmol/10^9 spermatozoa) in cattle bulls with 100 IU of catalase and 4.42 ± 0.18 (nmol/10^9 spermatozoa) in cattle bulls with 200 IU of catalase (Asadpour *et al.,* 2011).

In present study MDA level of post thaw semen in catalase added group (Table 2) was 1.62 ± 0.04 which is lower than the finding of Paudel *et al.,* (2010) and 100 IU of catalase by Bansal and Cheema (2016) whereas higher than the finding of 200 IU of catalase by Bansal and Cheema (2016) and Asadpour *et al.,* (2011), though the finding of other scientists could not be compared with our finding due to disparity in method of evaluation and units of measurement.

Catalase

The catalase levels (Mean ± SE) post-thaw semen samples in control group and catalase added group were 1.18 ± 0.36 and 1.46 ± 0.40, respectively (Table 2). Perusal of the table 2 it was observed that catalase levels in post-thaw semen samples differed significantly ($p<0.05$) between control and catalase group.

The catalase activity (µmol H_2O_2 decomosed/ min/ 10^8 spermatozoa) in post thaw semen has been reported as 1.16 ± 0.82 (U/ml) in Qinchuan bulls (Zhao *et al.,* 2015), 23.36 ± 0.25 in rainy season, 24.25 ± 0.30 winter season and 24.22 ± 0.56 in summer season, respectively in buffalo bull (Sharma *et al.,* 2016). In the present study catalase activity (µmol H_2O_2 decomosed/ min/ 10^8 spermatozoa) in post thaw semen of control group (Table 2) was 1.18 ± 0.31 which was higher than finding of Zhao *et al.,* (2015) whereas lower than the finding of Sharma *et al.,* (2016). There was reduction in the catalase activity with the level of freezing. CAT activity reduced at post thaw stage when compared to pre freeze stage (Lone *et al.,* 2017). CAT is an enzymatic antioxidant found in all living organisms exposed to oxygen, which decomposes harmful peroxides and converts them into water and oxygen (Chelikani *et al.,* 2004).In the present study, in the Catalase added group the level of Catalase (µmol H_2O_2 decomosed/ min/ 10^8 spermatozoa) in post thaw semen (Table 2) was 1.46 ± 0.40. There was reduction in the catalase activity with the level of freezing. CAT activity reduced at post thaw stage when compared to pre freeze stage (Lone *et al.,* 2017).

Superoxide dismutase (SOD)

The SOD levels (Mean ± SE) post-thaw semen samples in control group and catalase added group were 0.95 ± 0.06 and 1.42 ± 0.05, respectively (Table 2). Perusal of the table 2 it was observed that SOD levels in post-thaw semen samples differed significantly ($p<0.05$) between control and catalase group.

The level of Superoxide dismutase (Unit/ 10^8 spermatozoa) in post thaw semen have been reported as 104.02 ± 26.34 (µkat/g protein) in cattle bull (Sariozkan *et al.,* 2009a), 7.2 ± 1.8 (U/g protein) in cattle bull semen (Sariozkan *et al.,* 2009b), 3.50 ± 0.19 in cattle and 1.98 ± 0.09 in buffalo bulls (Nair *et al.,* 2006) and 0.16 ± 0.03 (units/mg protein) in buffalo bulls (Lone *et al.,* 2017). The level of Superoxide dismutase (Unit/ 10^8 spermatozoa) in present study in post thaw semen of control group (Table 2) was 0.95 ± 0.06 which was lower than the finding of Nair *et al.,* (2006), however it was higher than the finding of Lone *et al.,* (2017). Superoxide is a free radical which is converted to oxygen and hydrogen peroxide by the dismutation action of antioxidant enzyme superoxide dismutase (Tariq *et al.,* 2015).

In the present study, the level of Superoxide dismutase (Unit/ 10^8 spermatozoa) in post thaw semen of catalase added group (Table 2) was 1.42 ± 0.05. In previous studies the post thaw semen has been reported as 501.74 ± 20.39, 307.21 ± 6.5, 504.47 ± 12.5 (Unit/10^9 spermatozoa) in three different bulls with 100 IU of catalase and 902.4 ± 9.3, 175.39 ± 2.17, 638.89 ± 3.71 (Unit/10^9 spermatozoa) in three different bulls with 200 IU of catalase (Bansal and Cheema, 2016).

In present study the level of Superoxide dismutase of post thaw semen in catalase added group (Table 1) was 1.42 ± 0.05, which is lower than the finding of Bansal and Cheema (2016). Asadpour *et al.,* (2011) stated addition of CAT was ineffective on the post thawing viability of spermatozoa while Roca *et al.,* (2005) reported that the addition of CAT enzyme to semen extender improves post thaw sperm viability and fertility.

In conclusion, the present study with Catalase (100 IU/ml) as the semen additive may be used for the cryopreservation of the semen. The parameters of semen quality improved in the catalase added cryopreserved semen in comparison of control group. The oxidative stress to the semen is also minimized after addition of the catalase. The *in vitro* fertility assessed by hypo-osmotic swelling test also indicates for the better quality and fertility in catalase, however, the larger number of the semen samples coupled with field fertility trials should be done in the cattle population for its final validation.

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