



Effect of extenders and sperm concentration on migration efficiency of Malabari buck spermatozoa in bovine cervical mucus[#]

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Abstract

The study evaluated the effect of extenders and sperm concentration on the migration efficiency of Malabari buck spermatozoa in bovine cervical mucus. Fresh semen ejaculates collected on six different days from three adult healthy Malabari bucks were pooled and divided into four groups based on the extender used (tris-egg yolk-based or liposome-based extender) and rate of extension (200 or 400 million motile spermatozoa per 0.5 mL) (n=6 per group) and subjected to cryopreservation. After freezing and thawing, Malabari buck spermatozoa cryopreserved in liposome-based extender travelled significantly ($p<0.05$) greater distance in bovine cervical mucus compared to those preserved in tris-egg yolk-based extender when packed in French medium straws with 200 million progressively motile spermatozoa. The corresponding values with 400 million progressively motile spermatozoa also showed the same trend. It was also observed that Malabari buck semen extended with 200 million progressively motile spermatozoa was significantly ($p<0.05$) better in terms of migration efficiency in bovine cervical mucus compared to that with 400 million progressively motile spermatozoa in both the extenders.

Keywords: Malabari buck spermatozoa, bovine cervical mucus, liposome-based extender, migration efficiency

Artificial insemination using cryopreserved semen is widely practised in Malabari goats. Even though the longevity and transportability of the cryopreserved semen are better, cryopreservation-induced biochemical, ultra-structural and functional damages to the spermatozoa

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could affect fertility. The composition of the extender is an important factor in deciding the extent of cryodamage to the spermatozoa. Egg yolk-based extenders are presently used for the cryopreservation of buck semen. A liposome-based extender is an alternative to this. Liposome is a nanovesicle with a single lipid bilayer which is chemically defined, standardized, and had low sanitary risks (Pillet *et al.*, 2012). Liposomes, with their contents of phospholipids and saturated and unsaturated fatty acids, are reported to abate the damage to spermatozoa caused by the freeze-thaw process (Purdy and Graham, 2015). It is also reported that the freezability and fertility of buck semen were influenced by the concentration of spermatozoa per dose (Nuti, 2007).

The migration of spermatozoa in oestrus cervical mucus could be used as a measure the ability of spermatozoa to migrate through the female genital tract in order to fertilize the oocytes. The distance travelled by vanguard spermatozoa of Malabari buck semen in bovine cervical mucus was also reported to be positively correlated with progressive motility, viability, and functional membrane integrity of spermatozoa (Bhai, 2021). The study was carried out to evaluate the post-thaw quality of Malabari buck semen extended to 200 and 400 million progressively motile spermatozoa per 0.5 mL in liposome-based and tris-egg yolk-based extenders.

Materials and methods

The study was conducted during the period of July 2021 to September 2022 using semen ejaculates collected from three adults, healthy Malabari bucks aged two to three years and with a body weight of 42 to 46 kg, maintained with uniform feeding, housing and managerial conditions in the Artificial Insemination Centre, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy.

The fresh semen ejaculates were pooled and divided into four groups as follows.

Group I (n=6): Semen was extended with a tris-egg yolk-based extender at the rate

of 200 million progressively motile spermatozoa per dose (French medium straw).

Group II (n=6): Semen was extended with a liposome-based extender at the rate of 200 million progressively motile spermatozoa per dose (French medium straw).

Group III (n=6): Semen was extended with tris-egg yolk-based extender at the rate of 400 million progressively motile spermatozoa per dose (French medium straw).

Group IV (n=6): Semen was extended with a liposome-based extender at the rate of 400 million progressively motile spermatozoa per dose (French medium straw).

Extension of semen samples from Group I and Group III included stepwise glycerolization in three steps at 10 min intervals at 5°C such that fully extended semen samples contained seven per cent of glycerol. After four hours of equilibration, the straws of the four groups were subjected to vapour freezing in a styrofoam box for 10 min and stored in liquid nitrogen (Behera, 2015; Bhai, 2015). After 24h of storage, the straws from all four groups were thawed in a water bath at 37°C for 60 sec.

A cervical mucus penetration test was carried out using the protocol proposed by Kumar *et al.* (2015) with modifications. Bovine cervical mucus was aseptically aspirated from the external cervical os of the oestrus cows (after 10h of the beginning of estrus) using a sterile plastic AI sheath fitted to a 10 mL syringe. The cervical mucus was filled into microcapillary tubes of 75 mm in length. The semen ejaculate of 250 µL was taken in a microcentrifuge tube, sealed with parafilm and a hole was made in the parafilm to hold the capillary tube. Sperm mucus interaction was allowed by introducing mucus filled capillary tube into the tube carrying semen and holding them in a vertical position in a water bath maintained at 37°C for 30 min. Thereafter, sperm migration was assessed at 200x magnification under a phase contrast microscope using a graduated slide. The migration efficiency was measured as the distance in millimeters travelled by vanguard spermatozoa. Treatment means were compared with one way ANOVA using SPSS (Statistical

package for social studies) software version 24.0.

Results and discussion

After freezing and thawing, Malabari buck spermatozoa cryopreserved in liposome-based extender travelled significantly ($p < 0.05$) greater distance (21.50 ± 0.04 mm) in bovine cervical mucus compared to that preserved in tris egg yolk-based extender (19.50 ± 0.04 mm) when packed in French medium straws with 200 million progressively motile spermatozoa. When the spermatozoan concentration was changed to 400 million progressively motile spermatozoa also, Malabari buck spermatozoa cryopreserved in liposome-based extender travelled significantly ($p < 0.05$) greater distance (16.00 ± 0.07 mm) compared to that preserved in tris-egg yolk-based extender (13.16 ± 0.04 mm). This is in agreement with the results of Kumar *et al.* (2015), where the average distance travelled by vanguard buffalo spermatozoa cryopreserved in a liposome-based extender was more compared to that in tris-egg yolk-based extender when incubated in bovine cervical mucus at 38°C for 60 min. But Martinez *et al.* (1997) observed that the distance travelled by vanguard spermatozoa of cryopreserved goat semen in tris-egg yolk-based extender was 14.3 mm when incubated at 39°C for 7 min.

Variations in the migration efficacy of spermatozoa may be due to differences in the age, species, breed, nutrition, management of the males, season and the differences in the quality of cervical mucus collected from estrus cows. Galli *et al.* (1991) carried out a bovine cervical mucus penetration test as a means for evaluating frozen-thawed bovine semen. Vanguard spermatozoa travelled an average distance of 28.5 ± 15.1 mm and 18.1 ± 12.6 mm when incubated at 37°C and 21°C for 90 min, respectively. The distance travelled by vanguard spermatozoa of bull and buffalo in bovine cervical mucus was 34 mm and 31 mm, respectively (Kumaresan *et al.*, 2000).

Being nanoparticles, liposomes might have efficient delivery of phospholipids and cholesterol to the buck spermatozoa. Thus, they might have prevented damage of the axoneme and mitochondrial apparatus of spermatozoa

more effectively and maintained comparatively better migration efficiency in bovine cervical mucus. Significantly higher ($p < 0.05$) migration efficiency of spermatozoa in bovine cervical mucus was observed at the sperm concentration of 200 million progressively motile spermatozoa per 0.5 mL than that of 400 million in both the extenders.

L-aromatic amino oxidase is released from the dead and damaged spermatozoa during the freeze-thaw process. This enzyme mainly targets L-phenyl alanine which is abundantly present in egg yolk and leads to the increased production of reactive oxygen species resulting in the destabilization of the membrane and internal structure of live spermatozoa (Chatterjee and Gagnon, 2001). The liposome-based extender can compensate for this demerit of egg yolk-based extender. Similarly, at low sperm concentrations, such toxic reactive oxygen species might have been more diluted compared to high sperm concentrations and resulted in the maintenance of better post-thaw quality. The effect of extenders and concentration of spermatozoa on Bovine cervical mucus penetration distance by spermatozoa is presented in Table 1

Table 1. Effect of extender and sperm concentration per dose on cervical mucus penetration distance by Malabari buck spermatozoa after thawing, mm (n=6)

Groups	Cervical mucus penetration distance (mm) (Mean $\pm$ SE)	F value
Group I	19.50 ± 0.04^b	48.498
Group II	20.50 ± 0.04^a	
Group III	13.1 ± 0.04^d	
Group IV	16.00 ± 0.07^c	

Group I: Tris-egg yolk based extender at 200 million sperms

Group II: Liposome based extender at 200 million sperms

Group III: Tris-egg yolk based extender at 400 million sperms

Group IV: Liposome based extender at 400 million sperms

Values with different superscripts within column differ significantly ($p < 0.05$)

Conclusion

Migration efficiency of Malabari buck spermatozoa in bovine cervical mucus was significantly better when cryopreserved in a liposome-based extender than in tris-egg yolk-based extender. Significantly better results were also obtained with 200 million progressively motile spermatozoa per 0.5 mL compared to 400 million progressively motile spermatozoa per 0.5 mL.

Conflict of interest

The authors declare that they have no conflict of interest.

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