Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Assessment of Cattle Bull Semen Preservability Using Tris Extender Enriched with *Turmeric* Extract

Muhammad M. Arboud [1], Reham S. Waheeb[1], Reda I. El-Sheshtawy[2] and Gamal A. El-Amrawi[1]
[1]*Theriogenology Dept., Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.*
[2]*Animal Reproduction and Artificial Insemination Dept., National Research Centre, Cairo, Egypt.*

THE objective was to evaluate the effect of tris citric acid fructose egg yolk (TCFY) extender supplemented with turmeric extract on cattle bull semen preservability. Pooled bull semen were extended with Tris extender enriched with 0 µl turmeric extract /5ml (control), 100µl /5ml (TT1), 200 µl /5ml (TT2), 300 µl /5ml (TT3) The semen samples were added to reach a final sperm concentration 60 × 10^6/ml. Extended semen were subjected to semen freezing protocol. Semen parameters and conception rate were carried out. The post cooling semen characteristics revealed apparent improvement in sperm motility, alive and sperm abnormalities for all concentrations, sperm membrane integrity (HOST) was significantly ameliorated in TT1 and TT2, while acrosome integrity was significantly enhanced in TT1 when compared to the control. The post thawing results exhibited significant improvement in sperm motility for all concentrations and significant amelioration in sperm membrane integrity (HOST) for all concentrations when compared to the control. Sperm abnormalities for all the concentrations and the control treatment were indifferent. Acrosome integrity, in TT1 and TT2, did not significantly differ than the control while TT3 was significantly decreased when compared to the control and the other concentrations. Conception rate was the best in all concentrations if compared to the control. It is concluded that, in cooled and post- thawed semen, the superior semen quality was attained in TT1 and TT2. Conception rate was the best in all concentrations especially inTT_3

Keywords: Cattle, Semen, Preservation, Turmeric, Tris.

Introduction

Bull semen freezing frequently exerts an oxidative stress hazard on sperm due to their overwhelming the total antioxidant capacity and hence the spermatozoal membrane become more liable to an oxidative damage [1] that affect the membrane integrity [2].

Improvement of semen cryopreservation of the bulls is a great objective, this could be achieved through supplementation of the extended semen with antioxidants. Plant extracts are considered a major category to fulfill this purpose. Phytochemicals as antioxidants have a strong preservative effect for cellular viability and metabolic function of frozen bovine spermatozoa [3]. Recently, the phyto-products has gained interest worldwide upon using as supplements. They have been used as plant supplement which enhance the healthy status. Turmeric extract contains curcumin which is a main ingredient acting as antioxidant in semen extenders [4].

Turmeric is a useful plant. Curcumin is a phytochemical having antioxidant and anti-inflammatory effect and is extracted from the rhizome of turmeric longa. Curcumin is demonstrated to have a protective effect for spermatozoa in vitro depending on its

Corresponding auther: Reda I. El-Sheshtawy, E.mail: rielsheshtawy@gmail.com. Tel. 01099952962
(*Received* 09/04/2020, *accepted* 06/07/2020)
DOI. 10.21608/ejvs.2020.27051.1168

concentration where low concentrations improved sperm motility while high concentrations decreased sperm motility [5]. Curcumin is a polyphenolic insoluble in water that scavenges free radicals [6] through decreasing generation of reactive oxygen species (ROS), as H_2O_2 and nitrite. Addition of curcumin to fresh bull semen significantly increased sperm output after thawing [7]. Administration of curcumin to male rodents improved testicular function and fertility [8,9].

Curcumin is the principal of curcuminoid of turmeric (*Curcuma longa*), a member of ginger family. Curcuminoids are natural phenols responsible for turmeric is yellow colour [10]. Turmeric extract contain curcumin with other curcuminoids and essential oils which were found to be bioactive [11]. The objective was to evaluate the effect of tris citric acid fructose egg yolk (TCFY) extender supplemented with turmeric extract on cattle bull semen preservability.

Materials and Methods

Preparation of semen extenders

TRIS base extender: Tris-citric acid-fructose diluent (TCF) was prepared according to Foote et al. [12] . 20% whole egg yolk (TCFY) was added.

Preparation of turmeric extract: 4 gm turmeric powder + 60 ml ethanol in a test tube. The turmeric powder was purchased from the Ministry of Agriculture.

4 gm turmeric powder + 60 ml distilled water in another tube. Using stirrer for mixing in each tube, filtration. The filtrate is left at 40°C for 24 hrs till evaporation. The residues in both tubes were mixed together and dissolved in 2 ml tris and kept as a stock solution. The residues were mixed in order to have both the alcoholic and aqueous extracts.

Turmeric enriched extender [TEE]: Four tubes (each contain 5 ml TCFY). The first tube contains 0 turmeric extract and kept as a control. The other three tubes contain turmeric extract as follows (100 , 200 and 300 µl /5 ml, v/v).

Semen Collection and Initial Evaluation

Semen from five mature cattle bulls kept at Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used. Ejaculates were collected using artificial vagina at weekly intervals for 18 weeks. Semen samples were initially evaluated for subjective sperm motility, morphology and sperm concentration. Ejaculates fulfilling minimum sperm motility (70%) and normal sperm morphology were pooled in order to to exclude the bull effect. Semen was hold for 10 minutes at 37°C in the water bath before dilution.

Semen processing

Semen samples were diluted with TCFY extender and used as control and other aliquots of pooled semen samples were diluted with TCFY extenders containing the different concentrations of turmeric extract to reach concentration of 60 million sperm/ml. Extended semen was cooled slowly (approximately for 2 hrs) to 5°C and equilibrated for 2 hrs. Semen was packed into 0.25 ml polyvinyl French straws. After this period, the straws were placed horizontally on a rack and frozen in vapor 4 cm above liquid nitrogen surface for 10 minutes and were then plunged into the liquid nitrogen [15].

Evaluation of Semen Quality Parameters

The assessment was implemented post cooling and on freeze- thawed bull spermatozoa. Frozen straws were thawed at 37°C/ 1 minute. The parameters studied were subjective semen characteristics (motility, alive, abnormality, hypoosmotic swelling test (HOST) and acrosome status) [13].

In vivo fertility rate (CR)

Two hundred and ninety cows were inseminated with the TT post-thawed semen and with the post-thawed semen extended in TCFY (control group). Pregnancy was recorded by rectal palpation after 2 months from insemination. The inseminated cows were used via the cooperation in Beni-Suef Governorate. CR was computed according to the equation:

$$CR = \frac{\text{no.of conceived cattle}}{\text{total no.of inseminated cattle}} \times 100$$

Statistical analysis

Statistical analysis data were analyzed using the SPSS [14] computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different parameters between control and additives replications. Significant difference between means was calculated using Duncan test at $P<0.05$.

Results

The post cooling semen characteristics revealed apparent improvement sperm motility, alive and sperm abnormalities in all concentrations, sperm membrane integrity (HOST) significantly (*P<0.001*) ameliorated in TT1 and TT2 and acrosome integrity significantly (*P<0.044*) enhanced in TT1 when compared to the control.

The post thawing results exhibited significant (*P<0.0001*) improvement in sperm motility in all concentrations and significant *(P<0.0001)* amelioration in sperm membrane integrity (HOST) in all concentrations if compared to the control and the best was in TT2. Sperm abnormalities were kept in all concentrations when compared to the control. Acrosome integrity was enhanced (P<0.026) in TT1 and TT2 when compared to the control, while TT3 was significantly decreased when compared to the control and the other concentrations. Conception rate was the best in all concentrations except the control.

TABLE 1. Effect of Tris extender enriched with Turmeric extract on cattle bull semen quality post-cooling (Mean±SE).

Diluent	Motility	Alive	Abnormalities	Host	Acrosome
TT_1	91.67 ± 1.00^a	91.33 ± 1.86^a	8.00 ±.58^a	64.00 ±1.0^c	85.33 ± .33^b
TT_2	89.33 ± 2.33^a	91.00 ± 2.08^a	7.33 ± 0.33^a	68.33 ± 3.33^c	84.33 ± 1.20^{ab}
TT_3	86.67 ± 1.67^a	90.67 ±.67^a	8.33 ± 0.33^a	55.00 ±2.89^b	81.33 ±1.33^a
Control	87.33 ± 1.45^a	89.67 ± 1.45^a	8.33 ±0.88^a	45.00 ± 2.89^a	81.00 ± 1.0^a
Total	88.58± o.80	90.67±.71^a	8.0±0.28	58.08±2.93	83.00±0.72
p-value	0.320	0.893	0.596	0.001	0.044

Different letter superscripts indicate a significant difference between means within column using the multiple range Duncan's test at P<0.05. TT denotes Tris Turmeric .

TABLE 2. Effect of tris extender enriched with Turmeric extract on the post- thawed extended cattle bull semen (Mean±SE) .

Diluent	Motility	Alive	Abnormalities	Host	Acrosome
TT_1	61.66±1.66^b	68.33±1.66^b	8.00±0.57^a	52.33±1.45^c	82.67±1.45^b
TT_2	61.66±.1.66^b	80.00±2.88^c	7.00±0.58^a	61.66±1.67^d	81.66±1.66^b
TT_3	66.66±1.66^b	83.33±1.66^c	7.66±0.33^a	42.33±1.45^b	76.33±.88^a
Control	36.66±1.66^a	54.33±1.45^a	8.00±0.57^a	25.00±2.88^a	80.33±.33^b
Total	56.66±3.60	71.50±3.53	7.66±0.26	45.33±4.18	80.25±.88
p-value	.000	.000	.528	.000	.026

Means bearing different superscripts between different extenders and differ at 5% level of probability. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG), TT1 (TrisT1), TT2 (TrisT2), TT3 (TrisT3).

TABLE 3. Effect of Tris extender enriched with Turmeric extract on a field conception rate test in cattle bulls.

Treatment	In vivo fertility rate (CR %)
TT_1	77.6%
TT_2	75.6%
TT_3	79 %
Control(TCFYG)	40.2%

Discussion

Sperm cryopreservation is of an extreme interest [16]. According to Gadea et al. [17], Uysal & Bucak [18] and Bucak et al. [19] decreasing the sperm stresses after cooling, freezing and thawing and thereby enhancing sperm livability and potentiality of fertilization is attained by adding cryopreservatives in the semen diluent [17, 18, 19]. Cryopreservation causes chemical, physical, and mechanical injures to sperm membranes [20], which are related to temperature changes, over accumulation of reactive oxygen species (ROS), conversions in the transition from the lipid phase, and osmotic stress [21, 20]. Also the extra release of ROS results in oxidative damage that includes morphological changes of the spermatozoal membranes, decrease of intracellular ATP levels in the sperm cells with consequent lowered motility and livability of frozen spermatozoa [23, 24].

Recently, there is a great worldwide interest with the beneficial synergistic effects of natural supplements and their multiple ingredients as compared to the single active fractions [25]. Semen freezing causes damage to spermatozoa leading to reduction in semen quality [20], but it is essential to conserve the supergenetic characters of our local breeds of bulls. Semen freezing is associated with cryodamage caused by overproduction of oxygen free radicals [24], so, the natural additive to the extender ameliorates the antioxidant effect and consequently improving the fertilizing capacity of frozen spermatozoa [22]. The post cooling, post thawing semen characteristics and conception rate in our study were improved upon using Tris enriched with Turmeric as a cryoprotectant in the bull semen extender. These results come in accordance with Glombik et al. [5] who demonstrated that curcumin has a protective effect for spermatozoa in vitro depending on its concentration where low concentrations improved sperm motility while high concentrations decreased sperm motility. Also, our results are compatible with the findings of Bucak et al. [7] who recorded that supplementation of Curcumin prior to cryopreservation process ameliorated semen quality. Curcumin is the major extract of turmeric, it is a lipophilic polyphenol insoluble in water and scavenge free radicals, significantly inhibit the generation of (ROS) [4]. Curcumin significantly increase the sperm content of GSH, thus improving the antioxidant capacity of the semen extender [7]. Curcumin shows antioxidant activity through binding with egg and soyphosphatidyl choline which in turn binds divalent metal ions and has antibacterial and antiviral effects [24]. The antioxidant effect of curcumin is referred to its unique conjugated structure which includes two methoxylated phenols and an enol form of b-diketone, this structure revealed ideal free radical trapping ability as a chain breaking antioxidant [27]. Turmeric contains essential oils. The polyunsaturated fatty acids in the essential oils interact with sperm membrane rendering it more stable and resistant to cold shock during cryopreservation [28]. It could be concluded that, in cooled and post- thawed semen, the superior semen quality was attained in TT1 and TT2.. Conception rate was the best in all concentrations especially inTT_3.

Acknowledgment

The authors are appreciating to the National Research Centre for the financial support and to the cooperation of Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt.

Conflict of interest

The authors declare that they have not any conflict of interest

References

1. El-Sisy, G.A., El-Nattat, W.S. and El-Sheshtawy, R.I., Buffalo semen quality, antioxidants and peroxidation during chilling and cryopreservation. *Online J. Vet. Res.*, **11**(2), 55-61 (2007).

2. Awda, B.J., Mackenzie-Bell, M. and Buhr, M.M., Reactive oxygen species and boar sperm function. *Biol. Reprod.,* **81**(3), 553-561 (2009).

3. Câmara, D.R., Mello-Pinto, M.M.C., Pinto, I.C., Brasil, O.O., Nunes, J.F. and Guerr , M.M.P. Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. *Small Rumin. Res.*, **100**(1), 44-49 (2011).

4. Petruska, P., Marcela Capcarova, M. and Sutovsky, P. Antioxidant supplementation and purification of semen for improved artificial insemination in livestock species. *Turk. J. Vet. Anim. Sci.,* **38**(6), 643-652 (2014).

5. Głombik, K., Basta-Kaim, A., Sikora-Polaczek, M., Kubera, M., Starowicz, G. and Styrna, J. Curcumin influences semen quality parameters and reverses the di (2-ethylhexyl) phthalate (DEHP)-induced testicular damage in mice. *Pharmacological Reports*, **66**(5), 782–787(2014).

6. Sharma, O.P. Antioxidant activity of curcumin and related compounds. *Biochem Pharmacol*., **25**, 1811–1812 (1976).

7. Bucak, M.N. , Baspinar, N., Tuncer, P.B., Coyan, K., Sariozkan, S., Akalin, P.P., Buyukleblebici, S. and Kucukgunay, S. Effects of curcumin and dithioerythritol on frozen-thawed bovine semen. *Andrologia*, **44** (Suppl. 1), 102–109 (2012).

8. Sahoo, D.K., Roy, A. and Chainy, G.B. Protective effects of vitamin E and curcumin on L-thyroxine-induced rat testicular oxidative stress. *Chem. Biol. Interact*, **176**(2-3),121–128 (2008).

9. Mathuria, N. and Verma, R.J. Ameliorative effect of curcumin on aphlatoxin-induced toxicity in serum of mice. *Acta Pol. Pharm*., **65**, 339–343 (2008).

10. Nelson, K.M., Dahlin, J.L., Bisson, J , Graham, J., Pauli, G.F and Walters, M.A. The Essential Medicinal Chemistry of Curcumin. *J. Med. Chem.,* **60**(5),1620-1673 (2017).

11. Kulkarni, S.J., Maske, K.N.,Budre, M.P. and Mahajan, R.P., Extraction and purification of curcuminoids from Turmeric (*Curcuma longa* L.) *Int. J. Pharmacol. Pharmaceut. Technol.*, **1**(2), 81-84 (2012).

12. Foote, R. H, Brockett, C.C. and Kaproth, M.T.. Motility and fertility of bull sperm in whole milk extender containing antioxidants. *Anim. Reprod. Sci*., **71**,13-23 (2002).

13. Salisbury, G.W., VanDemark, N.L. and Lodge, J.R., Semen evaluation: In "Physiology of Reproduction and Artificial Insemination of Cattle." 2nd edition. W.H. Freeman & Compagny, San Francisco, USA., pp. 400-427. (1978).

14. SPSS. (2005) SPSS v.14.0 for Windows Evaluation Version Release. 14.0.0,.

15. Khan, M.I. and RandIjaz, A. Assessing undiluted, diluted and frozen–thawed Nili-Ravi buffalo bull sperm by using standard semen assays. *Ital. J. Anim . Sci.*, **6**, 784–787 (2007).

16. Medeiros, C.M. , Forell, F., Oliveira, A.T. and Rodrigues, J.L., Current status of sperm cryopreservation: Why isn't better. *Theriogenology*, **57**(1), 327-344 (2002).

17. Gadea, J., Gumbo, D., Novass, C., Zquezf, A.Z., Grullol, A. and Gardo, G.C. Supplementation of the dilution medium after thawing with reduced glutathione improves function and the in vitro fertilizing ability of frozen thawed bull spermatozoa. *Int . J .Androl.*, **31**(1), 40-49 (2007).

18. Uysal, O. and Bucak, M.N., Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. *Acta Vet. Brno*., **76**(3), 383-390 (2007).

19. Bucak, M.N., Atessahin, A. and Yuce, A., Effect of anti-oxidants and oxidative stress parameters on ram semen after the freeze-thawing process. *Small Rumin. Res.,* **75**(2-3), 128-134. (2008).

20. Watson, P.F., The causes of reduced fertility with cryopreserved semen. *Anim. Reprod. Sci.,* **60-61**,481-492 (2000).

21. Câmara, D.R., Mello-Pinto, M.M.C., Pinto, I.C., Brasil, O.O., Nunes, J.F. and Guerr, M.M.P., Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. *Small Rumin. Res.*, **100**(1), 44-49 (2011).

22. Ortega Ferrusola, C., González Fernández, L., Morrell, J.M. , Salazar Sandoval, C., Macías García, B. and Rodríguez-Martinez, H. Lipid peroxidation, assessed with BODIPY-C11, increases after cryopreservation of stallion spermatozoa, is stallion dependent and is related to apoptotic-like changes. *Reproduction*, **138**(1), 55-63. (2009).

23. Baumber, J., Ball, B.A., Gravence, C.G., Medina, V. and Davies-Morel, M.C., The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. *J. Androl*., **21**(6), 895-902. (2000).

24. Agarwal, A . , Prahakaran, S.A. and Said T.M.. Prevention of oxidative stress injury to sperm. *J. Androl.* , **26**(6) , 653-660 (2005)

25. Seeram, N.P., Adams, L.S. , Hardy, M.L. and Heber, D., Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects. *J. Agric. Food Chem*., **52**, 2512–2517. (2004).

26. Bhowmik, D., Chiranjib, K. P. Kumar, S., Chandira, M. and Jayakar, B., Turmeric: *A Herbal and Traditional Medicine Archives of Applied Science Research*, **1** (2) 86-108 (2009).

27. Bagchi, A ., Extraction of Curcumin IOSR Journal of Environmental Science, *Toxicol. Food .Tech*. **1**(3) , 1-16 (2012).

28. Singh, A.K., Singh, V.K., Narwade, B.M., Mohanty, T.K. and Atreja, S.K. Comparative quality assessment of buffalo (Bubalus bubalis) semen chilled (5°C) in egg yolk-and soya milk-based extenders. *Reprod. Domest.. Anim*., **47**(4), 596-600 (2012).

تقييم كفاءة الحفظ بالتجميد للسائل المنوى لطلائق الابقار باستخدام التريس المدعم بمستخلص القرقم

محمد عربود[1] , ريهام وهيب[1]* , رضا الششتاوي[2] و جمال العمراوي[1]
[1]قسم التوليد - كلية الطب البيطري - جامعة الاسكندرية - الاسكندرية - مصر.
[2]قسم التكاثر الحيوانى والتلقيح الصناعى-شعبة البحوث البيطرية - المركز القومي للبحوث - القاهرة - مصر.

الهدف من هذا البحث هو تقييم كفاءة حفظ السائل المنوى المخفف بالتريس والمدعم بمستخلص القرقم . تم تخفيف السائل المنوى المجمع بمخفف التريس فقط ككنترول بالاضافة الي التريس المدعم بمستخلص القرقم بتركيزات مختلفة من المخزون (100 µl /5ml 300 µl /5ml 200 µl /5ml/ 100د\ق0ml/5). تم عمل تبريد وتجميد للسائل المنوى المخفف. أظهرت النتائج أن خصائص السائل المنوى المبرد والمجمد تحسنت صفاته مقارنة بالكنترول وكذلك نسبة الحمل .و الخلاصة أن أفضل النتائج بعد التجميد والاذابة كانت فى التركيزات (TT_1,TT_2،) وتحسنت نسبة الحمل فى كل التركيزات خاصة فى TT_3.