

Effect of Different Glycerol Concentration on Sperm Morphology of Cryopreserved Surti Buck Semen

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Abstract

The study was carried out on Surti bucks maintained at Livestock Farm Complex (LFC), College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari. The objective of the study was to assess the effect of different glycerol concentration on sperm morphology of frozen thawed Surti buck semen. Total 32 ejaculates from two adult Surti bucks (16 ejaculates/buck) were collected and the semen collected from both the bucks were pooled in each replicate. Immediately after neat semen evaluation, the semen was divided into five equal aliquots and extended with TEYCF diluter, having 5 % (T1), 5.5 % (T2), 6 % (T3), 6.5 % (T4) and 7 % (T5) of glycerol concentration. Subsequently, according to various groups the extended semen was loaded in 0.5 ml French medium straws and cryopreserved. The post thaw abnormal sperm count (%) found was lowest in T4 (13.81±0.26) followed by T3 (14.75±0.45), T5 (15.25±0.58), T2 (16.63±0.36) and T1 (18.06±0.51) groups. In conclusion, 6.5 % glycerol is an optimum for cryopreservation of Surti buck semen to maintain the sperm morphology.

Keywords: Abnormal Sperm Count, Cryopreservation, Glycerol, Morphology and Surti Buck.

Introduction

Goat farming acts as an important tool in social development for rural small farmers, because of easy adaptability of this animal species in adverse climatic and nutritional conditions. In changing livestock scenario goat farming has a promising future. Goat husbandry plays a significant role in the economy and nutritional security of landless, small and marginal farmers. Notwithstanding the fact that the goat population has shown steady growth over the years, very little structured effort has been taken for genetic improvement at the farm level (Sundararaman and Edwin, 2008). Considering the poor production potential of goats, the need for genetic improvement via implementation of Artificial Insemination (AI) from superior sires through semen preservation is crucially required. It is possible with semen preservation, an important tool to conserve the valuable germplasm to improve the genetic quality of farm animals. Goat semen can be preserved either at room temperature temporarily, at refrigerated temperature for 24-48 hours (Ferdinand *et al.*, 2012) or cryopreserved (Beltran *et al.*, 2013) for long term storage. But, the major problem with this technique is the reduction in the quality of semen during freezing and thawing.

A cryoprotectant is included in a cryopreservation medium to minimize the physical and chemical stresses resulting from the cooling, freezing and thawing of sperm cells. Cryoprotectants are classified as either penetrating (glycerol or dimethyl sulfoxide) or non-penetrating (milk or egg yolk). A penetrating cryoprotectant is membrane permeable and acts both intra as well as extracellularly. After a short period of time, the cryoprotectant and water equilibrate and result in similar intracellular and extracellular concentrations (Amann, 1999; Sathe and Shipley, 2015). The penetrating cryoprotectants also cause membrane lipid and protein rearrangement, which results in increased membrane fluidity, greater dehydration at lower temperatures and therefore, an increased ability to survive cryopreservation (Holt, 2000). Many researchers suggested the different levels of glycerol concentrations for cryopreservation of goat semen at optimum level in various breeds (Anakkul *et al.*, 2013; Kulaksiz *et al.*, 2013; Sikarwar *et al.*, 2015; Rasad *et al.*, 2017; Sharma *et al.*, 2020; Akhondzadeh *et al.*, 2023).

Surti goat especially reared for the milk as well as meat purpose by the South Gujarat's people as a source of income for their livelihood. Surti goat is a medium sized dual-purpose breed and mostly confined to small towns and cities situated on the western coastal belt of the South Gujarat. The constant decline in Surti males of superior genetic merit due to extensive culling and unintended breeding (inbreeding) emphasizes the significance of superior male conservation and their use for AI. The available literature is unambiguous about the importance of glycerol during sperm cryopreservation but, the optimal level seems to vary between species and breeds. Hence, the present study was conducted to assess the effect of different glycerol concentration on sperm morphology of cryopreserved Surti buck semen

Materials and Methods

Preparation of Clarified Tris Egg Yolk Citrate Fructose (TEYCF) Diluter

The TEYCF diluter was prepared on the day of experiment by adding 10% egg yolk in previously prepared Tris-citric acid-fructose buffer in sterile flask. The mixture was thoroughly mixed with magnetic stirrer for five minutes followed by centrifugation for 4 minutes at 3000 rpm in centrifuge machine and the supernatant from each tube was obtained carefully in a sterile glass bottle to use as a clarified TEYCF diluter.

Collection of Semen

A total two apparently healthy Surti bucks above 1 year of age maintained under healthy and hygienic conditions and well-fed at LFC, College of Veterinary Science and Animal Husbandry, Navsari were selected. The bucks were trained to donate the semen in artificial vagina using female as a dummy. After completion of the training period, semen was collected regularly by artificial vagina twice a week from each buck for up to 8 weeks. Total 32 semen ejaculates (16 from each buck) were collected. To eliminate individual buck variability and increase semen quantity, the ejaculates from both bucks were pooled during each semen collection.

Experimental Groups

The TEYCF diluter so prepared was divided into five equal aliquots and supplemented with 5%, 5.5%, 6%, 6.5% and 7% of glycerol followed by thorough mixing with magnetic stirrer and stored at 37°C for further use as semen

diluter. The neat semen was divided into five equal aliquots and extended with TEYCF dilutor having concentrations of glycerol as 5% (T1), 5.5% (T2), 6% (T3), 6.5% (T4) and 7% (T5). After grouping, the semen samples were examined for initial evaluation of abnormal sperm count (%) for all the groups. Extended semen was filled in previously marked 0.5 ml French medium straw (IMV Technologies, France) using micropipette having final concentration of 50×10^6 sperm/straw.

Freezing of Semen

All the loaded straws were laid on a floating rack (Minitube, Germany) and placed in a refrigerator at 4°C for equilibration about 4 hrs. After equilibration, the floating rack holding the straws were placed in a manual vapour freezing unit (Minitube, Germany) for 7 minutes in a way that straws remain 4 cm above the liquid nitrogen in vaporous phase. After completion of freezing the straws were directly and quickly plunged into liquid nitrogen container. The pre-freezing and post thaw abnormal sperm count (%) was evaluated for all the groups.

Sperm Morphology

Sperm morphology was determined by the evaluating the abnormal sperm count. To determine the percentage of abnormal spermatozoa, smear was prepared on pre-warmed clean glass slide by gentle mixing of a drop of diluted sample with a drop of eosin-nigrosin stain. After drying the smear, it was examined under oil immersion. A minimum of 200 sperms were counted from different fields and the normal and abnormal sperms were counted and recorded separately. The total sperm abnormalities in percentage were calculated by following formula-

$$\text{Percentage of abnormal sperm (\%)} = \frac{\text{No. of abnormal sperm counted}}{\text{Total No. of abnormal sperm counted}} \times 100$$

Statistical Analysis

The data pertaining to various aspects was suitably tabulated and analyzed using R-3.3.2 software. The differences among the parameter means were performed using appropriate statistical methods viz., ANOVA and DNMRT (Duncan's New Multiple Range Test). The mean differences were considered significant at $p < 0.01$ and $p < 0.05$.

Results and Discussion

The initial, pre-freezing and post thaw abnormal sperm count in all the groups are presented in Table 1. The initial abnormal sperm count (%) found just after dilution differed non-significantly between various groups. However, abnormal sperm count (%) found at pre-freezing stage was lowest in T4 (11.13 ± 0.39) followed by T3 (12.06 ± 0.4), T5 (12.31 ± 0.28), T2 (12.88 ± 0.45) and T1 (13.88 ± 0.56) groups. Similarly, the post thaw abnormal sperm count (%) recorded at 24 hrs. after cryopreservation was lowest in T4 (13.81 ± 0.26) followed by T3 (14.75 ± 0.45), T5 (15.25 ± 0.58), T2 (16.63 ± 0.36) and T1 (18.06 ± 0.51) groups.

Table 1: Effect of different glycerol concentrations on abnormal sperm count (%) in Surti buck semen (Mean $\pm$ SE)

Groups N=16/group	Abnormal sperm count (%)			Overall (n=48)	F value	P value
	Initial	Pre-freezing	Post thaw			
T1	9.31 ± 0.36^a	$13.88 \pm 0.56_A^b$	$18.06 \pm 0.51_A^c$	$13.75 \pm 0.59_A$	81.056**	0
T2	9.06 ± 0.36^a	$12.88 \pm 0.45_{AB}^b$	$16.63 \pm 0.36_B^c$	$12.85 \pm 0.5_{AB}$	93.217**	0
T3	8.81 ± 0.19^a	$12.06 \pm 0.4_{BC}^b$	$14.75 \pm 0.45_{CD}^c$	$11.88 \pm 0.41_{BC}$	66.041**	0
T4	8.88 ± 0.26^a	$11.13 \pm 0.39_C^b$	$13.81 \pm 0.26_D^c$	$11.27 \pm 0.34_C$	64.761**	0
T5	8.94 ± 0.28^a	$12.31 \pm 0.28_{BC}^b$	$15.25 \pm 0.58_C^c$	$12.17 \pm 0.44_{BC}$	60.181**	0
Overall (n=80)	9.00 ± 0.13^a	12.45 ± 0.21^b	15.70 ± 0.26^c	--	262.573**	0
F value	0.444	5.701**	13.816**	4.211**	--	--
P value	0.776	0	0	0.003	--	--

^{a-c} Means with different superscript between column (between various stages of cryopreservation) differ significant at $p < 0.01$;

^{A-B} Means with different subscript within columns (between groups) differ significant at $p < 0.01$; ** $p < 0.01$; T1 - 5% glycerol; T2 - 5.5% glycerol; T3 - 6% glycerol; T4 - 6.5% glycerol; T5 - 7% glycerol

Conclusion

The findings of present study is in close agreement with the findings of Sharma *et al.* (2020), who reported significantly ($p<0.05$) lower sperm morphological abnormalities in semen frozen with 6% ($7.93\pm0.28\%$) as compared to 7% ($9.18\pm0.69\%$) glycerol supplemented group in Gaddi bucks. However, Kulaksiz *et al.* (2013) reported significantly ($p<0.001$) lower post thaw abnormal spermatozoa percentages in G5 (5% glycerol) group for Angora (34.3 ± 1.43 vs. $37.0\pm1.61\%$), Kilis (30.8 ± 1.40 vs. $38.8\pm1.14\%$), and Saanen (40.8 ± 1.08 vs. $45.3\pm0.84\%$) bucks as compared to G7 (7% glycerol) group. The findings were in close contrast with the present findings.

Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

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