©South African Society for Animal Science
Peer-reviewed paper: 10th World Conference on Animal Production

Effect of cryoprotectant on the cryopreservation of South African Kolbroek pig semen

M.H Mapeka[1,2], K.C. Lehloenya[2#], T.L. Nedambale[1] and B. Sutherland[2]
[1] Agricultural Research Council, Germplasm & Reproductive Biotechnologies, Private Bag X2, Irene 0062, South Africa
[2] Tshwane University of Technology, Department of Animal Sciences, Private Bag X680, Pretoria 0001, South Africa

Abstract

The study evaluated the effect of different cryoprotectants on post-thaw survival and motility of Kolbroek sperm. Semen from Kolbroek boars was collected with the gloved hand technique. Ejaculates were diluted with Beltsville thawing solution (BTS) at a ratio of 1 : 1 prior to freezing. Semen was diluted with egg yolk tris; thereafter, one of the three cryoprotectants (14% glycerol, 14% DMSO or 7% glycerol + 7% DMSO) were added. Diluted samples were then loaded into 0.5 mL straws and cooled with a programmable freezer. Thereafter the semen straws were plunged directly into liquid nitrogen (-196 °C) and stored for 48 h. Frozen straws were thawed at 39 °C for a minute and evaluated for sperm motility and survival at 0, 30, 60 and 90 min post-thaw. The post-thaw sperm survival frozen using glycerol as a cryoprotectant was significantly higher immediately after thawing, compared to DMSO, however, similar to the combination of glycerol and DMSO. There was no significant difference on motility rate immediately (0 min) post-thaw between the three cryoprotectants. Sperm cryopreserved with glycerol exhibited a significantly higher percentage motility at 30, 60 and 90 min post-thaw than in the other cryoprotectants. Based on sperm motility, glycerol was a better cryoprotectant for cryopreservation of Kolbroek boar sperm.

Keywords: Indigenous South African pig, cryoprotectant, swine, spermatozoa, sperm
[#] Corresponding author: lehloenyakc@tut.ac.za

Introduction

Kolbroek is an indigenous South African pig breed. The name Kolbroek originated from the name of the ship called Coalbrook which was wrecked on the Cape coast in 1778 (Ramsay *et al.*, 1994). Kolbroek pigs have superior genetic traits for biodiversity. It is extremely hardy and survives under harsh conditions (Ramsay *et al.*, 1994). The use of sperm from Kolbroek boars may potentially contribute to cross-breeding programmes for improving adaptability traits of pigs. It has been widely demonstrated that cryopreservation leads to a decrease in sperm motility in many animal species even when measured objectively by computer-aided methods (Thurston *et al.*, 2001; Dorado *et al*., 2005; Martinez *et al.*, 2005). Pig sperm are also no exception, and are reported to be highly sensitive to cryopreservation procedures. When frozen-thawed pig semen is used for artificial insemination, farrowing rates are reduced by 10 – 25 %, and litter sizes by one to three piglets when compared to artificial insemination with fresh semen (Waterhouse *et al*., 2006). There is also a variation in freezing tolerance between breeds and between male variations within breeds (Holt, 2000). It is therefore necessary to find a suitable cryoprotectant that can protect Kolbroek boar sperm during cryopreservation, which will minimise damage to Kolbroek sperm as a result of cryopreservation. The aim of this study was to determine and evaluate the effect of different cryoprotectants on sperm survivability and motility of Kolbroek pigs.

Materials and Methods

This trial was conducted at the Agricultural Research Council, Irene. Three South African indigenous Kolbroek boars with ages ranging from nine to 10 months were utilized in this study. The boars were housed in individual cages for routine semen collection and cryopreservation purposes. Semen was collected from each Kolbroek boar by the gloved hand technique, as outlined by Roca *et al.* (2004). The sperm-rich fraction was collected using a thermo flask containing warm water (39 ºC) and a glass beaker covered with a gauze filter to separate the gel fraction from sperm-rich fraction (Roca *et al.*, 2004). Within two hours of collection semen was transported to the laboratory for evaluation of pH, sperm concentration, sperm motility and

©South African Society for Animal Science

percentage live sperm. A drop of 20 µL of fresh semen was placed on a microscopic slide and evaluated with the aid of a phase contrast microscope for percentage of motile and live sperm. Sperm concentration was determined by means of a Spermacue photometer (Minitub®).

After evaluation, semen was pooled and diluted with Beltsville thawing solution (BTS) at a ratio of 1 : 1 v/v and equilibrated in a controlled temperature laboratory at 22 ºC for one hour. Semen was then transferred to a controlled rate freezer (Model CL5500; Biogenics, Napa, C.A.) to cool in vapour from 22 ºC to 15 ºC at a rate of -0.05 ºC/min for three hours. After the cooling period, semen samples were centrifuged at 800 x g for 15 min. Following centrifugation, the sperm pellet was recovered and extended with egg yolk citrate base (EYC) extender at a ratio of 1 : 1, and placed back into the contolled rate freezer for further cooling (15 ºC to 5 ºC at 0.11 ºC/min). Thereafter, semen was extended with egg yolk citrate base (EYC) extender containing one of the three cryoprotectant treatments (%14 glycerol, %14 dimethylsuloxide (DMSO) or the combination of glycerol and DMSO (7% glycerol + 7% DMSO). After dilution semen was loaded into 0.5 mL polyvinyl chloride straws and placed back into the controlled rate freezer and further coolled from 5 °C to -4.5 °C at the rate of 1 °C/min for 90 min. The straws were then plunged into liquid nitrogen (-196 °C) for storage. The straws were thawed after two days at a temperature of 38 ºC for one minute and evaluated with the aid of a phase contrast microscope for percentage motility and live sperm, pH and sperm concentration. Data were analysed using analysis of variance (ANOVA). Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at 5% level of significance (Snedecor & Cochran, 1980).

Results

Table 1 presents the post-thaw percentage survival and motility of Kolbroek boar sperm. The percentage motility sperm immediately (0 min) after thawing, was not influenced (P >0.05) by the type of cryoprotectant used. The post-thaw percentage live sperm of semen frozen using glycerol was significantly (P <0.05) higher immediately after thawing, compared to semen cryoprotected with DMSO; however,

Table 1 Post-thaw sperm survival and motility of Kolbroek boar semen (means ± s.e.)

Parameters	Treatment		
	Glycerol	DMSO	Glycerol + DMSO
Raw semen %			
Survival	85 ± 16.9	85 ± 16.7	83 ± 16.5
Motility	90 ± 17.8	90 ± 17.6	90 ± 17.4
0 min Post-thaw			
Survival	61 [a] ± 12.1	21 [b] ± 6.7	35 [b] ± 10.7
Motility	48 ± 10.0	24 ± 9.0	28 ± 8.8
30 min Post-thaw			
Survival	46 [a] ± 13.9	8 [b] ± 5.9	18 [b] ± 5.8
Motility	33 [a] ± 10.7	10 [b] ± 7.9	8 [b] ± 2.1
60 min Post-thaw			
Survival	25 [a] ± 10.3	1 [b] ± 0.9	8 [b] ± 2.5
Motility	11 [a] ± 6.7	1 [b] ± 0.9	5 [ab] ± 1.6
90 min Post-thaw			
Survival	13 [a] ± 7.5	0 [b] ± 0.4	3 [b] ± 1.6
Motility	10 [a] ± 6.7	0 [b] ± 0.4	3 [b] ± 1.9

[a,b] values with different superscript within a row differ significantly (P <0.05).

©South African Society for Animal Science

similar to that of semen cryoprotected with a combination of glycerol and DMSO. On the other hand, the survival rate of spermatozoa at 30, 60, 90 min post-thaw was significantly higher ($P < 0.05$) in semen cryopreserved with glycerol than DMSO, and the combination of glycerol and DMSO. For all cryoprotectants, the survival and motility rates of Kolbroek semen decreased with storage time post-thaw.

Discussion

The present study demonstrated that the post-thaw motility and survival of Kolbroek boar sperm were more retained at 0 min in semen cryopreserved with 14% glycerol compared to 14% DMSO. The results suggest that glycerol is suitable for cryopreserving Kolbroek semen, and yields better results than DMSO and a combination of glycerol plus DMSO. This observation is in agreement with the findings of Curry (2000), where the cryoprotective effects of glycerol were most evident at higher concentrations in domestic animal species. However, maximal post-thaw motility rates were also achieved with different concentrations of glycerol (Wilmut & Polge, 1977; Fiser *et al.*, 1993; Buhr *et al.*, 2001). Dimethyl sulfoxide resulted in a decreased motility at 0, 30, 60, 90 h post-thaw compared to the other treatments. Although DMSO has been successfully used to cryopreserve semen from several species (Kashiwazaki *et al*., 2006), it was not an effective cryoprotectant for cryopreservation of Kolbroek sperm. It has been reported that DMSO can damage the plasma membranes due to its high toxicity to cells (Shuyang & Woods, 2004). This suggests that the degree of damage caused by DMSO during freezing may vary with different species, with pig sperm being more sensitive to cryodamage. However, from this study the addition of glycerol to DMSO provided an additional protection to Kolbroek boar sperm, as the sperm survival rate immediately post-thaw was similar between glycerol and the combination of glycerol and DMSO. These results suggest that for Kolbroek semen, if DMSO is to be used, it should be combined with glycerol. This study also demonstrates that semen quality slowly declines during processing and cryopreservation. This may suggest that cryoprotectants affected the sperm function during and after cryopreservation therefore leading to decreased survival and motility of boar sperm. Similar observations have been reported in other pig breeds (Almlid & Johnson, 1988). From the results of this study it can be recognized that the percentage of sperm survival and motility of South African indigenous pigs were normal and can be used in breeding programmes.

Conclusion

This study demonstrated that glycerol on its own or in combination with other cryoprotectants can be used to cryopreserve Kolbroek sperm. Investigating the mechanism involved during semen cryopreservation and finding better cryoprotectants with standardized concentration levels will assist in optimising sperm viability and motility post-thaw, so that samples are of acceptable quality to be used in breeding programmes.

Acknowledgements

The authors wish to thank the National Research Foundation and Agricultural Research Council for the financial support in this study.

References

Almlid, T. & Johnson, L.A., 1988. Effects of glycerol concentration, equilibration time and boar spermatozoa frozen in straws 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., Baily, J.L. & Curtis, E.F., 2001. Cryopreservation in different Concentrations of glycerol alters boar sperm and their membranes. J. Androl. 22, 961-969.

Curry, M.R., 2000. Cryopreservation of semen from domestic livestock. Rev. Reprod. 5, 46-52.

Doraldo, J., Rodr´tuez, I., Hidalgo, M. & Sanz, J., 2005. Computer-assisted analysis of goat sperm motility and velocity before and after cryopreservation. Reprod. Dom. Anim. 40, 401-402.

Fiser, P.S., Fairfull, R.W., Hansen, C., Panich P.L., Shrestha, J.N.B. & Underhill. L., 1993. The effect of warming velocity on motility and acrosomal integrity of boar sperm as influenced by the rate of freezing and glycerol level. Mol. Reprod. Dev. 34, 190-195.

Holt, W.V., 2000. Fundamental aspects of sperm cryobiology: The importance of species and individual differences. Theriogenology 53, 47-58.

©South African Society for Animal Science

Kashiwazaki, N., Okuda, Y., Seita, Y., Hisamatsu, S., Soroki, S., Shino, M., Masaoka, T. & Inomata, T., 2006. Comparison of glycerol, lactamide and dimethyl sulfoxide as cryoprotectants of Japanese white rabbit spermatozoa. J. Reprod. Dev. 52, 511-516.

Martinez, P.F., Garcia-Marcias, V., Alvarez, M., Herraez, P., Anel, L. & De paz, P., 2005. Sperm subpopulations in Iberian red deer epididymal sperm and their changes through the cryopreservation process. Biol. Reprod. 72, 316-327.

Ramsay, K., Harris, L. & Kotze, A., 1994. Landrace breeds: South Africa's indigenous and locally developed farm animals: Farm Animal Conservation. Pretoria.

Roca, J., Gil, M.A., Hernandez, M., Parrilla, I., Vazquez, J.M. & Martinez, E.A., 2004. Survival and fertility of boar spermatozoa after freeze-thawing in extender supplemented with butylated hydroxytoluene. J. Andro. 25, 397-405.

Shuwang, He. & Woods, C., 2004. Effects of dimethyl sulfoxide and glycine on cryopreservation: Induced damage of plasma membranes and mitochondria to striped bass (Morone saxatilis) sperm. Cryobiol. 48, 254-262.

Thurston, L.M., Watson, P.F., Mileham, A.J. & Holt, W.V., 2001. Morphologically distinct sperm subpopulations defined by Fourier shape descriptors in fresh ejaculates correlate with variation in boar semen quality following cryopreservation. J. Andr. 22, 382-394.

Waterhouse, K.E., Hofmo, P.O., Tverdal, A. & Miller, R.R., 2006. Within and between breed differences in freezing tolerance and plasma membrane fatty acid composition of boar sperm. Reprod. Fertil. 131, 887-894.

Wilmut, I. & Polge, C., 1977. The low temperature preservation of boar spermatozoa. 3. The fertilizing ability of frozen and thawed boar semen. Cryobiology 14, 483-491.