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Effect of Freezing Rate on Quality of Cryopreserved Goat Spermatozoa Using a Programmable Freezer

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Abstract: *A total of 45 ejaculates from nine Beetal bucks collected by artificial vagina, washed and extended in Tris were frozen in 0.5 ml French straws using a programmable freezer to study the effect of three freezing rates on freezing in liquid nitrogen. Fifteen ejaculates were used for each of the three freezing rates. The straws were thawed in a water bath at 40°C for 20 seconds. Sperm motility, live sperm, incidence of intact acrosome and HOST-reacted sperm were studied after collection, equilibration and freezing by conventional methods. The sperm motility, live sperm, incidence of intact acrosome and HOST-reacted sperm in the semen samples used for freezing by different freezing rates did not differ significantly after collection and after equilibration. However after freezing, sperm motility and live sperm were significantly ($P<0.05$) higher for Freezing rate II than for Freezing rates I and III but they did not differ significantly between Freezing rate I and Freezing rate III. After freezing, incidence of intact acrosome and HOST-reacted sperm did not differ significantly between freezing rates.*

Key words: *Beetal goat, Freezing rate, Frozen semen*

I. Introduction

Freezing protocol has been reported to cause comparatively more damage to sperm than other stages (dilution, cooling and equilibration) of cryopreservation. Buck sperms do not seem to be well-adapted in enduring cooling to low temperatures and there is a reduction of their post thaw viability and consequently fertility. Cooling rate of diluted semen from temperature just above 0°C can significantly influence the survival of spermatozoa after freezing and thawing [1]. The hazards of cryopreservation of goat semen could possibly be lowered by optimizing the freezing rates that could improve the viability and fertility of frozen thawed semen. Keeping in view the above facts, the present study was planned to find out the effect of different freezing rates on quality of cryopreserved Beetal goat semen.

II. Materials And Methods

A total of nine Beetal bucks aged one to two years maintained at Goat Research Station, Assam Agricultural University, Burnihat were used in the study. The bucks were thoroughly examined for sexual and general health before selection. Semen was collected from each buck once / twice a week with the help of a standard artificial vagina using a restrained doe as a mount. Two false mounts were allowed before the collection of semen. Immediately after collection, the semen was evaluated for volume, mass activity and initial sperm motility. The ejaculates having volume 0.5 ml or more, mass activity (0 to 4+ scale) 3 + or more and initial sperm motility 70 per cent or more were only used for the study. The collection tubes containing semen were placed in a beaker containing warm water (35°C). The ejaculates found suitable were diluted (1:5) with Tris buffer of P^H 6.8 consisting of 2.422g Tris, 1.36g citric acid, 1.0g fructose and 100ml triple glass distilled water maintained at 35°C and then again placed in a beaker containing water at 35°C. All the constituents of the extender except egg yolk were mixed and kept overnight at 5ºC. On the following morning of semen collection

it was warmed up to room temperature and the egg yolk was added. The diluted semen samples placed in the beaker containing water at 35°C were transferred to a thermo flask and then brought from Goat Research Station, Burnihat to the laboratory in the College of Veterinary Science, Khanapara at a distance of 14 KM by a motor vehicle. On arrival in the laboratory, the seminal plasma was separated by centrifugation (washing) at room temperature for seven minutes at 3000 RPM. The supernatant fluid was discarded and the centrifugate was extended (1: 5) with fraction A of Tris extender. For extension, the initial volume of semen prior to washing was taken into consideration. The room temperature of the laboratory was recorded and the initially extended semen was cooled gradually to 5°C @ 1°C per 3 min in a cold handling cabinet with the help of crushed ice cubes. The fraction B of Tris extender cooled at 5°C was then added (amount equal to fraction A) in two steps at an interval of 15 minutes to the initially extended semen. The extended semen was then maintained at 5°C in the cold handling cabinet for 4 hours of equilibration. French medium straws (0.5 ml) and polyvinyl alcohol powder of different colours were used for filling and sealing the extended semen. Fifteen ejaculates (Two from each of six bucks and one each from the remaining three bucks) were assigned to study the quality of frozen semen under each of three freezing rates *viz.*, Freezing rate I (+4˚C to -5˚C @ 4˚C/min, -5˚C to -110˚C @ 25˚C/min and -110˚C to -140˚C @ 35˚C/min), Freezing rate II (+4˚C to -12˚C @ 4˚C/min, -12˚C to -40˚C @ 40˚C/min and -40˚C to -140˚C @ 50˚C/min) and Freezing rate III (+4˚C to -10˚C @ 5˚C/min, -10˚C to -100˚C @ 40˚C/min and -100˚C to -140˚C @ 20˚C/min). Freezing of semen was carried out in a programmable freezer* adopting one of the three freezing rates in serial succession on consecutive days of freezing. Immediately after freezing, the straws were collected in a goblet containing liquid nitrogen and transferred to liquid nitrogen container for storage. After 16 hours of storage in liquid nitrogen, the frozen semen was thawed in warm water at 40ºC for 20 sec. After equilibration and after freezing each semen sample was evaluated for sperm motility, live sperm and intact acrosome by conventional method, and HOST-reacted sperm [2]. The data generated from this experiment were organized and processed for further analysis. Total repetition was 45 [3 freezing rate × (6 animals × 2 ejaculates + 3 animals × 1 ejaculate)]. Descriptive statistics were performed to calculate the mean, standard error and percentage. A one-way ANOVA test was performed to obtain the difference in sperm motility, live sperm, intact acrosome and HOST-reacted sperm between freezing rates. Statistical significant differences of parameters between freezing rates were compared by Fisher's Least significant difference test. All statistical analysis was performed using SPSS software package (version 19.00; SPSS Inc., Chicago, IL, USA).

III. Results And Discussion

The sperm motility, live sperm, intact acrosome and HOST-reacted sperm in the semen samples used for freezing by different freezing rates did not differ significantly after collection and after equilibration (Table 1). However after freezing, sperm motility and live sperm differed significantly ($p<0.05$) between freezing rates. This is an agreement with the findings of [3] in Korean native bucks who observed significant differences in sperm motility and live sperm between fast and slow freezing methods using Tris yolk glycerol extender. [4] found significant differences in viability of ram spermatozoa between two freezing curves. On the contrary, [5] observed no significant difference in viability of frozen thawed ram sperm between slow and fast freezing. In the present study, the post thaw sperm motility (69.73 ± 1.19%) and live sperm (75.37 ± 1.36%) were the highest in Freezing rate II. The present value of sperm motility and live sperm was close to that reported by [6] in Beetal, but was higher than that recorded by [7] in Beetal. This might be due to differences in freezing rate, freezing method, quality of semen used, season of experiment, osmolarity, composition of freezing media and method of estimation. Critical difference test revealed that the sperm motility and live sperm after freezing was significantly ($p<0.05$) higher for Freezing rate II than for Freezing rates I and III. Present finding was similar to the observation of [3] who reported that the percentage of sperm motility and live sperm in rapid freezing method was significantly higher than for two different slow freezing methods. The sperm motility and live sperm after freezing did not differ significantly between Freezing rates I and III. The higher percentage of live sperm observed after freezing with Freezing rate II affirmed the observations on sperm motility in the present study.

The highest incidence of intact acrosome after freezing occurred in freezing rate III (82.33 ± 1.48%). The values recorded in the study was higher than that recorded by [7] in Beetal. On the other hand, the incidence of intact acrosome in this study was lower than that reported by [6] in Beetal. This might be due to difference in breed, freezing rate and freezing method. The incidence of intact acrosome after freezing did not differ significantly between freezing rates. This is in agreement with the findings of [8] in Majorera goat who found no significant difference in respect of intact acrosome between two freezing protocols. On the contrary, [9] found significantly higher frozen-thawed ram spermatozoa with intact acrosome in samples processed by controlled slower rate of cooling as compared to uncontrolled cooling prior to programmable freezing of semen in straws and opined that this might be attributed to lesser structural damage due to slower rate of cooling.

The post thaw HOST-reacted sperm obtained was higher than that reported in goat by other workers [10]. The sperm motility, live sperm and intact acrosome recorded in the present study was much higher than

that observed by the other workers who also estimated HOST-reacted sperm and this might account for the higher value of HOST-reacted sperm in the present study. The highest HOST-reacted sperm after freezing (66.73 ± 1.41%) was recorded in Freezing rate I. The mean HOST-reacted sperm after freezing did not differ significantly between freezing rates.

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IV. Conclusion

The results obtained in the present study led to the conclusion that out of the three freezing rates, Freezing rate II (4°C per minute from +4 to -12°C, 40°C per minute from -12 to -40°C and 50°C per minute from -40 to -140°C) was superior as significantly higher sperm motility and live sperm were obtained with it.

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Table 1. Sperm Motility, Live Sperm, Intact Acrosome and Host-Reacted Sperm of Beetal Buck Semen in Tris Extender Using Three Freezing Rates

	Freezing rate I			Freezing rate II			Freezing rate III		
	After collection	After equilibration	After freezing	After collection	After equilibration	After freezing	After collection	After equilibration	After freezing
Sperm motility (%)	86.07±1.37	79.67±1.28	66.07b±0.69	85.67±1.36	80.60±1.53	69.73a±1.19	85.67±1.39	79.20±1.40	66.47b±1.04
Live sperm (%)	91.67±1.21	85.37±1.25	70.67b±0.75	92.43±1.14	86.37±1.40	75.37±1.36	90.93 ±1.18	84.87± 1.23	71.83b±1.24
Intact acrosome (%)	97.60 ±0.46	90.13±0.85	82.23±1.23	98.10±0.32	89.67±1.13	81.33 ±1.11	96.20 ±0.82	88.33±1.29	82.33±1.48
Host-reacted sperm (%)	83.13±1.50	75.07±1.33	66.73±1.41	84.63±1.75	74.97±1.36	66.50 ±1.91	84.10±1.28	74.87±1.24	66.57±1.79

Means bearing different superscript in a row differ significantly ($p<0.05$)