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Exogenous catalase improves the goat seminal attributes at refrigeration temperature

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ABSTRACT

The objective of the present study was to evaluate the potential benefit of catalase (CAT) on sperm functional parameters (motility, viability, membrane integrity and acrosomal integrity) during liquid storage of buck spermatozoa at 0 h, 24 h, 48 h, 72 h and 96 h. Semen samples from bucks were pooled to avoid individual effect and diluted with tris-egg yolk-fructose extender with catalase (0 U/ mL (control), 200 U/mL, 400 U/ mL, 600 U/ mL and 800 U/ mL) at a final concentration of 400 million/mL. No significant difference was observed between treatments at 24 h with respect to motility, viability and plasma membrane integrity. However, acrosome integrity of control was significantly higher as compared to other concentrations. Motility, viability, plasma membrane and acrosome integrity were observed significantly highest for CAT @ 600 U/mL in comparison to other concentrations at 48 h, 72 h and 96 h of liquid storage at refrigeration temperature. The seminal parameters declined with the progression of storage time and remained over 50% even after 72 h at refrigeration temperature in CAT @ 600 U/mL. It can be concluded that the addition of CAT @ 600 U/mL in semen diluent along with 20% egg yolk reduced the detrimental effects of cooling on motility, viability, plasma membrane and acrosome integrity.

Keywords: Buck, Catalase, Refrigeration temperature, Semen analysis

Preservation of gametes of goat breed is important in order to sustain the unique qualities of the breed and to prevent the dilution of valuable germplasm by indiscriminate mating. This can be achieved by adopting Artificial Insemination (AI) technique which allows faster transmission of genetic merit through diluted semen. Transport of semen from collection centres to distantly situated farms is necessary to impregnate large number of does with semen of superior bucks. This can be achieved by reducing the metabolic activities of spermatozoa by lowering the temperatures.

Liquid storage of buck semen is an economical and practical alternative to frozen semen as it involves less technical efficiency and financial set up. Buck spermatozoa is sensitive to cold shock. Cooled semen suffers from reduced motility and morphological integrity and declined survival ability in the female reproductive tract leading to decrease in fertility and increased embryonic loss compared to fresh semen. These damages are less pronounced in diluted and chilled semen than in frozen–thawed semen (Aisen *et al.* 2002). Reactive oxygen species (ROS) is the main culprit behind these damages that leads to oxidative stress further resulting in increased rates of lipid peroxidation and consequent loss of sperm motility during long term storage (Aitken 2017). The use of a suitable extender is necessary to achieve successful liquid storage of spermatozoa and to improve sperm quality (Allai *et al.* 2018). Various additives have been added to extenders to maintain motility and fertilizing capacity and to preserve the integrity of the sperm membrane (Ranjan *et al.* 2014, 2015, 2017; Gangwar *et al.* 2014, 2015). Mostly, these protectants possess antioxidant activity and either reduce the process of oxidation (Pietta 2000), or regulate, suppress or prevent the formation of ROS (Maneesh and Jayalekshmi 2006). Several studies have been carried out about the liquid storage of goat semen (Ranjan *et al.* 2009b, Saraswat *et al.* 2012, 2014) with or without additives. The addition of catalase as an antioxidant into semen extenders improved semen quality in ram (Gungor *et al.* 2018), bovine (Anzar *et al.* 2011) and equine (Baumber *et al.* 2002). Unlike other farm species, the effect of ROS on goat sperm in addition to cryoinjuries encounter lethal interactive losses. However, few reports are available on this aspect in goat.

AI is considered to be an effective tool in crossbreeding programs of non-descript indigenous goats with superior germplasm to enhance productivity in the developing countries including India (Abu *et al.* 2008). Freezing and thawing of buck semen causes changes in sperm motility, morphology including damage to mitochondria, plasma membrane and acrosome intactness thereby, reducing the fertilizing ability of spermatozoa. The presence of high content of polyunsaturated fatty acids, plasmalogens and sphingomyelin is responsible for the flexibility and

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functional ability of sperm cells (Sanocka and Kurpisz 2004). Lipid composition of sperm plasma membrane is a major determinant of mobility characteristics, cold sensitivity, overall viability (Hammerstedt *et al.* 1990) and membrane integrity (Tran *et al.* 2017). Insemination with liquid semen has improved the reproductive efficiency as well as economic profit in the goat industry. However, the major drawback of liquid semen is short term storage for 3–7 days (Kadirvel *et al.* 2019). Therefore, the present study has been planned to reinstate the oxidant-antioxidant balance in the system by the addition of catalase in extender for increasing the life of semen in refrigerated conditions. The objective of present work was to examine the effect of catalase supplementation in diluent on seminal attributes during liquid storage of goat semen at refrigeration temperature during different hour intervals.

MATERIALS AND METHODS

All chemicals were obtained from Sigma Chemicals Co. (St. Louis, MO, USA) unless otherwise stated.

Extender preparation: The basic semen extender was composed of 3.604 g tris, 1.902 g citric acid, 1 g fructose, 200 mg streptomycin, 120 mg penicillin and 200 mL triple distilled water. The pH was set at 6.8. For preparing working solution, 20% egg yolk was added freshly to the basic extender and mixed well.

Semen collection and evaluation: Sexually mature bucks, managed under semi-intensive system were used in the study. Semen samples were collected twice a week using artificial vagina after stimulating with an oestrous doe. Immediately after collection, volume, colour, consistency and mass motility of ejaculates were assessed. Ejaculates with mass motility +4 and above were pooled and divided into 5 equal aliquots. Each sperm pool was extended with tris-egg yolk-fructose diluent containing 0 U/mL (control), 200 U/mL, 400 U/mL, 600 U/mL and 800 U/mL catalase concentration. The diluted semen samples were stored in glass tubes at 5°C up to 96 h. Sperm evaluations were performed immediately after dilution (fresh and diluted) and after 24 h, 48 h, 72 h and 96 h of storage at refrigeration temperature. During the study, all the experimental protocols met the Institutional Animal Care and Use Committee regulations.

Motility assessment: Diluted semen (10 µl) was placed on a clean grease free warm slide (37°C) with cover slip and observed under 400× magnification of phase contrast microscope. The progressive motility was calculated by considering the average values of two independent experts.

Sperm vitality assessment: The live and dead spermatozoa were calculated by using Eosin – Nigrosine stain as described by Hancock (1951). The dead sperm cell takes eosin colour, while those which are alive before staining do not take any colour.

Acrosomal integrity assessment: The acrosomal integrity of spermatozoa was assessed by using Giemsa stain (Watson 1975). A thin smear of diluted semen was prepared on a clean grease free slide and air dried. The slide was immersed in Hancock's fixative for 1 h at 38°C. The slides washed in running water and air dried. The slides were immersed in Giemsa stain (6 mL Giemsa (from stock solution), 4 mL Sorrenson buffer and 90 mL triple distilled water) for 2 h at 38°C. After staining, the slides were washed in water and dried. The slides were examined for acrosome morphology under oil immersion objective of the microscope. A total of 100 sperms were counted and the percentage of acrosomal alterations was calculated.

Functional membrane integrity assessment: The membrane integrity of spermatozoa was examined by hypo osmotic swelling test as described by Revell and Mrode (1994) and modified by Ranjan *et al.* (2009a). Diluted semen (10 µl) was added to 1 mL of hypo-osmotic solution (75 mOS) in microtubes and incubated at 38°C for 2 h. Immediately after incubation, semen (10 µl) was placed on a glass slide, covered with coverslip and examined under 400× magnification, using a phase contrast microscope. A total of 100 spermatozoa were counted in at least 5 different fields. Besides total coiling, a strong coiling was the description given when the tail became much coiled.

Experimental design: Catalase (CAT, 25 mg; 11700 U, SRL) was used at 5 concentration, viz. [control (non-supplement), 200 U/mL, 400 U/mL, 600 U/mL and 800 U/mL]. The progressive motility, live cell count, functional plasma membrane integrity and acrosome integrity were evaluated immediately after dilution and at 24 h, 48 h, 72 h and 96 h after liquid storage at refrigeration temperature.

Statistical analysis: The sperm evaluation data were analyzed using two–way ANOVA. Statistical analysis was performed using SPSS Package 16 (IBM® SPSS Statistics Software). Differences were considered significant at a level of $P<0.05$.

RESULTS AND DISCUSSION

There were no significant differences in motility, live cell count and HOS at 24 h of liquid storage at refrigeration temperature. However, the acrosome integrity at 24 h was significantly highest ($P<0.05$) in control group followed by 800 U/mL concentration group (Table 1).

At 48 h, the group having CAT @ 600 U/mL concentration showed highly significant ($P<0.05$) values of motility, live cell count, HOS and acrosome integrity. Similarly, at 72 h and 96 h, the group supplemented with 600 U/mL CAT showed significant ($P<0.05$) values for motility, live cell count, HOS and acrosome integrity (Table 1).

All the experimental groups showed a continuous decrease in motility, live cell count, membrane integrity and acrosome integrity with the progression of time. The highest motility was observed in case of control at 24 h; whereas at 48 h, 72 h and 96 h, the group supplemented with 600 U/mL catalase showed highest motility (Table 1). The live cell count was highest in case of group with 200 U/mL catalase at 24 h; while at 48 h, 72 h, and 96 h the group containing 600 U/mL catalase recorded with highest value for live cell count. The membrane integrity of 200 U/

Table 1. Seminal qualities after supplementation of different concentration of Catalase at different hours of refrigeration (Mean±SE)

Time	Concentration (Catalase U/mL)	Motility %	Live %	HOST %	Acrosome %
0	0	82.85±1.84	66.74±3.77	62.69±3.98	72.58±3.69
24	0	72.14±1.48^{a}	74.45±2.33^{a}	71.64±3.08^{a}	74.99±1.36^{a}
	200	70.00±1.88^{a}	75.11±2.71^{a}	74.96±2.31^{a}	73.07±2.14^{ab}
	400	70.00±2.18^{a}	71.37±1.72^{a}	72.14±1.73^{a}	71.61±1.66^{ab}
	600	70.00±2.43^{a}	71.41±1.83^{a}	72.03±2.12^{a}	72.03±2.26^{ab}
	800	64.28±4.68^{a}	68.74±3.53^{a}	67.07±4.55^{a}	66.73±3.92^{b}
48	0	53.57±1.79^{b}	58.08±2.65^{b}	54.96±1.77^{b}	54.46±1.30^{c}
	200	59.28±2.76^{ab}	64.08±2.87^{ab}	62.07±3.52^{ab}	61.76±2.09^{b}
	400	60.71±3.35^{ab}	65.02±3.22^{ab}	64.12±3.80^{ab}	63.22±2.60^{b}
	600	65.71±2.29^{a}	70.29±2.35^{a}	70.21±3.20^{a}	71.92±2.56^{a}
	800	58.57±1.79^{ab}	65.00±1.32^{ab}	61.10±2.40^{ab}	59.84±1.94^{bc}
72	0	44.28±2.02^{b}	48.64±2.13^{b}	49.20±2.40^{b}	49.37±2.50^{bc}
	200	45.00±1.88^{b}	50.86±2.38^{b}	52.21±2.04^{b}	50.17±1.57^{bc}
	400	42.14±2.64^{b}	48.21±2.21^{b}	48.20±2.76^{b}	44.56±3.00^{b}
	600	53.57±1.42^{a}	57.79±1.72^{a}	60.43±1.75^{a}	58.13±1.79^{a}
	800	47.14±1.01^{b}	51.74±0.90^{b}	53.03±1.10^{b}	52.58±1.20^{ab}
96	0	32.85±1.84^{c}	36.25±2.72^{c}	37.78±2.38^{c}	37.43±1.88^{c}
	200	35.71±2.^{97bc}	41.58±3.07^{bc}	41.27±2.59^{c}	40.47±2.07^{bc}
	400	34.28±1.70^{bc}	39.72±2.21^{bc}	39.49±2.50^{bc}	39.11±1.74^{c}
	600	49.28±2.97^{a}	55.56±3.02^{a}	59.31±2.49^{a}	57.40±1.96^{a}
	800	40.71±1.30^{b}	46.12±1.97^{b}	46.99±1.91^{b}	45.92±1.92^{b}

$^{a-c}$Within a column, value with different superscript differ significantly ($P<0.05$).

mL concentration group was highest at 24 h whereas, 600 U/mL concentration group showed highest membrane integrity at 48 h, 72 h and 96 h. At 24 h, the acrosome integrity was highest for control group while the group supplemented with 600 U/mL catalase showed the highest values for acrosome integrity at 48 h, 72 h and 96 h.

The results showed that supplementation of tris-egg yolk-fructose diluent by 600 U/mL catalase increased semen motility, viability, membrane integrity and acrosome intactness in comparison to control and other concentrations (200 U/mL, 400 U/mL and 800 U/mL) ($P<0.05$) at 48 h, 72 h and 96 h. After 24 h freezing, all groups showed almost similar pattern (non-significant) for motility, live cell count and membrane integrity except acrosome integrity where control group showed significant differences. The seminal parameters declined by the passage of time and remained over 50% after 72 h.

The results revealed that addition of catalase has improved the motility, live cell count, membrane integrity and acrosome integrity of buck semen and thus it protects the structures and functions of spermatozoa efficiently. Thus, it may enhance the quality of semen by preserving efficiently at refrigeration temperature. Many scholars reported that catalase has beneficial effects on preservation of mammalian sperm and improves the functional parameters of spermatozoa (Gungor *et al.* 2018).

It has been reported that the quality of chilled semen decreased with time and remained suitable for use up to 30 h as judged by motility and morphology (Urata *et al.* 2001). The reduction in sperm motility during liquid storage of semen over the period might be attributed to decline in membrane integrity and thereby initiating the acrosome reaction during storage (Kadirvel *et al.* 2019). The plasma and acrosomal membrane integrity, ultrastructure and biochemical components of sperm also get affected during liquid storage because of lower temperature (Gaczarzewicz *et al.* 2015). The gradual reduction in the seminal parameters by the time of storage might be due to the overproduction of ROS from continuously increasing number of dead sperm cells that cannot be checked by catalase as its concentration was fixed. Also, overdosage of catalase causes high fluidity of plasma membrane above desired point, making sperm more prone to oxidative stress induced damage. Falchi *et al.* (2018) reported a significant increase in ROS production from 48 to 96 h in ram semen stored at 4°C. The changes in the morphological and functional parameters of sperm such as motility, viability, plasma membrane and acrosome integrity during liquid storage have been described in goats (Ranjan *et al.* 2009b, Saraswat *et al.* 2012), ram (Falchi *et al.* 2018), boar (Li *et al.* 2018), pig (Kadirvel *et al.* 2019), bovine (Akhter *et al.* 2011), mithun (Perumal *et al.* 2013a, 2013b) and equine (Brogan *et al.* 2015).

Based on the results of the present study, we inferred that the addition of catalase (600 U/mL) significantly reduced the detrimental effects of cooling on motility, viability, membrane integrity and acrosomal integrity of buck sperm after 72 h. The goat keepers will get higher benefits by using semen stored at 5°C and supplemented with CAT @ 600 U/mL Liquid storage of goat semen at 5°C may represents an inexpensive and practical alternative to frozen semen if AI is performed under field conditions because of ease in handling as the use of frozen semen requires expensive and invasive tools and trained personnel. Insemination with liquid semen could improve the reproductive efficiency as well as economic profit in the goat industry.

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