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Effect of different glycerol levels in tris-based extender on quality of frozen yak semen

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Artificial insemination is one of the important biotechnological tools for rapid genetic improvement of livestock. At present, in India, AI is commonly used for breeding of cattle and buffaloes. Attempts are in progress to make this technique popular in other species like goat, sheep, pig, etc. In India, AI in yak is at its infancy and practiced only under farm condition and efforts are underway to popularize the technology among the yak rearers. Glycerol is well known as a cryo-protectant widely used during freezing of almost all mammalian semen. The level of glycerol may be varying in extender to obtain the best result after freezing of semen of different species (Veerabramhaiah *et al.* 2011, Sansone *et al.* 2000, Farshad *et al.* 2009.) Addition of glycerol in the semen extender leads to changes in the size and shape of the ice crystals formed and thereby reduces their mechanical destructions. It binds with water and markedly decreases the freezing point of solutions leading to less ice formations in its presence at any given temperature. Glycerol for freezing of bull semen remains the standard for comparison for any cryoprotective agents. No substitute has yielded satisfactory results. Therefore, glycerol remains the widely used cryo-protectant for semen freezing. Hence, the present study was carried out to know the best level of glycerol in tris extender while freezing yak semen.

Four healthy yak bulls aged between 3 to 4.5 years were used in the present study. The yak bulls were maintained at the institutional farm of ICAR-National Research Centre on Yak, Dirang, located at West Kameng district of Arunachal Pradesh at an altitude of 2750 above msl.

Artificial vagina method was applied to collect twenty ejaculates from each of four bulls at weekly intervals and initial evaluations were done immediately after collection of semen as per standard methods. Ejaculates having minimum of volume 1ml, mass activity 3^{+} and initial sperm motility 70% were diluted in tris extender containing 5, 6.4 and 7.5% glycerol by split sample technique to find out best level of glycerol for freezing of yak semen. Filling and sealing of straws was done at room temperature followed by cooling @1°C/3 minutes rate in 1.5 h. An equilibration period of 4 h was followed before vapour freezing and final storage in liquid nitrogen. The samples were analysed for sperm motility, live sperm, HOST reacted spermatozoa, acrosomal changes, alanine transferase (ALT) and aspartate transferase (AST) both after equilibration and freezing. The statistical difference between the groups was analysed as per Snedecor and Cochran (1994).

The mean sperm motility, live sperm and HOST-reacted sperm in fresh yak semen immediately after collection were 76.00±0.86, 81.85±1.03 and 75.45±0.88% respectively. Sperm motility, live sperm count and HOST-reacted sperm at different stages of processing and freezing in tris extender containing 5, 6.4 and 7.5% glycerol are presented in Table 1.

The percentage of sperm motility, live sperm and HOST-reacted sperm differed significantly ($P<0.01$) between glycerol levels, between stages and due to glycerol level × stage interaction. Critical difference test revealed that the post-thaw sperm motility and HOST-reacted sperm were significantly ($P<0.05$) higher in tris extender containing 5 and 6.4% glycerol than in 7.5% glycerol, difference between 5 and 6.4% glycerol being non significant. Critical difference test, further showed that the live sperm per cent after freezing was significantly ($P<0.05$) higher for 5% glycerol than for 7.5% glycerol but the differences between 5 and 6.4%, and between 6.4 and 7.5% glycerol with extender were not significant. The critical difference test revealed that sperm motility, live sperm and host-reacted sperm declined significantly ($P<0.05$) at each stage of processing and freezing of semen.

It was observed in the present study that the level of glycerol in the extender significantly influenced the sperm motility, live sperm and HOST-reacted sperm both after equilibration and after freezing of yak semen. Similar observation was also reported by earlier workers on post thaw semen quality in different species (Jainudeen and Dass, 1982 and Dutta 1989). On the contrary, others did not

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observe any significant difference in sperm motility after equilibration and after freezing in different species (Becker *et al.* 1977 and Sinha 1989). The diversity of opinion of different workers could be attributed to species variation and differences in composition of the extender which is important in determining the effects of equilibration periods.

The percentage of sperm motility, live sperm and HOST-reacted sperm were significantly (P<0.05) higher in tris extender containing 5% glycerol than containing 7.5% glycerol both after equilibration and after freezing but the difference between 5 and 6.4% glycerol was non significant. Similar findings were also reported by earlier workers on bull (Mortimer *et al*. 1976) and buffalo bull semen (Jainudeen and Dass 1982). Higher post-thaw sperm motility was observed in skim milk extender with 5.5% glycerol than with 4 and 7% glycerol (Negoita and Otel 1975), in egg yolk sodium citrate extender with 5% glycerol than with 10 and 11% glycerol (Mortimer *et al.* 1976), in egg yolk citrate and reconstituted skim milk extender with 5% glycerol than with 3% glycerol (Bandyopadhyay *et al.* 1974), in lactose glycerol egg yolk extender with 5% glycerol than with 9% glycerol (Crabo *et al.* 1980) and in tris egg yolk glycerol extender with 5% glycerol than with 3 or 7% glycerol (Jainudeen and Dass 1982). Moreover, Graham *et al.* (1978) reported that glycerol levels above 6% were detrimental to post-thawing survival of spermatozoa.

On the contrary, a higher percentage of glycerol in the extender was also recommended by other workers in different species of animals (Unal *et al.* 1978 and Prakash *et al*. 1986). The diversity of opinion of different workers might be due to differences in extender composition, method of glycerolization, equilibration period and freezing process and/ or species of animals used in different studies.

The percentage of sperm motility, live sperm and HOST-reacted sperm differed significantly (P<0.01) due to glycerol level × stage of processing and freezing of yak semen. This indicated that the main effects were not independent.

The mean incidence of total acrosomal changes in tris extender containing 5, 6.4 and 7.5% glycerol was 7.50±0.53, 6.95±0.48 and 6.15±0.46 after equilibration and 12.25±0.57, 12.55±0.54 and 12.75±0.61 after freezing. The corresponding values for ALT (IU/l) was 6.37±0.79, 6.61±0.86 and 6.78±0.71 respectively after equilibration, and 8.28±0.75, 9.45±0.92 and 8.60±0.76 respectively after freezing and for AST (IU/l) was 4.83±0.59, 5.72±0.69 and 5.93±0.61 after equilibration and 8.93±0.83, 9.79±0.82 and 8.62±0.90 after freezing.

In the present study, the total incidence of acrosomal changes was not significantly affected by glycerol level in tris extender. On the contrary, others observed significantly higher percentage of intact acrosomes in frozen buffalo bull semen for 3 or 5% glycerol than that for 7% glycerol (Jainudeen and Dass 1982), in frozen buck semen for 5% glycerol than that for 7% glycerol (Sinha 1989).

The total incidence of acrosomal changes, extracellular release of ALT and AST did not differ significantly between

Table 1. Per cent sperm motility, live spermatozoa and HOST-reacted sperm (mean*±SE) of yak semen in tris extender containing three levels of glycerol at different stages of processing and freezing

Stage	Glycerol level									
	Per cent sperm motility				Per cent live spermatozoa			Per cent HOST-reacted sperm		
	5%	6.4%	7.5%	Overall mean	5%	6.4%	7.5%	Overall mean	5%	6.4%	7.5%	Overall mean
Fresh semen	76.00^{a}±0.86	76.00^{a}±0.86	76.00^{a}±0.86	76.00^{A}±0.49	81.85^{a}±1.03	81.85^{a}±1.03	81.85^{a}±1.03	81.85^{A}±0.59	75.45^{a}±0.88	75.45^{a}±0.88	75.45^{a}±0.88	75.48^{A}±0.50
After equilibration	67.75^{b}±0.92	65.00^{b}±1.20	61.75^{c}±1.27	64.83^{B}±0.72	75.30^{b}±1.18	73.85^{bc}±1.07	71.30^{c}±1.17	73.48^{B}±0.68	68.05^{b}±0.87	66.2^{bc}±0.71	64.70^{c}±0.77	66.33^{B}±0.48
After freezing	54.00^{d}±1.23	52.25^{d}±1.17	43.75^{e}±0.80	50.00^{C}±0.85	62.15^{d}±1.42	60.15^{de}±1.41	54.25^{e}±1.14	58.85^{C}±0.87	54.10^{d}±1.51	52.70^{d}±1.35	47.10^{e}±1.01	51.30^{C}±0.84
Overall	65.92^{A}±1.31	64.42^{A}±1.41	60.50^{B}±1.81		73.10^{A}±1.27	71.95^{B}±1.35	69.13^{C}±1.61		65.8^{A}±1.32	64.80^{A}±1.35	62.42^{B}±1.60	

[a,b,c,d,e]Means bearing different letter superscripts differ significantly (P<0.05). [A,B,C]Means bearing different letter superscripts differ significantly (P<0.05) within row and within column.

glycerol levels and due to glycerol level × stage interaction but differed significantly (P<0.01) between stages.

SUMMARY

Present study was conducted to evaluate different levels of glycerol (5, 6.4 and 7.5%) in tris extender after equilibration and freezing on yak semen quality. The percentage of sperm motility, live sperm and HOST-reacted sperm differed significantly (P<0.01) between glycerol levels, between stages and due to glycerol level × stage interaction. Critical difference test revealed that the post thaw sperm motility, live sperm and HOST-reacted sperm were significantly (P<0.05) higher in tris extender containing 5.0 and 6.4% glycerol than in 7.5% glycerol, difference between 5.0 and 6.4% glycerol being non significant. The total incidence of acrosomal changes, extracellular release of ALT and AST did not differ significantly between glycerol levels and due to glycerol level × stage interaction but differed significantly (P<0.01) between stages. It may be concluded that 5 or 6.4% glycerol in tris extender is better for cryopreservation of yak semen in comparison to 7.5% glycerol.

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