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EFFECT OF DIFFERENT AGES AND SEASONS ON QUALITY OF THE DROMEDARYCAMELSPERMATOZOASTORED AT 5°C

By

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ABSTRACT

Semen was collected, evaluated, extended with Lactose-yolk-citrate (LYC) extender and stored at 5 °C for 3 days. Sperm penetration into she-camel cervical mucus, during incubation at 37ºC for 4 hours in different seasons and ages of the camels was recorded.

The results revealed that, the percentages of sperm motility and storagability were significantly (P<0.05) higher in the breeding season of the dromedary camel at 5 to 10 years than non-breeding season and at 15 to 20 years old, during storage at 5°C. However, the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa were significantly (P<0.05) lower in the breeding season of the male dromedary camels at 5 to 10 and 10 to 15 years than the non-breeding season and at 15 to 20 years old. The advancement of storage time at 5°C for 3 days was significantly (P<0.05) decreased the percentage of sperm motility, while significantly (P<0.05) increased the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa either the breeding or non-breeding season with the different ages of the male dromedary camels. The penetrating ability of spermatozoa into she-camel cervical mucus was significantly (P<0.05) better during the breeding than non-breeding season. The advancement of incubation at 37ºC for 4 hours was significantly (P<0.05) decreased the penetrating ability of spermatozoa into she-camel cervical mucus during the breeding and non-breeding seasons. In conclusion we can recommend to collect and store the dromedary camel semen at 5°C for the artificial insemination (AI) programs.

Keywords:

Dromedary camel, Semen, Storagability, Penetration score.

INTRODUCTION

In the literature, information about the stimuli of the onset of the breeding season in the dromedary camel, are rather conflicting. Some studies showed short daylight appeared to be the stimulus to seasonality **(Merkt *et al*., 1990 and Musa *et al*., 1990)**. Full reproductive potential of the male camel is reached at 5-6 years **(Novoa, 1970)**. However, **Al-Qarawi *et al*. (2001)** reported that, the first ejaculate that contains higher concentrations of spermatozoa is produced at 6 years old in dromedary camel. Physiological capacity may increase up to 10 years, then remains at a more or less constant of fairly high level until 18-20 years of age **(Zeidan and Abbas, 2004 and Matter, 2018)**.

Moreover, the breeding activity in male dromedary camels in nomadic herds starts at five to six years of age and continues until 14 to 15 years, with some minor differences according to breed and geographical location **(El-Wishy 1988).** Although reproductive management of females can influence parameters such as age at first service, conception rate, calving rate and intercalving interval **(Khanna, 1990)**. Less information is available about management of male breeding activity.

Epididymal spermatozoa have been used in many laboratories because they are easier to get in some special species, cryopreserved epididymal spermatozoa are now used for intercytoplasmic sperm injection (ICSI) in human insemination **(Patrizio, 2000)**. Epididymal spermatozoa have been obtained and individual variation in cryoprotect toxicities have been studied for African antelope **(Loskutoff *et al*., 1996).**

Achievement of the high reproductive activity partially depends on the success of Artificial Insemination (AI) which in turn is dependent on quality of semen obtained and its capacity for dilution and storage with minimum loss of fertilizing ability **(Wilson,1984).** Generally, the live spermatozoa can be prolonged for several days on chilled storage (2-5 ºC). However, satisfactory fertility results are not always achieved after, as little as, one day of storage **(Murase *et al*., 1990 and Zeidan *et al*., 2001).**

The present study was carried out to study the effects of age and season of the year on the dromedary camel semen quality, during storage at 5 °C for 3 days. The penetrating ability of spermatozoa into she-camel cervical mucus during incubation at 37ºC for 4 hours was also assessed.

MATERIAL AND METHODS

The present study was conducted in the Laboratory of Physiology, Department of Animal Production, Faculty of Agriculture, Al-Azhar University, and Cairo,Egypt. The practical work was carried out in Zagazig and Belbies Abattoris, Sharkiya Governorate, during the period from June, 2015 to May 2016.

Experimental animals:

Fourty-six male dromedary camels (*Camelus dromedarius*) testes aging 5-20 years old and 400 to 600 kg body live 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.

The temperature–humidity index (THI) was estimated according to Livestock and Poultry Heat Stress Indices **(LPHSI, 1990)** as the following formula:

THI=db°F-(0.55-0.55×RH/100) (db°F-58.00).Where,db°F=drybulb temperature in Fahrenheit and RH=relative humidity. The obtained values of THI were classified as follow: less than 72 = absence of heat stress, 72 to < 74 = moderate heat stress, 74 to < 78 = severe heat stress and over 78 = very severe heat stress.

Camels sperm collection:

Epididymal spermatozoa was collected immediately after slaughtering. A total number of 46 clinically normal testes were collected during the breeding (n=23) and non-breeding (n=23) seasons, respectively.

Sperm recovery:

The processing of the samples was carried out directly at arrival to the laboratory, as soon as, possible. Genitalia were dissected, isolating the epididymis and vas deferens from its corresponding testes. Sperm recovery was carried out on 46 epididymis.

Semen extension:

Epididymal semen samples were collected, pooled and evaluated for each camel in the different seasons and ages. Semen was extended with Lactose-yolk-citrate (LYC) extender according to **Musa *et al.* (1992)**. The final extension rate was 1ml semen:4ml extender.

Chilling of semen at 5°C:

The test tubes containing extended semen were placed in a 500 ml beaker containing water at 30°C with a thermometer in order to facilitate periodic checking of the temperature during cooling period. Another test tubes containing extended semen only were placed in the beaker to maintain the extended temperature similar to that of semen (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. The cooled spermatozoa were kept at 5°C for up to 0, 1, 2 and 3 days. After each storage time (0, 1, 2 and 3 days), percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, and acrosome damage of spermatozoa, were recorded. Enzymatic activity (ALT and AST enzymes) were also determined during storage at 5°C for up to 3 days according to **Reitman and Frankle (1957).**

1. Epididymal sperm characteristics:

Epididymal spermatozoa were collected directly after slaughtering from the body of epididymis (corpus of epididymis).Each corpus of epididymis region was cut to allow the escape of its contents in buffered citrate solution (0.5 ml NaCl) for the determination of semen characteristics immediately after collection.

1.1. Camel sperm motility (%):

Generally, camel sperm motility (%) was detected as an oscillatory motion the flagellum, but not progressive due to the viscous materials according to **Tibary and Anouassi (1997)**. Sperm motility was estimated by adding one drop of the diluted fresh semen with physiological saline (0.9% sodium chloride) on the dry, clean and pre-warmed (37°C) glass slide. With regard to extended semen, the percentage of sperm motility was determined using one drop of the extended semen after each storage period. The drop of the extended semen was covered by a warmed cover slip and immediately examined using high power magnification (40x).

1.2. Dead camel spermatozoa (%):

The eosin/nigrosin staining procedure was carried out by dissolving 1.67 gm eosin and 10 gm nigrosine in distilled water up to100 ml according to **Hackett and Macpherson (1965)**. The percentage of dead spermatozoa was calculated from 200 spermatozoa which were counted in each slide in the different microscopical fields using a hand counter.

1.3. Camel sperm morphology (%):

The morphological abnormalites of spermatozoa (%) were determined in the same smears

prepared live/dead spermatozoa ratio.

1.4. Acrosome damage of spermatozoa (%):

Assessment of the percentages of acrosome damage of spermatozoa and acrosome damage (%) were 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 (xl00).

1.5. Chromatin damage of spermatozoa (%):

Toluidine blue staining was performed as the method described by **Erenpreiss *et al.* (2004)**. Smears was fixed in ethanol for 3 mins.Thereafter smears were hydrolyzed for 20 min in 1 Mm hydrochloric, rinsed in distilled water and air-dried. One droplet of 0.025% Toluidine blue in Mcllvaine 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 to have normal chromatin, while spermatozoa stained with dark blue to violet were considered to have damaged chromatin.

1.6. Sperm-cell concentration ($\times 10^6$/ml):

The spermatozoa were counted using haemocytometer according to **Salisbury *et al*.** as follow

a. Semen was drawn to 0.5 mark of the special pipette used for RBC's count and diluting (normal saline 3% sodium chloride) fluid was drawn in 101 mark to give 200 times dilution of the semen in the pipette. The pipette was then gently rotated for proper mixing. Then the haemocytometer was charged with the diluted fluid.

b.The total number of spermatozoa counted in five large squares (four corners and one central) using the low or high power. Total number of spermatozoa of 5 squares were counted and added four zero and the result expressed as million per cubic millimeter of semen.

1.7. Sperm penetration (Score):

Sperm penetration into she-camel cervical mucus with different ages of the male camel was assessed. Cervical mucus was obtained from she-camel. A portion of the mucus was sucked into polyethylene sealed tubes with 2 mm internal diameter to provide a column of 6 cm length. During breeding and non-breeding season with the different ages camel semen was

collected and extended with Lactose-yolk-citrate extender as described by **Musa *et al*. (1992)** and then placed into 2 ml cuvettes (1 ml each). The tubes containing the mucus were inserted (open end) into the cuvettes containing the extended semen and incubated at 37ºC for up to 4 hours. Sperm penetration was judged as the rank score as the method described by **Eskin *et al*. (1973) and Hanson *et al*. (1982)**.

Statistical analysis:

Data were statistically analyzed by ANOVA in Factorial design using General Linear Model (GLM) procedure of SAS **(SAS, 2000).** Duncan's New 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. Penetration score was analyzed by Chi-square test.

RESULTS AND DISCUSSION

1. Camel semen quality during storage at 5°C:

1.1. Percentage of sperm motility:

Data presented in (Table 1) showed that, the effect of season of the year on the percentages of sperm motility during storage at 5ºC was significantly (P<0.05). The percentages of sperm motility during the breeding season was significantly (P<0.05) increased as compared with non-breeding season, during storage at 5°C for up to 3 days. The highest (P<0.05) value of the percentage of motile spermatozoa was recorded, during the breeding season, while the lowest (P<0.05) value was recorded during non-breeding season (Table1). These results are in agreement with those of **Zeidan *et al.* (2008) and Abdalla *et al*. (2011)** in the dromedary camels. These changes in sperm motility are consistent with the idea that there is a transition of semen quality from high in the breeding to low in non-breeding season. In addition, the changes in photoperiod is the principle environmental cue of the changes in the testosterone levels. Decreasing day length in autumn and winter was stimulate gonadal activity, while increase of day light length in spring and summer have the reverse effect **(Pelletier and Ortavant, 1975 and Linocln and Peet,1977).**

In respect to age, the effect of ages on the percentage of sperm motility was significantly (P<0.05). The highest (P<0.05) values of sperm motility were recorded with the camels at 5-10 years and at 10-15years, while the lowest value was recorded with the camels at 15- 20 years (Table1).

The prolongation of storage at 5°C for up to 3 days decreased significantly (P<0.05) the percentage of sperm motility of the dromedary camels either the breeding or non-breeding season (Table1). These results are in agreement with those of **Ahmadi (2001) and Zeidan (2002)**. Similar trend was reported by **Zeidan *et al.* (2008)** in camel spermatozoa

These findings may be due to the increase of sperm motility causes an increase in sperm metabolic activity, consequently, increase of lactic acid production which in turn exerts a toxic effect on the sperm cells or attributed to decrease in the content of adenosine triphosphate which activated spermatozoa apparently ability of resynthesizing accompanied with a precipitous fall in the rate of fructolysis **(Mann and Lutwak - Mann, 1981)**.

Table (1): Mean percentages of motile camel spermatozoa, with different age and seasons of the year, during storage at 5^{o}C for up to 3 days.

Storage time (day)	Breeding season			Mean	Non-breeding season			Mean
	Age (year)				Age (year)			
	5-10	10-15	15-20		5-10	10-15	15-20	
0	66.41 ±0.94	64.82 ±0.87	56.42 ±0.66	62.55 ±2.48^{A}	57.92 ±0.53	56.81 ±0.64	53.61 ±0.48	56.11 ±1.05^{A}
1	57.39 ±0.86	53.90 ±0.65	48.25 ±0.58	53.18 ±2.39^{B}	45.76 ±0.48	45.97 ±0.61	46.14 ±0.37	45.95 ±0.89^{B}
2	45.64 ±0.65	46.25 ±0.48	40.8 7±0.37	44.25 ±1.38^{C}	40.85 ±0.51	38.70 ±0.47	27.62 ±0.19	35.72 ±3.34^{C}
3	30.87 ±0.42	30.78 ±0.19	25.19 ±0.29	28.94 ±1.53^{D}	21.47 ±0.18	17.65 ±0.13	12.45 ±0.12	17.19 ±2.13^{D}
Mean	50.07 ±0.87^{a}	48.93 ±0.43^{a}	42.68 ±0.61^{b}	47.23^{A}	41.50 ±0.45^{a}	39.78 ±0.53^{a}	34.95 ±0.28^{b}	38.74^{B}

A-D: Means with the different superscripts in the same column, differ significantly (P<0.05).
a-b: Means with the different superscripts in the same row, differ significantly (P<0.05).
n= 46 animals.

1.2. Percentage of dead spermatozoa:

Data presented in (Table 2) showed that, the percentage of dead camel spermatozoa as affected by the breeding and non-breeding seasons was significantly (P<0.05). The highest (P<0.05) value of the percentage of dead camel spermatozoa was recorded during non-breeding season,while the lowest (P<0.05) value was recorded during the breeding season. These results are in agreement with those of **Maiada (2011)** who found that, the percentage of dead camel spermatozoa during non-breeding season in hot-humid months was significantly (P<0.05) higher compared with hot-dry months or the breeding season,during storage at 5°C for up to 3 days.

In respect to age, the effects of ages on the percentage of dead camel spermatozoa were significant (P<0.05). The highest values of percentage of dead spermatozoa were recorded with camels at 15-20 years, while the lowest value was recorded with camels at 5-10 years and 10-15years.

The prolongation of storage at 5°C for up to 3 days was significantly (P<0.05) increased the percentage of dead spermatozoa of the dromedary camels either the breeding or non-breeding season (Table 2). The percentage of dead spermatozoa increased significantly (P<0.05) as time of storage increased. These results are in agreement with those of **Zeidan *et al.* (2008) and Maiada (2011)** in camel spermatozoa.

Table (2): Mean percentages of dead camel spermatozoa (%) with different ages and seasons of the year, during storage at 5^{o}C for up to 3 days.

Storage time (day)	Breeding season			Mean	Non-breeding season			Mean
	Age (year)				Age (year)			
	5-10	10-15	15-20		5-10	10-15	15-20	
0	17.82 ±0.12	19.27± 0.38	20.47 ±0.43	19.18 ±0.76^{D}	31.65 ±0.28	32.46 ±0.42	36.81 ±0.78	33.64 ±0.59^{D}
1	20.92 ±0.23	22.68 ±0.47	27.15 ±0.65	23.58 ±0.85^{C}	35.12 ±0.35	35.77 ±0.38	39.53 ±0.84	36.80 ±0.26^{C}
2	25.78 ±0.58	27.54 ±0.52	31.66 ±0.74	28.3 2±0.74^{B}	38.9 ± 0.52	39.81 ±0.27	48.37 ±0.86	42.36 ±0.65^{B}
3	30.54 ±0.65	33.42 ±0.78	42.16 ±0.92	35.37 ±0.49^{A}	45.38 ±0.65	47.11 ±0.18	53.15 ±0.92	48.54 ±0.78^{A}
Mean	23.76 ±0.34^{b}	25.72 ±0.28^{b}	30.36 ±0.47^{a}	26.61^{B}	37.76 ±0.41^{b}	38.78 ±0.64^{b}	44.46 ±0.58^{a}	40.33^{A}

A-D: Means with the different superscripts in the same column, differ significantly (P<0.05).

a-b: Means with the different superscripts in the same row, differ significantly (P<0.05).

1.3. Percentage of sperm morphology:

Data presented in (Table 3) showed that the percentage of abnormal spermatozoa as affected by the breeding and non-breeding seasons was significantly (P<0.05). The percentage of abnormal camel spermatozoa showed to be highest (P<0.05) in non-breeding season as compared with the breeding season, during storage at 5°C for up to 3 days.The highest (P<0.05) value of the abnormal camel spermatozoa was recorded in non-breeding seasons, while the lowest (P<0.05) value was recorded during the breeding season (Table 3). Similar trend was reported by **El-Maghraby (2007)** in goats. These results are in agreement with those of **Maiada (2011)** who found that, the highest (P<0.05) value of the camel abnormal spermatozoa was recorded during non-breeding season, while the lowest (P<0.05) value was recorded during the breeding season.

The advancement of storage at 5°C for up to 3 days showed significantly (P<0.05) increased the percentage of abnormal camel spermatozoa either the breeding or non-breeding season (Table 3). The percentage of abnormal spermatozoa increased significantly (P<0.05) as time of storage increased. Similar trend was reported by **Zeidan *et al.* (2008) and Maiada (2011)** in camel spermatozoa.

Table (3): Mean percentages of abnormal camel spermatozoa with different ages and seasons of the year, during storage at 5^{o}C for up to 3 days.

Storage time (day)	Breeding season			Mean	Non-breeding season			Mean
	Age (year)				Age (year)			
	5-10	10-15	15-20		5-10	10-15	15-20	
0	12.82 ±0.16	14.53 ±0.24	19.71 ±0.15	15.68 ±0.12^{D}	26.42 ±0.23	28.15 ±0.16	30.8 ±0.28	28.47 ±0.75^{D}
1	15.70 ±0.22	1635 ±0.38	21.35 ±0.28	17.80 ±0.16^{C}	28.65 ±0.61	30.24 ±0.27	38.46 ±0.43	32.45 ±0.82^{C}
2	17.24 ±0.13	19.47 ±0.16	24.18 ±0.35	20.29 ± 0.18^{B}	30.84 ±0.65	32.16 ±0.48	47.24 ±0.51	36.74 ±0.61^{B}
3	24.87 ±0.19	25..11 ±0.19	30.46 ±0.42	26.81 ±0.23^{A}	34.13 ±0.52	38.45 ±0.53	52.78 ±0.64	41.78 ±0.38^{A}

Mean	**17.65 ±0.16^{b}**	**18.86 ±0.16^{b}**	**23.92 ±0.28^{a}**	**20.14^{B}**	**30.01 ±0.86^{b}**	**32.25 ±0.62^{b}**	**42.33 ±0.53^{a}**	**34.86^{A}**

A-D: Means with the different superscripts in the same column, differ significantly (P<0.05).

a-b: Means with the different superscripts in the same row, differ significantly (P<0.05).

n= 46 animals.

In respect to age, the effect of ages on the percentage of abnormal spermatozoa was significant (P<0.05). The lowest values of abnormal spermatozoa (Table 3) were recorded in the camel at 5-10 years and 10-15years, while the highest value was recorded in the camel at 15- 20 years.

None of the available literature studied on the effect of the breeding and non-breeding season with different ages on the percentage of abnormal camel spermatozoa during storage at 5^{o}C.

1.4. Percentage of acrosome damage of spermatozoa:

Data presented in (Table 4) showed that, the percentage of acrosome damage of spermatozoa as affected by the breeding and non-breeding seasons was significantly (P<0.05). The highest (P<0.05) value of the acrosome damage of spermatozoa was recorded during non-breeding season, while the lowest (P<0.05) value was recorded during the breeding season.

From another point of view, **Lenz *et al.* (1977) and Jones and Stewart (1979)** indicated that extension and cooling of bull semen to 5°C caused acrosome swelling in about 50% of the spermatozoa. Subsequent freezing and thawing caused considerable ultrastructural changes to the acrosomes disruption of the plasma and outer acrosome membranes and dispersion of the acrosome contents and middle pieces (breakage of the plasma membrane and a reduction in the electron density of the mitochondrial matrix) of a high proportion of spermatozoa. This may be due to the higher proportion of sperm motility of spermatozoa recovered during winter and autumn seasons with the different times of incubation at 37°C for up to 2 hours, similar to that reported by **El-Gaafary (1987).**

Maiada (2011) found that, the highest (P<0.05) value of the acrosome damage of the dromedary camels spermatozoa was recorded during non-breeding season, while the lowest (P<0.05) value was recorded during the breeding season during storage at 5°C for 3 days. Similar trends were reported by **Zeidan *et al.* (2008) and Abdalla *et al.* (2011**). Moreover, storage of semen at low temperatures caused structural damage as a result of cold shock. The changes involved damage to the plasma membrane over the acrosome and the outer acrosome membrane and damage to the plasma membrane of the middle piece. These changes

are followed by a decrease in the proportion of spermatozoa with intact acrosomes and an increase in the release of enzymes into extracellular medium. Therefore, the morphological characteristics of sperm acrosomes and enzymes concentration in the extracellular medium, with initial motility gives the best indication, so far of initial quality, especially for frozen semen **(Zeidan *et al.*, 1998).**

Table (4): Mean percentages of acrosome damage of the male dromedary camel spermatozoa, with different ages and seasons of the year, during storage at 5°C for up to 3 days.

Storage time (day)	Breeding season			Mean	Non-breeding season			Mean
	Age (year)				Age (year)			
	5-10	10-15	15-20		5-10	10-15	15-20	
0	3.84 ±0.11	5.16 ±0.08	8.94 ±0.13	5.98 ±0.09^{D}	19.23 ±0.15	19.78 ±0.73	23.75 ±0.43	20.92 ±0.16^{D}
1	5.43 ±0.13	7.62 ±0.12	12.65 ±0.10	8.56 ±0.06^{C}	20.35 ±0.28	21.46 ±0.18	27.18 ±0.28	22.99 ±0.18^{C}
2	6.78 ±0.17	10.73 ±0.18	17.82 ±0.12	11.77 ±0.11^{B}	21.94 ±0.34	23.35 ±0.24	30.12 ±0.64	25.13 ±0.15^{B}
3	12.94 ±0.23	14.37 ±0.16	26.73 ±0.31	18.01 ±0.14^{A}	28.14 ±0.38	30.11 ±0.35	34.86 ±0.78	31.03 ±0.23^{A}
Mean	7.24± 0.16^{b}	9.47 ±0.12^{b}	16.53 ±0.10^{a}	11.08^{B}	22.41 ±0.31^{b}	23.67 ±0.19^{b}	28.97 ±0.34^{a}	25.01^{A}

A-D: Means with the different superscripts in the same column, differ significantly (P<0.05).

a-b: Means with the different superscripts in the same row, differ significantly (P<0.05).

In respect to age, the effects of ages on the percentage of acrosome damage were significant (P<0.05). The lowest (P<0.05) values of acrosome damage of spermatozoa was recorded with the camels at 5-10 years and 10-15years, while the highest (P<0.05) value was recorded with the camels at15- 20 years.

The prolongation of storage at 5°C for up to 3 days increased significantly (P<0.05) the percentage of acrosome damage of spermatozoa of the dromedary camels either the breeding or non-breeding season. The percentage of acrosome damage of spermatozoa increased significantly (P<0.05) as time of storage increased. Similarily, **Zeidan *et al.* (2008) and Abdalla *et al*. (2011**) found that the prolongation of storage at 5°C for up to 3 days increased

significantly (P<0.05) the percentage of acrosome damage of spermatozoa of the dromedary camels either the breeding or non-breeding seasons. Similar trend was reported by **El-Gaafary *et al.* (1993)** in hamster, **Maxwell and Stojanov (1996)** in ram and **Zeidan *et al.* (2008)** in camel spermatozoa.

1.5. Percentage of chromatin damage of spermatozoa:

Data presented in (Table 5) revealed that, the percentage of chromatin damage of spermatozoa in the dromedary camels were significantly (P<0.05) decreased during the breeding season as compared with the non-breeding season. Similar trends were reported by **Matter (2018)** in the dromedary camel spermatozoa. There are many fluctuation in damaged DNA spermatozoa such as imperfect of spermatogenesis process; apoptosis, reactive oxygen species, in vitro handling, and type of extender and cryopreservation stress **(Baiee *et al*., 2017).**

Table (5): Mean percentages of chromatin damage of the male dromedarycamel spermatozoa, with different ages and seasons of the year, during storage at 5°C for up to 3 days.

Storage time (day)	Breeding season			Mean	Non-breeding season			Mean
	Age (year)				Age (year)			
	5-10	10-15	15-20		5-10	10-15	15-20	
0	2.38 ±0.02	2.81 ±0.06	3.74 ±0.07	2.97 ±0.05^{D}	3.18 ±0.08	3.11 ±0.09	6.21 ±0.11	4.16 ±0.08^{D}
1	3.16 ± 0.08	4.23 ±0.08	6.37 ±0.10	4.58 ±0.07^{C}	5.31 ±0.10	5.17 ±0.10	8.63 ±0.13	6.37 ±0.42^{C}
2	5.25 ±0.09	5.14 ±0.12	7.45 ±0.11	5.94 ±0.11^{B}	8.24 ±0.12	8.19 ±0.16	11.86 ±0.16	9.43 ±0.17^{B}
3	8.72 ±0.11	8.16 ±0.14	10.26 ±0.15	9.04 ±0.16^{A}	14.86 ±0.15	12.37 ±0.19	16.58 ±0.24	14.60 ±0.26^{A}
Mean	4.87 ±0.06^{b}	5.08 ±0.11^{b}	6.95 ±0.14^{a}	5.63^{b}	7.39 ±0.16^{b}	7.21 ±0.13^{b}	10.82 ±0.24^{a}	8.64^{a}

A-D: Means with the different superscripts in the same column, differ significantly (P<0.05).

a-b: Means with the different superscripts in the same row, differ significantly (P<0.05).

Lioyd *et al*. (2012) confirmed that sperm DNA integrity was better in commercial diluent could be significantly increased DNA fragmentation during at 5 ^{0}C for up to 48 hours.

Similar trends were reported by **Khalifa *et al*. (2013) and Asmaa (2018)** in ram spermatozoa. However, **Pardana *et al*. (2016)** found that, the percentage of sperm chromatin integrity of dog spermatozoa was not significantly different between extender type, during storage at 5^0C . The prolongation of storage at 5^0C for three days was significantly (P<0.05) increased the percentage of chromatin damage of camel spermatozoa in different breeds either the breeding or non-breeding season. These results are partially agreement with those of **Asmaa (2018)** in ram spermatozoa.The phenomenon may be due to the decrease of adenosine triphosphate which activated apparently ability of resynthesizing.This was accompanied with precipation fall in the rate of fructolysis, consequently, increased chromatin damage **(Mann and Lutwak-Mann, 1981).**

3. Sperm penetration into cervical mucus as affected by season and age during incubation at 37ºC for up to 4 hours:

Fig. (1) shows that, the penetrating ability of spermatozoa into she-camel cervical mucus significantly (P<0.05) better during the breeding than non-breeding season. However, the advancement of incubation time at 37ºC for up to 4 hours significantly (P<0.05) decreased the penetrating ability of spermatozoa into she-camel cervical mucus during the breeding and non-breeding season. Similarly, the penetration score of spermatozoa was significantly (P<0.05) decreased with the advancement time of incubation at 37°C for up to 12 hours in camels at 5 to 10 years. **Aitken *et al.* (1983)** found a close correlation between human movement of spermatozoa and their penetrating ability into cervical mucus. **Murase *et al.* (1990)** reported that, the duration of sperm motility and penetration distance in the mucus closely correlated to the pregnancy and conception rate. Similar findings were recorded by **Zeidan (2002)** in the dromedary camels. **Abd El-Salaam *et al*. (2011)** found that, the use of lactose-yolk citrate (LYC) extender in penetrating ability of the extended spermatozoa into she-camel cervical mucous was insignificant.

In respect to age, the effect of ages Fig. (1) on the penetrating ability of spermatozoa into she-camel cervical mucus was significantly (P<0.05) better with the camel at 5-10 and 10-15 years than 15-20 years.

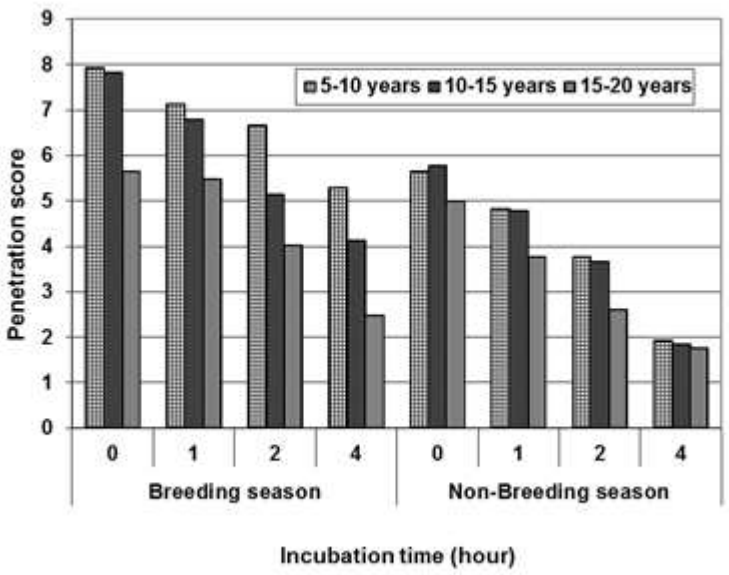

Fig. (1): Penetration score of the extended dromedary camel spermatozoa into she-camel cervical mucus in breeding and non-breeding season with different age, during incubation at 37ºC for up to 4 hours.

In conclusion it can be recommended to collect and storage of the dromedary camel semen at 5°C and frozen camel spermatozoa for artificial insemination (AI) programs especially during non-breeding season. Particularly, in the desert regions where liquid nitrogen may not be available for freezing and storage of frozen semen for long time to come through the non-breeding season under Egyptian environmental conditions.

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تاثير العمر وموسم السنة على جودة السائل المنوى للجمال اثناء الحفظ على درجة 5 م^0

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الملخص العربى

أجريت الدراسة على عدد 46 ذكرناضج وبالغ جنسياً من ذكور الإبل العربية تتراوح أعمارها من 5 – 20 سنة ، وتتراوح أوزانها من 400 – 600 كيلو جرام لدراسة تأثير مواسم السنة على صفات السائل المنوى الذى تم الحصول عليه من البربخ فى الإبل العربية فى المواسم والأعمار المختلفة حيث تم تخفيف السائل المنوى لذكور الإبل العربية المخفف بمخفف Lactose – Yolk – Citrate (LYC) وحفظه على درجة حرارة (5) درجة مئوية لمدة 3 أيام ، كما تم قياس مقدرة الحيوانات المئوية على النفاذية داخل مخاط عنق الرحم فى النوق خلال موسم النشاط والخمول الجنسى فى الإبل ذات الأعمار المختلفة.

واظهرت النتائج زيادة النسبة المئوية لحركة الحيوانات المنوية بعد التخفيف معنويًا (على مستوى 0.05) فى ذكور الإبل العربية عند عمر 5-10 سنة و 10-15 سنة مقارنة بالإبل عند عمر 15-20 سنة أثناء الحفظ على درجة حرارة 5 درجة مئوية لمدة 3 أيام سواء فى موسم النشاط أوالخمول الجنسى. انخفاض النسبة المئوية لكل من الحيوانات المنوية الميتة والشاذة وتالفة الأكروسوم فى ذكور الإبل العربية معنويًا (على مستوى 0.05) فى موسم النشاط الجنسى مقارنة بموسم الخمول الجنسى بعد التخفيف والحفظ على درجة حرارة 5 درجة مئوية لمدة 3 أيام ، بينما كان هناك زيادة فى النسبة المئوية لكل من الحيوانات المنوية الميتة والشاذة ، وتلف الاكروموسوم معنويًا (على مستوى 0.05) فى ذكور الإبل العربية عند عمر 15-20 سنة مقارنة بالإبل عند عمر 5-10 و 10-15 سنة سواء فى موسم النشاط أو الخمول الجنسى بينما تحسنت مقدرة الحيوانات المنوية على اختراق مخاط عنق الرحم للنوق معنويًا (على مستوى 0.05) وذلك أثناء التحضين على درجة حرارة 37^{o}C لمدة 4 ساعات ، وعند عمر 5-10 سنة و 10-15 سنة عنها عند عمر 15-20 سنة سواء فى موسم النشاط أو الخمول الجنسى. فى حين انخفضت مقدرة الحيوانات المنوية للإبل عند عمر 15-20 سنة على النفاذية داخل مخاط عنق الرحم للنوق مقارنة بالإبل عند عمر 5-10 أو 15-20 سنة سواء فى موسم النشاط أو الخمول الجنسى مع تقدم زمن الحفظ.

وبناءً عليه يمكن جمع وحفظ السائل المنوى للإبل العربية على درجة حرارة 5 درجة مئوية واستخدامها فى برامج التلقيح الاصطناعى وخاصة فى عمر 5 -15 سنة لرفع نسبة الخصوبة فى المناطق الصحراوية .