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Cryopreservation of Indigenous bull semen (Zobawng) of Mizoram: Effect of fructose on extender

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Abstract
A total of 36 ejaculates were collected from three indigenous bulls (Zobawng) of Mizoram. The semen samples were evaluated to find out the effects of fructose i.e. Tris egg yolk citric acid fructose glycerol (TEYCAFG) and Egg yolk sodium citrate glycerol (EYSCG) extenders on freezability of indigenous bulls' (Zobawng) semen of Mizoram in terms of sperm motility, per cent live sperm, HOSST reacted spermatozoa and acrosomal integrity. The mean sperm motility, per cent live sperm, HOSST and acrosomal integrity in TEYCAFG extender at pre and post freezing were (69.53±0.31 and 45.36±0.59%), (76.36±0.35 and 53.03±0.45%), (67.91±0.45 and 44.92±0.56%) and (75.17±0.58 and 63.25±0.56%) respectively, and in EYSCG extender were (45.67±0.48 and 33.08±0.36%), (54.56±0.41 and 38.08±0.28%), (47.86±0.44 and 34.61±0.40%) and (57.22±0.31 and 45.89±0.48%) respectively. The mean motility, live sperm, HOSST and acrosomal integrity in both TEYCAFG and EYSCG extenders were significantly (P<0.01) higher at pre-freezing when compared to post freezing and the mean values of the said parameters were significantly (P<0.01) higher in TEYCAFG extender than EYSCG. In conclusion, the Tris egg yolk citric acid fructose glycerol (TEYCAFG) is good for freezing of indigenous bulls' (Zobawng) semen of Mizoram.

Keywords: Zobawng bull semen, Mizoram, freezing, fructose

1. Introduction
Mizoram is one of the North Eastern state of India, covered mainly hilly landscape. The state is blessed with a cattle breed which is small in size with cylindrical type of body [1]. The native name of the local cattle of Mizoram is called as Zobawng ('zo' means high land and 'bawng' means cattle) i.e. cattle of hills. No studies on their semen characteristics had been done till date.

Fructose, a ketonic monosaccharide generates intracellular ATP to provide energy to the spermatozoa [2]. Fructose supplement in extenders improves sperm motility, acrosome reaction as well as sperm penetration [3]. Fructose improves the motility of spermatozoa [4], viability is improved [5, 6]. Sperm quality is greatly influenced by the addition of fructose in coconut water semen extender [6]. The present study was designed to find out the effects of fructose i.e. Tris egg yolk citric acid fructose glycerol (TEYCAFG) and Egg yolk sodium citrate glycerol (EYSCG) extenders on freezability of indigenous bulls' (Zobawng) semen of Mizoram.

2. Materials and methods
2.1 Experimental animals
The study was conducted with three indigenous bulls of Mizoram (2- 4 years old) maintained in Livestock Farm Complex, College of Veterinary Sciences & A. H., CAU, Selesih, Aizawl, Mizoram. The bulls were maintained under hygienic condition, with proper supply of feed and drinking water and managerial regime under intensive system of management in well-ventilated sheds. They were thoroughly examined for sexual and general health before selection.

2.2 Semen collection
A total of 36 ejaculates were collected with the help of a standard Artificial Vagina (AV) which was cleaned, sterilized and properly assembled before the collection of semen and the

temperature inside AV maintained at 45 °C. The dummy was kept in a crate and the bull was allowed to approach and mount. Two false mounts were allowed before the collection of semen. After the bull was sufficiently stimulated to jump over the dummy, when the penis protrudes from the bull, it was directed into the AV to collect the semen into a graduated semen collection tube. After the collection of semen, to avoid cold shock, the vial containing the semen was placed into a flask containing water at 37°C and then taken to the laboratory for processing and evaluation. Collection of semen was done twice a week.

2.3 Preparation of extender

Tris egg yolk citric acid fructose glycerol (TEYCAFG) was prepared using 2.422g Tris, 1.36g citric acid, 1.g fructose, 73.6 ml double distilled water, 1,00,000 IU penicillin, 100mg streptomycin, 6.4ml Glycerol and 20ml Egg yolk.

Egg yolk sodium citrate glycerol (TEYCAFG) was prepared using 2.9 g Sodium citrate dehydrate, 73.6 ml double distilled water, 1,00,000IU penicillin, 100mg streptomycin, 6.4ml Glycerol and 20ml Egg yolk.

2.4 Semen processing

The ejaculates were diluted (1:5) in TEYCAFG and EYSCG extender. Both the extenders were prepared in two fractions fraction A (50 ml) and fraction B (50 ml). The semen was extended with half of the final dilution, the fraction A of each of the extender at 35^0C and place the conical flask in a beaker containing water at 35˚C. Initial volume of semen was taken into consideration for extension. Gradual cooling of then semen was carried out in a cold handling cabinet (5 °C @ 1 ºC per 3 minutes). The fraction B of each of the extender maintained at 5 ºC was then added (amount equal to the fraction A) at 15 minutes interval to the initially extended semen in three steps. The diluted semen was kept for 4 hours in cold handling cabinet at 5^0C for equilibration. Semen was filled in 0.5 ml French straws. The straws were filled during equilibration period by suction at 5°C. The straws were then sealed using polyvinyl alcohol powder and put in water (5 ˚C) for about 1 hour for hardening the plug. The straws were then removed from water and then dried using towel (5 ˚C) and then placed on freezing rack. The liquid nitrogen level in the freezer was kept 1 cm below the grill in LNR A9000 and was kept closed for 5 minutes to stabilize the liquid nitrogen gas inside the freezer. The freezing rack was removed from the cold handling cabinet and then placed it on the grill inside the liquid nitrogen freezing container (freezer) LNR A9000 for 10 minutes. After that, the straws were collected and put in a goblet containing liquid nitrogen and then the goblet was plunged into liquid nitrogen for storage.

2.5 Sperm assessment

The progressively motile sperm [7], per cent live sperm [7], HOSST [8] and acrosomal integrity [9] of indigenous bull (Zobawng) semen of Mizoram were estimated after pre-freezing (5˚C) and post-freezing (-196˚C).

2.6 Statistical analysis

Statistical analysis was done using SPSS software and the paired t-test was employed to compare the values.

3. Results and Discussion

3.1 Effect of fructose in the extenders on freezing of Indigenous bull semen

3.1.1 Motility

The mean sperm motility in TEYCAFG and EYSCG extender was 69.53±0.31 and 45.67±0.48 per cent after equilibrium respectively. The corresponding values after freezing were 45.36±0.59 and 33.08±0.36 respectively. (Table 1; Fig. 1). Critical difference test revealed that there was significant difference (P<0.01) in sperm motility between pre-freezing and post-freezing in both extenders. The sperm motility in between TEYCAFG and EYSCG extender differed significantly (P<0.01) both at pre-freezing and post-freezing stages. The mean sperm motility in TEYCAFG extender was significantly (P<0.01) higher than in EYSCG extender both at pre-freezing and post-freezing stages. There was no significant difference between sperm motility in EYSCG extender at pre-freezing and that of post-freezing in TEYCAFG extender. Application of fructose into extender improves the motility of spermatozoa [5]. Kankrej bulls' semen motility was good in semen extended with fructose [10]. Comparative study between bulls' semen extended with and without fructose in whole milk revealed significantly higher (P<0.01) result in extender with fructose [11].

3.1.2 Live sperm

The post equilibrium sperm motility in TEYCAFG and EYSCG extender was 76.36±0.35 and 54.56±0.41 per cent respectively. The mean live sperm percentage after freezing using TEYCAFG and EYSCG extender was 53.03±0.45 and 38.08±0.28 per cent respectively. (Table 1). Critical difference test revealed that the live sperm at pre-freezing and post-freezing stages differed significantly (P<0.01) in between TEYCAFG and EYSCG extender. The mean live sperm count in TEYCAFG extender was much higher than in EYSCG extender both at pre-freezing and post-freezing stages. No significant difference between sperm motility was seen in EYSCG extender at pre-freezing and that of post-freezing in TEYCAFG extender. Viability is greatly improved by the addition of fructose into extender [5]. Semen Tris-citrate-fructose-yolk-glycerol (TFYG) extender was significantly (P<0.01) higher than in Biociphos extender both at pre-freezing and post freezing [12].

3.1.3 HOSST

After equilibrium, the mean HOSST reacted sperm in TEYCAFG and EYSCG extenders were 67.91±0.45 and 47.86±0.44 per cent respectively. The HOSST positive sperm in TEYCAFG and EYSCG extender post-freezing was found to be 44.92±0.56 and 34.61±0.40 per cent respectively. (Table 1). Critical difference test revealed that the HOSST reacted spermatozoa in TEYCAFG extender was significantly (P<0.01) higher than in EYSCG extender at both pre and post freezing stages. It also revealed that HOSST reacted spermatozoa was significantly (P<0.01) higher at pre-freezing than post freezing in both the extenders. The mean per cent HOSST reacted spermatozoa of bull semen in between Tris-citrate-fructose-yolk-glycerol (TFYG) extender and Biociphos extender was found to be non significant [13, 12]. This might be due to composition effect.

3.1.4 Acrosomal integrity

The mean percentage of intact acrosome in TEYCAFG and EYSCG extender after equilibrium was 75.17±0.58 and 57.22±0.31 per cent respectively and at post-freezing in TEYCAFG and EYSCG extender was 63.25±0.56 and 45.89±0.48 per cent respectively. (Table 1). On critical difference test it was observed that percentage of intact acrosome was significantly (P<0.01) higher in TEYCAFG than in EYSCG extender at both pre and post freezing stages. The incidence of intact acrosome was higher significantly (P<0.01) at pre-freezing than post freezing in both the extenders. The per cent intact acrosome in TEYCAFG

extender post-freezing was significantly (P<0.01) than in EYSCG extender at pre-freezing. The mean per cent intact acrosome of bull semen in between Tris- citrate-fructose-yolk-glycerol (TFYG) extender and Biociphos extender was found to be non significant [13, 12]. This might be due to composition effect.

Table 1: Per cent sperm motility, live sperm, HOSST and acrosomal integrity (Mean ± SE) of semen in different extenders during freezing process of indigenous cattle of Mizoram, (N= 36)

Parameters	Extenders	Pre-freezing	Post-freezing	t-value
Sperm motility	TEYCAFG	69.53±0.31^{a}	45.36±0.59^{b}	48.33**
	EYSCG	45.67±0.48^{b}	33.08±0.36^{c}	23.20**
	t-value	41.38**	17.87**	
Live sperm	TEYCAFG	76.36±0.35^{a}	53.03±0.45^{b}	52.59**
	EYSCG	54.56±0.41^{b}	38.08±0.28^{c}	35.52**
	t-value	40.18**	28.29**	
HOSST	TEYCAFG	67.91±0.45^{a}	44.92±0.56^{b}	35.67**
	EYSCG	47.86±0.44^{c}	34.61±0.40^{d}	20.94**
	t-value	31.68**	14.898**	
Acrosomal integrity	TEYCAFG	69.53±0.31^{a}	45.36±0.59^{b}	48.33**
	EYSCG	45.67±0.48^{b}	33.08±0.36^{c}	23.20**
	t-value	41.38**	17.87**	

** Significant at (P<0.01)
Mean bearing different lowercase alphabets in superscript showed significant difference (P<0.01)

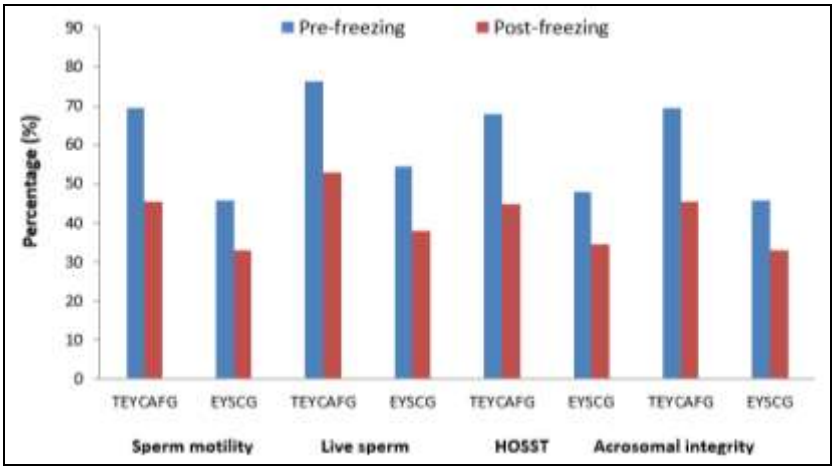

Fig 1: Per cent sperm motility, live sperm, HOSST and acrosomal integrity of semen in different extenders during freezing of indigenous cattle of Mizoram

4. Conclusion

In conclusion, the Tris egg yolk citric acid fructose glycerol (TEYCAFG) is good for freezing of indigenous bulls' (Zobawng) semen of Mizoram.

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