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Research article

Freezability of buffalo semen by using tris extender supplemented with different concentrations of LDL

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Abstract

The present study aimed at displaying the effect of LDL, as a cryopreservative, on the quality of the frozen-thawed buffalo semen. For buffalo semen extension, Tris-Citric acid-Fructose-Egg yolk extender was used as a control. Instead of Egg yolk, LDL fraction extracted from the Egg yolk would be added to the extender at6, 8, 10, and 12 % providing a concentration of 60 million sperm/ml. From the obtained results, the best semen quality was observed with the addition of 12% LDL to the frozen semen extender, as expressed by the highest sperm motility (32.50±1.50 %), sperm livability (72.00± 1.82 %), sperm cell membrane (69.10 ±1.84 %) and DNA (98.90 ±0.91 %) integrities as well as the lowest sperm abnormalities (09.10 ±1.48 %). From the present study, the use of LDL at a concentration of 12% in buffalo frozen semen extender might be recommended to have better sperm motility, sperm livability and sperm abnormalities as well as sperm cell membrane and DNA integrities.

Key words: Buffalo, Semen, Freezability, Extender, Egg yolk, LDL

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1. Introduction

Artificial insemination (AI) is widely extended, for genetic improvement, after banking of the frozen semen [1].However, the genetic impact in AI industry is limited by the efficiency of semen production. About 40-50 % of viable sperm lose their functional integrity during the freezing-thawing process [2, 3]. The cryo-damage induced by the cryopreservation could be minimized and significantly improved by inventing the optimal cooling rate and efficient cryoprotectant [4, 5]. It has been reported that the low density lipoprotein (LDL) is protective against cold shock, and hence, improves the sperm motility, acrosome and plasma membrane integrity as well as better protection against the sperm DNA fragmentation[6-9]. LDL has a cryo-protective property by adhering to the sperm cell membrane [10-12] and reduces the sperm membrane modifications [6,12] during the freezing-thawing process. Although cryopreservation of buffalo spermatozoa has been performed routinely,

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improvement of the existing cryopreservation protocols for buffalo spermatozoa is still targeted. The present study aimed at displaying the effect of LDL, in buffalo frozen semen extender, on the post-thaw sperm motility, sperm livability, acrosome and sperm cell membrane and DNA integrities.

2. Materials and Methods

Semen was collected from five mature buffalo-bulls by using an artificial vagina in Abbasia Frozen Semen Center, General Organization for Veterinary Services, Ministry of agriculture, Egypt. Immediately after collection, semen samples were kept in a water bath at 37° C for at least 5 min before evaluation. Only semen samples with at least 70% initial sperm motility and 80% normal sperm would be used for freezing. For extension, Tris-Citric acid-Fructose-Egg yolk extender [13] was used as a control. Instead of Egg yolk, LDL fraction extracted from the Egg yolk [6], would be added to the extender at6, 8, 10, and 12 %providing a concentration of 60 million sperm/ml. After freezing and thawing of semen in water bath at 37°C for 30 sec, evaluation of the sperm motility, livability and abnormalities [14],the sperm membrane integrity by using the HOST [15] and the sperm DNA integrity by using the Acridine orange stain [16] were adopted as described by Shahba[17]. The obtained data were tabulated and computed for statistical analysis by SPSS® program, version 10 (1999) to assess, where appropriate, Mean ± SEM, ANOVA and LSD for the different sperm parameters at different concentrations of LDL when compared to those in the control.

3. Results and Discussion

Results

As shown table 1, with the exception of the sperm abnormalities, the present results did not show any significant difference in the post-thaw sperm motility and sperm livability as well as the sperm cell membrane and DNA integrities. The best semen quality was observed with the addition of 12% LDL to the frozen semen extender, as expressed by the highest sperm motility (32.50 ± 1.50 %), sperm livability (72.00± 1.82 %), sperm cell membrane (69.10 ±1.84 %) and DNA (98.90 ±0.91 %) integrities as well as the lowest sperm abnormalities (09.10 ±1.48 %).

Table 1. Effect of the LDL content of buffalo frozen semen extender on the quality of buffalo frozen spermatozoa after thawing at 37°C for 30 sec.

LDL content	Sperm parameters (%)				
	Motility	Livability	Abnormality	membrane integrity	DNA integrity
Egg yolk	31.50±2.36^{a}	71.80±1.65^{a}	10.00± 1.00^{b}	65.20 ±1.92^{a}	98.40 ± 0.73^{a}
6%	29.50±1.90^{a}	69.80±1.51^{a}	13.20± 1.20^{a}	66.10 ±2.00^{a}	97.70 ± 1.00^{a}
8%	28.50±1.50^{a}	70.10±2.57^{a}	15.80 ±0.82^{a}	64.10 ±2.58^{a}	97.90 ± 0.68^{a}
10%	31.00±2.08^{a}	69.40±1.63^{a}	11.60 ±1.40^{b}	65.50 ±2.63a	97.20 ±0.68^{a}
12%	32.50±1.50^{a}	72.00±1.82^{a}	09.10 ±1.48^{b}	69.10 ±1.84^{a}	98.90 ±0.91^{a}

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

Discussion

LDL has been approved to be the effective part of egg yolk in cryopreservation of semen [8,18-20].The present study revealed that LDL addition to buffalo frozen semen extender offered slight better protection than egg yolk, a finding which came in agreement with some previous reports [6,7, 9, 21] indicating that LDL possesses remarkable cryopreservative properties. This effect was clear, especially when added at a concentration of 12% as expressed in terms of the sperm motility, livability, and abnormalities as well as the sperm cell membrane and DNA integrities. However, Munoz et al. [22] and Amirat-Briand et al. [23] noticed that the use of 8% LDL in Tris extender was more effective in the preservation of motility and plasma membrane integrity of bull spermatozoa. It has been suggested that LDL promotes incorporation of phospholipids and cholesterol into sperm membrane[12] and building a complex with seminal plasma proteins and making them unavailable to function in the cell membrane [12,24].

From the present study, the use of LDL at a concentration of 12% in buffalo frozen semen extender might be recommended to have better sperm motility, sperm livability, and sperm abnormalities as well as sperm cell membrane and DNA integrities.

References

1. Cardellino, R., I. Hoffmann and K.A. Tempelman.. First report on the state of the world's animal genetic resources: views on biotechnology as expressed in country reports, In: Proceedings of the FAO/IAEA International Symposium on the Applications of Gene-based Technologies for Improving Animal Production and Health in Developing Countries 2003; 12–14.
2. Prathalingam, N.S., W.V. Holt, S.G. Revell, S. Mirczuk, R.A. Fleck and P.F. Watson. Impact of antifreeze proteins and antifreeze glycoproteins on bovine sperm during freeze–thaw. Theriogenology 2006; 66: 1894–1900.
3. Celeghini, E.C.C., R.P. Arruda, A.F.C. Andrade, J. Nascimento, C.F. Raphael and P.H.M. Rodrigues. Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. Anim. Reprod. Sci. 2007; 100: 1–13.
4. Holt, W.V. Basic aspects of frozen storage of semen. Anim. Reprod. Sci. 2000; 62: 3–22.
5. Johnson, L.A., K.F. Weitze, P. Fiserand W.M.C. Maxwell. Storage of boar semen. Anim. Reprod. Sci. 2000; 62: 143–172.
6. Moussa, M., V. Martinet, A. Trimeche, D. Tainturier and M. Anton.. Low-density lipoproteins extracted from hen egg yolk by an easy method cryoprotective effect on frozen–thawed bull semen. Theriogenology 2002; 57: 1695–1706.
7. Amirat-Briand, L., D. Tainturier, L. Jeanneau, C. Thorin, O. Gerard, J. Courtens and M. Anton.. Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with optidyl, a commercial egg yolk extender. Theriogenology 2004; 61: 895–907.
8. Hu, J.H.; Q.W. Li, G. Li, X.Y. Chen, H. Yang, S.S. Zhang and L.Q. Wang.. The cryoprotective effect on frozen–thawed boar semen of egg yolk low density lipoproteins. Asian-Aust. J. Anim. Sci. 2006; 19: 486–490.
9. Al-Ahmad, M.Z.,G. Chatagnon, L. Amirat-Briand, M. Moussa, D. Tainturier, M. Anton and F. Fieni .. Use of Glutamine and Low Density Lipoproteins Isolated from Egg Yolk to Improve Buck Semen Freezing. Reprod. Dom. Anim. 2008(In press).
10. Demianowicz, W. and J. Strezek. The effect of lipoprotein fraction of egg yolk on some of the biological properties of boar spermatozoa during storage of the semen in liquid state. Reprod. Domest. Anim. 1996; 31: 279–280.

11. Anton, M., V. Martinet, M. Dalgalarrondo, V. Beaumal, E. David-Briand and H. Rabesona.. Chemical and structural characterization of low-density lipoproteins purified from hen egg yolk.Food Chem. 2003; 83: 175–183.
12. Bergeron, A., M.H. Crête, Y. Brindle and P. Manjunath. Low-density lipoprotein fraction from hen's egg yolk decreases the binding of the major protein of bovine seminal plasma to sperm and prevents lipid efflux from the sperm membrane, Biol. Reprod. 2004; 70: 708–717.
13. Foote, R.H. Fertility of bull semen at high extension rates in Tris buffered extenders. J. Dairy Sci. 1970; 53: 1475–1477.
14. 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.
15. Jeyendran, RS, H.H. Van Derv Ven, M. Perez-Pelaes, B.G. Craboand L.J.D. Zaneveld. Development of an assay the functional integrity of human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil. 1984; 70:219-28.
16. Tejada R.I., J.C. Mitchell, A. Norman, J.J.Marik and S. Friedman. 1984. A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil. Steril. 42:87–91.
17. Shahba, M.I. Sudy of some factors affecting efficiency of frozen buffalo semen. M.S.Sc. Thesis, Benha Univ. 2010.
18. Varela Junior, A.S., C.D. Corcini, R.R. Ulguim, M.V.F. Alvarenga, I. Bianchi, M.N. Corrêa, T. Lucia Jr and J.C. Deschamps. Effect of low density lipoprotein on the quality of cryopreserved dog semen. Anim. Reprod. Sci. 2009; 115: 323–327.
19. Hu, J.H, J. Zhong-Liang, L.v.Rui-Kai, L. Qing-Wang, Z. Shu-Shan, Z. Lin-Sen, L. Yao-Kun and L. Xin. The advantages of low-density lipoproteins in the cryopreservation of bull semen. Cryobiology 2011; 62: 83–87.
20. Iaffaldano, N., M. Di Iorio, M.P. Rosato and A. Manchisi. Cryopreservation of rabbit semen using non-permeable cryo-protectants: Effectiveness of different concentrations of low-density lipoproteins (LDL) from egg yolk versus egg yolk or sucrose. Anim. Reprod. Sci. 2014; 151: 220–228.
21. Jiang, Z.L.,Q.W. Li, W.Y. Li, J.H. Hu, H.W. Zhao and S.S. Zhang.. Effect of low density lipoprotein on DNA integrity of freezing–thawing boar sperm by neutral comet assay. Anim. Reprod. Sci. 2007; 99: 401–407.
22. Munoz, O. V., L. Amirat-Briand, T. Diaz, L. Va´squez, E. Schmidt, S. Desherces, M. Anton, D. Bencharif and D. Tainturier. Effect of semen dilution to low-sperm number per dose on motility and functionality of cryopreserved bovine spermatozoa using low-density lipoproteins (LDL) extender: Comparison to Triladyl® and Bioxcell®. Theriogenology 2009; 71: 895–900.
23. Amirat-Briand, L., D. Bencharif, O. Vera-Munoz, S.Pineau, C. Thorin, S. Destrumelle, S. Desherces, M. Anton, M. Jouan,E.Shmitt and D. Tainturier.. In vivo fertility of bull semen following cryopreservation with an LDL (low density lipoprotein) extender: Preliminary results of artificial inseminations. Anim. Reprod. Sci. 2010; 122: 282–287.
24. Manjunath, P., V. Nauc, A. Bergeron and M.Menard.. Major proteins of bovine seminal plasma bind to the low-density lipoprotein fraction of hen's egg yolk. Biol. Reprod. 2002; 67: 1250–1258.