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EFFECT OF VARIOUS TYPES AND LEVELS OF CRYOPROTECTANTS ON SPERM MOTILITY DURING FREEZING PROCESSES OF EGYPTIAN BUFFALO SEMEN.

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ABSTRACT

This study was carried out at El-Gemmezah Animal Production Research Station, belonging to Animal Production Research Institute, during the period from November 2006 to January 2007 to investigate the effect of adding different types and levels of cryoprotectants on sperm viability during different freezing processes of buffalo semen. Five sexually mature buffalo bulls aged 7-10 years were used for semen collection by means of an artificial vagina. Ejaculates were obtained from each buffalo bull twice/week for 10 collection weeks (100 ejaculates). The main extender used for semen dilution was Egg Yolk-Citrate-Tris with different types (glycerol, GL; dimethyl sulfoxide, DMSO and ethylene glycol, EG) and levels (5, 7 and 10% for each) of cryoprotectants. Then, semen was frozen in liquid nitrogen and thawed at 37°C/30 sec. Percentage of progressive motility of spermatozoa was determined pre- and post-dilution, post- equilibration period for 4 or 6 h, post-thawing after 24 h freezing period. Results showed that in post-diluted semen, sperm motility was the highest (72.9%, P<0.05) with GL, followed by DMSO (98.8%), while, EG showed the lowest motility (63.6%). The differences between DMSO and each of GL and EG were not significant. Increasing level of cryoprotectant resulted in gradual reduction in sperm motility, being significant (P<0.05) only by increasing the level from 7 to 10%, whereas sperm motility slightly decreased from 70.7% at a level of 5% to 69.3% at a level of 7%, while it decreased (P<0.05) to 65.2% at a level of 10%. The effect of interaction between type and level of cryoprotectants on sperm motility in post-diluted semen was not significant. In post-equilibrated semen, sperm motility with GL or DMSO was higher (68.7 and 64.8%, respectively, P<0.05) than that with EG (557%). Sperm motility with GL or DMSO did not differ significantly. Increasing level of cryoprotectant from 5 to 7% resulted in slight and insignificant reduction in sperm motility from 65.1 to 64.9%. This reduction was significant (P<0.05) by increasing the level from 7 to 10%, being 59.2% at a level of 10%. Sperm motility was higher (P<0.05) with 6 than 4 hours (58.9 vs. 67.2%) as equilibration period. Only the effect of interaction between type and level of cryoprotectants was significant (P<0.001) on sperm motility in post-equilibrated semen. In post-thawed semen, sperm motility was the highest (36.4%, P<0.05) with GL, moderate (29.7%) with DMSO and the lowest (9.5%) with EG. Increasing level of cryoprotectant from 5 to 7% resulted in slight and insignificant increase in sperm motility in post-thawed semen from 27.4 to 28.8%. Sperm motility sharply decreased (P<0.05) by increasing the level from 7 to 10%, being 17.6% at a level of 10%. The effect of interaction of type with level of cryoprotectants was not significant on sperm motility in post-thawed semen.

The current study concluded that glycerol was the best cryoprotectant as compared to dimethyl sulfoxide and ethylene glycol when it was added at a level of 7% in Tris-based extender used for freezing Egyptian buffalo bull semen.

Keywords: Buffalo semen, glycerol, dimethyl sulfoxide, ethylene glycol, motility.

INTRODUCTION

In a successful organization of artificial insemination, it is important to obtain good quality semen collected from male and in turn high fertility. In Egypt, AI is still practiced on a limited scale. The implementation of AI in buffaloes using liquid semen has been passing problems in respect of preservation.

During semen freezing process, the removal of pure water from diluent to form ice and the resultant increased concentration of diluent in residual liquid are the major physical chemical consequences of freezing. The level and type of cryoprotectants in semen diluent influence these events and their effects on the sperm cells during freezing (Gil *et al.*, 2000, Watson, 2000 and Jorge *et al.*, 2003). Therefore, the cryoprotectants were added to extenders to maintain the sperm for damage during freezing process (Singer *et al.*, 1995).

Glycerol, has been the most widely used as a cryoprotective agent for spermatozoa (Abdel-Khalek *et al.*, 2008). Glycerol penetrates the sperm cell membrane, replacing part of its free water, thus reducing the harmful concentration of intracellular electrolytes during freezing (Mann and White, 1957). Conflicted results were obtained by many authors on the effect of different levels of glycerol on sperm motility of frozen semen (Salamon and Maxwell, 2000). Dimethyl sulfoxide (DMSO) has been reported to have a cryoprotective effect on bull spermatozoa (Snedeker and Gaunya, 1970). While, Molinia *et al.* (1994) revealed that ethylene glycol (EG) exhibited a cryoprotective effect. Rodrigues *et al.* (2004) found that EG diffuse a cross cell membrane in exchange for cell water. This displacement of water by cryoprotectants, in addition to freezing point depression, decreases the possibility of intracellular ice formation and maintains cell volume during freezing, avoiding damage (Demirci *et al.*, 2002).

Therefore the current study aimed to investigate the effect of adding different types (glycerol, dimethyl sulfoxide and ethylene glycol) and levels (3, 5 and 8% for each cryoprotectant) of cryoprotectants on sperm viability during different freezing processes of buffalo semen.

MATERIALS AND METHODS

This study was done at El-Gemmezah Animal Production Research Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, in co-operation with Animal Production Department, Faculty of Agriculture, Mansoura University, during the period from November 2006 to January 2007.

Five sexually mature buffalo bulls aged 7-10 years and weighed 550-620 kg were used for semen collection. Bulls were fed formulated diets on the basis of recommendation of APRI for adult buffalo's bull requirements. All bulls were fed daily ration composed of 8 kg concentrate feed mixture (CFM), fresh berseem (14 kg) and rice straw (4 kg). Animals were housed individually under semi-open sheds. The CFM was composed of

32% undecorticated cotton seed cake, 26% wheat bran, 22% yellow maize, 12% rice bran, 5% linseed meal, 2% vines, 0.5% limestone and 0.5% NaCl. The ration was given individually to all bulls at 8.0 a.m. and 3.0 p.m., while, fresh water and mineral blocks were available for all bulls at all day times.

Semen was collected by means of an artificial vagina set up at optimal conditions to induce a good ejaculatory thrust. At the time of collection, buffalo bull was used as a teaser. One false mount had been always allowed before collection of the first ejaculates. Ejaculates were obtained from each buffalo bull twice/week early in the morning (7 a.m.) for 10 collection weeks (100 ejaculates). Immediately after collection, the ejaculates were transferred to the laboratory and were placed in a water bath at 37°C and care was taken to avoid exposure of the semen to any unfavorable conditions during or after collection. Ejaculates taken from the five bulls (only with ≥70% sperm motility) on each collection day were pooled and divided into 9 portions for dilution.

The main extender used for semen dilution was Egg Yolk-Citrate-Tris extender with different types and levels of cryoprotectants as shown in Table (1). So, 9 extenders with 3 types of cryoprotectants, 3 levels of each cryoprotectant were used in this study.

Table (1): Composition of extenders used in this study with different types and levels of cryoprotectants.

Ingredient	Type of extender								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
Tris buffer (ml)*	75	73	70	75	73	70	75	73	70
Glycerol (ml)	5	7	10	-	-	-	-	-	-
DMSO (ml)	-	-	-	5	7	10	-	-	-
EG (ml)	-	-	-	-	-		5	7	10
Fresh egg yolk	20 ml								
Pencillin	1000 IU/ml								
Streptomycin	1 mg/ml								

*** Tris buffer was composed of 3.028 g Tris, 1.678 g citric acid and 1.25 g glucose dissolved in 100 ml distilled water.**

At each collection time, each of the nine semen portions was diluted with each type of extender (T1-T9) and further processed for freezing, whereas semen of each treatment was extended at rate 1: 10 semen to diluent in heated (37°C) Tris-based extender. Each type of the Tris-based extender was gently mixed and warmed up to 37°C in a water bath during processing of semen extension. The vial containing the extended semen were placed in a water bath at 37°C, and then placed into a refrigerator at 5°C for 4 hours for gradual cooling as an equilibration period of spermatozoa.

Semen extended with each type of extender before filling in straw was always kept in iced water bath to keep its temperature at 5°C while semen packed in straws was placed in a cooled ice chest.

At the end of the equilibration period for 4 hours, the extended semen in each tube was filled by semen automatic filling machine in French straw of 0.25 ml capacity (containing about $20x10^6$ motile sperm/straw). The semen-filling machine connected with computer and printer, which print all the data

on the straws such as bull number, breed and freezing date. The filled straws were sealing by heat.

The extended packed semen was transferred into processing container and located horizontally in static nitrogen vapor 4 cm above the surface of liquid nitrogen for 10 minutes, then the straws were placed vertically in a metal canister and immersed completely in liquid nitrogen container for storage at -196°C for 24 h, thereafter the frozen semen was thawed by dipping the frozen straws into a water bath at 38°C for 30 seconds. Frozen straws were with drawn from the liquid nitrogen container and semen was thawed at 37°C/30 sec.

Percentage of progressive motility of spermatozoa was determined by placing two semen aliquots on warm glass slides (37°C) and examined under light microscopy. The percentage of motile sperm was estimated to the nearest 5%. Percentage of sperm motility was estimated during different following phases of cryopreservation process:

- Initial motility pre-dilution
- Post-dilution (pre-cooling for equilibration period)
- Post- equilibration period (pre-freezing) for 4 h
- Post-thawing after 24 h freezing period

The reduction in each sperm motility post each process was calculated Also, recovery rate of motility in post-thawed semen was calculated as:

Reduction rate (%) =

(Initial motility percent/Post motility percent) x 100

Recovery rate (%) =

{Post-thawed motility (%)/Post-diluted motility (%)} x 100.

The effects of type and level of cryoprotectants and equilibration period on percentage or reduction rate of sperm motility were statistically analyzed as a factorial design according to Snedecor and Cochran (1982) using SAS (2000). Duncan's Multiple Range Test (Duncan, 1955) was used to separate the means when the effect was significant at P<0.05. The percentage values of sperm progressive motility were subjected to arcsine transformation before performing the analysis of variance.

RESULTS AND DISCUSION

Sperm motility in post-diluted semen:

Results presented in Table (2) show significant (P<0.05) effect of both type and level of cryoprotectants on sperm motility in post-diluted semen. Post-dilution, semen diluted by extenders containing glycerol significantly (P<0.05) showed the highest percentage (72.9%) of sperm motility, followed that contained dimethyl sulfoxide (DMSO), being 98.8%, while, semen diluted with extender containing ethylene glycol (EG) showed the lowest sperm motility percentage (63.6%). However, the differences in sperm motility between semen diluted with DMSO and each of glycerol and EG were not significant.

As affected by cryoprotectant level, results show that increasing level of cryoprotectant resulted in gradual reduction in sperm motility. This reduction was significant (P<0.05) only by increasing the level from 7 to 10%, whereas

sperm motility slightly) decreased from 70.7% at a level of 5% to 69.3% at a level of 7%, while it significantly decreased to 65.2% at a level of 10% (Table 2).

Table (2): Effect of type and level of cryoprotectants on sperm motility percentage (X±SE) in post-diluted semen.

Type of Cryoprotectant	Level of cryoprotectant (%)			Overall mean
	5	7	10	
GL	74.3±28.0	74.3±28.0	70.0±20.4	72.9±15.8[a]
DMSO	67.1±25.3	70.0±26.4	69.3±26.1	68.8±15.0[ab]
EG	70.7±26.7	63.6±24.0	56.4±21.3	63.6±13.87[b]
Overall mean	70.7±15.4[a]	69.3±15.1[a]	65.2±14.2[b]	-

a and b: Means denoted within the same row or column with different superscripts are significantly different at P<0.05). GL: Glycerol EG: Ethylene glycol

The effect of interaction between type and level of cryoprotectants on sperm motility in post-diluted semen was not significant indicating a negative effect of increasing level of each type of cryoprotectant on sperm motility and the superiority of glycerol at a level of 5 or 7% in improving sperm motility of buffalo spermatozoa in post-diluted semen (Table 2).

The reduction rate in sperm motility in post diluted semen showed consistent trend with that of sperm motility percentage, but the reduction rate in sperm motility with glycerol at a level of 7% was the lowest rate (8.8%) as compared to the other additives (Table 3).

Table (3): Effect of type and level of cryoprotectants on reduction rate (%) of sperm motility (X±SE) in post-diluted semen proportional to initial motility.

Type of Cryoprotectant	Level of cryoprotectant (%)			Overall mean
	5	7	10	
GL	10.4±3.91	8.8±3.43	14.1±5.29	11.1±2.42[b]
DMSO	17.5±6.59	10.6±3.99	16.4±6.21	14.8±3.23[b]
EG	13.2±4.99	21.9±8.27	30.7±11.60	21.9±4.78[a]
Overall mean	13.7±2.98[b]	13.8±3.00[b]	20.4±4.46[a]	-

a and b: Means denoted within the same row or column with different superscripts are significantly different at P<0.05). GL: Glycerol EG: Ethylene glycol

Sperm motility in post-equilibrated semen:

Results presented in Table (4) show that the effect of type and level of cryoprotectants as well as equilibration period on sperm motility in post-equilibrated semen was significant. Sperm motility in post-equilibrated semen diluted with extenders containing glycerol or DMSO was significantly (P<0.05) higher (68.7 and 64.8%, respectively) than that contained EG (557%). However, sperm motility in semen diluted with glycerol or DMSO did not differ significantly.

Increasing level of cryoprotectant from 5 to 7% resulted in slight and insignificant reduction in sperm motility from 65.1 to 64.9%. However, this reduction was significant (P<0.05) by increasing the level from 7 to 10%, being 59.2% at a level of 10% (Table 4).

As affected by equilibration period, sperm motility was significantly (P<0.05) higher with 6 than 4 hours as equilibration period (58.9 vs. 67.2%, Table 4).

Table (4): Effect of type and level of cryoprotectants, and equilibration period on percentage and reduction rate of sperm motility (X±SE) in post-thawed semen.

Item	Sperm motility (%)	Reduction rate (%)*
Type of cryoprotectant:		
GL	68.7±1.13[a]	4.1±2.91
DMSO	64.8±1.02[a]	5.1±1.53
EG	55.7±2.20[b]	11.7±2.14
Level of cryoprotectant (%):		
5	65.1±1.11[a]	6.4±2.31
7	64.9 ±1.54[a]	5.5±1.98
10	59.2±2.01[b]	8.9± 1.84
Equilibration period (h):		
4	58.9±1.39[b]	6.9±2.18
6	67.2±1.20[a]	7.0±1.89

[a and b]: Means denoted within the same row or column with different superscripts are significantly different at P<0.05). GL: Glycerol EG: Ethylene glycol * Proportional to sperm motility in post diluted semen

The effect of interaction was significant (P<0.001) on sperm motility in post-equilibrated semen only between type and level of cryoprotectants. This was reflected in the highest sperm motility for semen diluted with extender containing 7% glycerol as compared to the other levels. On the other hand sperm motility showed gradual increase by increasing level of DMSO and gradual reduction by increasing level of EG, showing an opposite trend of change with increasing their levels (Fig. 1). However, the interaction effects of each of level and type of cryoprotectants with equilibration period or of type x level x period were not significant.

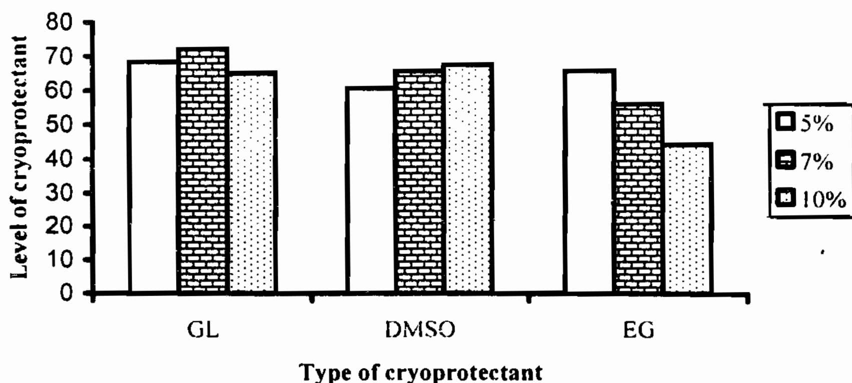

Fig. (1): Effect of interaction between type and level of cryoprotectants on sperm motility percentage.

It is worthy noting that the effects of type and level of cryoprotectants as well as equilibration period on reduction rate of sperm motility during equilibration period were not significant, although the lowest rates of reduction was indicated with glycerol and at level of 7%. However, nearly similarity in reduction rate of sperm motility was found for equilibration period (Table 4).

Sperm motility in post-thawed semen:

Data in Table (5) show that the effect of type and level of cryoprotectants on sperm motility in post-thawed semen was significant ($P<0.001$). Sperm motility in post-thawed semen was significantly ($P<0.05$) the highest (36.4%) in semen diluted with extenders containing glycerol, moderate (29.7%) with DMSO and the lowest (9.5%) with EG.

Increasing level of cryoprotectant from 5 to 7% resulted in slight and insignificant increase in sperm motility in post-thawed semen from 27.4 to 28.8%. However, sperm motility significantly ($P<0.05$) showed sharp decrease by increasing the level from 7 to 10%, being 17.6% at a level of 10% (Table 5).

Table (5): Effect of type and level of cryoprotectants on percentage and reduction rate of sperm motility (X±SE) in post-thawed semen.

Item	Sperm motility (%)	Reduction rate (%)*	Recovery rate (%)
Type of cryoprotectant:			
GL	36.4±2.30[a]	44.7±2.51[a]	49.9
DMSO	27.9±2.04[b]	33.8±2.41[b]	40.6
EG	9.5±1.98[c]	11.9±2.87[c]	14.5
Level of cryoprotectant (%):			
5	27.4±1.25[a]	33.9±1.20[a]	38.8
7	28.8±1.39[a]	34.7±1.34[a]	41.6
10	17.6±2.18[b]	21.8±2.04[b]	27.0
Effect of interaction (Type x level):			
GL x 5%	33.6±1.25	41.2±1.23	45.2
GL x 7%	47.1±1.57	57.9±1.46	63.4
GL x 10%	28.6±2.01	34.9±1.97	40.9
DMSO x 5%	34.3±1.57	42.6±1.45	51.1
DMSO x 7%	30.0±1.36	34.5±1.36	42.9
DMSO x 10%	19.3±2.08	24.3±1.95	27.8
EG x 5%	14.3±2.14	17.8±2.07	20.3
EG x 7%	9.3±1.89	11.6±1.80	13.4
EG x 10%	5.9±1.74	6.3±1.69	9.4
Overall mean	24.6±1.16	30.1±1.14	

a and b: Means denoted within the same column for each classification with different superscripts are significantly different at $P<0.05$).

GL: Glycerol EG: Ethylene glycol. * proportional to sperm motility in post diluted semen.

The effect of interaction of type with level of cryoprotectants was not significant on sperm motility in post-thawed semen, reflecting the highest sperm motility for semen diluted with extender containing 7% glycerol as compared to the other levels. On the other hand sperm motility had the lowest percentages with 10% EG (Table 5).

It is of interest to note that the reduction rate in sperm motility during freezing was associated with percentage of sperm motility. Higher reduction in sperm motility was in negative relationship with percentage of sperm motility in pre-frozen semen. The trend of change in reduction rate and recovery rate of sperm motility was similar to that in sperm motility percentage (Table 5). The pronounced increase in reduction rate with glycerol or 7% level inspite of higher sperm motility percentage may indicate the importance of presence of higher concentration of motile spermatozoa in semen pre-freezing.

DISCUSSION

The present study indicated significant ($P<0.05$) effect of type and level of cryoprotectants on sperm motility in post-diluted semen, being the best with glycerol and 7% level, respectively. The interaction effect of type with level of cryoprotectants indicated the superiority of adding 7% glycerol in Tris-based extender on sperm motility in post-diluted buffalo bull semen. It is of interest to note similar reduction rate in sperm motility in post diluted semen as affected by type and level of cryoprotectants. However, the lowest rate of reduction in sperm motility with glycerol at a level of 7% (interaction effect), may indicate a beneficial effect of 7% glycerol on sperm motility in post-diluted semen. It is worthy noting that nearly similar results were obtained in post-equilibrated and post-thawed semen.

In earlier reports, several authors indicated that glycerol is the most commonly used protective substance in diluents for freezing semen. Its cryoprotective function occurs through its penetrating and physiochemical properties and by protecting sperm cells against injury during the crystallization phase (Smith *et al.*, 1951 and Morris and Farrant, 1972). The optimal glycerol concentration in semen diluent is related to its final concentration relative to the concentration of spermatozoa (Colas, 1975).

The negative effect of increasing glycerol level to 10% on sperm motility percentage indicated in this study was stated in bovine semen by Bocker *et al.* (1977), who found that glycerol at a level of 11% significantly ($P<0.01$) reduced semen quality. Also, Abdelhakeam (1988) found that the glycerol concentration above 8% was contributed greatly to progressive decrease in spermatozoa survival. According to many authors, increasing glycerol concentration over 8% has a toxic effect due to the decrease in cryosurvival of spermatozoa (Arriola, 1982 and Chen *et al.*, 1989).

In this respect, Fahy (1986) reported that optimal glycerol level for freezing have been between 2.25 and 9% with many studies demonstrating toxicity beyond this concentration. Inspite of the positive effect of glycerol at a level of 7% as achieved in our study, The level of glycerol in semen diluent depends on cooling and freezing rate, diluent composition, method of glycerol addition and in particular on it's osmotic pressure (Salamon and Maxwell, 2000). Also, the glycerol concentration may be influenced by the egg yolk level in diluent. Watson (1995) reported that increased concentration of egg-yolk may reduce the required concentration of glycerol.

In nearly accordance with the present results, Chinnaya and Ganguli (1980) noticed that the best level of glycerol in maintaining sperm motility was

6% for egg yolk-citrate (23%), 6% for citric acid-whey (21.4%). While, higher level (8%) was needed for Tris (24.4%) extenders. Also, Pondit (1984) found that glycerol added at a level of 7% gave higher (P<0.01) sperm motility in post-thawed semen diluted with egg yolk-citrate diluent than did 5% glycerol. In buffalo semen, El-Azab *et al.* (1984) found better motility percentage at 3-7% glycerol in the Tris and sodium citrate diluents than at 10% glycerol.

In our study, sperm motility was affected significantly (P<0.05) by equilibration period, being higher with 6 than 4 hours, but the effect of interaction between equilibration period and each of type or level of cryoprotectants on sperm motility was not significant. Slavic (1982) observed that the penetrating activity of spermatozoa depended on the concentration of glycerol and equilibration time.

The moderate results presented in our study of DMSO as compared to glycerol and EG was related to that DMSO as an antioxidant has been also reported to have a cryoprotective effect on bull spermatozoa (Snedeker and Gaunya, 1970) and its beneficial effects may related to that DMSO (140 mM) could minimize the deleterious effect of hydroxyl radicals on sperm viability (Baiardi *et al.*, 1997). Also, Fujihara and Koga (1984) observed that incubation of roosters semen with DMSO significantly decreased the production of lipid peroxides in spermatozoa. In addition, 2% DMSO preserved the post-thaw sperm motility when ram semen pellets were frozen at 150 to 160°C on cooled aluminum plate (Varnavski and Turbin, 1974). In human semen, De Lamirande and Gagnon (1992) recorded a significant reduction in the concentration of hydroxyl radicals and a pronounced improvement in sperm viability after *in vitro* treatment of spermatozoa with DMSO.

The recorded recovery rate of post-thaw motility in this study was 40.6% with DMS. In this respect, Castellini *et al.* (1992) and Viudes and Vicente (1996) found that the best post-thawing recovery rates of sperm motility was 53.8% using DMSO without glycerol. It is of interest to note that increasing level of DMSO had negative effect on sperm motility in post-thawed semen. Similar finding was reported by Windsor and White (1995), who observed that the mitochondrial membrane potential of spermatozoa after freezing and thawing with 250 mM DMSO was significantly higher than that of spermatozoa after freezing and thawing with 4% glycerol. Recently, Abd El-Salam (2002) and Zeidan *et al.* (2002) found that 2% glycerol, 4% DMSO increased significantly (P<0.01) percentage of post thawing motility and freezing ability of spermatozoa but decreased percentage of acrosomal damage. In similar trend with the present positive results of glycerol and DMSO as compared to EG, Weitze (1977) reported that post-thawing motility of spermatozoa was higher in diluents containing DMSO or glycerol than in diluents without cryoprotectants.

The EG may be protect spermatozoa from freezing injury by mechanism similar to glycerol, but the effect of glycerol on protecting living cells of spermatozoa at lower freezing temperatures is better than EG. In bull semen extender, the aim of adding EG as cryoprotectant was to prevent ice crystal formation during freezing to fully prevent ice crystallization. The addition of less ethylene glycol would be necessary (Anchrodoguy et al.,

1987) as indicated in this study. Rodrigues *et al.* (2004) found that EG diffuse across cell membrane in exchange for cell water. This displacement of water by cryoprotectants. In addition, it decreases the possibility of intracellular ice formation and maintains cell volume during freezing, avoiding damage (Demirci *et al.*, 2002).

The observed low sperm motility in post-diluted and post-equilibrated semen as well as low recovery rate of motility in post-thawed semen may be due to higher level used from EG. This finding may explained from the results of Awad (1998), who reported that the percentages of post-thawing motility decreased with 6 than 3% EG. Also, Khalifa (2005) found that the percentage of post-thawing motility and sperm recovery decreased significantly by increasing EG level from 1 to 2 and from 2 to 3%. Moreover, Molinia *et al.* (1994) reported that increasing the level of EG decreased post-thawed motility and acrosome integrity of spermatozoa. In our study the lowest level of EG was 5%. This phenomena may be due to the effect of toxicity of the cryoprotectant, which dose not only prevent the use of fully protective level of penetrate in diluent, but also causes further cryo-injury to sperm. In this respect, Azell *et al.* (1989) found high toxicity of EG compared to glycerol, which may be due to that spermatozoa are permeable to EG than glycerol.

According to the foregoing results, the current study concluded that glycerol was the best cryoprotectant as compared to dimethyl sulfoxide and ethylene glycol when it was added at a level of 7% in Tris-based extender used for freezing Egyptian buffalo bull semen.

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تأثير استخدام أنواع ومستويات مختلفة من مواد الحماية على حيوية الحيوانات المنوية أثناء تجميد السائل المنوي لطلائق الجاموس المصري

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** معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية

أجريت هذه الدراسة في محطه بحوث الإنتاج الحيواني- الجميزه - معهد بحوث الإنتاج الحيواني - مركز البحوث الزراعية الفترة من نوفمبر ٢٠٠٦ إلى ينـاير ٢٠٠٧ وتهـدف هـذه الدراسة لتقييم استخدام أنواع و مستويات مختلفة من مواد الحماية على حيوية الحيوانات المنويـة خلال المراحل المختلفة من تجميد السائل المنوي للجاموس المصري. استخدم في هذه الدراسـة ٥ طلائق جاموس ناضجة بتراوح عمرها من ٧-١٠ سنوات وتم جمع السائل المنوي منها مـرتين أسبوعيا بواسطة المهبل الصناعي واستخدم في هذه الدراسة مخفف Tris مع مستويات مختلفة من مواد الحماية وهي الجليسرول - داي ميثايل سلفوكسيد (DMSO) وايثيلين جليكول (EG) علـى التوالي وكانت المستويات ٥ - ٧ و ١٠٪ لكل مادة حماية. تم تجميد السـائل المنـوي باسـتخدام النيتروجين السائل والاساله على درجة حرارة ٣٧°م لمدة ٣٠ ثانية بعـد فتـرات تـوازن ٤ أو ٦ ساعات. قدرت الحركة بعد التخفيف وبعد فترات التوازن وكذلك بعد التجميد والاساله.

ويمكن تلخص النتائج فيما يلي:

١. بعد التخفيف كانت نسبة الحركـة للحيوانـات المنويـة ٧٢,٩٪ للجليـسرول - ٦٨,٨٪ لـ DMSO بينما كانت ٦٣,٦٪ لـ EG . كانت الاختلافات بين مواد الحماية بعد التخفيـف غير معنوية ولكن كان الاختلاف معنوي مع زيادة مستوي الإضافة من ٧ الـى١٠٪ (عنـد مستوى معنوية ٥٪). كان تأثير التداخل بين أنواع مواد الحماية و مستواها غير معنوي على حركة الحيوانات المنوية.
٢. بعد فترات التوازن سجلت حركة الحيوانات المنوية ارتفاعا ملحوظا مع الجليسرول بالمقارنـة بالـ DMSO , EG حيث بلغت ٦٨,٧٪ للجليسرول , ٦٤,٨٪ لـ DMSO بالمقارنـة بالاثيلين جليكول ٥٥,٧٪ . عند زيادة مستويات الإضافة من ٧ إلى ١٠٪ لوحظ انخفاض من ٧٠,٧٪ (عند مستوى معنوية ٥٪) إلى ٦٩,٣٪ عند مستوى ٧٪ وكان الانخفـاض معنـوي (عند مستوى معنوية ٥٪) وصل الانخفاض إلى ٦٥,٢٪ عند مـستوى ١٠٪ ولـوحظ عـدم وجود اختلافات معنوية بين الجليسرول و DMSO. مع زيادة المستوى من ٥ إلى ٧٪ كانت نسب الانخفاض غير معنوية (٦٥,١ و ٦٤,٩٪ - على الترتيب).
٣. بعد الإسالة سجلت حركة الحيوانات المنوية مع الجليسرول أعلى القيم (٣٦,٤٪) عند مـستوى معنوية ٥٪ وكانت متوسطه (٢٩,٧٪) مع DMSO والأقل (عند مستوى معنوية ٥٪) مـع الاثيلين جليكول (٩,٥٪). سجلت حركة الحيوانات المنوية ارتفاعا مـن ٢٧,٤ إلـى ٢٨,٨٪ (عند مستوى معنوية ٥٪) مع زيادة المستوى من ٥ إلى ٧٪ وكان هناك انخفاض معنـوي (عند مستوى معنوية ٥٪) إلى ١٧,٦% مع زيادة المستوى من ٧ إلى ١٠٪ . و وكان تـأثير التداخل بين كل مواد الحماية ومستوياتها غير معنوي.

تشير هذه الدراسة إلى حدوث تحسن في حركة الحيوانات المنوية في الـسائل المنـوي لطلائق الجاموس المصري مع مستوى ٧٪ جليسرول مع استخدام مخفف الترس مع صفار البيض أثناء التجميد.