The Indian Journal of Animal Reproduction; 29(1): 14-18; June 2008

Research Article

Effect of Glycerol Concentration on Freezability of Buck Semen

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ABSTRACT

Thirty six ejaculates from 6 Black Bengal bucks were diluted at the rate of 1:10 in TEYCAFG (Tris Egg Yolk-citric acid Fructose Glycerol) extender having 3 different concentrations of glycerol TC (Total glycerol) 4.0, 6.4 & 9.0 percent. The semen was frozen in straws and stored for three months.The seminal characteristics were assessed at monthly intervals. The present investigation revealed that the motility, live sperm count and sperm abnormalities for 4, 6.4 and 9 per cent glycerol extenders at zero hour freezing were 37.53 ± 0.28, 42.12 ± 0.31, 36.63 ± 0.35, 66.32 ± 1.15, 64.61 ± 0.70, 61.63 ± 0.68 and 8.21 ± 0.26, 9.13 ± 0.34 and 10.27 ± 0.27, respectively. The motile sperm per cent was found to be the highest (39.14 ± 0.29, 38.82 ± 0.33 and 38.42 ± 0.39) for TC 6.4 as compared to TC 4.0 and TC 9.0 for the 1st, 2nd and 3rd months of storage. Further, it was observed that the live sperm percentage was highest in TC 4.0 (61.91 ± 0.67, 60.25 ± 0.51 and 59.50 ± 0.64) for the first three months of storage, respectively as compared to TC 6.4 and TC 9.0. Similarly, highest percentage of sperm abnormalities was encountered in TC 9.0 (12.30 ± 0.31, 13.33 ± 0.32 and 14.15 ± 0.28) against the corresponding values for TC 6.4 and TC 4.0 for the three months of observation.

Key Words: Glycerol, TRIS, Freezing, buck

INTRODUCTION

In a country like India, Artificial Insemination has been widely adopted in cattle with much success, where as the use of same practice in the field of goat production is very low. Limited research work on goat semen preservation has been conducted (Deka and Rao, 1987; Purohit *et. al.* 1992 and Singh *et. al.* 1992). The present investigation as a part of successful AI programme in goat breeding.was planned to evaluate the freezability of semen of Black Bengal bucks in Tris dilutor with different levels of glycerol and its consequent evaluation following storage for three months.

MATERIALS AND METHODS

A total of 36 ejaculates (twice in a week) were collected from six healthy Black Bengal bucks maintained at goat breeding farm of Orissa University of Agriculture and Technology by modified artificial vagina for goat (Rath *et. al.* 1971). Freshly collected semen was evaluated for mass activity, individual motility, sperm concentration (Heamocytometric method), live sperm (Eosin-Nigrosin stain) and abnormal sperm percentage (Rose-Bengal stain). Semen thus collected was divided in three parts and diluted at the rate of 1: 10 with Tris Egg Yolk-citric acid Fructose Glycerol (TEYCAFG) extender having three different levels of glycerol (4.0, 6.4 & 9.0 percent) at 37°C. Each part was filled into (0.25 ml) straws and after being extended at 37°C in water bath slowly cooled down to 20°C by adding ice water in about 45 minutes. All the instruments, straws, balls and towels were precooled and the straws were filled and sealed mechanically in a

cold handling box maintained at 20°C. The straws were equilibrated at 5°C for 5 hours after which these were freezed to – 196°C step wise. Pre-freezing estimation of motility, livability and abnormality of spermatozoa was done from the straws of each treatment group. At zero hour of freezing, straws from each group were examined for the above parameters after being thawed in warm water at 37°C for 15 seconds. Finally, the stored semen straws in liquid nitrogen were evaluated for motility, livability and abnormality as per the routine methods at the end of 1st, 2nd and 3rd months of storage. Data obtained were statistically analysed as per methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSIONS

In the present findings the average volume, mass motility, initial motility, sperm concentration and percentage of abnormality were found to be 0.73 ± 0.03 ml, 3.88 ± 0.05, 77.40 ± 0.38, 2640.00 ±98.60 x 10^6, 84.61 ± 1.50 and 3.63 ± 0.29 per cent, respectively. The percentage of sperm motility, livability and abnormality at pre-freezing and zero hour freezing with different percentages of glycerol in Tris egg Yolk Citric acid fructose glycerol extender are presented in (table 1) and their correlations in (table 2). It was observed that in pre-freezing stage *i.e.* at 5 °C after 5 hours of equilibration time, the spermatozoan motility was the highest (75.32 ± 0.25 %) when glycerol level in the diluent was 4 % and lowest (72.83 ± 0.33 %) at 9 % glycerol level. The present finding is in close agreement with Despande and Mehta (1991) who found highest pre-freezing motility (77.61 ± 0.92 %) with 4 – 5 % glycerol in TEYCAFG extender where as Deka and Rao (1987) observed highest value with 6.4 % glycerol in the same diluent at pre freezing stage. Similarly, Fiser and Fairfull (1986) and Deka and Rao (1987) found better sperm post thaw motility with 5 – 7.5 % glycerol in TEYCAFG extender. However, there exist highly significant differences between motility in pre freezing and zero hour freezing of the diluents irrespective of different percentages of glycerol in dilutor which indicates that the process of freezing reduces the metabolic rate in post freeze state suggesting irreversible changes due to cold shock. Comparison between TC-4 and TC-6.4 showed no significant difference at pre freezing period with respect to motility, livability and abnormality. Similarly, no significant difference could be observed in livability and abnormality where as it was significantly ($p \leq 0.01$) different for motility. The same type of observation was also evident between TC-4 and TC-9 for aforesaid parameters in pre freezing condition. However, a highly significant ($p \leq 0.01$) difference could be observed for sperm abnormality, but the motility and livability was only significant ($p \leq 0.05$).

Comparison between TC-6.4 and TC-9 showed highly significant difference (pd"0.01) between motility, where as livability and abnormality were different at 5 % level. The zero hour freezing between these diluents indicated significant difference ($p \leq 0.01$) between motility and sperm abnormality ($p \leq 0.05$). On the contrary, no significant difference could be observed for livability.

The percentage of live spermatozoa was found to be higher (82.90 ± 0.41 and 66.32 ± 1.15) in diluent containing 4 % glycerol both at pre freezing and zero hour freezing and lowest in 9 % glycerol extender. The survival rate of buck spermatozoa as observed in the present investigation in Tris diluent with 4 % glycerol coincides with the observations of Andersen (1969). Also significant difference ($p \leq 0.05$) was seen in all the three percentages of glycerol in diluent with respect to motility of spermatozoa which might be due to failure of spermatozoa to regain full metabolic activity and livability because of disruption of cell membranes and loss of intracellular constituents. Moreover, at pre-freeze highly significant ($p \leq 0.01$) difference was found between TC-4 and TC-9 in pre freezing where only a significant difference ($p \leq 0.05$) was between TC- 6.4 and TC – 9 at, where as only a significant difference ($p \leq 0.05$) was between TC-4 and TC-9 during zero hour freezing.

The percentage of morphologically abnormal spermatozoa was found to be higher (5.04 ± 0.27 and 10.27 ± 0.77) in TC-9 both at pre freezing and zero hour freezing respectively and the lowest (4.12 ± 0.21 and 8.21 ± 0.26 % respectively) in TC-4. This finding agrees with the work of Deka and Rao (1986). Statistically, the difference was highly significant ($p \leq 0.01$) between pre-freezing and zero hour freezing which might be probably due to severe cryo-injuries during the process of freezing and thawing. But by comparing different percentages of glycerol in dilutor or extender, it was observed that in both pre freezing and zero hour freezing, TC-4 and TC-9 differed significantly ($p \leq 0.01$)

The effect of storage on different sperm parameters (table 1) and their correlations (table 3) indicates that in all the three dilutors, there was decrease in motility at the end of first month of storage when compared to their zero hour freezing (Table 1). Subsequent reduction in motility for the second and third month of storage followed the same pattern with least decline in TC-6.4 followed by TC-4 and TC-9. Statistical analysis showed a highly significant ($p \leq 0.01$) difference in TC-4 and TC-9 between first and third months of storage and was non significant for TC-6.4 between the months of storage. Further, when the effect of glycerol percentage in dilutors are compared between the same month of storage, it was evident that there exist a highly significant ($p \leq 0.01$) difference between all the three dilutors in first, second and third months of storage except between 4 and 9 % glycerol in 2nd month (pd"0.05) and non significant in 3rd month for the same glycerol percentage.

The live sperm percentage of diluted semen stored for 3 months was found to be lesser than at zero freezing. This decline was very sharp from zero hour to first month of storage than in second and third months which indicates that during the first month the sperms get adjusted to the environment and death of sperm was minimized during the subsequent months. Therefore, no significant difference was observed in all the three dilutors between different months of storage (table

Table 1: Seminal attributes of Black Bengal bucks in extenders with different levels of Glycerol at pre freezing, zero hour freezing and during storage

Glycerol per cent in TEYCAFG extender	Sperm Motility(percent)				Live sperm(percent)				Sperm Abnormality(percent)			
	Hours of Freezing	Month of Storage			Hours of Freezing	Month of Storage			Hours of Freezing	Month of Storage		
	Pre-freeze	Zero hour	1st	2nd	3rd	Pre-freeze	Zero hour	1st	2nd	3rd	Pre-freeze	Zero hour	1st	2nd	3rd
TC(4.0%)	75.32± 0.23	37.53± 0.28	33.72 ± 0.35^{a}	32.71± 0.35 ab	31.25± 0.41^{b}	82.90± 0.41	66.32± 1.15	61.91 ± 0.67	60.25± 0.51	59.50 ± 0.64	4.12± 0.21	8.21± 0.26	9.51± 0.22	9.65± 0.28	9.74± 0.56
TC(6.4 %)	74.91± 0.37	42.12± 0.31	39.14± 0.29^{a}	38.82± 0.33 a	38.42± 0.39 a	81.91± 0.25	64.61± 0.70	58.31 ± 0.45	56.92± 0.51	54.52 ± 1.12	4.53± 0.24	9.13± 0.34	9.91± 1.15	11.53 ± 0.27	12.16 ± 0.19
TC(9.0 %)	72.83± 0.33	36.63± 0.25	31.62± 0.27^{a}	30.81± 0.33 ab	29.50± 0.39^{b}	80.25± 0.36	61.63± 0.68	50.24 ± 1.94	49.72± 1.38	49.41 ± 0.54	5.04± 0.27	10.22± 0.27	12.3 ± 0.31^{a}	13.33 ± 0.32^{ab}	14.15 ± 0.28^{b}

Table 2. Test of significance by Fischer's 't' test between pre freezing and zero hour freezing for semen qualities with different percentage of glycerol

Between groups	Category	Motility	Livability	Abnormality
Pre freezing and Zero hour freezing	TC-4	47.63**	9.07**	14.82**
	TC-6.4	40.42**	11.33**	4.97**
	TC-9	170.00**	13.75**	14.92**
TC-4 & TC-6.4	Pre freezing	0.53^{NS}	1.46^{NS}	1.78^{NS}
	Zero hour freezing	4.98**	0.75^{NS}	1.95^{NS}
TC-4 & TC-9	Pre freezing	3.36**	3.63**	4.06**
	Zero hour freezing	2.37*	2.04*	4.46**
TC-6.4 & TC-9	Pre freezing	2.85**	2.63*	2.14*
	Zero hour freezing	6.78**	1.72^{NS}	2.58*

*$p \leq 0.05$ **$p \leq 0.01$ ^{NS}Non significant (TC-4 Glycerol 4%, TC-6.4 Glycerol 6%, TC- 9 Glycerol 9%)

1). However, highly significant ($p \leq 0.01$) difference was found between 4, 6.4 and 9 Percent of glycerol in dilutors during each month of experiment period except between TC-4 & TC-6.4 (first & third month) and TC-6.4 and TC-9 (third month), where the difference was only significant ($p \leq 0.05$).

The morphological abnormality of sperm in stored semen sample was found to have increased from zero hour of freezing. But the increase was sharper during first month of storage for sperm motility and livability. However, no significant differences were observed in all the three dilutors between the months of storage except between first and third month in dilutors having 9 % glycerol where the difference was highly ($p \leq 0.01$) significant. Again comparison between the dilutors within the same month, showed that a non significant difference existed in all the groups except between TC-4 & TC-6.4 (third month) and TC-4 & TC-9 (second month) where the difference was highly significant ($p \leq 0.01$).

The present findings in relation to storage of semen with dilutors having different concentrations of glycerol for a period of three months are in partial agreement with the findings of Pintado and Perez (1992). Thus, it indicates that various concentrations of glycerol in Tris diluent

Table 3. Fischer's 't' test between dilutors of different glycerol percentage for different seminal attributes over different months of preservation.

Seminal attributes	Period (month)	TC-4 X TC-6.4	TC-4 X TC-9	TC-6.4 X TC-9
Motility	First	7.10**	2.84**	11.15**
	Second	7.39**	2.44*	9.91**
	Third	7.70**	1.83^{NS}	9.92**
Livability	First	2.44**	3.94**	2.81**
	Second	2.66**	4.10**	2.79**
	Third	2.29*	6.86**	2.36**
Abnormality	First	0.32^{NS}	6.79**	1.87^{NS}
	Second	1.65^{NS}	1.40^{NS}	3.64**
	Third	3.69**	6.16**	1.16^{NS}

* $p \leq 0.05$ ** $p \leq 0.01$ ^{NS} Non significant

Table 1: Seminal attributes of Black Bengal bucks in extenders with different levels of Glycerol at pre freezing, zero hour freezing and during storage

influenced the quality of spermatozoa during freezing due to diverse cryo-protecting action of different levels of glycerol as semen additive.

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