ASSESSMENT OF BUFFALO SEMEN PRESERVABILITY UPON USING TRIS-EGG YOLK EXTENDER ENRICHED WITH DIFFERENT CONCENTRATIONS OF TAURINE

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ABSTRACT

Cryopreservation is connected with increased undesirable premature sperm capacitation, reduced lifespan and fertility results. Taurine acts as an antioxidant improving the semen quality after cryopreservation. The objective of the current study is to assess the effect of addition of different levels of taurine amino acid to tris-extender on bull sperm cryo-preservability. Methods: The collected semen samples were pooled and diluted with tris-citric acid-fructose egg yolk extender (control, 0% taurine) and different concentrations of taurine (10 mM, 20 Mm, 30 Mm, 40 Mm, 50 Mm, 60 Mm, 70 Mm, 80 Mm, 90 Mm and 100 mM) to final concentration of 60 x10^6 motile sperm/ ml. Diluted semen was processed for freezing and evaluated for sperm parameters. Results: Data showed that taurine enriched extender improved sperm parameters during cooling. Post thawing sperm motility %, membrane integrity %, Alive sperm % improved and the percentages of abnormal sperm was lowered upon using taurine enriched extender. Conclusion: It could be concluded that, most the concentrations of taurine in Tris–citrate egg yolk extender ameliorated the post cooling and post freezing semen quality in buffalo bulls.

Keywords: *Bubalus bubalis*, buffaloes, semen, freezing, taurine

INTRODUCTION

Artificial insemination is a breeding technology widely applied in buffalo husbandry using frozen semen. However, frozen semen exhibit declined results of IVF which induced by temperature changes, sperm cell dehydration, freezing and thawing (Medeiros *et al*., 2002). Cryopreservation is connected with increased undesirable premature sperm capacitation, reduced lifespan and fertility results (Medeiros *et al*., 2002). In bovine semen, dead spermatozoa induce ROS production via an aromatic amino acid oxidase catalyzed reaction (Upreti *et al*., 1998). Although over-production ROS alter sperm functionional and enzymatic activities (Baumber *et al*., 2000), low concentrations of it are required for maintenance of the capacitation and fertilizing potential of spermatozoa.

Freezing and thawing processes are a potential source for ROS over-production which adversely affect sperm quality, antioxidant status and fertility (Aitken *et al*., 1998; Bilodeau *et al*., 2001). Sperm cells are subjected to lipid peroxidation

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due to its high contents of polyunsaturated fatty acids (PUFA), resulting in impairment of motility, membrane integrity, fertilizing capacity and metabolic alterations of sperm (Cassani *et al*., 2005). Tris-based diluents are commonly used for semen freezing in farm animals including buffaloes (Purdy, 2006). Taurine were indicated to be beneficial additive to the freezing extender of bull (Chen *et al*., 1993; Sarıozkan *et al*., 2009; Uysal *et al*., 2007), ram (Bucak *et al*., 2007; Bucak *et al*., 2008), goat (Purdy, 2006), donkey (Bottrel *et al*., 2018) and human (Seify *et al*., 2019) to improve the semen cryo-survival.

Taurine acts as a potent antioxidant (Chen *et al*., 1993; Foote *et al*., 2002), interact with membrane phospholipids, creating hypertonic media, dehydrate water and so decreasing ice crystal formation within sperm cell during freezing. (Liu *et al*., 1998; Storey *et al*., 1998). The effect of including taurine in buffalo semen extender on sperm post-cooling and frozen thawed semen quality has not been sufficiently interpreted. Therefore, this study aimed to evaluate the effect of addition of different concentrations of taurine to buffalo semen extender on sperm freezability and quality parameters.

MATERIALS AND METHODS

The present plan of work was approved by the Medical Research Ethics Committee of the National Research Centre, Cairo, Egypt (Ethical Approval Certificate no. 18 212).

Semen collection: semen samples were collected from Five buffalo-bulls belonging Ministry of Agriculture, Egypt using an artificial vagina at weekly intervals for 5 weeks. These samples were evaluated directly for volume, concentration and sperm motility and semen samples with more than 70% motility and 80% normal spermatozoa were proceed to freezing procedure. To avoid individual bull variations the ejaculates were pooled kept for 10 minutes in a water bath adjusted at 37^{o}C before dilution.

Semen processing

Semen samples were diluted in a Tris–citrate fructose egg yolk extender (TCFY) containing 20% egg yolk and 7% glycerol at 37^{o}C (de Paz *et al*., 2010; Roof *et al*., 2012) and the final conventions of spermatozoa were adjusted to be 60x10^{6} motile sperm mL^{-1}. Taurine was added at various concentrations to tris extender (10 to 100 mM, 10 stepwise increase) and the control tubes free of taurine (0). The tubes were cooled gradually within 2 h to reach up to 5^{o}C and equilibrated for 4 h. 0.25 mL polyvinyl French straws (IMV, France) were used for semen packing.

The packed semen straws were arrange horizontally on a rack and subjected liquid nitrogen (LN_2) for 10 minutes and dipped stored in liquid nitrogen at -196^{o}C directly.

Semen evaluation

Sperm motility, viability, abnormality, membrane integrity and acrosome integrities in cooled and frozen-thawed semen were evaluated. Thawing of Frozen straws were done at 37ºC for 30 seconds using water bath.

Sperm motility

Motility was evaluated using phase contrast microscope (Olympus Optical Co. Ltd., Japan) to the nearest 10%.

Live and abnormal spermatozoa (%)

Eosin-Nigrosin stained smears were

examined for viability and abnormalities% of sperm according to Sidhu and Guraya (1985).

Sperm membrane integrity

Sperm membrane integrity was examined using the hyposmotic swelling test (Jeyendran *et al*., 1984). Two hundred sperm cells were examined to calculate the % spermatozoa with swollen/intact plasma membrane.

Intact normal acrosome percent

Smears stained with Giemsa stain were examined to evaluate acrosome integrity according to Watson (1975).

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA), Duncan test to assess significant differences in all the semen characteristics among all groups using SPSS (2005) Version 14.0 for Windows. Differences with values of $P<0.05$ were considered to be statistically significant.

RESULTS

Effect of taurine enriched extender on buffalo sperm characteristics during chilling

Data in Table 1 showed that sperm motility was significantly ($P<0.0150$) kept high during cooling with taurine enriched extender at the concentration of 50, 60, 70, 90 and 100 mmol/L (91.67, 86.67, 88.33, 93.33% and 86.67%, respectively) compared to the control (88.33%). Additionally, taurine had significantly ($P<0.0125$) improved alive sperm % at the concentration of 50 (94.00%) and intact spermatozoa membrane % at the concentration of 40, 50 and 60 (84.00, 83.00 and 79.33%, respectively), also the use of taurine had significantly ($P<0.0001$) lowered the percentages of abnormal sperm compared to the control at all the concentrations used in the current study.

Effect of taurine enriched extender on buffalo post thawing sperm characteristics

Data in Table 2 illustrated that post thawing sperm motility % was kept significantly ($P<0.0573$) higher with taurine enriched extender at the concentration of 20, 50, 60, 70, 90 and 100 mmol/L (56.25, 60.00, 58.75, 56.25, 60.00 % and 57.50%, respectively) compared to the control (42.50%). Also, membrane integrity % was significantly ($P<0.0001$) increased at the concentration of 10, 50, 60, 70, 80 and 100 mmol/L (58.50, 57.50, 65.25, 69.75, 62.00 % and 62.25%, respectively) compared to the control (49.00%). While, alive sperm % was significantly ($P<0.0293$) improved at the concentration of 60 mmol/L (75.75 %). On the other hand, the use of taurine had significantly ($P<0.0001$) lowered the percentages of abnormal sperm % at the concentrations of 20 mmol/L (5.25%) when compared to the control (8.00%) while the other concentrations didn't illustrate any effect.

DISCUSSION

A variety of antioxidants are found in the spermatozoa and seminal plasma as represented by antioxidant enzymes (SOD, CAT and GSH). Their antioxidant capacity is insufficient and gradually declines on extending the freezing process, so antioxidant supplements should be added to the semen extender (Bilodeau *et al*., 2001). Taurine has a beneficial effect in improving CAT level and consequently enhancing the antioxidant effect

(Bucak *et al*., 2007).

In the current study, taurine enrichment to the freezing extender improved semen characteristics as represented by post- thaw sperm motility, sperm membrane integrity, acrosome status and viability. Our results agreed with Reddy *et al*. (2010) who recorded improved semen parameters by the effect of taurine.

In the present study, addition of 50 to 100 mM concentrations of taurine improved sperm motility while addition of 50 mM taurine gave the best alive and intact sperm percent of the cooled semen and all concentrations of taurine significantly lowered the percent of abnormal sperms as compared to the control. The evaluation of viability, acrosome and membrane integrity are important as motility only is insufficient for sperm evaluation post freezing. Our results regarding the post-thawed semen revealed improved sperm motility and sperm membrane integrity at 50 to 100 mM concentration of taurine, alive sperm improved at 60 mM and sperm abnormalities significantly reduced at 20 mM. Sperm motility ameliorated also at concentration 20 mM and sperm membrane integrity enhanced also at 10 mM taurine. These improved results were compatible to that obtained in buffalo (Reddy *et al*., 2010) and ram (Alvarez *et al*., 1983).

Cryopreservation process lead to over accumulation of oxygen free radicals that lead to decreased motility, membrane integrity and fertilizing potential (Cassani *et al*., 2005). Spermatozoa are susceptible to oxidative stress which can be minimized by supplementing the semen extender with antioxidants (El-Sheshtawy *et al*., 2017). Many studies clarified the improving effect of taurine on post- thaw semen quality (Bucak *et al*., 2007; Sarıozkan *et al*., 2009). Taurine is a sulfuric amino acid that plays an important role as non enzymatic intrinsic scavenger of the oxygen free radicals, so protect sperm from oxidative damage during cryopreservation (Sarıozkan *et al*., 2009; Saleh and Agarwal, 2002). Thus, increasing motility and longevity (Alvarez and Storey, 1983; Holmes *et al*., 1992; Chen *et al*., 1983).

Taurine addition may exert cryoprotective effect on the functional integrity of acrosome and mitochondria with subsequent generation of energy from intracellular stores of ATP that lead to improved sperm motility (Bucak *et al*., 2007). It could be concluded that, most the concentrations of taurine in Tris-citrate egg yolk extender ameliorated the post cooling and post freezing semen quality in buffalo bulls.

Abbreviations

LN_2: Liquid nitrogen; IVF: *in vitro* fertilization; ROS: reactive oxygen species; PUFA: polyunsaturated fatty acids; LPO: lipid peroxidation; TCFY: Tris-citric acid-fructose egg yolk; HOST: hypoosmotic swelling test; ANOVA: one-way analysis of variance; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione reduced; ATP: adenosine triphosphate

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Table 1. Effect of taurine-tris extender on sperm quality parameters in cooled buffalo bull semen.

Mmol/L	Percentage				
	Sperm motility	Membrane integrity	Alive sperm	Abnormal sperm	Acrosome integrity
0 (Control)	88.33±1.67^{abc}	70.33±3.18^{cde}	86.67±2.03^{cd}	18.67±0.67^{a}	91.33±2.33^{a}
10	85.00±2.89^{bcd}	66.33±2.91^{de}	85.00±2.89^{d}	7.33±0.88^{bcd}	91.33±0.67^{a}
20	85.00±2.89^{bcd}	72.33±1.86^{bcd}	87.33±1.45^{bcd}	6.67±1.33^{bcd}	92.67±0.33^{a}
30	80.00±2.89^{d}	62.67±2.60^{e}	85.33±3.28^{d}	8.67±0.67^{bc}	91.67±0.88^{a}
40	83.33±1.67^{cd}	84.00±2.65^{a}	89.67±0.88^{abcd}	9.33±0.88^{b}	92.33±1.45^{a}
50	91.67±0.88^{ab}	83.00±2.08^{a}	94.00±1.53^{a}	5.00±1.15^{d}	92.67±0.33^{a}
60	86.67±1.67^{abcd}	79.33±1.67^{ab}	92.67±1.76^{abc}	5.67±0.33^{cd}	92.00±1.53^{a}
70	88.33±1.67^{abc}	65.33±2.85^{de}	92.67±0.88^{abc}	10.00±1.15^{b}	93.33±0.67^{a}
80	85.00±2.89^{bcd}	75.00±3.51^{bc}	91.00±1.53^{abcd}	9.00±1.00^{bc}	91.67±0.88^{a}
90	93.33±1.67^{a}	66.67±2.03^{de}	93.33±1.67^{ab}	9.00±0.58^{bc}	92.33±1.45^{a}
100	86.67±1.67^{abcd}	75.00±1.53^{bc}	91.67±1.20^{abc}	8.33±1.76^{bc}	90.67±1.33^{a}
F-cal	3.01	8.29	3.12	12.52	0.39
Sig	0.0150	0.0001	0.0125	0.0001	0.9392

Different superscripts within column indicate a significant difference between means using the Duncan multiple range test at $P<0.05$.

Table 2. Effect of taurine-tris extender on sperm quality parameters in frozen buffalo bull semen.

Mmol/L	Percentage				
	Sperm motility	Membrane integrity	Alive sperm	Abnormal sperm	Acrosome integrity
0 (Control)	42.50±2.50^{c}	49.00±5.32^{e}	71.25±2.39^{bc}	8.00±1.29^{bcd}	81.75±1.18^{a}
10	52.50±3.23^{abc}	58.50±3.38^{bcd}	74.00±0.82^{abc}	11.75±1.11^{a}	79.75±1.11^{a}
20	56.25±4.27^{ab}	55.00±1.29^{cde}	74.50±1.50^{ab}	5.25±0.48^{e}	82.00±1.22^{a}
30	46.25±2.39^{bc}	55.75±2.17^{cde}	73.75±1.25^{abc}	7.75±0.48^{cde}	81.75±0.48^{a}
40	51.25±2.39^{abc}	50.25±1.03^{de}	72.00±1.15^{abc}	9.00±0.71^{bc}	81.25±1.25^{a}
50	60.00±5.00^{a}	57.50±2.10^{bcd}	74.00±0.82^{abc}	7.75±0.85^{cde}	80.75±0.48^{a}
60	58.75±4.73^{ab}	65.25±2.29^{ab}	75.75±0.75^{a}	6.25±0.48^{de}	83.50±0.50^{a}
70	56.25±4.27^{ab}	69.75±1.70^{a}	72.25±1.03^{abc}	10.25±1.11^{abc}	81.25±0.48^{a}
80	53.75±3.75^{abc}	62.00±1.58^{abc}	70.25±1.18^{c}	10.50±0.65^{ab}	80.75±0.85^{a}
90	60.00±5.00^{a}	55.00±1.29^{cde}	70.25±0.48^{c}	11.75±1.03^{a}	81.75±1.44^{a}
100	57.50±4.33^{ab}	62.25±3.09^{abc}	71.00±0.71^{bc}	10.25±0.25^{abc}	82.00±0.91^{a}
F-cal	2.07	5.82	2.39	6.71	0.97
Sig	0.0573	0.0001	0.0293	0.0001	0.4901

Different superscripts within column indicate a significant difference between means using the Duncan multiple range test at $P<0.05$.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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