IMPACT OF GLYCINE BETAINE ON COOLED CAMEL SEMEN QUALITY AND FERTILITY RATE

Zeidan, A. E. B.[1], A. M. Amer[1], Dalia S. A. Al-Tahan[2], Liza A. Abdel-Rafaa[1]
[1]Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.
[2]Animal Health Research Institute, Agricultural Research Center, Mansoura Laboratory, Egypt.
Corresponding author: A. M. Amer, Email: doctoramer69@gmail.com

ABSTRACT

The purpose of the current investigation was to determine the impact of adding various concentrations of glycine betaine (GB) to spermatozoa from Maghrebi camels that had been extended with a lactose-yolk-citrate (LYC) extender and kept at 5^{o}C for three days. Using an artificial vagina, semen was extracted from five camels and prolonged with LYC extender free-GB medium (first medium), or at 100 mM, 200 mM, or 300 mM GB for second, third, and fourth media, respectively. The ultimate sperm concentration was 100x10^{6} sperms per ml, and she camels that were inseminated using extended semen from first, second, third and fourth groups were determined after 45 days. The percentage of motile camel spermatozoa extended with LYC extender added to 100 or 200 mM GB were considerably greater (P<0.05) compared to 300 mM GB or free-GB media, according to the results. However, upon storage time, the proportion of dead, aberrant, damaged acrosomes and chromatin as well as the activity of the AST and ALT enzymes were considerably (P<0.05) lower than before. With or without the GB medium, the quality of the camel semen considerably (P<0.05) decreased when storage time was extended. Additionally, the first, second, third, and fourth media's respective. Fertility rates for one day of the artificially inseminated dromedary she-camels were 31.57, 52.63, 47.61, and 26.31%. In conclusion, GB addition to the cooled camel spermatozoa at 100 or 200 mM, while being stored at 5 ^{O}C, was found to enrich the camel sperm quality and fertility rates.

Keywords: Camel spermatozoa, Glycine betaine, sperm quality, fertility

INTRODUCTION

The lack of animal meat and milk in Egypt can be resolved by increasing camel output. Egypt is home to the Sudani, Maghrebi, Fellahi, and Al-Mowalled camel breeds. While the Al-Mowlled camel breed is far more suited as a farm and desert animal, the Al-Fellahi camel breed predominates in the Nile delta region. For the purpose of producing meat and milk, Al-Sudani and Al-Maghrebi camel breeds were raised (Wilson, 1984). Through breeding programs for farm animals, artificial insemination (AI) is regarded as one of the most significant and expedient ways in contemporary technology for the application of genetic improvement. By introducing and extending the best genetics of chosen camels into several female animals, this was accomplished.

In general, live spermatozoa can be kept refrigerated (2–5 ^{O}C) for several days. However, even after just one day of storage in the dromedary camels, good reproductive outcomes are not always obtained (Zeidan *et al*., 2001 and Matter, 2019). The success of AI, which in turn depends on the quality of the acquired semen and its capability for dilution and storage with minimal loss of fertilizing potential, is a necessary condition for achieving high reproductive levels (Tibary and Anouassi, 1997).

The GB compound betaine is a quaternary amine and trimethyl derivative of the amino acid glycine (N, N-dimethylglycine) that protects plants against salt stress (Storey and Wyn Jones, 1977) and modifies cellular reactions to osmotic stress (Petronini et al., 1992). The effects of GB on dromedary and camel spermatozoa fertility rates have not been studied, although it has been demonstrated that GB improves the quality of cryopreserved ram spermatozoa (Sanchez-Partida et al., 1998). However, even one day of storage does not produce good fertility findings (Murase et al., 1990 and Zeidan et al., 2001).

During storage at 5°C for up to 3 days, the current study sought to determine the impact of adding GB at concentrations of 100, 200, or 300 mM to cooled camel spermatozoa on semen quality and enzymatic activity. The fertility rates of camel spermatozoa that were cooled at 5°C for up to one day and then mixed with various concentrations of GB were also noted.

MATERIALS AND METHODS

The experimental study was conducted between December 2020 and April 2021 at Matrouh Animal Production Research Station, Camel Studies and Production Development Center, Matrouh Governorate (500 kilometers from Cairo), North West of Egypt which belong to the Animal Production Research Institute at Dokki, Giza, Egypt.

The goal of the experiment was to find out how adding glycine betaine (GB) to cooled camel spermatozoa at concentrations of 100, 200, or 300 mM affected the quality and enzymatic activity of dromedary camel semen while it was kept at 5 °C for up to three days. The fertility rates of camels that were artificially inseminated with spermatozoa that had been stored at 5 °C for up to adays with or without GB were estimated.

1. Experimental Animals

This study used five male dromedary camels (*Camelus dromedaries*), weighting between 500 and 600 kg live body weight and ranging in age from 6 to 10 years. All of the camels were in good health, clinically free from external or internal parasites, and had a long history of reproducing successfully in the herd.

2. Feeding and Management

The camels' daily ration was calculated according to Banerjee (1988). During the breeding season, an average of 35 kg of Egyptian Clover (*Trifolium alexandrinum*) and 7 kg of rice straw were supplied per head every day. During the dry season (non-breeding season), each camel received roughly 2 k.g of commercial concentrate combination, 2 kg of Egyptian clover hay and 9 kg of rice straw daily. All the camels got free access to clean and fresh water.

The camels were kept in a yard with a shared food basin and a shared covered watering trough on a concrete floor. In a tight space, camels could freely move around.

3. Camel Semen Collection

According to Abd El-Raouf et al. (1975), twelve ejaculates from each camel bull were collected using an artificial vagina (AV) between the hours of 8:00 and 10:00 in the morning. The technique described by Zeidan (2002) was carried out using a modified artificial vagina (30 cm long and 5 cm internal diameter, IMV, France). The inner liner's temperature was controlled at 45–50 °C while AV was filled with water at 50–55 °C.

4. Features of Sperm

4. 1. Sperm motility (%)

Due to the fluid materials, camel sperm motility (%) was generally observed as an oscillating motion of the flagellum (Agarwal et al., 2004). Using the procedure outlined by Salisbury et al. (1978), the percentage of sperm motility was assessed using one droplet of the diluted semen after each storage period.

4. 2. Dead spermatozoa (%)

According to Hackett and Macpherson (1965), eosin / nigrosin staining process was performed by dissolving 1.67 gm of eosin and 10.00 gm of nigrosin in distilled water for up to 100 ml.

4. 3. Abnormal spermatozoa (%)

Watson (1975) found that the same smears used to calculate the percentage of live/dead spermatozoa contained abnormal spermatozoa (%) based on their morphology.

4. 4. Spermatozoa with acrosome damage (%)

According to Watson (1975), the percentages of acrosome damage (%) were assessed.

4. 5. Spermatozoa chromosome damage (%)

Toluidine blue staining was carried out using the technique described by Erenpreiss et al. (2004). Smears were fixed for 1 minute in ethanol-acetic glactial (3:1, v/v), and then for 3 minutes in

70% ethanol. Smears were rinsed in distilled water, air-dried, then hydrolyzed for 20 min in 1 Mm HCL. Each smear was covered with one drop of 0.025% Toluidine Blue in Mcllvaine buffer (Sodium citrate-phosphate) at 4.0 pH. Smears were examined using a light microscope at a 1000x magnification. 300 sperm cells from each sample were examined to determine the proportion of chromatin damage. Spermatozoa stained in the range of green to light blue were thought to have normal chromatin, whilst those stained in the range of dark blue to violet were thought to have damaged chromatin.

4. 6. Aspartate and Alanine Aminotransferase (U/10^6 Spermatozoa)

According to Reitman and Frankel (1957), the activity of the aspartate and Alanine-aminotransferase enzymes was assessed colorimetrically using the QCA Kit, Amposta, Spain.

4. 7. Fertility rate

In order to induce ovulation using the technique outlined by Anouassi et al. (1994), 78 she-camels were intramuscularly injected with 5000 IU of human chorionic gonadotropin (hCG) (1 ml-ampules packaged by EPICO, A.R.E.). According to Musa et al. (1992), the ovulation normally happens 24 to 36 hours later.

If she-camels were observed to be exhibiting estrous indications, inseminations were performed same day (the mature Graafian follicle has a diameter of 1.5 to 3.0 cm). Following an hCG injection, she-camels were artificially inseminated 48 hours later using the technique outlined by Tibary and Anouassi (1997).

A sexually mature she-camel weighing 500–600 kg was randomly assigned to one of four groups for artificial insemination. The groups were as follows:

1- She-camels in Group 1 (n-19) were artificially inseminated using 2 ml of chilled, GB-free semen containing $100x10^6$ motile spermatozoa.
2- She-camel insemination was performed artificially on group 2 (n-19) using chilled semen (2 ml) containing $100x10^6$ motile spermatozoa and 100 mM GB.
3- She-camel insemination was performed artificially on group 3(n-21) using chilled semen (2 ml) containing $100x10^6$ motile spermatozoa and 200 mM GB.
4- She-camel insemination was performed on group 4(n-19) using chilled semen (2ml) containing $100x10^6$ motile spermatozoa and 300 mM GB.

The cervix was stabilized per-rectum before the insemination pistol was inserted into the vagina and into both uterine horns to deposit semen. By palpating the uterus, a pregnancy was identified two months (60 days) following the day of insemination.

5. Statistical Analysis

The General Linear Model (GLM) algorithm of SAS software (SAS, 2006) was used to statistically analyze the data using a two-way design (ANOVA). The multiple range tests by Duncan (1955) wre employed to find substantial variations in means. Prior to statistical analysis, percentage values were converted to arc-sin values. The t-test was used to compare the means on an individual basis. The Chi-square test was used to examine the fertility rate.

RESULTS

1. Camel Semen Quality

In comparison to 300 mM GB or free-GB media, the mean percentages of motile camel spermatozoa supplemented with various concentrations of GB (100 or 200 mM) exhibited considerably (P<0.05) increased (Table 1). Additionally, the percentage of camel spermatozoa that were motile reduced dramatically (P<0.05) as storage time at 5 ^{o}C advanced.

However, the mean percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa were significantly (P<0.05) lower than 300 mM GB or free-GB medium (Tables 2, 3, 4 and 5).

Table (1). Mean percentages of motile camel spermatozoa added with different Glycine betaine levels during storage at 5°C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	65.13±1.16	68.19±2.11	68.25±2.08	61.28±1.14	65.71±2.08^{A}
1	54.37±0.87	62.18±1.02	60.42±1.01	50.84±0.78	56.95±1.93^{B}
2	48.09±0.64	53.24±0.86	51.72±0.84	42.68±0.61	48.93±1.78^{C}
3	27.58±0.34	38.92±0.53	36.81±0.54	20.96±0.26	31.06±0.67^{D}
Mean	48.79±1.76^{b}	55.63±1.92^{a}	54.30±1.86^{a}	43.94±1.52^{c}	50.66

Table (2). Mean percentages of dead camel spermatozoa added with different Glycine betaine levels during storage at 5°C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	31.26±0.51	25.18±0.32	25.93±0.34	36.45±0.36	29.70±0.17^{D}
1	39.11±0.62	31.23±0.56	32.84±0.57	40.18±0.60	35.84±0.36^{C}
2	45.12±0.62	38.12±0.54	39.46±0.65	49.25±0.70	42.98±1.58^{B}
3	68.70±2.15	54.36±0.89	56.14±0.85	74.58±2.19	63.44±2.03^{A}
Mean	46.04±1.63^{b}	37.22±0.41^{c}	38.59±0.46^{c}	50.11±0.72^{a}	42.99

Table (3). Mean percentages of abnormal camel spermatozoa added with different Glycine betaine levels during storage at 5°C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	24.82±0.19	19.22±0.16	20.13±0.18	32.16±0.23	24.08±0.12^{C}
1	27.13±0.32	20.43±0.30	23.84±0.31	34.11±0.41	26.37±0.19^{C}
2	38.56±0.46	27.16±0.45	28.31±0.43	42.54±0.51	34.14±0.26^{B}
3	54.19±1.38	35.28±0.81	37.16±0.72	73.45±1.73	50.02±0.82^{A}
Mean	36.17±0.63^{b}	25.52±0.48^{c}	27.36±0.48^{c}	45.56±0.74^{a}	33.65

Table (4). Mean percentages of acrosome damage of the camel spermatozoa added with different Glycine betaine levels during storage at 5°C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	7.11±0.43	4.17±0.19	4.32±0.26	7.82±0.47	5.85±0.09^{C}
1	7.23±0.45	4.60±0.22	4.50±0.28	9.14±0.48	6.36±0.12^{C}
2	9.34±0.23	6.28±0.10	6.71±0.13	10.45±0.51	8.19±0.17^{B}
3	10.26±0.31	7.45±0.61	7.86±0.19	16.96±0.42	10.63±0.32^{A}
Mean	8.48±0.10^{b}	5.62±0.08^{c}	5.84±0.09^{c}	11.09±0.13^{a}	7.75

Table 5. Mean percentages of chromatin damage of the camel spermatozoa added with different Glycine betaine levels during storage at 5°C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	3.56±0.18	2.13±0.06	2.74±0.07	4.69±0.32	3.28±0.05^{C}
1	3.84±0.23	2.46±0.07	2.81±0.09	5.28±0.40	3.59±0.06^{C}
2	5.16±0.37	4.19±0.14	5.58±0.20	8.73±0.43	5.91±0.09^{B}
3	8.75±0.38	5.56±0.21	6.93±0.21	12.54±0.35	8.44±0.11^{A}
Mean	5.32±0.07^{b}	3.58±0.05^{c}	4.51±0.06^{c}	7.81±0.10^{a}	5.30

In all above Tables :

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

2. Enzymatic Activity

According to Tables 6 and 7, camel spermatozoa were diluted with LYC diluent added with 100 or 200 mM GB medium compared with 300 mM GB or free-GB medium during storage at 5°C for up to 3 days had significantly (P0.05) decreased levels of the enzymes aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT).

Table (6). Activity of Aspartate-aminotransferase enzyme (U/10^6 spermatozoa) of the camel spermatozoa added with different levels of Glycine betaine, during storage at 5^{o}C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	47.36±1.18	40.25±0.89	42.56±1.05	54.23±1.64	46.10±0.72^{C}
1	49.71±1.27	46.52±1.82	46.73±1.18	57.42±1.92	50.09±0.74^{C}
2	58.23±1.97	49.16±1.94	52.18±1.96	62.36±2.04	55.48±0.96^{B}
3	74.52±2.16	56.18±1.93	58.24±1.96	85.64±2.78	68.64±2.63^{A}
Mean	57.45±1.07^{b}	48.02±0.76^{c}	49.92±0.76^{c}	64.91±2.48^{a}	55.07

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

Table (7). Activity of Alanine-aminotransferase enzyme (U/10^6 spermatozoa) of the camel spermatozoa added with different levels of Glycine betain during storage at 5^{o}C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)				Mean
	Control	100	200	300	
0	40.76±0.28	36.28±0.31	37.59±0.32	43.12±0.62	39.43±0.62^{C}
1	41.37±1.15	37.26±0.75	39.65±0.82	46.82±1.25	41.27±0.68^{C}
2	46.28±1.36	46.35±1.36	47.16±1.23	58.47±1.86	49.56±0.74^{B}
3	60.29±2.16	57.86±1.84	58.42±1.92	74.28±2.08	62.71±2.13^{A}
Mean	47.17±0.87^{b}	44.43±0.86^{c}	45.70±0.94^{c}	55.67±0.64^{a}	48.24

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

3. Fertility Rate

Fertility rates of the cooled camel spermatozoa with LYC diluter containing 100 and 200 mM GB (Table 8) enhanced significantly (*P<0.05*) with LYC diluter containing 100 mM GB (52.63 %) or 200 mM GB (47.17 %) compared to 300 mM GB (26.31 %) or the cooled semen without GB addition GB (31.57 %).

Table (8). Fertility rate of the dromedary she-camel artificially inseminated with the cooled camel semen added with different levels of Glycine betaine (GB) during storage at 5^{o}C for up to one day.

Type of the camel semen	No. of she-camel inseminated	No. of she-camel conceived	Fertility rate (%)
Cooled semen without GB addition	19	6	31.57 ^{b}
Cooled semen added with 100 mM GB	19	10	52.63 ^{a}
Cooled semen added with 200 mM GB	21	10	47.61 ^{a}
Cooled semen added with 300 mM GB	19	5	26.31 ^{b}

a and b denoted within the same column with different superscripts are significantly different at (*P<0.05*).

DISCUSSION

Camel semen is the male generative fluid, since it contains the male gametes. It is deposited into the vagina of she-camels or collected by artificial vagina for storage or artificial insemination. In general, semen consists of two portions (spermatozoa and *seminal* plasma). Evans and Maxwell (1987) recorded that, the temperature of semen at ejaculation is about (37.5 ^{o}C); hence exposure of

semen to above this temperature increase the metabolic rate, loss of energy reserves and decreases the life span of spermatozoa. Moreover, temperature above 45 ^{o}C will kill spermatozoa.

Betaine belongs to the group of substances known as compatible solutes and both mammalian kidney cells and bacteria use unique betaine transporters to internalize the molecule, especially in hyperosmotic conditions (Nakanishi et al., 1990). Once within the cell, suitable solutes aid in controlling internal osmolality with little negative impact on enzyme activity or other cellular processes (Petronini et al., 1992). When spermatozoa are extended in buffered yolk medium, chilled and maintained at around 5^{o}C until insemination, the temperature of the stored semen affects spermatozoa metabolism (Anderson, 1945). Conversely, lower temperatures make storage last longer by slowing down cellular metabolism, however spermatozoa frequently experience cold shock at temperatures between 0 and 20 ^{o}C (Watson, 1981; White, 1993)

According to Zhang et al. (2001) in bull spermatozoa and Ahmadi (2020) in dromedary spermatozoa, adding GB at concentrations of 100 or 200 mM to camel spermatozoa increases their percentage of motile spermatozoa when stored at 5^{o}C for up to 3 days. Additionally, the presence of GB in the camel semen diluter was assessed for its ability to inhibit the development of reactive oxygen species (ROS), which kill spermatozoa.

Therefore, sperm damage is caused by excessive levels of ROS brought on by lipid peroxidation. The storage of camel spermatozoa in egg yolk diluter is problematical because seminal plasma contains phospholipase A, an enzyme that coagulates egg yolks which is toxic to spermatozoa (Roy, 1957; Iritani and Nishikawa (1963); Aamdal et al.; 1965; and Roca et al., 1992). This enzyme is produced by the Cowber's glands.

The increased activity of antioxidant enzymes like AST and ALT enzymes, which may be brought on by GB addition and raise the seminal plasma's capacity to alleviate oxidative stress. It may be the cause of these enhancements of the camel spermatozoa. In general, through hydration interactions, GB can preserve the three-dimensional structure of complex molecules like RNAse that have been thermally destabilized in the presence of urea (Burg and Peters, 1998).

Our findings showed that adding GB at concentrations of 100 or 200 mM to the cooled camel spermatozoa with LYC extender at 5°C increased significantly (*P<0.05*) the percentages of motile spermatozoa while decreasing significantly (P 0.05) the percentages of dead, abnormal, and acrosome damage, chromatin damage, as well as the activity of the AST and ALT enzymes. When diluted semen was combined with 100 or 200 mM GB, the proportion of motile camel spermatozoa increased significantly (*P<0.05*). Whereas with 300 mM GB or free-GB medium, they decreased (*P<0.05*) (Tables 1 to 7).

Similar results were discovered by Ahmadi (2020) in camel spermatozoa and by Zhang et al. (2001) in bull spermatozoa. It's interesting to note that the percentage of motile camel spermatozoa reduced significantly (P<0.05) when storage at 5°C was prolonged, whereas sperm damage and AST and ALT enzyme activity rose significantly (*P<0.05*) in dromedary camel spermatozoa (Ahmadi, 2020).

According to Zhang et al. (2001) and Ahmadi (2020), when storage time rose, the proportion of damaged sperm and the activity of the AST and ALT enzymes both significantly increased (P<0.05).

In the camel spermatozoa, Shannon and Curson (1972) found that sperm damage was a source of amino acid oxidase, which produces H_2O_2 as a result, increasing the percentage of sperm damage and functional membrane as storage time increased (Zeidan et al., 2001 and Ahmadi, 2020).

These outcomes could be the result of an overdose of GB or free-GB medium, which can harm the antioxidant system, decrease glutathione peroxidase activity, increase lipid peroxidation, and ultimately cause the death of sperm cells (Mezes and Salyi, 1994). They could also be the result of increased cellular stress, which is accompanied by a decrease in protein synthesis and an increase in protein degeneration (Hackett and Macpherson, 1965).

Additionally, there were changes in the fertility rate between cooled semen added with 100 or 200 mM GB compared to free-GB medium or 300 mM GB that were substantially different (*P<0.05*) (Table 8). The proportion of motile spermatozoa stored in LYC extender during storage at 5 ^{o}C, on the other hand, showed that GB increased camel spermatozoa survivability. According to Sanchez-Partida et al. (1998), GB may be improving sperm quality by stabilizing membrane phospholipids.

Furthermore, compared to free-GB (31.57%) and 300 mM GB (26.31%), the fertility rate in she-camels inseminated with cooled camel spermatozoa supplemented with 100 (52.63%) or 200 mM GB (47.61) was considerably ($P<0.05$) greater.

These outcomes may be attributed to the fact that spermatozoa supplemented with GB at concentrations of 100 or 200 mM survive longer when stored at 5 ^{o}C for up to one day than those added with 300 mM GB or free-GB medium (Zhang et al., 2001) in the case of bull spermatozoa and Ahmadi (2020) in the case of dromedary camel spermatozoa.

CONCLUSION

In conclusion, camel spermatozoa maintained at 5°C were extended with Lactose-Yolk-Citrate extender combined with 100- or 200-mM Glycine betaine (GB). Camel sperm motility, acrosome integrity, chromatin integrity, sperm ultrastructure, and eventual fertility rate were measured. Therefore, sperm quality was improved for artificial insemination programs in dromedary she-camels as long as the viability and fertility of camel spermatozoa supplemented with GB at concentrations of 100 or 200 mM.

REFERENCES

Aamdal, J., Lyngset, O. and Fossum, K. 1965.Toxic effect of lysolecithin on sperm. Nord. Vet. Med., 17: 633-639.

Abdel-Raouf, M.F., Fatah El-Bab, M.R. and Owaida, M.M. 1975. Studies on reproduction in the camel (*Camelus dromedarius*) V. Morphology of the testes in relation to age and season. J. reproduction and fertility, 43 (1): 109-116. https://doi.org/10.1530/jrf.0.0430109

Agarwal, V.K., Ram, L., Rai, A.K., Khanna, N.D. and Agarwal, S. P. 2004. Physical and biochemical attributes of camel semen. J. Camel Sci., 1: 25-30.

Ahmadi, E. A. A. 2020. Effect of age on sperm survivability of the camel spermatozoa supplemented with Glycine betaine during storage at 5 ^{o}C. J. Egyptian Vet. Med. Assoc., 80(2): 237-260.

Anderson, J. 1945. The semen of animal and its use for artificial insemination. Technical Communication (Imperial Bureau of Animal Breeding Genetics), Edinburgh, UK, Printed by Oliver and Boyd,

Anouassi, A., Tibary, A., Adnani, M. and Sghiri, A. 1994. Preovulatory phase characterization in *Camelus dromedarius* and induction of ovulation. Proc. of conference organized by FIS in Niamey, Niger, 159-167.

Banerjee, G.C. 1988. Feeds and Principles of Animal Nutrition. Primalani for Oxford and IBH Publishing Co. 66 Janpath, New Delhi, India.

Burg, M. B. and Peters, E. 1998. Effects of glycine betaine and glycerophosphocholine on thermal stability of ribonuclease. American J. Physiol., 274: 762-765.

Duncan, D. B. 1955. Multiple ranges and multiple F-tests. Biometrics, 11: 1-42.

Erenpreiss, J., Jepson, K., Giwercman, A., Tsarev, I., Erenpreisa, J. and Spano, M. 2004. Toluidine blue cytometry test for sperm DNA conformation: Comparison with the flow cytometric sperm chromatin structure and TUNEL assays. Hum. Reprod., 19: 2277-2282.

Evans, G. and Maxwell, W.C.M. 1987. Salamons Artificial Insemenation of Sheep and Goats. Butterworth, Sydney (Australia), p194.

Hackett, A.J. and Macpherson, J.W. 1965. Some staining procedures for spermatozoa. Canadian Vet. J., 6: 55-62.

Iritani, A. and Nishikawa, Y. 1963. Studies on the egg yolk coagulating factor in goat semen.III. Release of some acids accompanied by the coagulating phenomena. Japan J. Anim. Reprod., 8: 109- 112.

Matter, M.A.S. 2019. Reproductive and physiological studies on the male dromedary camels during breeding and non-breeding seasons under Egyptian condition. MSc. Thesis, Fac. Agric., Al-Azhar University, Cairo, Egypt.

Mezes, M. and Salyi, G. 1994. Effect of acute selenium toxicosis on the lipid peroxide status and the glutathione system of broiler chickens. Acta Vet. Hung., 42:459-463.

Murase, T., Okuda, K. and Sato, K. 1990. Assessment of bull fertility using a mucus penetration test and a human chorionic gonadotropin stimulation test. Theriogenology, 34: 801-812.

Musa, B., Sieme, H., Merkt, H. and Hago, B.E.D. 1992. Artificial insemination in dromedary camels. Proc. 1st Intern. Camel Cong., Dubai, U.A.E., 179-182.

Nakanishi, T., Turner, R.J. and Burg, M.B. (1990). Osmoregulation of betaine transport in mammalian renal medullary cells. American. J. Physiol., 258:106-107.

Petronini, P.G., De Angelies, E.M., Borghetti, P., Borghetti, A.F. and Wheeler, K.P. 1992. Modulation by betaine of cellular responses to osmotic stress. Biochem. J., 282*:* 69-73.

Reitman, S. and Frankel, M. (1957). A colorimetric method for determination of semen oxaloacetic and glutamic pyruvic transaminases. Anim. Clin. Pathol. J., 16: 28-56.

Roca, J., Martinez, E., Sanchez-Volverd, M. A., Ruiz, S. and Vazquoz, S. M. 1992. Seasonal variation of semen quality in male goats: study of sperm abnormalities. Theriogenology, 38: 115-125.

Roy, A. 1957. Egg yolk coagulation enzymes in the semen and cowper,s gland of the goat. Nature, 179: 318- 319.

Salisbury, G.W., Van Demark, N.L. and Lodge, J.R. 1978. Physiology of Reproduction and Artificaial Insemination of Cattle. WH. Freeman and Company, San Francisco, USA.

Sanchez-Partida, L.G., Setchell, B.P. and Maxwell, W.M.C. 1998. Effect of compatible solutes and diluent composition on the post-thaw motility of ram sperm. Reprod. Fertil. Dev., 10: 347-57.

SAS 2006. SAS Users Guide. Statistical Analysis System. Inst. Inc. Cary, NC, USA.

Shannon, P. and Curson, B. 1972. Toxic effect and action of dead sperm on diluted bovine semen. J. Dairy Sci., 55: 614-620.

Storey, R. and Wyn Jones, R.G. 1977. Quaternary ammonium compounds in plants in relation to salt stress. Phytochemistry, 16: 447-53.

Tibary, A. and Anouassi, A. 1997. Theriogenology in Camelidae.1st Ed., Ministry of Agriculture and Information, U.A.E.

Watson, P.F. 1975. Use of a giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Rec., 97: 12-15.

Watson, P.F. 1981. The effect of cooled shock on sperm cell membranes. In The Effects of Low Temperatures on Biological Membranes Eds., G. J. Morris and A. Clarke, Academic Press, London. 189- 218

White,I.G. 1993. Lipids and calcium uptake of sperm in relation to cold shock and preservation: a review. Reprod. Fertil. Dev., 5: 639- 58.

Wilson, R.T. 1984. The camel. Longman, London, pp. 83-101.

Zeidan, A.E.B. 2002. Semen quality, enzymatic activities and penetrating ability of spermatozoa into she- camel cervical mucus as affected by caffeine addition. J. Camel Practice and Res., 9 : 153-161.

Zeidan, A.E.B., Habeeb, A.A.M., Ahmadi, E.A., Amer, H.A. and Abd El-Razik, M.A. 2001. Testicular and physiological changes of the male dromedary camels in relation to different ages and seasons of the year. Proc.2nd Intern. Conf. Anim. Prod. and Health in Semi – Arid Areas, El-Arish, North Sinai, Egypt., 147-160.

Zhang, B.R., Buhr, M., Kroetsch, T. and Leibo, S.P. 2001. Glycine betaine improves survival of fresh bovine spermatozoa. Reprod. Fertil. Dev., 13: 187-192.

تاثير اضافة الجلايسين بيتان على جودة السائل المنوى المبرد للجمال ومعدلات الخصوبة

علاء زيدان[1] ، احمد عامر[1] ، داليا الطحان[2] ، ليزا عبد الرافع[1]

[1] معهد بحوث الانتاج الحيوانى – مركز البحوث الزراعية –الجيزة مصر

[2] معهد بحوث الصحة الحيوانية_ مركز البحوث الزراعية_ معمل المنصورة- مصر

الملخص العربي

اجريت هذه التجربة لمعرفة تاثير اضافة تركيزات مختلفة من الجلايسين بيتين الى السائل المنوى للجمال المخفف بمخفف اللاكتوز ستريت اثناء الحفظ على درجة حرارة 5 مئوية لمدة ثلاثة ايام حيث تم تجميع السائل المنوى بالمهبل الصناعى من خمسة ذكور ابل بصحة جيدة وتم تخفيفه بمخفف اللاكتوز سترات بنسبة 1 : 3 وبنسبة تركيز 100 × 10^6 حيوان منوى/ 100 ملل سائل منوى مخفف وتم تقسيم السائل المنوى المخفف الى اربعة مجاميع اضيف اليها الجلايسين بيتين بنسب صفر كنترول و 100 و200 و300 ملليمول/ 100 مللى سائل منوى مخفف بالترتيب وتم حفظ السائل المنوى المخف والمحتوى على الجلايسين بيتين للمجاميع الاربعة على درجة حرارة 5 مئوية لمدة ثلاثة ايام وتم تقدير جودة السائل المنوى يوميا كما تم التلقيح الصناعى للنوق بالسائل المنوى من المجموعات الاربعة فى اليوم الاول من الحفظ وتم جسها بعد 45 يوما لمعرفة نسبة الخصوبة وقد تحصلنا على النتائج التالية :-

كانت النسبة الحيوية للحيوانات المنوية اعلى بدرجة معنوية $P<0.05$ بينما كانت النسبة المئوية للحيوانات المنوية الشاذة والحيوانات المنوية مشوهة الاكروسوم ومشوهة الكروماتين وكذلك كان نشاط انزيمى ال AST وال ALT منخفضة بنسبة معنوية $P<0.05$ وذلك فى مخفف اللاكتوز ستريت المضاف اليها الجلايسين بيتين بنسبة 100 او 200 ملليمول مقارنة مجموعة الكنترول اوالمحتوية 300 ملليمول وقد ادت زيادة مدة الحفظ الى حدوث تدهور فى جودة السائل المنوى بدرجة معنوية $P<0.05$ سواء فى المجموعات المحتوية على الجلايسين بيتين او الخالية منه الكنترول.

كانت النسبة المئوية للخصوبة للنوق الملقحة صناعيا بالسائل المنوى المخفف بمخفف اللاكتوز والمضاف اليه الجلايسين بيتين من المجاميع الاربعة هى 31.57% و52.63% و47.61% و26.31% للمجموعة الاولى والثانية والثالثة والرابعة بالترتيب.

من خلال ما تقدم يمكننا ان نوصى باضافة الجلايسين بيتان بتركيز 100 او 200 ملليمول الى السائل المنوى المخفف بمخفف السترات عند حفظه على درجة حرارة 5 مئوية لمدة ثلاثة ايام لاستخدامه فى التلقيح الصناعى للنوق .

الكلمات الدالة: الإبل، الخصوبة، الجلايسين بيتان، التلقيح الصناعى.