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Effect of catalase in Deccani ram semen preservation at refrigeration temperature

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Abstract

The objective of this experiment was to study the effect of Catalase in Deccani ram semen preservation. The semen parameters like motility, viability, HOST, acrosomal integrity and sperm abnormalities were evaluated at refrigeration temperature (5 °C) after 24, 48, 72 hrs of post collection and dilution. Ten ejaculates per ram were collected by artificial vagina making a total of 60 ejaculates from six Deccani rams and extended with Egg yolk citrate. Catalase (CAT @ 100 IU) added to each ml of extended semen and control group (without any addition of antioxidant). The present experiment results reveal that addition of catalase 100 IU improved sperm cell parameters such as motility, livability, HOST and acrosomal integrity and also decreased sperm abnormalities when compared to the control group during liquid storage of semen up to 72 hrs at refrigerator temperature (5 °C). In the present study, all semen parameters significantly ($P \le 0.05$) differ between test and control groups.

Keywords: Deccani rams, catalase, liquid semen preservation

Introduction

Preservation of ram semen is most important thing in the artificial insemination (Bucak *et al.*, 2008)^[6]. The spermatozoa were protected by the extender in the low temperature preservation process and reduce the cold shock effects. With addition of some components to the extender like antioxidants are showed better results in preservation process. In cold storage the quality of semen is decreased due to production of reactive oxygen species due to oxidative stress. The mammalian spermatozoa are sensitive to ROS because it is made up of poly unsaturated fatty acids (Aitken *et al.*, 2016)^[2]. The semen naturally have some antioxidants but due to dilution rate their concentration may lowered which is not sufficient to protect from reactive oxygen species. Synthetic antioxidants supplementation in extender can reduce the oxidative stress.

Materials and Methods

For this study, the rams are selected from the Livestock Farm Complex (LFC), C. V. Sc, Rajendranagar, PVNRTVU, and Hyderabad during May to October, 2020. Six healthy rams aged between 2-4 years with average body weight around 45 kg were selected and maintained under intensive loose housing system. A total of ten semen ejaculates were collected from each ram by using artificial vagina (AV).

Experimental design

The collected semen was randomly divided into two groups as egg yolk citrate with antioxidants (Catalase@ 100U/ml), egg yolk citrate without antioxidants (control).

Dilution and preservation

Immediately after collection of semen some parameters like volume, colour, consistency and mass motility were recorded semen was diluted with egg yolk citrate contains Part A (2.9 gm of Sodium citrate dihydrate, Streptomycin @1000 μ g/ml and Penicillin @ 1000 IU/ml in 100 ml of distilled water) 80 ml and part B Egg yolk (20%) and preserved at 5 °C.

Evaluation parameters Sperm concentration

Concentration was expressed by using improved Neubauer counting chamber after 1:200 dilution of semen with a dilution fluid (2% eosin y-solution dissolved in 1% (v/v) formalin, Saturated Nacl solution (1% w/v), Distilled water) and expressed in millions/ml.

Individual motility

It is observed under 40X magnification and expressed in terms of percentage of progressively motile (0-100) sperm's.

Live and dead spermatozoa

In this procedure eosin–nigrosin was used for livability and the percentage of live spermatozoa was determined by counting at least 200 spermatozoa.

Abnormality

Abnormality was done with rose Bengal stain (3%) technique as per method Pervage *et al.* $(2009)^{[19]}$.

Acrosomal integrity

The acrosomal damage was studied with Giemsa stained smears.

Hypo osmotic swelling test

Hypo-osmotic solution 150 milliosmol/L (0.735 g sodium citrate trihydrate, 1.351 g fructose in 100 ml of distilled water) was mixed with extended semen (0.1 ml) in a test tube.

Statistical analysis

Result data analyzed by using ANNOVA with the help of statistical software SPSS 16 version according to Snedecor and cohran (1994). The post hoc analysis was performed using Duncan's multiple range tests. The level of significance ($P \le 0.05$) was set at the data are presented in the table as mean \pm SEM.

Results: The average scrotal circumference, ejaculatory volume, colour, consistency, mass activity and concentration were showed in a table.

Table 1: Show the semen characteristics

Semen characteristics	Mean ± S.E (n=60)
Scrotal circumference (cm)	24.3
Semen volume (ml)	0.67±0.02
Semen concentration (millions/ml)	9327.46 ± 378.92
Colour	Creamy white
Consistency	Thick creamy
Mass activity (0-5 score)	3.4±0.06 ^a

Values in parenthesis are the no. of ejaculates collected from the six Deccani rams. After immediate collection of semen parameters studied are individual Motility, livability, abnormalities, acrosomal integrity and HOS-test values are showed in Table No. 2

Table 2:	Semen	parameters
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Semen parameters	Mean ± S.E (n=60)			
Motility (%)	85.63±0.30			
Viability (%)	84.35±0.23			
Sperm abnormalities (%)	1.68±0.11			
HOST (%)	82.35±0.24			
Acrosome integrity (%)	81.48±0.28			

After 24, 48 and 72 hrs of post collection, the evaluated parameters are showed in Table No. 3

Table 3: Incubation period and Individual motility

Incubation period	Individual motility		Livability		Abnormality		Acrosomal integrity		Host	
	Catalase	Control	Catalase	Control	Catalase	Control	Catalase	Control	Catalase	Control
24 hrs	77.40±0.41ª	64.98±0.34 ^b	75.93±0.40 ^a	63.58±0.42 ^b	2.88±0.11ª	3.88 ± 0.15^{b}	73.71±0.42 ^a	60.78±0.44 ^b	74.96±0.41ª	62.58±0.42 ^b
48 hrs	68.50±0.47 ^a	54.71±0.39b	66.76±0.47 ^a	53.03±0.47 ^b	4.91±0.17 ^a	6.95 ± 0.19^{b}	65.05 ± 0.48^{a}	50.05±0.49 ^b	65.76±0.48 ^a	51.70±0.42 ^b
72 hrs	60.05±0.62ª	43.30±0.53b	58.51±0.54ª	41.58±0.48 ^b	6.71±0.19 ^b	10.86±0.29ª	55.91±0.56 ^a	38.81±0.53 ^b	56.58±0.65ª	40.43±0.47b

There is a significant ($P \le 0.05$) difference between control and catalase groups in all seminal parameters

The average ejaculate volume (ml) of semen in Deccani sheep was 0.67 \pm 0.02 ml with a range of 0.51 \pm 0.02 ml to 0.83 \pm 0.05. There was a significant ($P \le 0.05$) difference between the rams. The overall mean of sperm concentration was 9327.46 \pm 378.92 millions/ml with range from 7599.00 \pm 556.48 to 11147.00 \pm 566.51 millions/ml. There was a significant (P \leq 0.05) difference between the individual animals in the concentration of spermatozoa. There was no significant difference between the mass motility and colour of spermatozoa among the animals. The overall mean value of individual motility percentage in neat semen was 85.63 ± 0.30. Analysis of variance revealed that there is a significant $(P \le 0.05)$ variation in individual motility percentage among rams. The overall mean value of live sperm percentage in neat semen was 84.35 \pm 0.239. Analysis of variance ($P \le 0.05$) revealed that there is significant variation in live sperm percentage among rams. The overall mean percentage of total abnormalities in the neat semen of Deccani rams was $1.68 \pm$ 0.11 and there no significant effect on this attribute. The overall mean value of acrosomal integrity percentage in neat semen was 81.48 ± 0.28 . Analysis of variance did not revealed any significant (P≥0.05) variation among individual rams. The overall sperm positive to HOS-test percentage in neat semen was 82.35 ± 0.24 . Ram wise analysis of variance revealed that the individual ram had no significant effect on this attribute.

At 24, 48 and 72 hrs of preservation all parameters showed highest in group I compared to group II except abnormalities whereas abnormalities was highest in control group. There is a significant (P \ge 0.05) difference between group I and group II.

Discussion

The mean scrotal circumference of Deccani rams of present study was in accordance with Horro breed of rams (Galmessa *et al.*, 2000) ^[8], whereas lower scrotal circumference was noticed in West African dwarf rams (Oyeyemi *et al.*, 2009) ^[16]. The difference in the scrotal circumference might be due to factors like body size, plane of nutrition, weather and breed.

The volume in present study is in accordance with Jayaganthan *et al.* (2015) ^[13] in Muzzafarnagari rams. Lower values of ejaculate volumes were reported by Ibrahim *et al.* (1997) ^[11] in local and crossbreed (local X Chios) rams, George *et al.* (2003) ^[9] in Garole rams. Collected semen Colour is in agreement with the reports of Mehta *et al.* (1972) ^[15], Ihukwumere *et al.* (1990) ^[12], Dabas *et al.* (1997) ^[7]. The present study of mass motility is in agreement with the reports of Alcay *et al.* (2014) ^[3] in Kivircik and Awassi rams, Rajashri (2016) ^[20], Anil M (2017) ^[4], Rajashri *et al.* (2016) ^[20] in Deccani ram semen. Higher values were reported by Ahmed E *et al.* (2017) ^[1] in indigenous breed of rams, Sherif

A, et al. (2020)^[21] in Barki breed of rams. Concentration of semen in this study showed similar results to that of Rajashri (2016)^[20] in Deccani rams, Anil M (2017)^[4], Rajashri et al. (2016)^[20] in Deccani rams. The individual motility values are in accordance with the results of Hossain et al. (2016) [10], Pankaj Kumar (2018) [17]. Lower values of mean individual motility percentage were reported by Al-Samarrae (2009)^[18]. About livability, the present findings were in accordance with the results of Bharti et al. (2007) [5] in Chhotanagpuri rams, Toppo (2013) [23], Pankaj Kumar et al. (2018) [17] in Bangladeshi rams. Compared to our study, higher spermatozoa abnormalities were recorded by Anil.M (2017) ^[4]. Acrosomal integrity and HOS-test results were in accordance with Kurmi (2014) [14] where as higher values were reported by Pankaj Kumar et al. (2018)^[17] in bangladesi rams, Rateb et al. (2020)^[21] in Barki rams.

After 24, 48 and 72 hrs of preservation, all parameters recorded higher values in Catalase containing group. Individual motility values were in accordance with Camara *et al.* (2011) Del Prete *et al.* (2019). Whereas Peruma *et al.* (2013) found that inclusion of Catalase into TEYC diluent improved the motility up to 24 hrs. Sperm livability Percentage in this study is in accordance with Del Prete *et al.* (2019) and Hakoueu *et al.* (2019) in Holstein bull. Acrosomal Integrity values after different hours of incubation were in accordance with Peruma *et al.* (2013). In Catalase group, sperm abnormalities are reduced and are in accordance with Hakoueu *et al.* (2019). Hypo-osmotic Swelling (HOST) Test results in this study are in accordance with Hakoueu *et al.* (2019) in Holstein bull.

Conclusion

The result of the present experiment shows that addition of Catalase to Deccani ram semen improved the percentage of sperm motility, sperm viability, plasma membrane integrity, acrosomal integrity when stored at refrigeration temperature (5°C) compared to the control. Thus on the basis of the present results, 100 IU of catalase may be recommended for supplementation in 1 ml of EYC extender diluted semen for liquid ram semen storage at (5°C). Catalase containing extender showed better results.

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