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FREEZABILITY, SOME BIOCHEMICAL CHANGES AND FERTILITY RATE OF MALE DROMEDARY CAMEL SPERMATOZOA ADDED WITH GLYCINE BETAINE

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ABSTRACT

Eight Fellahi camels (<5-10 years old) were used in the present study. Semen was collected and diluted with Lactose-yolk-citrate (LYC) extender and cooling to 5°C. Glycerol was added at a level of 6% and left at 5°C added with glycine betaine (GB) at levels of 100 or 200mM for 2 hrs as equilibration period and then packaging and freezing in straws (0.5 ml). After freezing, frozen-semen was thawed in a water bath at 37°C for 40 seconds and incubated at this temperature for 3 hrs. The percentages of post-thaw motility, freezability and fertility rates and some biochemical changes in the male camels were recorded added with GB, during thawing-incubation at 37°C for 3 hrs.

The obtained results revealed that, the percentages of post-thaw motility and freezability were significantly (P<0.05) higher,while the percentages of acrosome damage and chromatin damage were significantly (P<0.05) lower of the frozen-thawed camel spermatozoa added with GB than free-GB medium during thawing incubation at 37°C. The advancement of thawing-incubation at 37°C was significantly (P<0.05) lower the percentages of post-thaw motility and freezability, while was significantly (P<0.05) higher the percentages of acrosome damage and chromatin damage of the dromedary camel spermatozoa during thawing-incubation at 37°C for 3 hrs. Leakage of aspartate-aminotransaminase (AST), alanine-aminotransaminase (ALT) and malondialdhyde (MDA) enzymes into the extracellular medium was significantly (P<0.05) lower in the frozen-thawed camel spermatozoa added with GB than free-GB medium during thawing-incubation times at 37°C for 3 hrs. The conception rates were significantly (P<0.05) increased of she-camels artificially inseminated in the fresh semen (75.00%) as compared to frozen-thawed semen (free-GB medium, (37.50%), frozen-thawed semen added with 100 mM GB (56.25%) and 200 mM GB

(52.94%). In conclusion, post-thaw motility (%), freezability (%), enzymatic activity and fertility rates (%) of the camel spermatozoa added with GB were better than free GB medium.

Keywords:

Camel semen, Freezability, Enzymes, Glycine betaine, Fertility rate.

INTRODUCTION

Progress in the artificial insemination (AI), semen preservation and related techniques in camelidae has been slow in comparison to other livestock species. As there is relatively limited information in the literature on anatomy and physiology of the male and female camels.

In order to identify the way it goes for success of the artificial insemination in camels **(Zhao, 1995 and El-Bahrawy, 2005).**

Pervious experiments have shown that ovulation in both dromedary and Bactrian camels can be induced by depositing semen in the uterus **(Musa *et al.*,1992).**

Maximizing the use of outstanding sires in artificial breeding depends on many factors. Developing and extender that preserves the high fertility of unfrozen semen for a long time would help increase the overall conception rate, encourage more complete use of semen and make semen from outstanding sires more widely available in management systems using fresh semen **(Foote and Bratton, 1960).**

Media known to prolong the survival of fresh bovine spermatozoa contain complex buffers and varying levels of egg yolk, glucose, glycine, glycerol, citrate and other salts **(Foote and Bratton, 1960).** It has long been recognized that, the electrolyte composition, buffering agent and osmotic pressure of the solution in which the spermatozoa are suspended significantly affect spermatozoa morphology and function.

Glycine betaine (GB), called betaine, is a quaternary amine, a trimethyl derivative of the amino acid glycine *(N,N,N,-trimethylglycine),* which protects plants against salt stress **(Storey and Wyn Jones, 1977),** modulates cellular responses to osmotic stress **(Petronini *et al.*, 1992)** and has been used as a cry protectant **(Lindeberg *et al.*, 1999).** It has been shown that GB enhances the quality of cryopreserved ram spermatozoa **(Sanchez-Partida *et al.*, 1992 and 1998)** and equine spermatozoa **(Trimeche *et al.*, 1999),** but its effects on the camel spermatozoa have not been studied.

The objective of the present work was to study the effect of Glycine betaine (GB)

addition to the cooled semen on post-thaw motility, freezability, acrosome damage, chromatin damage and some biochemical changes in the dromedary camel spermatozoa, during thawing-incubation at 37°C. Conception rates (%) of the dromedary she-camels artificially inseminated with or without GB were also estimated.

MATERIAL AND METHODS

The experimental work was carried out in the Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkiya Governorate, Egypt, during the period from November to December, 2016.

1. Materials:

1.1. Experimental animals:

Eight Fellahi camels *(Camelus dromedarius)* aging < 5-10 years old and 400 to 600 kg live body weight were used in the present study. All camels were in healthy condition and clinically free from external and internal parasites with a sound history of fertility in the herd and 65 she-camels at <5-10 years were used for conception rate.

1.2. Feeding and management:

The rations offered to camels were calculated according to **Banerjee (1988).** Two types of rations were used as follows:

Green season (from December to May): The average amounts given per head/daily were 35Kg Egyptian clover *(Trifolium alexandrinum)* and 7 kg rice straw.

Dry season (from June to November): Each camel was received about 2 Kg commercial concentrate mixture, 2 Kg Egyptian clover hay and 9 Kg rice straw daily. Clean fresh water offered freely to all camels were housed in a yard which was provided with common feeding trough and a concrete floor provided with common sheltered water trough. The camels could move freely in enclosed area.

2. Methods:

2.1. Camel semen collection:

Seven ejaculates from each camel were collected on several occasions from an intact in the male camel using artificial vagina (AV) (30 cm long and 5 cm internal diameter, IMV, France) as the method described by **Zeidan (2002)** containing warm water (50^0C). Semen was evaluated and diluted within 45 mins after collection. The ejaculate usually comes in fractions.

Fresh camel semen that has a jelly-like consistency is left for liquefaction for about 30-60 mins to make the sperm attained motility.

2.2. Semen extension:

Semen samples were collected, pooled and evaluated for each camel (Fellahi, < 5-10 years old) and then diluted with lactose-yolk-citrate (LYC) extender (2.9 g sodium citrate dehydrate, 0.04 g citiric acid anhydrous, 1.25 g lactose and 10 ml egg-yolk, per 100 ml distilled water, 500 I.U/ml Penicillin and 500µg Streptomycin Sulphate) according to **Musa *et al.* (1992).** The dilution rate was 1 ml semen: 3ml extender according to **Musa *et al.* (1992).**Semen samples were immediately diluted with fraction (A) without cryoprotectant (glycerol) and kept at 25-30°C for liquefaction in water bath for 45 mins, where semen samples were shaked thoroughly at this time. Thereafter, the mixture was transported in glass containers to a cooled chamber cabinet at 5°C for 1.5 to 2 hrs. Thereafter, fraction (B) with cryoprotectant (6% glycerol) was added according to **Musa *et al.* (1992)** at 5°C to reach a final concentration of 3% glycerol. Diluted semen with 3% glycerol was left in the cooled chamber for 2 hours as equilibration period **(Tibary and Anouassi, 1997).** Final dilution rate with the freezing extender to a concentration of 100 x 10^6/ml and packaging in small straws (0.5ml).

2.3. Chilling of semen at 5°C:

The test tubes containing diluted semen were placed in a 500 ml beaker containing water at 30^0C with a thermometer in order to facilitate periodic check of the temperature during cooling period. Another test tubes containing diluted semen only were placed in the beaker to maintain the extended temperature (all the test tubes were covered with dark plastic sheath). The beaker was placed in a refrigerator and gradually cooled till their temperature reached to 5°C during a period of 1.5-2.0 hours according to **Salisbury *et al.* (1978).** After each thawing-incubation at 37°C (0, 1, 2 and 3 hrs), percentages of post-thaw motility, freezability, acrosome damage and chromatin damage of spermatozoa added with GB were recorded. The leakage of AST,ALT and MDA enzymes into the extracellular medium was also recorded.

2.4. Glycine betaine addition to the cooled camel semen:

Semen samples were collected, evaluated and diluted with the lactose-yolk citrate (LYC) extender. The diluted spermatozoa were cooled slowly by keeping the containing tubes at room temperature in water bath and put at a 5^0C through 1.5-2 hrs. After cooling, the cooled semen was divided into three media. The first medium was similar to a non-glycine betaine

(Woko pure Chemical Industries, Lte, Japan) and kept as a control medium. The second and third media were contain diluted cooled semen with 100 and 200 mM Glycine betaine, respectively according to **Zhang *et al.* (2001).** Assessment of survival rate of spermatozoa was determined at 0, 1, 2 and 3 hrs, during thawing-incubation at 37°C.

2.5. Semen packaging and freezing procedure

Semen packaged in Straws (0.5 ml) was placed on a rack using an automatic mini-tube filling and speeding machine (Type MPP 133) at known distances above the surface of liquid nitrogen. Freezing rates can be modified by adjustment of the elevation of the straws. The straws were placed on a platform which is lowered stepwise toward the liquid nitrogen surface. The temperature inside the samples is monitored by a thermocouple which guides the movement of the platform.

Packaged straws were exposed to liquid nitrogen vapors for 20 minutes (allowing them to reach a temperature of – 120^0C then plunging into liquid nitrogen)

Freezing procedure on a wire grid placed above liquid nitrogen was done in four steps as the method described by **Tibary and Anouassi (1997)** as follows:

Step 1: 3 minutes at 3 cm, corresponding to - 5°C.

Step 2: 2 minutes at 2 cm (-75°C).

Step 3: 1 minute at 1 cm (-175°C).

Step 4: plunging into liquid nitrogen (-196°C).

2.6. Thawing rate:

Frozen semen in straws was thawed out by placing them in a water bath set at 37°C for 40 seconds **(Tibary and Anouassi, 1997).**

1. Frozen-thawed semen quality:

1.1. Sperm motility (%):

Generally, camel sperm motility (%) was determined as an oscillatory motion the flagellum, but not progressive due to the viscous materials according to **Tibary and Anouassi (1997).** With regard to frozen-thawed semen, the percentage of post-thaw motility was determined using one drop on the dry clean and pre-warmed (37°C) glass slide of the thawed semen after each thawing-incubation (0, 1, 2 and 2 hrs). The drop of the frozen-thawed semen was covered by a warmed cover slip and immediately examined using high power magnification (400x). Freezability (%) of spermatozoa was estimated as the method described by **Patt and**

Nath (1969), Salhab and Merilan (1991) and Zeidan (1994).

1.2. Acrosome damage of spermatozoa (%):

Assessment of the percentages of acrosome damage of spermatozoa (%) was done according to **Watson (1975).**

The percentages of acrosome damage of spermatozoa were calculated for 100 spermatozoa observed at random on each slide using oil immersion lens (x1000).

1.3. Chromatin damage of spermatozoa (%):

Toluidine blue staining was performed as the method described by **Erenpreiss *et al.* (2004).** Smears were fixed in ethanol for 3 mins. Thereafter, smears were hydrolyzed for 20 mins in 1 mM hydrochloric, rinsed in distilled water and air-dried. One droplet of 0.025% Toluidine blue in McIlvaine buffer (Sodium citrate-phosphate) at 4.0 pH was placed over each smear and then cover slipped. The percentage of chromatin damage was determined by evaluating 300 sperm-cells in each slide. Spermatozoa stained with green to light blue were considered as normal chromatin, while spermatozoa stained with dark blue to violet were considered to have damaged chromatin.

2. Biochemical analysis in seminal plasma (U/10^6 spermatozoa):

After each thawing-incubation times (0, 1, 2 and 3 hrs), frozen-thawed semen was centrifuged for 15 minutes at 8000 RCF. Seminal plasma was separated and stored at-20^0C until assay of enzymes. Activity of aspartate-aminotransaminase (AST), alanine-aminotransaminase (ALT) and malondialdehyde (MDA) enzymes were recorded using commercial kit (Biodiagnostic, Egypt) and spectro-photometer 21 (Spectroliv-UV Auto, LIU-2602, Labomed, USA) as the method described by **Reitman and Frankle (1957) and Ohkawa *et al.* (1979).**

3. Fertility rate (%):

Semen is deposited into the uterus using an insemination gun to the internal cervical. Insemination has to be done using a porcine rubber insemination tube heated to 38°C to prevent cold shock **(Chen *et al.*, 1990).** When frozen semen is used, a second insemination is performed 24 hours after the first one.

She-camels were injected intramuscularly with 3000 IU of human chorionic gonadotropin (hCG) in the form of Preface (1ml-ampules packed by EPICO, Egypt, under licence from I.F. Serna, Rome, Italy) to induce ovulation **(Anouassi *et al.*, 1994).** She-camels were artificially inseminated approximately 48 hrs post hCG injection. Semen was divided into four media. The first medium was artificially inseminated with the fresh semen. The second medium was

similar to non-glycine betaine fraction. The third and fourth groups were consisting of 100 mM and 200 mM glycine betaine, respectively.

She-camels (5 to 10 years of age and 400-500 kg live body weight) were divided into four groups:

Group 1 (n=16): She-camels were artificially inseminated with the fresh semen (2ml) containing $100x10^6$ motile spermatozoa.

Group 2 (n-16): She-camels were artificially inseminated with the frozen-thawed semen (2ml) containing $100x10^6$ motile spermatozoa without GB.

Group 3 (n=16): She-camels were artificially inseminated with the frozen-thawed semen (2ml) containing $100x10^6$ motile spermatozoa with 100 mM GB.

Group 4 (n=17): She-camels were artificially inseminated with the frozen-thawed semen (2ml) containing 100×10^6 motile spermatozoa with 200 mM GB.

Pregnancy rate was diagnosed at 60 days after the date of insemination.

3. Statistical analysis:

Data were statistically analyzed by two-way design (ANOVA) using General Linear Model (GLM) procedure of SAS **(SAS, 2006).** Duncan's Multiple Range Test **(Duncan, 1955)** was used to detect significant differences among means. Percentage values were transformed to arc-sin values before being statistically analyzed. Conception rates were analyzed using Chi-square test.

The following model used was as follows:

$Y_{ijk} = \mu + G_i + Tj + (G_i) \times Tj) + e_{ijk}$

Y_{ijk}= is the observed value of the dependent variable determined from a sample taken from each animal.

μ= is the overall mean.

G_i= is the fixed effect of glycine betaine concentration.

T_j= is the fixed effect of thawing - incubation time.

$(G_i \times T_j)$= is the first – order interaction between glycine betaine and thawing- incubation.

e_{ijk}= is the residual error.

RESULTS

1. Frozen-thawed semen quality:

1.1. Post-thaw motility and freezability of the dromedary camel spermatozoa (%):

Data presented in (Tables 1, 2) revealed that the effect of GB addition at levels of 100 and 200 mM on the percentages of post-thaw motility and freezability were significantly (P<0.05) higher in the camel spermatozoa than free-GB medium, during thawing-incubation at 37°C for up to 3 hrs.

Table (1): Mean percentages of post-thaw motility in the camel spermatozoa added with glycine betaine during thawing – incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	43.11±0.76	52.37±1.12	50.63±1.03	48.70±0.82^{A}	0.001
1	32.84±0.25	40.78±0.72	39.46±0.68	37.69±0.29^{B}	0.002
2	19.72±0.16	28.16±0.22	28.04±0.21	25.30±0.18^{C}	0.006
3	8.67±0.10	12.74±0.16	11.35±0.14	10.92±0.12^{D}	0.001
Overall mean	26.08±0.21^{b}	33.51±0.29^{a}	32.37±0.24^{a}	30.65	

a-b Values with different superscripts within a row are significantly different (P<0.05).

A-D Values with different superscripts within a column are significantly different (P<0.05).

Table (2): Mean percentages of freezability in the camel spermatozoa added with glycine betaine during thawing – incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	61.58±1.18	74.81±2.06	72.32±2.01	69.57±1.62^{A}	0.001
1	46.91±0.61	58.25±1.72	56.37±1.14	53.84±1.08^{B}	0.001
2	28.17±0.23	40.22±0.36	40.05±0.28	36.14±0.24^{C}	0.002
3	12.38±0.14	18.20±0.21	16.21±0.19	15.60±0.16^{D}	0.004
Overall mean	37.26±0.29^{b}	47.87±0.62^{a}	46.24±0.59^{a}	43.79	

a-b Values with different superscripts within a row are significantly different (P<0.05).

A-D Values with different superscripts within a column are significantly different (P<0.05).

$$\text{Freezability (\%)} = \frac{\text{Post-thaw motility}}{\text{Initial motility}} \times 100$$

It is of interest to note that, the prolongation of thawing-incubation times at 37°C decreased significantly ($P<0.05$) the percentages of post-thaw motility and freezability in the camel spermatozoa without free-GB medium or with GB medium (Tables 1, 2).

1.2. Acrosome and chromatin damage in the dromedary camel spermatozoa (%):

Data presented in (Table 3) and 4 showed that, the effect of GB addition at levels of 100 and 200 mM was significantly ($P<0.05$) decressed the percentages of acrosome and chromatin damage of the frozen-thawed camel spermatozoa as compared to free-GB medium with the successive thawing-incubation times at 37°C for up to 3 hrs.

The prolongation of thawing-incubation times at 37°C increased significantly ($P<0.05$) the percentages of acrosome and chromatin damage in the camels spermatozoa with GB medium or without GB medium (Table 3, 4).

Table (3): Mean percentages of acrosome damage in the camel spermatozoa added with glycine betaine during thawing- incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	8.26±0.28	5.12±0.14	5.35±0.16	6.24 ± 0.29^{C}	0.001
1	10.42±0.61	5.63±0.23	6.78±0.27	7.61 ± 0.34^{BC}	0.004
2	15.68±0.84	6.37±0.29	7.43±0.30	9.82 ± 0.42^{B}	0.002
3	24.79±1.03	12.94±0.76	15.02±0.82	17.58 ± 0.91^{A}	0.001
Overall mean	14.78 ± 0.82^{a}	7.51 ± 0.32^{b}	8.64 ± 0.30^{b}	10.31	

a-b Values with different superscripts within a row are significantly different ($P<0.05$).

A-C Values with different superscripts within a column are significantly different ($P<0.05$).

Table (4): Mean percentages of chromatin damage in the camel spermatozoa added with glycine betaine during thawing – incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	2.16±1.14	1.64±1.10	1.82±0.11	1.87 ± 0.12^{C}	0.002
1	2.91±0.17	1.89±0.15	2.11±0.16	2.30 ± 0.17^{BC}	0.070
2	4.52±0.29	2.75±0.19	3.09±0.21	3.45 ± 0.24^{B}	0.001
3	7.11±0.41	4.18±0.26	5.14±0.34	5.47 ± 0.36^{A}	0.001
Overall mean	4.017 ± 0.27^{a}	2.61 ± 0.18^{b}	3.04 ± 0.20^{b}	3.27	

a-b Values with different superscripts within a row are significantly different (P<0.05).

A-C Values with different superscripts within a column are significantly different (P<0.05).

2. Enzymatic activity of the male dromedary camel (U/10^6 spermatozoa):

Addition of GB at levels of 100 or 200 mM to the frozen-thawed camel spermatozoa decreased significantly (P<0.05) the amount of AST, ALT and MDA enzymes released into the extracellular medium as compared to free-GB medium (Tables 5,6and7).The advancement of thawing-incubation times at 37°C increased significantly (P<0.05) the amount of AST, ALT andMDA enzymes released into the extracellular medium as compared to free-GB medium with the successive thawing-incubation times at 37°C for up to 3 hrs (Tables 5, 6 and 7).

Table (5): Activity of aspartate-aminotransaminase enzyme (U/10^6/spermatozoa) in the dromedary camel spermatozoa added with glycine betaine,during thawing-incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	48.62±0.82	39.27±0.71	39.28±0.72	42.39 ± 0.74^{D}	0.001
1	54.70±0.87	44.38±0.73	45.12±0.75	48.06 ± 0.78^{C}	0.001
2	65.14±1.12	51.25±0.80	53.79±0.84	56.72 ± 0.92^{B}	0.001
3	80.25±1.34	60.14±1.02	62.36±1.16	67.58 ± 1.23^{A}	0.002
Overall mean	62.17 ± 1.03^{a}	48.76 ± 0.79^{b}	50.13 ± 0.78^{b}	53.68	

a-b Values with different superscripts within a row are significantly different (P<0.05).

A-D Values with different superscripts within a column are significantly different (P<0.05).

Table (6): Activity of alanine-aminotransaminase enzyme (U/106/spermatozoa) in the camel spermatozoa added with glycine betaine, during thawing - incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	36.51±0.28	27.18±0.14	27.03±0.18	30.24±0.23^{D}	0.002
1	43.72±0.46	32.45±0.21	33.24±0.24	36.47±0.26^{C}	0.001
2	52.46±0.58	39.52±0.43	39.56±0.41	43.84±0.49^{B}	0.001
3	66.25±0.73	48.19±0.50	50.12±0.56	54.85±0.61^{A}	0.001
Overall mean	49.73±0.48^{a}	36.83±0.29^{b}	37.48±0.34^{b}	41.35	

a-b Values with different superscripts within a row are significantly different (P<0.05).

A-D Values with different superscripts within a column are significantly different (P<0.05).

Table (7): Activity of malondialdhyde enzyme (U/10^{6}/spermatozoa) in the camel spermatozoa added with glycine betaine, during thawing – incubation at 37°C (Mean±SE).

Thawing-incubation (Hours)	Glycine betaine concentration (mM)			Mean	P-value
	Control	100	200		
0	29.17±0.46	21.38±0.32	21.75±0.33	24.10±0.35^{D}	0.001
1	38.46±0.51	26.17±0.40	27.83±0.41	30.82±0.63^{C}	0.002
2	46.35±0.62	32.46±0.48	33.40±0.56	37.40±0.71^{B}	0.002
3	59.20±0.75	40.84±0.49	42.13±0.53	47.39±0.64^{A}	0.004
Overall mean	43.29±0.60^{a}	30.21±0.52^{b}	31.27±0.58^{b}	34.92	

a-b Values with different superscripts within a row are significantly different (P<0.05).

A-D Values with different superscripts within a column are significantly different (P<0.05).

3. Fertility rate (%):

Conception rates of she-camels artificially inseminated with fresh semen, frozen-thawed semen (free-GB medium), frozen-thawed semen added with 100mM GB and frozen-thawed semen added with 200 mM GB were 75.00%, 37.50%, 56.25% and 52.94%, respectively with significantly (P<0.05) different (Table 8).

Table (8): Conception rates (%) of the dromedary she-camels artificially inseminated with fresh semen and frozen-thawed semen without and with GB medium (Mean±SE).

Type of semen	No. of she-camel inseminated	No. of she-camel conceived	Conception rate (%)
Fresh semen	**16**	**12**	**75.00^{A}**
Frozen-thawed semen (Free GB medium)	**16**	**6**	**37.50^{C}**
Frozen-thawed semen (With 100mM GB)	**16**	**9**	**56.25^{B}**
Frozen-thawed semen (With 200mM GB)	**17**	**9**	**52.94^{B}**

Means with different superscripts letters in the same column differ significantly (P<0.05).

DISCUSSION

As like in many other animals, artificial insemination (AI) is the most important technique to insure rapid genetic programs in camelidae. In addition, to its use for genetic improvement **(Tibary and Anouassi, 1997).**

Semen includes spermatozoa and seminal plasma **(Salisbury *et al.*, 1978).** Maintenance of sperm quality is achievable by using extender added with GB **(Zhang *et al.*, 2001).** The obtained results of the experimental work show for the first time that GB enhances the long-term maintenance of the frozen-thawed camel spermatozoa at concentrations of 100 and 200 mM being beneficial effect on post-thaw motility of spermatozoa. The inherent bull camel differences in the degree of spermatozoa cryoinjury did not affect the percentages of post-thaw motility and freezability of spermatozoa during thawing-incubation. The effect of media on liquid semen quality has been investigated in previous studies **(Foote and Bratton, 1960). Foote and Bratton (1960)** found also that a dramatic improvement in survival of spermatozoa with addition of 10% glycerol plus 20% egg yolk-glycine extender during storage at 5°C. **Lindeberg *et al.* (1999)** in stallion and **Sanchez-Partida *et al.* (1992)** in ram spermatozoa has been successfully cryopreserved in extenders containing GB. However, GB effectively maintained the percentage of post-thaw motility of ram spermatozoa in the extenders containing glycerol **(Sanchez-Partida *et al.*, 1998).** Moreover, the viability and

hypo-osmotic swelling ability of ram spermatozoa were lower than motility, even when glycerol was present with GB in the cryopreservation medium **(Ollero *et al.*, 1998).**

Our results showed that, the addition of GB to the extender at levels of 100 mM or 200 mM was significantly improved the percentages of post-thaw motility (Table 1) and freezability (Table 2) of the camel spermatozoa. In addition, fertility rates (Table 8) were significantly better with fresh semen and medium added with GB than free-GB medium which may due to the highly active of semen quality. However, percentages of acrosome damage and chromatin damage were significantly decreased in the extenders containing GB of in the dromedary camel spermatozoa with the successive thawing-incubation.

The percentages of acrosome damage (Table 3) and chromatin damage (Table 4) of camel spermatozoa in the present work showed significantly decreased with the extenders containing GB at levels of 100 or 200 mM as compared to free-GB medium **(Woelders *et al.*, 1997).** Betaine is a member of the family of compounds known as compatible solutes and bacteria, as well as mammalian kidney cells. Moreover, betaine also transporters to internalize the molecule, particularly in hyperosmotic environments **(Nakanishi *et al.*, 1990).**

Once inside GB into the cell, compatible solids to regulate internal osmolarity **(Petronini *et al.*, 1992).** GB can also preserve the three-dimensional structures of complex molecules like as RNAse subjected to thermal destabilization in the presence of urea **(Burg and Peters, 1998).** Complex protein macromolecules have a characteristics water-accessible surface area where local domain water is highly bond and GB is highly excluded from this monolayer **(Courtenay *et al.*, 2002).**

In the present study, chromatin damage in the dromedary camels significantly decrease with GB at levels of 100 or 200 mM as compared to free-GB medium which may be due to increased antioxidant capacity.

The results of the present work also revealed that storage of semen at 37°C or 0°C quickly diminished and also caused a faster diminution of the percentage of motile spermatozoa. In addition, lower temperature benefits longer storage by reducing the cellular metabolic rate, but cold shock often occurs spermatozoa are cooled rapidly from 0 to -20°C **(White, 1993).** The temperature of storing semen influences spermatozoa metabolism and survival. For artificial insemination using raw semen, spermatozoa are usually extended in a buffered yolk medium, cooled and held at 5°C until inseminated **(Anderson, 1945).**

From another point of view, **Lenz *et al.* (1977)** indicated that extension and cooling of bull spermatozoa to 5°C caused acrosome swelling in about 50% of the spermatozoa. Subsequent freezing and thawing, the ultrastructural changes to the acrosomes (disruption of the plasma and outer acrosome membranes and dispersion of the acrosome contents) and middle piece (breakage of the plasma membrane and a reduction in the electron density of the mitochondrial matrix) of a high proportion of the spermatozoa.

The amount of AST, ALT and MDA enzymes (Tables 5, 6 and 7) released into the extracellular medium was significantly lower in the extenders containing GB than free-GB medium of the dromedary camel spermatozoa, during different thawing-incubation at 37°C for 3 hrs. These findings may be explained by the amplified antioxidant enzymes activity; consequently GB addition to the extenders could increase the ability of seminal plasma to reduce the oxidative stress. In addition, the enzymatic activity in seminal plasma is a good indicator of semen quality because it measure sperm membrane stability **(Corteel, 1980).** Negative correlation was reported by **Pesch *et al.* (2006)** between AST enzyme and sperm volume. Furthermore, many studies have correlated with AST enzyme level in semen and sperm concentration **(Khokhar *et al.*, 1987). Al-Daraji *et al.* (2002)** confirmed that cell membrane was damaged AST and ALT enzymes released into the extracellular medium. Similar trends were recorded by **Zeidan *et al.* (2001)** in the dromedary camel spermatozoa. **Graham and Pace (1967)** found that,the AST and ALT enzymes released into the extracellular medium may reflect the incubation breakdown of the celluar sperm membrane during thawing-incubation. The leakage of MDA enzyme into the extracellular medium was significantly lower of the frozen-thawed camel spermatozoa added with GB than free-GB medium, which due to less concentration of polyunsaturated fatty acids **(Petruska *et al.*, 2014).** The GB may able also to prevent the increase of the enzymatic activity in the LYC extender may be attributed to the better protection of lactose to spermatozoa against osmotic shock than other sugars or due to the media available energy and the osmotic balance of the extender **(Foot, 1964).** In conclusion, the percentages of post-thaw motility, freezability, acrosome damage, chromatin damage, enzymatic activity and fertility rate in the dromedary camel spermatozoa showed significantly better with the LYC extender containing 6% Glycerol plus GB at levels of 100 or 200 mM and left at 5°C for 2 hrs as equilibration period. These encouraging results suggest that GB could be easily included in the freezing procedure to improve quality in the camel spermatozoa to enhance of fertility rates in farm animals.

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قدرة الحيوانات المنوية على التجميد وبعض التغيرات البيوكميائية ومعدل الأخصاب في الجمال العربية مع إضافة الجليسين بيتان

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الملخص العربي

أجريت هذه الدراسة على عدد 8 ذكور جمال فلاحي عمرها أكبر من 5-10 سنوات. تم جمع وتخفيف السائل المنوي بمخفف Lactose-yolk-citrate (LYC) وتبريده إلى درجة حرارة 5°م. تم إضافة الجليسرول بمستوى 6% وتترك على درجة حرارة 5°م لمدة ساعتين كفترة إتزان مع إضافة الجليسين بيتان (GB) بمستوى 100 و 200 مللي مول ثم تعبأ وتجمد في القصيبات البلاستيك (0.5 ml). بعد ذلك تم إسالة السائل المنوي بعد التجميد في الحمام المائي على درجة حرارة 37°م لمدة 40 ثانية وتحضن على هذه الدرجة لمدة 3 ساعات. تم تقدير كل من النسبة المئوية لحركة الحيوانات المنوية وكذا قدرتها على تحمل التجميد ومعدل الأخصاب وبعض التغيرات البيوكيمائية في ذكور الجمال.

أوضحت النتائج أن هناك تحسن معنوي (على مستوى 0.05) في النسبة المئوية لحركة الحيوانات المنوية للجمال بعد التجميد والإسالة وكذا قدرتها على التجميد ، بينما انخفضت بدرجة معنوية (على مستوى 0.05) النسبة المئوية لكل من شذوذ الأكروسوم والكروماتين مع إضافة الجليسين بيتان عن الخالية من الجليسين بيتان وذلك عند التحضين بعد الإسالة على درجة حرارة 37°م. إنخفضت النسبة المئوية لحركة الحيوانات المنوية وكذا قدرتها على تحمل التجميد بدرجة معنوية (على مستوى 0.05) مع زيادة النسبة المئوية لشذوذ الأكروسوم والكروماتين في الحيوانات المنوية للجمال عند الحفظ على درجة حرارة 37°م لمدة 3 ساعات. كذلك كان هناك إنخفاض معنوي (على مستوى 0.05) في معدل ارتشاح كل من إنزيم MDA, ALT, AST إلى البيئة الخارجية عند حفظ السائل المنوي بعد التجميد والإسالة على درجة حرارة 37°م لمدة 3 ساعات عند إضافة الجليسين بيتان مقارنة بالخالية من الجليسين بيتان. زيادة معدل الأخصاب بدرجة معنوية (على مستوى 0.05) في النوق الملقحة اصطناعيا بالسائل المنوي الطازج (75,00%) مقارنة بالسائل المنوي بعد التجميد والإسالة والخالية من الجليسين بيتان (37,50%) ، السائل المنوي بعد التجميد والإسالة مع إضافة 100 مللي مول جليسين بيتان (56,25%) وإلى (52,94%) مع إضافة 200 مللي مول جليسين بيتان. الموجز: كانت حركة الحيوانات المنوية (%)، قدرتها على تحمل التجميد (%) والنشاط الإنزيمي ومعدل الأخصاب في الحيوانات المنوية بعد التجميد والإسالة أفضل عند إضافة الجليسين بيتان عن الخالية من الجلسين بيتان.