DOI: 10.56511/JIPBS.2016.v3.i01.pp198-203

Journal of Innovations in Pharmaceuticals and Biological Sciences
www.jipbs.com

JIPBS

ISSN: 2349-2759

Research article

Improvement of buffalo semen freezability by using TRIS extender enriched with different concentrations of trehalose/sucrose

Shahba, M.I.[1], El-Sheshtawy, R.I.[1*], El-Azab, A.I.[2]

[1]Animal Reproduction and AI dept., Veterinary Researches Division, NRC, Dokki, Giza, Egypt.
[2]Theriogenology dept., Fac.Vet.Med., Benha Univ., Egypt.

Abstract

The present study was designed to display the role of Trehalose / Sucrose addition to Tris-Fructose-Egg yolk-Glycerol extender on the rate of freezability and post-thawed characters of buffalo frozen semen. For extension and freezing, buffalo semen samples were extended in Tris-Fructose-Egg yolk-Glycerol without the addition of Trehalose / Sucrose as a control (TFEG-C) and with the addition of different concentrations of Trehalose or Sucrose. The best sperm motility, sperm livability, sperm abnormality, sperm cell membrane and DNA integrities appeared with TFEG-T100 mM/l (33.50±1.50%, 69.40±2.11%, 11.60±0.67%, 64.00±2.76%, 96.50±0.92 and 11.60±0.67%, respectively) and TFEG-S50 mM/l (33.00±2.81%, 68.80±2.25%, 10.40±0.54%, 66.10±2.68% and 95.60±1.44%, respectively). From the present study, it can be concluded that addition of Trehalose (100 mM/l) / sucrose (50 mM/l) to Tris-Fructose-Egg yolk-Glycerol extender might help in improvement of the post-thawed characteristics of buffalo frozen semen.

Key words: Buffalo, Semen, Freezability, Extender, Trehalose, Sucrose.

***Corresponding Author: El-Sheshtawy, R.I.,** Animal Reproduction and AI dept., Veterinary Researches Division, NRC, Dokki, Giza, Egypt.

1. Introduction

It is well known that the composition of the extender, suitable cryoprotectants and optimal freezing and thawing rates are important factors for successful semen cryopreservation [1]. The quality of frozen semen is the most influencing factor for conception rate [2]. It has been reported that cryopreservation process leads to the generation of reactive oxygen species (ROS) that impair sperm motility, membrane integrity and fertilizing ability [3-5]. These changes are due to oxidative and osmotic stresses [6,7]. Accordingly, the beneficial effects of sugars in the extender, on the post-thaw sperm viability of mammalian species, have been suggested earlier [8, 9, 10]. Trehalose as a membrane-protecting disaccharide confers a greater cryoprotective capacity to the basic extender when added up to 100 mM. Such addition results in an improvement in the sperm motile activity and in vivo fertility [11]. Sperm plasma and acrosomal membranes are especially important with regard to survival following thawing and it is considered that there are changes on the

©JIPBS, All rights reserved

primary organelles caused by cryopreservation process. Naing et al. [12] stated that combination of monosaccharide (glucose) and disaccharide (trehalose) improved semen quality following cryopreservation and better improvement was observed when trehalose was supplemented with concentration 198.24 mM to the glucose extender in goat semen. A combination of 100 mM trehalose and 5% glycerol was an adequate combination for achieving post-thawing semen quality [13]. The addition of trehalose to the freezing extender leads to the reduction of cryo-damage of buffalo spermatozoa [14].

The present study was designed to display the role of Trehalose / Sucrose addition to Tris-Fructose-Egg yolk-Glycerol extender on the rate of freezability and post-thawed characters of buffalo frozen semen.

2. Materials and Methods

Semen was collected from five mature buffalo-bulls kept in Abbasia frozen semen Center, General Organization for Veterinary Services, Ministry of agriculture, Egypt, by using an artificial vagina. Immediately after collection, semen samples were held in a water bath adjusted at 37° C. After evaluation, only semen samples with at least initial sperm motility of 70%, and normal sperm of 80% were used for further processing. Visual motility, sperm livability, sperm abnormalities and sperm membrane integrity were assessed [15]. For extension, Tris-Fructose-Egg yolk-Glycerol (TFEG) was utilized as described by Foote [16]. Semen samples were pooled and divided into 7 fractions; one diluted with the basic control extender (TFEG-C) and other aliquots of pooled semen samples were diluted with TRIS-Fructose-Egg yolk-Glycerol (TFEG) extender containing the different concentrations of Trehalose or Sucrose according to Woelders et al. [17] and liu et al. [18] with some modifications, in order to provide a concentration of 60 million sperm/ml, as shown in table 1.

Table 1.Tris-Fructose-Egg yolk-Glycerol (TFEG) with the addition of different concentrations of trehalose (T) or sucrose (S).

Ingredients (g/100ml)	Experimental extenders					
	TFEG-T 50mM/l	TFEG-T 100mM/l	TFEG-T 200mM/l	TFEG-S 50mM/l	TFEG-S 100mM/l	TFEG-S 200mM/l
Tris	2.295	2.295	2.295	2.295	2.295	2.295
Citric acid	1.295	1.295	1.295	1.295	1.295	1.295
Fructose	0.999	0.999	0.999	0.999	0.999	0.999
Glycerol %	7.300	7.300	7.300	7.300	7.300	7.300
Trehalose	1.700	3.400	6.800	-	-	-
Sucrose	-	-	-	1.700	3.400	6.800
Egg yolk %	20	20	20	20	20	20

All media contained 0.475 g/L sodium penicillin, 0.8 g/L streptomycin sulfate

After freezing and thawing in water bath adjusted at 37 ˚C for 30 sec., estimation of the post-thawed sperm motility, livability and abnormalities [19] as well as the sperm membrane integrity by using the HOST [20] and DNA integrity by using Acridine orange staining technique [21] were adopted as described by Shahba [22].

The obtained data were tabulated and computed for statistical analysis, where

appropriate, according to the SPSS® program version 10 (1999). Mean ± SEM, ANOVA and LSD were calculated to deduce the effect and the most efficient concentrations of Trehalose / Sucrose on the post-thawed characteristics of buffalo spermatozoa.

3. Results and Discussion

Results

The present results (Table, 2) revealed significant differences in the post-thawed characteristics of buffalo semen with the addition of Trehalose/Sucrose to the extender. The best sperm motility, sperm livability, sperm abnormality, sperm cell membrane and DNA integrities appeared with TFEG-T 100mM/l (33.50±1.50%, 69.40±2.11%, 11.60±0.67%, 64.00±2.76%, 96.50±0.92 and 11.60±0.67%, respectively) and TFEG-S50 mM/l (38.00±2.00%, 72.10±2.42%, 10.10±0.52%, 66.60±2.13% and 98.10±0.66%, respectively).

Discussion

The current study indicated the presence of some improvement in characteristics of buffalo frozen semen, a finding which came in agreement with that observed in some earlier reports [9, 23, 24].

Table 2. Effects of different trehalose and sucrose concentrations on sperm assessment parameters of frozen buffalo semen (Means ± SEM)

Extender	Sperm parameters (%)				
	Motility	Livability	Abnormality	Membrane integrity	DNA integrity
TFEG-C	34.00±1.94^{b}	70.80±1.86^{a}	09.70±0.49^{c}	57.30±2.27^{b}	97.00±1.06^{a}
TFEG-T50mM/l	32.50±1.53^{b}	68.00±1.71^{a}	14.40±0.82^{a}	62.10±3.31^{a}	87.20±8.60^{c}
TFEG-T100mM/l	33.50±1.50^{b}	69.40±2.11^{a}	11.60±0.67^{b}	64.00±2.76^{a}	96.50±0.92^{a}
TFEG-T200mM/l	30.50±1.57^{b}	66.60±1.90^{b}	12.60±0.67^{a}	59.20±3.00^{b}	95.00±0.93^{b}
TFEG-S50mM/l	38.00±2.00^{a}	72.10±2.42^{a}	10.10±0.52^{b}	66.60±2.13^{a}	98.10±0.66^{a}
TFEG-S100mM/l	33.00±2.81^{b}	68.80±2.25^{a}	10.40±0.54^{b}	66.10±2.68^{a}	95.60±1.44^{b}
TFEG-S200mM/l	28.50±1.50^{a}	68.10±2.21^{a}	13.30±1.23^{a}	62.00±3.70^{a}	96.70±0.97^{a}

Values within the same column with different letters differed significantly at least at $P < 0.05$

It has been found that the improved quality of frozen semen, on addition of trehalose or sucrose to the extender, is due to the osmotic changes reducing all injury caused by ice crystallization as non-permeable substances rendering hypertonic media enough to decrease intracellular freezable water [11, 25]. Trehalose / Sucrose interact with the membrane phospholipids and proteins providing the membrane more flexibility against cryo-injuries [26-29]. Sugars also act as a source of energy for spermatozoa by generating energy from intracellular ATP leading to improved post-thaw sperm motility [23,30,31]. A synergistic effect between glycerol as cryoprotectant and trehalose as a non-permeating cryoprotectant in semen extender is more beneficial than of single cryoprotectant [32]. In the present study, addition of Trehalose / Sucrose to buffalo frozen semen extender appeared to have nearly the same effect on the post-thawed quality of buffalo spermatozoa, a finding which came in accordance with that observed in semen cryopreservation of buffalo [31], ram [33] and boar [1]. However, in some previous studies adopted on semen cryopreservation of ram [11, 34, 35], goat [36] and bull [17, 37], there was a

greater cryoprotectant capacity for trehalose than that for sucrose as indicated by the sperm motility and sperm cell membrane integrity after thawing.

From the present study, it can be concluded that addition of Trehalose (100 mM/l) / Sucrose (50 mM/l) to Tris-Fructose-Egg yolk-Glycerol extender might help in improvement of the post-thawed characteristics of buffalo frozen semen.

References

1. Malo, C., L. Gil, N. Gonzalez, I. Cano de Blas and R.E. Espinosa. Comparing sugar type supplementation for cryopreservation of boar semen in egg yolk based extender. Cryobiology 2010; 61:17–21.
2. Saacke, R.G., S. Nadir, J.C. Dalton, J.H. Bame, J.M. De Jarnette, S. Degelos. Accessory sperm evaluation and bull fertility: an update. Proc. 15th Tech. Conf. Artif. Insem. and Reprod. Nat'l. Assoc. Animal Breeders, Columbia, 1994 MO, 57-67.
3. Bilodeau, J.F., S. Blanchette, C. Gagnon and M.A. Sirad. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol. Reprod. Dev. 2000; 55:282–288.
4. Gadea, J., E. Selles, M.A. Marco, P. Copy, C. Matas, R. Romar, and S. Ruiz. Decrease in glutathione content in boar sperm cryopreservation. Effect of the addition of reduced glutathione to the freezing and thawing extenders. Theriogenology 2004; 62: 690–701.
5. Hu, J.H., L.S. Zan, X.L. Zhao, Q.W. Li, Z.L. Jiang, Y.K. Li and X. Li. Effects of trehalose supplementation on semen quality and oxidative stress variables in frozen–thawed bovine semen. J. Anim. Sci. 2010; 88:1657–1662.
6. Hammerstedt, R.H., J.K. Graham and J.P. Nolan. Cryopreservation of mammalian sperm: what we ask them to survive. J. Androl. 1990; 11: 73–88.
7. Watson, P.F. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reprod. Fertil. Dev. 1995; 7: 871–891.
8. Garcia, M.A. and E.F. Graham. Development of a buffer system for dialysis of bovine spermatozoa before freezing. II. Effect of sugars and sugar alcohols on post-thaw motility. Theriogenology 1989; 31: 1029–1037.
9. Molinia, F., G. Evans, P. Quintana Casares, and W. Maxwell. Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim. Reprod. Sci. 1994; 36: 113–122.
10. Garde, J.J., A.A. del Olmo, J. Soler, G. Espeso, M.Gomendio and E.R. Roldan. Effect of egg yolk, cryoprotectant, and various sugars on semen cryopreservation in endangered Cuvier's gazelle (Gazella cuvieri). Anim. Reprod. Sci. 2008; 108: 384–401.
11. Aisen, E.G., V.H. Medina and A. Venturino. Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. Theriogenology 2002; 57: 1801–1808.
12. Naing, S.W., H. Wahid, K. Mohd Azam, Y. Rosnina, A.B. Zuki, S. Kazhal, M.M. Bukar, M. Thein, T. Kyaw and M.M. San. Effect of sugars on characteristics of Boer goat semen after cryopreservation Anim. Reprod. Sci. 2010; 122; 23–28.
13. Najafi A, M. Zhandi, A. Towhidi, M. Sharafi, S.A. Akbari and M.M. Khodaei. Trehalose and glycerol have a dose-dependent synergistic effect on the post-thawing quality of ram semen cryopreserved in a soybean lecithin-based extender. Cryobiology 2013; 66:275-282.
14. Shiva Shankar Reddy, N., G. Jagan Mohanarao and S.K. Atreja. Effects of adding taurine and trehalose to a tris based egg yolk extender on buffalo (Bubalus bubalis) sperm quality following cryopreservation. Anim. Reprod. Sci. 2010; 119 (3-4): 183-190.
15. Graham, E.F., M.K.L. Schmehl and M. Maki-Lauria. Some physical and chemical methods of evaluating semen. Proceeding

of the 3rd NAAB Technology Conference of Artificial Insemination and Reproduction, Milwaukee, W.I., National Association of Animal Breeding, Columbia, Mo. 1970.
16. Foote, R.H. Fertility of bull semen at high extension rates in Tris buffered extenders. J. Dairy Sci. 1970; 53: 1475–1477.
17. Woelders, H., A. Matthijs and B. Engel. Effects of trehalose and sucrose, osmolality of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing, Cryobiology 1997;35: 93–105.
18. Liu, Z., R.H. Foote and C.C. Brockett. Survival of bull sperm frozen at different rates in media varying in osmolarity. Cryobiology. 1998; 37: 219–230.
19. Campbell, R.C, H.M. Dott and T.D. Glover. Nigrosin-Eosin as a stain for differentiating live and dead spermatozoa. J. Agric. Sci. 1956; 48: 1-8.
20. Jeyendran RS, H.H. Van Derv Ven, M. Perez-Pelaes, B.G. Crabo and L.J.D. Zaneveld. Development of an assay the functional integrity of human sperm membrane and its relationship to other semen characteristics. J. Repro. Fertile. 1984; 70:219-28.
21. Tejada, R.I., J.C. Mitchell, A. Norman, J.J. Marik and S. Friedman. A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil. Steril. 1984; 42:87–91.
22. Shahba, M.I. Study of some factors affecting efficiency of frozen buffalo semen. M.S.Sc. Thesis, Benha Univ. 2010.
23. Aboagla, E.M. and T. Terada. Trehalose-enhanced fluidity of the goat sperm membrane and its protection during freezing. Biol. Reprod. 2003; 69: 1245–1250.
24. Hu, J.H., Q.W. Li, G. Li, Z.L. Jiang, S.H. Bu, H. Yang and L.Q. Wang. The cryoprotective effect of trehalose supplementation on boar spermatozoa quality. Anim. Reprod. Sci. 2009; 112:107–118.
25. Yildiz, C., K.M. Aksoy and T. Tekeli. Influence of sugar supplementation of the extender on motility, viability and acrosomal integrity of dog spermatozoa during freezing. Theriogenology 2000; 54:579–585.
26. Iwashi, H., K. Obuchi, S. Fuji and Y. Komatsu. The correlative evidence suggesting that trehalose stabilizes membrane structure in the yeast Saccharomyces cerevisiae. Cell Mol. Biol. 1995; 41:763–769.
27. Dalimata, A.M. and J.K. Graham. Cryopreservation of rabbit spermatozoa using acetamide in combination with trehalose and methyl cellulose. Theriogenology 1997; 48: 831-841.
28. Bucak, M.N., A. Ateşşahin, Ö. Varışlı, A. YÜce, N. Tekin and A. Akçay. The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen: microscopic and oxidative stress parameters after the freeze–thawing process. Theriogenology. 2007; 67: 1060–1067.
29. Tuncer, P.B., T.D. Umut, B. Serhat, Ö. Taner, C.K. Erdem and E. Halil. Effects of different doses of trehalose supplementation in egg yolk extender in frozen–thawed Angora buck semen. Small Rum. Res. 2013; 113:383-389.
30. Fernandez-Santos, M.R., F. Martinez-Pastor, V. Garcia-Macias, M.C. Esteso, A.J. Soler, P. de Paz, L. Anel, and J.J. Garde. Extender osmolality and sugar supplementation exert a complex effect on the cryopreservation of Iberian red deer (Cervus elaphus hispanicus) epididymal spermatozoa. Theriogenology 2007; 67: 738–753.
31. Reddy S.S.N., G. Jagan Mohanarao and S.K. Atreja. Effects of adding Taurine and Trehalose to a Tris based egg yolk extender on buffalo (Bubalus bubalis) sperm quality following cryopreservation. Anim. Reprod. Sci. 2010; 119: 183–190.
32. Blanco, J.M., J.A. Long, G. Gee, D.E. Wildt and A.M. Donoghue. Comparative cryopreservation of avian spermatozoa: benefits of non-permeating osmoprotectants and ATP on turkey and crane sperm cryosurvival. Anim. Reprod. Sci. 2011; 123: 242–248.

33. Tonieto, R.A., K.L. Goularte, G.D.A. Gastal, R.S. Schiavon, J.C. Deschamps, T. Lucia Jr. Cryoprotectant effect of trehalose and low-density lipoprotein in extenders for frozen ram semen. Small Rum. Res. 2010; 93: 206–209.
34. Jafaroghli, M., B. Khalili, A. Farshad and M. Zamiri. The effect of supplementation of cryopreservation diluents with sugars on the post-thawing fertility of ram semen. Small Rumin. Res. 2011; 96: 58–63.
35. Pelufo, V., M.F. López Armengol, V. Malcotti, A. Venturino and E.G. Aisen. Effects of glycerol and sugar mixing temperature on the morphologic and functional integrity of cryopreserved ram sperm. Theriogenology 2015; 83: 144–151.
36. Aboagla, E.M. and T. Terada. Effects of the supplementation of trehalose extender containing egg yolk with sodium dodecyl sulfate on the freezability of goat spermatozoa. Theriogenology 2004; 62: 809–818.
37. Chen, Y., R.H. Foote and C.C. Brockett. Effect of sucrose, trehalose, hypotaurine, taurine, and blood serum on survival of frozen bull sperm. Cryobiology 1993; 30: 423–431.