USE OF GLYCEROL AT 8% CONCENTRATION DURING CYROPRESERVATION IS OPTIMAL FOR THE POST-THAW QUALITY OF CAUDA EPIDIDYMAL SPERMATOZOA OF RAM

T. AHMED[1]* AND R. ISLAM[2]

Division of Animal Reproduction, Gynaecology and Obstetrics
S. K. University of Agricultural Sciences and Technology (K), Srinagar-190 006

Received: 28.07.2016 Accepted: 28.08.2016

ABSTRACT

The cauda epididymal spermatozoa of ram recovered using incision method and showing progressive motility ≥70% were pooled and divided into aliquots before extension in Tris-Citric acid-Fructose-Egg yolk extender using glycerol at 4% (G4), 6% (G6) and 8% (G8) concentration as cryoprotectant. At post-thaw stage, the sperm motility was higher (P<0.05) for G8 than G4. At pre-freeze stage, the hypoosmotic swelling test (HOST) reacted spermatozoa were higher (P<0.05) for G8 and G6 than G4. However, at post-thaw stage, the HOST reacted spermatozoa were higher (P<0.05) for G8 than both G6 and G4. In conclusion, 8% glycerol provides better sperm motility and sperm membrane integrity and is recommended for the freezing of ram cauda epididymal spermatozoa.

Keywords: Cryopreservation, Glycerol, Post thaw, Ram, Spermatozoa

INTRODUCTION

The recovery and subsequent cryopreservation of cauda epididymal spermatozoa from the animals has increased attention towards the conservation of various species (Foote, 2000). The cryopreservation of sperm induces an irreversible loss of important constituents and mechanical disarrangements that dramatically change the plasma and acrosome membranes of the cell resulting in decreased motility and viability of the spermatozoa (Ahmed *et al.,* 2016). Amongst the cryoprotectants used to prevent cryoinjuries, the most common cryoprotectant in use is glycerol and was used for ram spermatozoa (Holt, 2000 and Morrier *et al*., 2002). Glycerol as a cryoprotective agent in extender at 10% concentration was detrimental for ram spermatozoa (Salamon and Maxwell, 1995). Others reported no difference in post-thaw motility of cauda epididymal spermatozoa from brown bear using 4% and 8% glycerol (Anel *et al*., 2010). Therefore, the present study was planned to standardize the glycerol concentrations for the cryopreservation of ram cauda epididymal spermatozoa.

MATERIALS AND METHODS

From the slaughtered adult healthy rams at a local abattoir, about 18 testicles were collected and were transported in an ice chest to the semen processing laboratory for the further processing and spermatozoa recovery. In the laboratory, the testicles were cleaned by removing the additional tissues with the help of scissors. The recovery of spermatozoa from the cauda epididymis was done in Tris buffer using incision method (Lone *et al*., 2012). After recovery, the concentration of sample was determined by using photometer and the progressive motility was determined by using standard procedure. The samples showing motility ≥70% were pooled and subsequently equally divided into four aliquots. Each aliquot was extended with Tris-Citric acid-Fructose-Egg yolk [TCFEY; TCF buffer 76% (v/v) + egg yolk 20% (v/v) + penicillin G sodium 800 i.u./ml + streptomycin sulphate 1 mg／ml] extender but with different concentrations of glycerol (G4, 4% v/v; G6, 6%; G8, 8%). The quality of extended spermatozoa from each cauda epididymis was determined by

[1]M.V.Sc. Scholar, [2]Associate Professor; *touqeer_taz@yahoo.co.in

measuring % sperm motility (Lone *et al*., 2012), % live sperm (Lone *et al*., 2012) % intact acrosomes (Lone *et al*., 2012) and hypoosmotic swelling test (HOST; Vasquez *et al*., 2013).

Six semen samples from each group with ≥70% motility were cryopreserved using standard procedures till further post-thaw analysis. The frozen semen straws were thawed in warm water at 37ºC for 15 s. The post thaw spermatozoa were evaluated for % progressive motility, % live sperm, % intact acrosome and HOST. The data were analyzed statistically by using one-way analysis of variance (ANOVA) for comparison between the different glycerol levels. If an effect was noted, *post hoc* analysis was performed using Duncan's multiple range tests. The data pertaining to sperm quality between pre-freeze and post-thaw stage were compared by Paired samples T-test with the help of statistical software SPSS version 16.

RESULTS AND DISCUSSION

In the present study, the mean concentration (million/ml) of pool spermatozoa sample collected from cauda epididymis was 564.47±39.49 (462.94 to 665.99). At all levels of glycerol, the percent sperm motility decreased (P<0.05) from pre-freeze to post-thaw stage, however, at post-thaw stage, the percent sperm motility was higher for G8 than G4 (P<0.05) as well as for G8 compared to G6 (P>0.05, Table 1). These results are in agreement with the previous reports in epididymal spermatozoa retrieved from Iberian red deer (Martinez-Pastor *et al*., 2006), ram epididymal (Alvarez *et al*., 2012) and ejaculated spermatozoa (Mehar and Noori, 2013).

At pre-freeze and post-thaw stage, the percentage of live sperm count and intact acrosome was similar (P>0.05) amongst glycerol concentrations (G8, G6 and G4, Table 1). Others reported ethylene glycol (2.5%) as a good croprotectant for the freezing of ram epididymal spermatozoa in comparison to other cryoprotective agents viz. dimethylacetamide, dimethyl sulfoxide (DMSO), glycerol and propylene glycol at 2.5%, 5% and 10% (Vasquez *et al*., 2013). However, in line with the present study, another study reported higher post-thaw percentage of motile sperm using glycerol, compared to sperm frozen using ethylene glycol or DMSO (Kundu *et al*., 2000).

The percentage of HOST reacted sperm percentage declined (P<0.01) from pre-freeze to post-thaw for all levels of glycerol in TCFEY extender (Table 1). At pre-freeze stage, the HOST reacted spermatozoa were higher (P<0.05) for G8 and G6 than G4 (Table 1). Furthermore, at post-thaw stage, the HOST values were higher (P<0.05) for G8 than G6 and G4 (Table

Table 1: Effect of glycerol concentration (4, 6 or 8%) in extender on cauda epididymal spermatozoa parameters (mean±SEM) **during cryopreservation**

% Glycerol	Pre-freeze	Post-thaw	Pre-freeze	Post-thaw
	Sperm motility, %		Live sperm, %	
G8	70.83±1.54^{A}	56.67±4.01^{bB}	79.77±1.76^{A}	69.59±2.83^{B}
G6	68.33±1.05^{A}	50.83±6.38^{abB}	76.66±0.83^{A}	66.75±2.80^{B}
G4	65.83±2.01^{A}	36.67±4.77^{aB}	75.44±1.22^{A}	62.44±4.11^{B}
	Intact acrosome, %		HOST reacted sperm, %	
G8	91.09±0.88^{A}	77.10±5.69^{B}	84.98±1.55^{bA}	68.10±3.40^{bB}
G6	90.20±0.65^{A}	80.54±2.53^{B}	82.82±0.60^{abA}	53.34±4.50^{aB}
G4	88.87±0.78^{A}	76.82±5.05^{B}	80.78±0.42^{aA}	44.98±4.50^{aB}

Means with different superscripts in a column (a, b) and row (A, B) within a parameter differ significantly (P<0.05)

1). This indicated that higher glycerol percentage (G8) could maintain better membrane integrity than lower levels of glycerol (G4 and G6). Many authors have highlighted that epididymal spermatozoa could be resilient to different glycerol concentrations, possibly due to greater osmotic resistance (Monteiro *et al*., 2011), because of which epididymal spermatozoa show greater resistance to the toxic effects of glycerol and take more benefit from its protective effects (Alvarez *et al*., 2012). Other reports indicated better cryoprotection using low levels of glycerol (4-6%), when added in combination with other cryoprotectants (Mehar and Noori, 2013).

It could be concluded from the current study that glycerol at the rate of 8% should be used as cryoprotectant in Tris-Citric acid-Fructose extender for cryopreservation of ram cauda epididymal spermatozoa.

REFERENCES

Ahmed, T., Islam, R., Lone, F.A. and Malik, A.A. (2016). Effect of washing on the post-thaw quality of cryopreserved ram epididymal spermatozoa. *Vet. World.,* **9**(5): 519-523.

Alvarez, M., Tamayo-Canul, J., Martínez-Rodriguez, C., Lopez-Uruena, E., Gomes-Alves, S., Anel, L., Martinez-Pastor, F. and Paz, P. (2012). Specificity of the extender used for freezing ram sperm depends of the spermatozoa source (ejaculate, electroejaculate or epididymis). *Anim. Reprod. Sci.*, **132**: 145-154.

Anel, L., Gomes-Alves, S., Alvarez, M., Borragan, S., Anel, E., Nicolas, M., Martinez-Pastor, F. and de Paz, P. (2010). Effect of basic factors of extender composition on post thawing quality of brown bear electroejaculated spermatozoa. *Theriogenology*, **74**: 643-651.

Foote, R.H. (2000). Fertilizing ability of epididymal sperm from dead animals. *J. Androl.*, **21**: 355.

Holt, W.V. (2000). Basic aspects of frozen storage of semen. *Anim. Reprod. Sci.*, **62**: 3-22.

Kundu, C.N., Chakraborty, J., Dutta, P., Bhattacharyya, D., Ghosh, A. and Majumder, G.C. 2000. Development of a simple sperm cryopreservation model using a chemically defined medium and goat cauda epididymal spermatozoa. *Cryobiology*, **40**: 117-125.

Lone, F.A., Islam, R., Khan, M.Z. and Sofi, K.A. (2012). Effect of Different Egg Yolk-Based Extenders on the Quality of Ovine Cauda Epididymal Spermatozoa during Storage at 4°C. *Reprod. Domest. Anim.,* **47**: 257–262.

Martinez-Pastor, F., Garcia-Macias, V., Alvarez, M., Chamorro, C., Herraez, P., de Paz, P. and Anel, L. (2006). Comparison of two methods for obtaining spermatozoa from the cauda epididymis of Iberian red deer. *Theriogenology,* **65**: 471-485.

Mehar, M.R.A. and Noori, H. (2013). Effect of different levels of l-Glutamine and glycerol on freezing of ram spermatozoa. *Small Rumn. Res*., **115**(1-3): 103-107.

Monteiro, G.A., Papa, F.O., Zahn, F.S., Dellaqua Jr, J.A., Melo, C.M., Maziero, R.R.D., Avanzi, B.R., Alvarenga, M.A. and Guasti, P.N. (2011). Cryopreservation and fertility of ejaculated and epididymal stallion sperm. *Anim. Reprod. Sci.*, **127**: 197-201.

Morrier, A., Castonguay, F. and Bailey, J.L. (2002). Glycerol addition and conservation of fresh and cryopreserved ram spermatozoa. *Canadian J. Anim. Sci.*, **82**: 347-356.

Salamon, S. and Maxwell, W.M.C. (1995). Frozen storage of ram semen I. Processing, freezing, thawing and fertility after cervical insemination. *Anim. Reprod. Sci.*, **37**: 185-249.

Vasquez, J., Florentini, E.A., Camargo, L.A., Gonzales, J. and Valdivia, M. (2013). Hypo osmotic swelling test in epididymal ram (*Ovis aries*) spermatozoa. *Livest. Sci.*, **157**: 618-622.