

## **FREEZING OF BUCK SEMEN WITH DIFFERENT GLYCEROL CONCENTRATIONS\***

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Application of frozen semen technology in breeding of does is a great promise for faster propagation of superior germplasm. But lack of effective freezing technology for buck semen is one of the major bottlenecks in implementing large scale Artificial Insemination in goats. Realising the importance of goats in our country the present investigation was undertaken with the objective of studying the effect of different concentrations of glycerol on post thaw motility of frozen buck semen.

### **Materials and Methods**

Semen ejaculates from five Malabari crossbred bucks aged one to three years maintained at Artificial Insemination Centre, College of Veterinary and Animal Sciences, Mannuthy, were utilized for this study. Two ejaculates each were collected twice weekly from the bucks during the period from January 1994 to April 1995. Based on the

preliminary evaluation, samples with more than 70 per cent initial motility were used for freezing trials. Tris extenders were prepared as shown in Table 1. Semen samples, before dilution were kept in a water bath maintained at 37°C.

Table 1 Composition of semen extender

Ingredients	Non-glycerolated Tris Extender II
Tris (hydroxymethyl) amino methene (g)	1.21
Citric acid (g)	0.67
Fructose (g)	0.50
Egg yolk (ml)	5
Benzyl pencillin (iu)	25000
Streptomycin (mg)	25000
Agha dist ad (ml)	50

Glycerol was added at 4,5,6 and 7% to prepare glycerolated extenders.

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The samples were extended ten times with Tris buffer, centrifuged at 3000 rpm for five minutes and the supernatant fluid was pipetted out. Made up the extended volume by adding Tris buffer, repeated the centrifugation for five more minutes and the clear supernatant fluid was removed. The sediment, after double washing, was extended five times the original volume (1:5) with non-glycerolated Tris extender. Semen with non-glycerolated Tris extender was divided into four equal parts in four sterilized test tubes.

Tris extenders containing 4,5,6 and 7% glycerol were taken in separate test tubes, in volume equal to non-glycerolated fraction. Diluted semen and extenders were kept in the cold handling chamber for two hours. Then, the glycerolated fraction of the extender was mixed and added to the semen with non-glycerolated extender in three equal parts at 15 minutes intervals. Motility of semen from all test tubes were assessed. Semen with each combination of glycerol was filled in different coloured straws (0.5 ml) equilibrated for 4 hrs, frozen in liquid nitrogen vapour for eight minutes, placed in the precooled goblets and plunged into liquid nitrogen. The goblets along with straws were transferred into the storage container. After 12 hours the post-thaw motility was studied for each group of straws, after thawing them in water at 30°C for 30 seconds.

Insemination using semen frozen with six per cent glycerol (minimum of 35 per cent post-thaw motility) was done in a limited number of does.

## Results and Discussion

Average percentage of sperm motility of buck semen after washing in Tris buffer was  $76.71 \pm 0.79$  (Table 2). Ritar and Salamon (1982), Perez (1985) and Memon *et al.* (1985) reported that percentage sperm motility was higher in semen sample without seminal plasma than in sample with seminal plasma. Deka and Rao (1987) and Haunhorst (1990) opined that there was no significant reduction in sperm motility by centrifugation.

Maximum percentage motility after glycerolisation was observed in six per cent glycerolated semen, in accordance with the findings of Deka and Rao (1986) Sinha *et al.* (1992) and Sinha *et al.* (1993). There was highly significant ( $P < 0.01$ ) reduction in percentage motility after centrifugation to percentage motility after glycerolisation, probably due to the interaction between spermatozoa and glycerol.

There was highly significant reduction in percentage motility after freezing ( $P < 0.01$ ). This may be due to the death of weak spermatozoa from thermal shock and internal ice formation. During the physical change in the media, the sperms are subjected to high stress and ice formation leading to mechanical injury in certain cases (Mathew, 1984).

In this study it was seen that post-thaw motility was better with six per cent, glycerolated extender. This is in accordance with the findings of Deka and Rao, (1986), Sinha *et al.* (1992) and Sinha *et al.* (1993).

Table 2 Average spermatozoa motility percentage before and after freezing in extender containing different levels of glycerol (average of seven)

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation			Motility percentage after freezing with different level of glycerol				
				4%	5%	6%	7%	4%	5%	6%	7%
1	0.90	++++	78.57	68.57	68.57	71.42	64.28	30.71	36.42	46.42	41.42
2	0.71	++++	80.00	68.57	70.00	72.14	68.57	27.85	39.28	48.57	36.42
3	0.67	++++	74.28	64.28	64.28	68.57	65.71	27.85	29.28	34.28	24.28
4	0.98	+++(+)	75.00	64.28	64.28	61.42	62.85	31.42	37.14	40.00	31.42
5	0.80	++++	75.71	66.42	65.71	65.71	62.85	32.85	30.00	40.71	34.28
Average	0.81	++++	76.71 ± 0.79*	66.42 ± 1.15*	66.57 ± 1.15*	67.85 ± 1.39*	64.85 ± 1.38*	30.14 ± 1.78*	34.42 ± 1.87*	42.00 ± 1.84*	34.71 ± 1.72*

\* P < 0.01

Chauhan and Anand (1990) reported maximum post-thaw motility in seven per cent glycerolated semen, where as Purohit *et al.* (1992) observed it in five per cent and Deshpande and Mehta (1991) in four per cent glycerolated semen.

Fifty seven does were inseminated with frozen semen out of which twenty seven conceived (47.36%). Out of 27 does conceived, 25 does kidded (43.85%) and two does aborted. Kidding percentage reported earlier by other workers were 42.5% (Carteel *et al.*, 1974), 60.8% (Lawrenz, 1986) and 25% (Park *et al.*, 1989). Presumably, differences in managemental practices, methods of processing and freezing, fertility and skill of inseminators may account for variation in fertility rates in different investigations.

### Summary

Effect of different concentrations of glycerol on post-thaw motility and fertility of frozen buck semen was studied using semen from five Malabari crossbred bucks. Average percentage sperm motility after washing in Tris buffer was  $76.71 \pm 0.79$ . Maximum percentage motility after glycerolisation ( $67.85 \pm 1.39$ ) and freezing ( $42.0 \pm 1.84$ ) was obtained in six per cent glycerolated semen. There was highly significant reduction in motility percentage after glycerolisation and freezing. Conception percentage and kidding percentage were 47.36 and 43.85 respectively with 6% glycerolated samples.

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