Assiut Vet. Med. J. Vol. 60 No. 142 July 2014

EFFECT OF TREHALOSE, CYSTEINE AND HYPOTAURINE ON BUFFALO BULL SPERM FREEZABILITY, ULTRASTRUCTURE CHANGES AND FERTILIZING POTENTIALS

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Received at: 15/4/2014

Accepted: 21/6/2014

ABSTRACT

Cryopreservation induces sublethal damage to the spermatozoa, which leads to reduce their fertile life. The objective of the present study was to investigate the effect of trehalose, cysteine and hypotaurine on freezability, ultrastructure and in vitro fertilizing potentials of the buffalo spermatozoa. Buffalo spermatozoa were cryopresreved with Tris egg yolk extender containing 7% glycerol suplemmented with100 mM trehalose, 100 mM trehalose + 5 mM cysteine, 100 mM trehalose + 25 mM hypotaurine, 100 mM trehalose + 5 mM cysteine + 25 mM hypotaurine or Tris-based extender only (control). Cryopresreved spermatozoa were assessed for post-thawing sperm motility; viability and acrosomal integrity, ultrastructure changes, biochemical activity and in vitro fertilizing potentials. The current results clearly indicated that adding a mixture of 100 mM trehalose , 5 mM cysteine and 25 mM hypotaurine to Tris extender significantly improved ($P<0.05$) post-thawing sperm motility, viability index and maintained acrosomal integrity following cryopreservation (63.33±7.27%, 155.83±21.06 and 12.33±2.73%, respectively) compared with the control spermatozoa (38.33±4.41%, 94.17±12.28 and 25.33±3.49%, respectively). The current results illustrated that addition of combination of additives protected the plasma membrane, acrosomal region and mitochondria and maintained the ultrastructure integrity of the cryopresreved spermatozoa compared with the control spermatozoa. Additionally, the current results revealed that combination of trehalose, cysteine and hypotaurine significantly increased the total antioxidant capacity level (TAC), superoxide dismutase activity (SOD) and glutathione reductase activity (GSH) and reduced significantly the lipid peroxidation rate (0.60±0.89 mμ/ml, 70.00 ±5.78 U/L, 81.66±4.41 U/L and 7.33±1.86 nmol/ml, respectively) compared with the control extender (0.23±0.04 mμ/ml, 34.67±6.74U/L, 51.67±14.82 U/Land 20.67±3.84 nmol/ml, respectively). Furthermore, a mixture of trehalose, cysteine, hypotaurine significantly improved ($P<0.05$) the rate of in vitro fertilization, cleavage and embryo development to morula and blastocyst stages (62.66±6.28, 53.54±3.89, 23.87±6.28 and17.94±2.57%) compared with the control extender (31.19±4.42, 18.47±5.04, 8.36±1.91 and .42±1.71%, respectively). Therefore, the present results revealed that addition of a mixture of trehalose, cysteine and hypotaurine to the freezing extender could improve semen quality and reduce cryodamage of the buffalo bull spermatozoa.

Key words: *Trehalose, cysteine and hypotaurine, ultrastructure and in vitro fertilizing potentials.*

INTRODUCTION

Semen cryopreservation offers many advantages to the livestock industry, particularly in conjunction with allowing the widespread dissemination of valuable genetic material by means of artificial insemination (Bucak *et al.*, 2009). The success of an AI program depends on the proper management of semen collection, storage and use (Leboeuf *et al.*, 2000). Although, many protocols have been developed for semen cryopreservation, sperm cryosurvival rate is still not optimum in the buffalo. Cryopreservation induces some irreversible damages in sperm cells (Medeiros *et al.*, 2002). Factors responsible for these damages includes; changes in temperature, ice formation, access of reactive oxygen species and lipid peroxidation, alterations in sperm membrane, toxicity of cryoprotectants and osmotic stress which reduces the post thaw quality of semen (Watson, 2000). To

keep the cell alive during cryopreservation process, plasma membrane is a key component that must be maintained (Aboagla and Terada, 2003). The plasma membrane of mammalian spermatozoa contains high concentrations of polyunsaturated fatty acids, which make it susceptible to reactive oxygen species (ROS) induced peroxidative damage with a subsequent loss of sperm functions (Lenzi *et al.*, 2002). There are several substances used to protect sperm plasma membrane during cryopreservation, the most important of them are sugars and antioxidants.

Sugars have several functions in sperm extenders, including providing energy substrate for the sperm cell during incubation (Fukuhara and Nishikawa, 1973), maintaining the osmotic pressure of the diluents (Aboagla and Terada, 2003), acting as a cryoprotectant and decreasing the extent of cell injury by reducing the intracellular ice formation (Liu *et al.*, 1998). Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) is a non-reducing disaccharide consisting of two glucose moieties joined together by an alpha-1, 1 glucosidic bond (Patist and Zoerb, 2005). It has stabilizing effect on both cellular protein and plasma membrane (Aboagla and Terada, 2003) and reducing the cell injury by ice crystallization (Molinia *et al.*, 1994). Recently, authors suggested modern mechanisms of trehalose as it may have antioxidant action (Hu *et al.*, 2010 and Reddy *et al.*, 2010).

The inclusion of antioxidants in the cryopreservation media has improved the quality of semen against ROS-induced damage (Badr *et al.*, 2009 and Sariozkan *et al.*, 2009). Various antioxidants have been used for the purpose such as hypotaurine and cysteine.

Cysteine is a thiolic compound which is a precursor in the biosynthesis of intracellular glutathione. It penetrates the plasmatic cell membrane easily and has indirect radical scavenging properties (Uysal and Bucak, 2007). Moreover, cysteine has a cryoprotective effect on the functional integrity of axosome and mitochondria integrity (Bilodeau *et al.*, 2001 and Bucak and Uysal, 2008).

Hypotaurine is a precursor of taurine which exists in mammalian spermatozoa and it is essential for sperm functions, such as, motility, fertilizing ability and early embryonic development (Guerin *et al.*, 1995). Moreover, hypotaurine plays role in cell proliferation, viability, osmo-regulation, prevents injuries induced by oxidants in many tissues, protects of sperm membrane against lipid peroxidation (Chesney, 1985 and Huxtable, 1992), modulates Ca2+ uptake (Singh *et al.*, 2012) and inhibits protein phosphorylation (Kumar and Atreja, 2012).

Therefore, this study was aimed to assess the effect of trehalose, cysteine and hypotaurine on cryopreservation, ultrastructure changes, antioxidant capacity and in vitro fertilizing potentials of buffalo spermatozoa.

MATERIALS and METHODS

Semen collection and processing:

Semen samples were collected from six fertile buffalo bulls. Only semen samples at least 70 % initial motility and 800 $x10^6$ sperm cells/ml were used. After collection, semen samples were pooled, split into 5 portions and diluted at 30°C with Tris-based extender supplemented with 100 mM trehalose, 100 mM trehalose and 5 mM cysteine, 100 mM trehalose and 25 mM hypotaurine, 100 mM trehalose + 5 mM cysteine + 25 mM hypotaurine or Tris-based extender only (control). The extended semen was cooled to 5°C throughout 60 minute in a cold cabinet. The cooled semen was loaded into 0.25 ml French straws, and then suspended into liquid nitrogen vapor inside foam box before immersed into liquid nitrogen. Frozen semen straws were thawed in a water bath at 37°C for 30 second. Post-thawing sperm motility, viability index and acrosomal integrity were assessed according to Mohammed *et al.* (1998).

Biochemical analysis:

All biochemical measurement of the cryopresreved spermatozoa was carried out using the spectrophotometer. Extracellular aspartate-aminotransferase (AST); alanine-aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes concentration during cryopreservation was assessed according to Tietz (1976). Total antioxidant capacity level (TAC) was measured at 532 nm according to Cortossa *et al.* (2004). Lipid peroxidation was estimated by the end point generation of malondialdehyde according to Cortassa *et al.* (2004). SOD activity was measured at 560 nm and expressed as units per liter according to Flohe and Otting (1984). GSH content of sperm was measured at 412 nm and expressed as units per liter according to Sedlak and Lindsay (1968).

Ultrastructure analysis of the cryopresreved spermatozoa:

The ultrastructure changes occurred for the cryopresreved spermatozoa were evaluated by transmission electron microscopy (TEM). Straws from each treatment were washed three times by centrifugation at 1000 rpm for 5 min using PBS (Phosphate Buffered Saline). The frozen-thawed semen was prefixed for 2-3 h with PBS containing 2% glutaraldehyde, washed three times by centrifugation at 1000 rpm with PBS (pH 7.4) for 5 min at 4°C and post-fixed in 1% osmium tetroxide for 1-2 h at 4°C (Boonkusol, 2010). Spermatozoa were dehydrated in Ascending grade of ethanol (50,70,90 and100) and proplim oxide for one hour and embedded in epon resin. Ultrathin sections were cut

using the Leica EM UC6 ultramicrotome and stained with uranylacetate and lead citrate. Randomly fields were examined by a transmission electronic microscope (JEOL-EM-100 S at 80 Kv at VACSERA- Electron Microscopy Unit) and photographed for further analysis.

Evaluation of in vitro fertilizing potentials of the treated semen:

In vitro matured oocytes were washed three times in the fertilization media and incubated at 39°C in an atmosphere of 5% CO_2 in air with maximum humidity for 2 hours before insemination. Three straws from each treatment were thawed in a water bath at 37°C for 30 sec. The most motile spermatozoa were separated by swim up technique in the fertilization medium, modified Tyrode's Albumin-Lactate-Pyruvate (TALP) containing 6 mg/ml bovine serum albumin (BSA) for 1 hour as recorded by Parrish *et al.* (1988). The uppermost layer of the medium containing the most motile spermatozoa was collected and washed twice by centrifugation at 800 xg for 10 minutes. The sperm pellet was resuspended in the fertilization TALP medium containing 10 µg /ml heparin. After appropriate dilution, 2 µl (final concentration 2 $x10^6$ sperm cells/ml) of sperm suspension was added to the fertilization drops, containing in vitro matured oocytes. Gametes were co-incubated in the fertilization drops under sterile mineral oil for 6 hour at 39°C in an atmosphere of 5% CO_2 in air with maximum humidity. After 6 hours some oocytes were stained using aceto-orcein stain to evaluate in vitro fertilization rate (Totey *et al.*, 1992). Other oocytes were further cultured for 7 days, in modified synthetic oviductal fluid media supplemented with amino acids (SOFaa), to evaluate the in vitro embryo development according to Badr (2009).

Statistical analysis:

All data were analyzed by using Costat Computer Program (1986) Cottort Software, and were compared by the least significant difference least (LSD) at 5% levels of probability. The results were expressed as means ±SE.

RESULTS

The current results clearly indicated that adding a mixture of 100 mM trehalose , 5 mM cysteine and 25 mM hypotaurine to Tris extender significantly improved (P<0.05) post-thawing sperm motility , viability index and maintained acrosomal integrity following cryopreservation (63.33±7.27%, 155.83±21.06 and 12.33±2.73%, respectively) compared with the control spermatozoa (38.33±4.41%,94.17±12.28 and 25.33±3.49%, respectively).

Table 1: Effect of adding trehalose, cysteine and hypotaurine to the semen extender on the buffalo spermatozoa freezability.

Treatment	Cooling motility	Post-thawing motility	Viability index	Acrosomal integrity
Control	73.33±7.27[a]	38.33±4.41[b]	94.17±12.28[b]	25.33±3.49[a]
100 mM trehalose	73.33±6.01[a]	51.66±7.27[ab]	115.00±22.57[ab]	14.33±2.40[b]
100 mM trehalose+ 5 mM cysteine	81.67±4.42[a]	51.67±4.42[ab]	141.66±13.42[ab]	15.00±2.65[b]
100mM trehalose +25 mM hypotaurine	78.33±7.27[a]	53.33±6.01[ab]	127.17±16.61[ab]	14.06±2.18[b]
100mM trehalose+ 5mM cysteine+25 mM hypotaurine	81.67±6.01[a]	63.33±7.27[a]	155.83±21.06[a]	12.33±2.73[b]

[a,b]Values with different letters in the same column are significantly different (*P<0.05*).

respectively) compared with the control extender (0.23±0.04 mµ/ml, 34.67±6.74U/L, 51.67±14.82 U/L and 20.67±3.84 nmol/ml, respectively). Moreover, data presented in table 3 clarified that, addition of a mixture of 100 mM trehalose, 5 mM cysteine and 25 mM hypotaurine to Tris extender maintained sperm cell membrane integrity and this appeared through reduction of AST, ALT and ALP enzymes leakage (59.00±9.55, 16.50±5.31 and 12.33±2.02 U/L, respectively) compared with the control extender (102.33±7.89, 31.00±4.62 and 21.67±1.32U/L, respectively).

Data regarding the effect of adding trehalose, cysteine and hypotaurine to the freezing extender on the TAC capacity, SOD, GSH activity and lipid peroxidation of the cryopreserved semen are presented in table 2. In vitro provision of semen extender with a mixture of 100 mM trehalose, 5 mM cysteine and 25 mM hypotaurine to Tris extender significantly (P<0.05) increased the TAC capacity, SOD and GSH activity and diminished lipid peroxidation (LPO) of the frozen-thawed semen (0.60±0.89mµ/ml, 70.00±5.78 U/L, 81.66±4.41 U/L and 7.33±1.86 nmol/ml,

Table 2: Effect of addition of trehalose, cysteine and hypotaurine to the semen extender on the total antioxidants capacity (TAC) Superoxide dismutase (SOD), Glutathione reductase (GSH) and the lipid peroxidation (LPO) rate.

Treatment	TAC	SOD	GSH	LPO
Control	0.23±0.04^{b}	34.67±6.74^{b}	51.67±14.82^{b}	20.67±3.84^{a}
100 mM trehalose	0.51±0.09^{a}	48.33±6.02^{ab}	70.00±5.78^{ab}	11.33±1.85^{b}
100 mM trehalose+ 5 mM cysteine	0.56±0.8^{a}	61.67±7.27^{a}	78.33±7.28^{ab}	10.33±2.02^{b}
100mM trehalose +25 mM hypotaurine	0.58±0.11^{a}	51.67±10.15^{ab}	71.66±7.27^{ab}	9.66±2.02^{b}
100mM trehalose+ 5mM cysteine+25 mM hypotaurine	0.60±0.89^{a}	70.00±5.78^{a}	81.66±4.41^{a}	7.33±1.86^{b}

^{a,b}Values with different letters in the same column are significantly different ($P<0.05$).

Table 3: Effect of addition of trehalose, cysteine and hypotaurine to the semen extender on the enzymatic level of the cryopreserved buffalo semen.

Treatment	AST	ALT	AKP
Control	102.33±7.89^{a}	31.00±4.62^{a}	21.67±1.32^{a}
100 mM trehalose	75.00±10.41a^{b}	14.67±1.45^{b}	14.66±2.19^{ab}
100 mM trehalose+ 5 mM cysteine	71.66±13.65^{ab}	18.66±3.84^{b}	15.66±2.03^{ab}
100mM trehalose +25 mM hypotaurine	73.33±11.01^{ab}	15.66±3.28^{b}	19.66±3.84^{ab}
100mM trehalose+ 5mM cysteine+25 mM hypotaurine	59.00±9.55^{b}	16.50±5.31^{b}	12.33±2.02^{b}

^{a,b}Values with different letters in the same column are significantly different ($P<0.05$).
AST: aspartate-aminotransferase ALT: alanine-aminotransferase ALP: alkaline phosphatase

Electron microscopy images of sagital sections of the frozen thawed buffalo sperm cells treated with a mixture of trehalose, cysteine and hypotaurine illustrated a well defined and intact plasma membrane and homogenous mitochondria content (Fig. 4, 5, 8 and 9). Intact outer and inner acrosomal membranes and high-quality mitochondrial dense electron spaces with appeared transverse cristae. Meanwhile, frozen semen in the control group showed, swollen plasma membrane segmentation of the outer acrosomal membrane and swollen acrosome and severe degeneration marked vacuolation in the mitochondria with complete absence of the transverse cristae (Fig. 1, 2, 3, 6, 7 and 10).

Data concerning the effect of replenishing of semen extender with trehalose, cysteine and hypotaurine to the freezing extender on the in vitro fertilizing potentials and embryo development are presented in table 4. A combination of 100 mM trehalose, 5 mM cysteine and 25 mM hypotaurine yielded a significant (P<0.05) increase in the fertilization and cleavage rates and the development to the morula and blastocyst stages (62.66±6.28, 53.54±3.89, 23.87±6.28 and 17.94±2.57 %, respectively) compared with the control (31.19±4.42, 18.47±5.04, 8.36±1.91 and 3.42±1.71%, respectively).

Table 4: Effect of adding trehalose, cysteine and hypotaurine to the semen extender on the in vitro fertilization and embryo development rates.

Treatment	No. oocytes	Fertilization rate	Cleavage Rate	Morula stage	Blastocyst stage
Control	61	31.19±4.42^{b}	18.47±5.04^{b}	8.36±1.91^{b}	3.42±1.71^{b}
100 mM trehalose	54	51.65±7.04^{ab}	46.52±12.64^{ab}	18.66±2.45^{ab}	11.34±3.57^{ab}
100 mM trehalose+ 5 mM cysteine	63	51.31±10.02^{ab}	43.22±6.70^{ab}	19.22±3.28^{ab}	13.02±6.16^{ab}
100mM trehalose +25 mM hypotaurine	68	57.28±9.45^{a}	46.47±15.65^{ab}	19.03±3.45^{ab}	13.07±2.07^{ab}
100mM trehalose+ 5mM cysteine+25 mM hypotaurine	67	62.66±6.28^{a}	53.54±3.89^{a}	23.87±6.28^{a}	17.94±2.57^{a}

^{a,b}Values with different letters in the same column are significantly different ($P<0.05$).

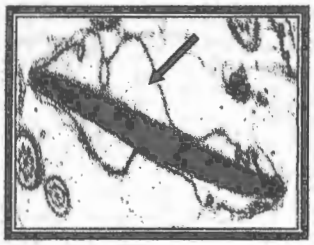

Fig. 1

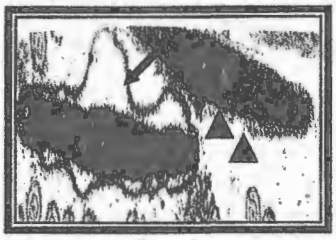

Fig. 2

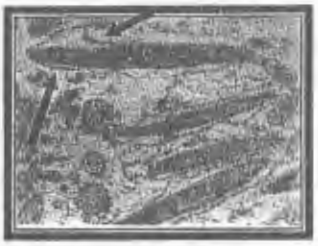

Fig. 3

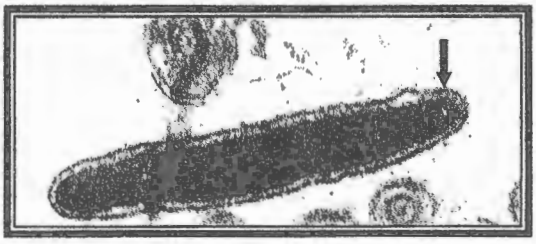

Fig. 4

Fig. 5

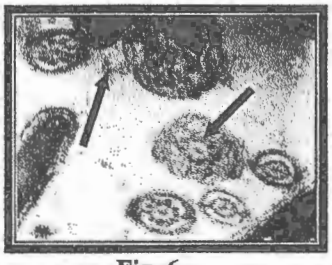

Fig. 6

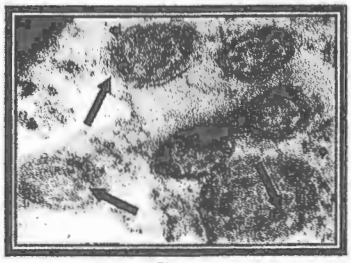

Fig. 7

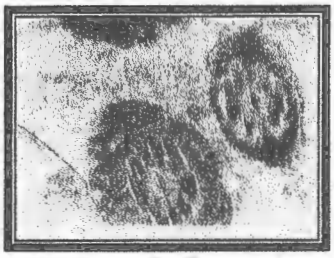

Fig. 8

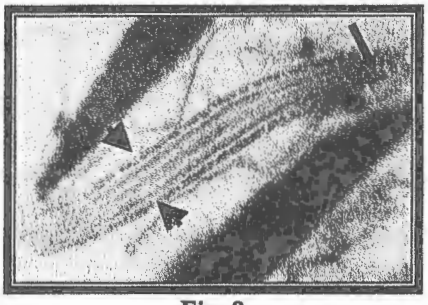

Fig. 9

Fig. 10

Fig. 1&2: Electron micrograph for a sagital section in the sperm head from a frozen-thawed semen sample of control group showing swollen plasma membrane (PM) (arrows) with intact outer acrosomal membrane and the nucleus content (N) is homogenous in the electron density. **Fig 2.** There is complete loss of the plasma membrane, segmentation of the outer acrosomal membrane (OAM) (arrow heads) and swollen acrosome (X 14000). **Fig. 3:** Showing swollen plasma membrane (PM) with intact outer and inner acrosomal membranes and the nucleus content (N) is not homogenous in the electron density. Sometimes there are multiple electron dense materials just underneath the plasma membrane that may be destructed material of the outer acrosomal membrane (arrows) (X 10000).

Fig. 4&5: Electron micrograph for a sagital section in the sperm head from frozen-thawed semen sample treated with a mixture of trehalose, cysteine and hypotaurine illustrating intact plasma membrane (PM) and the nucleus content (N) is homogenous in the electron density. Also, outer and inner acrosomal membranes are intact and the subacrosomal space is evident (arrow) (X 15000).

Fig. 6: Electron micrograph of a cross section in the neck region (note the presence of mitochondria in different orientation) of sperm from a frozen-thawed semen sample of control group showing severe degeneration (marked vacuolation) in the mitochondria that contained electron-translucent spaces with complete absence of the transverse cristae and some mitochondria are completely disappeared (arrows) (X 20000). **Fig. 7:** Electron micrograph of a cross section in the mid-piece region (note the presence of mitochondria in different orientation) of the tail from control samples illustrating light to moderate vacuolation of the mitochondria that contained electron-translucent spaces with complete absence of the transverse cristae (arrows) (X 25000). **Fig. 8:** Electron micrograph of a cross section in the mid-piece region of the tail from a frozen-thawed semen sample treated with trehalose, cysteine and hypotaurine illustrating good mitochondrial dense electron spaces with appeared transverse cristae (X 25000).

Fig. 9: Electron micrograph of a longitudinal section in the mid-piece (arrow heads) and end-piece regions of the tail from a frozen-thawed semen sample treated with trehalose, cysteine and hypotaurine showing marked dense electron mitochondrial matrix parallel along the axonemal complex and the plasmal membrane appeared thin and with less electron density at the end-piece (arrow) (X 5000). **Fig. 10:** Electron micrograph of a longitudinal section in the mid-piece (arrow heads) and end-piece regions of the tail from a frozen-thawed semen sample of the control group showing mild vacuolation of the mitochondrial matrix (mild electron translucent spaces) along the axonemal complex and the plasmal membrane appeared thin and with less electron density at the end-piece (arrow). Also, there is marked degenerated mitochondria appeared in cross section in lowered left field (X 8000).

DISCUSSION

The current results clearly indicated that supplementation of a mixture of trehalose; cysteine and hypotaurine to Tris extender prior to cryopreservation augmented the post-thaw semen quality, antioxidant activity, in vitro fertilizing potentials and preserved the integrity of the fine structure of the spermatozoa. These results are in consistent with the findings of Chen *et al.* (1993), Funahashi and Sano (2005), Anghel *et al.* (2010), Jafaroghli *et al.* (2011) and Shin-Ae *et al.* (2012). The authors showed that supplementation of taurine or trehalose to Tris extender increased the post-thaw semen motility, viability and membrane integrity and significantly decreased the rate of lipid peroxidation of the cryopresreved spermatozoa. This study indicated that there is a synergistic action among trehalose, cysteine and hypotaurine. However, the exact mechanism of sperm protection by amino acids has not been understood and remains unclear. Variety of hypotheses and peculations has been proposed by various authors to explain the protective mechanism of trehalose, cysteine and hypotaurine during cryopreservation. Hu *et al.* (2009), Tonieto *et al.* (2010) and Memona *et al.* (2011) suggested that the improvement of the cryopresreved semen quality may be due to action of trehalose and cysteine that creates the plasma membrane less vulnerable to cryo-damage during freezing and thawing process. Moreover, the presence of trehalose in extenders is likely to modulate membrane fluidity by inserting itself into membrane phospholipids and maintain membrane integrity during dehydration conditions (Woelders *et al.*, 1997). These theories about the effect of trehalose, cysteine and hypotaurine on the integrity of the plasma membrane are explained by the current electron microscope and biochemical results. The electron microscope images indicated that the integrity of the cell plasma membrane, outer and inner acrosomal membranes integrity, mitochondrial dense electron spaces and the homogeneity of the nuclear content was preserved in the sperm cells diluted with Tris extender supplemented with a mixture of trehalose, cysteine and hypotaurine. Likewise, a mixture of trehalose, cysteine and hypotaurine reduced the enzymes leakage during cryopreservation, indicating the intactness of the sperm cell membrane.

Recently some studies suggested that trehalose, cysteine and hypotaurine may have antioxidant properties (Badr *et al.*, 2010, Hu *et al.*, 2010 and Reddy *et al.*, 2010). These observations may explain the results of the current study that emphasized supplementation a mixture of trehalose cysteine and hypotaurine in semen extender increased significantly TAC level, SOD and GSH activity and decreased significantly the lipid peroxidation of the cryopresreved spermatozoa compared with the control semen. Therefore, the advantageous effect of trehalose, cysteine and hypotaurine on the cryopreserved buffalo spermatozoa may be attributed to their ability to preserve the stability of biomembranes, scavenge ROS and protect sperm cell from toxic oxygen metabolites causing lipid peroxidation of sperm plasma membrane. Mammalian spermatozoa are susceptible to LPO which destroys the structure of the lipid matrix of spermatozoa membrane, due to the attacks of ROS formed from reduction of oxygen, during cryopreservation. The damage of lipid matrix finally causes loss of membrane integrity, membrane deterioration-due to membrane phase transitions, decreased sperm motility, loss in fertility and damage of the sperm DNA (Cassani *et al.*, 2005).

Because of the results of the current study that indicated the beneficial effect of trehalose, cysteine and hypotaurine on diminishing the acrosomal damage, lipid peroxidation and enhancing the membrane integrity and their antioxidant activities during cryopreservation, it would ultimately enhance the fertilizing potentials of the cryopresreved spermatozoa.

In conclusion, results emerging from this study clearly demonstrated that supplementation of semen extender with trehalose, cysteine and hypotaurine exerted valuable effects on the quality and the in vitro fertilizing potentials of the frozen-thaw buffalo spermatozoa. These constructive effects appeared due to improvement of the antioxidant activities and diminishing of the lipid peroxidation of the cryopresreved spermatozoa.

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دراسة تأثير التريهالوز ، السيستين والهيبوتورين علي قابليه حيامن الجاموس للتجميد، التركيب الدقيق وكفاءته الإخصابية معمليا

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أجري هذا البحث لدراسة تأثير اضافة التريهالوز ، السيستين والهيبوتورين الي ممدد السائل المنوى علي قابلية حيامن الجاموس للتجميد والتركيب الدقيق والتغيرات الكمياوية وكذا قدرته الاخصابية معمليا. تم تجميع عينات السائل المنوي من ستة طلائق وبعد تقييم السائل المنوي معمليا تم تمديده في ممدد التريس المضاف اليه ١٠٠ ملليمول تريهالوز ، ١٠٠ ملليمول تريهالوز +٥ ملليمول سيستين ، ١٠٠ ملليمول تريهالوز + ٢٥ ملليمول هيبوتورين ،١٠٠ ملليمول تريهالوز +٥ ملليمول سيستين +٢٥ ملليمول هيبوتورين أو ممدد التريس فقط (المجموعة الضابطة) وبعد تبريد وتجميد الحيامن بالنظام الفرنسي تم تقيمه من حيث نسبه الحركه الأماميه والحيويه وتشوهات القلنسوه وكذا اثر التجميد علي التغير في التركيب الدقيق للحيامن والتغيرات الكمياوية وكذلك قدرته الاخصابيه معمليا. ولقد أوضحت نتائج الدراسة الحالية أن تجميد السائل المنوى الجاموسي في ممدد مضاف اليه ١٠٠ ملليمول تريهالوز +٥ ملليمول سيستين +٢٥ ملليمول هيبوتورين نتج عنة زيادة معنوية كبيرة في نسبة الحركة الأمامية بعدالإسالة، معدل الحيوية ونسبة المحافظة علي غشاء القلنسوة (٦٣.٣٣% ، ١٥٥.٨٣و ١٢.٣٣% علي التوالي) مقارنة بالمجموعة الضابطة (٣٨.٣٣% ، ٩٤.١٧و ٢٥.٣٣% علي التوالي). كما حافظ علي سلامة الغشاء البلازمي وغشاء القلنسوة للحيامن وسلامة وانتظام الميتوكوندريا بالمقارنة بالمجموعة الضابطة. كذلك نتج عنة زيادة معنوية كبيرة فى مستوي مضادات الأكسدة الكلية ومستوى انزيم السوبر أوكسيد ديسميوتيز وانزيم الجلوتاثيون المختزل (٠.٦٠ mμ/ml ، ٧٠.٠٠٠ U/L و٨١.٦٦ U/L علي التوالي) مقارنة بالمجموعة الضابطة (٠.٢٣ mμ/ml ، ٣٤.٦٧ U/L و٥١.٦٧ U/L علي التوالي) وانخفاض معنوي كبيرة في معدل أكسده الدهون (٧.٣٣ nmol/ml) مقارنة بالمجموعة الضابطة (٢٠.٦٧nmol/ml). كما أوضحت نتائج الإخصاب المعملى أن تجميد السائل المنوى في ممدد مضاف اليه ١٠٠ ملليمول تريهالوز +٥ ملليمول سيستين +٢٥ ملليمول هيبوتورين نتج عنة زيادة معنوية كبيرة في نسبة إخصاب البويضات وكذلك قدرتها علي الإنقسام والنمو إلي الطور التوتى وطور البلاستوسيست (٦٢.٦٦ ، ٥٣.٥٤، ٢٣.٨٧و ١٧.٩٤% علي التوالي) مقارنة بتلك التى تمديدها في المجموعة الضابطة (٣١.١٩ ، ١٨.٤٧، ٨.٣٦و ٣.٤٢% علي التوالي) ومن خلال نتائج الدراسة الحالية يمكن أن نستنتج أن إضافة ١٠٠ ملليمول تريهالوز +٥ ملليمول سيستين +٢٥ ملليمول هيبوتورين الي ممدد التريس يلعب دورا هاما وكبيرا في تحسن وظائف السائل المنوي الجاموسي المجمد ويبدو ذلك من خلال قدرتها علي مقاومة التأثير الضار لعمليات الأكسدة أثناء التجميد وكذلك المحافظة علي سلامة الحامض النووي للحيامن.