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Research Article

Effect of Glutamine Addition to Freezing Extender on Crypreserved Semen Parameters and Subsequent Fertility of Egyptian Buffalo Bull

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Abstract

Objective: The aim of this study was to evaluate the effect of glutamine addition to the tris-extender on the quality of cryopreserved buffalo spermatozoa and subsequent fertility. **Methodology:** Semen ejaculates from six clinically normal and sexual mature Egyptian buffalo bulls were collected once weekly and pooled. Qualifying ejaculates were split into 6 aliquots for dilution (80×10^6 spermatozoa) with tris egg yolk extender containing 0, 20, 40, 60, 80 and 100 mM glutamine. The extended semen was packed into 0.25 mL French straws using a semen filling machine. Semen samples were cooled and equilibrated before cryopreservation. **Results:** The results showed that sperm progressive motility and viability of Egyptian buffalo bulls were significantly higher ($p<0.05$) in extenders containing 20, 40 and 60 mM glutamine than control and other glutamine extenders. The further increase in glutamine to 100 mM caused a significant reduction ($p<0.05$) in sperm progressive motility and viability. The addition of glutamine led to improve sperm abnormalities diluted in the all extenders tested. Addition of 20 or 40 mM glutamine significantly increased post-thawed acrosome and plasma membrane integrity. The highest fertility rate was recorded for spermatozoa diluted in extenders containing 20 mM glutamine (72.7%) followed by 40 mM (64.3%) and 60 mM glutamine (60%). **Conclusion:** It's concluded that the addition of 20-60 mM glutamine to tris-based extender improved the post-thaw quality of buffalo bull spermatozoa and subsequent fertility.

Key words: Glutamine, buffalo semen, cryopreservation, fertility

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Buffaloes are an important aspect of animal production in Egypt, about 90% of the buffalo populations are in herds of less than 5 animals owned by small farmers. Artificial Insemination (AI) has been used for increasing genetically the production potential of animals, whereas application of AI with frozen-thawed spermatozoa has been restricted in buffalo due to low freezing ability and fertility of buffalo spermatozoa[1]. Thus, improvement of buffalo sperm cryopreservation may enhance the breeding programs of this valuable farm animal. Freeze-thawing of the spermatozoa is associated with oxidative stress[2] which accelerates the production of Reactive Oxygen Species (ROS) molecules[3] due to plasma membrane lipid peroxidation[4]. Also, it has been observed that buffalo sperm are more sensitive to oxidative stress as compared to cattle spermatozoa[5,6] which may be due to higher contents of polyunsaturated phospholipids present in sperm membrane[7]. Over production of ROS molecules increases the damage to functional and structural integrity of the buffalo sperm during freezing-thawing process[5]. Moreover, freeze-thaw cycle reduces the level of indigenous antioxidant in mammalian semen[8]. Therefore, to protect the sperm integrity during freeze-thawing of buffalo spermatozoa, an extra-antioxidants supplementation is recommended[9]. Amongst the amino acids used for freezing animal semen, glutamine has proven to be effective in several species such as in man[10], stallions[11] and bull[12]. The objective of this study was to assess the effect of addition glutamine in semen extender on quality of frozen-thawed buffalo semen and its subsequent fertility.

MATERIALS AND METHODS

Animals and semen collection: The experiment was conducted at the International Livestock Management training Center (ILMTC), Sakha belonging to the Animal Production Research Institute, Ministry of Agriculture, Egypt. A total of 72 ejaculates from six mature Egyptian buffalo bulls aged 3-4 years with known fertility were collected by an artificial vagina one weekly for 12 weeks during October-December (Winter season). The volume of ejaculates was measured in a conical tube graduated at 0.1 mL intervals and sperm concentration was determined by means of Neubaur Heamocytometer according to the procedures described by Sansone *et al.*[7]. Ejaculates fulfilling minimum standard of sperm motility (75%) and sperm morphology (80%) were processed for freezing. The ejaculates were pooled in order to have sufficient semen to make a replicates and to eliminate bull effect.

Semen processing: Immediately after collection, the ejaculates were immersed in warm water bath at 37°C until the laboratory evaluation. Semen assessment was performed within 10 min. The reference of cryopreservation extender was tris (Buffer oxford Lab-Chem., India)-egg yolk (20% EY, control) containing 20 mL of egg yolk, 3.025 g tris (hydroxyl methyl amino-methane), 1.675 g citric acid, 0.75 g glucose, 7 mL glycerol, 0.25 g lincomycin, 0.005 g streptomycin and completed with up to 100 mL bi-distilled water. The glutamine (Sigma Chem. Aldrich, USA) was added to the tris-egg yolk based extender at different concentrations (0 "Control", 20, 40, 60, 80 and 100 mM, respectively). Qualifying semen ejaculates were split into 6 aliquots and diluted with the different experimental extenders at 37°C in order to provide concentration of 80 million of motile spermatozoa per milliiter. The diluted semen was cooled slowly to 5°C within 2 h and equilibrated for 4 h at the same temperature. Semen was packed into 0.25 mL French straws using a semen filling machine and kept on liquid nitrogen vapors 4 cm above its surface for 10 min. Then, straws were plunged into the liquid nitrogen, stored for one month. For thawing after one month of storage, straws were dipped into a water bath at 37°C for 30 sec, then the percentages of sperm progressive motility, viability, abnormal morphology, plasma membrane and acrosome integrity were determined.

Semen evaluation: Sperm progressive motility was estimated using phase contrast microscope (×400 magnification) by placing semen sample on pre-warmed (37°C) glass slide and covered with a cover slip. Sperm plasma membrane integrity (%) was assessed using hypo-osmotic swelling test (HOST) as outlined by Jeyendran *et al.*[13]. One hundred spermatozoa were assessed and the percentage of spermatozoa with curled tail (swollen intact plasma membrane) was calculated. Sperm viability, abnormalities and acrosome integrity were determined by dual staining procedure[14] using trypan blue (Sigma T 8154) with Giemsa stains (Sigma GS 500).

Fertility trial: The fertilizing ability of spermatozoa from each extender was calculated based on the rectal palpation at 45-60 days post insemination of 65 Egyptian buffalo cows. Buffalo cows were artificially inseminated with one insemination dose (20×10^6) of frozen samples with different glutamine levels and control one.

Statistical analysis: The experimental data were statistically analyzed using the general model program[15]. Data of semen parameters was subjected to repeated measurement according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + S(P)_{ijk} + (T*P)_{ij} + E_{ijk}$$

Where:

Y_{ijk} = Any observation
μ = Overall mean
T_i = The fixed effect of the estimate
P_j = The fixed effect of sampling period (1, 2,.....)
$S(P)_{ijk}$ = Sample within period
$(T*P)_{ij}$ = The fixed effect of the interaction between Period of sampling and treatment
E = Error

The least square mean was calculated by modulating the data higher than 70% and less than 30%. Differences with $p<0.05$ were considered significant.

RESULTS

Progressive motility and viability of Egyptian buffalo bull spermatozoa were significantly higher ($p<0.05$) in extenders containing 20, 40 and 60 mM glutamine than those with 0, 80 and 100 mM at all stages of cryopreservation (Fig. 1 and 2).

The farther increase in glutamine to 100 mM caused a significant reduction in the progressive motility and viability of buffalo spermatozoa as compared with control and other glutamine extenders at all stages of cryopreservation. The addition of glutamine improved sperm abnormalities at all concentrations tested (from 20-100 mM) at any stage of cryopreservation (Fig. 3).

The addition of 20, 40 and 60 mM glutamine to freezing extender was more effective than the other glutamine levels

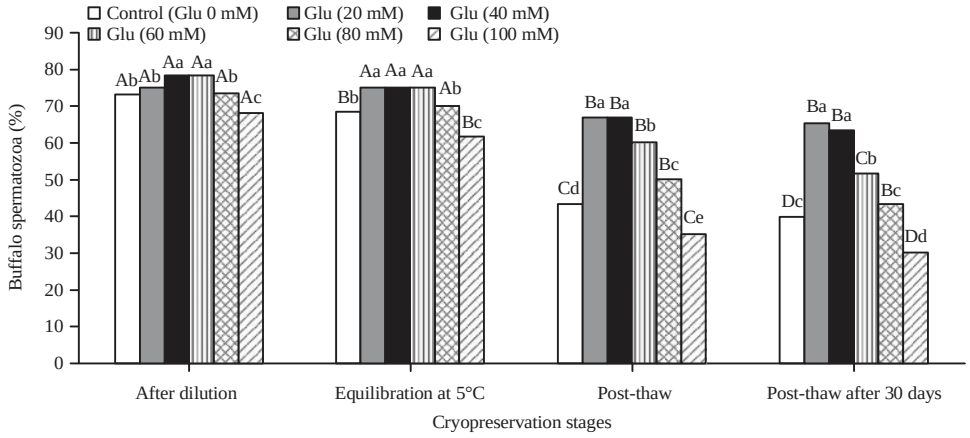

Fig. 1: Progressive motility of buffalo spermatozoa (%) at different stages of cryopreservation. The values with different letters are significantly different from one another ($p<0.05$) between treatments (a, b, c,...) and between cryopreservation stages (A, B, C,...), respectively

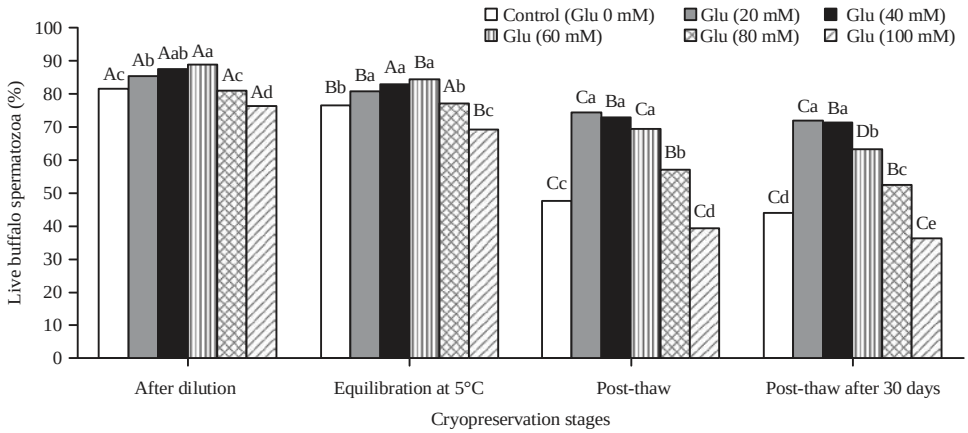

Fig. 2: Live buffalo spermatozoa (%) at different stages of cryopreservation. The values with different letters are significantly different from one another ($p<0.05$) between treatments (a, b, c,....) and between cryopreservation stages (A, B, C,...), respectively

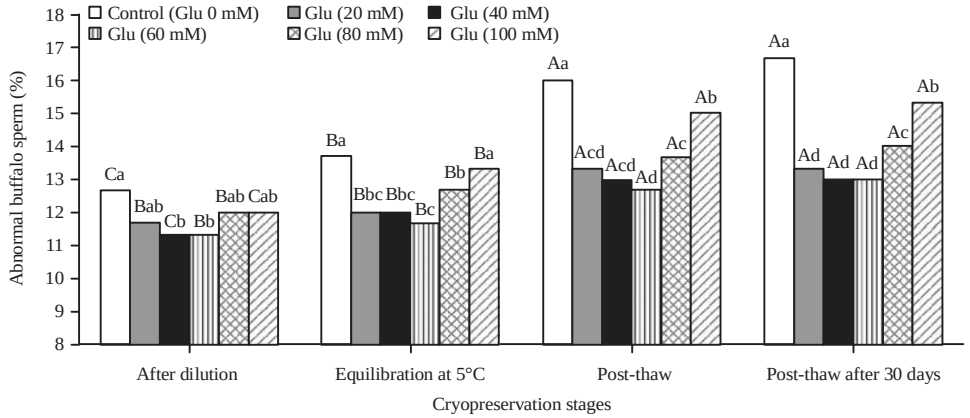

Fig. 3: Abnormal buffalo sperm (%) at different stages of cryopreservation. The values with different letters are significantly different from one another ($p<0.05$) between treatments (a, b, c,....) and between cryopreservation stages (A, B, C,...), respectively

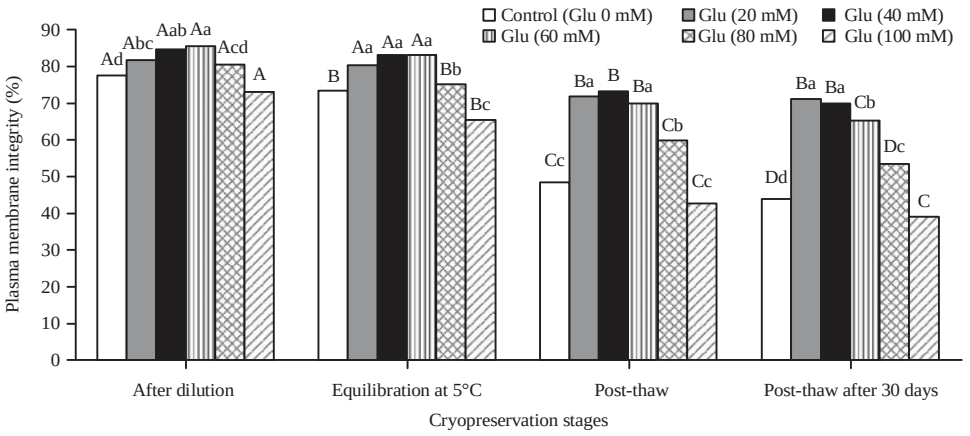

Fig. 4: Plasma membrane integrity (%) of buffalo sperm at different stages of cryopreservation. The values with different letters are significantly different from one another ($p<0.05$) between treatments (a, b, c,...) and between cryopreservation stages (A, B, C,...), respectively

on decreasing of sperm buffalo abnormalities after cooling and freezing-thawing process. The percentages of acrosome and plasma membrane integrity showed higher ($p<0.05$) in extender containing 60 mM after dilution and equilibration while, adding 20 and 40 mM glutamine significantly ($p<0.05$) increased sperm acrosome and membrane integrity at post-thawing process (Fig. 4 and 5). Whereas, the increase of glutamine to 100 mM led to a significant reduction in both acrosome and membrane integrity of buffalo spermatozoa in compared with that extended in other glutamine concentrations and control extenders.

The fertility rate was statistically higher ($p<0.05$) for spermatozoa diluted in extender containing 20 mM glutamine (72.7%) followed by that diluted in 40 mM (64.3%) and 60 mM (60%) extenders in compared with those diluted in 80 (50%), 100 mM (30%) and 0 mM control (40%) (Fig. 6).

DISCUSSION

The increase in sperm sensibility to oxidative stress and cryodamage affect spermatozoa quality, shortening their life span resulting in a reduction in sperm motility, viability, antioxidant enzyme activity and fertilizing capacity during extending, freezing and thawing process[16]. Frozen-thawed bull semen is more easily peroxidized than fresh semen. Additionally, intracellular antioxidant capacity in sperm decreases following freezing-thawing process[17].

In the present study, progressive motility of buffalo bull spermatozoa was high when the extender was supplemented with 20-60 mM glutamine than that in control one. The further increase in glutamine concentration to 100 mM in extender significantly decreased sperm motility percentage. These results are inline with that in a previous study on

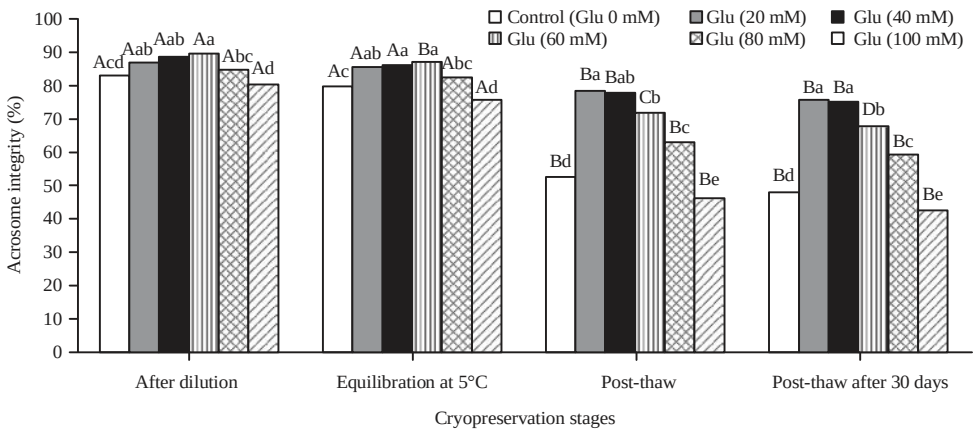

Fig. 5: Acrosome integrity (%) of buffalo sperm at different stages of cryopreservation. The values with different letters are significantly different from one another ($p<0.05$) between treatments (a, b, c,...) and between cryopreservation stages (A, B, C,...), respectively

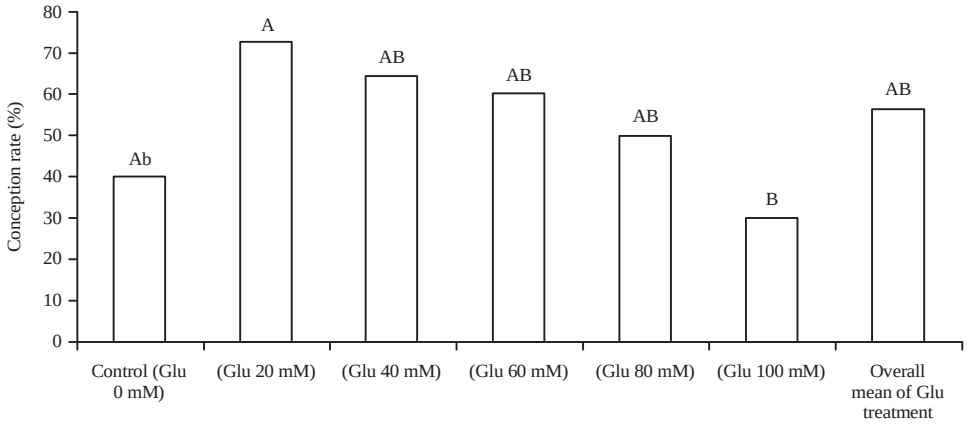

Fig. 6: Conception rate (%) of buffalo sperm. The values with different letters are significantly different from one another ($p<0.05$) between treatments (A, B, C,...)

cryopreserved stallion semen which indicated that glutamine addition to the INRA 82 extender significantly increased the motility parameters of stallion spermatozoa after freezing and thawing for the concentration of 30-80 mM and become ineffective when added at concentration of 120 and 160 mM resulted in a significant decrease in sperm motility[18]. Also, El-Sheshtawy *et al.*[19] noticed that 25 mM glutamine offered protection for frozen-thawed buffalo spermatozoa but the higher concentration of glutamine (50 mM) in extender did not improve the results whereas, the 100 mM concentration significantly decreased sperm motility. It is believed that glutamine improves the progressive motility of buffalo bull spermatozoa by reducing the Reactive Oxygen Species (ROS) in the semen extender which are responsible for reducing lipid peroxidation of bio-membrane system that associated with sperm motility during the freeze-thawing process[20-22]. Sperm viability and intact acrosome are responsible to achieve capacitation, acrosome reaction and final fertilization[14,23]. In the present study, viability of buffalo bull spermatozoa was higher in samples cryopreserved in tris-egg yolk extender containing 20-60 mM glutamine in compared to the control extender, whereas, the presence of 100 mM significantly decreased sperm viability. In addition, higher intact acrosome percentages were observed in cryopreserved buffalo bull spermatozoa due to addition of glutamine from 20-60 mM. These results were in agreement with that of Bucak *et al.*[24] in Ovine semen and Ansari[20] in buffalo semen. Bucak *et al.*[25] suggested that the addition of amino acid to the semen extender maintain the viability of buffalo spermatozoa by coping with ROS levels through increasing the intracellular activity of antioxidants. Glutamine has an extracellular mechanism of action. An increase in the

concentration glutamine in extender leads to increase in osmotic pressure[26]. When the concentration gets too high, spermatozoal motility was compromised and this was confirmed in the present study. Watson and Martin[27] suggested that a dual glutamine action was seen: The first is extracellular and cryoprotective, the second is intracellular and metabolic with the intervention of glutamine in the Krebs cycle via glutamine and α-cetoglutate. In the present study, all glutamine concentrations in extenders causes a significant decrease in abnormal and sperm plasma membrane damage at all stages of cryopreservation process compared to the control. Current results with respect to sperm parameters are in agreement with that of Trimeche[11] on Poitou jackass, Li *et al*.[28] on cynomonolgus monkey, Khlifaoui *et al*.[29] on stallion, El-Sheshtawy[19] and Topraggaleh *et al*.[30] on buffaloes and Sariozkan[31] on rabbit. With respect to fertility results based on 45-60 day rectal palpation after AI, the addition of glutamine at doses ranging from 20-60 mM in semen extender led to have a higher fertility rates compared to the control. While, adding 100 mM had adversely effect on fertility rate. These findings disagree with that of previous studies where there was no improvement in fertility rate when certain additives were added in semen extender[32,33,20,34].

CONCLUSION

The present results concluded that the addition of glutamine at doses ranging from 20-60 mM in tris freezing extender not only improved post-thaw motility of buffalo bull spermatozoa but also maintained sperm viability, abnormalities, acrosome and plasma membrane integrity during freezing-thawing process and enhanced the fertilizing ability of frozen-thawed buffalo spermatozoa.

REFERENCES

1. Andrabi, S.M.H., 2009. Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. Reprod. Domestic Anim., 44: 552-569.
2. Bilodeau, J.F., S. Chateerjee, M.A. Sirrard and C. Gagnon, 2000. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol. Reprod. Dev., 55: 282-288.
3. Baumber, J., B.A. Ball and J. Linfor, 2005. Assessment of the cryopreservation of equine spermatozoa in the presence of enzyme scavengers and antioxidants. Am. J. Vet. Res., 66: 772-779.
4. Kankofer, M., G. Kolm, J. Aurich and C. Aurich, 2005. Activity of glutathione peroxidase, superoxide dismutase and catalase and lipid peroxidation intensity in stallion semen during storage at 5° C. Theriogenology, 63: 1354-1365.
5. Kumaresan, A., M.R. Ansari and A. Garg, 2005. Modulation of post-thaw sperm functions with oviductal proteins in buffaloes. Anim. Reprod. Sci., 90: 73-84.
6. Nair, S.J., A.S. Brar, C.S. Ahuja, S.P.S. Sangha and K.C. Chaudhary, 2006. A comparative study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Anim. Reprod. Sci., 96: 21-29.
7. Sansone, G., M.J.F. Nastri and A. Fabbrocini, 2000. Storage of buffalo (*Bubalus bubalis*) semen. Anim. Reprod. Sci., 62: 55-76.
8. Stradaioli, G., T. Noro, L. Sylla and M. Monaci, 2007. Decrease in glutathione (GSH) content in bovine sperm after cryopreservation: Comparison between two extenders. Theriogenology, 67: 1249-1255.
9. Ansari, M.S., B.A. Rakha, N. Ullah, S.M.H. Andrabi, M. Khalid and S. Akhter, 2011. Effect of L-cysteine in tris-citric egg yolk extender on post-thaw quality of Nili-Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. Pak. J. Zool., 43: 41-47.
10. Renard, P., G. Grizard, J.F. Griveau, B. Sion, D. Boucher and D. Le Lannou, 1996. Improvement of motility and fertilization potential of postthaw human sperm using glutamine. Cryobiology, 33: 311-319.
11. Trimeche, A., J.M. Yvon, M. Vidament, E. Palmer and M. Magistrini, 1999. Effects of glutamine, proline, histidine and betaine on post-thaw motility of stallion spermatozoa. Theriogenology, 52: 181-191.
12. Amirat-Briand, L., D. Bencharif, O. Vera-Munoz, H.B.H. Ali and S. Destrumelle *et al*., 2009. Effect of glutamine on post-thaw motility of bull spermatozoa after association with LDL (low density lipoproteins) extender: Preliminary results. Theriogenology, 71: 1209-1214.
13. Jeyendran, R.S., H.H. van der Ven, M. Perez-Pelaez, B.G. Crabo and L.J.D. Zaneveld, 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fert., 70: 219-228.
14. Kovacs, A. and R.H. Foote, 1992. Viability and acrosome staining of bull, boar and rabbit spermatozoa. Biotechnic Histochem., 67: 119-124.
15. SAS., 1999. Statistical Analysis System User's Guide. Statistical Analysis Institute Inc., Carry, NC., USA.
16. Hammerstedt, R.H., 1993. Maintenance of bioenergetic balance in sperm and prevention of lipid peroxidation: A review of the effect on design of storage preservation systems. Reprod. Fertil. Dev., 5: 675-690.
17. Trinchero, G.D., M.A. Affranchino, L.M. Schang and M.T. Beconi, 1990. Antioxidant effect of bovine spermatozoa on lipid peroxidation. Comp. Biol., 8: 339-350.
18. Trimeche, A., P. Renard, D. Le Lannou, P. Barriere and D. Tainturier, 1996. Improvement of motility of post-thaw Poitou jackass sperm using glutamine. Theriogenology, 45: 1015-1027.

19. El-Sheshtawy, R.I., G.A. El-Sisy and W.S. El-Nattat, 2008. Use of selected amino acids to improve buffalo bull semen cryopreservation. Global Veterinaria, 2: 146-150.
20. Ansari, M.S., B.A. Rakha, M.F. Malik, S.M.H. Andrabi and N. Ullah *et al*., 2016. Effect of cysteine addition to the freezing extender on the progressive motility, viability, plasma membrane and DNA integrity of Nili-Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. J. Applied Anim. Res., 44: 36-41.
21. Urata, K., H. Narahara, Y. Tanaka, T. Egashira, F. Takayama and I. Miyakawa, 2001. Effect of endotoxin-induced reactive oxygen species on sperm motility. Fertil. Steril., 76: 163-166.
22. Garg, A., A. Kumaresan and M.R. Ansari, 2008. Effects of hydrogen peroxide (H_2O_2) on fresh and cryopreserved buffalo sperm functions during incubation at 37°C *in vitro*. Reprod. Domestic Anim., 44: 907-912.
23. Tartaglione, C.M. and M.N., Ritta, 2004. Prognostic value of spermatological parameters as predictors of *in vitro* fertility of frozen-thawed bull semen. Theriogenology, 62: 1245-1252.
24. Bucak, M.N., A. Atessahin and A. Yuce, 2008. Effect of anti-oxidants and oxidative stress parameters on ram semen after the freeze-thawing process. Small Rumin. Res., 75: 128-134.
25. Bucak, M.N., P.B. Tuncer, S. Sariozkan, N. Baspinar and M. Taspınar *et al*., 2010. Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: Antioxidants protect DNA integrity against cryodamage. Cryobiology, 61: 248-253.
26. Bencharif, D., L. Amirat-Briand, J. Le Guillou, A. Garand and M. Anton *et al*., 2013. Canine-chilled sperm: Study of a semen extender made with low-density lipoproteins from hen egg yolk supplemented with glutamine. Reprod. Domestic Anim., 48: 258-266.
27. Watson, P.F. and I.C.A. Martin, 1975. The influence of some fractions of egg yolk on the survival of ram spermatozoa at 5° C. Aust. J. Biol. Sci., 28: 145-152.
28. Li, Y., W. Si, X. Zhang, A. Dinnyes and W. Ji, 2003. Effect of amino acids on cryopreservation of cynomolgus monkey (*Macaca fascicularis*) sperm. Am. J. Primatol., 59: 159-165.
29. Khlifaoui, M., I. Battut, J.F. Bruyas, G. Chatagnon, A. Trimeche and D. Tainturier, 2005. Effects of glutamine on post-thaw motility of stallion spermatozoa: An approach of the mechanism of action at spermatozoa level. Theriogenology, 63: 138-149.
30. Topraggaleh, T.R., A. Shahverdi, A. Rastegarnia, B. Ebrahimi and V. Shafiepour *et al*., 2014. Effect of cysteine and glutamine added to extender on post-thaw sperm functional parameters of buffalo bull. Andrologia, 46: 777-783.
31. Sariozkan, S., S. Ozdamar, G. Turk, F. Canturk and A. Yay, 2014. *In vitro* effects of L-carnitine and glutamine on motility, acrosomal abnormality and plasma membrane integrity of rabbit sperm during liquid-storage. Cryobiology, 68: 349-353.
32. Sariozkan, S., M.N. Bucak, P.B. Tuncer, P.A. Ulutas and A. Bilgen, 2009. The influence of cysteine and taurine on microscopic-oxidative stress parameters and fertilizing ability of bull semen following cryopreservation. Cryobiology, 58: 134-138.
33. Sarıozkan, S., P.B. Tuncer, M.N. Bucak and P.A. Ulutas, 2009. Influence of various antioxidants on microscopic-oxidative stress indicators and fertilizing ability of frozen-thawed bull semen. Acta Vet. Brno, 78: 463-469.
34. Buyukleblebici, S., P.B. Tuncer, M.N. Bucak, A. Eken, S. Sariozkan, U. Tasdemir and B.U. Endirlik, 2014. Cryopreservation of bull sperm: Effects of extender supplemented with different cryoprotectants and antioxidants on sperm motility, antioxidant capacity and fertility results. Anim. Reprod. Sci., 150: 77-83.