Journal of Animal Research: v.6 n.5, p. 859-862. October 2016

DOI: 10.5958/2277-940X.2016.00110.8

Effect of Different Glycerol Levels on Quality of Frozen Semen of Mizo Local Boar

A.K. Das, N. Ahmed*, K. Lalrintluanga, F.A. Ahmed, M.A. Ali, P.K. Subudhi, D. Deuri and D.J. Kurmi

Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Aizawl, Mizoram, INDIA

**Corresponding author: N Ahmed; Email: nekibahmeds@gmail.com*

Received: 20 April, 2016 **Accepted:** 28 August, 2016

ABSTRACT

A total of 24 ejaculates were obtained from 3 Mizo local boars (Zovawk), were used by split sample technique for evaluating the effect of four glycerol levels on quality of semen extended with Lactose egg yolk glycerol (LEYG) extender at equilibration and after freezing. The sperm motility at equilibration and after freezing was significantly higher ($P<0.01$) for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels. The live sperm at equilibration and after freezing was significantly higher ($P<0.01$) for 3 percent glycerol levels than for 2 and 4 percent glycerol levels, and for 2 percent than for 3 percent glycerol level. The Hypo Osmotic Sperm Swelling Test (HOSST) reacted spermatozoa at equilibration and after freezing was significantly ($P<0.01$) higher for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels, and for 2 percent than for 3 percent glycerol level. The acrosomal integrity at equilibration and after freezing was significantly ($P<0.01$) higher for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels, and for 2 percent than for 3 percent glycerol level. In conclusion, preservation of boar semen in LEYG extender using 3 percent glycerol found to be superior.

Keywords: Boar semen, glycerol, Lactose egg yolk glycerol, freezing

Small scale pig rearing predominates throughout the North Eastern Region including Mizoram. In the region, pig rearing is one of the integral parts of the life. An average Mizo (people of Mizoram) consumes about 14 kg of meat annually, against the national per capita consumption of meat of 7.5 kg (Statistical Handbook Mizoram 2010). Therefore, farmers have tremendous opportunities to develop by this sector. However, Non-availability of good numbers of superior quality breeding male, high cost of transportation in hilly terrain, and lack of awareness about artificial insemination (AI) techniques forced the small holder pig breeder to choose natural service by one or two boar maintained in their village (Kumaresan *et al*., 2009). It leads to inbreeding and decline in growth and reproductive performance of pig population.

Artificial insemination (AI) technique could be beneficial for meeting the demand of improved pig germplasm at farmers' door step and might help in faster propagation of elite germplasm. Mostly, liquid stored semen or fresh semen is used for AI in commercial swine herds (Wagner and Thibier 2000). But it has disadvantage as it can't be stored for a longer duration. Therefore, freezing and thawed semen will fulfill this gap. Nevertheless, the success of boar semen cryopreservation is relatively variable. Therefore, the manipulation of boar semen requires special consideration during cryopreservation process. Moreover, till date no study has been conducted on Mizo local boar semen (Zovawk).

Considering the fact, the present study was aimed to evaluate the semen quality of Mizo local boar at different glycerol levels during preservation.

MATERIALS AND METHODS

A total of 24 ejaculates obtained from 3 Mizo local boars (Zovawk) were used by split sample technique for the study. Split ejaculates were extended with Lactose egg yolk glycerol extender to evaluate the effect of 3 glycerol levels (2, 3 and 4 percent) on quality of frozen semen. Prior to use, the Lactose egg yolk glycerol extender was

centrifuged at 3000 rpm for 10 minutes and the supernatant was used. Each ejaculate was split into 3 parts and was diluted at the rate of 1:1 with the same extender.

French medium straws were filled with extended semen of different glycerol levels and sealed by polyvinyl alcohol. The straws were then placed in a tray containing water at 5ºC to ensure proper hardening of seal. Semen was then equilibrated for 1 hour at 5ºC. Just before the end of 1 hour equilibration the straws were taken out from water and wiped dry by using pre-cooled (5ºC) towel. After drying, straws were freezed above liquid nitrogen vapour and stored in liquid nitrogen container. After 24 hours of storage, frozen semen was then thawed in warm water at 50ºC for 12 seconds.

All samples were evaluated for sperm motility, live sperm, HOSST reacted sperm and intact acrosomal at equilibration and after freezing.

The data was analyzed statistically by using software SPSS version 17.0.

RESULTS AND DISCUSSION

The results of analysis of variance of sperm motility, live sperm, HOSST-reacted spermatozoa and intact acrosome at different stages of processing and freezing in Lactose egg yolk glycerol (LEYG) extender with different glycerol level in indigenous pigs of Mizoram are presented in Tables 1 and 2 respectively.

Sperm Motility

The mean sperm motility at equilibration and after freezing in LEYG extender containing different glycerol levels are given in Table 1.

Statistical analysis revealed that the sperm motility at equilibration and after freezing was significantly higher (P<0.01) for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels. Between 2 and 4 percent glycerol level there was no significant difference in LEYG extender after freezing. The mean sperm motility in this study was in agreement with the findings of other (Eriksson *et al.*, 2002).

Live Sperm

The mean live sperm at equilibration and after freezing in LEYG extender containing 2, 3 and 4 percent glycerol level are presented in Table 1.

Critical difference test revealed that the live sperm at equilibration and after freezing was significantly higher (P<0.01) for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels, and for 2 percent than for 3 percent glycerol level. The mean live sperm in LEYG extender with different glycerol levels (2, 3 and 4 percent) dropped significantly (P<0.01) during freezing. Present experiment state that highest live sperm after freezing in LEYG extender with 3 percent glycerol level was 43.95±0.42 percent. The highest post thawing sperm motility observed in the present study was well comparable with that of Chanapiwat *et al.* (2008) and Khan *et al.* (2012).

HOSST-Reacted Spermatozoa

The mean HOSST-reacted spermatozoa at equilibration and after freezing in LEYG extender containing 2, 3 and 4 percent glycerol was given Table 2.

Critical difference test revealed that the HOSST reacted spermatozoa at equilibration and after freezing was

Table 1: Percent sperm motility and live sperm in boar semen at different stages of processing and freezing in different glycerol level

Stage	Sperm motility (%)			Live sperm (%)		
	Glycerol level			Glycerol level		
	2 %	3 %	4%	2 %	3 %	4%
At equilibration	62.65 ± 0.57 ^{b}	64.70 ± 0.41 ^{c}	58.90 ± 0.35 ^{a}	64.20 ± 0.59 ^{b}	67.35 ± 0.57 ^{c}	60.65 ± 0.68 ^{a}
After freezing	35.55 ± 0.72 ^{b}	43.42 ± 0.44 ^{c}	34.65 ± 0.62 ^{a}	40.02 ± 0.32 ^{b}	43.95 ± 0.42 ^{c}	36.55 ± 0.72 ^{a}

** Significant at (P< 0.01), Means bearing different superscript along a row differ significantly.

Table 2: Percent HOSST-reacted spermatozoa and intact acrosome in boar semen at different stages of processing and freezing in different glycerol level

Stage	HOSST-reacted spermatozoa (%)			Intact acrosome (%)		
	Glycerol level			Glycerol level		
	2 %	3 %	4%	2 %	3 %	4%
At equilibration	66.25 ± 0.38 [b]	68.55 ± 0.46 [c]	60.55 ± 1.08 [a]	66.70 ± 0.44 [b]	69.55 ± 0.45 [c]	62.55 ±0.64 [a]
After freezing	41.80 ± 0.46 [b]	46.67 ± 0.54 [c]	37.05 ± 0.54 [a]	42.82 ± 0.30 [b]	47.97 ± 0.36 [c]	39.12 ± 0.35 [a]

** Significant at (p<0.01), Means bearing different superscript along a row differ significantly.

significantly (P<0.01) higher for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels, and for 2 percent than for 3 percent glycerol level. The mean HOSST reacted spermatozoa in LEYG extender at 2, 4 percent and 2, 3 percent glycerol level was significant (P<0.05). Khan *et al*. (2012) also observed the same findings in LEYG extended semen.

Intact Acrosome

The mean acrosomal integrity at equilibration in LEYG extender containing 2, 3 and 4 percent glycerol was 66.70±0.44, 69.55±0.45 and 62.55±0.64 percent respectively. The corresponding values after freezing were 42.82±0.30, 47.97±0.36 and 39.12±0.35 percent (Table 2). Statistical analysis revealed that the acrosomal integrity at equilibration and after freezing was significantly (P<0.01) higher for 3 percent glycerol levels, than for 2 and 4 percent glycerol levels, and for 2 percent than for 3 percent glycerol level. The mean acrosomal in LEYG extender with different glycerol levels (2, 3 and 4 percent) dropped significantly (P<0.1) during freezing. This findings support the reports of Almid and Johnson (1988) who recorded higher incidence of intact acrosome in semen frozen in LEYG extender with 3 percent glycerol level. The decreased in the mean percentage of intact acrosome with increase in glycerol level from 3 to 4 percent in the present study was similar with the findings of earlier reports (Graham and Crabo 1992; Buhr *et al*., 2001). Glycerol in high concentration was known to affect the fertilizing capacity in both unfrozen and frozen boar semen (Wilmut and Polge 1974).

CONCLUSION

From the current study it can be concluded that preservation of boar semen in LEYG extender using 3 percent glycerol found to be superior. This advanced reproductive biotechnology can be incorporated to improve fertility among the swine population.

ACKNOWLEDGEMENTS

All the authors acknowledge the Dean, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University for providing necessary supports to carry out the research work.

REFERENCES

Almid, T. and Johnson, L.A. 1988. Effect of glycerol concentration, equilibration time and temperature of glycerol addition on post thaw viability of boar spermatozoa frozen in straws. *J. Anim. Sci.,* **66**: 2899-2905.

Buhr, M.M., Fiser, P., Bailey, J.L. and Curtis, E.F. 2001. Cryopreservation in different concentration of glycerol alters boar sperm and their membranes. *J. Andro.,* **22**(6): 961-969.

Chanapiwat, P., Kaeoket, and Tummaruk, P. 2008. L-cysteine supplementation improved qualities of cryopreserved boar semen. Proc. 15th Congress of FAVA, Bangkok, Thailand, pp. 165-168.

Eriksson, B.M., Petersson, H. and Martinez-Rodriguez, H. 2002. Effect of freezing and thawing rates on the post-thaw viability of boar spermatozoa frozen in flat packs and Maxi-straws. *Anim. Reprod. Sci.,* **63**: 205-220.

Graham, E.F. and Crabo, B.G. 1992. Some factor influencing the freezing of boar spermatozoa. In VIIth. *Int. Cong. Anim. Reprod.* A.I., Munich, pp. 324-325.

Khan, M.H., Naskar, S., Nath, K.C., Deka, B., Bardoloi, R.K., Bhuyan, D. and Kumar, S. 2012. Effect of different extender on cryopreservation of Hampshire and crossbred boar semen. *Indian J. Anim. Sci.,* **82**(1).

Kumaresan, A., Bujarbaruah, K.M., Pathak, K.A., Das, A. and Bardoloi, R.K. 2009. Integrated resource-driven pig production systems in a mountainous area of Northeast India: production practices and pig performance. *Tropic. Anim. Hlth. Prod.*, **41**: 1187–1196.

Statistical Handbook Mizoram 2010. The 18th quinquennial Livestock census 2007. http://dipr.mizoram.gov.in/uploads/documents/document3.pdf.

Wagner, H.G. and Thibier, M. 2000.World statistics for artificial insemination in small ruminants and swine. *Proc 14th ICAR, Stockholm, Sweden*, **15**(2): 3.

Wilmut, I. and Polge, C. 1974. The fertilizing capacity of boar semen stored in the presence of glycerol at 20. 5 and -75ºC. *J. Reprod. Fert.*, **38**: 105-113.