Egypt. J. Vet. Sci. **Vol. 55,** No. 3, pp. 607-614 (2024)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Cryoprotective Effects of Tris Taurine Cholesterol-Loaded-Cyclodextrins Extender on Buffalo Bull Spermatozoa.

Reda Ibrahim EL Sheshtawy

Animal Reproduction and AI Dept., Veterinary Research institute, National Research Centre, Dokki, Egypt

The purpose of the current investigation was to assess the impact of Tris-extender enriched with a mixture of taurine and different concentrations of Cholesterol-loaded-cyclodextrins(CLC)on preservability of buffalo semen.

Semen was extended in a Tris–citrate glycerol egg yolk extender. Different concentrations of CLC (1.0, 1.5 and2mg mL-1) and fixed concentration of Taurine (60mM) were added into the tubes containing the extended semen in Tris-Citrate-Fructose glycerol (TCFG) diluent with 20% egg yolk .The control tubes were TCFG containing (zero clc and zero taurine). The straws comprising the control (TCFG with zero CLC and zero Taurine) and the experimental extenders (TCFG containing different concentrations of CLC and the fixed concentration of Taurine) were exposed to the freezing process. In Tris taurine Cholesterol-loaded cyclodextrins (TTC) .Extended semen was evaluated initially, post cooling and post freezing. Post cooling sperm motility, Alive sperm , sperm abnormalities and acrosomal percent were kept in all extenders as the control. Sperm membrane integrity (HOST) was considerably superior in all extenders if compared to the control. Post – thawing, sperm motility was significantly higher in $TTC_1$and TTC_3. Living spermatozoa was markedly higher in all extenders relative to the control. Sperm morphological abnormalities were notably inferior in TTC_1 relative to the control. Sperm membrane integrity was extensively improved in all extenders as compared to control. Acrosomal integrity percent was reserved in all extenders relative to control. It could be fulfilled that, in cooled semen, Sperm membrane integrity was significantly higher in all extenders and other Sperm parameters were kept unchanged in comparison with the control. The post – thawed parameters and conception rate exhibited that, the superior was given with the first concentration (TTC_1) .

Keywords: Buffalo, semen, Taurine, CLC, Freezing.

Introduction

Artificial insemination is mainly the regular assisted biotechnological mean applied to enhance animal productivity [1]. AI aim to control animal reproduction especially when using semen from progeny tested proven sires to guarantee improves super genetic and production parameters [2]. Moreover, semen freezing depends on the kind of diluent and its inclusions used to ameliorate the sperm cells stabilization among the freezing - thawing procedures [3].

The reduced fertility of bulls used in AI program is a causative factor for great economic losses especially when these bulls are genetically superior [4].

Buffalo spermatozoa are sensitive to cryopreservation process and exhibited poor freezability and fertility as compared to cattle [5] .Freezing of buffalo semen exerts many stresses with cryoinjury of the cells[6].In this regard spermatozoal deterioration of the sperm membranes takes place upon cooling of cells from room temperature to 1ºC and below 18ºC, the sperm membranes suffer a phase transition into gel state as a result of efflux of

*Corresponding author: Elsheshtawy, Reda e-mail rielsheshtawy@gmail.com. Phone: 01099952962.
(Received 30/08/2023, accepted 10/11/2023)
DOI: 10.21608/EJVS.2023.217336.1525

phospholipids leading to increased membrane permeability and cell death[7].

Taurine cryoprotectant was enriched in the freezing diluent of bovines[8,9,10], swine[11-13], sheep[14], buck[15]and dog semen[16,17] to ameliorate the sperm quality post freezing. The antioxidant taurine exerts its effect through permeating the spermatozoal membrane and inhibit fatty acids peroxidation ,thus protecting the sperm cells from the excessive release of the oxygen free radicals[8,18] ,interacting with membrane lipids, creating hypertonic media, with subsequent cellular osmotic dehydration and so declining the degree of cell deterioration via ice crystals formation [19 ,20].

Cholesterol / phospholipids ratio of the plasma membrane is a major cause of loss of sperm membrane fluidity and stability during sperm freezing[21,22].Cholesterol decreases the lipid phase conversion temperature of the membranes , keeping their fluidity at lower degrees of temperature ,thus minimizing the sperm membrane injury [23].Cholesterol added to the extender during the freezing process is incorporated into the sperm membrane improving its fluidity[24,7].Cyclodextrin compounds are cyclic hepatosaccharides having a hydrophobic center [7] and can transport cholesterol into the sperm membrane[25]cholesterol loaded into cyclodextrin and added to bovine semen before the freezing process ameliorates sperm membrane integrity and sperm motility [7].

Material and Methods

Buffalo bulls

Four buffalo bulls (3.5-5.0 years) maintained at the Semen Freezing Center, Ministry of Agriculture, Egypt, were selected for semen collection. The animals were maintained on good nutrition and managemental care. The bulls were in good general health situation and weigh 600 to 800 kg .During summer, the animals were reserved cool and relaxed through sprinkling water at least three to four times per day, sheltered from direct wind, housed in a place with easy environment with minimum humidity, fed throughout cool day hours, and had a plenty of cool drinking water. They were fed 6 kg dry matter+2 kg tibn and 3.5 kg dried barseem/animal/day in summer and 6 kg dry matter+2 kg tibn and 28 kg barseem/animal/ day in winter. Temperature humidity index was 72-78.

Experimental groups

Group 1(TTC_1): Tris taurine cholesterol loaded cyclodextrin (Tris containing 1mgCLC and 60mM Taurine)

Group 2(TTC_2): Tris taurine cholesterol loaded cyclodextrin (Tris containing 1.5mgCLC and 60mM Taurine)

Group 3(TTC_3):Tris taurine cholesterol loaded cyclodextrin (Tris containing 2mgCLC and 60mM Taurine)

Control(TCFYG): Tris-citrate-fructose-egg yolk-glycerol (Tris containing zero CLC and Zero Taurine)

Semen collection and initial evaluation

Semen samples were obtained from bulls using a prepared artificial vagina every week for five weeks. The semen specimens were primarily assessed for the ejaculate volume using graduated test tube, sperm concentration using of the Neubaur haemocytometer (Thoma rulling) and sperm forward motility. The final semen specimens with more motility higher than 70% and 80% normally morphological spermatozoa were chosen for the freezing protocol. Pooling of the ejaculates was performed to get enough semen and to get rid of the individual bull effect. The collected semen was hold for ten minutes at 35ºC in the water bath prior to dilution and then assessed for sperm forward motility, livability, percent of morphological abnormalities, membrane and acrosome status prior to the freezing process.

Preparation of Cholesterol-loaded cyclodextrins (CLC)

Cholesterol-loaded cyclodextrins (CLCs) were prepared as described by Purdy and Graham [7]. Briefly, 1 g of methyl-cyclodextrin was dissolved in 2ml of methanol in a glass test tube. In a second glass test tube, 200mg of cholesterol was dissolved in 1ml of chloroform and a 0.45 ml aliquot of this cholesterol solution was added to the methyl-cyclodextrin solution. The solution of cyclodextrin and cholesterol was mixed and the solvents then removed using a stream of nitrogen gas. The resulting crystals were stored at 22 ◦C until further use.

Semen freezing

Semen specimens were diluted (1:7by volume) in a Tris–citrate glycerol egg yolk extender at 35ºC [26,27] to attain sixty million forward motile spermatozoa/mL. Diverse concentrations of CLC (1.0, 1.5 and 2mg mL^{-1}) and fixed concentration of Taurine (60mM) were supplementary into the tubes containing the extended semen .The control tubes contain Tris with (Zero Taurine and zero ClC). These tubes were slowly refrigerated (roughly for two hours) to 5 ºC and exposed to equilibration for four hours. Diluted semen was filled into 1/4 mL polyvinyl French straws (IMV, France). Post equilibration time, the straws were put in a horizontal manner on a special rack and frozen in a vapour four cm on top of liquid nitrogen (LN2) for ten minutes and plunged immediately in liquid nitrogen at -196 ºC for storage[28].

Semen characteristics

Frozen semen straws were exposed to thawing at 37 ºC for thirty seconds in a water bath for microscopical estimation. The criteria investigated were sperm forward motility, sperm membrane integrity (HOST) , Alive sperm, sperm morphological abnormalities and normal acrosomal percent in both refrigerated and frozen semen.

Sperm forward motility

Sperm forward motility was examined using phase contrast microscope (Olympus Optical Co. Ltd., Japan). Visual motility was microscopically examined with closed circuit television [29] .

Alive and abnormal morphological spermatozoa (%)

The sperm livability and morphological abnormalities were examined by means of eosin-Nigrosin stained smears as recorded by Revell and Mrode [30].

Sperm membrane status

Sperm membrane status was evaluated using the hyposmotic swelling analysis [31] .200 sperm cells were counted and the percent of spermatozoa having swollen coiled tails (intact sperm membrane) was estimated.

Normal acrosomal percentage

Acrosomal status was assayed by means of Giemsa stain as postulated by Watson [32].The intact acrosome percent was calculated for two hundred sperm cells that were investigated using an immersion lens (×1000) by phase contrast microscope.

Assay of oxidant/antioxidant parameters

Semen was gathered then centrifuged at 2773 ×g for 5 min at 40C using a cooling centrifuge (Sigma 3-18KS, Germany). The seminal plasma was separated and stored at -80 ºC. The level of total antioxidant capacity (TAC) in the seminal plasma was assayed as the method described by Koracevic et al. [33] and lipid peroxidation levels as malondialdehyde (MDA) according to the technique of Satoh [34]using test kits of Biodiagnostic Co., Egypt. All assays were carried out by Double Beam UV/Visible Spectrophotometer, Model T80, UK.

In vivo fertility rate (CR)

No. of buffalo females (n=290) were artificially inseminated with the TTB post-thawed semen and with the post-thawed semen extended in TCFY (control group). Pregnancy was detected by rectal palpation after sixty days post insemination. The artificially inseminated cows were allowed via the cooperation with Bani-Suef Veterinary Organization. CR was estimated according to the equation:

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

Statistical study

Data were analyzed by one-way ANOVA analysis of variance , then by Duncan test to calculate considerable variations in all the criteria amongst all groups, with SPSS Version 14.0 for Windows SPSS [35]. Differences with values of $P<0.05$ were measured to be statistically significant.

Results

In Tris taurine Cholesterol-loaded cyclodextrins (TTC) Post cooling , sperm motility and acrosome percent were kept in all concentrations as the control. Alive sperm and sperm abnormalities were kept in $TTC_1$and TTC_2 as the control. Sperm membrane integrity (HOST) was significantly (P<.005) higher in all concentrations if compared to the control (Table 1). Post – thawing, sperm motility was significantly (P<.005) higher in $TTC_1$and TTC_3, alive sperm was significantly (P<.002) higher in all concentrations if compared to the control. Sperm abnormalities were significantly (P<.008) lower in TTC_1 relative to the control . Sperm membrane integrity (HOST) was significantly (P<.000) improved in all concentrations if compared to the control. Acrosome percent were kept in all concentrations as the control (Table 2). The conception rate was the best in TTC_1 (Table 3) **.** TTC_1revealed higher TAC (P<.031) and lower MDA (P<.001) if compared to the control and other experimental groups (Table 4).

Discussion

Artificial insemination (AI) is measured as the principal mean for spreading of the superior genetic characteristics to ameliorate the genetic structure of the livestock [28, 36].

Variable antioxidant ingredients are available in the sperm cells and seminal plasma and categorized as antioxidant enzymes (GSH, CAT, SOD). Their antioxidant capability is inadequate and regularly decreases through the freezing process, therefore antioxidants should be enriched in the semen diluent [37].Taurine has a valuable consequence in increasing Catalase(CAT) intensity and as a result improving the antioxidant capacity [14].

TABLE 1. Effect of Tris extender enriched with taurine and CLC on the cooled extended buffalo bull semen (Mean±SE)

Diluent	Motility	Alive	Abnormalities	HOST	Acrosome
TTC_1	91.66±1.66^{a}	91.66±1.66^{c}	6.33±.66^{a}	89.90±.83^{b}	88.33±.33^{b}
TTC_2	91.66±1.66^{a}	86.00±1.00^{b}	7.33±.33^{a}	78.88±1.62^{b}	81.33±1.33^{a}
TTC_3	91.66±1.66^{a}	80.33±.33^{a}	13.33±1.33^{b}	82.42±1.50^{b}	88.66±1.85^{b}
Control	88.33±1.66^{a}	88.33±1.66^{bc}	6.66±.33^{a}	56.61±8.93^{a}	85.00±5.00^{ab}
Total	90.83±.83	86.58±1.36	8.41±.92	76.95±4.22	85.90±1.30
p-value	.441	.002	.001	.005	.081

Means bearing different superscripts between different extenders and differ at 5% levels of probability. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG); TTC_1 ($TrisTC_1$); TTC_2($TrisTC_2$); TTC_3($TrisTC_3$) .

TABLE 2. Effect of Tris extender enriched with Taurine and CLC on the post - thawed extended buffalo bull semen (Mean±SE)

Diluent	Motility	Alive	Abnormalities	HOST	Acrosome
TTC_1	58.33±1.66^{b}	91.00±1.00^{c}	8.33±.33^{a}	63.62±1.62^{b}	85.66±1.20^{a}
TTC_2	45.00±2.88^{a}	89.00±2.08^{bc}	9.33±.33^{ab}	63.13±.59^{b}	83.33±1.66^{a}
TTC_3	55.00±2.88^{b}	85.66±.66^{b}	10.33±.33^{b}	73.40±2.36^{c}	82.66±1.20^{a}
Control	43.33±1.66^{a}	80.66±.66^{a}	10.33±.33^{b}	57.90±.15^{a}	87.50±2.50^{a}
Total	50.41±2.17	86.58±1.29	9.58±.28	64.51±1.80	84.54±.86
p-value	.005	.002	.008	.000	.656

Means bearing different superscripts between different extenders and differ at 5% and 1% levels of probability. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG); TTC_1($TrisTC_1$); TTC_2($TrisTC_2$); TTC_3($TrisTC_3$).

TABLE 3. Effect of Taurine and different concentrations of CLC enriched extender on a field conception rate test in buffalo

Treatment	No of inseminated females	No of conceived females	In vivo fertility rate (CR%)
TTC_1	80	53	66.3%
TTC_2	85	47	55.3%
TTC_3	70	40	57.1 %
Control(TCFYG)	55	30	54.5%

TABLE 4 . Effect of Tris extender enriched with Taurine and CLC on Antioxidant concentration-TAC (mM) and MDA concentration (µM) (Mean±SE)

Diluent	TAC	MDA
Control (Tris extender)	0.24±0.01^{a}	8.87±0.13^{c}
TTC_1	0.31±0.01^{b}	7.05±0.05^{a}
TTC_2	0.24±0.02^{a}	8.20±0.12^{b}
TTC_3	0.27±0.01^{ab}	9.35±0.35^{c}
p-value	.031	.001

Semen freezing process results in excessive release of oxygen free anions with consequent lowering sperm motility, membrane integrity and fertilizing potential [38].

Sperm cells are liable to peroxidative deterioration that may be reduced via adding antioxidants to the semen diluent [39, 40].

In this investigation, addition of taurine to the semen freezing diluents enhanced sperm parameters manifested by post- thawing forward spermatozoal motility, spermatozoal membrane fluidity, acrosome integrity and liveability. The results of the present study are compatible with Reddy *et al.* [41] who postulated enhanced semen quality by the action of taurine.

In this study, supplementation of all concentrations of CLC combined with 60mM taurine improved semen characteristics and addition of 1.0 mg mL^{-1} CLC gave the best semen quality post freezing relative to the control. The pregnancy rate (CR) was superior at 1.0 mg mL^{-1} CLC and this result is compatible with the best sperm motility at this recorded concentration. Taurine enhanced the post-thawing semen quality in buffalo [41], ram[42], goat [15] and boar spermatozoa [13].

Numerous studies documented the ameliorating action of taurine on freeze post- thawing sperm parameters (14,9).Taurine is a sulfonic amino acid having a vital role as non enzymatic scavenger of the ROS, thus, protecting sperm cells from peroxidative deterioration among the freezing process [9, 43].

Taurine addition may exert cryoprotective effect on the metabolic activity of mitochondria and acrosome with consequent energy generation from intracellular ATP stores with improved sperm forward motility. The assessment of liveability, membrane and acrosome status are very important as sperm motility simply is unsatisfactory for spermatozoal evaluation post freeze-thawing.

Cholesterol/phospholipid ratio is the principal factor influencing sperm membrane fluidity and integrity especially during cryopreservation and taurine lowers cholesterol efflux that takes place from the sperm membrane [21, 22] .

The results agreed with purdy and Graham [7] in bulls , Moore *et al*.[44] in equine and Moce *et al*. [45] in ram who reported improved semen quality post freezing on adding CLC before the freezing process.However, our results are antagonist to those reported by Zahn *et al*. [46]in equines who recorded lowered semen quality post freezing on adding Cholesterol-loaded cyclodextrins .

The findings of the current study come in accordance with that recorded by Rajoriya *et al*.[47]who postulated that buffalo bull spermatozoa incubated with CLC (3mg/120 × 10^6) before the freezing process improved sperm freezability. Moraes *et al*.[48] reported improved percentage of post thaw viability and increased number of sperm that bind to zona pellucid in CLC addition to stallion sperm freezing .

The present improvement in sperm quality post freezing due to addition of CLC to the extender prior to freezing may be due to the protection of the sperm membrane from damage that occur during cryopreservation[48].Cholesterol added to the bull or equine semen before sperm freezing is permeated into the sperm membrane preserving its fluidity and integrity [24, 7].

The total cholesterol content of the sperm that supplies maximum cryosurvivability, is 2-3times superior than the control sperm and when cholesterol elevated to 4-5 times than that in the control, it becomes hazardous to the sperm cells [7].

The improved conception rate in (TTC_1) may be due to the best post-thaw freezing sperm motility, improved total antioxidant capacity (TAC) and lowest malondialdehyde (MDA) observed in our study.

These results are compatible with Bucak *et al*. [14] in ram semen and with the findings of Mahmoud et al. [49] who clarified that spermatozoal motility is considered an obvious marker for sperm parameters, and imminent relations were set up between sperm motility and each of membrane fluidity and sperm morphological abnormality. In this regard, Li *et al*. [50] documented significant correlation between sperm motility and IVF. Vale [51] recorded a pregnancy rate superior than fifty percent as an excellent result post artificial insemination with frozen- thawed spermatozoa in buffaloes . Al Naib *et al*. [52] classified bulls with pregnancy rate of nearly fifty percent to be considered of high fertility, and the sperm of these bulls tends to be more capable in passing through artificial mucus and having a high potential for fertilizing oocyte *in vitro* .

The improvement of sperm quality treated with cholesterol loaded cyclodextrin may be due to decrease in capacitation status [53,54], reduced oxidative stress [55] or enhanced antioxidant levels [56]. Studies have also revealed that CLC treatment of semen leads to increase in the percentage of noncapacitated sperm [53,57] and better freezability of sperm may be due to higher cholesterol: phospholipid ratio [54] helps in membrane stability and decrease in capacitation changes[53]. Methyl β-cyclodextrin solubilizes exogenous cholesterol in the medium, presumably facilitating its incorporation following the concentration gradient with subsequent inceased sperm membrane integrity with subsequent increased semen freezability [58] maintain the integrity of the mitochondrial matrix, probably due to the increase in the intramembrane cholesterol ratio[59].

Conclusion

It could be concluded that, in cooled semen, Sperm membrane integrity was significantly higher in all concentrations if compared to the control and other Sperm parameters were reserved in all concentrations as the control. The post – thawed semen parameters and conception rate exhibited that, the superior was given with the first concentration (TTC_1).

Ethical Approval

The experimental plan was approved by the Medical Research Ethics Committee of the National Research Centre, Dokki, Egypt and its registration number is 19/104 and its date is 10/10/2019.

Authorship

The author had performed all the items of the experimental design, the collection of semen, the diluting concentrations, the freezing process, semen evaluation and the preparing of the manuscript.

Acknowledgement

The authors are extremely grateful to the National Research Centre and the Semen Freezing Center, General Organization for Vet. Services, Ministry of Agriculture, Abbasia, Egypt, for sponsoring and supporting this work. This work was supported by the National Research Centre [grant number: 10120801].

Conflict of interest

The authors announce that, there isn't any conflict of interest.

References

1. Bailey, J. L. , Lessard, C. , Jacques, J. , Br`eque, C. , Dobrinski, I. , Zeng, W. and Galantino- Homer, H. L. ,Cryopreservation of boar semen and its future importance to the industry. *Theriogenology*, **70**,1251–1259(2008).

2. Paramio, M. T. and Izquierdo, D. Assisted reproduction technologies in goats. *Small Rumin. Res.*, **121**, 21–26(2014).

3. Nur, Z., Zik, B., Ustuner, B., Sagirkaya, H. and Ozguden, C. Effects of different cryoprotective agents on ram sperm morphology and DNA integrity. *Theriogenology,* **73**,1267–1275(2010).

4. Kuroda, K., Fukushima, M. and harayama, H. Premature Capacitation of Frozen-Thawed Spermatozoa from Subfertile Japanese Black Cattle. *J. Reprod. Develop.*, **53**, 1079-1086(2007).

5. Andrabi, S.M.H. Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. *Reprod. in Dom. Anim.*, **44** , 552–569 (2009).

6. Morris, G.J., Faszer, K., Green, J.E., Draper, D., Grout, B.W. and Fonseca, F. Rapidly cooled horse spermatozoa: loss of viability is due to osmotic imbalance during thawing, not intracellular ice formation. *Theriogenology*, **68**, 804–812 (2007).

7. Purdy, P.H. and Graham, J.K. Effect of adding cholesterol to bull sperm membranes on sperm capacitation, the acrosome reaction, and fertility. *Biol. Reprod.*, **71**, 522–527(2004).

8. Badr, M.R. , Azab, A.M.S and Rawash, Z.M. Effect of Trehalose, Cysteine and Hypotaurine on Buffalo Bull Sperm Freezability, Ultrastructure Changes and Fertilizing Potentials. *Assiut Vet. Med. J.,* **60,** 38-45 (2014).

9. Sarıozkan, S., Bucak, M.N., Tuncer, P.B., Ulutas, P.A. and Bilgen, A. The influence of cysteine and taurine on microscopic-oxidative stress parameters and fertilizing ability of bull semen following cryopreservation. *Cryobiology*, **58**, 134–138 (2009).

10. Uysal, O., Bucak, M.N., Yavas, I. and Varıslı, O. ,Effect of various antioxidants on the quality of frozen–thawed bull semen. *J. Anim. Vet. Adv.*, **6**, 1362–136 (2007).

11. Funahashi, H. and Sano, T. Selected antioxidants improve the function of extended boar semen stored at 10 ◦C. *Theriogenology*, **63**, 1605–1616(2005).

12. Gutierrez-Perez, O., Juarez-Mosqueda, M.L., Carvajal, S.U. and Ortega, M.E.T. Boar spermatozoa cryopreservation in low glycerol/trehalose enriched freezing media improves cellular integrity. *Cryobiology*, **58**,287–292 (2009).

13. Hu, J.H. , Li, Q.W. , Jiang, Z.L. , Yang, H. , Zhang, S.S. and Zhao, H.W. The cryoprotective effect of trehalose supplementation on boar spermatozoa quality. *Reprod. Dom. Anim*., **44**, 571-575 (2009).

14. Bucak, M.N., Ates, S., ahin, A., Varıs L, O., Yuce, A., Tekin, N. and Akcay, A. The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen: Microscopic and oxidative stress parameters after the freeze–thawing process. *Theriogenology*, **67**, 1060–1067(2007).

15. Bucak, M.N. and Uysal, O. The role of antioxidants in freezing of Saanen goat semen. *Indian Vet. J.*, **85**, 148-150 (2008).

16. Martins-Bessa,A., Rocha, A. and Mayenco-Aguirre, A.Effects of taurine and hypotaurine supplementation and ionophore concentrations on post-thaw acrosome reaction of dog spermatozoa. *Theriogenology*, **71**, 248–253(2009).

17.Michael, A., Alexopoulos, C., Pontiki, E., Hadjipavlou-Litina, D., Saratsis, P. and Boscos, C. Effect of antioxidant supplementation on semen quality and reactive oxygen species of frozen-thawed canine spermatozoa. *Theriogenology*, **68**, 204–212 (2007).

18. 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).

19. Liu, Z., Foote, R.H. and Brockett, C.C. Survival of bull sperm frozen at different rates in media varying in osmolarity. *Cryobiology*, **37**, 219–230(1998).

20. Pons-Rejraji, H., Vorilhon, S., Difrane, A.,, Dollet, S., Bourgne, C., Berger, M., Chaput, L., Pereira, B., Bouche, C., Drevet, J. R. and Brugnon, F. Beneficial effects of hypotaurine supplementation in preparation and freezing media on human sperm cryocapacitation and DNA quality. *Basic Clinic. Androl*., **31**, 26 (2021).

21. Harshan, H.M., Singh, L.P., Arangasamy, A., Ansari, M.R. and Kumar, S. Effect of buffalo seminal plasma heparin binding protein (HBP) on freezability and in vitro fertility of buffalo cauda spermatozoa. *Anim. Reprod. Sci.*, **93**, 124–133 (2006).

22. Srivastava, N., Srivastava, S.K., Ghosh, S.K., Singh, L.P., Prasad, J.K. and Kumar, A. Sequestration of PDC-109 protein improves freezability of crossbred bull spermatozoa. *Anim. Reprod. Sci.*, 54–62(2012).

23.Amann, R.P. and Pickett, B.W. Principles of cryopreservation and a review of cryopreservation of stallion spermatozoa. *J. Equine Vet. Sci. ,* **7**, 45–173(1987).

24. Combes,G.B., Varner, D.D., Schroeder, F., Burghardt, R.C. and Blanchard, T.L. ,Effect of cholesterol on the motility and plasma membrane integrity of frozen equine spermatozoa after thawing. *J. Reprod.Fertil.*, **56**, 127–132(2000).

25. Gimpl, G., Reitz, J., Brauer, S. and Trossen, C., Oxytocin receptors: ligand binding, signalling and cholesterol dependence. *Prog. Brain Res.*, **170**, 93-204 (2008).

26. de Paz, P., Esteso, M.C., Alvarez, M., Mata, M., Chamorro, C.A. and Anel, L. Development of extender based on soybean lecithin for its application in liquid ram semen. *Theriogenology*, **74**,663-671(2010).

27. Roof, D.J., Bowley, S., Price, L.L. and Matsas, D.J. Comparison of two commercial extenders for cryopreservation of goat semen without sperm washing. *Theriogenology*, **77**, 412–420 (2012).

28. Vishwanath, R. and Shannon, P. Storage of bovine semen in liquid and frozen state. *Anim. Reprod. Sci.*, **62**, 23-53 (2000).

29. Bearden H.J. and Fuquay, J. Applied Animal Reproduction. Reston, VA: Reston Publishing Co. Inc: ,158-160(1980).

30. Revell, S.G. and Mrode, R.A. An osmotic resistance test for bovine semen. *Anim. Reprod. Sci*., **36**,77-86(1994).

31. Jeyendran, R.S., Vander, Ven, H.H., Perez Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fertil*., **70**, 219-228(1984).

32. Watson, P.F. Use of giemsa stain to detect changes in the acrosome of frozen ram spermatozoa. *Vet. Record*., **97**, 12-15 (1975).

33. Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. and Cosic, V. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.*, **54**, 356–361(2001).

34. Satoh, K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica. Chimica. Acta*, **90**, 37-43(1978).

35. SPSSv.14.0 for Windows Evaluation Version Release, 14.0.0 (2005).

36. Durrant, B. S. The importance and potential of artificial insemination in CANDES (companion animals, non-domestic, endangered species) . *Thriogenology*, **71**,113-22 (2009).

37. Bilodeau, J.F., Blanchette, S., Gagnon, I.C. and Sirard, M.A. Thiols prevent H_2O_2- mediated loss of sperm motility in cryopreserved bull semen. *Theriogenology*, **56** (2), 275–286 (2001).

38. Cassani, P., Beconi, M.T. and O'Flaherty, C. Relationship between total superoxide dismutase activity with lipid peroxidation, dynamics and morphological parameters in canine semen. *Anim. Reprod. Sci.*, **86**(1-2), 163–173(2005).

39. Ball, B.A., Medina, V., Gravance, C.G. and Bumber, J. Effect of antioxidants on preservation of motility, viability and acrosomal integrity of equine spermatozoa during storage at 5°C. *Theriogenology*, **56**(4), 577–589 (2001).

40. Andreea, A. and Stela, Z . Role of antioxidant additives in the protection of the cryopreserved semen against free radicals. *Rom. Biotechnol. Lett*., **15** (3), 3–8 (2010).

41. Reddy, N.S.S., Mohanarao, G.J. and Atreja, S.K. Effects of adding taurine and trehalose to a tris-based egg yolk extender on buffalo (Bubalus bubalis) sperm quality following cryopreservation. *Anim. Reprod. Sci.*, **119**(3-4), 183–190 (2010).

42. Partyka, A., Rodak, O., Bajzert, J., Kochan, J. and Niżański,W. The Effect of L-Carnitine, Hypotaurine, and Taurine Supplementation on the Quality of Cryopreserved Chicken Semen. *Bio. Med. Res. International*, 1- 8 (2017).

43. Saleh, A. and Agarwal, A. Oxidative stress and male infertility: from research bench to clinical practise, *J. Androl*., **23**(6) ,737–752 (2002).

44. Moore, A. I., Squires, E. L. and Graham, J. K. Adding cholesterol to the stallion sperm plasma membrane Improves cryosurvival. *Cryobiology*, **51** (3), 241–249 (2005).

45. Mocé, E., Blanch, E., Tomás, C. and Graham, J.K. Use of cholesterol in sperm cryopreservation: present moment and perspectives to future. *Reprod. Dom. Anim.*, **45**(2), 57–66 (2010).

46.Zahn, F.S., Papa, F.O. and Dell'Aqua, Jr. J.A. Cholesterol incorporation on equine sperm membrane: Effects on post-thaw sperm parameters and fertility. *Theriogenology*, **58**, 237–240(2002).

47.Rajoriyaa, J.S., Prasadb, J.K., Ramtekeb, S.S., Perumalc, P., Ghoshb, S.K., Singh, M. P., M. and Srivastavad, N. Enriching membrane cholesterol improves stability and cryosurvival of buffalo spermatozoa. *Anim. Reprod. Sci.*, **164**, 72–81(2016).

48.Moraes, E.A., Matos, W.C.G., Graham, J.K., Ferrari, J r. W.D. Cholestanol-loaded-cyclodextrin improves the quality of stallion spermatozoa after cryopreservation. *Anim. Reprod. Sci*., **158**, 19-24 (2015).

49. Mahmoud, K.G.M , EL Sokary, A.A.E. Abou El Roos, M.E.A. Ghaffar, A.D.A. and Nawito, M. Sperm characteristics in cryopreserved buffalo bull semen and field fertility. *Iran. J. Appl. Sci.,* **3**, 777-783(2013).

50. Li, Y. D. Zeron, Y. and Roth, Z. Progressive motility – a potential predictive parameter for semen fertilization capacity in bovines. *Zygote*, **24**,70-82 (2016).

51-Vale, W.G., News on reproduction biotechnology in male. *Proceedings 5th World Buffalo Congress ,Royal Palace,* 103-123(1997).

52. Al Naib, A., Hanarahan, J.P., Lonergan, P. and Fair, S. In vitro assessment of sperm from bulls of high and low fertility. *Theriogenology*, **76**, 161–167 (2011).

53. Longobard, V. Z., Gianluig, A., Salzano, De C., Canditiis, A. Cammarano, De L. Luise, M.V. Puzio, G. and Neglia B. Gasparrini. Resveratrol prevents capacitation-like changes and improves in vitro fertilizing capability of buffalo frozen-thawed sperm. *Theriogenology*, **88**, 1-8 (2017).

54. Rajoriya, J.S., Prasad, J.K., Ramteke, S.S., Perumal, P., Ghosh, S.K., Singh, M., Pande, M. and Srivastava, N. Enriching membrane cholesterol improves stability and cryosurvival of buffalo spermatozoa. *Anim. Reprod. Sci.,* **164**,72-81 (2016).

55. Lone, S.A., Prasad, J.K., Ghosh, S.K., Das, G.K., Kumar, N., Balamurugan, B., Katiyar , R., Verma, M.R. Effect of cholesterol loaded cyclodextrin (CLC) on lipid peroxidation and reactive oxygen species levels during cryopreservation of buffalo (*Bubalus bubalis*) spermatozoa. *APJR*, **5**, 476-480 (2016a).

56. Lone, S.A., Prasad, J.K., Ghosh, S.K., Kumar, N., Bhat, S.A. and Das, G.K. Effect of cholesterol loaded cyclodextrin on activity of antioxidants during cryopreservation of buffalo (Bubalus bubalis) semen. *Indian J. Anim. Sci.,* **86**, 1255-1258 (2016b).

57.Lone, S.A., Mohanty, T.K., Bhakat, M.,Yadav, H.P., Parav, A.R., Dewry, R.K., Baitholu, R.K., Sinha, R. and kumar, P. Cholesterol-loaded cyclodextrin attenuates dilution effect and improves quality of bovine low sperm insemination doses during cryopreservation. *Andrologia*, **1,** 10 (2021).

58. Salmon, V. M., Leclerc, P. and Bailey, J. L. Cholesterol-Loaded Cyclodextrin Increases the Cholesterol Content of Goat Sperm to Improve Cold and Osmotic Resistance and Maintain Sperm Function after Cryopreservation. *Biol. Reprod*., **94**, 1-12 (2016).

59. Batissaco, L., Arruda, R.P., Alves, M. B. R., Torres, M. A., Lemes, K. M. Prado-Filho, R. R., Almeida,T.G.,deAndrade,A.F.C. and Celeghini, E.C.C. Cholesterol-loaded cyclodextrin is efficient in preserving sperm quality of cryopreserved ram semen with low freezability. *Reprod. Biol*., **20**,14-24(2020).

كفاءة حفظ الحيوانات المنوية لطلائق الجاموس باستخدام مخفف التريس تورين كولسترول لوديد سيكلودكسترين

رضـــا إبراهيـــم الششـــتاوي

قسم التكاثر في الحيوان والتلقيح الصناعي معهد البحوث البيطرية - المركز القومي للبحوث - الجيزة – مصر.

استهدفت الدراسة الحالية تقييم كفاءة حفظ الحيوانات المنوية لطلائق الجاموس باستخدام مخفف التريس تورين كولسترول لوديد سيكلودكسترين اثناء التبريد والتجميد.تم تخفيف السائل المنوى باستخدام مخفف التريس المحتوى على تركيزات مختلفة من كولسترول لوديد سيكلودكسترين(١و١,٥و٢ملجم/مل) والتورين(٦٠ مل مول)واحتوت أنابيب الكنترول على (٠و٠)من من كولسترول لوديد سيكلودكسترين والتورين . وقد تم تبريد السائل المنوى فى قصيبات وترك ٤ ساعات عند درجة٥ مئوية ثم تعريضه ١٠ دقائق وغمره فى النيتروجين السائل للتجميد .تم التقييم أظهرت النتائج تحسن فى صفات السائل المنوى بعد التبريد والتجميد وكانت أحسن النتائج فى التركيز الأول

الكلمات الدالة: الجاموس ، السائل المنوى، تورين، CLC ، تجميد.